In vivo hydraulic conductivity of muscle: effects of hydrostatic pressure

EL RASHEID ZAKARIA, JOANNE LOFTHOUSE, AND MICHAEL F. FLESSNER
Nephrology Unit, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

Zakaria, El Rasheid, Joan Lofthouse, and Michael F. Flessner. In vivo hydraulic conductivity of muscle: effects of hydrostatic pressure. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2774–H2782, 1997.—We and others have shown that the loss of fluid and macromolecules from the peritoneal cavity is directly dependent on intraperitoneal hydrostatic pressure (pip). Measurements of the interstitial pressure gradient in the abdominal wall demonstrated minimal change when pip was increased from 0 to 8 mmHg. Because flow through tissue is governed by both interstitial pressure gradient and hydraulic conductivity (K), we hypothesized that K of these tissues varies with pip. To test this hypothesis, we dialyzed rats with Krebs-Ringer solution at constant pip of 0.7, 1.5, 2, 2.2, 3, 4.4, 6, or 8 mmHg. Tracer amounts of 125I-labeled immunoglobulin G were added to the dialysis fluid as a marker of fluid movement into the abdominal wall. Tracer deposition was corrected for adsorption to the tissue surface and for local loss into lymphatics. The hydraulic conductivity of the tissue was measured using a micropipette and servo-null system. The technique requires immobilization of the tissue by a porous Plexiglas plate, and therefore a portion of the tissue is supported. In agreement with previous results, fluid flux into the unrestrained abdominal wall was directly related to the overall hydrostatic pressure difference across the abdominal wall (pip = 0), but the interstitial pressure gradient near the peritoneum increased only ∼40% over the range of pip of 1.5–8 mmHg (20–28 mmHg/cm). K of the abdominal wall varied from 0.90 ± 0.1 × 10⁻³ cm²·min⁻¹·mmHg⁻¹ at pip = 1.5 mmHg to 4.7 ± 0.43 × 10⁻³ cm²·min⁻¹·mmHg⁻¹ on elevation of pip to 8 mmHg. In contrast, for the same change in pip, the unsupported abdominal wall muscle supported on the skin side had a significantly lower range of fluid flux (0.89–1.7 × 10⁻⁴ vs. 1.9–10.1 × 10⁻⁴ ml·min⁻¹·cm⁻²) than pip = 1.5 mmHg to 4.7 ± 0.43 × 10⁻³ cm²·min⁻¹·mmHg⁻¹ on elevation of pip to 8 mmHg. In contrast, for the same change in pip, the unsupported abdominal wall muscle in anesthetized rats varies with the superimposed hydrostatic pressure within the peritoneal cavity.

interstitium; convection; transport; solvent drag; peritoneal cavity

THE STEADY-STATE FLOW of water through tissue can be described by Darcy's law (19, 26)

\[ Q = -K A \frac{dP}{dx} \]  

(1)

where \( Q \) is fluid flux into the tissue, \( K \) is tissue hydraulic conductivity, \( A \) is cross-sectional area for flow, and \( dP/dx \) is tissue hydrostatic pressure gradient, where \( x \) is distance. This equation demonstrates that the flow velocity or flux per unit area \( (Q/A) \) is directly proportional to the product of \( K \) and \( dP/dx \). Therefore, an increase in either \( K \) or \( dP/dx \) will result in an increase in flow of an incompressible fluid through tissue. \( K \) is a complex function of the interstitial void fraction \( (\theta_{int}) \), the fraction of total tissue volume that is available to interstitial water) and structural properties of the tissue (19, 20). \( \theta_{int} \) is a function of the local interstitial pressure, \( P \), and the compliance of the tissue (1, 13). Although there have been measurements of \( K \) in well-defined in vitro systems (5, 12, 17, 26), there are few in vivo determinations in the literature (15, 18).

We have previously shown (7, 8, 11) that the fluid loss from the peritoneal cavity of the rat into the surrounding abdominal wall muscle is driven by hydrostatic pressure gradients directed from the peritoneal surface (mesothelium) across the muscle layer toward the skin. This fluid movement is clinically observed in the therapeutic use of the cavity as a dialyzer (3, 8, 10, 31) and in the pathological condition of ascites (4). Recent measurements in the abdominal wall of rats dialyzed at a nominal intraperitoneal hydrostatic pressure \( (P_{ip}) \) of 3, 6, or 8 mmHg have shown that, despite proportional rises of \( Q \) with \( P_{ip} \) (11), the slope of the interstitial pressure versus distance profile at the peritoneal surface \( (dP/dx) \) changes very little among the pressures investigated (8). From these observations and Darcy's law, we hypothesize that \( K \) of the muscle varies with \( P_{ip} \).

To investigate this question, we dialyzed rats at constant levels of \( P_{ip} \) between 0.7 and 8 mmHg with a solution containing a large protein that acted as a marker of fluid movement. Fluid flux \( (Q/A) \) across the peritoneum into the abdominal wall muscle was calculated from the deposition of the marker into a given area of the wall. A micropipette-servo-null system was used to measure the \( P \) profile in the abdominal wall muscle, from which \( dP/dx \) at the surface was obtained. \( K \) for the flow through the surface of the tissue was calculated from Eq. 1 as a first approximation to the value for \( K \) flow through the entire tissue (11, 19, 26).

METHODS

Animals

All experiments were performed in ~200-g female Sprague-Dawley rats (Charles River Laboratories). Female rats are used in these studies because they do not grow as rapidly as male rats, and the sizes of the experimental subjects are more uniform. Animals had free access to water and standard rat chow until the morning of the experiment. Five animals were used for each pressure level investigated. All procedures were approved by the University of Rochester Committee on Animal Resources.
Materials

\(^{125}\)I-labeled immunoglobulin G (IgG; Amersham anti-rabbit IgG, no. IM-134, immunoadsorbed against rat antigens, 5–20 \(\mu\)Ci/\(\mu\)g antibody protein) was used as a marker of fluid movement. The free unbound \(^{125}\)I was removed by repeated dilution and centrifugation with Centrisulc 30 microseparators (Amicon) to ensure <1% of free \(^{125}\)I before initiation of the experiment. Integrity of the tracer label was checked by trichloroacetic acid precipitation of plasma and peritoneal fluid before and after each experiment, and the recovered tracer in the total urinary volume collected during the course of the experiment was <1% of the total radioactivity injected. Details of these techniques are documented in our previous publications (see Ref. 11).

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 tracheotomy was performed to reduce airway resistance. Two arterial lines were established using PE-50 catheters, a left carotid artery catheter to allow for continuous blood pressure measurements on a pressure measurement system (PE-102 Statham pressure transducer; Window Graf, Gould Valley Instruments, OH) and a tail artery catheter for blood sampling. A venous catheter was secured into the left external jugular vein to allow hydration of the animal with infusion of Krebs-Ringer bicarbonate solution from an infusion pump (Harvard Apparatus 22). The animal’s rectal temperature was continuously monitored and maintained between 35.5 and 38.5°C with a servo-controlled warming blanket (Harvard Apparatus) and an overhead heating lamp. The peritoneal cavity was exposed through a midline abdominal incision (~1.5 cm), and the hollow viscera (duodenum to rectum) were removed using the technique described in a previous publication (32). The siltlike abdominal incision was closed using a continuous suture after careful inspection to ensure that there was no bleeding. This maneuver was necessary so that fluid in the cavity had access to the entire abdominal wall. In earlier experiments we discovered that the hollow viscera would float in the solution within the cavity and rest against the abdominal wall, preventing contact between the solution and that portion of the peritoneum. With the aid of a trocar, a multihole catheter was placed through the abdominal wall into the peritoneal cavity and secured with a purse string. A medium-sized catheter was placed through one of the holes in the catheter to allow for fluid sampling. A venous catheter was inserted for collection of urine during the experiment.

Dialysis procedures

An experiment was initiated with infusion of the dialysis fluid (prewarmed to 37°C) in an amount sufficient to raise \(P_{\text{ip}}\) to 0.7–1.5 mmHg below the desired pressure. A reservoir containing the rest of the dialysis fluid was then connected to the three-way valve attached to the intraperitoneal catheter. The reservoir was maintained at the exact level above the right heart to produce the desired \(P_{\text{ip}}\), which was measured every 15 min by the glass capillary manometer. \(P_{\text{ip}}\) typically matched the desired level 15 min after initiation of the experiment. The dialysis fluid used in all experiments was 5% bovine serum albumin in Krebs-Ringer bicarbonate solution containing (in g/l) 6.92 NaCl, 0.35 KCl, 0.37 CaCl\(_2\), 2 H\(_2\)O, 2.1 NaHCO\(_3\), and 0.16 KH\(_2\)PO\(_4\) and 1.18 ml of 1 M MgSO\(_4\)·H\(_2\)O.

The osmolality of the solution was adjusted to 290 ± 5 mosmol/kg by addition of NaCl. The solution was filtered with a 0.45-\(\mu\)m membrane (Nalgene) and stored at 4°C. Approximately 50 \(\mu\)Ci of \(^{125}\)I-IgG and 0.01% of Evans blue were added to each solution before the experiment. Peritoneal fluid and blood were sampled at 30-min intervals.

A laparotomy was performed at the end of the experiment. The residual peritoneal fluid was collected using a syringe and needle with plastic tubing attached. The remaining fluid in the reservoir and tubing system was collected. Sample volumes and spillage volumes were carefully accounted for to estimate the absolute volume recovered. Tissue samples from all quadrants of the abdominal wall were cut from the carcass and labeled as upper, middle, and lower (three samples from each location) bilaterally. A total of 18 (~1 g each) tissue samples (virtually the entire anterior abdominal muscle) were cut from each carcass. The apparent peritoneal surface area of each sample was directly measured before placing the sample in a preweighed vial to determine its wet weight and the total counts of the labeled marker. The total counts were corrected for loss caused by lymphatic drainage from the tissue and for adsorption to the tissue surface. The corrected total counts divided by the concentration of labeled protein in the cavity provided an estimate of the total fluid flow across the area of peritoneum assayed. Details of these procedures are described in Calculations.

Tissues from the upper abdominal wall in the epigastric and costal regions showed very large protein deposition (approximately twice the rate of deposition in other portions of the abdominal wall), which was demonstrated by deep blue coloration, high radioactivity, and even gross edema in the subcutaneous plane. We attributed the marked difference in the upper portions of the abdominal wall to their proximity to the thorax and their exposure to additional stresses of diaphragmatic and chest wall motion of respiration. Because the forces for fluid flow into this tissue were apparently different from those of the remainder of the abdominal wall tissue, these data were not included in the overall analysis. When pooled together, all other portions of the abdominal wall typically had a relatively uniform concentration with an SD of 20–30%.

Measurements

Interstitial hydrostatic pressure. The hydrostatic pressure profile within the anterior abdominal muscle was measured by the technique of Wiig and colleagues (28) as modified by Flessner (8) to allow for in vivo measurements of interstitial hydrostatic pressure in the anterior abdominal wall muscle.

Briefly, a skin flap was made in the left anterior abdominal wall and a multihole (1- to 2-mm radius each) Plexiglas grid was affixed to the wall with cyanoacrylate tissue glue (see Fig. 1A). The grid was then anchored to two ring stands to isolate that part of the anterior abdominal wall from diaphragmatic motion (8). The supporting grid is necessary to prevent movement of the tissue in a plane perpendicular to the pipette; this movement results in breakage of the pipette tip (8). Figure 1A shows the cross-sectional appearance of the rat once the desired pressure is attained. Before instillation of the full volume, the dimensions of the cavity were symmetrical on the supported and unsupported sides. A micropipette filled with 0.5 M NaCl solution mounted on a micromanipulation and coupled to a servo-null system (model SA, Instrumentation for Physiology and Medicine, San Diego, CA) was precisely advanced, through one of the Plexiglas pores, in 200-\(\mu\)m increments within the tissue. The pressure at a given tissue depth was measured with a strain-gauge pressure transducer (Statham P23A) and recorded in the Gould Win-
Windowgraf became constant and equal to Pip or the micropipette resistance. Interstitial pressure was measured until the pressure recorded in the dowgraf pressure recording system. Interstitial pressure measurements were made until the pressure recorded in the Windowgraf became constant and equal to Pip or the micropipette resistance. The measured pressures were plotted versus distance from the peritoneal surface. It was assumed that the hydrostatic pressure gradient measured through a pore in the Plexiglas grid (open to atmospheric pressure, see Fig. 1B) across the portion of the abdominal wall supported by Plexiglas equals the profile of the contralateral unsupported side (see Fig. 1C). The presence of skin on the unsupported side has been shown previously not to affect the initial slope of the pressure profile (8). In a typical pressure profile, the pressure on the external tissue surface was zero, and the interstitial pressure 200-µm deep to this surface was 0.6 mmHg (SD of measurements is ±1 mmHg (8); this reflects typical muscle pressures (28)). However, Fig. 1B illustrates how the plate may affect overall fluid flow into the tissue that it supports. Because the pore area of the grid is <3% of the total area and the overall pressure gradient (Pip – 0) is exerted across the muscle and the plastic grid, flow to the supported tissue may be driven by a different pressure gradient than in the unsupported side.

Accuracy of pressure measurements. As stated in Interstitial hydrostatic pressure, the accuracy of the pressure measurements in the abdominal wall is ±1 mmHg, whereas the position of the pipette tip in the tissue is accurate to approximately ±200 µm. In none of the animals investigated in the present study was a negative pressure near the peritoneal surface measured. The fact that the pressure profile near the peritoneal edge is positive even at low Pip indicates a specific trend in the measurements despite the inaccuracy of the technique in measurements of <2 mmHg.

To test the assumption that the pressure profile measured through a pore in the Plexiglas across the restrained tissue is equivalent to that of the contralateral side, we performed additional control experiments in five rats in which we determined the pressure profile through pores that are two, three, and four times larger than the typical 2–3 mm. We chose 4.4 and 8 mmHg as nominal hydrostatic pressures in the cavity. The pipette was inserted into the tissue at an angle of ~80°–90° to minimize errors of tip position in the tissue. The pressure profile was measured through each size of pore and compared. The profile and its initial slope dP/dx for each of the various pores were compared and did not show a significant difference. The overall mean (±SD) of dP/dx was 20.4 ± 2.9 and 24.4 ± 2.3 mmHg/cm for Pip of 4.4 and 8 mmHg, respectively. We have also observed that the conversion through the pores of bovine serum albumin stained with Evans blue produced approximately the same apparent blue intensity in the tissue underlying the pore as in the contralateral unrestrained side.

Volume measurements. Dialysate volume changes were determined by weighing the total solution in the reservoir-tubing-cavity system before and after the experiment using a digital balance (Mettler PM 2500). The density of the solution was assumed to be 1 g/ml. Radioactivity in the peritoneal fluid, plasma, tissue samples, and urine was determined in a Beckman Gamma 8000 counter.

Adsorption of marker to peritoneum. Each new batch of 125I-IgG was tested for the degree of adsorption of the marker to the abdominal wall peritoneum. As published in our previous paper (11), adsorption is the process of binding of the IgG in the dialysis solution to the peritoneal surface in the first minute of an experimental dwell. Because it adds to the total counts recovered in the tissue but does not represent true convective transport into the tissue, the adsorbed counts per minute (cpm) per unit surface area must be subtracted from the total counts in the tissue to arrive at an estimate of the cpm deposited by fluid flow. For each new batch of labeled IgG, two rats were injected intraperitoneally with ~40 ml of the dialysis solution containing the labeled marker. The solution was allowed to dwell 1 min, and then it was recovered by laparotomy. The cavity was washed three times...
with solution that contained no labeled marker. Total cpm recovered versus total cpm injected was determined. Several sections of abdominal wall tissue were cut, and the apparent surface area of each was measured. Each section was then placed in a vial and counted in a gamma counter to determine the surface density of adsorbed protein (cpm/cm²). These measurements were averaged and divided by the concentration of IgG (cpm/ml) in the cavity to obtain the normalized surface density of the adsorbed protein.$^B$ad{	extsubscript{absorbed}}. For a given experiment with the concentration of labeled protein in the cavity ($C_{ip}$) the amount of adsorbed protein on the surface of the abdominal wall was equal to $B_{ad{	extsubscript{absorbed}}}$.

Lymphatic flow from abdominal wall. In previous experiments (9), we loaded a small portion of the abdominal wall with labeled IgG and determined its fractional rate of removal from the tissue, $k_{lymph}$ (min$^{-1}$) with the technique of Reed and colleagues (25). The value of $k_{lymph}$ was previously found to be 0.43 ± 0.12 × 10$^{-3}$ min$^{-1}$ (9). The local rate of removal (cpm/min) = $k_{lymph}$ × $C_{PM}$tissue, where $C_{PM}$tissue = the total labeled protein in the tissue minus the protein adsorbed to the surface.

Calculations

The overall peritoneal fluid loss rate at each nominal $P_{ip}$ was calculated as

$$\text{fluid loss rate} = \frac{V_{f}(0) - V_{f}(t)}{t_f}$$  \hspace{1cm} (2)

where $V_{f}(0)$ is the initial fluid volume before instillation, $V_{f}(t)$ is the total fluid volume recovered by the end of the experiment from the peritoneal cavity-reservoir-tubing system as corrected for sampling and spillage, and $t_f$ is the total dwell time.

The tissue hydraulic conductivity ($K$) can be calculated from a rearrangement of Eq. 1

$$K = \frac{Q}{A \frac{dp}{dx}}$$  \hspace{1cm} (3)

$^{125}$I-IgG was used as a volume indicator and as a marker for fluid transport. Its deposition into tissue was used to determine the total fluid transport across the peritoneum into the abdominal wall tissue. By dividing by the interstitial pressure gradient at the surface of the tissue (estimated from the slope of the P profile near the peritoneum), the value of K calculated with Eq. 3 yields an estimate of the hydraulic conductivity of the tissue immediately adjacent to the peritoneal cavity. This approach does not depend on precise knowledge of the fluid path through the tissue, nor does it require a balance on the fluid as it passes through the tissue. As noted in Adsorption of marker to peritoneum, the total protein in the tissue must be corrected for adsorption to the surface and for lymphatic removal of protein from the tissue. The amount of adsorption was determined for each batch of isotope, and tissue samples were corrected on the basis of their surface area. Lymphatic removal from the abdominal wall interstitium was estimated from the fractional rate of protein elimination ($k_{lymph}$), which was found to be 0.43 × 10$^{-3}$ min$^{-1}$ (9). The protein removed from the tissue was calculated by integrating over the duration of the experiment the product of $k_{lymph}$ times the tissue concentration times the tissue volume. The following equation was used to obtain an estimate of $Q_{corr}$

$$Q_{corr} = \frac{tissue\ CPM - CPM_{adsorbed} + CPM_{removed \ by \ lymph}}{duration \ of \ experiment \times C_{ip}^{avg}}$$  \hspace{1cm} (4)

where tissue $CPM$ is the apparent cpm in a specific section of tissue, $CPM_{adsorbed} = B_{ad{	extsubscript{absorbed}}} \times$ area of tissue section; and $CPM_{removed \ by \ lymph} = k_{lymph} \ \int_{t_{0}}^{t_{f}} C_{PM}tissue dt$, where $C_{PM}$tissue = tissue $CPM - CPM_{adsorbed}$. $C_{PM}^{avg}$ is the time-averaged concentration in the peritoneal cavity, which was found to be nearly constant during the experiment. $Q_{corr}$ was inserted into Eq. 3, along with the measured area (A) of each tissue sample and the average $dP/dx$ for the experiment to calculate K.

Statistics

All data are presented as means ± SE. Paired Student’s t-test was used to assess differences in fluid flux as calculated for each pressure level for unsupported tissue versus supported tissue. Differences were accepted as statistically significant at $P < 0.05$.

RESULTS

Figure 2 shows the relationship between the overall peritoneal fluid loss rate and $P_{ip}$. This overall peritoneal fluid loss rate appeared to increase with the rise of $P_{ip}$ for nominal $P_{ip}$ ranging between 0.7 and 8 mmHg.

The hydrostatic pressure gradients in the abdominal wall measured at different $P_{ip}$ are shown in Table 1. The slope from the first 600–800 µm of pressure profile data is labeled $dP/dx$ and provides an estimate of the driving force for flow at the peritoneal surface. With the accuracy of the servo-null micropipette technique being on the order of ±1 mmHg (7), the values of $dP/dx$ at pressures <2 mmHg must be qualified as “best estimates.” The pressure profiles at these lower pressures were always observed to decrease to zero before the skin side of the muscle was reached. However, the uncertainty in position of each reading (±200 µm) requires that we further qualify our estimates of $dP/dx$ and the resulting values for K. At $P_{ip}$ of 0.7 mmHg, the average pressure profile decreased to zero in a linear fashion over ~400 µm from the peritoneum. If the distance were 600 µm, $dP/dx$ would be ~11.6 mmHg/cm with $K = 1.6 \times 10^{-5}$ cm²·min$^{-1}$·mmHg$^{-1}$. On the other hand, $dP/dx$ would be ~20.1 mmHg/cm with $K = 2.6 \times 10^{-5}$ cm²·min$^{-1}$·mmHg$^{-1}$. These differences were accepted as statistically significant at $P < 0.05$.

Fig. 2. Overall peritoneal fluid loss rate as a function of $P_{ip}$. Peritoneal fluid volume decrease per hour for whole cavity appears directly proportional to $P_{ip}$ above $P_{ip}$ of 1.5 mmHg.
DISCUSSION

In the present study, using a unique animal model (rat anterior abdominal wall during peritoneal dialysis), we have determined the fluid flux from the peritoneal cavity into the muscle of the anterior abdominal wall simultaneously with the pressure gradient that drives the flow at the tissue surface. From these data, we have used Darcy’s law to calculate the hydraulic conductivity (K) of the tissue under a range of pressures, which are much higher than seen in normal physiology but are quite common in clinical therapeutic such as peritoneal dialysis (27). The specific hypothesis addressed by the present study is that K of the abdominal wall varies with P_{ip}. The study was designed to make the necessary measurements under controlled conditions, in which P_{ip} was held constant at the desired level for each individual experiment and K was determined under steady-flow conditions.
must be anesthetized. Pip may change with posture, completely unrestrained tissue and therefore cannot make the interstitial pressure measurements in the tissue to prevent breakage of the glass pipette used to enter the muscle. We have assumed that the tissue density is 1 g/cm³ and not constant in an awake rat that is free to move around its cage. However, anesthesia results in relaxation of muscle (23) and is well known to slow lymph flow and movement of albumin in muscle (25). Results of measurements of fluid flux from the peritoneal cavity into the abdominal wall will certainly be affected by the lack of movement and tone in the muscle, and despite the use of anesthesia, the interstitial pressure measurement requires further restraint of the tissue to prevent breakage of the glass pipette used to enter the muscle. We have assumed that the measured profile through pores in the support plate is equivalent to that of the unsupported tissue on the contralateral side of the abdominal wall, and we carried out control experiments to show that enlarging the size of the pores in the plastic support plate does not change the measured pressure profile. However, we cannot make the interstitial pressure measurements in completely unrestrained tissue and therefore cannot provide absolute proof of our assumption. Because of these factors, the results from our in vivo model must be carefully considered in the light of the limitations of the experimental preparation.

In the model, the water flux into the tissue is estimated by the clearance of tracer protein from the cavity into the tissue. By using this approach, we actually estimate K in the initial part of the tissue rather than the bulk of the entire tissue thickness. Because the calculations of K use the total fluid flux across the surface of the tissue, it is not necessary to follow the course of the water within the tissue to address our hypothesis. However, we can estimate the relative contribution of blood or lymph capillaries to the absorption of the fluid that enters the tissue. In separate experiments, we have established that at low Pip (0.7–1.5 mmHg), there is no tissue expansion (30). Therefore, the fluid flux of 0.0002 ml·min⁻¹·cm⁻² (Table 1) must be totally absorbed into lymph or blood. If we estimate the thickness of the tissue as 0.15 cm and assume that the tissue density is 1 g/cm³, the flow into the tissue = 0.0004 ml·min⁻¹·g tissue⁻¹ (0.00020/0.15). From our previous work (9), the estimated rate of lymph flow in this tissue = 0.00008 ml·min⁻¹·g tissue⁻¹. The ratio of the rate of fluid flow from the cavity into the tissue to the rate of lymph flow is 17.5. Because the extracellular space has not expanded, we presume that the remainder of the fluid transported into the tissue is taken up by blood capillaries. As Pip increases >1.5 mmHg, the rate of flow into the tissue will exceed the ability of the lymphatic and blood capillaries to transport the fluid from the tissue, and the tissue interstitium will expand (30).

Our estimation of K is strictly limited to the first 100 µm of the tissue below the surface. At this time we do not have data on the extracellular volume deep in the tissue, nor do we know the concentration of glycosaminoglycans (GAGs) including hyaluronan (hyaluronic acid; HA) in the tissue (concentration of interstitial macromolecules determines interstitial conductance). Although we believe the value for K deep in the tissue should be of the same order of magnitude of the value of K at the tissue surface for a given Pip without knowledge of these factors we cannot accurately extrapolate K to these locations.

Comparison With Other Values of Hydraulic Conductivity

Published values of interstitial conductivity span a range of three orders of magnitude, as shown in Table 1. The values for the hydraulic conductivity of abdominal wall muscle fall between those for mesentery and those for the subcutaneous plane. Each value in Table 2 must be viewed in the context of the specific tissue and the experimental conditions. Most of the data were collected in vivo in supported tissue preparations varying from the thoracic aorta (29) to the mesentery (17) to the sclera (6). In vivo data for normal tissues are available only for subcutaneous plane (15, 26) and the synovium of the knee joint (18, 24). All techniques require data about fluid flow versus pressure gradient.
Variation of $K$ with $P_{ip}$

From Darcy’s law, the steady-state fluid flux across a porous bed can be described. However, the tissue is neither a rigid porous bed nor a closed system, because the interstitium of this tissue is compliant and surrounds capillaries and lymphatics that can remove fluid from the tissue space. Preliminary measurements of the extracellular space demonstrate that for $P_{ip} \geq 1.5$ mmHg, the tissue space expands (30). In Fig. 3, we observe that the fluid flux into the abdominal wall varies linearly with increases in $P_{ip}$ above this pressure. Because the slope of the pressure profile near the peritoneal surface $dP/dx$ does not appear to change with increasing $P_{ip}$, the calculated values for $K$ parallel the increase in $Q/A$. The increased tissue fluid conductance with pressure is probably caused by a decreased interstitial resistance to fluid flow. Such changes in resistance may be attributable to changes in the number (surface area) and dimension of the “pores” or the fluid-rich channels spanning the interstitial matrix. Theoretically, interstitial $K$ depends on the interstitial void fraction ($\theta_if$), the wetted surface area per unit volume ($S$), and the Kozeny factor (G; Ref. 2).

$$K = \frac{\theta_if}{GS^2}$$  \hspace{1cm} (5)

The degree of tissue hydration directly affects $S$ and $\theta_if$. A doubling of $\theta_if$ would increase $K$ by a factor of 8 (23). However, this increase in $\theta_if$ may be offset by an increase in $S$ as more regions within the tissue matrix are available to water. $G$ depends on channel shape and tortuosity, and it likely changes with hydration as well.

Effect of hydration on tissue hydraulic permeability has been evaluated from in vitro studies in articular cartilage (21) and corneal stroma (16). In both tissues, a sharp decrease in $K$ was observed with a decrease in the tissue water content (16, 21). However, each tissue was supported on one or two sides and there was no direct measurement of $dP/dx$. Rather, $dP/dx$ was estimated by the pressure difference across the tissue divided by the tissue thickness. Both of these papers attribute the decrease in hydraulic permeability to the increase in GAG concentration that results from dehydration.

Few experimental studies have investigated the in vivo fluid flow in tissue under pressure load. McMaster (22) carried out careful experiments in which he monitored the flow of salt solutions through a small pipette connected to a 30-gauge needle inserted into the subcutaneous space of mice. He demonstrated no flow into the subcutaneous space at inflow pressures < 6.2 mmHg; above this threshold, there was a linear relationship between flow and pressure. McMaster further demonstrated in edematous subcutaneous tissue that there was no specific threshold for fluid flow into the tissue but that from very low pressures (~ 1 mmHg), a linear relationship existed between the imposed pressure and the rate of fluid flow (22). The same phenomenon was later observed by Guyton et al. (15), who measured the flow induced by a pressure differential between two catheters inserted in the subcutaneous space. They observed a very slow flow at small (2–3 mmHg) pressure differences but markedly increased flow at pressure differentials large enough to create edema. They estimated that edema increases tissue fluid conduc-

### Table 2. Selected literature data on tissue hydraulic conductivity

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Method</th>
<th>$K$, $cm^2 \cdot min^{-1} \cdot mmHg^{-1} \cdot 10^{-3}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsupported abdominal wall</td>
<td>3</td>
<td>0.9–4.7</td>
<td>Present study</td>
</tr>
<tr>
<td>Vitreous body</td>
<td>1, 3</td>
<td>16.80</td>
<td>5, 15</td>
</tr>
<tr>
<td>Subcutaneous plane</td>
<td>3, 3</td>
<td>10.08</td>
<td>15, 25</td>
</tr>
<tr>
<td>Mesentery</td>
<td>2, 3</td>
<td>1.52, 0.036</td>
<td>17, 25</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>2, 3</td>
<td>0.248, 0.045</td>
<td>12</td>
</tr>
<tr>
<td>Connective tissue lining an implanted capsule</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal lining</td>
<td>1</td>
<td>0.168</td>
<td>12</td>
</tr>
<tr>
<td>External lining</td>
<td>1</td>
<td>1.128</td>
<td>12</td>
</tr>
</tbody>
</table>

All data are presented as means ± SE. Method 1, extrusion of fluid under compressive loading; method 2, flow across tissue slice in vitro; method 3, transtissue flow in vivo.
range in the subcutaneous space by >100,000 times. Levick and co-workers (18–20, 24) have consistently found a linear increase in fluid flow conductance across synovial lining of rabbit knees when increasing intrarticular pressure above a yield point of ~6.6 mmHg. The common theme in all of these in vivo studies is the apparent threshold pressure or "yield point" above which the interstitial space swells and the resistance to fluid flow decreases. Our study demonstrates a threshold of 1.5 mmHg in the abdominal wall muscle, above which the flux of fluid into the tissue and its hydraulic conductivity increase.

We believe that the increase in K with pressure is caused in large part by the expansion of the interstitial space and the concomitant decrease in the macromolecular concentration of the interstitium. Price and colleagues (24) have recently studied the in vivo effects of increased intra-articular pressure on the GAG concentration and K of the rabbit knee synovium. Raising the pressure to pathological levels of 25 cmH2O increased the conductance of the synovium by a factor of five. The tissue concentration of fixed constituents (collagen and the sulfated GAGs, which would not be washed out by the convection) were reduced to 62–70% of control values by the pressure-induced flow. The authors concluded that the decrease in concentration of the fixed elements was caused by dilution from the influx of water and that the changes were more than sufficient to account for the increased conductivity. Unfortunately, the detailed constituent data, which have been reported for the synovium (24), are not yet available for the abdominal wall muscle.

Supported Versus Unsupported Tissue

Our experimental study revealed marked differences between supported and unsupported tissues with respect to fluid flux induced by PIP. These differences are attributed to the fact that rigid tissue support may provide a counterpressure across the abdominal wall, which results in a reduced pressure gradient across the abdominal wall (8). It has been shown that for a given pressure drop, the hydraulic permeability of slabs of arterial wall supported in a filtration cell against a rigid porous surface is an order of magnitude less than for vessels allowed to retain their normal geometry (29). Unfortunately, we cannot determine the pressure profile in the tissue directly under the Plexiglas plate, and therefore K for the supported tissue cannot be determined. Presumably such tissue exhibits lower K than unsupported tissue. The supported in vitro preparations also do not display the same threshold phenomenon as unrestrained in vivo tissues. The corneal stroma, for example, displayed constant values of K over pressure gradients from 0 to 250 mmHg/mm if the hydration of the tissue slice was constant. However, it was shown that the flow conductivity did vary directly with the degree of hydration (16).

In conclusion, we have carried out a unique set of experiments, which have demonstrated that K varies with PIP in unsupported abdominal wall muscle. When overall pressure difference across the model tissue was raised, we measured the pressure profile in the tissue and determined that the pressure gradient at the surface of the tissue did not change proportionately in spite of marked increase of fluid flux into the tissue. Significant differences in fluid flow occur in supported and unsupported tissues. We suspect that in unsupported tissue, the additional stress and influx of fluid likely result in changes within the tissue structure. In contrast, supported tissue does not experience the same forces and changes are relatively small. The next step in this research is the coupling of tissue hydration and determinations of K with the data on K.

This work was supported by grants from the Whitaker Foundation and National Institute of Diabetes and Digestive and Kidney Diseases Grant R29-DK 48479.

Address for reprint requests: M. F. Flessner, Box 675, 601 Elmwood Ave., Univ. of Rochester Medical Center, Rochester, NY 14642. Received 6 February 1997; accepted in final form 22 July 1997.

REFERENCES