In vivo diffusion of immunoglobulin G in muscle: effects of binding, solute exclusion, and lymphatic removal

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

Flessner, Michael F., Joanne Lofthouse, and El Rasheid Zakaria. In vivo diffusion of immunoglobulin G in muscle: effects of binding, solute exclusion, and lymphatic removal. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2783–H2793, 1997.—Previously, we demonstrated that immunoglobulin G (IgG), dissolved in an isotonic solution in the peritoneal cavity, transported rapidly into the abdominal wall when the intraperitoneal (ip) pressure was >2 cmH2O. We hypothesized that this was chiefly caused by convection and that diffusion of IgG was negligible. To investigate the role of diffusion, we dialyzed rats with no pressure gradient across the abdominal wall muscle for 2 or 6 h with an ip isotonic solution containing 125I-labeled IgG. At the end of the experiment, the animal was euthanized and frozen and abdominal wall tissue was processed to produce cross-sectional autoradiograms. Quantitative densitometric analysis resulted in IgG concentration profiles with far lower magnitude than profiles from experiments in which convection dominated. In other in vivo experiments, we determined the lymph flow rate to be 0.8 × 10^−4 ml·min^−1·g^−1 and the fraction of extravascular tissue (θx) available to the IgG to be 0.041 ± 0.001. An in vitro binding assay was used to determine the time-dependent, nonsaturable binding constant: 0.0065 min^−1 × duration of exposure. A non-steady-state diffusion model that included effects of θx, time-dependent binding, and lymph flow was fitted to the diffusion profile data, and the IgG diffusivity within the tissue void was estimated to be 2 × 10^−7 cm^2/s, a value much higher than that published by other groups. We also demonstrate from our previous data that convection of IgG through tissue dominates over diffusion at ip pressures >2 cmH2O, but diffusion may not be negligible. Furthermore, nonsaturable binding must be accounted for in the interpretation of tissue protein concentration profiles.

interstitial; transport; peritoneum; protein; mathematical model

THE TRANSPORT of high-molecular-weight proteins to and from the peritoneal cavity is important in clinical physiology and pathophysiology. During peritoneal dialysis (a dialysis technique that relies on solute and water exchange between a solution in the cavity and blood circulating in surrounding tissues), 5–15 g of protein are lost per day (13). Massive quantities of protein can be lost to the peritoneal cavity in conditions of severe hepatic failure or intra-abdominal malignancy. In recent years, researchers have attempted to combat the spread of metastatic colorectal or ovarian carcinoma with the use of intraperitoneal (ip) monoclonal antibodies, to which are bound high-energy radionuclides or cellular toxins (10). Understanding the mechanisms of protein transport through tissue could prove important in improving therapy, particularly in the case of regional immunotherapy for malignancy.

In previous work (18), we demonstrated that immunoglobulin G (IgG), dissolved in solution in the peritoneal cavity, transports into all surrounding tissues. We measured the concentration profiles in all tissues and attempted to fit a diffusive model to the data in the abdominal wall; this effort failed to produce realistic parameters. The presence of the solution resulted in an ip pressure of 3–4 cmH2O, and we have subsequently shown that pressures above a threshold of 2 cmH2O produce convection of the protein into the abdominal wall (19). In another set of experiments, we showed that there was considerable nonspecific binding of the IgG to muscle (17). All of these results implied that our protein concentration profiles resulted from a combination of diffusion and convection with possible effects of binding to tissue.

In this paper, our goal is to study in vivo diffusion of IgG through a defined tissue system. Because we already have data in the abdominal wall from presumably convection-dominated transport, we have employed the anterior abdominal wall muscle as a model tissue for this study to carry out a comparison between our previous results and diffusive transport. Our method minimizes convection of the protein from the cavity into the muscle by maintaining a zero pressure gradient across the abdominal wall. We use quantitative autoradiography (QAR) to determine tissue concentration profiles and the binding characteristics of the IgG to muscle. In addition, we have estimated the space within the tissue available to the protein (protein void space) and the lymphatic removal rate of IgG from the abdominal wall. We combine these measurements with a mathematical model to estimate the effective diffusivity of IgG in abdominal wall muscle.

THEORY

The concept used to model the tissue transport process is diffusion through a porous bed, which has a fixed void fraction (θx), the fraction of the tissue from which protein is not excluded, see Fig. 1). Protein enters from the peritoneal side of the abdominal wall and diffuses in the free state through the tissue. Free protein can be "removed" from the tissue void by either localized binding or lymphatics. If it is assumed that there is no convective movement of the protein across the abdominal wall (pressure gradient across wall = 0, see Fig. 1) and no metabolism of the protein during the experiment, the mass balance for unidirectional transport of unbound protein with binding and lymphatic removal is

\[
\frac{\partial (\theta_x C_v)}{\partial t} = \frac{\partial}{\partial x} \left[ D_{eff} \frac{\partial C_v}{\partial x} \right] - F_L - F_B
\]

(1)
Fig. 1. Tissue model concept used to formulate mathematical approach to diffusion across abdominal wall. Tissue is a porous media with a tissue void (\(\theta_s\)) through which macromolecule diffuses. Blood and lymph capillaries are distributed uniformly throughout tissue. Macromolecule does not penetrate cells or blood capillaries but can be transported from tissue by lymph flow. Solution containing antibody is kept in contact with peritoneal side on the left and the peritoneum defines the \(x = 0\) boundary for the concentration curve (total protein concentration in tissue \((C_{\text{tissue}})\)). Pressure across the abdominal wall is maintained at zero. \(C_{\text{PC}}\), protein concentration in peritoneal cavity; QAR, quantitative autoradiography; \(P_{\text{i.p.}}\), intraperitoneal pressure; \(P_{\text{skin}}\), pressure in skin; \(\Delta x\), change in distance from peritoneum.

\[ \theta_s \] is the solute or protein void fraction (fraction of total volume available to the protein within the tissue space). \(D_{\text{eff}}\) is the effective tissue diffusivity \((\text{cm}^2/\text{s})\) where \(D_V\) = diffusivity within the protein void fraction of tissue. \(C_v\) is the free protein concentration within the void fraction, normalized to \(C_{\text{PC}}(t = 0)\), where \(C_{\text{PC}}\) is protein concentration in solution in the peritoneal cavity and \(t\) is time \((\text{s})\). \(F_L\) is the lymphatic removal rate from tissue \((\text{mol protein} \cdot \text{cm}^3 \text{tissue}^{-1} \cdot \text{s}^{-1})\); transendothelial transfer directly to blood is assumed negligible. \(F_B\) is the rate of protein removal from the pool of unbound or free protein, and \(x\) is the distance from the peritoneum \((\text{cm})\). Values for \(F_L\), \(F_B\), and \(\theta_s\) are found experimentally as discussed in METHODS. \(\theta_s\) and \(D_{\text{eff}}\) are assumed constant for a given experiment. A mass balance of the total protein in the tissue is

\[ C_{\text{tissue}} = C_{\text{free}} + C_{\text{bound}} \]  

where \(C_{\text{free}} = \theta_s C_v\) = unbound protein in tissue, \(C_{\text{bound}}\) = concentration of bound protein in tissue = \(g C_{\text{free}}\), a function that is defined by experiment, and \(C_{\text{tissue}}\) = total protein concentration in tissue.

Boundary conditions are

\[ \begin{align*}
  & At \ t = 0 \quad C_{\text{tissue}}(x) = 0 \\
  & At \ t > 0, x = 0 \quad C_v = 1 \\
  & At \ x = x_1 \quad dC_v/dx = 0
\end{align*} \]

where \(x_1\) is the subcutaneous side of the abdominal wall muscle. In the second boundary condition, we assume that there is no boundary layer at the peritoneum and that the concentration measured from the solution in the cavity matches that at the peritoneal surface. In the third boundary condition, we assume that the slope of the tissue concentration profile approaches zero; this is demonstrated in the experiments. The set of equations was solved with an implicit finite-difference computational technique \((8)\) programmed in Fortran and computed with an Intel 486-based computer.

METHODS

Animals and surgical preparation. Sprague-Dawley female rats, 220–270 g, were used in all experiments. Anesthesia was induced with intramuscular injections of pentobarbital sodium. When the animal had lost its blink reflex, catheters were placed in the femoral artery (for blood pressure monitoring and sampling) and the femoral vein (for infusion of fluid or anesthetic). Blood pressure was monitored with a Microman blood pressure analyzer and Cobe CDX III transducer. The systolic blood pressure was noted to be > 100 mmHg during all experiments. A tracheostomy was performed. The animals were kept warm by a servo-controlled heating blanket and overhead heating lamp \((36 \pm 1°C)\). Euthanasia was carried out by an overdose \((10 \times \text{anesthetic induction dose})\) of pentobarbital sodium or an overdose followed immediately by decapitation to halt blood flow (used in QAR experiments). These techniques are documented in previous publications \((14–19)\).

Isotopic tracers and solutions. \(125\text{I}\)-labeled IgG \((\text{Amersham anti-rabbit IgG, no. IM-134, immunoabsorbed against rat antigens; 5–20 µCi/µg protein})\) was used as the test molecule in the diffusion and binding experiments and in the estimation of the void fraction of IgG \((\theta_s)\). This IgG was used as a marker of IgG transport because we have established a large body of data with it and have found that there is no specific binding to rat tissue, that the label is stable in the rat, and that its half-life is appropriately long \((460 \pm 8 \text{ min})\) for its molecular weight \((19)\).

In the estimation of \(\theta_s\), a second label was required to mark the vascular space. We utilized a second IgG \((\text{G-6638, Sigma Chemical})\) and labeled it with \(125\text{I}\) \((\text{purchased from Amersham})\) using Iodobeads \((\text{Pearce Chemical})\) and purified it by passing the solution over an ion-exchange column \((\text{Bio-Rad 1-XP})\). The plasma half-life of this IgG was approximately the same as that of IM-134.

All isotopic tracers before each experiment were checked for degradation and free iodote by trichloroacetic acid \((\text{TCA})\). If free iodote was > 1%, the solution was purified further by mixing it with saline and concentrating the mixture with a Centricon 30 microconcentrator \((\text{Amicon})\) by centrifugation \((\text{IEC Centra CL2})\). Dilution and concentration of the isotopic solutions were repeated until the free iodote was < 1% by TCA precipitation. At the conclusion of the experiment, samples of blood, urine, and peritoneal fluid were processed with TCA to ensure isotopic integrity. The tracer concentration of body fluids and tissue samples was determined by dividing the total counts per minute \((\text{measured with Beckmann 8000 gamma counter})\) by the sample volume. Details can be found in our previous publications \((17–19)\).

In addition to the above procedures, we performed additional experiments to check for label separation from the protein. Two animals underwent 3 h of dialysis to load the tissue with \(125\text{I}\)-IgG. Tissue samples of the abdominal wall were collected and macerated and subjected to a procedure similar to that used by Bratzler et al. \((6)\) to extract the labeled protein from the tissue. The extracted, labeled material displayed the same TCA-precipitable fraction of > 99% as the dialysis solution or plasma samples. We therefore concluded that the abdominal wall muscle lacks the capability to rapidly
break down the labeled protein during short-term experiments.

The experimental dialysis solution (solution A) consisted of an isotonic salt solution [Krebs-Ringer bicarbonate, which contains (in mol/l) 0.12 NaCl, 0.01 KCl, 0.0021 CaCl₂·2H₂O, 0.025 NaHCO₃, and 0.00028 KH₂PO₄ and 1.18 ml of 1 M MgSO₄·7H₂O to which 5% bovine serum albumin (BSA, no. A9312, Sigma Chemical) and 0.01% Evans blue dye (no. E2129, Sigma Chemical; the dye marks the peritoneum that makes contact with the fluid) were added. The 5% BSA solution has been used in our experiments to prevent a significant loss of total protein from the serum in this species of rats. Fluid in the cavity containing no protein has resulted in a 50% reduction in the total serum protein over 6 h (14). The pH of the solution was adjusted to 7.4, and osmolality was determined to be 290 ± 5 mosmol/kg.

Effect of albumin in solution on transport. Despite the fact that the peritoneal solution is isotonic, the 5% BSA solution may set up an oncotic difference between the bulk solution in the peritoneal cavity and the interstitial space of the rat (typically 2% Ref. 24). Prior experiments in our lab (19) demonstrated that fluid absorption from the cavity for 3 h at 4 cmH₂O of pressure was not affected by altering the BSA concentration between 0 and 5%. However, short-term (30 min) experiments in another rat species demonstrated a significant slowing of the rate of absorption by a solution containing 5% BSA (34). Although the experimental methods used in each of these studies were very different, neither study can truly resolve the question of the effect of a 2–3% difference in albumin on transport of the labeled IgG for two reasons. First, transport across the peritoneum is dependent on the total surface area exposed to the solution, and evidence exists (16) that only 25–30% of the dissected area of the peritoneum is touched by the fluid, even with the use of a large volume dwell. If the presence of the protein altered the surface tension of the fluid between surfaces in the cavity, there is the possibility that the peritoneal area of contact with the solution was changed as well. Secondly, the rate of uptake may vary from tissue to tissue, and changes in the contact area of specific tissues might change the total absorption rates. Experiments in intact animals cannot distinguish the specific surface areas that are touched by the peritoneal solution.

Because the assumption of no convection in the abdominal wall is important to the analysis of the data, we designed a specific set of experiments to address the hypothesis that a protein difference of 2–3% BSA between the solution in the cavity and the interstitial fluid does not cause a significant flow from the tissue to the cavity. To determine the effects of the dialysis solution on the fluid flux across the peritoneum of the abdominal wall, we carefully glued small plastic chambers to the serosal side of the anterior abdominal wall (without touching the peritoneum within the chamber) of anesthetized rats (n = 3). Details of the chamber construction and testing can be found in a previous publication (16). A predetermined volume of one of two solutions (Krebs-Ringer bicarbonate or Krebs-Ringer bicarbonate with 5% BSA, as specified in isotopic tracers and solutions) was initially placed into each chamber, with the height of the fluid at 1.4–1.5 cm. A small quantity of [³¹C]mannitol (0.2–0.3 µCi) was dissolved in each solution to provide a means to calculate the residual volume in the chamber at the end of an experimental period of 60 min. Volumes were determined by weight on a digital scale accurate to ±1 mg; the density of the solutions was assumed to be 1 mg/µl. After 60 min, the solution in the chamber was carefully drawn up into the original syringe and needle, and the total volume (weight) was redetermined. The chamber was then washed with the Krebs solution containing no labeled mannitol to determine the residual volume in the chamber and to prepare for the next 60-min dwell with the other solution (residual volume = (wash volume × wash fluid concn)/(chamber concn at t = 60 min)). Solutions were alternated several times in each animal, and data was taken from the second dwell period and thereafter (the initial dwell with either solution was used as a period of stabilization of the tissue after the tissue preparation). The resulting fluxes [calculated as (final volume – initial volume)/60 min × exposed surface area], µl·min⁻¹·cm⁻², mean ± SE] were, for the Krebs-Ringer solution, 0.30 ± 0.08 (n = 6) and, for the solution with 5% BSA, 0.37 ± 0.07 (n = 6). A one-way analysis of variance revealed no significant dependency on the solution type (P > 0.5). These results confirm our hypothesis that the 5% BSA solution does not significantly alter the flux of fluid from the tissue across the parietal peritoneum into the cavity because of a potential protein gradient between the tissue and the fluid in the cavity. Although no difference in fluid flux is noted in this model system with small changes in the oncotic pressure of the bulk fluid, the result may not apply to other serosal tissues. For the relative significance of the observed volume flux to diffusive transport of IgG, see DISCUSSION.

Protein binding assay. Because the IgG protein had been selected for its lack of specificity for rat antigens, our goal in these experiments was to determine the nonspecific binding. We used the technique of Del Vecchio et al. (11) to determine these characteristics in the abdominal wall of Sprague-Dawley rats for the 125I-IgG used in the diffusion experiments. Briefly, the abdominal wall of normal rats was rapidly frozen, and 8-µm sections were cut with a cryomicrotome and fixed to microscopic slides. All sections were preincubated for 30 min in a solution containing 2% BSA and 10% chicken serum (no. 6773, Sigma Chemical) to decrease binding of the labeled IgG to the glass slide. Separate fixed sections were then incubated in solutions (made up of the Krebs-Ringer bicarbonate solution containing 5% BSA) of increasing concentration of the labeled protein for 1, 10, 60, or 240 min to test the effect of time of exposure of the protein to the tissue (see Ref. 12). The solution was made up from the experimental dialysis solution containing 5% BSA. Each slide was then washed three times and incubated in a fourth tank of buffered saline for 30 min; on the basis of the characteristic diffusion time across the 8-µm section [D_total = L²/D₄th = (8 × 10⁻⁴ cm²)/(4 × 10⁻⁸ cm²/s) = 16 s; L = length, D = diffusivity], the 30-min incubation is more than sufficient for free IgG to diffuse from the tissue. Slides were then dehydrated with ethanol and air-dried. The amount of deposition was determined with QAR (see QAR). The measurements of the total binding of the protein were then correlated with the free concentration in the incubation tank and with the time of incubation of the tissue slice in a given concentration of tracer protein. These data permit the calculation of an apparent binding constant. Forward binding or association constants are typically found by using the technique outlined with minimal time in a saline bath. Dissociation constants are determined by carrying out the forward binding experiment, but the final incubation medium (saline) is replaced by a solution containing an overabundance of unlabeled IgG; the displacement of labeled IgG is measured versus time. Unfortunately, the unlabeled form of the test IgG was not commercially available, and therefore this portion of the binding assay could not be determined. Because the reverse constants for specific binding are typically two orders of magnitude less than the forward rate constants (12), we assumed that the determination of the apparent association constant would permit us to...
adequately describe the short-term (2–6 h) nonspecific binding of this immunoglobulin.

QAR. We have performed extensive studies with this technique, including dual-label QAR (17–18). QAR was used to determine the local concentration of each tracer in the tissue at the time of animal death.

Briefly, at the end of an experiment in which tissue profiles were to be measured, the following steps were taken in rapid succession: the animal was euthanized with an overdose of anesthetic and decapitation, the fluid was drained from the cavity, and the animal was rapidly frozen to prevent further transport of the tracer material. The anterior abdominal wall was cut from the carcass with an autopsy saw. Sections (20 μm) were obtained horizontally through this tissue with a Hacker-Bright cryomicrotome and were heat-dried. The sections were placed with standards (tissues with known isotope concentration) against X-ray film (Kodak Biomax MR) to produce autoradiograms. After development, the films were analyzed with a computerized densitometer (MCID) that measures optical density (OD) versus position in the tissue. The isotopic standards are used to construct a calibration curve (concentration vs. OD) to convert the unknown ODs from the tissue samples to concentration. After exposure, the tissue slices were stained with hematoxylin and eosin. By superimposing the autoradiogram over the tissue histology, we carefully determined the location of the reading, and a concentration vs. position curve (diffusion experiments) or mean concentration was obtained in a large area of the tissue (binding experiments). For the diffusion experiments, the serosal surface was used as the reference point, and concentration was plotted versus distance into the abdominal wall.

Experimental protocol: IgG diffusion through abdominal wall muscle. The diffusion of labeled IgG from the peritoneal cavity into surrounding muscle must be studied when the solvent drag or convection is near zero. This condition can be obtained by eliminating the forces for convection through use of isotonic solutions and by maintaining zero hydrostatic pressure difference across the abdominal wall (see Fig. 1 for conceptual model). We have previously shown that with the cavity at zero ip pressure, the tissue pressures in abdominal wall muscle of animals have been approximately zero (15).

However, because the standard deviation of the micropipette servo null technique is ±1 mmHg (15), we cannot rule out a small gradient of 1 mmHg into the tissue [0 – (–1 mmHg)]. Previous studies have demonstrated no significant convective flow from the cavity at these low pressures (19). In addition, the 5% BSA solution, used to maintain the total serum protein at a normal level, results in a gradient of albumin from 5% in the cavity to approximately one-half that value within the tissue. In none of our previous studies (17–19) have we observed the protein added to peritoneal fluid to result in fluid flow into the cavity or to diminish the flow of an isotonic solution from the cavity into tissue. Our chamber experiments (described in Effect of albumin in solution on transport) have confirmed these observations and determined that the addition of BSA to the dialysis fluid does not alter fluid movement significantly across the abdominal wall peritoneum. If we used a solution containing a lower albumin content, the serum protein concentration would continuously decrease, resulting in decreased oncotic pressure in the microcirculatory system. During the diffusion experiment with the 5% BSA solution, the BSA profile would likely be similar to the IgG profiles with the exception that the value for θs would likely increase. With these caveats, we assume that movement of protein into the wall in the direction perpendicular to the peritoneum under these conditions is caused by diffusion alone. The experiments were carefully conducted with an attempt at measurement of all factors that could influence the transport. However, the characteristics of this in vivo model system should be taken into account when the data are compared with the results of other studies.

After surgical preparation, a laparotomy was carried out and the viscera were retracted medially from both sides of the abdominal wall (see Fig. 2). This, in effect, formed a space between the viscera and the abdominal wall to ensure that the isotonic solution, containing the labeled protein, would be in continuous contact with the abdominal wall peritoneum. The cavity was left open to ensure that ip hydrostatic pressure was 0 cmH2O at the abdominal opening. Peritoneal and blood samples were collected every 30 min for 2 or 6 h (minimum of 3 animals per time period) to allow for measurable deposition of protein (17–19). The two experimental durations were performed to determine the diffusivity by matching concentration profiles at two different times; we assumed that tissue properties [void space diffusivity (Ds), θs] were constant over the entire 6 h. At the end of each experiment the animal was euthanized, and the final dialysate concentration of the labeled protein was determined. The carcass was frozen and processed for QAR to obtain concentration profiles within this tissue. The concentrations determined were total protein concentration (bound + free tracer), based on the total volume of the tissue (cells, interstitium, and intravascular space).

Experimental protocol: Lymphatic flow from abdominal wall. Proteins are removed from the intersitial space of tissue by lymphatics. Although the lymphatics of the abdominal wall are located in the tissue planes and in the subcutaneous space (27), we assume in our model that lymph flow is uniformly distributed in the tissue. Despite data showing that the protein turnover rate is quite slow in skeletal muscle (28), protein diffusion is also a slow process and resulting tissue profiles may be affected by tracer removal via the lymphatics. Therefore, the rate of lymph flow in the abdominal wall was estimated by using a technique similar to that of Reed et al. (28), which determines the rate of disappearance of a radiolabeled protein infused or injected into the extracellular space of the tissue. This experiment measures the...
outflow rate of protein, which is contained within a localized region of the abdominal wall interstitium.

Six animals were anesthetized and surgically prepared as described in Animals and surgical preparation. During initial experiments, it was found that the interstitial space of the abdominal wall could be loaded with radioactive tracer (125I-IgG) equally well from the subcutaneous side or the peritoneal side. Because the animal is more stable with a closed abdomen, in five of six experiments, loading of the tissue was performed as depicted in Fig. 3A. As shown, a plastic chamber (inner diameter 1 cm, with a flange, outer diameter 2 cm; height 4–6 cm) was affixed with cyanoacrylate glue to the subcutaneous side of the anterior abdominal wall muscle (16).

Solution A containing 5% BSA, Evans blue dye, and isotope (125I-IgG) was added to the chamber until the height of solution was at least 3 cm above the surface to cause a convection of the protein into the tissue. The chamber fluid was sampled hourly and mixed every 30 min. After 1–2 h, the solution was removed from the chamber, and it was washed out by rinsing with Krebs-Ringer solution. The chamber was carefully removed from the tissue, and residual counts on the surface were removed by washing with saline and making light contact with a gauze pad. Typically, the Evans blue dye, which is tightly bound to the BSA, showed staining in an area equal to the chamber base and through the tissue thickness. If gross edema had formed in the tissue, the preparation was not used. As noted above, urine was collected to check on excretion of low-molecular-weight labeled protein fragments and free iodine; in all experiments there was negligible excretion of low-molecular-weight labeled protein fragments not used. As noted above, urine was collected to check on excretion of low-molecular-weight labeled protein fragments and free iodine; in all experiments there was negligible excretion of low-molecular-weight labeled protein fragments not used.

To determine the turnover rate of the protein in the interstitium, a directional scintillation probe (Dosimeter Model 41) was mounted as illustrated in Fig. 3B, as close as possible to the tissue that was previously under the chamber. The localized labeled protein had a diameter approximately two-thirds of the shielded probe detection area. Significant localized transport beyond the area of detection of the probe was not observed. Great care was taken not to change the position of the probe with respect to the tissue surface. The shielded scintillation probe was connected to a digital ratemeter (Ludlum model 2241–2), and readings were taken every 15 min for 5–6 h. The raw counts per minute (cpm) were then corrected for background cpm, divided by the cpm at time 0, and plotted on semilog paper. The half-life of the labeled IgG (t_{1/2}) was then determined. The turnover rate or fractional removal rate was then calculated as

\[ k_{lymph} = \frac{0.693}{t_{1/2}} \]  

(3)

The local rate of lymphatic removal of the protein is \( F_L = k_{lymph}C_{tissue}/(C_{tissue}/C_{peritoneal cavity} + 1) \) (tissue), see Eq. 1. C_{tissue} is used in this expression because Eq. 3 is based on the turnover of all counts in the tissue, which includes both bound and free protein. An estimate of the tissue lymph flow rate can be derived from lymph flow = \( k_{lymph}C_{tissue} \) where \( k_{lymph} \) is the interstitial void fraction (ml/cm² tissue, see Experimental protocol: Solute void fraction). Because these experiments are carried out under anesthesia, rates will likely be lower than if they were carried out in awake animals (28).

Experimental protocol: Solute void fraction. To calculate the role of diffusion with the mathematical model (Eqs. 1–2), \( \theta_d \) must be determined. The solute void fraction, \( \theta_d \), is equivalent to the interstitial fraction, \( \theta_{if} \), for small solutes such as mannitol, EDTA, or inulin. To determine \( \theta_{if} \), we injected a bolus of [14C]mannitol intravenously into rats with either no fluid in the peritoneal cavity or a volume of the isotonic Krebs-Ringer with 5% BSA (after dwell time = 3 h). The [14C]mannitol was continuously infused to maintain a constant plasma concentration over 1 h. Ten minutes before the end of the experiment, [131I]-IgG was administered to mark the vascular space. At the conclusion of the experiment, the animal was given an overdose of pentobarbital sodium and rapidly decapitated and frozen. The abdominal wall tissue was collected, sliced, and processed for QAR as described in QAR. \( \theta_{if} \) was calculated as follows

\[ \theta_{if} = \frac{C_{tissue}}{C_{plasma}} \]  

(4)

We have found that the abdominal wall muscle at zero ip pressure with either fluid or no fluid in the cavity \((n = 3 \text{ each}, \text{ means } \pm \text{ SE}) \) has a vascular space fraction of 0.01 ± 0.00 and an extravascular space fraction of 0.19 ± 0.00 (no fluid in

![Fig. 3](image-url)
cavity) and 0.18 ± 0.02 (with fluid in the cavity), which by subtraction gives an interstitial void fraction of 0.17–0.18. The fact that the mannitol distribution space stays essentially the same after 3–4 h of exposure to fluid at zero pressure indicates that the interstitial hydration is relatively constant during these procedures.

Because of their size and molecular charges, proteins are excluded from more of the tissue than small solutes, and \( \theta_u \) for proteins is not necessarily equal to \( \theta_v \). One method that is used to estimate the protein void fraction includes the injection of one labeled protein intravenously (iv) 24 h before an injection of a second marker of the vascular space. To calculate the solute void space, it is necessary to assume that during the 24 h, the protein equilibrates with its void space in the extravascular compartment (2, 3). Labeled albumin is considered to come to steady state in skeletal muscle within 24 h and to reflect the distribution of native albumin (25). For IgG, this may not actually occur (23), and therefore our technique could possibly underestimate the true quantity.

After the second injection, the plasma is sampled to determine the concentration of both isotopes. The animal is euthanized, and the tissues are collected, weighed, and counted to determine the concentration of each isotopic label. The protein void fraction can be calculated from

\[
\theta_s = \frac{C_{\text{plasma}}^{125I}}{C_{\text{plasma}}^{131I}} - \frac{C_{\text{tissue}}^{125I}}{C_{\text{tissue}}^{131I}}
\]

where the first term on the right side of Eq. 5 equals the extracellular fraction of the “equilibrated” protein and the second term equals the intravascular fraction of the tissue.

On the first day, the rat was restrained in the prone position, and with sterile technique the dorsal side of its tail was surgically prepared, draped, and locally anesthetized with 1% lidocaine. One of the two veins in the tail was dissected out and catheterized with sterile PE-10 tubing. Approximately 50 µCi of the test protein (\(^{125}\text{I}\)-label) were injected iv via the tail vein catheter. The catheter was then withdrawn, the vein was tied off, and the wound was sutured closed. The animal was placed back into the cage with ad libitum access to water and food. On the second day, the animal was surgically prepared with iv and intra-arterial catheters, as noted above. The second labeled protein (\(^{131}\text{I}\)-IgG) was injected iv, and plasma samples at 5 and 10 min were obtained. The animal was then euthanized by pentobarbital overdose and decapitation, and triplicate tissue samples (100–500 mg) were collected from each section of the abdominal wall muscle. The tissue samples and plasma samples were then counted in a gamma counter with settings to exclude the lower-energy isotope. The samples were stored for 10 half-lives of \(^{131}\text{I}\) (2.5 mo) and then recounted for \(^{125}\text{I}\).

Calculations and statistics. All calculations were performed on a 486 Intel-based computer. Microsoft Excel (5.0) and NCSS (Provo, Utah) were used for calculations or estimation of statistical parameters. Tissue concentrations in the diffusion experiments were normalized by the concentration in the peritoneal cavity, which remained relatively constant throughout the experiment. Mean profiles were calculated for each experimental duration by pooling all normalized data from all animals. With values for \( F_F \), \( F_L \), and \( \theta_v \), fitting of the model was performed by varying \( D_v \). The upper limit of \( D_v \) was the value for IgG diffusivity in water. \( D_v \) was varied until good fits to the data were obtained by visual inspection. The sums of the squares of the differences between model output and data point for the full distance range and both experimental times were then calculated to ensure that they were at a minimum.

**RESULTS**

Lymph drainage from abdominal wall muscle. Six experiments were carried out, and the half-life of the protein in the tissue was ~1,610 min. Figure 4 illustrates the data and the mean values for 5 h. The mean turnover rate (\( k_{\text{lymph}} \)) or fractional removal rate was 0.43 ± 0.12 × 10\(^{-3}\) ml·min\(^{-1}\)·g tissue\(^{-1}\). A rough estimate of the actual lymph flow rate can be obtained by multiplying the turnover number by the fraction of interstitial space (\( \theta_I = 0.18 \text{ ml/cm}^3 \text{ tissue} \)). This results in an estimated lymph flow rate of 0.8 × 10\(^{-4}\) ml·min\(^{-1}\)·g tissue\(^{-1}\). The mean \( k_{\text{lymph}} \) is used directly in the equation for the mass removal rate due to lymphatics (\( F_F = k_{\text{lymph}} C_{\text{tissue}} \)).

Estimation of protein solute fraction. Six separate sections of the abdominal wall were used in each of six animals to determine an estimate of the average of the nonexcluded space in the tissue available to IgG. The overall mean (±SD) for the interstitial space available to the protein was 0.041 ± 0.001. The total extracellular space available to the IgG (\(^{125}\text{I}\)-IgG) was 0.050 ± 0.014, with the estimated intravascular space (\(^{131}\text{I}\)-IgG) of 0.009 ± 0.003.

Protein binding to abdominal wall muscle. Figure 5 shows that the apparent binding, as determined in the in vitro assay, has a linear dependence on the free concentration, which is typical of nonspecific tissue binding. In addition, the slope of the binding curve increases with time of exposure of the tissue to the protein. A least-squares correlation of the slopes of these four lines produces the following result

\[
C_{\text{bound}} = k_B \times (\text{duration of exposure}) \times C_{\text{free}}
\]

where \( k_B \) (binding constant) = 0.0065 min\(^{-1}\) (\( R^2 = 0.99 \)).
From Eq. 6, an expression can be derived for \( F_B \) (see Eq. 1)

\[
F_B = \frac{dC_{\text{bound}}}{dt} = k_B C_{\text{free}} \tag{7}
\]

Protein concentration profiles. Figure 6 displays the 2- and 6-h diffusion profiles of IgG concentration in the abdominal wall muscle (mean ± SD) normalized by dividing by the IgG concentration in the cavity at time 0. The values represent total tracer concentration in the tissue (\( C_{\text{tissue}} = \text{bound} + \text{free} \)). The protein concentration in the peritoneal cavity was essentially constant after injection into the cavity. Although the thickness of the abdominal wall muscle is \( 1.5-1.9 \text{ mm} \), only the first millimeter of tissue adjacent to the peritoneum was used in the analysis because of artifacts in the tissue caused by inadvertent removal of the skin from the subcutaneous side of the abdominal wall.

The relatively large variability is typical of these local measurements and mimics our previous results with QAR (17–18). The intercept of the curve at the y-axis nearly doubles over 6 h. If all the protein were unbound and if the transport were purely diffusion, the intercept at \( x = 0 \) (the peritoneum) would be the same. The large difference indicates that binding is likely a major factor in the observed transport of the protein.

Mathematical simulations of IgG diffusion. After substituting the expressions for \( \theta_s \), \( F_L \), and \( F_B \) into Eq. 1, we numerically solved the equation for the boundary conditions presented in THEORY. Diffusivity was varied until a good fit was observed for both 2- and 6-h data. Because our technique may underestimate the actual fractional tissue space available to the protein (see DISCUSSION), we increased the value of \( \theta_s \) to 0.050 and recalculated the concentration profiles. Figure 7 displays the data with the model calculations for both values of \( \theta_s \) and a \( D_v \) of \( 2.0 \times 10^{-7} \text{ cm}^2/\text{s} \). The sums of squares analysis showed nearly equal minima at either value of \( \theta_s \). The value for \( D_v \) corresponds to a \( D_{\text{eff}} \) of \( 8-10 \times 10^{-9} \text{ cm}^2/\text{s} \).

To illustrate the effect of binding on the concentration profiles at 2 and 6 h, Fig. 8 displays the free and bound protein concentrations and the calculated profiles of the free, bound, and total tissue concentrations for the case of \( \theta_s = 0.05 \) and \( D_v = 2.0 \times 10^{-7} \text{ cm}^2/\text{s} \). As shown in the figure, the profile for \( C_{\text{free}} \) (dotted curves) changes slightly from 2 to 6 h, and the two profiles are nearly superimposed near the peritoneal surface. The profile for \( C_{\text{bound}} \) (dashed profiles in Fig. 8) is below the \( C_{\text{free}} \) profile at 2 h, but it increases considerably over the next 4 h. The upward displacement of the tissue concentration curve from 2 to 6 h is therefore caused by binding of free protein that continues to diffuse into the tissue.

DISCUSSION

We have carried out a series of in vivo experiments designed to obtain data necessary for the quantitative description of diffusion of IgG in muscle. Our animal model permits the control of the major convective force in transport of protein through the tissue, the hydrostatic pressure difference across the abdominal wall. To minimize convection, we maintained the overall pressure gradient at zero. We then kept the peritoneal side of the abdominal wall in continuous contact with a solution containing labeled IgG. By rapidly freezing the
tissue at 2 or 6 h, we preserved the concentration profiles resulting from diffusion of IgG into the tissue. Quantitative autoradiography was used to determine the concentration profiles of Fig. 6. However, to use the diffusive mathematical model to describe the transport, we were required to determine the binding characteristics of the IgG, the rate of its removal from the abdominal wall, and an estimate of the actual fraction of tissue space that is available to the protein ($\theta_i$). Our study provides a unique set of data from a single in vivo tissue model. The assumptions and limitations in our methods are discussed below.

Protein binding. That binding is a significant factor in the diffusive transport of IgG through the abdominal wall is apparent from Fig. 6. Diffusion is a passive process in which molecules move from areas of high concentration to areas of low concentration. The free concentration of protein at the edge of the tissue can be no greater than the product of the concentration in the interstitial fluid (CPC) times the fraction of tissue available to the protein ($\theta_i$). Therefore, the intercept of concentration data at the peritoneal surface should not be greater than 0.04–0.05, nor should it change with time if $\theta_i$ (protein void fraction) within the tissue remains constant. In previous experiments we demonstrated that the ip pressure was required to surpass 2 cmH$_2$O before significant convection from the cavity into the abdominal wall (19). We have therefore assumed that with the hydrostatic pressure gradient across the abdominal wall equal to zero, there will be no significant influx of fluid into the muscle and no change in the protein void space. In addition, our determinations of the interstitial fluid fraction ($\theta_if$) have demonstrated that exposure of the abdominal wall tissue to fluid in the peritoneal cavity at zero pressure does not change $\theta_if$ from the value obtained with no fluid in the cavity.

A change in apparent protein space could result from binding, and we therefore determined the binding characteristics of the test IgG with a published in vitro assay (11), with modifications based on data from Dower et al. (12). The apparent binding curves (Fig. 5) suggested a nonsaturable, nonspecific binding of the IgG to the tissue. However, the slope of the binding curves was directly dependent on the time of exposure of the tissue to the labeled protein. Could this be caused by continued diffusion into the tissue from the bulk solution? A characteristic diffusion time for the 8-µm-thick sections can be derived from $T = (\text{thickness})^2/4D_{\text{eff}} = (8 \times 10^{-4} \text{ cm})^2/(0.8 \times 10^{-8} \text{ cm}^2/\text{s}) = 20 \text{ s}$. Therefore, diffusional transport would likely not explain the continued binding of the immunoglobulin. Unfortunately, the investigators who developed the antibody-tissue binding assay did not check for this effect of time of incubation, and therefore only data from a single incubation time are available for comparison. Dower et al. (12) studied specific binding of monoclonal antibodies (MAb) to antigens on cells grown in vitro in monolayers. The apparent nonspecific binding of different antibodies varies considerably, depending on the conditions of the assay. Although the true nature of nonspecific binding is unknown in most tissues, it is thought to involve IgG interaction with Fc receptors on normal cells (11) or to be caused by electrostatic effects in the tissue matrix (22). Nonspecific binding is observed under low-salt conditions similar to those used in our assay; it can be eliminated by solutions with high salt concentrations (22). However, to increase the salt concentration to 0.4–0.6 M would produce a very nonphysiological environment in the tissue sections and would not reflect what occurs in vivo. Although the test IgG has been processed with immunoadsorption to eliminate specific binding to a panel of rat antigens, there is the possibility that this polyclonal antibody has retained the ability to bind to elements in the muscle of the rat abdominal wall.

When we compared the 60-min curve with our previous result for MAb 96.5 (specifically binds to FEMX-2 cells, a human melanoma cell line), we found that the nonspecific binding to muscle or other tissues was far less (bound pmol/g = 0.042 x pmol/ml of antibody; Ref. 17). However, the nonspecific binding of MAb 96.5 was assayed with a large amount of unlabeled antibody present in the solution. On reexamination of our previous data (17), abdominal wall muscle and heart muscle, when incubated with tracer concentrations of MAb 96.5 for 60 min, produced a linear relationship with a slope of 0.25, similar to the 60-min line in Fig. 5, which has a slope of 0.39. Del Vecchio et al. (11) found a slope similar to ours when they determined the binding of MAB 96.5 to antigen-negative tumor cells by incubating the cells for 60 min; the slope of the bound versus free correlation was 0.25–0.5, close in magnitude to our measurement. Therefore, the method of the binding assay will greatly influence the quantitative result. We believe that our results reflect the in vivo binding of tracer quantities of IgG in the model transport system.

Lymph flow from abdominal wall muscle. An important factor to assess in a model transport tissue is the rate of protein removal from the tissue. In accordance with pore theory, we assume that transfer directly to blood across the wall of a blood capillary does not occur.
The chief means of removal of protein from tissue is via lymphatics, which are located at tissue planes of the abdominal wall (27). Because the rate of protein diffusion will likely be a slow process, we cannot assume that the relative removal rate from tissue is insignificant. We have assumed that the removal of protein from the tissue is governed by a process that is uniformly distributed in the tissue space. This is necessary because the model does not contain specific spatially related sinks for protein transport but distributes them uniformly. In addition, the form of the in vivo model restricts us from experimentally assessing the function of individual lymph capillaries.

Our experimental procedure is analogous to that of Reed et al. (28). Instead of a direct injection of protein-containing solution into the interstitium (28), we delivered the material into the tissue interstitium with convection, to a specific area that was slightly smaller than the diameter of the shielded scintillation probe. We then determined the elimination rate of IgG from the local tissue space by monitoring the total radioactivity in the tissue. Our turnover number or fractional removal rate is similar to that found by Reed et al. (28) for albumin in the hindleg muscle of an anesthetized rat (0.5 × 10⁻³ min⁻¹). Our estimate of the lymph flow is almost the same number as that found by Reed et al. (0.5 × 10⁻⁴ µl·min⁻¹·g tissue⁻¹; Ref. 28). Bill (5), who infused radiolabeled protein for 24–48 h to maintain a constant plasma concentration in awake rabbits, calculated a turnover rate of 0.0017 min⁻¹ for the triceps muscle, which is nearly four times the rate that we found. Anesthesia is known to slow lymphatic rates by a factor of four to five (28); the difference between our value and that of Bill is likely caused by this factor. We therefore conclude that the IgG fractional removal rate that we have determined in the anterior abdominal wall of the anesthetized rat is very similar in magnitude to that found in skeletal muscle by other investigators.

Protein void fraction. The interstitium can be described as a two-phase system in which a colloid-rich, water-poor phase is in equilibrium with a water-rich, colloid-poor phase. The colloid-rich phase consists of several mucopolysaccharides, which exclude solutes and, in particular, protein. These excluded solutes transport primarily through a tortuous, water-rich phase by diffusion and convection. Recent ultramicrospectrophotometric measurements of protein in the rat mesentery (23) have demonstrated the heterogeneity of the interstitial space between blood capillary and lymphatic. These investigators demonstrated variable concentration profiles of endogenous serum proteins in the tissue interstitium between blood capillaries and lymphatics. If these native proteins are not in equilibrium, it is unlikely that exogenous tracers will be at a uniform concentration in their void space even after circulating for 24–48 h in the blood. However, the overall tissue concentration may approach an in vivo steady-state concentration from which one can estimate the solute void space.

In vitro estimates of protein θₑ do approach true equilibrium in the tissue because the transport system is a closed one: there is no lymph removal of protein. The excised tissues are typically incubated in a medium containing the labeled marker until equilibration occurs. Using this method, Schultz and Armstrong (31) obtained an estimate of θₑ = 0.08 for albumin. Page and Bernstein (26) performed similar experiments in slices of cat heart muscle and obtained θₑ of 0.09 for inulin and 0.01–0.06 for dextrans with molecular weight of 150,000–180,000.

All in vivo experimental techniques used to estimate θₑ are based on the principle of equilibration between the plasma and the interstitium after sufficient time has passed after an intravenous injection/infusion of the substance under study. On the basis of the observations discussed above, “equilibration” of protein between the plasma and the interstitium may be unattainable. The lack of equilibration between the plasma and the saline void space in the tissue may lead to an underestimation of the true void space. On the other hand, binding of the protein to the tissue may tend to offset this effect. Although our technique mimics that of others, all of these data must be interpreted within the context of these limitations.

Bell and colleagues (3) injected labeled sucrose, albumin, and fibrinogen intravenously into dogs and determined the plasma equivalent space in the extracellular compartment after 24 h. In the interosseous muscle of the leg, the estimated θₑ was 0.20, 0.05, and 0.002 ml/g of tissue, respectively. Bill (5), on the other hand, infused labeled albumin and IgG to maintain a constant plasma concentration in rabbits for 48 h; he determined the tissue concentration versus time and divided tissue concentration by plasma concentration to obtain an equivalent plasma space. For albumin, a steady-state concentration was obtained in the triceps muscle after 24 h and the extravascular space was estimated as 26 µl/g tissue or a θₑ of 0.026 ml/g tissue. For IgG, the corresponding estimate of θₑ was 0.011 ml/g tissue. Other investigators have noted significant increases in θₑ with tissue hydration (2) or significant decreases with dehydration (29). Variation in species, tissue, and experimental preparation including degree of hydration may account for some of the variation in the measurements. A third group (33) infused labeled serum albumin into rats for several days and found the θₑ to be 26% of the interstitial space in the hindlimb muscle. This would correspond to a θₑ of 0.041 ml/g tissue (0.26 × 0.18 ml/g tissue, where θₑ = 0.18).

Our estimate of θₑ of 0.04 ml/g tissue is in the same order of magnitude as previously determined values. However, we would caution that all of these data should not be viewed as absolute determinations of the actual space but as a dynamic property that likely depends on the specific tissue, the degree of local tissue binding, and the state of hydration of the animal. We have previously shown (19) that water transport into the abdominal wall muscle is minimal at ip pressures <2 cmH₂O and that the θₑ in the abdominal wall muscle does not change after 4 h of exposure to fluid. Therefore,
it is unlikely that a swelling phenomena occurs in the space available to the protein (θu) over the course of the experiment. However, our measurements of θu were carried out in animals that had no large bulk of fluid in the peritoneal cavity. To obtain such data would be technically quite difficult in our in vivo model.

Model results: Estimates of IgG diffusivity. Although the model fits shown in Fig. 7 are quite reasonable given the uncertainty in the data, the lack of a perfect fit to the data may be caused by several factors. The model assumes an isotropic tissue bed; the abdominal wall has at least one tissue plane in its cross section. Lymphatic flow is averaged over the entire tissue; in reality, the lymphatics are located in tissue planes and in the subcutaneous space. Binding has been based on large cross-sectional analyses of the abdominal wall and has not been studied for variation with the abdominal wall. In addition, despite careful precautions to eliminate significant pressure forces external to the tissue, we cannot completely rule out local convection caused by pressure gradients within the tissue. These assumptions in the model are necessary because of limitations in our ability to obtain data that would justify more complex approaches.

Care must be taken to define the diffusivity of a molecule as it transports through a tissue. If the model of porous media is assumed, which has a nonexuded fraction of the tissue θu, the effective diffusivity for the whole tissue is $D_{\text{eff}} = \theta_u D_v$. $D_v$ equals the diffusivity within the tissue space available to the substance and takes into account the tortuosity of the path and any retardation by charged molecules surrounding the void space. Early estimates of $D_v$ for IgG in normal tissue were on the order of $1-2 \times 10^{-8}$ (32) to $2-4 \times 10^{-9}$ (9) cm²/s. The estimated diffusivity in water for IgG is $6 \times 10^{-7}$ cm²/s (9). These earlier techniques did not account for binding of the protein. The effects of specific binding on the transport of monoclonal antibodies have been explored by several authors (see Refs. 1, 21). All demonstrate that the total antibody concentration increases in tissue with increasing binding effects and that if free protein is not separated from bound in the analysis, the apparent transport can appear retarded in tumor tissue. Our findings mirror this effect for nonspecific binding of tracer quantities of IgG as well.

Recently, Jain’s group has reassessed their previous estimate of $D_v$ with the technique of fluorescence recovery after photobleaching (see Ref. 4). They have demonstrated in a mouse dorsal skinfold model containing human tumor xenografts that $\sim 30\%$ of the IgG that extravasated from the tumor circulation was rapidly pumped into vascularized normal muscle. The estimated tortuosity within the tissue space (equals the actual distance traveled divided by the shortest distance between two points, $\tau$) varies between 1.4 and 4.5 (7, 26). The average of all of these determinations is 2.5 (31) and might account for the difference between $D_v$ and $D_{\text{water}}$, where $D_v = D_{\text{water}}/\tau$.

IgG diffusion vs. convection. Because our original goal was to investigate the significance of the diffusive transport of IgG versus the transport under conditions of both diffusion and convection, we have replotted the results of Fig. 5 with data from an earlier study of IgG transport (18). Figure 9 displays concentration profile data from this study and from experiments in which the pressure gradient across the abdominal wall muscle was 3–4 cmH₂O. The profiles at 20 min and 200 min clearly demonstrate the marked effect that convection has on the transport of large proteins. The IgG in the earlier study was a monoclonal antibody (MAb 96.5) to a human melanoma cell line (FEMX-2) and had no specific receptors in normal rat tissue. The binding characteristics were not determined for different times of incubation, but for comparable incubation times (60 min), the nonspecific binding of MAb 96.5 was of the same order of magnitude as that of the IgG of this study (17).

From the model using the parameters that have been estimated from our data, we have calculated the diffusive flux of IgG across the peritoneal surface to be on the order of $2-3 \times 10^{-6}$ ml·s⁻¹·cm⁻². This flux is two orders of magnitude greater than the fluid flux that was determined in the chamber experiments that were presented in METHODS [water flux = $0.3 \times 10^{-6}$ ml·min⁻¹·cm⁻² or $0.005 \times 10^{-6}$ ml·s⁻¹·cm⁻²]; therefore, the small fluid flux that may be present at very low ip pressures should not interfere with the diffusion of IgG across the peritoneal of the abdominal wall. However, higher ip pressures increase the convection considerably and can have a significant effect on the transport process. From our previous work (18–19), we estimate the total IgG flux (convection and diffusion) into the abdominal wall at an ip pressure of 4 cmH₂O as $\sim 2 \times 10^{-5}$ ml·s⁻¹·cm⁻², which is an order of magnitude larger than the estimated diffusive flux from our experiments. The actual ratio of convective to diffusive flux may not be 10, however. This would assume that the tissue space remains unchanged when under pressure from fluid in the cavity. In unpublished studies, we have found that increasing the ip pressure to 4 cmH₂O
results in a doubling of the extracellular space in the abdominal wall. This would likely increase \( \theta_s \) and would therefore increase \( D_{\text{eff}} (D_{\text{eff}} = D_0 \theta_s) \), which would in turn increase the rate of diffusion. Fox and Wayland (20) observed significant increases in \( D_0 \) of serum albumin in the case of a hydrated mesentery (\( D_0 = 6 \times 10^{-7} \text{ cm}^2/\text{s} \)) versus the nonhydrated mesentery (\( 0.4 \times 10^{-7} \text{ cm}^2/\text{s} \)).

We conclude that convection, in general, dominates over diffusion, but we would caution that diffusion of macromolecules in tissue may not be negligible. More experimentation will be required to sort out the complex structural changes of the tissue space under a variety of stresses, the local flow regime, binding phenomena, and lymph flow.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant R29-DK48479–01 and a grant from the Whitaker Foundation.

Preliminary results of this paper were presented in abstract form at Experimental Biology 1996, Washington, DC, and at the 1996 Digestive and Kidney Diseases Grant R29-DK48479–01 and a grant from the Whitaker Foundation.

REFERENCES


