Transport of protein in the abdominal wall during intraperitoneal therapy. I. Theoretical approach

MICHAEL F. FLESSNER
University of Rochester Medical Center, Rochester, New York 14620
Received 27 September 2000; accepted in final form 26 February 2001

Flessner, Michael F. Transport of protein in the abdominal wall during intraperitoneal therapy. I. Theoretical approach. Am J Physiol Gastrointest Liver Physiol 281: G424–G437, 2001.—Intraperitoneal therapies such as peritoneal dialysis or regional chemotherapy use large volumes of solution within the peritoneal cavity. These volumes increase intraperitoneal hydrostatic pressure (Pip), which causes flow of the solution into tissues that surround the cavity. The goal of this paper is to integrate new experimental findings in a rigorous mathematical model to predict protein transport from the cavity into tissue. The model describes non-steady-state diffusion and convection of protein through a deformable porous medium with simultaneous exchange with the microcirculation and local tissue binding. Model parameters are dependent on local tissue pressure, which varies with Pip. Solute interactions with the tissue in terms of local distribution volume (solute void space), local binding, and retardation relative to solvent flow are demonstrated to be major determinants of tissue concentration profiles and protein penetration from the peritoneal cavity. The model predicts the rate of fluid loss from the cavity to the abdominal wall in dialysis patients to be 94 ml/h, within the observed range of 60–100 ml/h. The model is fitted to published transport data of IgG, and the retardation coefficient f is estimated to be 0.3, which markedly reduces the rate of protein penetration and is far lower than previously published estimates. With the value of f = 0.3, model calculations predict that Pip of 4.4 mmHg and dialysis duration of 24 h result in several millimeters of protein penetration into the tissue.

The model is based on a deformable porous medium and simulates diffusional and convective transport of protein and fluid. Mathematical models have been developed to predict the rate of protein transport, and these models have been fitted to published transport data of IgG. The model predicts that the rate of fluid loss from the cavity to the abdominal wall in dialysis patients is 94 ml/h, within the observed range of 60–100 ml/h. The model is fitted to published transport data of IgG, and the retardation coefficient f is estimated to be 0.3, which markedly reduces the rate of protein penetration and is far lower than previously published estimates. With the value of f = 0.3, model calculations predict that Pip of 4.4 mmHg and dialysis duration of 24 h result in several millimeters of protein penetration into the tissue.

THE PERITONEAL CAVITY is a natural site for introduction of drugs or for dialytic removal of substances from the circulation (38). It is potential space contained within the thin tissue called the peritoneum, which has a large surface area (11) and which adheres to a variety of well-perfused tissues. In peritoneal dialysis, 2–3 liters of sterile solution are instilled into the peritoneal cavity via a catheter through the abdominal wall. The 2- to 3-liter volumes used in typical therapy in the adult peritoneal cavity increase the intraperitoneal hydrostatic pressure from 0 (when cavity is empty) to 2–15 mmHg (30, 52). Figure 1 is a horizontal cut through the peritoneal cavity below the transverse colon, and it illustrates how the hydrostatic pressure gradient across the abdominal wall [intraperitoneal pressure − skin pressure (typically atmospheric)] can change through a decrease or increase of the intraperitoneal pressure. Fluid loss from the peritoneal cavity into the body of a human patient has been found to be directly proportional to the intraperitoneal hydrostatic pressure (24, 63) and amounts to 60–100 ml/h in healthy patients undergoing peritoneal dialysis with 2 liters of solution (7, 36, 46). This loss equals or exceeds the net volume recovery (obtained through the use of hypertonic solutions) in most patients and can be a major cause of dialysis failure.

In contrast to dialysis, intraperitoneal chemotherapy uses an intraperitoneal dialysis solution as a vehicle for therapeutic agents to treat infections or metastatic tumors on the peritoneal surface (53). This form of chemotherapy has recently included intraperitoneal immunotherapy with monoclonal antibodies and other macromolecules, which transport from the cavity into the surrounding tissue primarily by hydrostatic pressure-driven convection of therapeutic proteins into their target within the surrounding tissue space (16). Fluid flow induced by intraperitoneal hydrostatic pressure therefore makes up an important part of the transport of water and large solutes across the peritoneum during this clinical procedure as well as during dialysis.

A quantitative understanding of the driving forces and parameters that govern fluid transport may lead to strategies to minimize the fluid loss to the patient's body and to improve fluid recovery at the end of dialysis. A knowledge of the same process can also assist in our understanding and improvement of intraperitoneal therapy designed to introduce macromolecular medicines into metastatic intraperitoneal tumors. In prior work, we (17) developed a non-steady-state, unidirectional model in which the interstitium was assumed to be a rigid, porous medium. The model simulated diffusion and a distance-averaged rate of convection in the interstitium, with simultaneous blood capillary uptake in a tissue bed with uniformly dispersed blood capillaries. We fitted the model to in vivo concentration profiles of a small solute transporting from the abdominal cavity into tissue. Fluid transport is governed by the hydrostatic pressure gradient across the abdominal wall, which varies with the intraperitoneal pressure and the skin pressure (typically atmospheric). The model predicts that the rate of fluid loss from the cavity to the abdominal wall in dialysis patients is 94 ml/h, within the observed range of 60–100 ml/h. The model is fitted to published transport data of IgG, and the retardation coefficient $f$ is estimated to be 0.3, which markedly reduces the rate of protein penetration and is far lower than previously published estimates. With the value of $f = 0.3$, model calculations predict that Pip of 4.4 mmHg and dialysis duration of 24 h result in several millimeters of protein penetration into the tissue.
neal cavity into surrounding tissue primarily by diffusion (18, 20). However, we were unable to fit this simple model to tissue concentration profiles that resulted from the transport of IgG from the cavity (16). In subsequent work (61, 62), we found that the properties of the interstitium are highly dependent on the local tissue pressure \( P_T \), which varies with intraperitoneal pressure \( P_{ip} \) and that the tissue cannot be modeled as a rigid structure.

Seames et al. (48) developed a more complicated model of peritoneal transport during dialysis, but because of a lack of tissue-level data, these authors made several assumptions that were shown subsequently to be in error. Their theoretical model included diffusive and convective transport across the peritoneum and within the subperitoneal tissue. They considered the mesothelium of the peritoneum to be a semipermeable barrier with properties analogous to the capillary endothelium, and they applied pore theory (43) to model water and solute flow during dialysis with a hypertonic solution in the cavity. However, our experimental data (21) contradict this assumption and demonstrate that the mesothelium is an insignificant barrier to solutes and water flow. As a result of their assumption of a mesothelial barrier, Seames et al. predicted that during the first 2 h of dialysis, when the osmotic pressure in the cavity is high, the tissue pressure is below zero and the interstitial volume shrinks during hypertonic dialysis. Our data (62) have demonstrated that the interstitial volume is dependent on the hydrostatic pressure but independent of the osmotic pressure in the cavity. In their model, the coefficient of convection (hydraulic conductivity) was held constant. In contrast, our recent data in rats (61) have demonstrated that the hydraulic conductivity of the tissue varies fivefold over a clinically relevant range of intraperitoneal pressure. Seames et al. additionally assumed that molecules would flow at the same velocity as the solvent through the tissue, despite evidence that solutes are known to be retarded in their movement through mixtures of interstitial matrix components (32). Despite the common clinical observation of significant loss of protein from tissue to the cavity during dialysis (10) and our experimental data (16, 19) that demonstrated protein movement from the cavity to the surrounding tissue in proportion to the flow, Seames et al. assumed that movement of protein in the tissue was insignificant. Because they did not model protein movement in the tissue, neither the effect of binding nor lymph flow within the tissue was included in their model. Their model was able to simulate rates of small-solute transport during dialysis and to fit our previously published small-solute tissue concentration data (20), which result primarily from diffusion. However, their approach is inadequate to calculate the tissue concentrations or rates of transport of macromolecules, which are transported primarily by convection during large-volume intraperitoneal therapeutic procedures.

This paper presents a model that is based on experimental observations and attempts to correct the inconsistencies of previous theoretical models of fluid and solute transport across the peritoneum. The model focuses on the complex problem of macromolecular delivery from the cavity to the surrounding tissue and includes the processes of diffusion, hydrostatic pressure-driven convection, tissue binding, and macromolecular removal via lymphatics. The interstitial space and its transport parameters are variables, dependent on the local tissue interstitial pressure; they are derived from experimental work of our own laboratory and from the work of others. The model equations are solved numerically, and the sensitivity of the model to major parameters is tested by varying the parameters from their baseline and calculating the output. The model output is further compared with existing interstitial concentration profiles for immunoglobulin in normal tissue to demonstrate the need for more experimental data on specific parameters. Extensions of the model to predict events in longer-term intraperitoneal immunotherapy and dialysis applications are also discussed.

**MODEL FORMULATION**

Our research has emphasized the study of these forces in the abdominal wall, because it receives 30–50% of the total fluid flowing from the cavity and because the pressure forces exerted on the tissue can be easily controlled (15). Figure 2 is a conceptual model of the abdominal wall (see Fig. 1 for anatomic position of the tissue), across which the pressure can change through a decrease or increase of the intraperitoneal pressure \( P_{ip} \). The abdominal wall of the experimental animal is very accessible for the determination of tissue pressures and tissue concentrations of various marker substances (13, 16).

According to Darcy’s law, fluid flow through tissue (Q) has been shown to be directly proportional to the hydraulic conductivity \( K \), the cross-sectional area of tissue (A), and the tissue pressure gradient (\( \frac{dP_T}{dx} \), where \( x \) is distance) (33)

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

(1)
Rather than using $P_{it}$, the true interstitial pressure, we designate the tissue pressure by $P_T$, which is determined in rat experiments by a micropipette mounted on a precision designator. The micropipette is connected to a servo-null system and is used to create successive small chambers of free interstitial fluid (3- to 6-μm diameter × 50-μm length) within the tissue. Within each small chamber, the pipette-servo-null device is allowed to come to equilibrium with the local fluid pressure, and this is assumed to be equivalent to $P_{it}$. Further discussion of this approximation is contained in papers by Gilanyi and Kovach (28) and Wiig et al. (59).

Our theoretical formulation takes the practical approach that a model should include parameters that are either published or attainable from current experimental techniques. The mathematical model is based on our previous work (17) with modifications to take into account theories of convection (1, 48) and experimental findings (19, 22, 61, 62) in the transport process. The model focuses on hydrostatic pressure as the chief driving force for convection. Thermodynamic analyses (28) have suggested the importance of local colloid osmotic pressure to interstitial flow, and some researchers have included the effects of local colloid osmotic pressure in their mathematical models (34, 51). However, there are few in vivo data available for testing the significance of these concepts, and the determination of local changes of colloid osmotic pressure at multiple sites within the rat abdominal wall has not been performed.

The interstitium has been demonstrated to be compliant in several tissues (2). Interstitial compliance ($\Omega$) is defined as the “ratio of the change in interstitial fluid volume divided by the corresponding change in interstitial hydrostatic pressure” (2)

$$\Omega(P_T) = \frac{dV_{if}}{dP_T}$$

where $V_{if}$ is interstitial fluid volume. To estimate $\Omega(P_T)$ for a given tissue, $\theta_{if}$ is measured versus the interstitial pressure and the slope of the curve is determined. Typically, the shape of these curves is linear in dehydration (57, 58) but highly nonlinear above zero pressure (relative to atmospheric pressure) (42, 62). The compliance of abdominal wall muscle during hydration has been determined to be similar to that of skeletal muscle with magnitudes of 1.4–4.3 ml·mmHg$^{-1}$·100 g tissue$^{-1}$, depending on the average tissue pressure (62).

For intraperitoneal pressures above 3 mmHg in the rat, we have determined that the pressure profile is nearly linear across the entire abdominal wall (~2 mm thick) (13). Figure 3 illustrates these profiles and their initial slopes (d$P_T$/dx) at the peritoneal edge, which are equivalent to the convective driving force from the cavity (13). Note that the slopes in the tissue are approximately the same with magnitudes of $-20$ to $-25$ mmHg/cm of tissue (13, 61). Across the abdominal wall, therefore, pressures will be high at the peritoneum and low at the skin side (see also Figs. 1 and 2). This variation in pressure may cause the interstitial volume to be higher in the vicinity of the peritoneum and to decrease across the abdominal wall in accordance with the pressure profile in the tissue and the tissue compliance. We have also found (61) that the hydraulic conductivity of the abdominal wall tissue increases linearly with the mean tissue pressure. If we impose an $P_{ip}$ of 4.4 mmHg (6 cmH2O) across the abdominal wall, Fig. 4, using data from our previous studies (13, 61, 62), illustrates the pressure gradient and the corresponding extracellular space for the initial 2 mm of tissue adjacent to the peritoneum. This variation of $P_T$ and $\theta_{if}$ in the tissue results in variation of the tissue hydraulic conductivity and in the effective tissue diffusivity ($D_{eff}$)

$$D_{eff} = D_s \theta_{if}$$

where $D_s$ is the solute diffusivity in the tissue void and includes a correction for the tortuosity of the path and $\theta_{if}$ is
can be modeled as an infinite plane with isotropic properties. The abdominal wall is equivalent to a thin-walled shell, which unit with isotropic characteristics. It is assumed that the dominal wall, groups of muscles in tissue planes and sepa-

The solute void fraction (proportion of the total tissue that is available to the solute) and is $\leq \theta_{v}$. 

**Solute Balance**

The general balance equation for a solute transporting via unidirectional, non-steady-state diffusion and convection in the interstitial is given in the following

$$\frac{\partial (\theta_s C_s)}{\partial t} = \frac{\partial}{\partial x} \left[ D_{\text{eff}} \frac{\partial C_s}{\partial x} + C_s f K \frac{\partial \theta_s P_T}{\partial \theta_s} \right] + R_{\text{cap}} - R_{\text{bind}} - R_{\text{lymph}} - R_{\text{metab}} + R_{\text{gen}} \quad (4)$$

where $C_s = C_s(x, t)$ is free solute concentration within its tissue void; $t$ is time; $\theta_s = \theta_s(P_T)$ is solute void volume, which is the portion of the tissue volume within which the solute distributes, dependent on the local interstitial pressure ($P_T$); $D_{\text{eff}} = D_s(\theta_s)$, where $D_s$ is the solute diffusivity in the solute void volume and includes effects of tortuosity of the solute path; $f$ is the solute retardation factor (ratio of solute to solvent velocity); $K = K(P_T)$ is the tissue hydraulic conductivity; $R_{\text{cap}}$ is local solute exchange with distributed blood capillaries; $R_{\text{bind}}$ is net rate of binding of free solute to local tissue; $R_{\text{metab}}$ is local uptake of agent by cells; $R_{\text{lymph}}$ is local rate of lymphatic uptake and removal from tissue; and $R_{\text{gen}}$ is local rate of solute generation in tissue.

Equation 4 is derived from Eqs. 1 and 3 and from the model concept in Fig. 2. The first term of the right-hand side of Eq. 4 follows directly from the diffusion equation: diffusion velocity $=-D_{\text{eff}}(\partial C_s/\partial x)$. The second term is derived as follows. Within the interstitial space, from Eq. 1, the velocity of the solvent, $v_s$, equals $Q/(A \theta_0)\rho - (K \theta_0 \rho) (\partial P_T/\partial x)$. Because large solutes may not move with the same velocity as the solvent, the solvent flow is multiplied by the solute retardation factor $f$ to obtain a solute velocity $v_s^f = v_s f$. The mass flow equals $C_s v_s = C_s f v_s^f = C_s (-K \theta_0 \rho) (\partial P_T/\partial x)$. To relate the mass velocity within the interstitium to movement in the tissue as a whole, the interstitial velocity is multiplied by $\theta_s$, the fraction of the total tissue volume that the solute can occupy. The result is the second expression in the brackets of the right-hand side of Eq. 4.

Equation 4 encompasses several assumptions. In the abdominal wall, groups of muscles in tissue planes and separated by fascia (see Fig. 1) are assumed to form a single tissue unit with isotropic characteristics. It is assumed that the abdominal wall is equivalent to a thin-walled shell, which can be modeled as an infinite plane with isotropic properties including uniform tissue compliance and a uniform density of blood and lymphatic microvessels. Thus the model transport can be constrained to one direction ($x$), and the location of blood vessels can be simplified to a uniform density throughout the tissue. As mentioned above, the driving force for convection is assumed to be hydrostatic pressure alone. The various rates ($R_i$) are defined by separate rate equations. $P_T$ is assumed to be less than intracapillary hydrostatic pressure. [In therapeutic situations, pressures above the portal vein pressure collapse the vein and cause hemostasis in the gastrointestinal tract (unpublished observations).] The solute retardation factor $f$ is defined to have a range of 0–1 and must be derived from model fits to experimental data after all other parameters are established. Parameters $D_{\text{eff}}, K, f, \theta_s$, and $\theta_{v}$ are variable and are functions of the local $P_T$.

**Volume Balance**

The volume balance on the interstitial is as follows

$$\frac{\partial \theta_s}{\partial t} = \frac{\partial}{\partial x} \left[ K \frac{\partial P_T}{\partial x} \right] + F_{\text{cap}} - F_{\text{lymph}} \quad (5)$$

where $\theta_s$ is interstitial void volume, a function of $P_T$, as shown in Eq. 2, $F_{\text{cap}}$ is local volume flow to the blood capillaries, and $F_{\text{lymph}}$ is local volume flow to lymph capillaries.

**Rate Equations**

**Solute binding.** To model the movement of proteins through tissue, binding of the solute must be taken into account in the model. Actual tissue data typically consist of the total amount of labeled solute per unit volume of tissue, including bound and free species. The model variable $C_s$ equals the free concentration in the solute void volume, which is essentially a virtual space within the tissue in which the solute distributes within the total tissue volume. If there was no binding of the solute, the concentration measured in the tissue, $C_{\text{tissue}}$, would equal $C_{\text{free}} = C_{\text{free}}$. Because there is either specific or non-specific binding for all proteins, the more general definition for $C_{\text{tissue}}$ is as follows

$$C_{\text{tissue}} = C_{\text{free}} + C_{\text{bound}} \quad (6)$$

where $C_{\text{free}}$ is concentration of free solute in tissue = $C_{\text{free}}$ and $C_{\text{bound}}$ is concentration of bound solute in tissue, where $C_{\text{bound}} = g(C_{\text{free}}, C_{\text{bound}})$, a function defined by experiment. The general mass balance for the bound species is (8, 9)

$$R_{\text{bind}} = \frac{dC_{\text{bound}}}{dt} = B_f C_{\text{free}} - B_R C_{\text{bound}} \quad (7)$$

where $B_f$ is the forward (association) rate coefficient and $B_R$ is the reverse (dissociation) rate coefficient. Both of these coefficients must be derived from experimental data.

**Transendothelial transport.** The expressions for solute and water transport across the blood capillary barrier are taken from the multiple-pore theory of Rippe (47, 49). The details of this theory are given in the APPENDIX. Because this theory has been used extensively in simulations of the subperitoneal tissue (18, 20), and most of the blood capillary coefficients have been defined by Rippe (45, 49) for the muscle that surrounds the mammalian peritoneal cavity, the theory will be used in simulations for normal abdominal wall muscle. However, the author is well aware of new theory that supports the glycoelastic as a major barrier in the endothelium (25, 26). When those new models mature, they can be incorporated into this model, the chief goal of which is to simulate the interstitial convection of proteins.

If the solute of interest is a protein or macromolecule larger than albumin (~58,000 Da), pore theory constrains all
transport to convection through the “large pore” in the endothelial barrier. Because the intracapillary pressures are generally higher than the interstitial pressures, transport is generally one way, from the capillary lumen to the interstitial.

Equation A1 (see Appendix) can be simplified to

$$R_{\text{cap}} = R_{Lp} = C_p F_{lp}(1 - \sigma_{Lp}) \tag{8}$$

where $R_{Lp}$ is solute transport via large pores, $C_p$ is plasma concentration of protein, $F_{lp}$ is water transport via large pores, and $\sigma_{Lp}$ is the reflection coefficient for the large pore for the protein. For the short-duration simulations used in this paper, the solution of Eq. 4 for macromolecules does not depend significantly on the expression for $R_{\text{cap}}$ (see below).

Lymphatic transport from tissue. Lymph will be assumed to flow from the tissue at a constant rate equivalent to $F_{\text{lymph}}$, which must be derived by experiment. The rate of solute flow from the tissue space is given by

$$R_{\text{lymph}} = C_{\text{free}} F_{\text{lymph}} \tag{9}$$

Other rate equations. If the solute of interest can be taken up by cells, metabolized, or endogenously produced in the tissue, experimental data must be collected to define $R_{\text{metab}}$ and $R_{\text{gen}}$. In our experimental transport studies, exogenous solutes that are not produced in the experimental subject and that do not undergo significant metabolism are chosen to obviate the need for these additional data; these rate functions are therefore set to zero in the simulations in this paper.

Initial and Boundary Conditions After an Intraperitoneal Injection

Figure 2 illustrates the key initial and boundary conditions

at $x = 0$, $t = 0$: $C_x = C_{ip}(0)$, the intraperitoneal concentration at time “0,” $P_T(0) = P_{ip}$ is intraperitoneal pressure. These two conditions presume that there is no boundary layer on the peritoneal surface that affects concentration or pressure.

at $x = 0$, $t > 0$: $C_x = C_{ip}(t)$ (determined from experiment)

At $x > 0$, $t = 0$: $C_x = 0 \tag{10}$

At $x = x_1$, $t > 0$: $dC_x/dx = 0$ and $dP_T/dx \geq 0$ at $x_1$. Based on experimental data (see Fig. 3), $dP_T/dx$ at the abdominal wall edge ($x_1 \approx 0.2$ cm) at high pressures ($P_{ip} > 6$ mmHg) may not $= 0$

At $t = 0$, $C_{ip} = 0$; at $t > 0$, $C_{ip} = C_{ip}(t)$ (from experiment)

In the general case of a tissue that undergoes expansion, the value of the $x_1$ boundary is a variable. In the abdominal wall, the expansion of the whole tissue between $P_{ip}$ of 0 and 8 mmHg is <20% (62). If we assume that the expansion is equal in all directions, the $x_1$ value could possibly change by ~6%. Practically speaking, this magnitude of change cannot be measured in quantitative macro-autoradiographic data (16). For the model simulations in this paper, $x_1$ is assumed to be constant.

Model Solution and Parameter Estimation

Equations 4 and 5, along with rate equations for solute and water transport across the capillary and lymphatic endothelia (Eqs. 8 and 9), rate equations for binding (Eq. 7), appropriate transport parameters, and boundary conditions, can be solved numerically for values of $\theta_{tr}$ and $C_p$. If the intraperitoneal solution is isotonic, intraperitoneal concentration [C_{ip}(t)] is essentially constant for molecules >50,000 Da (15, 19, 23), whereas $C_{ip}(t)$ rises to 10% of $C_{ip}(0)$ over several hours (15, 19). We have collected data on $\theta_{tr}(P_{ip})$, which allows us to concentrate on the solution of Eq. 4 alone. The solution of Eq. 4 depends on the following: solute-specific parameters $\theta_{tr}$, $f$, $D_{lp}$, binding rate coefficients, and capillary large-pore reflection coefficient; the tissue-specific parameters $K$, $\theta_{tr}$, lymph flow rate, and capillary large-pore flow rate; and the local hydrostatic pressure gradient ($dP_T/dx$). Our laboratory has published data on several of these parameters as functions for $P_{ip}$ or $P_T$ (21, 61, 62).

To make use of existing data, the solute was chosen to be IgG with no specific binding to the abdominal wall muscle. The equation describing nonspecific binding of IgG to muscle is represented by Eq. 7 with $B_0 = 0$ and $B_1 = 1.08 \times 10^{-4}$ s$^{-1}$ (22).

From our previously published results (61, 62), expressions for $\theta_{tr}$ and $K$ will be derived. The expression for $K$ is as follows

$$K(P_T) = A_0 + A_1(P_T - 1.2) \tag{11}$$

where hydraulic conductivity coefficients $A_0$ and $A_1$ are 0.15 $\times 10^{-6}$ cm$^2$s$^{-1}$mmHg$^{-1}$ and 0.18 $\times 10^{-6}$ cm$^2$s$^{-1}$mmHg$^{-2}$ respectively, the units for $K$ are square centimeters per second per millimeter of mercury, and the units for $P_T$ are millimeters of mercury. $A_1$ will be varied to determine how sensitive the model output is to $K$.

The expression for $\theta_{tr}$ is as follows

for $P_T < 0.7$ mmHg, $\theta_{tr} = 0.19$ ml/g tissue;
for $0.7$ mmHg $\leq P_T \leq 4.2$ mmHg,
$$\theta_{tr} = B_0 + B_1(P_T - 0.7); \tag{12}$$
for $P_T > 4.2$ mmHg, $\theta_{tr} = 0.35$ ml/g tissue

where $B_0 = 0.19$ ml/g tissue and $B_1 = 0.046$ ml g$^{-1}$ mmHg$^{-1}$. $B_1$ is equivalent to the compliance of $O(P_{ip})$ of Eq. 2 and is varied in the sensitivity analysis to demonstrate the importance of this factor to macromolecular transport.

From Fig. 3, we observe that the average slope of the three $P_T$ curves is ~20 to ~25 mmHg/cm. Our previous determinations were made in anterior abdominal wall tissue, 15 min to 4 h after a steady-state $P_{ip}$ was obtained. We observed that $P_T(x)$ did not vary significantly with time, and we assume that a constant $P_{ip}$ will result in a constant $P_T(x)$ for $t > 0$. We therefore compute the tissue pressure profiles by the following

$$P_T = P_{ip} - 25x \tag{13}$$

where $P_{ip}$ is the intraperitoneal pressure (held constant at 2, 4.4, or 8.8 mmHg for this study) and $x$ is in centimeters. Previously, we measured the rate of lymph flow ($F_{\text{lymph}}$) to equal $1.33 \times 10^{-6}$ cm$^3$s$^{-1}$mmHg$^{-2}$. This value is varied in the analysis below to show that it does not significantly affect the convective flow of protein through the abdominal wall.

According to pore theory, solute transport of IgG occurs through the convective flow of protein through the abdominal wall. If the solute of interest can be taken up by cells, metabolized, or endogenously produced in the experimental subject and that do not undergo significant metabolism are chosen to obviate the need for these additional data; these rate functions are therefore set to zero in the simulations in this paper.

AJP-Gastrointest Liver Physiol • VOL 281 • AUGUST 2001 • www.ajpgi.org
order of −1 to −2 mmHg (flow out of the capillary). With overall transcapillary hydraulic conductivity ($L_{p,t}$) = $6 \times 10^{-5}$ ml·mmHg$^{-1}$·s$^{-1}$ and $c_{\text{L,P}} = 0.07$, the resulting $F_{\text{cap,L,P}}$ is $-6 \times 10^{-4}$ ml·s$^{-1}$·g tissue$^{-1}$. With the estimated value of $F_{\text{cap,L,P}}$ and $C(t)$, the contribution of the “backflow” of IgG from the plasma to the tissue can be calculated. In simulations with $F_{\text{cap,L,P}} = 6 \times 10^{-5}$ to $6 \times 10^{-4}$ ml·s$^{-1}$·g tissue$^{-1}$, no significant change in tissue concentration was observed.

As shown in Eq. 3, the effective diffusivity, $D_{\text{eff}}$, equals $D_0 \theta_s$. For a given tissue the diffusivity within the tissue distribution space of the solute, $D_0$, is assumed to be a constant. $D_0$ also takes into account the tortuosity of the path. Our previous experiments (22) and those of others (3) produced an estimate for $D_0$ of $2 \times 10^{-7}$ cm$^2$/s for IgG. This value is used as the baseline value in the sensitivity analysis and is varied to determine its effect on the transport of IgG.

There is still uncertainty concerning the fraction of tissue that makes up the distribution space for the solute. Expansion of the interstitial space from fluid influx, which occurs in the abdominal wall muscle during dialysis, only complicates the estimation of this parameter. We have estimated the apparent $\theta_s$ under conditions of $P_T = 0$ and mean $P_T = 4$ cmH$_2$O (22). We did this by injecting labeled IgG 24 h before a second injection of IgG with a different radioactive label. Ten minutes after the second injection, the animal was euthanized and the abdominal wall tissue was sampled and counted for the concentrations of each tracer. If it is assumed that the first tracer is in equilibrium with its volume of distribution within the tissue, its tissue concentration divided by the plasma concentration provides an estimate of the total distribution space volume (including the intravascular space). The second tracer is assumed to remain within the vascular space, and therefore the ratio of its tissue concentration to the plasma concentration provides an estimate of the intravascular space. The total distribution space minus the intravascular space produces an estimate of $\theta_s$. At $P_T = 0$, $\theta_s = 0.043–0.050$, whereas at $P_T = 4$ cmH$_2$O, $\theta_s$ increases to $0.07–0.08$ (unpublished data).

These estimates may in fact be much lower than the actual values, because Witte (see Ref. 60) has shown that it is unlikely that the interstitial protein concentration ever increases to 0.07–0.08 (unpublished data).

Table 1. Parameter variations: immunoglobulin G

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Parameter Name</th>
<th>Units</th>
<th>Base Value</th>
<th>Low Value</th>
<th>High Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_T$</td>
<td>Forward binding rate coefficient</td>
<td>s$^{-1}$</td>
<td>$1.083 \times 10^{-4}$</td>
<td>$0.217 \times 10^{-4}$</td>
<td>$5.415 \times 10^{-4}$</td>
<td>22</td>
</tr>
<tr>
<td>$A_1$ (eq. 11)</td>
<td>Tissue conductivity slope</td>
<td>cm$^2$·s$^{-1}$·mmHg$^{-2}$</td>
<td>$0.18 \times 10^{-6}$</td>
<td>$0.09 \times 10^{-6}$</td>
<td>$0.36 \times 10^{-6}$</td>
<td>61</td>
</tr>
<tr>
<td>$D_0$</td>
<td>Tissue diffusivity in void volume</td>
<td>cm$^2$/s</td>
<td>$0.2 \times 10^{-6}$</td>
<td>$0.1 \times 10^{-6}$</td>
<td>$1.0 \times 10^{-6}$</td>
<td>22</td>
</tr>
<tr>
<td>$f$</td>
<td>Solute retardation</td>
<td>Dimensionless</td>
<td>0.5</td>
<td>0.1</td>
<td>1.0</td>
<td>See text</td>
</tr>
<tr>
<td>$F_{\text{lymph}}$</td>
<td>Lymph flow rate</td>
<td>ml·s$^{-1}$·cm$^{-2}$</td>
<td>$1.33 \times 10^{-6}$</td>
<td>$0.266 \times 10^{-6}$</td>
<td>$6.65 \times 10^{-6}$</td>
<td>22</td>
</tr>
<tr>
<td>$P_{\text{in}}$</td>
<td>Intraperitoneal pressure</td>
<td>mmHg</td>
<td>4.4</td>
<td>2</td>
<td>8.8</td>
<td>Arbitrary</td>
</tr>
<tr>
<td>$d\theta_s/dP_T$</td>
<td>Compliance</td>
<td>cm$^{-2}$·mmHg$^{-1}$</td>
<td>0.046</td>
<td>0.025</td>
<td>0.092</td>
<td>62</td>
</tr>
<tr>
<td>$\theta_s$min</td>
<td>Minimum tissue void volume</td>
<td>cm$^2$</td>
<td>0.05</td>
<td>0.025</td>
<td>0.10</td>
<td>22</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
<td>s</td>
<td>3,600</td>
<td>1,800</td>
<td>10,800</td>
<td>Arbitrary</td>
</tr>
</tbody>
</table>
The value $\Psi$ therefore reflects the normalized change in model output divided by the normalized change in a particular parameter. By sampling at three time points and at four points in the tissue, we attempt to define an index that reflects overall changes in the output.

Figure 6 illustrates the model sensitivity to perturbations in the parameters listed in Table 1. The one parameter that did not affect the concentrations in the tissue significantly was the lymph flow rate. The results are not plotted, but $\Psi$ was $<$0.1. For $f$, $P_{ip}$, $\theta_s$, and $K$, the resulting $\Psi$ values are intuitive, given the structure of Eq. 4 and the fact that the macromolecule transports chiefly via convection. The sensitivity to $D_v$ demonstrates that the diffusion still plays a role in the tissue penetration of IgG and cannot be eliminated from Eq. 4. From Fig. 5, the diffusive flux across the peritoneal surface varies in magnitude from 3 to 30% of the convective flux. As shown below, binding has major effects on the solute diffusion and the shape of the tissue concentration curves. With decreases in binding, there are higher free IgG concentrations in the tissue and the diffusive flux actually reverses direction to diffusion from the tissue toward the peritoneal cavity. On the other hand, an increase in binding provides a “sink” in the tissue that decreases the free concentration within the tissue and speeds up the rate of diffusion into the tissue. The $\Psi$ values for $\theta_s$ are negative because a decrease in the interstitial volume will increase the solute velocity (see Eq. 4), and, provided the $\theta_s$ remains constant (somewhat artificial in this analysis because it will likely parallel the changes in $\theta_{ip}$), the rate of solute movement into the tissue will increase. Analogously, an increase in $\theta_{ip}$ with a constant $\theta_s$ results in a decrease in solute velocity and a decrease in the solute flux into the tissue.

Figure 7 demonstrates the effect of time on output of the model. As time increases beyond 60 min, a greater proportion of the total $C_{tissue}$ is due to $C_{bound}$. $C_{free}$ at any distance from the peritoneum can be found by subtraction: $C_{tissue} = C_{bound}$. Binding of protein to tissue is a major determinant of $C_{tissue} (-C_{free} + C_{bound})$. Figure 7 illustrates the effect of time on the concentration of bound IgG ($C_{bound}$) and the total concentration ($C_{tissue}$). Most of the observed IgG in the tissue after 3 h is bound through interaction with nonspecific sites in the tissue, as demonstrated in our previous 4-h binding studies of IgG to muscle slices (22). With longer time of the peritoneal solution at a constant $P_{ip}$ of 4.4 mmHg, the concentration rises at each point in the tissue and the protein gains access to deeper portions of the tissue. This points to the importance of dwell duration in the clinical delivery of macromolecules to the subperitoneal interstitium.

![Graph showing convective and diffusive fluxes at the peritoneal surface](image)

**Fig. 5.** Convective and diffusive fluxes at the peritoneal surface for the base values of parameters and for the perturbed values listed in Table 1. The dominance of convection over diffusion in the case of a large protein such as IgG is apparent. See text for full discussion. Downward arrows pertain to high values for the parameter. Upward arrows pertain to low values for the parameter.
Figure 8 shows what happens to the 60-min curves of $C_{\text{tissue}}$ when the binding within the tissue varies from 20% of the baseline to five times the baseline value. As binding increases, $C_{\text{tissue}}$ near the source (the peritoneal surface) increases dramatically and less solute is transported further into the tissue. At the extreme end of the binding spectrum, the concentration of IgG at the surface would be very high, with almost no penetration into the interior of the tissue. This has been termed the “binding site barrier” and may be present in some antibody-tumor interactions (27).

Because of the dominance of convection in the transport of IgG, factors that directly affect the rate of water flow ($K, P_T$) and the force of convection on the solute ($f$) should demonstrate significant effects on the tissue concentrations of IgG in the abdominal wall. Figure 9 demonstrates the effects of doubling or halving the hydraulic conductivity coefficient $A_1$ of Eq. 11, which changes the hydraulic conductivity ($K$) proportionately; the higher the $K$, the further protein penetrates into the tissue. The effects of pressure are shown in Fig. 10, in which $P_{\text{ip}}$ is varied from 2.0 to 8.8 mmHg. The $P_{\text{ip}}$ is transmitted to the tissue as demonstrated in Fig. 1 and sets up the pressure profile of $P_T$ (Fig. 2), which drives fluid flow from the cavity into the tissue. When $P_{\text{ip}}$ is ≤2 mmHg, there is no significant change in the interstitial volume or in $K$ (61, 62). With the increase in pressure to 4.4 mmHg, the tissue undergoes a linear expansion with doubling of the interstitial volume and proportionate increase in $K$. With the increase in $P_{\text{ip}}$ from 4.4 to 8 mmHg, the interstitial volume does not increase, but $K$ continues to increase; the lack of tissue expansion explains the similar $y$-intercept at $x =$
0, whereas the higher conductivity increases the rate of flow of water and protein through the tissue, resulting in deeper IgG penetration. Without experimental data, this nonlinear response of the tissue could not have been anticipated.

Less subtle are the effects of $f$ on protein transport. Figure 11 illustrates the effect of increasing rates of solute velocity through the tissue. Pure diffusion is shown at $f = 0$ in a curve that can be contrasted with almost any degree of convection. This parameter has a major effect on protein transport through the tissue and the depth of macromolecular penetration in the subperitoneum.

Significant variations in $D_v$ (Table 1) do not result in large changes in $C_{\text{tissue}}$ (see Fig. 12). This would likely change if the $\theta_s$ were higher and the magnitude of $D_{\text{eff}}$ ($D_v\theta_s$) were correspondingly higher. The large exclusion space in the tissue is a major factor in the rate of transport, as illustrated in Fig. 13. The nonexcluded volume is varied over three alternate ranges (low to high in ascending order). The larger the space available to protein, the more will transport, provided the driving forces and other coefficients do not change. Note that with the increase of $\theta_s$, the concentration at the peritoneum and throughout the tissue increases. A pure change in this variable, however, does not increase the depth of penetration for a given amount of transport time. Because $\theta_s$ is assumed to be a function of $\theta_t$, the isolated variation of $\theta_s$ is likely an unrealistic representation of the changing environment of the tissue.

Figure 14 demonstrates the effects of variation of $\theta_t$ from low to higher values. As the interstitial volume increases, transport increases. However, these simulations illustrate the direct effects of solute retardation factor $f$ on $C_{\text{tissue}}$ and the depth of penetration.

---

**Fig. 11.** Calculated IgG concentration profiles for $P_{\text{ip}} = 4.4$ mmHg demonstrate the direct effects of solute retardation factor $f$ on $C_{\text{tissue}}$ and the depth of penetration.

**Fig. 12.** Calculated IgG concentration profiles for $t = 60$ min: effect of solute void diffusivity ($D_v$). This demonstrates the lack of effect that diffusion has on transport of large-molecular-weight solutes (such as IgG) where convection dominates.

**Fig. 13.** Calculated IgG concentration profiles for $t = 60$ min: effect of solute void fraction ($\theta_v$) on model output. This demonstrates that, as the tissue expands and the space available to the solute increases, transport of IgG increases. Because other parameters such as $f$, $K$, and $P_{\text{ip}}$ are held constant, the depth of penetration does not vary.

**Fig. 14.** Calculated IgG concentration profiles for $t = 60$ min: effect of interstitial volume ($\theta_t$) on model output. See text for discussion.
lated curves are likely artifactual and represent unrealistic model output because \( \theta_s \) and \( K \) have been held constant. With the expansion of the interstitium, the volume available to the solute and the hydraulic conductivity would surely increase. As demonstrated in Figs. 9 and 13, the cumulative changes in \( K \) and \( \theta_s \) would bring about further changes in \( C_{\text{tissue}} \) and likely result in a greater depth of penetration and higher tissue concentrations close to the peritoneum.

**MODEL SIMULATION OF PREVIOUS DATA**

In previous work (14), we examined the penetration of IgG from an isotonic solution in the peritoneal cavity into the tissues surrounding the cavity of RNU-nude rats. These experiments were carried out with MAb 96.5, a monoclonal antibody specific for receptors on the FEMX2 human melanoma cell line. Because the rats had no tumor implants, the binding of the antibody was assumed to be nonspecific. Intraperitoneal volumes were scaled to produce pressures of 2.2–3.0 mmHg in the cavity throughout the 180-min experiment. Concentrations in the peritoneal cavity were found to be nearly constant. After euthanasia at the end of the experiment, the abdominal cavity was rapidly drained and the carcass was frozen to preserve the concentration profile in the surrounding tissue. Tissue samples were sliced with a cryomicrotome, dried, and placed against autoradiographic film. The autoradiograms were subsequently analyzed with a computerized densitometer to determine the profile (n = 44) displayed in Fig. 15.

As an initial test of the model as a quantitative tool, we have fitted the model to the data in Fig. 15. The \( P_T \) was set equal to 3 mmHg, and the parameters used in the simulations were tissue void diffusivity, hydraulic conductivity, interstitial volume, lymph flow, transendothelial transfer, and compliance of the tissue as displayed in Table 1. Three parameters required adjustments to improve the fit. The minimum tissue void volume (\( \theta_s \)) was set to 0.07, with the variation from the high-pressure side to the low-pressure side of 0.10 to 0.07; this is close to our unpublished observations. The solute retardation (\( f \)) was set to 0.3 to produce a better fit to the curve. We did not have binding data from these experiments and found that setting the binding coefficient to \( 8.66 \times 10^{-4} \) (8 times the number in Table 1) improved the fit considerably. The fitted curve is displayed in Fig. 15. The necessary adjustments in the three parameters demonstrate the importance of good experimental data on binding characteristics and the interstitial space available to the solute. The parameter \( f \) must typically be fitted to concentration profile data, as in Fig. 15, once all other parameters have been determined. In reality, there is likely a family of parameter values that could produce reasonable simulations of the data. However, this exercise demonstrates the utility of the mathematical approach in discovering data gaps and in planning experiments to fill these gaps.

**DISCUSSION OF THE MODEL APPROACH**

Theoretical Approach to Convection in Subperitoneal Tissue

This paper presents an integration of extensive in vivo data with theory to produce a new mathematical approach toward protein movement through the subperitoneal interstitium during dialysis or during a procedure designed to regionally treat the peritoneum. Previous predictive models have been based on assumptions that have subsequently been shown to be incorrect in experimental studies. Our model incorporates the nonlinear compliance of the abdominal wall muscle interstitium to predict the volume expansion over a clinically relevant range of intraperitoneal pressure (56, 62). The major driving force for convection has been shown to be \( P_T \), which is dependent on the pressure in the cavity (24). Because the mesothelium does not act as a membranelike barrier (21), the model is based on flow through porous media, and all parameters are varied on the basis of the hydrostatic pressure profile in the tissue. With a rise in \( P_T \), the tissue is predicted to expand, which causes \( \theta_f \) and \( \theta_s \) to increase in magnitude (22, 62). The model incorporates this extracellular expansion and appropriately increases the magnitude of the major transport coefficients of diffusion (\( D_{\text{eff}} \)) and convection (\( K \)) as well (22, 61). By varying model parameters and calculating the changes in concentration profiles and solute fluxes, the model sensitivity to specific parameters has been tested. We have demonstrated that IgG transport is most sensitive to factors that influence convection: \( P_T \), \( f \), \( K \), \( \theta_f \), and \( \theta_s \). We have also included effects of capillary transport, lymphatic transport, and local binding of protein to the tissue. We have demonstrated that for IgG, much of the total tissue protein concentration is bound, even if that binding is nonspecific. Although diffusion is not insignificant (especially in the early period of transport when the protein is just entering the tissue and the concentration gradient is large), we
have demonstrated that it has only small effects on the concentration profile after 1 h of transport.

In the model presented here, the solute retardation factor \( f \) has been demonstrated to be a major factor influencing the convective movement of proteins through tissue (see Figs. 5, 6, and 11). In our earlier model (17), \( f \) was assumed to equal 1 because of the lack of data. The factor is not even mentioned in the work of Seames et al. (48). In this paper, with other major parameters defined, the model has been fitted to previously published IgG concentration profiles, and \( f \) was estimated to be 0.3. This estimate means that 70% of the protein is retarded in its passage through the tissue, relative to the solvent flow. Although certain assumptions were made in these calculations, it is unlikely that the velocity of large solutes within the abdominal wall interstitium equals that of water flowing through the tissue. This result does not match the calculated value for \( f \) given by Swabb’s formula (50), which produces an estimate of \( f \) that is >1 for the interstitial hyaluronan concentration in the abdominal wall. Swabb’s formula was derived from the data of others (32) who studied the sedimentation of proteins in pure solutions of hyaluronan. Although hyaluronan is an important component of the interstitial matrix, other substances such as proteoglycan and collagen make up the interstitial matrix. Collagen is thought to form the scaffolding between cells via \( \beta_1 \)-integrins (41) and has recently been shown to be a major determinant of IgG transport resistance in solid tumors (37). The characteristics of the in vivo interstitium are therefore unlikely to be properly characterized by correlations involving only one of the components. There is a clear need for more detailed experimental data concerning the interstitial structure of various tissues and their transport characteristics. Parameters such as the solute void space have been determined properly in only a few tissues (55) and are very dependent on the degree of hydration of the tissue.

**Model Limitations**

To simplify the conceptual approach to the abdominal wall, the tissue has been assumed to have uniform properties of the interstitium and microvessel distribution. Tissue planes and fascial layers of connective tissue are neglected in the analysis of the data and in the mathematical model. Local tissue data (i.e., measurements vs. position in the data) are used to implement the model, but there has been no attempt to correlate irregularities in different concentration or pressure profiles with different layers of muscle groups. Variations in local tissue data in Fig. 15 have been smoothed out by averaging the profiles together. Lymphatic vessels are typically located at tissue planes (39), but these are modeled as uniformly distributed. All of these assumptions are in keeping with the practical approach of keeping the mathematics within the boundaries of the data. Although we are working toward better definition of the microvascular perfused surface area and distribution, we do not have the means at this time to specify the necessary parameters to justify a more detailed and complex approach.

A major assumption in the mathematical model is that transport parameters are solely dependent on interstitial hydrostatic pressure. This neglects possible effects of osmotic or oncotic pressure within the interstitium. Some authors (51) have proposed inclusion of the local osmotic pressure within the expression for the convective driving force. However, there is a lack of local (\( \pi_T \) vs. position) data to implement the proposed model scheme, and it is uncertain how to calculate the effects of osmotic gradients within the interstitium. Based on our experimental data at \( P_{ip} > 3 \) mmHg (62), the interstitial space doubles and the concentration of interstitial albumin and large interstitial matrix molecules must change. Indeed, experiments in which we dialyzed rats for 2 h at \( P_{ip} = 6 \) mmHg demonstrated an apparent movement of hyaluronan from the inner layer of abdominal wall muscle to the subcutaneous space (62). We are actively pursuing the question of how the loss or addition of hyaluronan will affect the transport of fluid and large solutes within the interstitium. When more data are available, the model will be updated to include these effects.

**Implications for Dialysis**

Despite the fact that this model does not include the tissue-level effects of a hypertonic solution in the cavity, it does provide insights into the effects of hydrostatic pressure in the peritoneal cavity. The pressure in the cavity will cause fluid and solutes to move from the cavity into the tissue surrounding the cavity, provided that there exists some pressure gradient. Because the major goal for peritoneal dialysis is to remove excess fluid and solute from the body, it is instructive to calculate an estimate of the rate of fluid movement into the abdominal wall in a human being with Eq. 1. Assuming a mean \( P_{ip} \) of 3.0 mmHg, Eq. 11 produces an estimate of \( K = 0.47 \times 10^{-6} \) cm²/(s·mmHg) and \( -dP/dx = 20 \) mmHg/cm (61) and the fluid flux across the abdominal wall peritoneum = \( 9.5 \times 10^{-6} \) ml/(s·cm²). To provide an estimate of the flow into the abdominal wall, it will be assumed that 50% of the area of contact is abdominal wall. The active area of fluid contact for the human peritoneal cavity during dialysis with a 2-l volume has been estimated recently to be 5,500 cm² (5). Multiplying the fluid flux times 50% of this area yields an estimate of fluid loss to the abdominal wall of 94 ml/h \( [9.5 \times 10^{-6} \) ml/(s·cm²) \( \times 2,750 \) cm² \( \times 3,600 \) s/h]. This estimate is of the same order of magnitude as the measurements in patients of 60–100 ml/h (7, 30, 35, 46); unfortunately, none of these authors measured \( P_{ip} \) and therefore the 3 mmHg value was assumed on the basis of our own measurements in normal patients (12). This calculation implies that the abdominal wall, because the full force of the \( P_{ip} \) is exerted across it, is the major recipient of fluid loss from the cavity. Although we do not know the pressure profiles in other peritoneal tissues that are inaccessible to direct measurement, we can speculate that the hollow viscera,
which are within the peritoneal fluid, may not experience the hydrostatic pressure gradients that exist in the abdominal wall and may be the major sources of osmotic ultrafiltration when hypertonic dialysis solutions are infused into the peritoneal cavity.

From the theoretical concept in Figs. 1 and 2 and from the dependence of convection into the abdominal wall on the tissue pressure profile, it was hypothesized that pressure applied to the skin side of the abdominal wall would decrease the overall driving gradient and decrease fluid movement. This maneuver successfully reduced fluid loss by 50% in rats (12), in which the abdominal wall is approximately the thickness of the pressure profile (~2 mm). However, it was unsuccessful in humans (12), presumably because the human abdominal wall is over a centimeter thick. Abdominal counterpressure would only compress the first few millimeters of external skin and subcutaneous space of the abdominal wall and would not eliminate the pressure profile on the inside of the wall.

**Implications for Regional Immunotherapy**

Figures 8 and 11 illustrate how interaction of the protein with the tissue may decrease penetration. In Fig. 8, increased protein binding results in higher concentrations close to the surface but decreases penetration into the deeper portions of the tissue. Fujimori and colleagues (27) have called this the "binding site barrier," which is even more pronounced in tumors that overexpress the ligand for the antibody. Antibodies with less binding will transport further into tumor but demonstrate lower total concentrations close to the peritoneal surface of the tumor. Although binding affects total tissue antibody concentration closest to the cavity, f has significant effects on transport throughout the tissue. As seen in Fig. 11, marked retardation (f = 0.1) results in a concentration profile very close to that of pure diffusion. With no solute retardation (f = 1), the concentrations throughout the tissue are markedly higher and the solute penetration distance doubles in 3 h from pure diffusion. This parameter has a major influence on IgG delivery to the interior of targeted tissue, but there is a dearth of data to properly define its value in normal or neoplastic tissues. We have demonstrated that the single correlation in the literature (50) is based on in vitro data and does not fit our in vivo data. Defining the values of f for tumor tissue will be a major effort of our laboratory in the future.

The influence of convection in the delivery of IgG to the interior of tissue is demonstrated by Figs. 9–11. Although f is determined by the protein interaction with the interstitial matrix and cellular components in the tissue, P_ip is determined by the volume infused and the size of the patient. P_ip directly affects P_T, which controls the magnitude of θ_ip, θ_sn, and K. The expansion of the abdominal wall has been shown to be a nonlinear function of P_T (62), but K is a linear function of P_T (61). To promote the penetration and delivery of macromolecules to subperitoneal tissue, the most practical method is to use maximal P_ip for the longest duration possible.

The model simulations illustrated in Fig. 7 demonstrate how increasing the time of a therapeutic dwell from 1 to 3 h at constant pressure in the peritoneal cavity improves the protein delivery to deeper portions of the tissue. The total tissue concentration rises dramatically and the penetration distance of the protein improves as time increases. Our previous work demonstrated little penetration after the same period of time for purely diffusive delivery of IgG (22). If an isotonic salt solution is infused into the peritoneal cavity, it would be absorbed into the tissues with time and P_ip would decrease, which would slow delivery of the protein. However, starch solutions are now available that are able to maintain their volume for up to 48 h (29). This type of solution could potentially maintain P_ip at a relatively constant value and facilitate the delivery of macromolecular agents. Figure 16 demonstrates the potential for antibody delivery of such solutions, assuming that P_ip is maintained nearly constant for 24 h. In this model simulation, f = 0.3 and P_ip = 4.4 mmHg; P_T and θ_ip are as in Fig. 4, and the other coefficients are as given in Table 1. C_ip will decrease as the protein transports into the tissue and therefore must be varied over the longer dwell period. If we assume a clearance (k) of ~90 ml/h (see estimate of fluid loss above) and a 3-l volume, the half-life (T_1/2) of the antibody in the cavity is ~23 h (k = 90 ml/h ÷ 3,000 ml = 0.03/h; T_1/2 = 0.693/0.03 h = 23 h). When C_ip is varied accordingly, the model predicts the concentrations of Fig. 16. The tissue pressure gradient causes convection of the antibody to ~2 mm, where transport becomes primarily diffusive. This causes a concentration "wave" to appear because of the buildup of protein in this region. The model predicts that, with sufficient time and pressure,
APPENDIX

Transendothelial Transport

The expressions for solute and water transport across the blood capillary barrier are taken from the multiple-pore theory of Rippe (47, 49)

\[
J_{a} = \sum_{j=1}^{2} J_{a,j} = a \sum_{j=1}^{2} \left[ \frac{\alpha_{j} F_{L}\left[ C_{T} - C_{P} \exp(-\gamma_{j}) \right]}{1 - \exp(-\gamma_{j})} \right] \tag{A1}
\]

and

\[
J_{a} = \sum_{j=1}^{3} J_{a,j} = a \sum_{j=1}^{3} \left[ \frac{\alpha_{j} \left[ P_{j} - P_{P} - \sum_{i=1}^{n} \sigma_{i,j}(\pi_{T,i} - \pi_{P,j}) \right]}{1 - \exp(-\gamma_{j})} \right] \tag{A2}
\]

where \( a \) is capillary surface area-density (area/unit volume of tissue), \( \gamma_{j} \) is Peclet number for pore \( j \)

\[
\xi_{o} \frac{D_{o}}{A_{o}} = \frac{\chi_{o}}{A_{o}}
\]

\( \xi_{o} \) is the ratio of pore-to-bulk solute diffusivity, \( \chi_{o} \) is the sieving coefficient of the pore, \( D_{o} \) is the solute diffusivity in free solution, \( A_{o}(A_{c}) \) is the ratio of total pore surface area per unit capillary area to thickness of the capillary wall, \( \alpha_{j} \) is the fraction of \( L_{p} \) that is due to pore \( j \), \( C_{p} \) is plasma concentration, \( L_{p} \) is the overall filtration coefficient for capillary, \( P_{p} \) is capillary pressure, \( \sigma_{j} \) is the solute reflection coefficient of the \( i \)th solute in the \( j \)th pore, \( \pi_{T,j} \) is osmotic pressure in interstitium of the \( i \)th solute, and \( \pi_{P,j} \) is osmotic pressure in the plasma. Details of these coefficients can be found in our previous publications (18, 20) and in those of others (40). Most of the blood capillary coefficients have been defined by Rippe (45, 49) for the muscle that surrounds the mammalian peritoneal cavity and will be used in simulations for normal abdominal wall muscle.

The following is an additional constraint

\[
\alpha_{SP} + \alpha_{LP} + \alpha_{CP} = 1 \tag{A3}
\]

where \( \alpha_{j} \) is the fraction of \( L_{p} \) that is due to pore \( j \) and the subscripts refer to the pore type: transcellular pore (CP), small pore (SP), or large pore (LP).

If the solute of interest is a protein or macromolecule larger than albumin (~58,000 Da), the theory constrains all transport to convection through the large pore. Equation A1 can be simplified to

\[
R_{LP} = C_{P} F_{LP} (1 - \sigma_{LP}) \tag{A4}
\]

where \( R_{LP} \) is solute transport via large pores, \( C_{P} \) is plasma concentration of protein, \( F_{LP} \) is water transport via large pores, and \( \sigma_{LP} \) is reflection coefficient for the large pore for the protein.

---

This work was supported by a grant from the Whitaker Foundation and Public Health Service Grants R29-DK-48479 and R01-CA-85984.

REFERENCES


Protein Transport in Abdominal Wall Muscle


AJP-Gastrointest Liver Physiol • VOL 281 • AUGUST 2001 • www.ajpgi.org