Model of albumin reabsorption in the proximal tubule

Matthew J. Lazzara and William M. Deen

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts

Submitted 13 January 2006; accepted in final form 4 September 2006


Normally, the small amount of albumin which passes through the glomerular capillary wall is almost completely reabsorbed in the proximal tubule, via an endocytic mechanism, but the reabsorptive process can be overwhelmed if the filtered load of albumin is too large. To examine the factors that control the fractional reabsorption of albumin (f), we developed a mathematical model which assumes saturable endocytosis kinetics with a maximum reabsorptive capacity, V\text{max}, and which includes the effects of flow and diffusion in the lumen. Limitations in albumin transport from the bulk tubule fluid to the endocytic sites at the bases of the microvilli had only a modest (8%) effect on the value of V\text{max} needed to fit micropuncture data on tubule albumin concentrations in rats. For moderate changes in filtered load, there was much greater sensitivity of f to SNGFR than to the albumin concentration of the filtrate (C\text{p}). A 50% increase in SNGFR was predicted to cause four- to fivefold increases in albumin excretion in rats or humans. For large increases in C\text{p}, as might result from defects in glomerular sieving, there was a threshold at which the reabsorptive process became saturated and f fell sharply. That threshold corresponded to sieving coefficients of 10^{-3} to 10^{-2}, the higher values occurring at reduced SNGFR. The predictions of the present model contrast with those of one proposed recently by Smithies (32), which does not include the effects of tubule flow rate.

tubule flow rate; protein microanalysis; endocytosis

ADVANCES IN MICROPUNCTURE sampling and protein microanalysis techniques in the early 1970s led to the first precise measurements of albumin concentration in the mammalian proximal tubule (11, 19, 25, 26, 34). It was found that tubule fluid in normal rats contains a small but measurable amount of albumin (typically, 20–30 μg/ml), which led to the conclusion that the glomerular barrier does not completely retain albumin and that a mechanism for albumin reabsorption exists. In fact, filtered albumin is normally almost completely reabsorbed by the proximal tubule (11, 25, 33, 34) via an endocytic mechanism (1, 3, 7, 37). The surface-expressed glycoproteins megalin and cubulin have been identified (3, 7), shown to colocalize in endocytic pits (37), bind albumin (3), and be necessary for the endocytosis of albumin by tubular epithelium (3, 7). Thus, as reviewed recently (2), binding to the megalin/cubulin complex and subsequent endocytosis is the principal mechanism for albumin removal from tubule fluid.

In addition to receptor expression and kinetics, the rate of albumin reabsorption will depend on physical processes within the tubule lumen. For cellular uptake to occur, albumin must diffuse and/or be convected radially from the bulk tubule fluid to the endocytic pits at the bases of the microvilli. Accordingly, mass transfer resistances in the open part of the lumen and in the intermicrovillar fluid potentially limit the overall rate of albumin reabsorption. Moreover, the axial flow rate will influence how rapidly the albumin concentration falls along the tubule. It follows that the tubule dimensions, axial and radial fluid velocity, and aqueous diffusivity of albumin all play some role. Indeed, proximal albumin reabsorption is analogous to a classical situation in chemical reactor engineering, in which a catalytic reaction occurs at the wall of a tubular reactor. It is well known that as the flow rate through such a reactor increases and the residence time of the reacting species decreases, the overall conversion of reactant tends to be reduced. Conversion also decreases if the reactant inlet concentration is increased enough to begin to saturate the catalytic binding sites. By analogy, if either SNGFR or Bowman’s space albumin concentration is abnormally elevated, we may expect a smaller fraction of the filtered load to be reabsorbed. Another feature of the tubular reactor is the potential for significant radial concentration gradients to develop when the rate of reaction at the wall is fast relative to diffusion from the bulk fluid. For very fast kinetics, the reactant concentration will fall almost to zero at the wall, and the overall rate will be determined entirely by diffusion. Thus, both radial and axial variations in albumin concentration in the proximal tubule might be important. Present in the proximal tubule but not in classical reactors are invaginations in the wall (microvilli) and radial fluid flow (water reabsorption). A desire to understand the previously unexplored interplay between endocytic kinetics and mass transfer limitations for albumin in the proximal tubule is what motivated the present study.

Mathematical models of the tubular reabsorption of solutes smaller than albumin have been developed to estimate kinetic rate constants and permeability coefficients (20, 30, 31, 35). Lingard et al. (20) used a model to estimate the relative magnitudes of diffusive and active transport of amino acids and glucose from the tubule lumen for a range of water fluxes and solute concentrations in the perfused tubule. Silbernagl and co-workers (30, 31, 35) applied their model to rat tubule perfusion data to elucidate differential kinetics for the absorption of specific amino acids. While the models developed by those authors may be accurate for small solutes, where diffusivities might be large enough to minimize luminal mass transfer limitations, their applicability to macromolecules such as albumin is more uncertain. Moreover, these previous models did not account for the effects of radial fluid flow.

More recently, Smithies (32) modeled the combined effects of glomerular defects and alterations in tubular reabsorption in determining the rate of albumin excretion and the onset of albuminuria. The calculations used a software package designed to simulate electrical circuits; because the model was not equation based, it is difficult to pinpoint each assumption.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Also, little use was made of the micropuncture data that are available on proximal tubule albumin concentrations. Nonetheless, that study points out the need to account for the interplay between glomerular filtration and proximal reabsorption, and the model yields testable predictions, as will be discussed later.

The objective of the present work was to develop a model for the tubular reabsorption of albumin, taking into account luminal mass transfer considerations as well as endocytic kinetics. The model is based on independently measured physiological and kinetic quantities and employs only one fitted parameter, the maximum uptake rate at receptor saturation ($V_{\text{max}}$). The paper is organized as follows. In Model Development, the governing equations are derived and sources for the parameter values are identified. Results includes a discussion of general trends in tubule reabsorption and application of the model to fit micropuncture data and infer a value of $V_{\text{max}}$. In Discussion, the model is compared with the results of Smithies (32), used to evaluate a controversial albumin retrieval hypothesis, and applied to certain pathophysiological situations in rats and humans.

Model Development

General considerations. The mammalian proximal tubule consists of convoluted and straight sections lined by an epithelial surface with \( \sim 40 \) microvilli/\( \mu \text{m}^2 \) of tubule surface area (23). The microvilli, which are \( \sim 2 \) \( \mu \text{m} \) in height (\( \sim 15\% \) of the total inner radius of the tubule lumen), divide the lumen into two regions, as shown in Fig. 1A. One region is an open inner core, and the other is an outer annular volume densely packed with microvillar projections. In developing the model, it is convenient to first consider the two regions separately. Beginning with the open lumen, a mass balance will be derived that relates variations in the bulk albumin concentration (\( C_b \)) with axial position (\( z \)) to the concentration of albumin at the tips of the microvilli (\( C_w \)). That relationship will involve the luminal mass transfer coefficient and the axial and radial fluid velocities. Then, an analysis of diffusion in the intermicrovillar fluid will be used to relate \( C_w \) to the concentration at the base of the microvilli (\( C_m \)), which is where protein reabsorption occurs (13). Finally, the two analyses will be joined to provide a set of equations governing \( C_b(z) \) that encapsulates all the kinetic and mass transfer considerations.

Mass transfer in the open lumen. Although the mammalian proximal tubule consists of convoluted and straight sections, we will represent it as a straight cylindrical tube of radius \( R \) and length \( L \), as depicted in Fig. 1B. For laminar flow through coiled tubes, eddies may develop that increase mass transfer to the wall, but with Reynolds numbers as low as in the proximal tubule (\( \sim 0.02 \)), that effect is negligible and the curvature may be ignored (10). Primary glomerular filtrate enters the tubule with an average axial velocity \( u_0 \) (equal to the SNGFR divided by the cross-sectional area of the tubule) and albumin concentration \( C_0 \). Because water is reabsorbed along the tubule, the local mean velocity \( u(z) \) decreases with increasing \( z \). Following standard engineering practice, the bulk concentration \( C_b(z) \) is defined as the velocity-weighted cross-sectional average (8). With this definition, and provided that axial diffusion is negligible (as is true for albumin in the proximal tubule), the axial flux of albumin at a given position along the tubule is \( u(z)C_b(z) \).

At steady state, the rate at which albumin enters a cylindrical volume of differential length \( \Delta z \) (Fig. 1B) must equal the rate at which it leaves. Thus,

\[
\pi R^2 u(z)C_b(z) = \pi R^2 u(z + \Delta z)C_b(z + \Delta z) + 2\pi R\Delta z N_r
\]

where \( N_r \) is the flux of albumin in the radial (outward) direction, evaluated at radial position \( r = R \) (the microvilli tips). Again following standard practice, we use a mass transfer coefficient \( k(z) \) to describe the diffusive part of that flux, with the corresponding driving force being \( C_b - C_w \). Accordingly,

\[
N_r = k(C_b - C_w) + v_w C_w
\]

where \( v_w \) is the radial fluid velocity at \( r = R \). The last term in Eq. 2 accounts for the outward albumin flux due to bulk flow. Combining Eqs. 1 and 2 and taking the limit as \( \Delta z \to 0 \) gives

\[
\frac{d}{dz} (uC_b) = -\frac{2}{R} [k(C_b - C_w) + v_w C_w]
\]

The mass balance for albumin that was just obtained requires specification of \( u(\z) \). A volume balance analogous to Eq. 1 results in

\[
\frac{du}{dz} = -\frac{2 v_w}{R}
\]

Because \( v_w \) is approximately independent of \( z \) (22), it follows that...
\[ u(z) = u_0 - \frac{2 v_w z}{R} \]  

Thus, a constant reabsorptive velocity implies a linear decline in mean velocity with axial position. Combining Eqs. 3 and 4 gives

\[ \frac{d C_b}{d z} = -\frac{2}{u R} (k - v_w) \left( C_b - C_w \right) \]  

where \( u(z) \) is evaluated from Eq. 5. Remaining to be specified are \( k(z) \) and \( C_w(z) \).

Uptake kinetics. To relate \( C_w \) to \( C_b \), we first recognize that the rate of albumin loss from the lumen equals the rate of cellular uptake at the base of the microvilli, where the concentration is \( C_m \) (Fig. 1A). As shown in Appendix, receptor-mediated endocytosis is expected to follow Michaelis-Menten kinetics. Accordingly, we write

\[ N_r = k(C_b - C_w) + v_w C_w = \frac{V_{\text{max}} C_m}{K_m + C_m} \]  

where \( V_{\text{max}} \) is the rate when the receptors are fully saturated and \( K_m \) is the concentration at half-saturation. For convenience, \( V_{\text{max}} \) is based on the cylindrical area corresponding to \( r = R \). To proceed further, we need a model for transport in the intermicrovillar region.

Diffusion in the intermicrovillar space. Because the height of the microvilli (\( h \)) is only \( \sim 15\% \) of \( R \), little error is introduced by using rectangular coordinates in this region, as in Fig. 1C. If the fractional surface area not occupied by microvilli (i.e., available for extracellular transport) is \( \epsilon \), then the radial velocity in the intermicrovillar space is approximately \( v_w/\epsilon \). The Peclet number based on this velocity, \( h \), and the diffusivity of albumin in water (\( D \)) is very small (\( \sim 0.04 \)), indicating that convection here is negligible. Accordingly, we equate the diffusive flux and uptake rate of albumin to obtain

\[ -\epsilon D \frac{dC}{dx} = \frac{V_{\text{max}} C_m}{K_m + C_m} \]  

where \( C(x) \) is the local concentration in the intermicrovillar fluid. Integrating Eq. 8 and applying the boundary condition that \( C(0) = C_w \) gives

\[ C(x) = C_w - \frac{x}{\epsilon D} \left( \frac{V_{\text{max}} C_m}{K_m + C_m} \right) \]  

Setting \( C(h) = C_m \) provides a relationship between \( C_w \) and \( C_m \), namely,

\[ C_m = C_w - \frac{h}{\epsilon D} \left( \frac{V_{\text{max}} C_m}{K_m + C_m} \right) = C_w - \frac{D_a C_w C_m}{K_m + C_m} \]  

The dimensionless parameter \( D_a \) (\( = V_{\text{max}} h / \epsilon D C_w \)), which is a Damkohler number, expresses the relative speeds of endocytic uptake and intermicrovillar diffusion. If the uptake rate is slow relative to diffusion (\( D_a \rightarrow 0 \)), Eq. 10 reduces to \( C_m = C_w \). In that case, the concentration gradient that develops in the intermicrovillar region is negligible and the uptake kinetics entirely limit the process. However, if uptake is fast (\( D_a \rightarrow \infty \)), diffusion becomes limiting and Eq. 10 reduces to \( C_m = 0 \), as described earlier for a tubular reactor with fast kinetics.

Additional reabsorptive pathways. Additional pathways for the reabsorption of albumin may exist at the base of the microvilli (27). To incorporate a second saturable kinetic rate process, Eqs. 7 and 10 must be rewritten. Equation 7 is readily modified by equating the net rate of transport in the open lumen to the sum of two reaction rates, as

\[ k(C_b - C_w) + v_w C_w = \frac{V_{\text{max1}} C_m}{K_{m1} + C_m} + \frac{V_{\text{max2}} C_m}{K_{m2} + C_m} \]  

To rewrite Eq. 10, we must similarly equate the rate of diffusion in the intermicrovillar region to the sum of reaction rates in Eq. 8. Integration of that new differential equation gives

\[ C_m = C_w - \frac{D_a_1 C_w C_m}{K_{m1} + C_m} - \frac{D_a_2 C_w C_m}{K_{m2} + C_m} \]  

where \( D_a_1 \) and \( D_a_2 \) are analogous to \( D_a \).

Mass transfer coefficient. The one remaining function to be specified is \( k(z) \). A well-known feature of mass transfer in a tube in which reaction or permeation begins at \( z = 0 \) is that \( k \) declines sharply with increasing \( z \) when \( z \) is small (“entrance region”) and approaches a constant when \( z \) is large (“fully developed region”) (8). The most thoroughly studied situations are ones where either the solute concentration at the wall or the solute flux at the wall are constant, and there is no flow across the tube wall (8). Results are not readily available for a water-permeable tube with saturable reaction kinetics, as for albumin in the proximal tubule. However, the type of boundary condition at the tube wall is known to have only a modest effect on the mass transfer coefficient. That is, the values of \( k \) for the constant concentration and constant flux cases, which turn out to be extremes that bracket the results for all other linear boundary conditions, differ by only \( \sim 20\% \). Also, the effects of radial flow on mass transfer in the proximal tubule are expected to be weak, based on the fact that the radial Peclet number (\( P_e = v_w R / D \)) for albumin is well below unity. A lengthy analysis shows that \( k(z) \) for the present situation should differ by at most a few percent from the classical result for a constant flux at the wall. Accordingly, we used the following piecewise expression, which closely approximates the constant flux results:

\[ \frac{k R}{D} = \begin{cases} 0.820 \xi^{-1.5} & \xi < 0.0175 \\ 1.635 \exp(-29.397\xi) + 2.182 & \xi \geq 0.0175 \end{cases} \]  

The dimensionless axial coordinate here is \( \xi = z/(R P_e) \), where \( P_e = 2u_b R / D \). As shown by Eq. 13, the length of tube over which \( k \) varies depends on \( R \) and on \( P_e \), which is the Peclet number based on axial velocity.

Solution method. The principal model consists of a system of three coupled equations, one differential (Eq. 6) and two algebraic (Eqs. 7 and 10), that together describe the changes in \( C_b \), \( C_w \), and \( C_m \) with \( z \). For calculations involving two reabsorptive pathways, the system consists of Eqs. 6, 11, and 12. Because there is only one differential equation, only one boundary condition \( [C_b(0) = C_a] \) is required to fully specify the problem. Solution of the differential-algebraic systems of equations was accomplished using Matlab v7.01. The method-
ology included defining a mass matrix for the system of equations and solution with solver ode15s.

**Model parameters.** Estimates of the kinetic parameters for albumin endocytosis are available from studies using either isolated rabbit proximal tubules or cultured monolayers of opossum kidney (OK) epithelial cells. By perfusing isolated rabbit proximal tubules with varying concentrations of tritiated albumin, collecting effluent, and applying a material balance, Park and Maack (27) identified two populations of binding sites, one of high affinity and low capacity ($K_m = 31 \mu g/ml$, $2\pi R V_{max} = 0.064 ng \cdot min^{-1} \cdot mm$ tubule length$^{-1}$) and one of low affinity and high capacity ($K_m = 1,200 \mu g/ml$, $2\pi R V_{max} = 3.7 ng \cdot min^{-1} \cdot mm^{-1}$). By measuring the accumulation of FITC-labeled BSA in cultured OK cells, Schwegler et al. (29) showed albumin endocytosis to be a saturable process with an apparent $K_m = 24 \mu g/ml$. To measure the binding affinity of albumin for its receptors, Gekle et al. (14) incubated confluent plates of OK cells with FITC-albumin on ice (to suppress endocytosis) and measured surface-bound albumin as a function of albumin concentration, identifying one binding site for albumin with $K_m = 20 \mu g/ml$. That this site was specific for albumin was demonstrated by the observation that excess unlabeled albumin inhibited binding of FITC-albumin, whereas no inhibitory effect was observed for excess conalbumin, $\alpha$-lactalbumin, and transferrin. Applying a similar technique but using $[^{125}\text{I}]$BSA, Brunskill et al. (5) identified two albumin binding sites, one of high affinity ($K_m = 155 \mu g/ml$) and one of low affinity ($K_m = 8,300 \mu g/ml$).

In summary, there is general agreement that there is a high-affinity site for albumin binding and internalization that half-saturates at albumin concentrations comparable to those reported in the rat proximal tubule (20–30 $\mu g/ml$). As shown in Table 1, we chose $K_m = 31 \mu g/ml$, the value reported by Park and Maack (27), as representative of this high-affinity site. A limited number of calculations were done also including a second site with $K_m = 1,200 \mu g/ml$ (the value reported by Park and Maack for the low-affinity pathway). As will be discussed, the $V_{max}$ for the high-affinity site found by Park and Maack (27) for perfused rabbit tubules is much too low to be consistent with in vivo micropuncture findings in the rat or measured albumin clearances in humans. The same is true even if the second low-affinity pathway is included. The baseline value of $V_{max}$ for rats in Table 1 (0.086 ng $\cdot s^{-1} \cdot mm^{-2}$) was obtained by fitting the rat micropuncture data of Tojo and Endou (33) to the model that includes a single reabsorptive mechanism, as will be discussed. Also from Tojo and Endou (33) is the albumin concentration in normal glomerular filtrate, $C_0 = 23 \mu g/ml$.

The remaining parameter estimates in Table 1 are straightforward. With the use of a luminal radius of 14 $\mu m$ for the rat (23) and an average microvillar height along the tubule of $h = 2 \mu m$ (23), the radius of the open inner region of the tubule was taken as $R = 12 \mu m$. Based on estimates of microvillus thickness and number density, we find that the fractional surface area not occupied by microvilli at $r = 12 \mu m$ is $\epsilon = 0.6$ (23). With the use of data from rabbits and rats (15, 36), the total length of the mammalian proximal tubule is approximately $L = 10 mm$. The SNGFR of 40 nl/min is typical of euvolemic rats (22). Based on SNGFR and $R$, the initial mean axial velocity is $u_0 = 1.5 mm/s$. With the use of SNGFR, $R$, and $L$, and the typical finding that 70% of filtered water is reabsorbed for any SNGFR (glomerular-tubular balance), the radial velocity at the wall is $V_w = 6.2 \times 10^{-4} mm/s$. Whenever SNGFR was changed in the simulations, $u_0$ and $V_w$ were altered in proportion, corresponding to fixed tubule dimensions and a constant fractional water reabsorption of 70%. Finally, using a Stokes-Einstein radius of 3.6 nm (17), the albumin diffusivity in water at 37°C is $9.5 \times 10^{-7} cm^2/s$. Unless stated otherwise, all simulations used the baseline parameter values in Table 1.

The calculations for humans in DISCUSSION make use of the dimensions listed in Table 1, along with estimates of SNGFR, $\Theta$, and $V_{max}$ in humans that are presented later.

### RESULTS

**Effects of $V_{max}$ on albumin concentration profile.** A key determinant of tubule albumin reabsorption is $V_{max}$, which defines the capacity for endocytic uptake. Plotted in Fig. 2 are axial variations in concentration predicted for $V_{max}$ values between 0.001 and 0.2 ng $\cdot s^{-1} \cdot mm^{-2}$. For the smallest values of $V_{max}$ ($\approx 0.02$ ng $\cdot s^{-1} \cdot mm^{-2}$), the rate of albumin uptake is so slow relative to the reabsorption of water that $C_b$ is predicted to increase with $z$ rather than decrease. Of particular interest is the curve for $V_{max} = 0.014$ ng $\cdot s^{-1} \cdot mm^{-2}$, which corresponds to the value reported by Park and Maack (27) for the high-affinity reabsorptive pathway in isolated rabbit tubules. As seen, that uptake capacity is insufficient to cause the albumin concentration to fall with increasing distance along the tubule, contrary to in vivo micropuncture observations in rats. This discrepancy may reflect species differences and/or the effects of tubule isolation. As seen in Fig. 2, a roughly threefold higher capacity ($V_{max} = 0.04$ ng $\cdot s^{-1} \cdot mm^{-2}$) is required to just overcome the competing effect of water reabsorption and cause $C_b$ to decrease slightly with increasing $z$. As will be discussed, the curve for $V_{max} = 0.086$ ng $\cdot s^{-1} \cdot mm^{-2}$ is closest to the situation for normal rats. Because there is not general agreement on its existence, and because it would play a role mainly at high concentrations, we did not include a second, low-affinity site for most of our calculations. Whereas addition of the low-affinity pathway reported by Park and Maack (27) to their high-affinity pathway does lower the final albumin concentration (to $\approx 13 \mu g/ml$), that lowering is not nearly enough to be in agreement with the data of Tojo and Endou (33).

**Evaluation of $V_{max}$ from rat micropuncture data.** To estimate $V_{max}$ for normal rats, we fit the model to the micropuncture data of Tojo and Endou (33) from Munich-Wistar rats. That study employed an innovative collection technique that minimized
the possibility of sample contamination by subcapsular fluid, and thus probably provides the best available set of measurements of $C_b$. Because albumin concentrations were reported as a function of tubule fluid/plasma inulin concentration ratios, the dependence of $C_b$ on $z$ can also be inferred. To do that, we converted the inulin ratios in Fig. 4 of Tojo and Endou (33) to $z$ by using the dimensions and flow rates in Table 1. Shown in Fig. 3 is a comparison between the data of Tojo and Endou (33) (with $z$ calculated as just described) and model results for $V_{\text{max}}/H^{10.05}_{18.528} = 0.086$ ng·s$^{-1}$·mm$^{-2}$. In this and the other figures, all parameter values not stated are those given in Table 1.

Mass transfer resistances. A novel aspect of the present analysis is the acknowledgment that albumin uptake may be limited, in part, by transport from the bulk tubule fluid to the bases of the microvilli, where endocytosis occurs. Given that albumin is reabsorbed more avidly than water, mass transfer resistances in the open lumen and the intermicrovillar spaces will tend to create radial concentration gradients, such that $C_m < C_w < C_b$. Thus, for any given axial position, the extent to which those concentrations differ reflects the importance of the mass transfer resistances. Shown in Fig. 4 are predictions of $C_m$, $C_w$, and $C_b$ vs. $z$ for rats. It is seen that the mass transfer resistances are sufficient to create noticeable differences in those concentrations. The value of $C_m$ is predicted to be 8% lower than $C_b$ near the beginning of the tubule ($z = 0.05$ mm) and 17% lower than $C_b$ at the end ($z = 10$ mm). The fraction of the concentration drop in each layer indicates the relative contribution of that layer to the overall mass transfer resistance. Because the mass transfer coefficient is unbounded at $z = 0$ (Eq. 13), the entire mass transfer resistance there is due to diffusion in the intermicrovillar region. For $z \geq 0.05$ mm, the open tubule lumen contributes $\sim 55\%$ and the intermicrovillar region contributes $\sim 45\%$. The reduction of $C_m$ relative to $C_w$ reflects the diffusional resistance of the intermicrovillar space, and the reduction of $C_w$ relative to $C_b$ reflects the mass transfer resistance in the open part of the lumen.
crovillar region 45% of the total mass transfer resistance. To the extent that a dense cell coat fills the intermicrovillar spaces, the albumin diffusivity will be lower than that in water and the resistance there will be even more prominent. However, there is at present no reliable way to estimate the local reduction in diffusivity, if any.

How much impact do the mass transfer resistances have on the estimation of $V_{\text{max}}$? This was addressed by artificially eliminating those resistances by increasing $k$ and $D$, and refitting a value of $V_{\text{max}}$ to the Tojo and Endou (33) data presented in Fig. 3. Increasing $k$ and $D$ each by a factor of 103, and thus eliminating radial gradients in albumin concentration, yielded $V_{\text{max}} = 0.079 \, \text{ng} \cdot \text{s}^{-1} \cdot \text{mm}^{-2}$, which is 8% lower than calculated previously. A reduction in $V_{\text{max}}$ is expected because the absence of radial gradients will tend to make $C_m$ larger, and a compensating change in $V_{\text{max}}$ is needed to give the same observed rate. We conclude that, unless there is a significant reduction in the albumin diffusivity in the intermicrovillar spaces, radial concentration gradients have only a modest effect on the estimation of the kinetic constants.

Effects of moderate changes in filtered load. Because albumin reabsorption is receptor mediated and therefore saturable, the fractional reabsorption of albumin ($f$) must decrease when the filtered load ($F = \text{SNGFR} \cdot C_0$) exceeds the uptake capacity of the tubule. However, moderate increases in $F$ (up to, say, a doubling) might occur through various combinations of increased SNGFR and increased $C_0$. Is the change in $F$ all that matters, or will the tubule respond differently to changes in flow rate and concentration? This issue is addressed in Fig. 5, in which $f$ is shown as a function of relative filtered load for three situations: changes in $C_0$ alone, changes in SNGFR alone, or simultaneous changes in the two that are suggested by the dynamics of glomerular filtration. The relative filtered load is $F/F_0$, where $F_0$ is the baseline value of $F$. The three kinds of changes in filtered load will be discussed in turn.

It is seen in Fig. 5 that, within the range of filtered loads considered ($0 \leq F/F_0 \leq 2$), selective changes in $C_0$ have very little effect on fractional albumin reabsorption. Indeed, a two-fold increase in $F$ causes only a 3% reduction in $f$. The effects of selective changes in SNGFR are much more striking, a doubling in filtered load leading in this case to a 19% reduction in $f$. The very different effects of concentration and flow rate can be understood by referring to the concentration profile in Fig. 3. As shown there, the albumin concentration normally begins at $\sim 20 \, \mu\text{g/ml}$, which is comparable to the $K_m$ for the reabsorptive mechanism, and falls steadily over the length of the tubule. Thus, under normal conditions, the reabsorptive pathway operates at less than half of its overall capacity. A doubling in $C_0$ shifts the curve upward at $z = 0$, but the excess capacity allows the rate of reabsorption to respond almost in proportion, so that $f$ remains nearly constant. In contrast, a selective increase in SNGFR will leave the initial concentration unchanged, while reducing the slope of the curve. (Note that $u$, which will increase if SNGFR increases, is in the denominator on the right-hand side of Eq. 6.) Thus, there is much less of an increase in the overall rate of reabsorption, which roughly follows the area under the curve.

The much greater sensitivity of albumin reabsorption to changes in SNGFR is essentially a residence-time effect. That is, whereas under normal conditions the tubule has excess reabsorptive capacity, the residence time is already slightly too short to utilize it fully, making $f$ slightly less than unity. Increases in SNGFR, which raise the axial velocity in the proximal tubule and therefore decrease the time that the fluid spends there, exacerbate this limitation. Conversely, decreases in SNGFR allow full utilization of the albumin reabsorptive capacity, so that $f \rightarrow 1$ as SNGFR $\rightarrow 0$ (Fig. 5).

The final situation considered in Fig. 5 is motivated by the fact that changes in SNGFR will tend to alter $C_0$. A well-known characteristic of ultrafiltration across synthetic membranes is that the sieving coefficient of a given macromolecule (filtrate concentration divided by retainate concentration) tends to vary inversely with the volume flux. Such a relationship has been demonstrated also for glomerular filtration of dextran (6), Ficoll (28), and various proteins (21) in rats. If the plasma albumin concentration is constant, changes in $C_0$ will parallel those in $\Theta$, the albumin sieving coefficient. The dependence of $\Theta$ on SNGFR was modeled by assuming that

$$\Theta = \frac{\Theta_\infty}{1 - (1 - \Theta_\infty) \exp(-c \cdot \text{SNGFR})}$$  \hspace{2cm} (14)

where $\Theta_\infty$ and $c$ are constants (18). This function implies that $\Theta \rightarrow 1$ for SNGFR $\rightarrow 0$ and $\Theta \rightarrow \Theta_\infty$ for SNGFR $\rightarrow \infty$, consistent with the usual sieving behavior in ultrafiltration. The constant $c$ in Eq. 14 for albumin was assumed to be the same as that for a Ficoll of equivalent Stokes-Einstein radius. Based on the Ficoll data of Rippe et al. (28), we found that $c = 0.0231 \, \text{min}/\text{nl}$. Using a plasma albumin concentration in rats of 3 g/dl and the value of $C_0$ from Table 1, we estimated that $\Theta = 7.7 \times 10^{-4}$ at SNGFR $= 40 \, \text{nl/min}$. This sieving coefficient and the aforementioned value of $c$ imply that $\Theta_\infty = 4.65 \times 10^{-4}$ for albumin in normal rats. It is worth noting that the sieving coefficient of $7.7 \times 10^{-4}$ inferred above agrees well with that.
reported by Lund et al. (21) for radiolabeled human serum albumin in rats, \(6.6 \times 10^{-4}\).

Returning now to the last curve in Fig. 5, and again assuming a plasma albumin concentration of 3 g/dl, Eq. 14 was used to compute specific combinations of SNGFR and \(C_0\) that correspond to various filtered loads. As shown, this made \(f\) somewhat more sensitive to changes in \(F\) than was the case for selective changes in SNGFR. The reason for this is that, to increase \(F\) by the same amount, a greater increase in SNGFR was needed when \(C_0\) was allowed to decrease according to Eq. 14. As already noted, for the moderate changes in filtered load in Fig. 5, SNGFR is the more important determinant of \(f\).

**Effects of large changes in filtered load.** In contrast to the moderate perturbations discussed above, order-of-magnitude increases in the filtered load of albumin can result only from similarly large increases in \(C_0\) and \(\Theta\) due to glomerulopathies. In chronic kidney diseases, there is a tendency for GFR to decrease, due to nephron loss and/or reductions in SNGFR. Accordingly, what is of most interest in this context is a greatly elevated \(\Theta\) combined with a normal or reduced SNGFR. Plotted in Fig. 6 are predictions of \(f\) as a function of \(\Theta\) for values of SNGFR equal to 100, 50, or 25% of baseline. As before, we assumed a plasma albumin concentration of 3 g/dl. At any given SNGFR, there is a threshold in \(\Theta\) beyond which \(f\) falls very rapidly. The lower the SNGFR, the higher is the threshold, the breakpoints in the curves ranging from about \(\Theta = 10^{-3}\) to \(10^{-2}\). In each case, the rapid fall in \(f\) indicates that the reabsorption process has been overwhelmed by the amount of filtered albumin. As noted in connection with Fig. 5, increased residence times in the tubule allow more of its reabsorptive capacity to be used, so that the breakpoints are delayed as SNGFR is reduced.

**Determinants of \(f\).** As shown in APPENDIX, \(f\) is governed mainly by two dimensionless groups, one related to binding affinity (\(\beta\)) and the other describing the capacity of the endocytic mechanism (\(\chi\)). Those dimensionless parameters are defined as

\[
\beta = \frac{K_m}{C_0}, \quad \chi = \frac{V_{\text{max}}}{S/F}
\]

where \(S\) is the inner tubule surface area (see also Eqs. A6 and A7). The dependence of \(f\) on these parameters (based on the full model, rather than the simplified one in APPENDIX) is illustrated in Fig. 7. In this plot each parameter has been normalized by its baseline value in rats (\(K_m = 1.35\) or \(\chi = 4.23\)). As \(\chi\) increases (increasing capacity), albumin reabsorption becomes more avid and \(f\) increases. As \(\beta\) increases (decreasing affinity), binding to the endocytic sites becomes inadequate to utilize the available capacity and \(f\) decreases. Given that \(\beta\) depends mainly on \(\chi\) and that the tubule dimensions appear only in the latter (through \(S\)), the effects of the assumed length and radius can be estimated from Eq. 16. For example, if the actual \(S\) were larger, then a smaller \(V_{\text{max}}\) would be needed to achieve the same \(\chi\) and therefore the same value of \(f\).

**DISCUSSION**

The model developed here demonstrates several important characteristics of proximal tubule albumin reabsorption, among the more interesting of which are predicted responses to alterations in filtered load. For moderate increases in filtered load, changes caused by increases in \(C_0\) alone are predicted to have a relatively modest effect on \(f\), because the tubule has enough endocytic capacity to increase albumin reabsorption in near proportion. Increased filtered load resulting from elevated SNGFR, however, is predicted to have a more profound effect on \(f\), a result of insufficient tubule residence time. For large increases in \(C_0\), of course, the reabsorptive capacity is eventually exceeded, causing \(f\) to fall sharply; the lower the value of SNGFR, the higher the concentration at which that occurs. Also demonstrated was the ability of a single kinetic pathway to explain observed axial variations in albumin concentration in the rat, although the required \(V_{\text{max}}\) was significantly larger than that reported for rabbits. Though important conceptually, and useful in testing alternative hypotheses concerning intrarenal albumin processing, mass transfer limitations seem to have only a modest effect on \(f\). The remainder of this section includes a comparison with a previous model, a discussion of how mass transfer limitations provide insight into a controversial theory of albumin sieving and reabsorption, and applications of the model to certain micropuncture and clearance results in rats and humans.

**Comparison with previous model.** A model that relates alterations in glomerular sieving to changes in albumin excretion was presented by Smithies (32). Although that model makes a number of predictions, we will focus on one that seems to delineate most sharply how it differs from that presented here. A prediction of that model is that a 50% reduction in SNGFR would result in a nearly twofold increase in \(C_0\) and that such an increase in \(C_0\) would result in albuminuria. Both elements of that prediction, glomerular and tubular, are inconsistent with those of the present model. With the use of Eq. 14, a 50% reduction in SNGFR (from 40 to 20 nl/min) should cause only a 63% increase in \(C_0\). Accordingly, we estimate that a 50% reduction in SNGFR will cause an 18% reduction in \(F\), rather than a filtered load that is nearly un-

![Fig. 6. Fractional reabsorption of albumin (f) vs. albumin sieving coefficient (\(\Theta\)) for various SNGFR levels (fractions of basal SNGFR). The plasma albumin concentration in each case is assumed to be 3 g/dl.](http://ajprenal.physiology.org/)
changed. Referring to the results presented in Fig. 5, our model predicts a slight increase in $f$ under these conditions. Given the reduction in $F$ and increase in $f$, it predicts a decrease in albumin excretion, rather than an increase. One important reason for the discrepancy between the two sets of results is that Smithies’ assumption that $f$ decreases sigmoidally with increasing albumin concentration, ranging from ~1 at normal concentrations to <0.1 when albumin concentrations increase sixfold, makes no allowance for the effects of tubule flow rate or residence time. Thus the results of changing SNGFR in that model are mediated by changes in $C_0$ alone, which we have shown to be less important (at moderate filtered loads) than residence-time effects. Moreover, the effects of selective changes in $C_0$ appear to be overestimated in the Smithies’ model. Looking again at the results in Fig. 5, the present model does not predict a significant reduction in $f$ for a twofold increase in $C_0$ alone. As discussed earlier, we find that for such small increases in $C_0$ the tubule has the capacity to increase the rate of reabsorption in near proportion. In summary, we think that deficiencies in both the glomerular and tubular parts of the Smithies model make it unreliable even for predicting directional changes in albumin excretion.

Mass transfer limitations on albumin retrieval. Although the mass transfer resistances in the tubule are apparently not dominant (as is true also for a well-designed tubular reactor), they do provide an interesting upper limit on how fast albumin can be processed by the epithelium. That is, even for arbitrarily rapid binding and internalization kinetics, the rate of albumin reabsorption cannot exceed the rate at which albumin is delivered to the endocytic sites by convection and diffusion. Thus, a preliminary version of the present model was used to examine the plausibility of the hypothesis that albumin passes through the glomerular barrier in fairly large amounts (i.e., as readily as uncharged Ficoll of similar size) and is mostly retrieved intact by the tubule epithelium. By setting $C_w = 0$ in Eq. 6, integrating the resulting expression, and using an average value of $k$ appropriate for a tube with constant wall concentration, we reported that no more than 80% of filtered albumin can be reabsorbed within the first millimeter of proximal tubule, no matter how fast the uptake kinetics (9). An improved estimate for the maximum amount of albumin reabsorption is possible with the present model, where the diffusional resistance within the intermicrovillar region is also considered. By increasing $V_{max}$ until $C_w → 0$, and using an expression for $k$ appropriate for a constant concentration boundary condition, we found that no more than ~60% of filtered albumin can be reabsorbed within the first millimeter of tubule. This lower value is consistent with our finding that the intermicrovillar mass transfer resistance is comparable to that of the open tubule lumen. The improved estimate of the rate of albumin reabsorption under mass transfer-limited conditions strengthens our previous conclusion, which is that the albumin retrieval hypothesis cannot be reconciled with the data in Fig. 3 or with the similar tubule fluid concentrations reported by others.

Application to rat pathophysiology. As an example of how the model can be used to interpret physiological data, we consider the results of Galaske et al. (12), who induced anti-GBM nephritis in male Wistar rats via intravenous injection of graded doses of rabbit anti-rat GBM antiserum. For brevity, only three of their groups will be considered: group 1 (control), group 2 (0.5 ml antiserum), and group 3 (1.0 ml antiserum). In each, micropuncture sampling of Bowman’s space and late tubule fluid allowed albumin processing by the proximal tubule to be quantitated, along with glomerular sieving. All three groups had very similar values of whole kidney GFR (0.9–1.1 ml·min⁻¹·100 g body wt⁻¹), and we assumed that SNGFR = 25 nl/min in each. Groups 1 and 2 had nearly identical values of $C_0$ (7.2–7.3 μg/ml), but $f$ was significantly lower in group 2 than in group 1 (0.71 vs. 0.92), suggesting that $V_{max}$ was reduced in nephritis. Assuming that was the only effect of nephritis on the tubule, we calculated a nearly twofold reduction in $V_{max}$ from 0.030 to 0.015 ng·s⁻¹·mm⁻². In group 3, where $C_0$ was markedly higher (61 μg/ml) and $f$ was reduced further (to 0.16), we found that $V_{max} = 0.007$ ng·s⁻¹·mm⁻², an additional twofold reduction relative to group 2. Thus, there appears to have been a marked, dose-dependent effect of anti-serum on the albumin reabsorption capacity of the proximal tubule.

Glomerular hyperfiltration and proteinuria. The results in Fig. 5 suggest that hyperfiltration alone might lead to noticeable albuminuria, without any abnormalities in the selectivity of the glomerular barrier or reabsorptive capacity of the tubule. The potential magnitude of that effect in rats is illustrated by assuming a 50% increase in SNGFR, and taking into account the inverse relationship between the albumin sieving coefficient and SNGFR. With the use of Eq. 14, increasing SNGFR from 40 to 60 nl/min is predicted to reduce $Θ$ by 20%. Thus, the increase in filtered load is estimated as 20% (i.e., less than the 50% increase in SNGFR). From the curve in Fig. 5 for simultaneous changes in SNGFR and $C_0$, it is found that $f$ decreases from 0.976 to 0.914 when $F/Θ_0$ increases from 1 (baseline) to 1.2. That corresponds to a fourfold increase in albumin excretion.

Fig. 7. Fractional reabsorption of albumin ($f$) as a function of dimensionless reabsorptive capacity ($χ$), for 3 values of the dimensionless binding parameter ($β$). The capacity and binding parameters are each normalized by their basal values, where $χ_0 = 4.23$ and $β_0 = 1.35$. The 3 curves correspond to $β/β_0 = 0.1$, 1, and 10, respectively.
Any such calculations for humans require some new parameter values. To estimate $V_{\text{max}}$ for humans, we assumed first that the normal albumin sieving coefficient equals the fractional clearance reported by Norden et al. (24) for patients with Dent’s disease (a form of Fanconi syndrome), in which proximal protein reabsorption is thought to be absent. That yields $\Theta = 7.7 \times 10^{-5}$, which is 10-fold lower than our estimate for normal rats. That sieving coefficient, together with a plasma albumin concentration of 3.5 g/dl, which is representative of healthy humans (4), yields $C_0 = 2.7 \mu g/mL$. The fractional reabsorption of albumin in normal humans was inferred then by using the GFR and urinary albumin excretion data reported by Blouch et al. (4) for controls; the result was $f = 0.986$, which is slightly larger than the 0.976 that we calculated for rats. Using the measured GFR and assuming a total of $1.4 \times 10^6$ nephrons, we estimated that SNGFR = 71 ml/min in healthy humans. Finally, with the use of the tubule dimensions in Table 1, the model yielded $V_{\text{max}} = 0.18$ mg-sec$^{-1}$-mm$^{-2}$, or approximately twice the value for rats. With these parameter values, the predicted effects of filtered load for humans (not shown) are very similar to those presented in Figs. 5 and 6 for rats. We used tubule dimensions based on rat and rabbit data, because we were unable to find reliable values for humans. If the human dimensions were found to be different, then $V_{\text{max}}$ would need to be adjusted to keep the product $V_{\text{max}}S$ the same, as discussed in connection with Fig. 7. The results to be discussed next would be unchanged, however.

Having estimated the parameters for humans, we return now to the discussion of hyperfiltration. Because the data for humans are inadequate to calculate the parameters in Eq. 14, we assumed that a 50% increase in SNGFR would cause $\Theta$ for humans to decrease by the same 20% as for rats. Thus, a 50% increase in SNGFR would result again in just a 20% increase in filtered load. With these inputs, the model predicts a reduction in GFR and urinary albumin excretion.

In conclusion, a model has been presented to show how the rate of albumin reabsorption in the proximal tubule is influenced by the kinetics of endocytosis, diffusion in the intermicrovillar spaces, and fluid flow and diffusion in the lumen. Using in vivo micropuncture and other data from the literature, all of the required parameter values could be estimated for normal rats. Thus, for rats, the effects on albumin reabsorption of such factors as Bowman’s space concentration and SNGFR could be predicted with reasonable confidence, and the interaction between albumin filtration and reabsorption explored. Although the parameter estimates were necessarily less direct, similar kinds of predictions could be made for humans. Some of the model predictions, such as the extreme sensitivity of fractional albumin reabsorption to variations in GFR (Fig. 5), may be testable using noninvasive (clearance) methods. We hope that this analysis will stimulate renewed interest in quantifying tubule protein reabsorption. The significance of urinary protein levels, an important clinical measure of renal health, can be understood fully only by appreciating the interplay between filtration and reabsorption.

**APPENDIX**

Validity of Michaelis-Menten kinetic model for receptor-mediated endocytosis of albumin. It has been customary to assume that the tubular reabsorption of albumin follows Michaelis-Menten kinetics. To determine whether that description of a saturable kinetic process is consistent with receptor-mediated endocytosis, we performed a steady-state analysis of a standard receptor ligand binding and trafficking model that includes the effects of reversible ligand association, complex internalization, receptor recycling, and degradation. An example of such a model is given in Hendriks et al. (16). Solving their Eqs. A-D at steady state for the four unknowns and setting the rate of ligand (in our case albumin) reabsorption equal to the product of the endocytosis rate constant ($k_r$) and the concentration of surface ligand receptor complexes ($C_r$), the rate of albumin endocytosis in terms of their model parameters is

$$k_r C_r = \frac{k k_p S L}{k_r k_f - k_r S f_c,}$$

(A1)

Parameters $k_r$ and $k_f$ are the forward and reverse rate constants for ligand binding to free receptor, respectively. Parameter $k_r$ is the rate constant for free receptor endocytosis, and $f_c$ is the fraction of free receptor exiting the endosome that is recycled to the plasma membrane; $k_r$ and $k_f$ are analogous to constants for ligand receptor complexes. $S_r$ is the rate of free receptor synthesis, and $L$ is the concentration of ligand. A slight rearrangement of Eq. A1 reveals that it has the same form as traditional Michaelis-Menten kinetics, with $V_{\text{max}}$ and $K_m$ being related to the receptor-ligand model parameters as

$$V_{\text{max}} = \frac{k k_p S}{k_r k_f - k_r S f_c}$$

(A2)

$$K_m = \frac{-k r f_c, k_r - k r f_c, k_r - k r f_c, + k a k_r + k a k_r + k a f_c + k a f_c}{k r k_f - k r f_c,}$$

(A3)

Thus the rate expression in Eq. 7 is fully consistent with an endocytic mechanism.

Dimensional analysis of simplified model. The parameters that have the most important effects on fractional albumin reabsorption are revealed by simplifying the model somewhat and making all quantities dimensionless. If the radial mass transfer resistance is neglected, then $C_m = C_w = C_p$. In that case, Eq. 3 can be rewritten as

$$\frac{d}{dz} (u C_b) = -\frac{2}{R} \left( \frac{V_{\text{max}} C_b}{K_m + C_b} \right)$$

(A4)

Defining dimensionless position and dimensionless albumin concentration as $X = zL$ and $\phi = C_b/C_0$, respectively, and with the use of Eq. 5 to evaluate $u$, the differential equation and initial condition for the simplified model become

$$\frac{d\phi}{dX} = \left( \frac{\phi}{1 - f e X} \right) f e - \frac{V_{\text{max}} S}{F} \frac{1}{(K_m/C_0 + \phi)}$$

(\phi(0) = 1)

(A5)

where $f e = 2\nu_w L/\eta_o R$ is the fractional water reabsorption, $S = 2\pi RL$.
is the inner tubule surface area, and $F = \pi R^2 u_s C_0$ is the filtered load of albumin. According to Eq. A5, $\delta(X)$ depends on just three dimensionless parameters, namely, $f_w$ and
\[
\beta = \frac{K_m}{C_0} \quad \text{(A6)}
\]
\[
\chi = \frac{V_{\text{max}} S}{F} \quad \text{(A7)}
\]
Because fractional albumin reabsorption is given by $f = 1 - (1 - f_w)\delta(1)$, the simplified model implies that $f$ is a function only of $f_w$ and the affinity and capacity parameters defined by Eqs. A6 and A7. Inasmuch as glomerular-tubular balance ensures that $f_w$ remains nearly constant, $f$ is a function mainly of $\beta$ and $\chi$, as shown in Fig. 7. A similar analysis of the full model (with radial mass transfer limitations) reveals that $f$ depends also on $\Pi$ and certain other dimensionless groups. However, those additional groups are less important than $\beta$ and $\chi$.

ACKNOWLEDGMENTS

The authors are grateful to Dr. K. Beers for computational advice, and to Dr. T. Meyer, Dr. B. Haraldsson, and Dr. J. Nystrom for suggestions on sources for human data.

GRANTS

This work was supported in part by National Institutes of Health Grant DK-20368.

REFERENCES


