A hydrodynamic mechanosensory hypothesis for brush border microvilli

P. Guo, A. M. Weinstein, and S. Weinbaum. A hydrodynamic mechanosensory hypothesis for brush border microvilli. Am J Physiol Renal Physiol 279: F698–F712, 2000.—In the proximal tubule of the kidney, Na\(^+\) and HCO\(_3\)\(^-\) reabsorption vary proportionally with changes in axial flow rate. This feature is a critical component of glomerulotubular balance, but the basic mechanism by which the tubule epithelial cells sense axial flow remains unexplained. We propose that the microvilli, which constitute the brush border, are physically suitable to act as a mechanosensor of fluid flow. To examine this hypothesis quantitatively, we have developed an elastohydrodynamic model to predict the forces and torques along each microvillus and its resulting elastic bending deformation. This model indicates that: 1) the spacing of the microvilli is so dense that there is virtually no axial velocity within the brush border and that drag forces on the microvilli are at least 200 times greater than the shear force on the cell’s apical membrane at the base of the microvilli; 2) of the total drag on a 2.5-μm microvillus, 74% appears within 0.2 μm from the tip; and 3) assuming that the structural strength of the microvillus depends on its length, the luminal fluid flow of 30 nL/min produces a deflection of the microvillus tip which varies from about 1 to 5% of its 90-nm diameter, depending on the microvilli length. The microvilli thus appear as a set of stiff bristles, in a configuration in which changes in drag will produce maximal torque.

glomerulotubular balance; mechanosensory mechanism; actin cytoskeleton; microvilli force and torque

Perhaps the most important characteristic of solute and water transport in the proximal tubule is the observation that reabsorption varies proportionally with delivered load, i.e., with the glomerular filtration rate (GFR) (14). In large measure this “glomerulotubular balance” derives from a “perfusion-absorption balance,” that is, the capability of the proximal tubule epithelial cells to sense changes in luminal flow rate and translate this signal into changes in volume reabsorption (53). This system is remarkably precise, since the fraction of filtered fluid reabsorbed by the proximal tubule is nearly constant over the entire physiological range of flow (48). In this report, we shall propose a new role for the microvilli as a mechanosensory system, which not only senses fluid shear and drag forces, but has the capability of greatly amplifying the mechanical stresses that are felt on the intracellular cytoskeleton. To quantitatively explore this hypothesis, we shall develop a mathematical model to predict the hydrodynamic forces and torques on the microvilli, their distribution along the length of the microvillus, and the bending deformation of the F-actin microfilaments in the microvilli due to this hydrodynamic loading. These hydrodynamic forces and torques will also be related to the flow-dependent spacing of the microvilli that has been measured (36).

The efferent limb of the control of proximal tubule fluid reabsorption appears to be reasonably well established. Volume reabsorption is driven by Na\(^+\) reabsorption, and this reabsorption rate is determined by Na\(^+\) entry across the luminal membrane in which the Na\(^+\)/H\(^+\) antiporter is the most important entry step (50). The afferent limb, namely the mechanism by which the proximal tubule senses axial flow rate, is unknown. In this regard attention has been focused on the brush border of proximal tubule cells, since this is the interface with the tubule lumen. These 2- to 3-μm, densely packed luminal projections not only greatly amplify luminal membrane surface area (52), they define a region near the luminal cell surface, whose geometry can change with changes in perfusion conditions (36). A number of workers have considered the possibility that changes in axial flow might produce changes in solute ion concentration within the brush border region and that such ion concentration changes could somehow be sensed by the cell (3). However, all model calculations attempting to estimate such solute gradients have indicated that this diffusion is so rapid as to preclude any significant concentration difference within the brush border (27).

One possibility, which has not been previously proposed or quantitatively explored, is that the microvilli can serve a mechanosensory role in the afferent limb of proximal tubule perfusion-absorption balance. This possibility is suggested by the axial cytoskeletal struc-
ture of the microvilli reported by Hassen and Hermann (20). Subsequent studies (44, 37) showed that each microvillus contains a distributed array of 6–10 long axial microfilaments of 7 nm diameter, which immunocytochemical analysis showed were F-actin microfilaments (5). These filaments, we hypothesize, would provide a structural rigidity to the microvilli that could enable them to resist bending when subject to hydrodynamic forces and thus be capable of serving as a mechanotransducer, much like the hair cells in the inner ear.

A logical mechanosensory candidate for most cells subject to fluid flow is fluid shear stress. There is an extensive literature on the biochemical and ultrastructural effects of fluid shear on cells since the study by Dewey et al. (11) first demonstrated that vascular endothelial cells could be grown in culture and exposed to fluid shear under carefully controlled conditions. A wide variety of ultrastructural and intracellular biochemical responses to fluid shear have been documented involving Ca$^{2+}$, second messengers, and NO release (10). Vascular endothelial cells are exposed to fluid shear stresses that are typically 10–20 dyn/cm$^2$ on the arterial side of the circulation and 1–2 dyn/cm$^2$ on the venous side. If the surface of the brush border cells did not have microvilli, then the fluid shear stress would vary from about 1 to 5 dyn/cm$^2$ for Poiseuille flow over the normal range of flow rates, which lies in the same range as for vascular endothelium. However, the presence of the microvilli produces a unique flow structure in the vicinity of the brush border, wherein the fluid shear stress at the base of the microvilli is several hundred times smaller than the drag forces on the microvilli themselves. The dynamics of this fluid flow and the forces generated by both the fluid shear stresses acting near the tips of the microvilli and the axial flow past the main body of the microvillus will be examined. In particular, we are interested in the force and torque (bending moment) distribution on each microvillus, and we hypothesize that it is the bending moment produced by these forces that acts as a mechanical transducer for the sensing of the axial fluid flow along the tubule. These hydrodynamic forces will then be used as input into an elastic model for the bending deformation of the actin cytoskeleton of the microvilli to predict the displacement of the microvilli tips. We speculate that this bending moment is transmitted to the cytoskeleton in the cell interior, where it is converted into a biochemical response.

METHODS

Ultrastructural Model

The idealized ultrastructural model for the proximal tubule is sketched at the cellular and tubular cross-sectional scales in Fig. 1. Figure 1A is a schematic of a proximal tubule cell from an S2 segment in the distal part of the convoluted tubule or the beginning of the straight segment (35). It is in this region that Maunsbach et al. (36) examined the effect of flow on proximal tubule ultrastructure. The cells in this segment are characterized by densely spaced microvilli, numerous mitochondria, and extensive interdigitation of the lateral membrane near the basal surface. The microvilli are even more dense and longer in the S1 segment and significantly less dense in the S3 segment, which comprises most of the straight tubule. Of particular interest in this study is how this difference in ultrastructure affects the flow, forces, and torques on the microvilli. Figure 1B is our macroscopic model of the entire tubule shown in transverse cross section. The luminal radius of the tubule excluding the microvilli is $R_L$, and the height of the brush border is $L$. From a hydrodynamic viewpoint the fluid flow is subtle, since the microvilli form a closely spaced array in which there is a small, but nonnegligible axial bulk flow through the microvilli, which is driven by the axial pressure gradient in the tubule lumen. In addition, there are two thin interaction layers, one near the microvilli tips and one at the base of the microvilli, where the fluid flow must adjust to satisfy the no-slip boundary conditions at the apical membrane of the proximal tubule cell in Fig. 1A or the fluid shear matching condition at the microvilli tips.
tion layer near the base of the microvilli determines the fluid shearing stress on the apical membrane of the proximal tubule cells and the interaction layer near the tips of the microvilli determines the forces on the tips and, as we show later, provides the dominant contribution to the hydrodynamic torque on the microvillus. A central question in determining the torque is the drag distribution. In essence, one wishes to determine how the small forces acting over most of the length of the microvilli due to the pressure-driven bulk flow compared with the much larger drag forces that are felt in the highly localized region near the microvilli tips.

Figure 2A is an enlarged sketch of the transverse section of the brush border showing the cross-sectional geometry of the microvilli. Ultrastructural studies have shown that the microvilli form a closely spaced hexagonal array (37) where the open gap, $\Delta$, between microvilli can vary significantly with fluid flow rate (36) and as previously noted with location on the S1, S2, and S3 segments. This hexagonal array will be further subdivided into repetitive periodic units, each containing the equivalent of a single microvillus, as shown in Fig. 2B. Ultrastructural studies (20, 44, 37) have shown the existence of longitudinal actin microfilaments of 6–7 nm diameter extending the length of the microvillus but no microtubules or intermediate filaments. In our model we assume that these actin filaments provide a structural rigidity that prevents the microvilli from deforming significantly during flow from a hydrodynamic standpoint. By this we mean that the microvilli can undergo small deformations in which the axial microfilaments might serve as strain transducers but that these small strains do not significantly affect spacing of the microvilli and hence the fluid dynamic forces and torques acting on them.

Mathematical Model

The hydrodynamic problem for the flow past the microvilli tips is a classic unsolved problem in the fluid mechanics literature. Rigorous hydrodynamic solutions have been obtained for the two-dimensional Stokes flow past periodic fiber arrays which are infinite in extent. One of the best known of these solutions, that of Sangani and Acrivos (46), describes the flow transverse to an infinite hexagonal array of circular fibers whose cross-sectional geometry is shown in Fig. 2A. The more difficult problem, which we wish to analyze in the present study, is the three-dimensional flow in the transition region in the vicinity of the microvilli tips. In particular, we wish to examine the flow transition that occurs between the shear flow in the tubule lumen and the fiber-containing region where the flow asymptotically approaches the behavior described by the Sangani and Acrivos solution. This region is of greatest concern to us since it is the primary determinant of the forces and torques on the microvilli.

There have been several prior studies that have examined related interface problems. The one that is closest to the present study is the model proposed by Mokady et al. (38) for the shear flow over an endothelial glycocalyx. In this model the endothelial glycocalyx is treated as a layer of porous matrix whose fibers are arranged in a periodic horizontal square array parallel to the underlying solid boundary and transverse to the flow direction. A numerical technique developed in Larson and Higdon (30) is used to solve the Stokes equations for viscous flow around each fiber and the decay in the average horizontal velocity as one penetrates the matrix layer. This problem differs from the present one in several fundamental aspects. In our problem the microvilli (the fiber array in Fig. 1B) are vertical rather than horizontal, the flow is confined in a circular cylinder rather than unbounded, the fiber fraction is much greater (this is typically 0.01 to 0.02 for the endothelial glycocalyx), and the interface is cylindrical rather than planar. Also, our fiber array is hexagonal rather than square, but this adds no further difficulty since the solutions in Sangani and Acrivos (46) for the infinite fiber array describe both fiber geometries.

The most important difference cited above is the fiber orientation. The force on a horizontal fiber transverse to the flow direction is uniform, whereas in our problem the most important feature is the variation of the force along the microvillus length. This type of problem has not been treated previously, to our knowledge. The presence of a confining cylindrical boundary at $R = R_0$ creates an axial pressure gradient that...
drives a small, but important, flow across the microvilli. The magnitude of this flow determines the relative importance of the drag forces over the main body of the microvillus as opposed to the drag force on the microvilli tips in the interface region described above. The problem for the detailed flow around the horizontal fibers in Mokady et al. (38) is two-dimensional, in contrast to the present flow geometry, which is fully three-dimensional. We thus seek a simplified solution approach in which we try to avoid the necessity for obtaining detailed solutions for the three-dimensional velocity field surrounding each microvillus. The key to this simplification is to devise an approach that first describes just the decay in the average velocity with distance from the microvillus tip and then to use this solution in a model that describes the local fluid flow around and the local variation of the axial force along the microvillus.

The simplified solution approach that we have adopted combines effective medium theory (Brinkman equation) and the rigorous hydrodynamic solutions in Sangani and Acrivos for Stokes flow past the infinite hexagonal fiber array. We first consider the solution to the axisymmetric flow problem sketched in Fig. 1B. This flow is divided into two regions, a core flow and a flow through the fiber (microvillus) array sketched in Fig. 2A. The flow in the core region or lumen is described by the Navier-Stokes equation for unidirectional axial flow in a circular cylinder. For this flow the inertial terms vanish, and the simplified equation can be written in axisymmetric (R, Z) coordinates as

\[
\frac{dP}{dz} = \frac{\mu}{R} \frac{\partial}{\partial R} \left( R \frac{\partial U_c}{\partial R} \right) \tag{1}
\]

where \(U_c\) is the fluid velocity in the core, and \(\mu\) is the fluid viscosity. In the margin region \(R_L < R < R_0\) with microvilli, we use a Brinkman equation for flow through a porous medium (6)

\[
\frac{dP}{dz} = \frac{\mu}{R} \frac{\partial}{\partial R} \left( R \frac{\partial U_m}{\partial R} \right) - \frac{\mu}{K_p} U_m \tag{2}
\]

Here \(U_m\) is the local average axial velocity in the microvillus array, and \(K_p\) is the two-dimensional Darcy permeability for an infinite fiber array. The expression for \(K_p\) will be derived shortly using the solution for the drag coefficient for an individual fiber in Sangani and Acrivos (46). Equation 2 differs from Eq. 1 in that it contains a distributed body force or Darcy term, \(\mu U_m/K_p\), due to the drag on the microvillus. The second term in Eq. 2 is the same as the viscous term in Eq. 1. This term is only important near boundaries or interfaces where the gradient in the axial velocity is large. In the region removed from boundaries, the Darcy term will dominate, except in the limit where the microvilli are sparse. Both the Stokes and Brinkman equations are linear, and thus it is a simple matter to include the radial component of the velocity to account for fluid absorption. This absorption also leads to a decay in the axial velocity and a decrease in the axial pressure gradient in the tubule lumen. This radial flow is not of importance in the present study, since it is directed along the axis of the microvillus and thus does not produce a drag or bending moment on the microvillus.

Equations 1 and 2 can be cast in dimensionless form by introducing the dimensionless variables

\[
R = R_0 \cdot r, \quad Z = R_0 \cdot z, \quad U_i = U_0 \cdot u_i, \quad P = P_0 \cdot p \tag{3}
\]

where the characteristic length, velocity, and pressure are defined by

\[
R_0 = R_L + L, \quad U_0 = \frac{Q}{A}, \quad P_0 = \frac{\mu U_0}{R_0}
\]

and \(i = c \text{ or } m\) depending on whether one is describing the core flow or brush-border flow, respectively. Here, \(R_L\) is the luminal radius, \(L\) is the height of the microvillus, \(Q\) is the flow in the tubule, \(A\) is the cross-sectional area of the tubule, and \(U_0\) is the average velocity.

The dimensionless form of Eq. 2 for axisymmetric flow

\[
\frac{dP}{dz} = \frac{1}{r \partial r} \left( r \frac{\partial u_m}{\partial r} \right) - \alpha^2 u_m \tag{4}
\]

contains a single dimensionless parameter \(\alpha\) given by

\[
\alpha = \frac{R_0}{\sqrt{K_p}} \tag{5}
\]

The denominator, \(\sqrt{K_p}\), in the definition of \(\alpha\) is a characteristic length describing the thickness of the fiber interaction layers at the base and tip of the microvilli. One can show, as did Tsay and Weinbaum (49), that this characteristic thickness is of the same order as the microvillus spacing \(\Delta\). Thus one anticipates that the solution of Eq. 4 will entail thin regions whose thickness is of order \(\Delta\) near the base and tip of the microvilli where there will be steep velocity gradients and the velocity must adjust to satisfy boundary and matching conditions. Since \(\Delta\) is of order 0.1 \(\mu m\) and \(R_0\) is typically 20–30 \(\mu m\) depending on flow rate, \(\alpha\) is of order several hundred. We will, therefore, be interested in the solutions to Eq. 4 in the large \(\alpha\) limit.

There are two boundary conditions, one at the center of the tubule, \(r = 0\), where we require that the velocity be symmetric

\[
\frac{\partial u_c}{\partial r} = 0 \tag{6}
\]

and one on the wall (apical membrane), \(r = 1\), where we require that the no-slip boundary condition be satisfied

\[
u_m = 0 \tag{7}
\]

In addition, we require that velocity and shear stress be continuous at the edge, \(r = r_L\), of the brush border

\[
u_m(r_L) = u_c(r_L), \quad \tau_m(r_L) = \tau_c(r_L) \tag{8}
\]

where \(\tau_m\) and \(\tau_c\) are given by \(\mu \times \partial U_m/\partial R\) and \(\mu \times \partial U_c/\partial R\), respectively.
Solution for Velocity Field and Mass Flow

The general solution to Eq. 4 is given by

\[ u_m(r) = \left( - \frac{1}{4} \frac{dp}{dz} \right) \left( C_1 \frac{I_0(\alpha r)}{I_0(\alpha)} + C_2 \frac{K_0(\alpha r)}{K_0(\alpha)} + 1 \right) \quad (9) \]

where \( I_0(r) \) and \( K_0(r) \) are zero-order modified-Bessel functions, \( dp/dz \) is the dimensionless pressure gradient, and \( C_1 \) and \( C_2 \) are arbitrary integration constants.

The general solution to Eq. 1, which satisfies boundary condition Eq. 6, can be written in dimensionless form as

\[ u_e(r) = \left( - \frac{1}{4} \frac{dp}{dz} \right) (r_L^2 - r^2) \left( \frac{1}{\alpha^2} \frac{dp}{dz} \right) C_3 \quad (10) \]

where \( C_3 \) is an integration constant.

The matching conditions, Eqs. 7 and 8, determine the unknown constants \( C_1, C_2, \) and \( C_3 \). These expressions for the \( C_i \) and their asymptotic expressions, in the limit \( \alpha > 1 \), are given by

\[ C_1 = - \frac{K_1(\alpha r_L)}{K_0(\alpha)} + \frac{\alpha r_L}{2} \quad (11A) \]

\[ C_2 = \frac{\alpha r_L}{2} - \frac{K_1(\alpha r_L)}{I_0(\alpha)} \quad (11B) \]

\[ C_3 = C_1 \cdot \frac{I_0(\alpha r_L)}{I_0(\alpha)} + C_2 \cdot \frac{K_0(\alpha r_L)}{K_0(\alpha)} + 1 = \frac{\alpha r_L}{2} + 1 \quad (11C) \]

The dimensional water flux in the tubule is given by

\[ Q = \int_{r_L}^{r_U} U_I \cdot 2\pi R dR = \int_{r_L}^{r_U} U_e \cdot 2\pi R dR \]

\[ + \int_{r_L}^{r_U} U_m \cdot 2\pi R dR \quad (12A) \]

Equation 12A is the integral of the velocity over the entire cross section of the tubule. Since the brush-border flow contributes very little to the total flux, the second integral describing this flux can be neglected, and Eq. 12A reduces to

\[ Q \approx \int_{r_L}^{r_U} U_e \cdot 2\pi R dR \]

\[ = U_0 \cdot \pi r_L^2 \cdot \left( \frac{1}{\alpha^2} \frac{dp}{dz} \right) \quad (12B) \]

From Eq. 11, \( C_3 \) is of \( O(\alpha) \), and the second term of \( Q \) in Eq. 12B is of order \( 1/\alpha \) smaller than the first. The first term in Eq. 12B is the same as for the Poiseuille flow in a tube of dimensionless radius \( r_L \). The second term in Eq. 12B describes a small bulk flow due to the small slip velocity at the microvillus tips.

By introducing the dimensionless water flux

\[ Q_0 = U_0 \cdot \pi r_L^2 \]

one can write the dimensionless pressure gradient to order \( 1/\alpha \) as

\[ - \frac{dp}{dz} \equiv q \left( \frac{1}{8} \frac{r_L^4}{\alpha^2} C_3 \right) \quad (13) \]

Here \( q \) is the dimensionless flux, \( Q/Q_0 \), and \( r_L \) is the dimensionless position of the edge of the brush border.

Darcy Permeability Coefficient \( K_p \)

The expression for the two-dimensional Darcy permeability coefficient can be derived by examining a periodic unit in the hexagonal microvillus array shown in Fig. 2B. The distributed or body force per unit volume, \( F \), is the Darcy term in Eq. 2.

The total pressure force acting on a periodic unit, ABCD, in Fig. 2B, whose length and height are \((2a + \Delta)\) and \((2a + \Delta) \sqrt{3}/2\), respectively, is

\[ - \frac{dp}{dz} \cdot (2a + \Delta) \cdot (2a + \Delta) \sqrt{3}/2 \quad (14) \]

whereas Darcy’s law requires that

\[ \frac{dp}{dz} = - \frac{\mu}{K_p} U_m \quad (15) \]

From Eqs. 14 and 15, the pressure force acting on the periodic unit ABCD can be written as

\[ - \frac{dp}{dz} \cdot (2a + \Delta) \cdot (2a + \Delta) \sqrt{3}/2 = \frac{\mu}{K_p} U_m \cdot (2a + \Delta) \cdot \sqrt{3}/2 \quad (16) \]

Note, however, from Fig. 2B, that this is also the local drag force \( F \) per unit length on a single microvillus, since the periodic unit for the hexagonal array comprises a single cylindrical fiber or microvillus. Thus from Eq. 16, \( F \) is given by

\[ F = - \frac{dp}{dz} \cdot (2a + \Delta) \cdot \sqrt{3}/2 = \frac{\mu}{K_p} U_m \cdot (2a + \Delta) \cdot \sqrt{3}/2 \quad (17) \]

and \( K_p \) can be written in terms of \( F \) as

\[ K_p = \frac{\mu U_m}{F} \cdot (2a + \Delta) \cdot \sqrt{3}/2 \quad (18) \]

Sangani and Acrivos (46) have obtained a numerical solution for the Stokes flow past the periodic fiber array in Fig. 2B. They showed that the dimensionless drag, \( F/\mu U_m \), can be expressed by

\[ \frac{F}{\mu U_m} = \ln \left( \frac{c^{-1/2}}{0.745 + c - \frac{1}{4} c^2 + O(c^4)} \right) \quad (19) \]
where \( c \), the solid fraction, is defined by

\[
c = \frac{\pi a^2}{(2a + D)^2} \frac{\Delta^3}{2} = \frac{\pi}{(2 + \Delta/a)^2} \frac{\Delta^3}{2}
\]  

(20)

The expression in Eq. 19 is valid for \( c < 0.4 \). For control flow conditions where \( \Delta = 74.1 \) nm and \( a = 45 \) nm, \( c = 0.27 \). From Eqs. 18, 19, and 20

\[
K_p = \frac{\mu U_m}{a^2} \frac{\pi}{F} \frac{\ln (c^{-1/2}) - 0.745 + c - \sqrt{c^2 + O(c^4)}}{4e}
\]

(21)

Equation 21 is rigorously valid only for two-dimensional flow. It is, however, also a reasonable approximation for the local drag along a microvillus where \( U_m \) is a function of \( R \). A rigorous justification of this approximation would require a much more complicated three-dimensional analysis similar to that in Tsay and Weinbaum (49) where the radial pressure gradient and velocity are considered.

**Drag and Shear Force per Unit Tubule Length**

The total drag force on the microvilli per unit tubule length is given by

\[
F_d = \int_{R_L}^{R_0} \left( -\frac{\mu U_m}{K_p} \right) \cdot 2\pi RdR
\]

(22)

Equation 22 is the integrated drag on all microvilli that lie in the annular region \( R_L < R < R_0 \) due to thin effective hydrodynamic resistance, the Darcy term \( \mu U_m/K_p \) in Eq. 2. Using Eqs. 1 and 2, one can write Eq. 22 in the equivalent form

\[
F_d = \int_{R_L}^{R_0} \left( -\frac{\mu U_m}{K_p} \right) \cdot 2\pi RdR = \int_{R_L}^{R_0} (\nabla P - \mu \nabla^2 U_m) \cdot 2\pi RdR
\]

= \int_{R_L}^{R_0} (\nabla P - \mu \nabla^2 U_i) \cdot 2\pi RdR

where \( i = c \) or \( m \) in the last integral. The latter integral can be readily evaluated

\[
F_d = \int_{R_L}^{R_0} (\nabla P - \mu \nabla^2 U_i) \cdot 2\pi RdR
\]

= \frac{\frac{dP}{dZ}}{R} \pi R_d^2 - \frac{\tau(R_0)}{2\pi R_0}

(23)

From Eq. 23 it is clear that the pressure drop per unit tubule length (first term on right-hand side of Eq. 23) is balanced by the shear force at wall, \( F_s \) (second term on right-hand side of Eq. 23), and the drag force, \( F_d \), on the microvilli per unit tubule length.

The shear force per unit tubule length is given by

\[
F_s = \tau(R_0) \cdot 2\pi R_0
\]

(24)

From Eq. 23, the ratio \( \lambda \) of the drag force to the shear force per unit tubule length is expressed by

\[
\lambda = \frac{F_d}{F_s} \left( \frac{d}{2\pi R_0} \right) = 1 - \frac{\alpha}{2} \left( \frac{I_1(\alpha) + \alpha}{I_0(\alpha) + \frac{\alpha}{2} K_1(\alpha r_L)} \right)
\]

(25)

Note that in Eq. 25, \( \lambda \) depends only on the brush border and microvillus geometry.

**Drag and Torque on a Single Microvillus**

To obtain the drag force distribution \( f_d \) acting on each microvillus, we divide the total drag on all the microvilli per unit length, \( F_d \) in Eq. 22, by \( n \), number of microvilli per unit length of tubule, and integrate between \( R_L \), the microvillus tip and any position \( R \)

\[
f_d = \frac{1}{n} \int_{R_L}^{R} \left( -\frac{\mu U_m}{K_p} \right) \cdot 2\pi RdR
\]

(26)

When \( R \) is equal to the radius of the tubule, Eq. 26 gives the total drag force acting on a single microvillus. From Eq. 26 the local force per unit length of microvillus is given by

\[
\frac{df_d}{dR} = \frac{1}{n} \int_{R_L}^{R} \left( -\frac{\mu U_m}{K_p} \right) \cdot 2\pi RdR
\]

(27)

The bending moment per unit length acting on the base of the microvillus is given by

\[
\frac{dT}{dR} = R \cdot \frac{df_d}{dR}
\]

where \( R = R_0 - R \) is the lever arm of the force element.

The integrated torque distribution is defined by

\[
T(R) = \int_{R_L}^{R} R \cdot df_d = \int_{R_L}^{R} \frac{1}{n} \left( -\frac{\mu U_m}{K_p} \right) \cdot 2\pi RdR
\]

(28)

\( T(R) \) is the torque acting on a single microvillus between its tip and any position \( R \). When \( R \) is equal to the radius of the tubule, Eq. 28 gives the total torque acting on a single microvillus.

**Elastic Model for Bending of Microvillus**

Fortunately, there is sufficient information on the structure of the F-actin cytoskeleton of the microvillus and the elastic properties of an individual actin filament to construct from first principles a model for its bending deformation due to its hydrodynamic loading. These ultrastructural studies summarized by Maunsbach (37) indicate that there are between 6 and 10 long axial microfilaments randomly distributed in each cross section. Most of the axial microfilaments are clustered in the central region of the microvillus cross section rather than near its periphery. In our idealized ultrastructural model shown in Fig. 3A, we have assumed that on average seven such filaments are arranged in an hex-
agonal array with six of the filaments equally spaced on a circle that is the half-radius of the cross section. This is a close approximation to the electron micrographs shown in Maunsbach (37). Each microfilament is 7 nm in diameter. Although the spacing of the transverse linker molecules, fimbrin and $\alpha$-actinin, is not known to our knowledge, it is reasonable to assume that their primary function is that of spacer molecules that hold the long axial filaments in a nearly parallel array. The bending moment on the microvillus due to the distributed hydrodynamic drag is, therefore, borne entirely by its seven axial elements. This structure is analogous to a reinforced concrete beam in which the bending resistance of the concrete between its axial steel reinforcing rods is neglected. Fig. 3B is a sketch showing the geometry of the deformed microvillus with just its central microfilament.

According to the elementary theory for the bending of beams (4), the deflection $y$ of the microvillus (see Fig. 3B), satisfies the fourth order equation

$$\frac{d^2}{dx^2} \left( EI \frac{d^2y}{dx^2} \right) = D(x) = \frac{df_\alpha}{dR}$$

Here $x = R_0 - R$, $E$ is the Young's modulus of the individual actin filaments, $I$ is the moment of inertia of the cross section, and $D(x)$ is the distributed axial load due to the hydrodynamic drag obtained from Eq. 27. $I$ depends on the orientation of the bending axis. However, one can show that for the geometry in Fig. 3A the moment of inertia varies insignificantly with any axis passing through the origin because of the hexagonal symmetry. Since the radius of an actin filament, $r_f$, is small compared with the radius of the microvillus $a$, the contribution of each filament to $I$ is the area of the filament $\pi r_f^2$ times the distance from the axis of rotation. Thus $I$ for the cross-sectional geometry in Fig. 3A is

$$I = 4 \cdot \pi r_f^2 \cdot \left( \frac{\sqrt{3}}{2} \frac{a}{2} \right) = \frac{3}{4} \pi r_f^2 \cdot a^2$$

The drag force distribution or the beam loading is given by Eq. 27. However, as will be shown in the results, this loading can be well approximated by the superposition of two simple loads, a uniform load, $q_m$, over most of the length of the microvillus and a concentrated force, $P_m$, acting at its tip. This allows us to write a greatly simplified expression for the loading, which enables us to integrate the beam equation analytically. This simplified loading distribution is given by

$$D(x) = P_m \delta(x - L) + q_m$$

Four boundary conditions are needed to define the boundary value problem for the deflection of the microvillus. At $x = 0$, the base of the microvillus, it is reasonable to assume that the longitudinal actin filaments are anchored to more rigid supporting structures in the intracellular cytoskeleton. These could be either more complex actin networks near the apical membrane, microtubules, or intermediate filaments. The nature of this anchoring is not yet known. If this anchoring is relatively inflexible and treated as a rigid support, then the displacement and slope of the microvillus relative to the vertical will vanish

$$y(0) = 0, \quad y'(0) = 0$$

At the free end of the beam, $x = L$, there is no bending moment, and the vertical shear force does not vanish. We require that
The solution to the boundary value problem defined by Eqs. 29, 31, 32, and 33 is given by

\[ y(x) = \frac{1}{EI} \left( \frac{1}{6} P_m (-x^3 + 3Lx^2) + \frac{1}{24} q_m (x^4 - 4Lx^3) + 6L^2x^2 \right) \]  

At the free end of the beam, \( x = L \), the maximum deflection is achieved. From Eq. 34, it is given by

\[ y(L) = \frac{1}{EI} \left( \frac{1}{3} P_m L^3 + \frac{1}{8} q_m L^4 \right) \]  

RESULTS

Velocity Field

In Fig. 4 we have plotted the velocity profiles in the tubule lumen, the transition layer in the vicinity of the microvilli tips and in the brush border. Three curves are required because the velocity scale varies so greatly between regions. This behavior is characteristic of solutions in the large \( \alpha \) limit. The solutions in Fig. 4 as well as Fig. 6 are for a control value of \( Q \) of 30 nl/min. The edge of the brush border is located at a dimensionless \( r = 0.847 \). The centerline velocity in the lumen is 1.66 mm/s, and the profile in the core is a parabola typical of Poiseuille flow except near the interface with the brush border, where the velocity drops to very small values over a distance of a few tenths of a micron as observed in Fig. 4B. At the edge of the brush border, \( r = 0.847 \), the velocity is only 4.11 \( \mu \)m/s or roughly 1/400 of the centerline velocity. The velocity then falls off rapidly as one enters the lateral spaces between the microvilli and, as shown in Fig. 4C, asymptotically approaches a nearly constant value of only 10 nm/s in the central region of the brush border. As seen in Fig. 4B the thickness of the transition region at the microvilli tip where the velocity decays to the nearly vanishing bulk flow velocity is \( \sim 0.011 \) in dimensionless \( r \) units. This corresponds to a distance of 0.180 \( \mu \)m in physical units or about 7% of the microvilli length. This is about 2.4 times the open gap between microvilli, which for the control flow is 74 nm. As observed in Fig. 4C, a transition layer of comparable thickness exists at the base of the microvilli where the velocity decays from the miniscule bulk flow velocity in the brush border to zero at the apical membrane, so as to satisfy no-slip conditions. Because of the miniscule magnitude of the bulk flow in the brush border, the velocity in Fig. 4C is plotted in nanometers per second, where the velocity at the edge of the boundary layer at the apical membrane is about 10 nm/s. There is a slight decrease in the bulk flow velocity across the brush border due to the curvature of the radial coordinate system. This small bulk flow is driven by the axial pressure gradient in the lumen of the tubule.

In Fig. 5 we have plotted the tip velocity vs. the open gap \( \Delta \) between microvilli and also the dimensionless drag coefficient given by Eq. 19. It is clear that there is a nearly linear relationship between the tip velocity and \( \Delta \). This linearity can easily be derived from asymptotic analysis, see the APPENDIX. This effective slip velocity at the microvilli tips gives rise to the second term in the expression for \( Q \) in Eq. 12B.

The miniscule bulk flow in the brush border contributes negligibly to the total flow in the tubule. However,
it plays a very important role in determining the total force on each microvillus and the torque that it experiences. Similarly, it might seem at first glance that the details of the velocity profile in the thin transition region near the tips of the microvillus are not significant. Since the forces in Stokes flow are proportional to the velocity, it is the integral of this velocity profile that determines the contribution of the tip region to the total force and torque on the microvillus. The central question is how this integrated drag on the tips of the microvilli compares with the much smaller forces due to the bulk flow but which act over most of the length of the microvillus.

**Drag vs. Shear Force**

With a bulk flow velocity in the brush border that is five orders of magnitude smaller than the centerline velocity in the tubule, one wonders how the shear force acting on the apical membrane at the base of the microvilli compares with the drag force on the microvilli. As noted earlier, nearly all previous studies on the effect of fluid forces on vascular endothelial cells have examined the effect of fluid shear on the cell’s cytoskeleton and intracellular biochemical responses. Since both these forces scale linearly with the velocity, the ratio of the drag force to the fluid shear force would be independent of the tubule flow rate, if the microvilli geometry did not change with flow rate. The ultrastructural study by Maunsbach et al. (36) revealed that the open separation between the microvilli increased from 62.1 nm to 90.4 nm as the tubule flow was increased from 5 to 45 nl/min. One is thus interested not only in the ratio of the drag to fluid shear force at control conditions, where this separation distance is 74.1 nm, but also how this ratio changes more generally with microvilli spacing. The results of Eq. 25 are plotted in Fig. 6. One observes that the ratio of the drag to fluid shear force increases from ~360 to 580 as the open spacing between microvilli decreases from 90.4 to 62.1 nm and that there is a rapid fall off in this ratio as the distance between microvilli increases. The density of the microvilli in the S3 segment is approximately one-quarter that in the S2 segment and thus could be twice the control value cited above, or 150 nm. Even for this large spacing, the ratio of the drag to shear force is nearly 200.

**Drag and Torque Distribution on a Single Microvillus**

In Fig. 7 we have plotted Eq. 26 for the integrated drag distribution starting at the microvillus tip, \( r = 0.847 \), to the base of the microvillus. One observes a sharp break in this curve at approximately \( r = 0.858 \). At this location the average velocity differs by less than 1% from the bulk flow velocity in the interior of the brush border. The integrated drag at this location is 73.8% of the total drag on the microvillus, although this drag acts on only the 7% (0.18 \( \mu \)m) of the microvillus length near its tip. In summary, the drag due to the bulk flow in the interior of the brush border contributes \( 1/4 \) of the total drag, and the flow near the tip contributes \( 3/4 \) of the total drag. This highly asymmetric distribution, as we see next, provides for a large amplification in the torque experienced by the microvillus. The total drag force on the microvillus for a control flow of 30 nl/min is \( 7.38 \times 10^{-3} \) pN.

In Fig. 8 we have plotted Eq. 28 for the integrated torque distribution starting at the microvillus tip proceeding to the base of the microvillus. One again observes a sharp break in this curve at approximately \( r = \) 0.858.
0.858. The integrated torque at this location is 86.2% of the total torque on the microvillus, although this torque, like the drag, acts only on the 7% of the microvillus length near its tip. In summary, the torque due to the bulk flow in the interior of the brush border contributes only about 1/7 of the total torque, and the flow near the tip contributes 6/7.

**Effect of Flow on Drag and Torque**

In Table 1 we have summarized the effects of changing the flow rate using the ultrastructural data provided by Maunsbach et al. (36). One observes that the drag force on the microvilli does not vary linearly with flow rate if the changes in lumen diameter and microvilli separation with flow rate are accounted for. In fact, one notes that there is only a slightly more than twofold increase in drag when the flow is increased from 5 to 45 nl/min. If we assume that the length of the microvilli do not change, then, since three-quarters of the drag acts near the tips of the microvilli, the torque should increase roughly in proportion to the fluid drag on the microvilli tips. This nonlinear behavior, which is observed in experiments with individual perfused tubules in situ, is associated with an increase in hydrostatic pressure in the lumen of the tubule. This increase in tubule lumen pressure will cause a distension of the perfused nephron, since neighboring nephrons will not be at elevated transepithelial hydrostatic pressure. In vivo, this distension probably does not occur, since neighboring nephrons would all be at the same transepithelial pressure.

**Bending Deformation of the Microvillus**

The bending deformation of the microvillus and the deflection of its tip are given by Eqs. 34 and 35, respectively. The two key parameters in these expressions are the moment of inertia, I, given by Eq. 30 and the Young's modulus, E, of the individual actin filaments in the microvillus cross section shown in Fig. 3A. Realistic values for both of these parameters are available. In calculating I, we have used the measured values, r_f = 3.5 nm and a = 45 nm (37). The Young's modulus for an F-actin microfilament has been estimated from its bending modulus. This has been determined from force measurements obtained by the micromanipulation of single actin filaments by Kishino and Yanagida (23). The calculated value for E is 1.44 × 10^9 dyn/cm^2. In Fig. 9 we have plotted the deformed shapes of microvilli of four different lengths from 1.5 to 3.0 μm for a control flow of 30 nl/min when the open gap Δ between microvilli is 74.1 nm. Equation 35 shows that the maximum deflection of the microvilli, which is primarily determined by the concentrated drag forces near its tip, is proportional to the third power of the microvilli length. This accounts for the large increase in the maximum deflection with microvilli length. For a microvillus of 2.5 μm length in an S2 segment, the tip deflection is 3.78 nm, whereas for a 1.5-μm microvillus, the tip deflection is about one-fifth this value. Thus, at a control flow rate of 30 nl/min, the maximum tip deflection varies from about 1 to 5% of the microvilli diameter 90 nm.

As noted earlier, while the lengths of the microvilli decrease as one proceeds from the S1 to the S2 and S3

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**Table 1. Effects of changing flow rate in individually perfused tubules**

<table>
<thead>
<tr>
<th>Flow rate, nl/min</th>
<th>Low Flow Rate</th>
<th>Control</th>
<th>High Flow Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal diameter, μm</td>
<td>5</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Distance between MV, nm</td>
<td>17</td>
<td>27.7</td>
<td>35.6</td>
</tr>
<tr>
<td>MV density, per μm²</td>
<td>62.1</td>
<td>74.1</td>
<td>90.4</td>
</tr>
<tr>
<td>Solid fraction</td>
<td>49.9</td>
<td>42.5</td>
<td>35.5</td>
</tr>
<tr>
<td>Permeability coefficient, K</td>
<td>0.3175</td>
<td>0.273</td>
<td>0.226</td>
</tr>
<tr>
<td>Young's modulus, E</td>
<td>1.93 × 10^-16</td>
<td>2.93 × 10^-16</td>
<td>4.76 × 10^-16</td>
</tr>
<tr>
<td>Total force on each MV, pN</td>
<td>972</td>
<td>955</td>
<td>839</td>
</tr>
<tr>
<td>Total torque on each MV, pN·μm</td>
<td>3.93 × 10^-3</td>
<td>7.40 × 10^-3</td>
<td>8.20 × 10^-3</td>
</tr>
<tr>
<td>Effective shear force, dyn/cm²</td>
<td>1.59 × 10^-2</td>
<td>1.59 × 10^-2</td>
<td>1.76 × 10^-2</td>
</tr>
<tr>
<td>Maximum deflection, nm</td>
<td>1.96</td>
<td>3.14</td>
<td>2.91</td>
</tr>
</tbody>
</table>

MV, microvilli. Ultrastructural data are from Maunsbach et al. (36).
segments, the spacing of the microvilli increase and $\Delta$ for the S3 segment is about twice that for the S2 segment (52). One can show from Eq. 23 that the hydrodynamic loading of an S3 microvillus at its tip, $P_m$, is roughly four times that for the S2 microvillus. This suggests that the length of the microvilli may decrease as their spacing increases so that the deflection of the microvilli tips does not vary greatly along the length of the tubule.

**DISCUSSION**

Glomerulotubular balance was first established in rat kidney via micropuncture (48), where spontaneous variation in glomerular filtration (over a range of flows from 15 to 60 nL/min) was accompanied by a constant fractional proximal reabsorption. During periods of low filtration, this aspect of proximal tubule transport ensures preservation of adequate delivery of sodium to the distal nephron (preserving acid and potassium excretion). The mechanisms for balanced tubular reabsorption appear to include both peritubular capillary effects and luminal factors (13, 14, 19, 53). With respect to peritubular factors, any increase in filtration fraction must result in an increased peritubular protein concentration, and increased peritubular capillary protein concentration enhances proximal reabsorption (7, 17). The mechanism is thought to involve changes in interstitial hydrostatic pressure (25, 26), with effects on both the tight junctions (31) and on the cells themselves. Nevertheless, a mathematical model of proximal nephron, which incorporated the observed effect of peritubular Starling forces to modulate reabsorption, demonstrated that the observed constancy of fractional reabsorption could only be achieved when alterations in luminal flow, independent of peritubular Starling forces, could also influence proximal reabsorption (51).

The impact of variation in luminal flow rate on tubular reabsorption has been termed “perfusion-absorption balance” (53). This effect has been best demonstrated in rat microperfusion studies (2, 18, 40, 43). With respect to specific solutes, flow-dependent reabsorption has been found to influence the transport of glucose (24), bicarbonate (1, 9, 33), and chloride (16, 54). One of the best illustrations of this phenomenon is the micropuncture data of Chan et al. (9), in which a threefold increase in luminal perfusion rate (with trivial changes in luminal HCO$_3^-$ concentration) produced a doubling of the rate of bicarbonate reabsorption. The underlying mechanism for flow-dependent changes in reabsorption has not been established. One consideration has been an unstirred layer effect within the brush border (42). The morphological observations of Maunsbach et al. (36) demonstrated that lower tubule flow rates are associated with diminished tubule distention and a compaction of the brush border microvilli. Nevertheless, model calculations indicate it is unlikely that there is any appreciable convective stirring within this pile (3), nor should the diffusion barrier between the bulk luminal fluid and the cell membrane pose any significant hindrance to Na$^+$/H$^+$ exchange (27). Two relatively recent studies raise the possibility that increases in axial flow velocity recruit new transporters into the luminal membrane. Preisig (41) examined recovery of cellular pH from an acute acid load in vivo. With increases in luminal flow rate, the pH recovery mediated by Na$^+$/H$^+$ exchange was enhanced. Maddox et al. (34) subjected rats to acute changes in vascular volume to obtain hypodemic, eu- volemic, and volume-expanded groups, with respective grouping according to decreased, normal, and increased GFR. When brush-border membrane vesicles were prepared from each of these groups and Na$^+$/H$^+$ kinetic parameters were assessed, it was found that the $V_{\text{max}}$ determinations stratified in parallel with GFR.

Ultimately, perfusion-absorption balance must derive from an afferent sensor of fluid flow rate in series with a cascade of effector steps which insert or activate new membrane transporters. The calculations of this report indicate that the microvilli that constitute the proximal tubule brush border are physically suitable to function as such a sensor. The second component of this system may well be the actin cytoskeleton, which is abundant within and beneath the proximal tubule brush border (35). Indeed, the cytoskeleton has been implicated in volume regulation in a variety of cells (39), and specifically in proximal tubule, cytoskeletal disruption impairs the cell solute loss (volume regulatory decrease) following hypotonic cell swelling (32). Direct interactions of the cytoskeleton with a variety of membrane transporters, including ion channels, co- transporters, and the Na$^+$/K$^+$-ATPase, have been identified (8). Thus specific interaction between the proximal tubule cytoskeleton and the apical cell membrane Na$^+$/H$^+$ exchanger remains a critical feature to demonstrate, to maintain plausibility for this scheme of signal transduction. Such a connection is provided by the proposal of Lamprecht et al. (29) in which the Na$^+$/H$^+$ exchanger is linked via Na$^+$/H$^+$ exchanger regulatory factor to ezrin, a kinase anchoring protein, which is attached to the actin cytoskeleton. Indeed, gross disruption of the actin cytoskeleton has recently been shown to impair function of the proximal tubule Na$^+$/H$^+$ exchanger, when expressed in other cells (28). It is not known, however, whether more subtle changes, such as perturbation of cytoskeletal stresses, can also affect this transporter. It must also be acknowledged that more extensive feedback loops may be important, from flow changes to membrane transporter density via gene activation. Such responses have been identified in renal tubular cells in culture, in which changes in shear stress have been implicated in increased number of microvilli and in increased abundance of integral membrane proteins (22). Obviously, this response cannot be invoked to account for acute (micropuncture) observation of perfusion-absorption balance.

The analysis in the present study indicates that from both a hydrodynamic and structural standpoint, the microvilli are ideally suited to serve as mechanotransducers. Previously, it has been widely believed that the
primary role of the brush border was to greatly amplify the luminal membrane area required for water and solute exchange. This does not explain why the microvilli have such striking uniformity in length, why the spacing between microvilli is so regular, why the microvilli decrease in length as their density decreases, what determines the density and distribution of the axial actin microfilaments in the microvilli, and how these microfilaments are anchored to the intracellular cytoskeleton near the apical membrane. These questions lead to a new view of the brush-border epithelial cells not only as a transport organ, but as a cell with specialized ultrastructural components that could have mechanosensory functions.

We first consider the hydrodynamic function of the brush border. Except for the thin interaction region near the microvilli tips, the axial velocity within the brush border is five orders of magnitude smaller than the average axial velocity within the lumen of the tubule. In contrast, one can estimate that if one-half the filtrate is reabsorbed along the entire length (1 cm) of the proximal tubule, then the radially directed average velocity along the axes of the microvilli is 0.24 μm/s. This is a tiny fraction (0.15 × 10⁻³) of the axial velocity in the lumen. The radially directed flow due to reabsorption is, therefore, about 24 times the axially directed bulk flow within the brush border, but this radial flow produces no drag or torque on the microvilli since it is directed along the microvillus axis. The miniscule axial bulk flow contributes negligibly to the total flow in the tubule, yet it determines the nature of the hydrodynamic forces on both the microvilli and the apical membrane.

The nearly vanishing axial bulk flow velocity in the brush border in Fig. 5C was previously predicted in Basmadjian et al. (3), using a semi-empirical Carman-Kozeny equation to estimate the hydraulic resistance. This semi-empirical relation was widely used before the more accurate solution of Sangani and Acrivos (46) (Eq. 19) was derived. In this earlier study Basmadjian and coworkers (3) derived an expression for the ratio of the flow in the brush border to the flow in the core. For control flow in the present study, this ratio is \(-5 \times 10^{-6}\), a value that is similar to the prediction in Basmadjian et al. (3) for a dense microvillus array. These investigators were primarily interested in unstirred layer effects due to the axial flow rather than the forces and torques due to the flow in the thin transition layer near the microvilli tips. This thin boundary layer, which is the primary focus of the present study, was neglected since it contributed insignificantly to the transport processes in the brush border.

Figure 6 shows that, despite the very small axial bulk flow through the brush border, the drag forces on the microvilli are at least 200 times greater than the shear forces acting on the underlying apical membrane. Proximal tubule epithelial cells would never be able to detect fluid shear forces in the same manner as vascular endothelial cells, although the flow rate in the tubule is quite similar to the flow rate in a blood vessel of comparable diameter. One of the remarkable features of the circulation is that, although the blood vessels undergo more than 20 generations of branching, the fluid shear stress is nearly constant throughout the entire arterial side of the vasculature (21). Furthermore, relatively modest changes in fluid shear stress are readily detected by endothelial cells (10). These wall shear variations lead to short-term biochemical responses and, in large vessels, endothelial-dependent adaptive remodeling after longer exposure to changes in wall shear stress (55). Proximal tubule epithelial cells thus need a different machinery to sense fluid flow rates, and hydrodynamic drag would appear to be a more logical candidate since it is several hundred times greater than fluid shear.

An unusual feature of the brush border microvilli is their remarkable local uniformity of length. Such uniformity would not be a prerequisite for a transport function associated with unstirred layer effects. It would, however, be essential if the tips of the microvilli all needed to sense the flow at the edge of the brush border. The detailed velocity profile near the tips of the microvilli in Fig. 4B provides an intriguing and rational argument for this structure. One observes that the axial velocity decays over a length that is less than 0.2 μm or roughly twice the open spacing Δ between the tips. A microvillus that is only slightly shorter than its neighbors would experience virtually no axial flow near its tip, and the hydrodynamic torque on this microvillus would be only about one-seventh that of its neighbors. There would be a small torque due to the bulk flow in the interior of the brush border, but as noted in Fig. 8, this torque is about one-seventh that due to the drag at the microvillus tips. The deflection of this microvillus would be much smaller than its neighbors (see Eq. 35).

The arguments just advanced for uniformity in microvilli length also apply for the unusual hexagonal regularity in microvilli spacing sketched in Fig. 2A. If this spacing were not regular, then each microvillus tip would experience a very different drag force. Figure 5 is a plot of Eq. 19 where the solid fraction, \(c\), has been related to the open spacing Δ between the microvilli using Eq. 20. It is evident from Fig. 5 that the dimensionless drag coefficient, \(F/\mu U\), on the microvillus tip is very sensitive to the spacing Δ and that the stimulus from each microvillus would vary greatly if their distribution were random. Such regularity in ultrastructure would only make sense if the microvilli were relatively stiff and their deformation due to hydrodynamic forces was small compared with their spacing.

It is clear from Fig. 5 that the drag force coefficient falls off rapidly as the open gap Δ increases. However, Fig. 5 also shows that the velocity at the microvillus tip, \(U_m(R_L)\), increases nearly linearly with Δ as the microvillus density decreases. The net result is that the drag force per unit length at the microvillus tips is relatively insensitive to Δ, and one can show there is a weak minimum (not shown) at Δ = 130 nm. The depth of the penetration of the tip boundary layer, however, also increases with Δ, so that the integrated drag force
on the tip increases as $\Delta$ decreases. This suggests that several competing factors determine the optimum spacing and length of the microvilli. The amplification of absorptive area along the apical surface decreases from 36 to 15 from the S1 to the S3 segment in mammalian tubules (37), and this correlates with volume reabsorption. However, a given amplification can be achieved by either long microvilli that are less dense or short microvilli that are more dense. It is here that mechanical forces could play an important role. In our hypothesized mechanosensory system, one would anticipate that either the torque on the microvillus or its tip deflection are the critical measures of flow rate. Thus, if one wanted to keep torque constant, then one could decrease microvillus length while increasing the separation of the microvilli so as to increase the value of $U_{\text{opt}}(R_L)$ and the drag at the microvilli tip. Similarly, a different flow-microvillus length relationship would emerge if one were to maximize deflection of the microvillus tip in Eq. 35. This optimal design requires further study.

From a mechanosensory viewpoint the stimulus could be either the bending deformation of the microvillus or the cytoskeletal structures that anchor the axial actin microfilaments to the intracellular cytoskeleton. We shall examine each of these possibilities. The model for microvillus bending provides insight into the first of these possibilities. The predicted deformations of the microvillus in Fig. 9 are based on realistic models of the actin microfilament structure in Fig. 3A, taken from Maunsbach (37), and a reliable estimate of the Young’s modulus of individual F-actin filaments in Kishino and Yanagida (23). The predicted maximum deflections of the microvilli at normal flow rates, 30 nl/min, are 1 to 5% of the microvilli diameter depending on microvilli length. The microvilli, therefore, act as remarkably stiff bristles whose deformation under flow is less than 0.2% of their length. This supports our initial assumption that the fluid flow through the microvilli and the resulting forces and torques can be calculated neglecting this deformation. Although this bending deformation is small, we shall show next that it is sufficient to produce an intracellular biochemical response.

There is a large literature on the biochemical response of cells grown on elastic substrates that are then subjected to stretch. These studies typically show that biochemical responses are elicited only for strains (change in length over initial length) that are 1% or greater. This could be the opening of stretch-sensitive ion channels in the membrane or the release of $\text{Ca}^{2+}$ from intracellular stores. When the microvillus bends, there is a relative motion of the axial actin filaments, and they slide past one another much like the pages of a paperback book when you bend its cover. The relative motion of the front and back cover is of the same order as the edge deflection and thus the relevant strain is the edge deflection divided by the total thickness of its pages. In the microvillus, this relative motion would be transmitted via cross-linking molecules such as ezrin, which link the actin cytoskeleton to proteins in the plasma membrane, or fimbrin and $\alpha$-actinin, which link the deforming actin fibers. The order of magnitude of the strain on these cross-linking molecules would be the tip deformation divided by the microvillus diameter, which was 1 to 5%. These strains fall in the range where intercellular biochemical responses are elicited on cells stretched in vitro. In the present case a plausible candidate for the membrane protein is NHE3, the Na$^+$/H$^+$ exchanger located in the microvillus membrane (29).

The second possibility is that the sensory stimulus is the torque exerted on the anchoring elements in the intracellular cytoskeleton. The ultrastructural arrangement of the microvilli is ideally designed to maximize this function. Nearly 90% of the torque is produced by drag forces acting at the microvilli tips. The long lever arm accentuates the moments on the intracellular cytoskeleton, and the stiffness of the microvilli allows one to transmit this torque without a large deformation that would produce tip-tip interaction. Our basic assumption in solving Eq. 29 is that the microvillus acts like a cantilever beam that satisfies a rigid end condition (32), much like the roots of a tree fasten its trunk to the ground.

The total drag force acting on the microvillus for a control flow of 30 nl/min is $7.4 \times 10^{-3}$ pN. This is about two orders of magnitude smaller than the drag forces applied by laser tweezers in moving proteins laterally in the plane of the membrane. Typically, forces less than 0.1 pN are too small to hold the proteins in the laser trap when the tail of the protein interacts with the underlying microfilaments in the membrane skeleton (45). Forces of the order of 0.5 pN will cause a large deformation of the F-actin microfilaments that form the fence of the microdomains that restrict the diffusion of the membrane proteins. A significant rebound of the protein is observed if it escapes from the optical trap at a microdomain boundary. Thus the hydrodynamic forces predicted by our model are small compared with the dragging forces that create large deformations of the membrane cytoskeleton. This is consistent with our hypothesis that the longitudinal actin filaments do indeed provide sufficient stiffness to prevent the microvilli from undergoing large strains and that the underlying cytoskeleton beneath the apical membrane, which provides the anchoring support, can withstand the bending moments at the base of the microvilli.

One of the most attractive features of this new model is that it is able to take full advantage of the available information that has been gathered over the past few decades on the ultrastructure of the microvilli, their actin cytoskeleton, and the physical parameters that determine both the hydrodynamic and elastic behavior. The limitation and uncertainty in the hydrodynamic model are minor since the spacing and regularity of the microvilli has been well documented. Similarly, the cytoskeletal model for the bending of the microvilli sketched in Fig. 3, which is idealized in the spacing of the axial actin filaments, is a quite realistic representation of the ultrastructure (37). The greatest
uncertainty in the prediction of the model lies in the available measurements determining the Young’s modulus $E$ of the individual actin filament. The estimation of $E$ has an order of magnitude uncertainty, since the experimental techniques have been based on measurements of the flexural rigidity $EI$ of a single actin filament subject to either micromanipulation or Brownian deformation (12, 15, 23) and not direct measurement of $E$, which is a derived quantity once the moment of inertia $I$ of the actin filament has been estimated. The measured values of $EI$ have varied from $15 \times 10^3$ pN·nm² (12) to $73 \times 10^3$ pN·nm² (15) with the measurement of $17 \times 10^3$ pN·nm² (23) used in our calculation being close to the former. Since $I$ varies as the fourth power of the effective radius of the filament, small variations in assumed cross-sectional geometry lead to substantial changes in $I$. In this study we have used the calculation in Satcher and Dewey (47), which assumes an effective radius of 3.5 nm, since this corresponds closely to the measurements reported in Refs. 35 and 44. This gives a value of $E$ of 0.14 GPa. If the effective radius had been reduced 2.5 nm, as assumed in Dupuis et al. (12), then $E$ would be a factor of four larger and the microvilli tip deflections a factor of four smaller.

The theoretical predictions of our model suggest a relatively simple critical experiment that can be performed to test the basic hypothesis that the microvilli serve a mechanosensory function, namely, to examine the effect of viscosity on reabsorption. If the flow rate is kept constant, but the viscosity of the perfusing fluid is increased, then the drag on the microvilli will increase and produce a larger drag on the microvilli tips. This should increase the torque on the microvilli and, if our hypothesis is correct, produce the same effect as increasing the flow rate. Additional biological information will be necessary, however, to formulate a complete signaling mechanism. Although the axial actin structure of the microvillus has been known since the early 1970s, there is no equivalent information on how this microfilament skeleton is anchored into the intracellular cytoskeleton. Finally, much more needs to be learned about the relationship between the sensing mechanism and the interactions that lead to the entry of $\text{Na}^+$ across the apical membrane. In particular, the specific interactions between the proximal tubule cytoskeleton and the apical cell membrane $\text{Na}^+/\text{H}^+$ exchanger need to be elucidated.

**APPENDIX**

**Slip Velocity at Microvillus Tip**

In the interior of brush border the bulk flow velocity is uniform, and there is no shear force at all. The dimensionless Brinkman Eq. 4 reduces to the Darcy equation, and the dimensionless bulk velocity, $u_m$, is given by

$$u_m = -\frac{1}{\alpha^2} \frac{dp}{dz} \quad (A1)$$

From Eq. 10 the dimensionless tip velocity is given by

$$u_{\text{tip}} = -\frac{1}{\alpha^2} \frac{dp}{dz} \cdot C_3 \quad (A2)$$

In the large $\alpha$ limit $C_3$ is given by Eq. 11C, and the dimensional slip velocity from Eq. 3 can be written as

$$u_{\text{tip}} = -\frac{K_p}{\mu} \frac{dp}{dz} \cdot \frac{\alpha_{rl}}{2} \quad (A3)$$

Using the definition of $\alpha$ and $\alpha_{rl}$, one can write Eq. A3 as

$$u_{\text{tip}} = -\frac{K_p}{2\mu} \frac{dp}{dz} \cdot \sqrt{K_p} \quad (A4)$$

Tsay and Weinbaum (49) have shown that the Darcy permeability coefficient $K_p$ is closely approximated by

$$K_p = 0.0572 \times a^3 \times \left(\frac{I}{a}\right)^{3.77} \quad (A5)$$

Substituting Eq. A5 into Eq. A4, the tip velocity is given by

$$u_{\text{tip}} = -\frac{K_p}{2\mu} \frac{dp}{dz} \cdot \sqrt{K_p} = K \cdot 1^3 \quad (A6)$$

where $K$ is a constant. Thus the tip velocity varies nearly linearly with $\Delta$.

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