Effects of pH and medullary blood flow on oxygen transport and sodium reabsorption in the rat outer medulla

Jing Chen,1 Aurélie Edwards,2 and Anita T. Layton1
1Department of Mathematics, Duke University, Durham, North Carolina; and 2Department of Chemical and Biological Engineering, Tufts University, Medford, Massachusetts

Submitted 5 October 2009; accepted in final form 19 March 2010

Chen J, Edwards A, Layton AT. Effects of pH and medullary blood flow on oxygen transport and sodium reabsorption in the rat outer medulla. Am J Physiol Renal Physiol 298:F1369–F1383, 2010. First published March 24, 2010; doi:10.1152/ajprenal.00572.2009.—We used a mathematical model of O2 transport and the urine concentrating mechanism of the outer medulla of the rat kidney to study the effects of blood pH and medullary blood flow on O2 availability and Na+ reabsorption. The model predicts that in vivo paracellular Na+ fluxes across medullary thick ascending limbs (mTALs) are small relative to tranacellular Na+ fluxes and that paracellular fluxes favor Na+ reabsorption from the lumen along most of the mTAL segments. In addition, model results suggest that blood pH has a significant impact on O2 transport and Na+ reabsorption owing to the Bohr effect, according to which a lower pH reduces the binding affinity of hemoglobin for O2. Thus our model predicts that the presumed greater acidity of blood in the interbundle regions, where mTALs are located, relative to that in the vascular bundles, facilitates the delivery of O2 to support the high metabolic requirements of the mTALs and raises the concentrating capability of the outer medulla. Model results also suggest that increases in vascular and tubular flow rates result in disproportional, smaller increases in active O2 availability and Na+ reabsorption. The load dependence of active Na+ transport may be partly insensitive to changes in GFR. Moreover, GFR and renal blood flow do not always vary proportionally, so that potential disequilibria in O2 supply and demand may also result from changes in the filtration fraction. One objective of this study is to determine the extent to which O2 consumption in the OM can adjust to O2 delivery. The availability of O2 in the OM is regulated by medullary perfusion (i.e., supply) and metabolic consumption (i.e., demand). Active Na+ reabsorption across the TAL, and therefore O2 consumption, varies in tandem with the glomerular filtration rate (GFR), so that an intrinsic regulatory system exists to maintain homeostasis of intrarenal oxygenation (14). However, the load dependence of active Na+ transport is likely stronger across the cortical TAL than across the medullary TAL, because under physiological conditions the mTAL luminal Na+ concentration is sufficiently high that carrier saturation is approximately attained. As a result, O2 consumption due to mTAL active transport may be partly insensitive to changes in GFR.

Both acute and chronic renal diseases are characterized by tissue hypoxia (5, 34, 39). The medullary thick ascending limb (mTAL) is particularly susceptible to anoxic damage given its high metabolic needs and its location in a region of limited O2 availability (6). In a recent theoretical study, we examined the impact of the complex structural organization of the rat outer medulla (OM) on O2 distribution (9, 10). Our mathematical model predicted that the OM architecture results in significant O2 tension (PO2) gradients in both the axial and the radial directions. The segregation of descending vasa recta (DVR), the main supply of O2, at the center and immediate periphery of the vascular bundles, helps to preserve O2 delivery to the inner medulla (IM). Consequently, mTALs, which are located away from the vascular bundles in an O2-poor region, operate on the brink of hypoxia. As a result, the model predicted that the concentrating capability of the OM is impaired to some extent. In the current study, we focus on the effects of pH and blood flow variations on O2 distribution in the OM.
mTAL, which should in turn enhance active NaCl reabsorption and the concentrating capability of the OM. Thus another objective of this study is to assess the importance of the Bohr effect on O₂ availability and NaCl reabsorption from the mTAL.

**MODEL DESCRIPTION**

We recently developed a detailed model of O₂ transport in the rat OM (9, 10), using the region-based approach first formulated by Layton et al. (27). In the region-based formulation, the structural organization of the OM is represented by means of four concentric regions centered on a vascular bundle: an innermost region containing the central vascular bundle (R1); a peripheral region of the vascular bundle (R2); a region neighboring the vascular bundle (R3); and the region most distant from the vascular bundle (R4). The radial organization of tubules and vasa recta with respect to vascular bundles is represented by specifying the fractions of the tubules and vasa recta assigned to each concentric region at each medullary level, as shown in Fig. 1.

Our model depicts the transport of water, Na⁺, urea, hemoglobin, and O₂ along and across tubules and vasa recta in the rat OM. As described previously, fluid flow and solute concentrations are determined by solving conservation equations in each tubule, vas rectum, and interstitial region; boundary conditions prescribe flows and concentrations at the corticomedullary junction for descending tubules and vessels, and at the OM-IM boundary for ascending tubules and vessels (10). Described below are the principal O₂ transport equations; all the other equations can be found in our previous work (10). We also highlight model parameters that are different from the previous study (10).

**O₂ Dissociation Curve**

The dissociation of oxyhemoglobin (HbO₂) into O₂ and deoxyhemoglobin (Hb) is modeled as a one-step reaction

\[ \text{HbO}_2 \quad \xrightarrow{k_{1}} \quad \text{Hb} + \text{O}_2 \]

where Hb represents one of the four heme groups in each hemoglobin molecule. The net volumetric rate of O₂ release by oxy-hemoglobin is thus given by

\[ R_{\text{O}_2} = k_1 \cdot C_{\text{HbO}_2} \cdot C_{\text{RBC}} \]  

where \( C_{\text{RBC}} \) denotes the RBC concentration of solute \( i \). Since this reaction is much faster than the diffusion of O₂, models of O₂ transport generally assume that even during O₂ unloading, the reaction proceeds essentially to completion. The O₂ dissociation curve is defined by

\[ P_{\text{O}_2} = \frac{1}{k_1} \left( \frac{1}{C_{\text{Hb}}} - 1 \right) \]

where \( P_{\text{O}_2} \) is the partial pressure of O₂ at the O₂ dissociation curve.

---

**Fig. 1.** Schematic representation of the region-based model. Displayed in A and B are the cross sections of the outer stripe (OS) and inner stripe (IS) of rat outer medulla (OM), respectively. In the model formulation, the four regions (R1–R4) have coincident centers; the display in this figure is intended to minimize the figure area. LDV, long descending vas rectum; SDVa and SDVb, 2 populations of short descending vasa recta; LAVa and LDL, 2 populations of long descending vasa recta; SAVa and SAVb, 2 populations of short ascending vasa recta; LDV, long descending limb of Henle’s loop; SDL, short descending limb; LAL, long ascending limb; SAL, short ascending limb; CD, collecting duct. The numbers represent the relative weight of interaction between a type of vessel or tubule and a given region (i.e., the parameter \( k_{i,R} \) in Eqs. 5, 7a, and 12). As shown in C, we differentiate between the red blood cell (RBC) and plasma compartments within vasa recta. The vascular endothelium and tubular epithelium are not explicitly distinguished in this model, but their thickness is taken into account in calculating cellular O₂ consumption. The arrows show the direction of the O₂ fluxes (see Eqs. 11, 13, and 14).
is at equilibrium, i.e., $E_{\text{RBC}}$ = 0. Experimentally, the O2 dissociation curve can be represented by the Hill equation over most of the physiological range of saturation (S) levels (18)

$$S = \frac{C_{\text{RBC}}}{C_{\text{RBC}} + C_{\text{HbO}_2}} = \frac{(C_{\text{RBC}}/C_{\text{S0}})^n}{1 + (C_{\text{RBC}}/C_{\text{S0}})^n}$$

(2)

where $C_{\text{S0}}$ is the O2 concentration (CO2) that yields a saturation of 0.5, and $n$ is an empirical constant (taken as 2.6). The relationship between CO2 and PO2 is given by $C_{\text{O2}} = \alpha_{\text{O2}} C_{\text{PO2}}$, where $\alpha_{\text{O2}}$ is the O2 solubility coefficient, estimated as 1.56 µM/mmHg in RBC and 1.34 µM/mmHg in plasma (11). Thus, when the Hill equation is formulated using $P_{\text{O2}}$ instead of $C_{\text{O2}}$, the parameter $C_{\text{S0}}$ is replaced with $P_{\text{S0}} = C_{\text{S0}}/\alpha_{\text{O2}}$.

Compatibility between the Hill equation and the equilibrium condition requires that at least one rate constant be variable. At low $P_{\text{O2}}$, the preferred approach is to fix the dissociation rate ($k_1$) and to vary the association constant ($k_{-1}$) as follows (18)

$$k_{-1} = \frac{k_1}{C_{\text{S0}} \left(1 - S \right)^{1-\frac{1}{n}}}$$

(3)

As described above, the O2 dissociation curve is shifted to the right under conditions of increased acidity. The magnitude of the Bohr effect can be estimated assuming that the Bohr coefficient, defined as $BC = d\log_{10} P_{\text{O2}}/d\log(\text{pH})$, is a constant, with a value of $-0.48$ (48). We therefore calculate the parameter $P_{\text{S0}}$ (in mmHg) as follows

$$\log_{10} P_{\text{S0}} = \log_{10}(26.4) - 0.48(\text{pH} - 7.4)$$

(4)

In the previous study (10), $P_{\text{S0}}$ was set to 26.4 mmHg, which corresponds to a pH of 7.4 and a temperature of 37°C (11). Here, we take into account experimental findings (reviewed above) which suggest that the pH in the vessels and tubules distant from the vascular core is the inner radius of tubule $i$ in contact with region R, $P_{\text{S0}}$ is the permeability of tubule $i$ to Na+, $C_{\text{R,N}}, C_{\text{i,N}}$, are the Na+ concentrations in region R and tubule $i$ respectively, and the normalized electrical potential difference is given by

$$\xi_{\text{i,N}}(x) = \frac{z_N F}{RT} \Delta V_{\text{R,i}}(x)$$

(6)

The parameter $F$ is the Faraday constant, $z_N$ is the valence of the Na+ ion, $RT$ is the product of gas constant and temperature, and $\Delta V_{\text{R,i}}$ is the electrical potential difference between region R and tubule $i$. Knowing that the sum of the transcellular and paracellular currents is zero, the transepithelial electrical potential difference is calculated at each step, as described in the APPENDIX. The permeability of mTAL to Na+ is set to 2.8 × 10^{-5} cm/s, consistent with mTAL Na+ permeability measurements in the rabbit and mouse (37).

The transcellular Na+ flux into tubule $i$ (i = SAL, LAL) is calculated as

$$j_{\text{i,N}}^{\text{active}}(x) = -2 \pi r_i(x) \sum_{R} \xi_{\text{i,N}}(x) \psi_{\text{i,N}}^{\text{active}}(x)$$

(7a)

$$\psi_{\text{i,N}}^{\text{active}} = \frac{V_{\text{max,i,N}} C_{\text{i,N}}(x)}{K_{\text{M,i,N}} + C_{\text{i,N}}(x)}$$

(7b)

where $V_{\text{max,i,N}}$ (in mol Na+·m^{-2}·s^{-1}) is the maximum rate of Na+ transport in tubule $i$, and $K_{\text{M}}$ is the Michaelis-Menten constant, taken as 70 mM (27). Below a critical $P_{\text{O2}}$ value (denoted by $P_{\text{C}}$), active O2 availability and the active Na+ transport rate is assumed to decrease linearly with $P_{\text{O2}}$, such that

$$V_{\text{max,i,N}} = \begin{cases} V_{\text{O2,max,i,N}} & P_{\text{O2}} \geq P_{\text{C}} \\ V_{\text{max,i,N}}(P_{\text{O2}}/P_{\text{C}}) & P_{\text{O2}} < P_{\text{C}} \end{cases}$$

(8)

where $V_{\text{O2,max,i,N}}$ is a constant, estimated as 10.5 mmol·cm^{-2}·s^{-1} in mTALs in the outer stripe, and 25.9 in mTALs in the inner stripe, based on the experimental results of Garg et al. (16). The value of $P_{\text{O2}}$ in Eq. 5 is taken as the average $P_{\text{O2}}$ between the mTAL lumen and the surrounding interstitium. The baseline value of $P_{\text{C}}$ is taken as 5 mmHg as described below.

Active and Basal O2 Consumption

Na+ is actively reabsorbed at the basolateral membrane by the Na+-K+-ATPase pump, the stoichiometry of which implies that the number of Na+ moles actively reabsorbed per mole of O2 consumed (also known as the $T_{\text{N}}/Q_{\text{O2}}$ ratio) is 18 under maximal efficiency. The
volumetric rate of O₂ consumption for active transport in tubule $i$ (denoted by $R_{i,Na}^{\text{active}}$, in mol O₂·m⁻³·s⁻¹) is thus determined as

$$R_{i,Na}^{\text{active}} = \frac{T_{i,Na}^{\text{active}}}{18}$$

where $T_{i,Na}$ (in mol Na⁺·m⁻³·s⁻¹) is the rate of active Na⁺ reabsorption in tubule $i$, and $A_{i,\text{cell}}$ is the cross-sectional area of the epithelial cell layer of $i$, perpendicular to the corticomedullary axis. It is calculated as $A_{i,\text{cell}} = \pi (r_i + \delta_i)^2 - r_i^2$, where $\delta_i$ is the thickness of the epithelial layer, taken as 8 μm in mTALs (22). The inner radius ($r_i$) of SAL is taken to decrease linearly from 10.5 μm at the corticomedullary junction to 5.0 μm at the boundary between the OM and IM; that of LAL remains fixed at 10.0 μm (27).

In addition to “active O₂ consumption” (that is, O₂ consumption for active Na⁺ transport), basal metabolism of interstitial cells, vascular endothelial cells, and tubular epithelial cells requires “basal O₂ consumption.” The volumetric rate of basal O₂ consumption in tubule or vas rectum $i$ ($R_{i,O2}^{\text{basal}}$ in mol O₂·m⁻³·s⁻¹) is calculated as

$$R_{i,O2}^{\text{basal}} = \frac{R_{i,O2}^{\text{max}}}{K_{MO2} + C_{i,O2}(x)}$$

(10)

The maximum volumetric rate of O₂ consumption ($R_{i,O2}^{\text{max}}$) is assumed to be the same in each compartment and is taken as 10 μM/s (10). The Michaelis-Menten constant ($K_{MO2}$) is taken as 5.4 μM, or 4 mmHg (10).

**O₂ Conservation Equations**

The model yields O₂ concentrations in the lumen of each type of vas rectum and tubule, and in the four interstitial regions, all along the corticomedullary axis (i.e., as a function of the axial coordinate x). Vascular and tubular walls are represented as single barriers; that is, the endothelium and epithelium are not explicitly modeled as separate compartments. The steady-state conservation equation for O₂ in the lumen of tubule $i$ is written as

$$\frac{\partial [F_{i,v}(x)C_{i,O2}(x)]}{\partial x} = J_{i,O2}(x) - [\theta_{i,\text{basal}}R_{i,O2}^{\text{basal}}(x) + \theta_{i,\text{active}}R_{i,O2}^{\text{active}}(x)]A_{i,\text{cell}}(x)$$

(11)

where $F_{i,v}$ is volumetric flow rate, and $J_{i,O2}$ denotes the transmural flux of O₂ into tubule $i$. Since we do not explicitly distinguish solute concentrations in the epithelial layer surrounding the lumen of tubule $i$, we assume that a fraction $\theta_{i,\text{basal}}$ (or $\theta_{i,\text{active}}$) of the amount of O₂ consumed for basal (or active) metabolism in that layer is taken from the lumen, with the rest [that is, $(1 - \theta_{i,\text{basal}})$ or $(1 - \theta_{i,\text{active}})$] being taken from the surrounding interstitial region. The fraction $\theta_{i,\text{basal}}$ is taken as one-half whereas the fraction $\theta_{i,\text{active}}$ is proportional to the relative PO₂ in the lumen, that is

$$\theta_{i,\text{active}} = C_{i,O2}(x) / \left[ C_{i,O2}(x) + \sum R C_{R,O2}(x) \right]$$

(12)

where $C_{R,O2}$ is the interstitial CO₂ in region $R$. Equation 11 represents O₂ conservation in the tubular lumen. In the vascular lumen, we distinguish between the plasma and the RBC compartments (Fig. 1C) and therefore write two separate O₂ conservation equations, one for plasma and one for RBCs

$$\frac{\partial [F_{i,v}(x)C_{i,O2}(x)]}{\partial x} = J_{i,O2}(x) - \theta_{i,\text{basal}}R_{i,O2}^{\text{basal}}(x)A_{i,\text{cell}}(x)$$

(13)

$$\frac{\partial [F_{i,v}(x)C_{i,O2}(x)]}{\partial x} = J_{i,O2}(x) - R_{i,O2}^{\text{RBC}}A_{i}^{R}(x)$$

(14)

where $F_{i,v}$ and $F_{i,v}^{R}$, respectively, denote the plasma and RBC water flow rate in vessel $i$, and $J_{i,O2}$ and $J_{i,O2}^{R}$, respectively, denote the net O₂ flux entering the plasma and RBC compartments of vessel $i$. The cross-sectional area of the RBC compartment ($A_{i}^{R}$) is calculated based on the cross-sectional area of the lumen of $i$ ($A_{i}$) and the hematocrit

$$A_{i}^{R} = A_{i} \left( \frac{F_{i}^{R}}{F_{i}^{P} + F_{i}^{R}} \right)$$

(15)

Note that when Eqs. 13 and 14 are applied specifically to short vas recta (i.e., DVR and AVR that terminate or originate within the OM), the number of which changes along the corticomedullary axis, variables must be expressed as a function of both $x$ and $y$, where $x$ denotes the medullary depth at which the flow or flux is evaluated, and $y$ denotes the medullary depth reached by the terminus of the short vas rectum being considered ($0 \leq x \leq y \leq L$).

Other model equations, together with boundary conditions, can be found in the APPENDIX of Ref. 10.

**Parameter Values**

Parameters related to O₂ transport are summarized in Table 1. All other values can be found in our previous study (10). The OM-to-systemic hematocrit ratio has been estimated as ~ 90–95% (35). Based on a number of whole blood hematocrit measurements in rats (30, 38, 46, 47), OM hematocrit is taken as 45%.

To determine the total RBC water and solute fluxes in the capillaries that traverse in the direction perpendicular to the corticomedullary axis, we assume that the average radial distance of the capillary path from region $R_i$ to region $R_j$ is given by the distance between the midpoints of $R_i$ and $R_j$. The midpoint of $R_1$ is the midpoint between

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-saturation in Hill equation, $P_50$ (pH = 7.4)</td>
<td>26.4 mmHg</td>
</tr>
<tr>
<td>Bohr coefficient, BC</td>
<td>-0.48</td>
</tr>
<tr>
<td>Hill equation parameter, $n$</td>
<td>2.6</td>
</tr>
<tr>
<td>Dissociation constant $k_1$</td>
<td>49 s⁻¹</td>
</tr>
<tr>
<td>Oxygen solubility coefficient in plasma</td>
<td>1.34 μM/mmHg</td>
</tr>
<tr>
<td>Oxygen solubility coefficient in RBC</td>
<td>1.56 μM/mmHg</td>
</tr>
<tr>
<td>Maximum volumetric rate of basal O₂ consumption, $R_{i,O2}^{\text{basal}}$</td>
<td>10 μM/s</td>
</tr>
<tr>
<td>Michaelis-Menten constant for basal O₂ consumption, $K_{MO2}$</td>
<td>4 mmHg</td>
</tr>
<tr>
<td>Maximum rate of Na⁺ active transport across mTAL, $V_{\text{max,x,Na}}$</td>
<td>Outer stripe: 10.5 nmol Na⁺·cm⁻²·s⁻¹</td>
</tr>
<tr>
<td></td>
<td>Inner stripe: 25.9 nmol Na⁺·cm⁻²·s⁻¹</td>
</tr>
<tr>
<td>Michaelis-Menten constant for Na⁺ active transport, $K_{MO2}$</td>
<td>70 mM</td>
</tr>
<tr>
<td>Oxygen permeability of vascular and tubular walls</td>
<td>0.04 cm/s</td>
</tr>
<tr>
<td>Hematocrit at corticomedullary junction</td>
<td>0.45</td>
</tr>
</tbody>
</table>

RBC, red blood cells; mTAL, medullary thick ascending limb of Henle’s loop.
its center and perimeter. The midpoint of R2 is given by the midpoint between the perimeters of R1 and R2; the midpoints of R3 and R4 are determined analogously.

RESULTS

Base-Case Results

Using base-case parameter values, we determined the overall supply of O2 to the OM, given by the total molar flow rate per nephron of O2 and HbO2 entering descending vessels and tubules at the corticomedullary junction (i.e., \( x = 0 \)), as 50.22 pmol-min\(^{-1}\)-nephron\(^{-1}\), or \( 8.37 \times 10^{-13} \) mol-s\(^{-1}\)-nephron\(^{-1}\). Of that supply, the model predicts that 79.8% is consumed in the OM.

We solved the model equations to obtain water flow and solute concentration profiles in all tubules, vessels, and concentric regions. Figure 2 shows Po\(_2\) profiles in each class of tubules and vessels and in each region: Fig. 2, A–D, shows results for tubules and vessels associated with the vascular bundle region (R1 and R2) and the interbundle regions (R3 and R4), respectively. Tubules and vessels are assigned to the

![PO2 profiles in tubules, vasa recta, and concentric regions.](image)

Fig. 2. PO\(_2\) in tubules, vasa recta, and concentric regions. A–D: regions R1, R2, R3, and R4; tubules are assigned to the region with which they are in contact for 50% or more of their inner stripe length. E: PO\(_2\) profiles in the interstitium of the 4 regions. Vertical dotted lines mark the boundary between the OS and IS, and \( x/L \) denotes the ratio of the axial coordinate to total length of outer medulla.
region with which they are in contact for half or more of their inner stripe length. Figure 2E shows PO2 profiles in each concentric region.

As discussed further below, the PO2 profiles are qualitatively similar to those reported in our previous study (10), where detailed descriptions and explanations can be found. The main findings are highlighted below. Our model predicts substantial variations in O2 availability between the four regions. Interstitial PO2 is predicted to be low in the interbundle regions, i.e., in R3 and R4, where it hovers between 3.9 and 12.8 mmHg between the mid-inner stripe and the mid-inner stripe. Interstitial PO2 is low in these outer regions because whereas they contain most of the mTALs, which have the highest O2 requirements, they do not include any O2-supplying vessels. (In the outer stripe, half of the LAL and 1/2 of the SAL reside in R3, and the remainder of the SAL resides in R4; in the inner stripe, half of the LAL and three-quarters of the SAL reside in R3, and the remainder of the SAL resides in R4.)

The model predicts an increasing osmolality gradient along the corticomedullary axis in all regions, tubules, and vasa recta (except in the prebend segment). Figure 3 shows the fluid osmolality profiles in the collecting duct (CD) and in the interstitial regions, as a function of medullary depth. In R1, the core of the vascular bundle, osmolality increases from 317 to 596 mosmol/kgH2O, i.e., by a factor of 1.88. In R3, where the majority of the mTALs are located, osmolality increases from 327 to 666 mosmol/kgH2O, i.e., by a factor of 2.03. Indeed, the model, consistent with well-established evidence (41), indicates that the axial osmolality gradient of the OM is generated and maintained by active, outwardly directed transmural transport of Na+ from thick segments. As interstitial fluid osmolality increases along the OM, water is absorbed from the CD; CD tubular water flow rate decreases by 51%, from 6.1 to 3.0 nl/min. As a result, CD tubular fluid osmolality increases from 309 to 650 mosmol/kgH2O along the OM, i.e., by a factor of 2.10.

Energy Efficiency of mTAL Cells

In our previous study, the number of Na+ moles reabsorbed per mole O2 consumed (also known as the TNa/O2 ratio) was taken as 24 for the base case, 50% higher than the value suggested by Na+-K+-ATPase stoichiometry under maximal efficiency, so as to account for paracellular transport and contributions of ions and transporters not represented in the model (10). In the present study, the ratio of paracellular to transcellular Na+ transport is not set a priori. Under maximal efficiency, the consumption of 1 mol of O2 yields 6 mol of ATP, the hydrolysis of which drives the extrusion of 6 × 3 = 18 mol of Na+ across basolateral Na+-K+-ATPase pumps. Hence, the number of moles of Na+ actively reabsorbed through the transcellular pathway per mole of O2 consumed for active transport is taken as 18 in our simulations. Paracellular Na+ transport, which is driven by the transmural electrochemical potential difference (Eq. 5), is considered separately. The mTAL tight junction is assumed to be cation selective, so that the lumen-positive transepithelial electrical potential (denoted $V_{te}$ below) favors Na+ reabsorption from the mTAL lumen. However, when interstitial Na+ concentration exceeds mTAL luminal Na+ concentration, as occurs in the OM, the transepithelial concentration gradient acts in the opposite direction; namely, it favors Na+ secretion into the mTAL lumen across the paracellular pathway. The net paracellular flux of Na+ across the mTAL is zero when the chemical and electrical contributions to the electrochemical potential exactly balance each other. From Eq. 5, this happens when

$$\frac{C_{R,Na}}{C_{mTAL,Na}} = \exp(-\xi_{mTAL,Na}) = \exp\left(-\frac{-\Delta V_{R,Na}}{RT}\right) = \exp\left(-\frac{-\Delta V_{mTAL}}{25.7}\right) \quad (16)$$

where $\Delta V_{R,Na}$ (in mV) is the electrical potential difference between interstitial region R and the mTAL lumen, that is, the opposite of $V_{te}$. If the interstitial-to-lumen Na+ concentration ratio is below the limiting value calculated in Eq. 16, Na+ is passively reabsorbed across the paracellular pathway; however, if the ratio exceeds that limiting value, the electrochemical gradient favors back-leak into the lumen.

We calculated $V_{te}$ based upon charge balance in the open-circuit epithelium (see the APPENDIX). Baseline values of $V_{te}$ range between 18.0 and 25.2 mV in the outer stripe, and between 4.1 and 25.1 mV in the inner stripe, as illustrated in Fig. 4. The $V_{te}$ values we predict under in vivo conditions are generally higher than in vitro estimates, which range between 3 and 10 mV (21, 37), but are similar to other modeling predictions (44).

To facilitate the analysis of the TNa/O2 ratio predicted by the model, we first examined the Na+ concentration profiles and the interstitium-to-lumen Na+ concentration ratios for the mTALs. Predicted Na+ concentration profiles are shown in Fig. 5A for the two populations of mTALs (SAL and LAL) and their surrounding interstitium (R2, R3, and R4). For simplicity, results are shown and discussed below only for SALa, which is the TAL associated with the short loop of Henle that has a prebend segment (27); results for SALb, the TAL associated with the short loop of Henle that does not include a prebend segment (27), are qualitatively similar. In both the SAL and LAL, luminal Na+ concentration progressively decreases toward the corticomedullary boundary, as the mTAL cells vigorously pump Na+ out of the lumen. Near the OM-IM boundary, the LAL Na+ concentration is predicted to be higher than
that of SAL (297 mM in LAL and 243 mM in SAL at the OM-IM boundary). However, the water flow rate is lower in the LAL (5.3 nl/min in LAL, 6.7 nl/min in SAL), which results in a lower Na\textsuperscript{+} flow rate (1,574 pmol/min in LAL, 1,628 pmol/min in SAL). Consequently, Na\textsuperscript{+} concentration decreases faster toward the corticomedullary boundary in the LAL than in the SAL, and near the inner-outer stripe boundary, the SAL Na\textsuperscript{+} concentration eventually exceeds that of the LAL.

From these Na\textsuperscript{+} concentration profiles, we computed interstitial-to-lumen Na\textsuperscript{+} concentration ratios for the SAL and LAL as a function of medullary depth, as shown in Fig. 5B. Using Eq. 16, we also calculated the limiting Na\textsuperscript{+} concentration ratios at which the chemical and electrical contributions to the electrochemical potential balance each other and the paracellular flux is zero. Throughout the OM, the interstitial-to-lumen Na\textsuperscript{+} concentration ratio for the LAL is below that limiting value; that is, the paracellular flux drives Na\textsuperscript{+} reabsorption. The same is true for the SAL, except near the OM-IM boundary, where \( V_{te} \) is sufficiently low that the inward-directed concentration gradient exceeds the outward-directed electrical potential gradient, and the paracellular flux drives Na\textsuperscript{+} secretion into the SAL lumen.

Based on the mTAL transcellular and paracellular Na\textsuperscript{+} fluxes predicted by the model, we computed an “effective” \( \frac{T_{Na}/Q_{O2}}{\text{ratio}} \), defined as the net number of Na\textsuperscript{+} moles reabsorbed (through transcellular and paracellular pathways) per mole of \( O_2 \) consumed for active transport, averaged over the mTAL length. (Recall that the number of Na\textsuperscript{+} moles actively reabsorbed, that is, across the transcellular pathway, per mole of \( O_2 \) consumed is set to 18, according to Na\textsuperscript{+}-K\textsuperscript{+}-ATPase stoichiometry.) As expected from the net direction of the paracellular flux, the effective \( \frac{T_{Na}/Q_{O2}}{\text{ratio}} \) is >18: it is predicted as 20.6 for LAL and 20.3 for SAL. However, the effective \( T_{Na}/Q_{O2} \) ratios remain close to 18 because paracellular fluxes are small relative to transcellular fluxes.

Effects of \( V_{te} \) on Energy Efficiency

As described in the APPENDIX, the mTAL lumen-positive, transepithelial potential difference is calculated based on charge conservation in the open-circuit epithelium. Specifically, \( V_{te} \) is a function of the total-to-Na\textsuperscript{+} transcellular current ratio, the absolute value of which is denoted \( \alpha \). If the transcellular flux of K\textsuperscript{+} were zero, and if the Cl\textsuperscript{−}:Na\textsuperscript{+} transcellular flux ratio was equal to 2.0, \( \alpha \) would be equal to 1. Based on a recent model of ionic transport across the mTAL (44, 45), in the base case we assumed that \( \alpha \) equals 0.65 in the outer stripe and 0.35 in the inner stripe (see the APPENDIX). To examine the impact of \( V_{te} \) on model results, we varied the value of the parameter \( \alpha \).

If \( \alpha \) is taken as 0.35 throughout the OM, the predicted \( V_{te} \) ranges from 4.1 to 25.4 mV, with an average value of 14.2 mV (the average was computed over length and over LAL and SAL). The predicted \( \frac{C_{R,Na}}{C_{mTAL,Na}} \) ratios for LAL and SAL, and the corresponding limiting ratios, are shown in Fig. 6. Under those conditions, the model predicts that paracellular fluxes are lower than in the base case (compare the difference between \( \frac{C_{R,Na}}{C_{mTAL,Na}} \) and the limiting ratio), and the effective \( \frac{T_{Na}/Q_{O2}}{\text{ratio}} \) is calculated as 20.2 for LAL and 19.4 for SAL.

If \( \alpha \) is taken as 0.65 throughout the OM, the predicted \( V_{te} \) ranges from 5.3 to 41.5 mV, with an average value of 23.1 mV. The \( \frac{C_{R,Na}}{C_{mTAL,Na}} \) ratios, shown in Fig. 6, and the paracellular fluxes are predicted to be higher than in the base case, and
the effective \( T_{Na}/QO_2 \) ratio is calculated as 23.0 for LAL and 22.2 for SAL. If \( \alpha \) is further increased and taken as 1.0 throughout the OM, the predicted \( V_e \) varies between 6.5 and 58.1 mV, with an average value of 32.4 mV. Under these conditions, the effective \( T_{Na}/QO_2 \) ratio is calculated as 26.1 for LAL and 25.3 for SAL.

Effects of pH on mTAL Na\(^+\) Active Transport

An increase in blood CO2 level or a decrease in pH reduces the binding affinity of Hb for O2, a phenomenon known as the Bohr effect. In the base case, we assumed that within the vascular bundles blood has a neutral pH; i.e., blood pH = 7.4 in R1 and R2. In the interbundle regions, we assumed a lower blood pH. Compared with other tubules, mTALs have a greater CO2 production rate because of their higher metabolism. A fraction of that CO2 is then converted to H\(^+\) and HCO\(_3^-\), thereby lowering the pH of the surrounding fluid. We also assumed that the HCO\(_3^-\) concentration in the extrabundle AVR is lower than that in the intrabundle vasa recta. Given these assumptions, in the base case the blood pH in R3 and R4 was set to 7.2.

In the first set of parameter studies, we sought to assess the effects of blood pH on O\(_2\) distribution, mTAL Na\(^+\) active transport, and the concentrating capability of the model OM. We first fixed the pH in R1 and R2 to 7.4, and varied the pH in R3 and R4, denoted \( \text{pHR}_{\text{R3, R4}} \). The effects on mTAL Na\(^+\) transport are summarized in Fig. 7A (case 1), which shows the total amount of Na\(^+\) reabsorbed (active and passive) along the LAL and SAL as a function of \( \text{pHR}_{\text{R3, R4}} \). Figure 7B (case 1) displays CD tubular fluid osmolality at the OM-IM boundary, a key measure of the concentrating effect, as a function of \( \text{pHR}_{\text{R3, R4}} \). As illustrated, mTAL Na\(^+\) transport and the concentrating capability of the model OM increase as the blood in the interbundle region becomes more acidic. When the pH of the interbundle regions is lowered, the affinity of Hb for O2 decreases, and more O2 is released from capillary RBCs, thereby raising the PO2 of the interstitial fluid in R3 and R4. For \( \text{pHR}_{\text{R3, R4}} = 6.5 \), PO2 in R3 and R4 is 4.2 and 11.2 mmHg at the mid-inner stripe (x = 0.65L, where L is the length of the OM), higher than the base-case values of 3.9 and 9.6 mmHg; for \( \text{pHR}_{\text{R3, R4}} = 7.5 \), PO2 in R3 and R4 is 3.7 and 7.9 mmHg at the mid-inner stripe. As a result, an acidic interbundle environment yields more vigorous mTAL Na\(^+\) active transport, particularly in the deep inner stripe where O\(_2\) is limited. Enhanced Na\(^+\) reabsorption raises the fluid osmolality of the interbundle.

**Fig. 6.** Interstitial-to-mTAL lumen Na\(^+\) concentration ratios for 2 different values of \( \alpha \): 0.35 and 0.65. The parameter \( \alpha \) represents the absolute value of the total-to-Na\(^+\) transcellular current ratio. Solid lines, predicted interstitial-to-mTAL lumen Na\(^+\) concentration ratios; dashed lines, critical ratio values, at which net paracellular Na\(^+\) fluxes are zero. A: SAL. B: LAL. In these simulations, \( \alpha \) is taken to be constant throughout the OM, whereas in the base case, \( \alpha = 0.65 \) in the outer stripe and 0.35 in the inner stripe. The lumen-positive \( V_e \) across mTAL increases with increasing \( \alpha \).

**Fig. 7.** Effects of blood pH on total mTAL Na\(^+\) reabsorption (A) and CD tubular fluid osmolality at the OM-IM boundary (B). In case 1, variations in blood pH in R3 and R4 are shown; blood pH in R1 and R2 is fixed at 7.4. The concentrating capability of the OM increases as blood in the interbundle region becomes more acidic, thereby facilitating O2 dissociation from oxyhemoglobin near the mTALs. In case 2, variations in blood pH in R1 and R2 are shown; blood pH in R3 and R4 is fixed at 7.2. The concentrating capability of the OM decreases as blood in the vascular bundle region becomes more acidic, thereby reducing O2 delivery to the interbundle region.
interstitium, increases water reabsorption from the CD, and raises CD tubular fluid osmolality. The CD tubular fluid osmolality is predicted to be 695 mosmol/kgH₂O at the OM-IM for pH₃,₄ = 6.5. 6.9% higher than the base-case value of 650 mosmol/kgH₂O; conversely, a lower value of 615 mosmol/kgH₂O is obtained for pH₃,₄ = 7.5.

In the next set of parameter studies, we fixed the pH in R3 and R4 to the base-case value of 7.2 and varied the pH in R1 and R2, denoted pH₁,₂. As shown in Fig. 7 (case 2), mTAL Na⁺ reabsorption and the concentrating capability of the model OM decrease as blood in the vascular bundle region becomes more acidic. When the pH at the core and immediate periphery of the vascular bundle is lowered, more O₂ is released from capillary RBCs in those central regions, thereby decreasing the amount of O₂ that is delivered to the interbundle regions to support the O₂ requirements of the mTALs. Thus, as pH₁,₂ decreases from 7.5 to 6.5, CD tubular osmolality at the OM-IM boundary decreases from 655 to 566 mosmol/kgH₂O.

In another set of simulations, we assumed a uniform pH throughout the entire OM. The total amount of mTAL Na⁺ reabsorbed and the concentrating capability of the model, summarized by the CD tubular fluid osmolality at the OM-IM boundary, are shown in Fig. 8 as a function of blood pH. As blood pH increases from 6.5 to 7.0, the dissociation of HbO₂ within the vascular bundle is progressively limited and more O₂ is delivered to the interbundle regions where its demand is highest. As a result, the concentrating capability of the model OM increases, with the CD tubular fluid osmolality at the OM-IM boundary increasing from 593 to 637 mosmol/kgH₂O. However, further increases in blood pH reduce the amount of O₂ released by the RBC to the interbundle regions, thereby lowering the interstitial PO₂ and reducing mTAL Na⁺ active transport. Consequently, the CD tubular fluid osmolality at the OM-IM boundary decreases to 616 mosmol/kgH₂O at pH = 7.5.

These results suggest that an acidic interbundle environment, where the mTALs are located, together with pH-neutral vascular bundles, facilitates the delivery of O₂ to support the high metabolic demands of the mTALs and raises the concentrating capability of the OM.

**Effects of O₂ Availability on mTAL Na⁺ Active Transport**

The extent to which ATP production by anaerobic glycolysis in rat mTAL cells can maintain a substantial fraction of tubular function remains uncertain (see the DISCUSSION). The baseline model configuration assumes that the contribution of anaerobic metabolism in mTAL cells is negligible. In the deep OM, the average PO₂ in the mTAL lumen and in the surrounding interstitium falls below the critical PO₂, i.e., the extracellular PO₂ at which O₂ utilization becomes limited. As a result, the mTAL Na⁺ active transport rate is assumed to decrease (Eq. 8). Reported Pc values range from 1 to 20 mmHg (3, 12, 14, 33). Our baseline value was chosen as 5 mmHg.

Given the wide range of reported Pc values, we examined the effect of the critical PO₂ on medullary Na⁺ reabsorption and the concentrating capability of the model OM. The results, summarized as the total amount of mTAL Na⁺ reabsorbed and the CD tubular fluid osmolality at the OM-IM boundary, are shown in Fig. 9. As the critical PO₂ varies from 1 to 20 mmHg, we observed an increase in the length of the mTAL segments along which the surrounding PO₂ is sufficiently low, relative to Pc, that Na⁺ active transport rate is reduced. As a result, the total amount of Na⁺ reabsorbed along the mTAL decreases by 18.6%, from 281 to 229 pmol-min⁻¹-nephron⁻¹. The interstitial fluid Na⁺ concentration and osmolality consequently decrease, less water is reabsorbed from the OMCD, and CD osmolality decreases by 15%, from 674 to 573 mosmol/kgH₂O.

In the next set of simulations, we sought to quantify the degree to which anaerobic metabolism could increase the concentrating capability of the model OM. To achieve that goal, we assumed that as the averaged (interstitial and mTAL lumen) PO₂ decreases between 5 and 0 mmHg, anaerobic metabolism supplies an increasingly larger fraction of the energy needed to actively transport Na⁺ across mTALs, so that in the absence of any O₂ supply, anaerobic metabolism produces enough ATP to sustain an active Na⁺ transport that is a fraction a (where 0 ≤ a ≤ 1) of the maximum rate when O₂ supply is abundant. With this assumption, Eq. 8 becomes

\[
V_{\text{max,mTAL,Na}} = \begin{cases} 
V_{\text{max,mTAL,Na}}^{\text{a}} & \text{if } P_{O_2} \geq P_c \\
V_{\text{max,mTAL,Na}}^{\text{a}} \left[ \frac{P_{O_2}}{P_c} + a \left( 1 - \frac{P_{O_2}}{P_c} \right) \right] & \text{if } P_{O_2} < P_c
\end{cases}
\]

(17)
In the base case, which assumes negligible anaerobic metabolism, \( a = 0 \); when \( a = 1 \), the model simulates the idealized case in which mTAL Na\(^+\) active transport rate is independent of O\(_2\) supply.

Figure 10 shows the total amount of mTAL Na\(^+\) reabsorbed and CD tubular fluid osmolality at the OM-IM boundary as a function of the critical PO\(_2\) (\( P_c \)), below which O\(_2\) consumption becomes limited by O\(_2\) availability (Eq. 8).

In the base case, which assumes negligible anaerobic metabolism, \( a = 0 \); when \( a = 1 \), the model simulates the idealized case in which mTAL Na\(^+\) active transport rate is independent of O\(_2\) supply.

Figure 10 shows the total amount of mTAL Na\(^+\) reabsorbed and CD tubular fluid osmolality at the OM-IM boundary as a function of the anaerobic metabolism contribution, given by the coefficient \( a \) (Eq. 17). In the base case, which assumes negligible anaerobic metabolism, \( a = 0 \); in the idealized case, in which mTAL Na\(^+\) active transport rate is taken to be independent of O\(_2\) supply, \( a = 1 \).

Effects of Vascular and Tubular Flows on O\(_2\) Distribution in the OM

The load dependence of tubular transport suggests that renal O\(_2\) consumption may vary in parallel with the GFR. As discussed by Evans et al. (14), this constitutes an intrinsic regulatory system for maintaining homeostasis of intrarenal oxygenation. However, medullary blood flow (MBF) and GFR do not always vary in the same proportion, and changes in medullary O\(_2\) supply and O\(_2\) consumption are not necessarily commensurate. In the following set of studies, we investigated the synergy between MBF, GFR, and OM distribution of O\(_2\).

To that end, we simulated variations in MBF and GFR by varying the flow entering DVR (\( Q_{DVR} \)) and that entering descending limbs and CDs (\( Q_{DL,CD} \)), respectively.

In all simulations, the fraction of O\(_2\) supplied to the OM that is consumed in the IM was taken as 5% (10), the base-case value. This percentage was not varied even in the simulations where DVR, DL and CD flows were altered; without an explicit representation of the IM, it is difficult to predict how that fraction would vary. However, in a previous study, we found that the effect of IM boundary conditions on model predictions is minimal (28).

Figure 11 displays the O\(_2\) consumption-to-supply ratio obtained for the cases where \( Q_{DVR} \) and \( Q_{DL,CD} \) were simultaneously varied by \( \pm 5\% \), \( \pm 10\% \), \( \pm 15\% \), \( \pm 20\% \), and \( \pm 25\% \). These results indicate that the O\(_2\) consumption-to-supply ratio decreases at higher O\(_2\) supply.

When both \( Q_{DVR} \) and \( Q_{DL,CD} \) increase by 25%, O\(_2\) is more plentiful throughout the medulla. At the junction between the inner stripe, where anaerobic metabolism allows mTAL Na\(^+\) active transport rate to occur at a significantly higher rate.

**Effects of Vascular and Tubular Flows on O\(_2\)**

**Distribution in the OM**

The load dependence of tubular transport suggests that renal O\(_2\) consumption may vary in parallel with the GFR. As discussed by Evans et al. (14), this constitutes an intrinsic regulatory system for maintaining homeostasis of intrarenal oxygenation. However, medullary blood flow (MBF) and GFR do not always vary in the same proportion, and changes in medullary O\(_2\) supply and O\(_2\) consumption are not necessarily commensurate. In the following set of studies, we investigated the synergy between MBF, GFR, and OM distribution of O\(_2\).

To that end, we simulated variations in MBF and GFR by varying the flow entering DVR (\( Q_{DVR} \)) and that entering descending limbs and CDs (\( Q_{DL,CD} \)), respectively.

In all simulations, the fraction of O\(_2\) supplied to the OM that is consumed in the IM was taken as 5% (10), the base-case value. This percentage was not varied even in the simulations where DVR, DL and CD flows were altered; without an explicit representation of the IM, it is difficult to predict how that fraction would vary. However, in a previous study, we found that the effect of IM boundary conditions on model predictions is minimal (28).

Figure 11 displays the O\(_2\) consumption-to-supply ratio obtained for the cases where \( Q_{DVR} \) and \( Q_{DL,CD} \) were simultaneously varied by \( \pm 5\% \), \( \pm 10\% \), \( \pm 15\% \), \( \pm 20\% \), and \( \pm 25\% \). These results indicate that the O\(_2\) consumption-to-supply ratio decreases at higher O\(_2\) supply.

When both \( Q_{DVR} \) and \( Q_{DL,CD} \) increase by 25%, O\(_2\) is more plentiful throughout the medulla. At the junction between the inner stripe, where anaerobic metabolism allows mTAL Na\(^+\) active transport rate to occur at a significantly higher rate.
outer and inner stripes, PO2 is 3.9 mmHg (24.9%) higher in R2 relative to the base case, 3.6 mmHg (79.5%) higher in R3, and 5.8 mmHg (53.3%) higher in R4. At the mid-inner stripe, PO2 is 1.0 mmHg (12.0%) higher in R2 relative to the base-case, 1.4 mmHg (36.8%) higher in R3, and 2.0 mmHg (21.0%) higher in R4 (Fig. 12). The MBF-induced PO2 elevation, especially in the interbundle region, increases ATP production and therefore the maximum rate of active Na+ transport ($V_{\text{max,mTAL,Na}}$; see Eq. 8), which in turn raises O2 consumption. However, the impact of PO2 on $V_{\text{max,mTAL,Na}}$ is small in the outer stripe, where PO2 is generally above the critical, rate-limiting value. For its part, the GFR-induced increase in mTAL luminal Na+ flow rate slows the rate at which Na+ concentration decreases along the mTAL flow direction; in turn, the higher Na+ concentrations are expected to increase carrier occupancy (see Eq. 7b), thereby enhancing the Na+ active transport rate and O2 consumption. The latter effect is nevertheless minimal, because in the OM the mTAL luminal Na+ concentration is much larger than the Michaelis constant ($K_{M,Na} = 70$ mM), which is the concentration at which the transport rate is exactly half of $V_{\text{max,mTAL,Na}}$. Since the mTAL Na+ active transport rate is largely insensitive to luminal concentration, the impact of GFR on carrier saturation is small. Overall, the model predicts that, because the increase in mTAL active Na+ transport is not proportionally to the increase in O2 supply, the medullary O2 consumption-to-supply ratio is reduced from 79.8 to 72.7%.

Conversely, as $Q_{\text{DVR}}$ and $Q_{\text{DL/CD}}$ decrease in tandem, the OM O2 consumption-to-supply ratio increases slightly until it reaches a plateau at 81.3%, which is only 1.9% higher than the base-case ratio (Fig. 11). Indeed, at sufficiently low O2 supply, mTAL active Na+ transport is reduced along much of the OM; therefore, an increase in O2 supply that raises interstitial PO2 and mTAL luminal PO2 significantly enhances O2 consumption and mTAL active Na+ transport throughout the OM. Thus, at sufficiently low O2 supply, O2 supply and consumption scale approximately proportionally.

In the context of the concentrating mechanism, increasing MBF and GFR gives rise to several competing effects. First, as previously noted, higher MBF increases medullary PO2, which, taken in isolation, increases Na+ active transport in the deep OM. Also, higher GFR increases Na+ flow along the mTAL, and the resulting higher mTAL tubular Na+ concentrations also act to increase Na+ reabsorption. However, the latter effect is minimal as described above. Additionally, higher MBF and GFR, simulated in the model by increases in descending limb, CD, and DVR flow rates at the corticomedullary boundary, present a larger load to the concentrating mechanism. (Here, “load” is taken to mean a descending tubular or vascular fluid flow that must be concentrated by the concentrating mechanism.) The load effect appears to be dominant and yields a decrease in the concentrating effect: as MBF and GFR are both increased by 25%, CD osmolality at the OM-IM junction drops from 650 mosmol/kgH2O (in the base case) to 604. Conversely, if both $Q_{\text{DVR}}$ and $Q_{\text{DL/CD}}$ decrease by 25%, the competing effects (i.e., lower O2 availability and decreased concentrating load) approximately balance each other, resulting in a small increase in CD osmolality to 659 mosmol/kgH2O.

In the next set of simulations, we considered the scenario in which $Q_{\text{DVR}}$ and $Q_{\text{DL/CD}}$ do not vary simultaneously. As reviewed by Navar et al. (32), both vasoconstrictors and vasodilators alter the filtration fraction. Intravenous administration of the nitric oxide synthase inhibitor L-NAME was found to increase the filtration fraction by up to 45% in anesthetized rats (26). Infusion of ANG II in the renal artery of dogs on normal and high-sodium diets raised the filtration fraction by 17 and 33%, respectively (40). Conversely, the ANG II antagonist DuP 753 (losartan) decreased the filtration fraction by 10% in anesthetized rats (15). Hence, vasoactive agents most likely also alter the $Q_{\text{DVR}}$-to-$Q_{\text{DL/CD}}$ ratio.

Accordingly, we performed simulations in which $Q_{\text{DVR}}$, and therefore medullary O2 supply, was varied by $\pm 5$, $\pm 10$, $\pm 15$, and $\pm 25$, whereas $Q_{\text{DL/CD}}$, remained constant. The model predicts O2 consumption-to-supply ratios that are hardly distinguishable from the ratios shown in Fig. 11, which were obtained for simultaneous variations in $Q_{\text{DVR}}$ and $Q_{\text{DL/CD}}$. That is, the O2 consumption-to-supply ratio is primarily determined by $Q_{\text{DVR}}$. In particular, the O2 consumption-to-supply ratio decreases at higher O2 supply. Consider the case where $Q_{\text{DVR}}$ is increased by 25%. At the mid-outside stripe, PO2 is 4.1 mmHg higher in R2 relative to the base case, 4.9 mmHg higher in R3, and 6.4 mmHg higher in R4; at the mid-inner stripe, those differences are 1.0, 1.4, and 2.0 mmHg, respectively. Given that in the base case, PO2 is above its critical, rate-limiting...
value in most of the OM, the PO₂ increase enhances active Na⁺ reabsorption in the deep OM only (see above), and the O₂ consumption-to-supply ratio decreases to 72.4%. In this case, however, Na⁺ reabsorption increases relative to the Na⁺ tubular load (which is fixed), and the urinary concentrating capability of the OM improves: CD osmolality at the OM-IM junction increases from 650 to 670 mosmol/kgH₂O. Conversely, if \( Q_{\text{OVR}} \) decreases by 25% and \( Q_{\text{DLUO}} \) remains constant, the O₂ consumption-to-supply ratio increases slightly from the base-case value of 79.8 to 81.3%, and the OM concentrating capability is significantly impaired: CD osmolality at the OM-IM junction drops from 650 to 559 mosmol/kgH₂O.

**DISCUSSION**

We have extended a mathematical model of O₂ transport in the OM of the rat kidney, which we recently developed (10), to include an explicit representation of paracellular Na⁺ transport across the mTAL walls that is driven by electrical potential and concentration gradients. The model was used to determine the relative contribution of transcellular and paracellular pathways to Na⁺ reabsorption, to assess the effect of blood pH on O₂ distribution and mTAL Na⁺ transport, and to examine the effects of tubular and vascular load on O₂ consumption in the OM.

**Comparison with Previous Study**

The present study is based on our previous model of O₂ transport in the rat OM (10). To examine the effects of pH on O₂ transport and mTAL Na⁺ reabsorption, the current model includes the pH dependence of the O₂ dissociation curve (see Eq. 4), and the explicit consideration of Na⁺ paracellular fluxes across the mTAL (see Eqs. 5 and 6). Other parameter values that differ between the two models were discussed in **MODEL DESCRIPTION**. The predicted PO₂ profiles of the two models are qualitatively similar (compare Fig. 2 in the current study and Fig. 3 in Ref. 10). One notable difference is that along most of the inner stripe, the predicted PO₂ in R4 is substantially higher (~50% higher at the mid-inner stripe) in the present model than in the previous model. This difference may be attributed to the higher acidity assumed in the present model, which increases the dissociation rate of HbO₂. Another notable difference is that the current model predicts steeper gradients in the Na⁺ concentration profiles (compare Fig. 5A in the current study and Fig. 5B in Ref. 10). The more rapid decrease in mTAL Na⁺ concentration in the present model can be attributed to the higher mTAL Na⁺ reabsorption rate, given the higher interbundle PO₂. The higher mTAL Na⁺ reabsorption in turn increases the osmolality gradient along the CD, the fluid osmolality of which is predicted as 650 mosmol/kgH₂O at the OM-IM boundary, compared with 580 in the previous model (10).

**Energy Efficiency of mTAL Cells**

The number of Na⁺ mol reabsorbed per mole of O₂ consumed, also known as the \( T_{\text{Na}}/Q_{\text{O}_2} \) ratio, has been estimated as 36:1 in the mTAL (17). This estimate is based on a model of NaCl transport across the TAL which posits that for each Na⁺ ion actively transported through the cell, one Na⁺ is transported through the paracellular pathway without additional energy expenditure (37). This model has been challenged by Kiil and Sejersted (21), who noted that under in vitro conditions, peritubular and luminal Na⁺ concentrations are typically equal, whereas in vivo, the interstitial-to-lumen Na⁺ concentration ratio is generally much higher than 1. Thus, in vivo, the paracellular (or passive) Na⁺ flux across mTAL is expected to be small and to sometimes favor back-leak of Na⁺ into the lumen.

Our results support these predictions: the model suggests that paracellular fluxes are small relative to transcellular fluxes and may drive Na⁺ secretion across SAL in the deep OM. Since fluid flows, and therefore interstitial-to-lumen Na⁺ concentration ratios, differ significantly between LAL and SAL, electro-diffusive forces across LAL and SAL also vary significantly. Overall, our model predicts that paracellular transport favors net Na⁺ reabsorption across LAL and most of the SAL. The effective \( T_{\text{Na}}/Q_{\text{O}_2} \) ratios are predicted to be 20.6 for LAL and 20.3 for SAL.

The applicability of the paracellular flux computations and \( T_{\text{Na}}/Q_{\text{O}_2} \) predictions is somewhat limited by the lack of an explicit representation of Cl⁻ and K⁺ transport. To predict the mTAL transmural electrical potential gradient, we had to assume the fractions of the transcellular current that are carried by those two ions (see the **APPENDIX**). Our baseline estimates of these fractions are based on a recent model of ionic transport across the TAL (44, 45). As shown in Fig. 6, model predictions are sensitive to those fractional values. Despite this limitation, the current model is the first to represent, in a mathematical model of the urine concentrating mechanism of the rat kidney, the effect of O₂ availability on mTAL NaCl transport, and it could serve as a platform for more sophisticated models that include detailed representation of the mTAL epithelium.

**Bohr Effect**

Our representation of the HbO₂ dissociation process (Eq. 1) is approximate. Hb has four O₂ binding sites, and each O₂ molecule that is added to the Hb molecule modifies its binding affinity and reaction rate. A more complex mathematical model of the O₂ dissociation curve was formulated by Adair (1), and kinetic studies have sought to quantify how its parameters vary with the Bohr effect (13). Other O₂ dissociation curve equations have been formulated since (reviewed by Buerk and Bridges in Ref. 7). These alternate models generally involve many parameters and seldom allow PO₂ to be explicitly expressed as a function of saturation. The approach we used here has the advantage of being mathematically simpler, while still providing a satisfactory fit of the O₂ dissociation curve over physiological saturation ranges (18) and allowing for incorporation of the Bohr effect.

Extrapolation of vasa recta pH measurements in the IM (20, 25) suggest that blood pH may be higher in the vascular bundles than in the surrounding regions. The Bohr effect states that an increase in blood CO₂ level or a decrease in pH reduces the binding affinity of hemoglobin for O₂ and increases the availability of O₂. Our model predicts that a high intrabundle pH limits dissociation of HbO₂ within the vascular bundles and preserves O₂ delivery to the interbundle regions (see Fig. 7). Conversely, a lower pH in the interbundle regions favors the dissociation of HbO₂ therein, thereby substantially raising the local PO₂ and enhancing Na⁺ reabsorption from mTALs in the deep
OM. Thus the high interbundle P O_{2} that results from this radial pH gradient is predicted to increase the concentrating capability of the model OM.

**O_{2} Homeostasis**

As reviewed by Evans et al. (14), until recently the homeostasis of intrarenal oxygenation was thought to depend exclusively on the balance between O_{2} delivery (i.e., renal perfusion) and O_{2} consumption. The flow (i.e., GFR) dependence of O_{2} consumption was seen as the predominant mechanism for maintaining a constant renal P O_{2}. Evans et al. pointed out, however, that such a balance would be difficult to sustain when, for instance, the filtration fraction is altered by vasoactive agents, or when the bioavailability of nitric oxide changes. Recent evidence suggests that diffusional arterial-to-venous (AV) O_{2} shunting in the cortex, which blunts the delivery of O_{2} to the renal medulla, constitutes another significant O_{2} homeostatic mechanism and that changes in AV shunting that are linked to renal blood flow could help maintain P O_{2} when renal blood flow varies within physiological limits (14).

Our model does not explicitly represent the cortex and therefore cannot account for AV O_{2} shunting. Thus we focused our investigations on the correlation between OM O_{2} supply and consumption. What our simulations show is that, at sufficiently high medullary O_{2} supply, OM O_{2} consumption does not increase proportionally with O_{2} delivery, even when MBF and GFR vary in tandem (Fig. 11). In the deep OM, where mTAL Na^{+} reabsorption is rate limited by O_{2}, the higher interstitial P O_{2} that results from a higher MBF increases the rate of mTAL active Na^{+} transport. As a result, O_{2} consumption in the inner stripe is strongly dependent on MBF. However, in the upper OM, where O_{2} is relatively plentiful, mTAL Na^{+} active transport rate is largely insensitive to variations in O_{2} supply and luminal Na^{+} concentration. Thus O_{2} consumption in the outer stripe is largely insensitive to variations in both MBF and GFR. Our model therefore predicts that higher O_{2} and Na^{+} delivery to mTAL do not raise active O_{2} consumption and Na^{+} transport proportionally to the increases in MBF and GFR.

In contrast, higher GFR and MBF, which increase the amount of descending fluid flows that must be concentrated, have a substantially negative effect on the concentrating mechanism of the model OM, which is driven by the single effect provided by mTAL active Na^{+} reabsorption. That negative effect is, to a large extent, not compensated by the positive effect of the increased O_{2} supply. As a result, our model predicts that CD tubular fluid osmolality at the OM-IM junction, a key indicator of the concentrating capability of the model OM, varies significantly with, and in an opposite direction to, variations in tubular and vascular load.

**Anaerobic Metabolism**

Results from studies on the importance of anaerobic metabolism in mTAL differ significantly depending on the species examined. Measurements of glucose uptake and lactate formation in the rabbit kidney indicate that under anaerobic conditions, the rate of glycolysis is high in the OM; the increase in glycolysis following a reduction in P O_{2} almost compensated for the decrease in oxidative metabolism in maintaining cellular ATP levels in OM slices (29). In the mouse nephron, however, the specific contribution of anaerobic metabolism to maintaining cellular ATP was found to be low in the mTAL (43). Studies in the rat nephron showed that all distal segments produce significant amounts of lactate and that addition of antimycin A, an inhibitor of oxidative metabolism, can increase mTAL lactate production by a factor of 15 (2).

Taken together, these studies suggest that perhaps the relative contribution of anaerobic glycolysis to ATP production in the mTAL varies from species to species. It is conceivable that the rat mTAL and the rabbit mTAL may be capable of substantial ATP production by anaerobic glycolysis, whereas the mouse mTAL may not. If that is indeed the case, there would appear to be an interesting correlation between the capacity for anaerobic metabolism in the OM and the structural organization of the OM. The mouse has a complex medulla, in which the descending limbs of the short loops of Henle reach into the vascular bundle in the inner stripe. That short descending limb position allows O_{2} to diffuse from the O_{2}-rich interstitium of the vascular bundle into the descending limb tubular fluid. As that fluid enters the SAL, the P O_{2} may be sufficiently high to maintain tubular function in the absence of anaerobic metabolism. In contrast, the rabbit has a simple medulla, in which the short descending limb does not enter the vascular bundle. The position of the rabbit short descending limb suggests that its luminal P O_{2} (and later that of the SAL) may be low, relative to that in the mouse, which may render a substantial amount of glycolysis necessary.

The inner stripe position of the rat short descending limb lies, in some sense, between those of the mouse and the rabbit (23). Like the mouse, the rat has a complex medulla. However, in the rat inner stripe, the short descending limb enters only the periphery of the vascular bundle. Thus the O_{2} diffusive flux into the short descending limb may be lower in the rat than in the mouse. We speculate that the capacity for anaerobic glycolysis of the rat mTAL may lie between those of the mouse and the rabbit.

Significant ATP production from anaerobic metabolism would help to explain some experimental findings in the rat OM that has undergone hypertrophy. It has long been observed that a high-protein diet induces hypertrophy in the OM of the rat kidney (4, 31), increases the epithelial volume of mTALs, which may be accompanied by a corresponding increase in Na^{+}-K^{+}-ATPase activity (42) and active Na^{+} transport, and enhances the urine-concentrating capability (19). However, our previous modeling study suggested that, along much of the mTAL in a hypertrophied OM, luminal P O_{2} is below the level at which sufficient active Na^{+} transport can be supported solely by aerobic metabolism (9). Thus, in the absence of sufficient anaerobic metabolism to compensate for the reduction in aerobic active transport, it is not clear that the concentrating capability of a hypertrophied OM can be significantly improved. We obtained similar results using the current model (not shown) together with the assumption of no anaerobic metabolism. Thus we believe that the possibility of significant glycolysis in rat mTAL cells, especially when the cells have undergone hypertrophy, should not be dismissed.

In summary, our model suggests that in vivo paracellular Na^{+} fluxes across mTAL are small relative to transcellular Na^{+} fluxes and that paracellular fluxes favor reabsorption of Na^{+} from the lumen along most of the mTAL segments. If the interbundle region, where mTALs are located, is more acidic than the vascular...
bundtles, $O_2$ delivery to mTALs will increase, which will then raise the concentrating capability of the OM. Our simulations also suggest that $O_2$ supply and demand generally do not vary proportionally in the OM.

**APPENDIX**

**Determination of mTAL Transepithelial Voltage Difference**

Under open-circuit conditions, the sum of the total transcellular ($I_{\text{trans}}^{\text{tot}}$) and paracellular $I_{\text{para}}^{\text{tot}}$ currents across tubule $i$ ($i = \text{LAL, SAL}$) is zero, i.e., $I_{\text{trans}}^{\text{tot}} + I_{\text{para}}^{\text{tot}} = 0$. Since the paracellular current is predominantly carried by $Na^+$ and $Cl^-$ ions, we have

$$I_{\text{trans}}^{\text{tot}} + I_{\text{para}}^{\text{Cl}} = -I_{\text{trans}}^{\text{tot}} = -I_{\text{para}}^{\text{Cl}}$$

(A1)

where $I_{\text{trans}}^{\text{tot}}$ and $I_{\text{para}}^{\text{tot}}$ represent the transcellular and paracellular currents carried by ion $k$ across tubule $i$ ($i = \text{LAL, SAL}$). At the luminal membrane, $Na^+$ and $Cl^-$ enter the cell via the $Na^+-K^+-2Cl^-$ transporter NKKC2 in a 1:2 ratio. In addition, some $Na^+$ enters via the $Na^+/H^+$ exchanger NHE3. A recent model of the mTAL cell (44) suggests that the $Cl^-$-$Na^+$ transcellular flux ratio is $\sim 1.65$ in the outer stripe and $1.75$ in the inner stripe. The model also predicts that in the inner stripe, the $K^+$ transcellular flux is negligible ($\sim 40\%$ of the $Na^+$ transcellular flux). We therefore assume that the total-to-$Na^+$

trancellulausal current ratio is equal to $1 - 1.65 = 0.65$ in the outer stripe, and $1 + 0.40 - 1.75 = -0.35$ in the inner stripe. With these assumptions, Eq. A1 can be rewritten as

$$I_{\text{trans}}^{\text{tot}} + I_{\text{para}}^{\text{Cl}} = -I_{\text{trans}}^{\text{tot}} = \alpha I_{\text{trans}}^{\text{tot}} = \alpha z_{\text{Na}} F I_{\text{trans}}^{\text{tot}}$$

(A2)

where the parameter $\alpha = 0.65$ in the outer stripe, and $-0.35$ in the inner stripe. The paracellular $Na^+$ and $Cl^-$ currents across $i$ ($i = \text{LAL, SAL}$) can be expressed as

$$I_{\text{trans}}^{\text{tot}} = 2 \pi r_i \sum_k k_{i,k} F_i \Delta V_{R,i}$$

(A3)

$$I_{\text{para}}^{\text{Cl}} = 2 \pi r_i \sum_k k_{i,k} F_i \Delta V_{R,i}$$

(A4)

where

$$\xi_{i,Na} = -\xi_{i,Cl} = \frac{F}{RT} \Delta V_{R,i}$$

(A5)

For simplicity, the $x$ dependence of the variables has been omitted in Eqs. A3–A5. Since $Cl^-$ transport is not explicitly described in this model, we assume that the concentration of $Cl^-$ is equal to that of $Na^+$ everywhere. The paracellular permeability of the mTAL to $Na^+$ and $Cl^-$ is taken as 2.8 and $1.4 \times 10^{-5}$ cm/s, respectively (45). Substituting Eqs. A3–A5 into Eq. A2 yields an implicit equation for the transepithelial voltage difference across tubule $i$ ($i = \text{LAL, SAL}$), namely, $\Delta V_{R,i}$. Thus, given the transepithelial flux of $Na^+$ across $i$ at each position $x$ along the corticomedullary axis, $\Delta V_{R,i}$ is determined using Eq. A2 and the paracellular flux of $Na^+$ across $i$ is subsequently determined using Eq. A3.

**GRANTS**

This work was supported by National Institutes of Health Grant DK53775 to A. Edwards and National Science Foundation Grant DMS-0701412 to A. T. Layton.

**DISCLOSURES**

No conflicts of interest are declared by the authors.

**REFERENCES**


