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Maximum Urine Concentrating Capability in a Mathematical Model of the Inner Medulla of the Rat Kidney

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Abstract

In a mathematical model of the urine concentrating mechanism of the inner medulla of the rat kidney, a nonlinear optimization technique was used to estimate parameter sets that maximize the urine-to-plasma osmolality ratio (U/P) while maintaining the urine flow rate within a plausible physiologic range. The model, which used a central core formulation, represented loops of Henle turning at all levels of the inner medulla and a composite collecting duct (CD). The parameters varied were: water flow and urea concentration in tubular fluid entering the descending thin limbs and the composite CD at the outer-inner medullary boundary; scaling factors for the number of loops of Henle and CDs as a function of medullary depth; location and increase rate of the urea permeability profile along the CD; and a scaling factor for the maximum rate of NaCl transport from the CD. The optimization algorithm sought to maximize a quantity E that equaled U/P minus a penalty function for insufficient urine flow. Maxima of E were sought by changing parameter values in the direction in parameter space in which E increased. The algorithm attained a maximum E that increased urine osmolality and inner medullary concentrating capability by 37.5% and 80.2%, respectively, above base-case values; the corresponding urine flow rate and the concentrations of NaCl and urea were all within or near reported experimental ranges. Our results predict that urine osmolality is particularly sensitive to three parameters: the urea concentration in tubular fluid entering the CD at the outer-inner medullary boundary, the location and increase rate of the urea permeability profile along the CD, and the rate of decrease of the CD population (and thus of surface area) along the cortico-medullary axis.

Keywords

Urine concentrating mechanism; Countercurrent system; Osmoregulation; Optimization; Inverse problem; NaCl transport; Urea transport

1 Introduction

Most mammals can produce a urine that is substantially more concentrated than blood plasma (Beuchat, 1996). The excretion of such a urine, which is said to be hypertonic, allows a mammal to maintain a nearly constant blood plasma osmolality when water consumption is reduced. Hypertonic urine is produced by the urine concentrating mechanism (UCM), which is localized in the renal medulla. In most mammals, the renal medulla has two sections: an outer medulla (OM) and an inner medulla (IM). When a mammal is producing concentrated urine, an increasing osmolality gradient is maintained in all tubules and vessels along the cortico-

medullary axis of the OM by means of active NaCl transport from specialized renal tubules (viz., thick ascending limbs, TALs) and by means of a countercurrent configuration of renal tubules and blood vessels. Along the cortico-medullary axis of the IM, an additional gradient, larger than that in the OM, is generated when highly concentrated urine is needed. The IM gradient also appears to depend on a countercurrent configuration of tubules and vessels, but other details of the IM concentrating mechanism remain controversial: decades of sustained experimental and theoretical effort have failed to definitively identify the source of the IM gradient (Layton, 2002; Sands and Layton, 2007; Stephenson, 1992). However, it is generally accepted that in both the OM and the IM, water is absorbed from the collecting duct (CD) system, in excess of the absorption of solutes, thereby concentrating the CD tubular fluid, which passes from the papillary tip of the IM as hypertonic urine.

Mathematical models of the UCM typically involve a large number of parameters; these parameters are needed to characterize the morphological and transport properties of renal tubules and vessels. The values of some of these parameters exhibit substantial variability or are based on experimental techniques that introduce uncertainty; some parameters have not been measured at all. To assess the impact of parameter variability, parameters may be identified that result in model predictions that meet specified criteria. The aim of the present study was to conduct a model optimization study, for parameter values within reasonable ranges, on a published model of the UCM in the IM of the rat kidney (Layton et al., 2004).

As we have previously noted (Marcano-Velázquez and Layton, 2003; Marcano et al., 2006), one can distinguish between those model studies on the UCM that have sought to investigate sensitivity to parameters by varying one parameter at a time (e.g., Layton et al. (2000), Layton and Layton (2005b), Layton et al. (2004), and Wexler et al. (1991)), and those that have incorporated algorithms that allow multiple parameters to vary simultaneously in an attempt to optimize a measure of model performance (e.g., Breinbauer (1988), Breinbauer and Lory (1991), Kim and Tewarson (1996), Tewarson (1993b), Tewarson (1993a), and Tewarson and Marcano (1997)). In the present study, as in our studies of the avian UCM (Marcano-Velázquez and Layton, 2003; Marcano et al., 2006), we applied the latter approach, because it allows one to identify the synergistic, and perhaps nonlinear effects of interacting parameters, which an organism may be able to adjust and coordinate to meet its functional objectives. In the avian kidney, where only one solute, NaCl, is believed to play a fundamental role in the UCM, concentrating capability is modest relative to that in most mammals, and the principles of the UCM are believed to be well-understood (Layton et al., 2000). However, in the mammalian kidney, two solutes, NaCl and urea, may be essential for the UCM, and fundamental aspects of the mechanism remain to be elucidated (Sands and Layton, 2007). Therefore, parameter studies in models of the mammalian UCM are substantially more challenging.

In the present study, model performance was assessed by maximizing the ratio of urine osmolality to blood plasma osmolality (denoted by “ U/P ”) while urine flow and model parameters were maintained within plausible normal physiologic ranges. Effectiveness E was defined to equal U/P minus a penalty parameter for urine flows that deviate substantially from those measured in experiments. To maximize E , a number of distinct starting points in parameter space were randomly chosen from a uniform distribution; each point was used as a starting point for a separate run of an optimization algorithm. Each such run included multiple iterations in which the model equations were solved; the problem of finding such a solution is called the “direct problem.” Each successive iteration predicted a direction in parameter space, and a corresponding parameter set, that increased E . Normally, those parameter sets converged to a parameter set that corresponded to a local (or possibly global) maximum value for E . Finally, the largest maximum arising from the random starting points was taken as an approximation to the global maximum.

2 Mathematical model: the direct problem

2.1 Model equations

The model equations were based on water and solute conservation. To model interactions among the renal tubules, we used a central core (CC) formulation (Stephenson, 1972): the vasculature, interstitial fluid, and interstitial cells were merged into a single tubular compartment, the CC. Then the descending thin limbs (DTLs) of Henle's loops, the ascending thin limbs (ATLs) of Henle's loops, and the composite CD exchanged water and solutes with the CC. The CC can be regarded as a tubule that has axial flow which represents the net blood flow that arises from fluid absorbed from renal tubules. Axial diffusion within the CC has frequently been used to represent the impact of non-ideal countercurrent exchange within the vascular flow (see, e.g., Stephenson (1972); Layton et al. (2004)). However, for simplicity, diffusion was not included in the present model. The DTLs, ATLs, CD, and CC (indexed by $i = 1, 2, 3$, and 4, respectively) were represented by rigid tubules. Figure 1 shows a schematic diagram of the CC model of the rat IM.

Because short loops of Henle turn back in the inner stripe of the OM, mostly within a narrow band near the OM-IM boundary (Han et al., 1992), only long loops are included in our model. We assumed that 12,667 long loops of Henle (one-third of a total of 38,000 loops of Henle) and 7,300 CDs extend into the IM (Han et al., 1992). Long loops of Henle are of differing lengths and thus turn back at differing levels along the IM. This configuration can be represented by means of continuously distributed model loops (Layton, 1986); in a distributed-loop model, tubular concentration profiles in loops turning at differing levels are assumed to differ, and transmural fluxes are weighted according to the rate at which loops turn at each level. Two space variables are used for the DTLs and ATLs: x denotes the level of the IM and y indexes a loop by the level at which it turns; both x and y range along the IM from $x = 0$ to $x = L$, with $x \leq y$, where L denotes the length of the IM. The fraction of loops $w_l(x)$ that have length greater than or equal to x , based on experimental findings (Han et al., 1992), is approximated by

$$w_l(x) = \left(1 - 0.95a^{1/16} \left(\frac{x}{L}\right)^2\right) e^{-2.8a^2(x/L)}, \quad (1)$$

where a is a scaling factor for the loop population distribution (Layton et al., 2004). The case of $a = 1.0$ corresponds to the base-case distribution; as a increases more loops turn back near the OM-IM boundary; and as a decreases to 0 more loops reach to the tip of the papilla. The negative of the derivative of w_l , $-w'_l(y)$, is the rate at which the DTLs turn to become ATLs at level y .

At time t , in a DTL or ATL ($i = 1$ or 2) reaching to IM level y , the water flow rate at level x is denoted by $F_{iv}(x, y, t)$, and the transmural water line flux (i.e., the water transport rate per unit tubular length) is denoted by $J_{iv}(x, y, t)$, taken positive for transport into the tubule. Analogous function notation and function arguments are used for other loop variables. The model represents Na^+ and urea concentrations in all tubules and the CC; a nonreabsorbable solute, denoted NR, is assumed to be present only in significant amounts in the tubular fluid of the CD. A chloride ion Cl^- , not explicitly represented, is assumed to accompany each sodium ion. Na^+ , urea, and NR are indexed by $k = 1, 2$, and 3, respectively.

Water conservation in a DTL or an ATL ($i = 1$ or 2) reaching to level y is represented by

$$\frac{\partial}{\partial x} F_{iv}(x, y, t) = J_{iv}(x, y, t). \quad (2)$$

Solute conservation in a DTL or an ATL ($i = 1$ or 2) is represented by

$$\begin{aligned} \frac{\partial}{\partial t} C_{i,k}(x, y, t) = & \frac{1}{A_i(x, y)} \left(-F_{iv}(x, y, t) \frac{\partial}{\partial x} C_{i,k}(x, y, t) \right. \\ & \left. - C_{i,k}(x, y, t) J_{iv}(x, y, t) + J_{i,k}(x, y, t) \right) \end{aligned} \quad (3)$$

For a limb of a loop of Henle reaching to level y , $C_{i,k}(x, y, t)$ is the concentration of solute k , $A_i(x, y)$ is the cross-sectional area of the limb, and $J_{i,k}(x, y, t)$ is the transmural line flux of solute k , taken positive into the tubule. The three terms inside the parentheses on the right-hand side of (3) arise from axial intratubular solute advection, transmural water transport, and transmural solute transport, respectively. The first two terms arise from the spatial derivative of the solute flow, i.e., the derivative of $F_{iv}(x, y, t) C_{i,k}(x, y, t)$ with respect to x .

Transmural water and solute transport through the epithelium that forms the walls of renal tubules is represented by a single-barrier formulation that summarizes transcellular transport. Thus transmural water flux (which is driven by the transmural osmolality difference) across a DTL or an ATL ($i = 1$ or 2) reaching to level y is represented by (Kedem and Katchalsky, 1958)

$$J_{iv}(x, y, t) = 2\pi r_i(x, y) P_{f,i}(x, y) \bar{V}_w \sum_{k=1,2} \phi_k (C_{i,k}(x, y, t) - C_{4,k}(x, y, t)), \quad (4)$$

where $r_i(x, y)$ is the radius of a tubule (DTL or ATL) reaching to level y , $P_{f,i}$ is the transmural osmotic water permeability coefficient, $\bar{V}_w$ is the partial molar volume of water (0.018136 cm³/mmole at 37°C, see p. B-152 and p. F-5 in (Weast, 1974)), and ϕ_k is the osmotic coefficient of solute k : 1.84 for NaCl and for NR, and 0.97 for urea (Weast, 1974). We assumed that the reflection coefficient for transepithelial solute transport equals one, as is now thought to be the case for most solutes transported in mammalian renal tubules (Sands, 2002); thus no reflection coefficient appears above in (4) or below in (5).

The transmural solute flux into a DTL or an ATL is given by (Friedman, 1986)

$$\begin{aligned} J_{i,k}(x, y, t) = & -2\pi r_i(x, y) \left(P_{i,k}(x, y) (C_{i,k}(x, y, t) - C_{4,k}(x, y, t)) \right. \\ & \left. + \frac{V_{\max,i,k}(x) C_{i,k}(x, y, t)}{K_{M,i,k} + C_{i,k}(x, y, t)} \right), \end{aligned} \quad (5)$$

where $P_{i,k}$ is the solute permeability of tubule i to the k -th solute, and $V_{\max,i,k}$ is the maximum transport rate per unit tubular area for inward-directed active transport of solute k , which was assumed to have the form of Michaelis-Menten kinetics with Michaelis constant $K_{M,i,k}$.

The CD system is modeled as a single tubule extending along the whole IM, as is the CC. Thus, the water and solute conservation equations for CD and CC ($i = 3$ or 4) are analogous to (2) and (3), respectively, and are obtained by omitting the argument y and letting $0 \leq x \leq L$; in addition, $k = 1, 2$, and 3 in the CD. The CD transmural fluxes are analogous to (4) and (5); CC fluxes are described below.

To account for the successive coalescences of CDs along the IM *in vivo*, which result in a decrease in CD population as function of increasing IM depth (Han et al., 1992), the model CD radius was multiplied by

$$w_{CD}(x) = \left(1 - 0.9616b^{1/16} \left(\frac{x}{L}\right)^2\right) e^{-2.5b^2(x/L)}, \quad (6)$$

where b is a scaling factor for the CD population distribution. The case $b = 1$ corresponds to the base-case distribution (Layton et al., 2004). As b increases, more CDs coalesce in the initial portion of the IM.

The transmural water and solute fluxes into the CC arise from the total transmural fluxes from the loops of Henle and CD. The total transmural line fluxes $\bar{J}_{iv}(x, t)$ and $\bar{J}_{is}(x, t)$ from DTLs or ATLs at level x are given by an integral of the fluxes from loops turning at level x or beyond, weighted by the rate at which the loops turn, i.e., weighted by $-w'_l$; thus,

$$\bar{J}_{i,k}(x, t) = \int_x^L J_{i,k}(x, y, t) (-w'_l(y)) dy, \quad (7)$$

where $i = 1$ or 2 , and $k = v, 1$, or 2 . The equations for transmural water and solute fluxes into the CC are given by

$$J_{4,k}(x, t) = -(\bar{J}_{1,k}(x, t) + \bar{J}_{2,k}(x, t) + n_{CD} J_{3,k}(x, t)), \quad (8)$$

where n_{CD} denotes the number of CDs per long loop of Henle (i.e., per loop of Henle reaching into the IM), and $k = v, 1$, or 2 .

To complete the system of model equations, boundary conditions must be specified for water flows and solute concentrations in the DTLs and the composite CD at the OM-IM boundary, i.e., at $x = 0$; for the ATLs at the loop bends; and for the CC at the medullary tip, i.e., at $x = L$. For the DTLs, $C_{1,2}(0, y, t)$ and $F_{1v}(0, y, t)$ are specified for $t \geq 0$, and $C_{1,1}(0, y, t)$ is computed, using $C_{1,2}(0, y, t)$, to obtain a DTL inflow osmolality of 673 mOsm/(Kg H₂O) (from the formula that osmolality is the sum of the concentrations weighted by their respective osmotic coefficients: $\sum_{k=1,2} \phi_k C_{1,k}(0, y, t)$). We assumed that the boundary fluid flow into the DTL that reaches to level y is given by

$$F_{1v}(0, y, t) = \gamma \left(F_{1vS} + (F_{1vL} - F_{1vS}) \frac{y}{L} \right), \quad (9)$$

where the term in parentheses linearly interpolates between F_{1vS} and F_{1vL} , fluid flows of shortest and longest DTL at the OM-IM boundary, respectively, and where γ is a positive parameter. The case $\gamma = 1$ corresponds to the base-case value of the OM-IM boundary flow of the DTLs. At each loop bend, the DTL is contiguous with the ATL; thus, $C_{2,k}(y, y, t) = C_{1,k}(y, y, t)$, and $F_{2v}(y, y, t) = -F_{1v}(y, y, t)$, where flow is taken positive in the increasing x direction. For the CD, $C_{3,2}(0, t)$, $C_{3,3}(0, t)$, and $F_{3v}(0, t)$ are specified for $t \geq 0$, and $C_{3,1}(0, t)$ is computed, using $C_{3,2}(0, t)$ and $C_{3,3}(0, t)$, to obtain a CD inflow osmolality of 673 mOsm/(Kg H₂O) (the same inflow osmolality as is computed for the DTLs). Because the CC is closed at the medullary tip, there is no axial flow of water at $x = L$, i.e., $F_{4v}(L, t) = 0$. This leads to the following ordinary differential equation (ODE) for $C_{4,k}(L, t)$:

$$\frac{\partial}{\partial t} C_{4,k}(L, t) = \frac{1}{A_4(L)} (-C_{4,k}(L, t) J_{4v}(L, t) + J_{4,k}(L, t)) . \quad (10)$$

At steady state, this equation implies that $C_{4,k}(L)$ equals the ratio of local solute absorption to water absorption, i.e., that

$$C_{4,k}(L) = \frac{J_{4,k}(L)}{J_{4v}(L)}, \quad (11)$$

where the dependence on time t has been removed.

2.2 Model parameters

Recent experimental studies have provided new insight into the functional anatomy and transport properties of long loops of Henle in rat IM (Layton et al., 2004; Pannabecker et al., 2004; Pannabecker and Dantzler, 2004). Based on these studies, two classes of long-looped nephrons were distinguished in the model: (1) those having loops that extend no more than 1 mm into the IM, and (2) those that extend further than 1 mm. Each loop in the first class was assumed to have two IM segments in its DTL. The first segment of such a DTL (denoted LDL_s) was assumed to have no permeability to water or NaCl, and a moderate urea permeability. The second segment of such a DTL, the prebend segment (denoted PBE, for prebend enlargement), was assumed to have the same properties as the associated ATL (denoted ATL_s): no permeability to water, a high permeability to NaCl, and a low urea permeability.

The DTLs in the second class of loops were assumed to have three distinct IM segments. The first segment (denoted LDL2), which spans the initial 40% of each DTL, was assumed to have a high permeability to water, based on the presence of the aquaporin-1 transporter; the second segment, denoted LDL3, and which spans most of the remainder of each DTL, was assumed to have a moderate water permeability (Chou and Knepper, 1992), though it has little or no aquaporin-1; and the prebend segment was assumed to have the same transport properties as the ATL. Both the ATL and the LDL3 were assumed to have low urea permeabilities, as suggested by studies in which urea transport proteins were not found in loops of Henle reaching beyond the first millimeter of the IM (Layton et al., 2004). All segments of the DTLs in the second class of loops were assumed to be impermeable to NaCl and to have a low urea permeability. The ATLs were assumed to be water impermeable and highly permeable to NaCl. In both classes of loops, the prebend segment was assumed to have a length of 166.7 μm.

The CD urea permeability was assumed to be 1 (in units of 10⁻⁵ cm/s) in the initial model IM, for 0 ≤ x ≤ λ L , where 0 ≤ λ < 1. For the terminal IM segment (i.e., for λ L < x ≤ L), CD urea permeability was assumed to increase exponentially, according to the formula

$$P_{3,2}(x, t) = \widehat{P}_0 + (\widehat{P}_1 - \widehat{P}_0) (e^{\alpha(x/L - \lambda)} - 1) / (e^{\alpha(1 - \lambda)} - 1), \quad (12)$$

where $\widehat{P}_0$, $\widehat{P}_1$, the initial and terminal CD urea permeabilities, were set to 1 and 110, respectively, and α = 7. CD urea permeability profiles for λ = 0.25, 0.50, and 0.75 are shown in Figure 2A. The profile for λ = 0.50 corresponds to the base case and was chosen by Layton et al. (2004) to be consistent with experiments in antidiuretic rats showing high urea permeabilities in the terminal CD (Kato et al., 1998; Sands and Knepper, 1987; Sands et al., 1987).

The CD Na^+ maximum transport rate ($V_{\max,3,1}$) was assumed to be $\beta \times 9 \text{ nmol cm}^{-2} \text{ s}^{-1}$ for the initial 30% of the model CD. The transport rate $V_{\max,3,1}$ then linearly decreased to $\beta \times 2.5 \text{ nmol cm}^{-2} \text{ s}^{-1}$ for the next 20%, and linearly decreased to $0 \text{ nmol cm}^{-2} \text{ s}^{-1}$ along the remainder of the CD. The $V_{\max,3,1}$ profile is shown in Figure 2B for $\beta = 0.5, 1.0$, and 1.5 . The case $\beta = 1.0$ corresponds to the base case and was chosen to ensure that substantial urea was absorbed from the CD and that the solute load reaching the terminal CD was consistent with experimental evidence for moderately antidiuretic rats (Layton et al., 2004). (Absorption of NaCl from the CD promotes osmotic water absorption from the CD, which raises the urea concentration of CD tubular fluid and thus promotes diffusive urea absorption from the CD.) The Michaelis constant for CD Na^+ active transport was set to 40 mM (Greger and Velázquez, 1987).

The model parameters were assigned to one of two sets: the parameters in one set, denoted S_f , were held fixed during the optimization process, and the parameters in the other set, denoted S_v , were allowed to vary within prescribed ranges. Table 1 exhibits values for the parameters in the set S_f . References for the parameters can be found in the study by Layton et al. (2004).

3 Numerical methods

3.1 The direct problem

In the steady-state formulation of the model, the time derivative vanishes in (3) and a tubular fluid flow direction was assumed for each tubule. A Newton-based method (Layton and Layton, 2002) was used to solve the resulting ODEs. That method updates variables along each tubule's flow direction by using the trapezoidal rule to integrate the conservation equations (2) and (3). At each medullary level, a nonlinear system consisting of the discretized equations was solved by means of Newton's method. In each iteration, values for the tubule variables were updated by using the CC solute concentrations from the previous iteration Equations (see (4) and (5)); then the CC variables were updated using the current values of the tubule concentrations. The iterations were repeated until a stopping criterion was satisfied.

3.2 Optimization problem

The maximization of UCM effectiveness E was formulated as a nonlinear optimization problem, under the constraints that the model equations were satisfied and the model parameters were within known or plausible experimental ranges. UCM effectiveness E was defined by

$$E(\mathbf{z}) = (U/P)(\mathbf{z}) - \rho(F_{3v}(L; \mathbf{z}) - F_{3v}^E)^2, \quad (13)$$

where U/P is the ratio of urine osmolality to plasma osmolality, F_{3v} is the model urine flow, F_{3v}^E is an experimental value of the urine flow, ρ is a positive penalty parameter for the urine flow difference term, and $\mathbf{z}$ denotes the vector containing the parameter values that are allowed to vary, i.e., parameters from the set S_v . To make E dimensionless, the penalty parameter ρ was considered to be in units of $\text{min}^2 \text{ nl}^{-2}$. The penalty term in the definition for E was formulated to reduce the difference between the model's urine flow rate and an experimental value for that rate. Urine osmolality was taken to be the sum of the products of the osmotic coefficients and the solute concentrations at the terminus of the CD, i.e., $\sum_{k=1,2,3} \phi_k C_{3,k}(L)$.

The experimental value of the urine flow F_{3v}^E was taken to be $0.0658 \text{ nl min}^{-1} \text{ nephron}^{-1}$ (based on a urine flow of $5 \mu\text{l min}^{-1}$ per rat (Atherton et al., 1968) and $38,000$ nephrons per kidney (Han et al., 1992)); the base value of ρ was taken to be $30 \text{ min}^2 \text{ nl}^{-2}$. Hai and Thomas (1969) reported a plasma osmolality of $314 \pm 13.2 \text{ mOsm/(kg H}_2\text{O)}$; we used a plasma

osmolality of 310 mOsm/(kg H₂O) to be consistent with the value used previously (Layton et al., 2004).

The optimization problem can be posed as

$$\text{maximize } E(\mathbf{z}) \quad (14)$$

$$\text{subject to } \mathbf{z}_\ell \leq \mathbf{z} \leq \mathbf{z}_u, \quad (15)$$

where the inequality relating the vectors $\mathbf{z}_\ell$, $\mathbf{z}$, and $\mathbf{z}_u$ holds element-by-element.

3.3 Optimization algorithms

To solve the optimization problem (14)–(15), we used a reduced-gradient algorithm and a quasi-Newton algorithm (Murtagh and Saunders, 1978), in conjunction with solutions of the direct problem (2)–(11) to evaluate the UCM effectiveness function E ; we call this procedure “Optimization Algorithm 1” (OA1). To implement the reduced-gradient algorithm and the quasi-Newton algorithm, we used the package *Modular In-core Nonlinear Optimization System (MINOS)*, version 5.5 (Murtagh and Saunders, 1998); a description of our adaptation of OA1 was previously provided (Marcano-Velázquez and Layton, 2003).

The OA1 requires an initial set of parameters to begin the iterations. Because OA1 is locally convergent, to identify or closely approximate a global optimum, the parameter space was explored by initializing OA1 with 60 different initial parameter sets randomly selected from a uniform distribution. For each parameter set, OA1 searched nearby parameter space for a direction in which effectiveness E increased; if such a direction could be found, a new set of parameters was chosen along the direction vector. Iterations continued until a stopping criterion was met. The parameter set that yielded the largest value of E was selected. To explore the parameter space in search of a global optimum, the following algorithm, which we call “Optimization Algorithm 2” (OA2), was used:

1. From a uniform distribution, m_1 starting points in parameter space were randomly selected.
2. On each such point, n iterations of OA1 were applied, to find a new point in parameter space, which has a value of E no less than that obtained for the starting point.
3. From these new points, the m_2 significantly distinct points corresponding to the largest m_2 values of E were selected.
4. Using each of these m_2 points as a starting point, OA1 was applied until a stopping criterion was met.

The values of m_1 , n , and m_2 were 60, 3, and 5, respectively.

The direct problem, OA1, and OA2 were all programmed in FORTRAN 77 and executed in double precision mode on a computer having two Intel® Xeon™ 3-GHz processors and 2 GB of RAM. A spatial discretization of 300 subintervals was used along the model IM, from $x = 0$ to $x = L$; thus 300 discrete loops of Henle were represented, each having a loop bend at a distinct numerical grid point.

4 Results

The optimization problem was solved by means of OA2 to seek parameter sets that corresponded to optimal antidiuretic function as assessed by maximizing effectiveness E . A selected set of model parameters, which we believe may have a significant impact on IM concentrating capability, and for which experimental evidence may be substantially uncertain or must be inferred indirectly, were allowed to vary. The following parameters were varied by $\pm 15\%$ relative to their base-case values: DTL tubular fluid inflow rate and urea concentration (at $x = 0$), the scaling factors for the loops of Henle and the CD population distributions, and the CD tubular fluid inflow urea concentration (at $x = 0$). Because measured urea permeabilities in CD vary substantially (Sands and Layton, 2007), as well as active transport rates of NaCl from CDs (Weinstein, 1998), related model parameters were allowed to vary by ranges exceeding 15%: the location of the beginning of increasing urea permeability and the scaling factor for Na^+ active transport were varied, relative to their base-case values, by $\pm 30\%$ and $\pm 50\%$ respectively. Furthermore, because the CD water inflow at the OM-IM boundary, which has never been measured, has a direct effect on the UCM effectiveness E , CD water inflow was also allowed to vary by a larger range, viz., from 2 to 4 nl/min.

Table 2A shows the base-case values and ranges of the parameters that were allowed to vary during optimization via OA2, and the optimal values attained by those parameters. In the optimum case, most parameters assumed values at the extrema of their prescribed ranges; the two exceptions were CD water inflow and the CD population distribution scaling factor, which took on values interior to their ranges.

To better explain the results that follow, it is useful to summarize the processes involved in the UCM of the model (a more detailed description can be found in Layton et al. (2004)). The model represented a UCM that operates on principles similar to those of the “passive” hypothesis proposed by Kokko and Rector (1972) and by Stephenson (1972). The diffusion of urea from the CDs into the IM interstitium, and the accompanying water that is absorbed from the CDs, sustains a decreased interstitial NaCl concentration; that lowered concentration promotes sustained diffusive absorption of NaCl from NaCl-permeable segments of the long loops of Henle. The absorbate from the CDs and loops is carried back toward the OM by ascending vasa recta, while ATLs carry fluid that is dilute, relative to the local interstitium, toward the OM. The mixing of NaCl and urea in the IM interstitium raises its osmolality (and thus the osmolality of the model’s CC), which further promotes water absorption from the CDs and increases CD tubular fluid osmolality. The mixing of the NaCl and urea was regarded by Kokko and Rector (1972) and by Stephenson (1972) as involving the passive processes of urea and NaCl diffusion from the CDs and ATLs, respectively, rather than a process of active transepithelial transport that required metabolic energy.

Figure 3A1 shows base-case osmolality profiles from that model in the CD, CC, and the longest loop. Osmolality increases with increasing medullary depth, except in the ATL near the OM-IM boundary and in the prebend segment. Base-case osmolality profiles for loops of Henle reaching to 20, 40, 70, and 100% of the IM depth (which correspond to lengths of 1.0, 2.0, 3.5, and 5.0 mm, respectively) are shown in Figure 3A2. Tubular fluid osmolality decreases rapidly along the prebend segment and along the nearby portion of the ATL, owing to the abrupt increase in NaCl permeability and to the transepithelial gradient that favors NaCl diffusion from the loop tubular fluid into the interstitium. That transepithelial NaCl concentration gradient, exhibited in the NaCl concentration profiles in Fig. 4A1, is maintained by urea absorption from the CD, which promotes a high CC urea concentration relative to the CC NaCl concentration; see Fig. 4A2. Base-case fluid flow rates, taken to be positive when flow is in the direction of increasing medullary depth, are shown in Fig. 5A1 for the CD and CC, and composite fluid flow rates are shown for the DTLs and ATLs (fluid flow rates are given per

nephron, assuming 38,000 nephrons per kidney). Owing to the exponential rate at which loops turn back along the model IM, the total DTLs-to-CD flow ratio rapidly decreases with increasing depth, with CD flow ultimately exceeding flow in DTLs.

Results for the optimum case, corresponding to those given above for the base case, are shown in the right-hand columns of Figure sets 3, 4, and 5. For the optimum case, effectiveness E was 5.41, whereas E was 3.79 for the base case. The optimal DTL water inflow scaling factor, γ , assumed its upper bound value of 1.15, whereas DTL inflow urea concentration assumed its lower bound value of 25.5 mM. Both optimal values helped to maximize the objective function E (and increase U/P), in part, by raising the NaCl concentration in the tubular fluid that enters the ATL. That was achieved by an increased DTL water inflow and a reduced DTL inflow urea concentration, while the inflow fluid osmolality was kept constant; this not only resulted in an increased DTL inflow NaCl concentration but also an increased NaCl flow (i.e., an increased $F_{1,v}(0, y)C_1(0, y)$) in the fluid entering the DTLs. NaCl flow and concentration at the bend of the longest loop were 4.29% and 18.0% higher, respectively, than the base-case values. At the beginning of the transition to the PBE of the longest loop, the tubular fluid NaCl concentration is significantly higher in the optimum case than in the base case, as can be seen by comparing Figures 4A1 and 4B1. As a result, transmural NaCl flux is also significantly higher near the loop bend in the optimum case, as indicated by the steeper decrease in NaCl concentration, relative to the base case. The loop-bend NaCl concentration and transmural flux are similarly increased in the optimum case in other loops of Henle, as suggested by a comparison of the osmolality profiles in Figures 3A2 and 3B2.

In the optimum case, the urea concentration of the CD inflow attained its upper bound value of 362 mM, whereas λ , which scales the location at which CD urea permeability begins to increase, took on its lower bound value of 0.35. Both optimal parameter values increase urea absorption from the CD. In the base case, 67.8% of the urea is absorbed from the CD, whereas in the optimum case, 73.0% of the urea is absorbed; see Figs. 4A2 and 4B2. The optimal CD NaCl transport rate $V_{\max, \text{Na}^+}$ scaling factor reached its upper bound value of 1.5. As previously noted (under **Model Equations**), CD NaCl transport promotes water absorption from the CD, and as a result of the increased water absorption, the concentration of NR solute increased, from a base-case concentration of 118 mM at the tip of the papilla to an optimum-case concentration of 211 mM.

In the optimum case, UCM effectiveness E was increased by a larger loop population distribution scaling parameter a , i.e., by fewer loops of Henle reaching into the deep IM, relative to the base case. With a larger fraction of the loops turning in the initial IM, the load on the concentrating mechanism of the inner part of the IM was reduced, the cascade effect of overlapping loop bends was enhanced, and the urea concentration in the terminal portion of the CC was sufficient for the model to concentrate effectively via urea and NaCl mixing. A comparison of Figs. 5A1 and 5B1 shows that the DTLs-to-CD flow ratio decreases significantly faster as a function increasing depth in the optimum case than in the base case.

In the optimum case, the CD inflow rate was 2.97 nl/min, which was strictly interior to the prescribed range of 2 to 4 nl/min. If the model CD inflow rate is the only parameter varied, and if it is increased, then the urine flow rate is increased, while the urine osmolality is decreased. On the other hand, changes in individual parameters that increase U/P tend to decrease urine flow. Thus, to avoid an unrealistically low urine flow rate, which would incur a penalty and decrease UCM effectiveness E , CD inflow rate in the optimum case reached a value that was 23.75% higher than the base-case value. The optimal CD distribution scaling parameter b took on a value of 0.932 that was also interior to its prescribed range and that was 7.8% below the base-case value. With a smaller b , the CD radius, and thus its transport rates, were larger deep in the IM. As noted previously, larger CD urea permeability and larger active

NaCl transport rate tend to increase U/P . However, because decreased b results in increased effective CD water permeability, a corresponding increase in water absorption from the CD may produce a urine at an unrealistically low rate. Thus, the lower bound value for b , which may have further increased U/P , was not reached.

The base-case and optimal-case tubular fluid composition for the bend of Henle's loop and for urine are given in Table 2B. In the base case, urine NaCl, urea, and NR concentrations were 254, 598, and 118 mM, respectively, and urine flow rate was 0.0781 nl/min per nephron. For the optimal-case parameters, the model's urine NaCl, urea, and NR concentrations were 191, 1,030, and 211 mM, respectively, and urine flow rate was 0.0542 nl/min per nephron. All of these values are within our assumed experimental ranges, excepting only the urine urea concentration, which was 18 mM (~2%) above its assumed experimental range. Compared to the base case, the optimal urine urea and NR concentrations increased significantly, almost by factors of two, whereas urine NaCl concentration decreased. The increase in urine urea concentration can be attributed to the higher CD inflow urea concentration and to increased water absorption from the CD; the increase in urine NR concentration resulted from the increased CD water absorption and from the higher CD inflow rate (which raised the amount of NR inflow); and the decrease in urine NaCl concentration, which is, in large part, a result of the higher CD NaCl transport rate. These competing effects yielded an optimal urine osmolality of 1,738 mOsm/(kg H₂O), which corresponds to a 37.5% increase above the base-case value of 1,264 mOsm/(kg H₂O). For the prescribed CD inflow osmolality of 673 mOsm/(kg H₂O), the optimal osmolality increase corresponds to an 80% increase in the IM concentrating capability relative to the base-case IM concentrating capability: $(1,738 - 1,264) / (1,264 - 673) \times 100\% = 80\%$.

Because the generation of the osmotic gradient in model UCM depends on urea absorption from the CD, the composition of the CD tubular fluid at the OM-IM boundary, especially the urea concentration, can significantly impact model predictions. In the optimization study, CD inflow urea concentration was allowed to vary by $\pm 15\%$ from the mean value of 314.54 mM estimated in a model study by Layton et al. (2004); in the optimum case, the upper limit of 362 mM was reached, which is consistent with tissue-slice results by Hai and Thomas (1969), and which is within the range of experimental control values (278 ± 95 mM) that were measured for urea concentration in the tissue of the IM base (Kim et al., 2005). However, in rats given a urea-supplemented diet, a tissue urea concentration of 586 ± 109 mM was found in the IM base (Kim et al., 2005). Thus, a significantly higher CD inflow urea concentration is consistent with experiments. To explore the dependence of the model optimization results on the range of CD inflow concentrations, we conducted an additional set of optimization simulations, in which $C_{\text{urea}}(0)$ was varied by $\sim \pm 58\%$ from its base-case value, i.e., between 132 and 500 mM. All other parameters were allowed to vary within the same ranges as prescribed in Table 2A. With this relaxed constraint, a higher urine osmolality was obtained: 1,928 mOsm/(kg H₂O), which is 52.6% above base case, and 10.9% higher than the previous optimum case. Also, the model predicted urine NaCl, urea, and NR concentrations of 188, 1,272, and 189 mM, respectively. The model urine flow was $0.0527 \text{ nl min}^{-1} \text{ nephron}^{-1}$, slightly lower (by 2.95%) than the previous optimum case. Most parameters that were varied took on the same values as those shown in Table 2B under the column labeled "Optimum case." The exceptions were: CD inflow urea concentration reached a new upper bound value of 500 mM; the CD water inflow was 2.60 nl min^{-1} ; the CD urea permeability began its exponential increase at a later normalized location of $\lambda = 0.397$; and the scaling factor β for the CD population distribution was reduced to 0.854. For this simulation, the value of effectiveness $E(13)$ increased to 5.96 from the base-case value of 3.79, whereas the value of E corresponding to the optimal parameters reported in Table 2A was 5.41.

Indeed, if the only parameter change were an increase in CD inflow urea concentration, the result would be increased urea diffusion into the interstitium, increased NaCl absorption from ATLS and water absorption from the CD, and a resulting increase in urine osmolality. But in the optimized case, urea and water absorption from the CD were further augmented via increased CD transport permeabilities arising from a reduction in the CD population scaling factor. However, excessive water absorption from the CD would result in an exceedingly low urine flow rate, which would in turn increase the penalty term in the optimization function (13). Thus, to yield a reasonable urine flow rate in the optimized case, CD inflow rate increased, and the CD urea permeability exponential increase was begun deeper into the IM so as to avoid an excessive loss of urea and water into the interstitium of the initial IM.

With the optimized parameters for modified CD inflow urea concentration, the model predicted a urine osmolality, urine Na⁺ concentration, and urine flow that were within experimental ranges. However, the urine urea concentration was predicted to be 34% above the mean experimental value from Hai and Thomas (1969), and it accounted for 66% of the urine osmolality. The model's large deviation from experimental values may not be unrealistic, since urea concentrations can vary widely and since higher urea concentrations are correlated with higher urine osmolalities (Gamble et al., 1934; Fenton et al., 2006).

Any maximum of $E(z)$ for the optimization function given by (13) increases urine osmolality while maintaining urine flow within experimental values suggested by experiments. However, this optimization function is not the only reasonable function that might be applied in the context of the UCM. Indeed, the optimization function given by (13) does not well represent important regulatory objectives of the kidney, notably those related to NaCl conservation and urea elimination. It is plausible that a function that better represents those objectives might yield urine osmolalities and flows that are more consistent with those found in rats producing highly concentrated urine. Therefore, we constructed an alternative optimization function that sought to not only incorporate a penalty for water excretion, but which also approximated excretion rates (urine flow rates) for NaCl and urea as reported in experiments; that optimization function is

$$\bar{E}(z) = FWA(z) - \rho_s (F_{3s}(L; z) - F_{3s}^E)^2 - \rho_u (F_{3u}(L; z) - F_{3u}^E)^2, \quad (16)$$

where FWA is the free water absorption rate¹; $F_{3s}(L; z)$ and $F_{3u}(L; z)$ are the steady-state model values of the urine Na⁺ and urea flows, respectively; F_{3s}^E and F_{3u}^E are experimental (or target) values for the urine Na⁺ and urea flow rates, respectively; and ρ_s and ρ_u are penalty parameters. Note that FWA is in units of nl min⁻¹ whereas the solute flows are in units of pmol min⁻¹; to preserve consistency of units, ρ_s and ρ_u were considered to be in units of nl min pmol⁻².

The experimental values of the urine Na⁺ flow F_{3s}^E and urea flow F_{3u}^E were based on an experiment conducted by Gamble and coworkers (1934) in which rats were fed a chronic high-urea diet. By optimizing the function $\bar{E}(z)$ we sought to approximate results reported in "Period"

¹The free water absorption rate FWA is defined to be the osmolar clearance O_C minus the urine flow rate U_f . The osmolar clearance is defined by $O_C = U_f U/P$. In this equation, the product $U_f U$ is the osmolar rate of excretion of solutes in urine. Because $U_f U$ equals $O_C P$, O_C is the volume, per unit time, of water that is required to contain excreted solutes, per unit time, at blood plasma osmolality. Thus, the urine flow rate U_f equals $O_C - FWA$, where FWA , in an animal producing concentrated urine, is the rate of (solute free) water absorption that elevates the urine osmolality above blood plasma osmolality; i.e., FWA is the amount of water absorbed from renal tubules to produce a urine that is more concentrated than blood plasma (Jamison and Kriz, 1982; Wesson and Anslow, 1952). In the model the steady-state urine flow U_f is denoted by $F_{3v}(L; z)$, where z is the vector containing the parameter values that are allowed to vary; thus, $FWA(z) = (O_C(z) - F_{3v}(L; z)) = F_{3v}(L; z)((U/P)(z) - 1)$.

VIII of Table 1 from Ref. (Gamble et al., 1934). If one assumes 38,000 nephrons per rat kidney (Han et al., 1992), then results from Period VIII correspond to a urine Na^+ flow rate of $\sim 17 \text{ pmol min}^{-1} \text{ nephron}^{-1}$, and a urine urea flow rate of $\sim 212 \text{ pmol min}^{-1} \text{ nephron}^{-1}$. The values of ρ_s and ρ_u were taken to be 5.7×10^{-4} and $5.7 \times 10^{-3} \text{ nl min pmol}^{-2}$, respectively. The optimization problem given by (14)–(15) was solved (by means of OA2) with the optimization function $\bar{E}(\mathbf{z})$ given by (16) (instead of $E(\mathbf{z})$ given by (13)).

When the parameter bounds reported in Table 2 were used, the optimization results for the alternative optimization function gave a urine osmolality of $809 \text{ mOsm/KgH}_2\text{O}$, a urine flow of $0.398 \text{ nl min}^{-1}$ per nephron, and a FWA of $0.641 \text{ nl min}^{-1}$ per nephron. These results can be compared with those found by Gamble et al. (1934): a urine osmolality of $2,240 \text{ mOsm/KgH}_2\text{O}$, a urine flow of $0.128 \text{ nl min}^{-1}$ per nephron, and a FWA of $0.797 \text{ nl min}^{-1}$ per nephron. Thus the alternative optimization function gave neither a high urine osmolality nor a low urine flow rate that were close to values found by Gamble et al. (1934). (When FWA was replaced by the negative of the urine flow rate $-F_{3v}(L; \mathbf{z})$ in (16), the results were almost identical, both in this optimization calculation and in the others summarized below. We attribute the similarity in results to the following: by aiming for target values of Na^+ and urea flow, we are effectively setting the osmoles through the optimization function for Na^+ and urea. If NR flow is small, relative to Na^+ and urea, then $F_{3v}(L; \mathbf{z})$ times U is nearly fixed. Then since plasma osmolality is fixed, it must be that O_c (see footnote 1) is nearly fixed. Because by definition $FWA = O_c - F_{3v}(L; \mathbf{z})$, it follows that in this context minimizing $F_{3v}(L; \mathbf{z})$ and maximizing FWA are nearly the same.)

In an attempt to obtain higher urine concentrations, we allowed the concentration of urea in the CD at the OM-IM boundary to range up to 500 mM ; this may be justified by the supplementary urea that was fed to the rats in Period VIII by Gamble et al. (1934). The optimal model results obtained for the enlarged urea concentration range were: urine osmolality, $1,155 \text{ mOsm/KgH}_2\text{O}$; urine flow, $0.222 \text{ nl min}^{-1}$ per nephron; and FWA , $0.604 \text{ nl min}^{-1}$ per nephron. Thus urine osmolality was moderately increased, whereas the urine flow rate was substantially decreased. The elevated FWA for these simulations arises from high urine flow rather than a significant increase in urine osmolality.

In a mathematical model by Layton and Layton (2005a) that included preferential interactions of renal tubules in the outer medulla, simulations predicted an osmolality at the OM-IM boundary of $\sim 800 \text{ mOsm/KgH}_2\text{O}$. Based on this result, we set osmolality at the OM-IM boundary to be equal to $815 \text{ mOsm/kgH}_2\text{O}$ and used that value to compute the boundary values of Na^+ concentrations in the DTL and CD from the urea and the NR solute concentrations. Moreover, we let the CD urea concentration at the OM-IM boundary vary up to 600 mM . The results are shown in Table 3. Even with the elevated urea concentration and the elevated osmolalities at the OM-IM boundary, the optimum osmolality value was $1,575 \text{ mOsm/KgH}_2\text{O}$, far below the experiment, although the calculated optimum and the experimental urine flows and FWA 's were more similar in magnitude. The results from the alternative optimization function suggest that the IM concentrating mechanism, as represented in our model, does not have the rat's capability to produce urines of high osmolality while maintaining a moderately high urine flow rate.

5 Discussion

The model used in this study (Layton et al., 2004) was developed to help interpret emerging findings in the functional anatomy of the rat kidney IM (Pannabecker et al., 2004; Pannabecker and Dantzler, 2004). These findings, and subsequent studies (Pannabecker and Dantzler, 2006; Pannabecker and Dantzler, 2007), suggest that the rat IM is more highly structured than previously appreciated and that the long loops exhibit substantial functional heterogeneity.

Based on preliminary findings by Pannabecker and Dantzler (Layton et al., 2004), in which urea transport proteins were not found in loop segments below the first millimeter of the IM, the model assumed that urea permeabilities were smaller than has been inferred from experiments in isolated perfused tubules (DTLs and ATLs) of rat and hamster (Imai, 1977; Liu et al., 2001). These experiments exhibited substantial scatter, and we believe that the measurements may have been affected by heterogeneity among loop populations, by as yet undetected functional segmentation of loops with respect to urea transport, or by selective harvesting of IM tubular segments from near the OM-IM boundary.

The model concentrates urine by means of principles proposed by Stephenson (1972) and by Kokko and Rector (1972); their proposed mechanism has frequently been called the “passive mechanism.” However, this terminology is misleading, because the passive mechanism depends on the separation of urea from NaCl in the OM, which is driven by vigorous active transport of NaCl from the TALs, and by active transport of NaCl from the CDs of the IM. Therefore, we believe that it would be more appropriate to call this mechanism a “solute-separation, solute-mixing mechanism” (SSSM), as was been previously proposed (Layton et al., 2004). A number of alternatives to the SSSM have been advanced (for a summary, see Sands and Layton (2007)), notably those involving the peristalsis of the renal papilla (Knepper et al., 2003) and those involving a net generation of osmolytes in the IM (Hervy and Thomas, 2003). Although the nature of the UCM of the IM remains controversial, the SSSM is the most widely-accepted mechanism.

The optimization results in this study, using the optimization function (13), show that the concentrating capability of the model by Layton et al. (2004) can be substantially increased by varying a subset of parameters within reasonable ranges. By varying these parameters by $\pm 15\%$ from base-case values, the model produced a maximum urine osmolality of 1,738 mOsm/(kg H₂O), which is within a typical experimental range of $1,805 \pm 194$ mOsm/(kg H₂O) reported by Hai and Thomas (1969), and which is 37.5% above the model’s base-case value of 1,264 mOsm/(kg H₂O). That osmolality increase corresponds to an 80% increase in the IM concentrating capability, which is the percent increase in CD tubular fluid osmolality increase along the IM (i.e., from $x = 0$ to $x = L$). By allowing CD inflow urea concentration to vary by a larger range, an even higher maximum urine osmolality was obtained by our model: 1,928 mOsm/(kg H₂O), which corresponds to an osmolality increase of 52.5% above the base case. These results suggest that a rat attains high urine osmolality by means of the regulation and coordination of key parameters. However, experiments indicate that, under appropriate conditions, some rats can concentrate urine up to osmolalities of nearly 3,000 mOsm/(kg H₂O) (Beuchat, 1996), which appears to be beyond the capability of our model when it is constrained within physiologically plausible parameter ranges. Moreover, an alternative optimization function, (given by $\bar{E}(\mathbf{z})$ in (16)), which sought to more realistically represent renal regulatory objectives, also did not yield high urine osmolalities.

The experimental values for the urine flow rate (which was used in the optimization function (13)), the urine osmolality, and the solute concentrations were obtained from studies by Atherton et al. (1968), Atherton et al. (1969), and by Hai and Thomas (1969); corresponding experimental loop-bend tubular fluid values were obtained from Pennell et al. (1974). These studies involved rats in similar states of antidiuresis: the maximum urine osmolality reported by Hai and Thomas (1969), 1,805 mOsm/(kg H₂O), exceeded that reported by Pennell et al. (1974), 1,693 mOsm/kg H₂O. In our optimum case, the model predicted loop-bend tubular fluid osmolality and concentrations that exceed the upper bounds of the experimental values reported by Pennell et al. (1974); however, these optimum-case values may more closely approximate the corresponding values in the study by Hai and Thomas (1969), which were likely higher than the experimental measurements shown in Table 2 (but which were, unfortunately, not measured by Hai and Thomas (1969)).

The optimization results of the present study suggest, as in the study by Layton et al. (2004), that the CD population distribution and CD urea permeability play a significant role in optimizing UCM performance; see Table 2B. The objective function E (which seeks to make U/P large while also constraining the urine flow to be within the experimental range) was maximized by a combination of increased CD urea permeability (through decreased λ) and decreased CD distribution (through decreased b) such that the increase in CD urea permeability and the decrease in effective CD water permeability (owing to the decrease in CD surface area) were neither too gentle nor too steep. A balance was found when composite CD carried a urea-rich fluid at a sufficiently high flow rate into the deep IM.

The results in the present study predict a significant response of the IM concentrating capability to CD tubular fluid flow rate and solute composition at the OM-IM boundary. CD inflow rate and inflow urea concentration must be sufficiently high to deliver enough urea deep into the IM to drive a SSSM. However, an excessive CD inflow rate would exert too large a load on the IM UCM for it to produce a highly concentrated urine. Thus the effectiveness of the IM UCM is critically dependent on the UCM of the OM, which modifies the CD tubular fluid in the OM by means of a number of processes that affect water absorption from the CD, and both solute absorption from, and solute secretion into, the CD (Weinstein, 2000).

Because our model does not include a representation of the outer medulla and the short loops of Henle contained therein, nor of the cortex and the renal tubules that connect the loops of Henle to the CDs, our model does not permit a confirmation that the delivery of urea to our model CD (which corresponds to the CD of the inner medulla) is consistent with comprehensive mass balance of urea. However, calculations based on data from a study by Armsen and Reinhardt (1971) suggest that the urea delivery to our model CD is reasonable. (Mass balance of water and NaCl is a lesser concern, because water and NaCl are relatively plentiful in the glomerular filtrate compared to urea, and their inflow values in our model are reasonably consistent with values computed in recently-published models (Layton and Layton, 2005a; Layton and Layton, 2005b; Weinstein, 2000; Weinstein, 2002).)

If one assumes that the plasma urea concentration is 8 mM (Armsen and Reinhardt, 1971) and that the average SNGFR is 33 nl/min (Rouffignac, C. de and Bonvalet, 1970), then the filtered load of urea is 264 pmol/min/nephron. In moderately concentrating rats, Armsen and Reinhardt (1971) reported results in which the fraction of filtered urea load delivered to the late distal tubules of superficial nephrons ranged from 0.65 to 0.93 (their experiment No. 23). If one assumes an average fractional delivery of 0.79, then nearly 209 pmol/min/nephron is delivered to the late distal tubule of each nephron. (The average urea delivery may be higher if juxtamedullary nephrons, which are not accessible to micropuncture, have higher urea concentrations than the superficial nephrons that give rise to the distal tubules that are accessible to micropuncture.)

For the optimum case reported in Table 2, the urea concentration of the CD inflow has a concentration of 362 mM, and water flow per nephron is $(2.97 \text{ nl/min/CD})/(5.2 \text{ nephrons/CD}) = 0.57 \text{ nl/min/nephron}$ (where $5.2 = 38,000 \text{ nephrons}/7,300 \text{ CDs}$). This yields a urea flow of about 206 nl/min/nephron, which is less than the filtered load and also less than a typical delivery to the late distal tubule. Therefore, the urea delivery to the model CD is consistent with mass balance, provided that in vivo urea absorption between the late distal tubule and the inner medullary CD is not too large.

Using the optimization function $E(\mathbf{z})$, the largest optimum urea concentration that we compute for the CD inflow (for the case of an enlarged range of variation for urea concentration in the CD inflow) is 500 mM; the corresponding fluid inflow per nephron is $2.6/5.2 \text{ nl/min} = 0.50 \text{ nl/min/nephron}$. Thus the urea delivery to the model CD is 250 pmol/min/nephron. Although

this delivery somewhat exceeds that of the late distal delivery computed above, it is not unreasonable within the context of the range of experimental values for delivery of urea to the late distal tubule reported by Armsen and Reinhardt (1971).

In our optimization studies of the avian UCM (Marcano-Velázquez and Layton, 2003; Marcano et al., 2006), the measure of UCM efficiency was chosen to be the free-water absorption rate (Jamison and Kriz, 1982; Wesson and Anslow, 1952), divided by a quantity that was approximately proportion to the energy that was expended by the UCM for active transport of NaCl. In the present study, we have maximized the urine-to-plasma osmolality ratio (U/P), while maintaining a urine flow rate within a plausible physiologic range. In the present context, that ratio is more appropriate than the measure used in the avian studies, for three reasons. First, a high free-water absorption rate can be produced by means of a slightly concentrated urine at a sufficiently high urine flow rate (Marcano et al., 2006). Second, the energy required by the IM UCM is difficult to quantify, because that energy arises in part from the separation of urea and NaCl through processes localized in the OM, and because NaCl absorption from the CD in the IM arises from a mechanism not yet fully understood. Third, the aim of most model studies of the UCM of the IM is not to quantify efficiency, but to gain an understanding of how high urine osmolalities may be attained. By optimizing U/P , subject to a penalty when the predicted urine flow rate deviates from an experimental measurement, we are able to assess the potential of parameter variations to raise urine osmolality while maintaining a reasonable urine flow rate. The penalty imposed on the difference between the model urine flow rate and the experimental measure eliminates parameter sets that produce highly concentrated urine, but which absorb excessive water from the CD and, therefore, produce an unphysiologic urine flow rate.

A parameter sensitivity study was previously conducted on the current model (Layton et al., 2004). In that study, selected parameters were varied individually, and for each new parameter value, urine osmolality, urine flow, and free-water absorption rate were computed. The results in that study and in the present optimization study are generally consistent, but there are differences. For example, in the previous study, as CD inflow rate was increased from its base-case value, urine osmolality decreased monotonically, and urine flow rate and free-water absorption rate increased monotonically. Thus if one sought to increase U/P by varying CD inflow rate alone, that rate would be lowered. In contrast, the present study yielded an optimal CD inflow rate of 2.97 nl/min, which is 23.8% above base case but is well below the upper limit of 4.00 nl/min. Indeed, our optimization results suggest that to produce a highly concentrated urine while maintaining a physiologic urine flow rate, the synergy between three parameters—the CD inflow urea concentration, the location and exponential increase rate of the urea permeability profile along the CD, and the CD population rate—may be significant. Such complex parameter relationships and interactions are not likely to be revealed in analyses that consider the sensitivity to each parameter individually.

Tewarson et al. (1998) studied the UCM of the rat renal medulla using a nonlinear least-squares optimization technique. In that model (which we refer to as the TTM model), the optimization method identified a set of model parameters that maximized the osmolality increase along the CC. In the optimum case, the urine osmolality was computed to be 1,240 mOsm/(kg H₂O).² That their optimum urine osmolality was significantly lower than ours (1,738 mOsm/(kg H₂O)) is perhaps surprising, inasmuch as urine flow was not constrained in the TTM model, which theoretically allowed an extremely concentrated urine to be produced at a minimal rate. Moreover, tubular fluid osmolality at the OM-IM boundary was higher in the TTM model (758 mOsm/(kg H₂O)) than in ours (673 mOsm/(kg H₂O)). The differences between the TTM model

²When preferential interactions among renal tubules were represented, the maximum urine osmolality increased to 1,330 mOsm/(kg H₂O) (Tewarson et al., 1998).

results and the present study may arise, at least in part, from differing loop-of-Henle representations: in the TTM model, the loops of Henle were represented using one model loop with shunting. That shunting used was not made clear in the TTM model paper, but it was implemented as previously described by Wang and Tewarson (1993): the turning back of loops of Henle at various depths along the IM was represented by transferring a fraction of the DTL tubular fluid to the ATL at each medullary level. A major drawback of a shunt formulation is that the transport properties of the model DTL and ATL must be homogeneous at each medullary level. This has the unfortunate consequence that the LDL3 and PBE, both of which may play an important role in the UCM (Layton et al., 2004), cannot be represented. It should be noted, however, that the TTM study and the present work also differ in other assumptions, notably the use of higher loop urea permeabilities in the TTM model; but despite the higher urea permeabilities, urea concentrations in loop of the TTM model were similar to those obtained in the optimum case of the present study.

A shortcoming of our model, in both its base case and in the optimum case, is the prediction that the ATLs of sufficiently long loops carry fluid that is concentrated, relative to fluid at the same medullary level in their contiguous DTLs, from the IM into the OM (e.g., see Figs. 3B1 and 3B2). This behavior tends to reduce concentrating capability, and we believe it to be unlikely to occur in vivo, because of its wastefulness. A higher urea permeability in the portions of the longest ATLs that are near the IM base, in conjunction with a more comprehensive model formulation, might increase urea absorption from these ATLs and thereby make the UCM more effective.

The optimization results of the present study show that the concentrating capability of a model can be augmented by well-selected parameter sets; these results also suggest that in vivo the production of maximally concentrated urine depends critically on favorable combinations of morphological and transport properties; these combinations are likely regulated and coordinated by the organism. A more complete understanding of the UCM will require a model that is more faithful to the rat kidney. The present model lacks an explicit representation of the OM, which, as noted above, likely exerts a significant influence on the IM UCM; the model lacks an explicit representation of the medullary vasculature, which makes it difficult to accurately assess the impact of vascular countercurrent exchange; and the model uses a representation in which at each medullary level the CC solute concentration is uniform, whereas the three-dimensional structure of the IM almost certainly promotes preferential interactions among vessels and tubules (Pannabecker et al., 2004; Pannabecker and Dantzler, 2004; Pannabecker and Dantzler, 2006; Pannabecker and Dantzler, 2007).

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Figure 1.

Schematic diagram of the central core (CC) model formulation showing six representative loops of Henle and a composite collecting duct (CD); in the numerical formulation of the model, 300 loops of Henle were represented to approximate a continuously decreasing number of loops as a function of medullary depth x . LDL2_s, a descending thin limb (DTL) of a loop of Henle that turns within the first millimeter of the inner medulla (IM); LDL2_s does not contain significant aquaporin-1 (Pannabecker et al., 2004) and is therefore assumed to be water-impermeable. LDL2, a segment that makes up the first ~40% of a DTL that reaches beyond the first millimeter of the IM; this segment contains plentiful aquaporin-1. LDL3, a segment that makes up most of the remaining ~60% of the DTL, which does not contain significant aquaporin-1, but may be moderately permeable to water (Chou and Knepper, 1992). The prebend segment (PBE) is considered to have the same transport properties as the ATL (ascending thin limb), which has no significant aquaporin-1 and is assumed to be water-impermeable. The collecting duct (CD) is permeable to water and has varying degrees of urea permeability and NaCl active transport, as a function of x .

Figure 2. CD transepithelial transport properties. A, CD urea permeability as a function of IM depth for three values of λ . B, CD maximum Na^+ active transport as a function of normalized IM depth (i.e., x/L) for three values of β .

Figure 3.

Profiles of tubular fluid osmolality as a function of IM depth. Osmolality profiles for longest loop of Henle (DTL and ATL), collecting duct (CD), and central core (CC): A1, base-case parameters; B1, parameters that maximize E . Osmolality profiles for loops of Henle reaching to 20%, 40%, 70%, and 100% of the IM depth: A2, base-case parameters; B2, parameters that maximize E . Segments with flow direction from $x/L = 0$ to $x/L = 1$ (DTL and CD) are indicated with black solid curves whereas segments with flow direction from $x/L = 1$ to $x/L = 0$ (ATL and CC) are indicated with dashed curves. Because of overlapping, the total fluid carried by all the ATLs to the OM is dilute relative to the flow in other tubules at the OM-IM boundary.

Figure 4.

Profiles of tubular fluid concentrations in longest DTL, longest ATL, CD, and CC as a function of normalized IM depth. Sodium and CD nonreabsorbable solute concentration profiles: A1, base-case parameters; B1, parameters that maximize E . Dashed curve, CD nonreabsorbable solute (NR) concentration (NR concentration is 0 in loops of Henle and in CC). Urea concentration profiles: A2, base-case parameters; B2, parameters that maximize E .

Figure 5.

Profiles of tubular flow rate as a function of normalized IM depth. Aggregate fluid flow rate (per nephron, based on 38,000 nephrons/kidney) in DTLs and ATLs, and fluid flow rate (per nephron) in CD and CC: A1, base-case parameters; B1, parameters that maximize E . CD solute flow rates (per nephron): A2, base-case parameters; B2, parameters that maximize E .

Table 1

Fixed parameter values (S_f)

Description	Value
DTL diameter	$15 \rightarrow 11 \uparrow 13^* \mu\text{m}$
ATL diameter	$20 \rightarrow 13^* \mu\text{m}$
CD diameter	$20 \rightarrow 25^* \mu\text{m}$
Inner medullary length, L	5 mm
Prebend segment length, L_{PBE}	166.7 μm
Number of long loops of Henle	12,667
Number of CDs extending into the IM	7,300
Partial molar volume of water, V_w	$0.018136^{\dagger} \text{ cm}^3/\text{mmole}$
Osmotic coefficients of NaCl and NR, ϕ_k	$1.84^{\ddagger}$
Osmotic coefficient of urea, ϕ_2	$0.97^{\ddagger}$
Michaelis constant, K_M	40 mM
H_2O permeability, P_f ($\mu\text{m/s}$)	
LDL2 _s , PBE, ATL _s , ATL	0
LDL2	2200
LDL3	400
CD	750
Na^+ permeability, P_{Na} (10^{-5} cm/s)	
LDL2 _s , LDL2, LDL3	0
PBE, ATL _s , ATL	80
CD	1
Urea permeability, P_{urea} (10^{-5} cm/s)	
LDL2 _s , LDL3, PBE, ATL _s , and ATL	1
LDL2	13
CD	1 to 110
Na^+ active transport rates, $V_{\text{max,Na}}$ ($\text{nmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$)	
All loop segments	0
CD	$9 \rightarrow 2.5 \rightarrow 0$
Values at OM-IM boundary	
$F_v(0)$ (nl/min)	
Shortest DTL	$5.0^{\S}$
Longest DTL	$7.5^{\S}$
$C_{\text{NR}}(0)$ (mM)	
CD	20

* Right arrow ($\rightarrow$) indicates that the parameter varies linearly between the given values as medullary depth x increases; up arrow ($\uparrow$) indicates that parameter abruptly increases to the given value.

$\dagger$ At 37°C.

$\ddagger$ Dimensionless. NR, nonreabsorbable solute, which is assumed to be present in significant amounts only in the tubular fluid of the CD.

$\S$ These flows are approximately 1/5 of the SNGFR's reported by Rouffignac and Bonvalet (1970): 27.9 nl/min for superficial nephrons and 38.5 nl/min for juxtamedullary nephrons.

Table 2

Optimization study with function E (13)

(A) Parameters			
Description	Base case, value and range		Optimum case
Outer-inner medullary boundary values			
Scaling factor for DTL water inflow, γ	1.00 [0.85,1.15]		1.15
DTL $C_{\text{urea}}(0)$ (mM)	30.0 [25.5,34.5]		25.5
DTL $C_{\text{Na}^+}(0)$ (mM)	350.0 [347.6,352.4]*		352.4
CD $F_v(0)$ (nl/min)	2.40 [2.00,4.00]		2.97
CD $C_{\text{urea}}(0)$ (mM)	314.54 [267,362]		362
CD $C_{\text{Na}^+}(0)$ (mM)	180 [155,205]*		155
CD transport parameters			
Location where CD P_{urea} changes, λ	0.500 [0.350,0.650]		0.350
Scaling factor for CD $V_{\text{max}}, \text{Na}^+, \beta$	1.00 [0.500,1.50]		1.50
Population distribution scaling parameters			
Loops of Henle, a	1.00 [0.85,1.15]		1.15
CDs, b	1.00 [0.85,1.15]		0.932
(B) Simulation values and experimental measurements			
Description	Base case	Optimum case	Experiment [†]
Urine			
Osmolality (mOsm/(kg H ₂ O))	1,264	1,738	1,805±194
Na ⁺ concentration (mM)	254	191	158±53
Urea concentration (mM)	598	1,030	946±66
NR concentration (mM)	118	211	657
Flow (nl/min/nephron [‡])	0.0781	0.0542	0.0658
FWA (nl/min/nephron)	0.241	0.249	0.317
Bend of longest loop of Henle			
Osmolality (mOsm/(kg H ₂ O))	1,073	1,401	1,264±82
Na ⁺ concentration (mM)	466	550	475±34
Urea concentration (mM)	222	401	287±32
Flow (nl/min)	4.81	4.25	3.59±0.30

* Range of Na⁺ concentration variation corresponds to the extrema of the stipulated urea concentration range, under the assumption that inflow osmolality is fixed; see the paragraph in Section 2.1 that contains Eqn (9).

[†] Urine composition during antidiuresis in rats with a urine flow of ~5 $\mu\text{L}/\text{min}$ per rat (Atherton et al., 1968). Experimental data are mean values or estimated mean values ($\pm\text{SD}$ or $\pm\text{SE}$) from Hai and Thomas (1969), for urine osmolality and urea concentration; from Atherton et al. (1969), for urine Na⁺ concentration and NR concentration, which was calculated as though it arose entirely from electrolyte solutes such as KCl; and from Pennell et al. (1974), for composition and flow of Henle's loop fluid.

[‡] Based on 38,000 nephrons per kidney. NR, CD nonreabsorbable solute.

Table 3

Optimization study with function $\bar{E}$ (16)

(A) Parameters			
Description	Base case, value and range		Optimum case
Outer-inner medullary boundary values			
Scaling factor for DTL water inflow, γ	1.00 [0.85,1.15]		1.15
DTL $C_{\text{urea}}(0)$ (mM)	30.0 [25.5,34.5]		25.5
DTL $C_{\text{Na}^+}(0)$ (mM)	350.0 [424.7,429.5]*		429.5
CD $F_v(0)$ (nl/min)	2.40 [2.00,4.00]		3.81
CD $C_{\text{urea}}(0)$ (mM)	314.54 [267,600]		600
CD $C_{\text{Na}^+}(0)$ (mM)	180 [107,282]*		107
CD transport parameters			
Location where CD P_{urea} changes, λ	0.500 [0.350,0.650]		0.350
Scaling factor for CD $V_{\text{max}, \text{Na}^+}$, β	1.00 [0.500,1.50]		1.50
Population distribution scaling parameters			
Loops of Henle, a	1.00 [0.85,1.15]		1.15
CDs, b	1.00 [0.85,1.15]		0.850
(B) Simulation values and experimental measurements			
Description	Base case	Optimum case	Experiment [†]
Urine			
Osmolality (mOsm/(kg H ₂ O))	1,264	1,575	2,240
Na ⁺ concentration (mM)	254	93.0	134
Urea concentration (mM)	598	1,280	1,660
NR concentration (mM)	118	88.2	207
Flow (nl/min/nephron [‡])	0.0781	0.166	0.128
Na ⁺ flow (pmol/min/nephron)	19.8	15.4	17
Urea flow (pmol/min/nephron)	46.7	212	212
FWA (nl/min/nephron)	0.241	0.677	0.797
Bend of longest loop of Henle			
Osmolality (mOsm/(kg H ₂ O))	1,073	1,188	NA
Na ⁺ concentration (mM)	466	438	NA
Urea concentration (mM)	222	394	NA
Flow (nl/min)	4.81	5.68	NA

^{*} Range of Na⁺ concentration variation corresponds to the extrema of the stipulated urea concentration range, under the assumption that inflow osmolality is equal to 815 mOsm/(kg H₂O)); see the paragraph in Section 2.1 that contains Eqn (9).

[†] Experimental data are mean values or estimated mean values from Gamble et al., (1934), for urine osmolality, urine urea concentration, and urine Na⁺ concentration; NR concentration was calculated as though it arose entirely from electrolyte solutes such as KCl; information from the work of Gambel et al., (1934) is not available (NA) for the solute compositions and water flow rates at the bends of long loops of Henle.

[‡] Based on 38,000 nephrons per kidney. NR, CD nonreabsorbable solute.