Uphill Transport of Urea in the Dog Kidney: Effects of Certain Inhibitors *

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Summary. To study the renal medullary transport and accumulation of urea in dogs independent of water transport, we obliterated the medullary electrolyte gradient by a sustained ethacrynic acid diuresis. Infusions of urea were also given at various rates to vary urinary urea concentration. In the steady state, the kidneys were removed, and slices were analyzed for water, urea, and electrolytes. In every experiment in 15 dogs over a range of urinary urea concentration from 19 to 230 mmoles per L and urine flow from 0.5 to 9.7 ml per minute per kidney, an intrarenal urea gradient persisted, and urinary urea concentration was always lower than papillary water urea concentration. The magnitude of this uphill urinary–papillary gradient (mean ± SE = −21 ± 2.9 mmoles per L) was not affected by hemorrhagic hypotension or a nonprotein diet.

In 12 additional experiments begun similarly, inhibitors were infused into one renal artery. Both iodoacetate, an inhibitor of anaerobic glycolysis, and acetamide, an analogue of urea, markedly and significantly reduced both the intrarenal urea gradient and the uphill urinary–papillary gradient. In contrast, cyanide, an inhibitor of oxidative metabolism, had no observable effect on the urea gradients. The data are best explained by postulating an active transport system for urea in the medullary collecting duct deriving its energy from anaerobic glycolysis.

Introduction

It has long been recognized that urea plays a unique role in the renal concentrating mechanism...
(8) to water, is also presumed to increase the permeability of the collecting duct to passive urea transfer (9). Therefore, all urea transport is indirectly dependent on the maintenance of an intramedullary electrolyte gradient, which is responsible for the osmotic flow of water out of the lumen of the collecting duct, and thus creates the favorable urea gradient.

There are some species, such as the elasmobranch fishes (10), in which active tubular reabsorption of urea seems to exist. In the rat, protein depletion and mannitol diuresis can produce higher concentrations of urea in renal papillary water than in the final urine (5, 11, 12). These data have been interpreted as suggesting active transport of urea across the collecting duct. This concept recently has been supported by the micropuncture studies of Lassiter, Mylle, and Gottschalk (13), which revealed a higher urea $^{14}$C concentration in the vasa recta than in the collecting duct at the same level. However, the applicability of data on urea transport in the rat to other species, such as dog and man, has been questioned (5).

We designed the present study to test the hypothesis of passive urea transport in the dog's renal medulla by studying medullary urea accumulation in the steady state when the medullary electrolyte gradient was obliterated with ethacrynic acid. Our data revealed that under these conditions a medullary urea gradient was still maintained, and furthermore, an uphill concentration gradient for urea between final urine and papillary tip was present. The effects on this uphill urea gradient of iodoacetate, an inhibitor of anaerobic glycolysis; cyanide, an inhibitor of oxidative metabolism; and acetamide, a possible competitive inhibitor, were also investigated.

Methods

A total of 30 acute experiments were performed in 30 female mongrel dogs weighing 20 to 25 kg. For 1 week before the experiment, 27 animals were maintained on a standard diet containing more than 100 g protein per day and adequate minerals and vitamins. In three a synthetic nonprotein diet similar to that utilized by Manitius, Pigeon, and Epstein (14) was substituted for the standard diet. On the day before each experiment, water was withheld, and 5 U of Pitressin tannate in oil was administered. After the induction of light anesthesia with intravenous sodium pentobarbital, a midline abdominal incision was made, the ureters were isolated near their entrance to the urinary bladder, and polyethylene cannulas were inserted up to the pelvis of each kidney. A priming dose of creatinine was administered intravenously, followed by a sustaining infusion of isotonic saline at a rate of 1 ml per minute containing adequate amounts of creatinine for clearance determinations plus aqueous Pitressin given at a rate of 50 mU per kg per hour throughout the experiment. Cannulas were inserted into the femoral artery for blood sampling and into the femoral vein for subsequent administration of the various loading solutions. The following experimental modifications were then utilized.

Mannitol–urea diuresis. Three preliminary experiments were performed (dogs $U_1$, $U_2$, $U_3$) in which diuresis was induced by the intravenous infusion of 10% mannitol at a rate of 12 to 15 ml per minute. When total urine flow ($V$) from both kidneys reached 16 to 18 ml per minute, the mannitol was stopped and a solution of 8% urea in 75 mM saline was infused at a comparable rate until a stable diuresis was maintained for 45 to 60 minutes at V of 20 to 24 ml per minute. For the last few collection periods, the urine was collected separately from each kidney. The renal pedicles were then clamped, and both kidneys were rapidly removed and immediately frozen in a mixture of acetone and dry ice. They were subsequently sliced and analyzed for urea, electrolytes, and water as described below.

Ethacrynic acid diuresis alone. Thirty to 45 minutes after instituting the priming and sustaining clearance infusions, we gave an intravenous priming dose of ethacrynic acid of 1.25 mg per kg (dogs $D_1$, $D_2$, $D_3$, $D_4$). This was followed immediately by a sustaining infusion of ethacrynic acid in 5% dextrose and water administered at a rate of 1.25 mg per kg per hour for the remainder of the experiment. One to 2 hours after the priming dose of ethacrynic acid, when $V$ was stable and urinary osmolality was virtually isotonic for three to four 5-minute collection periods, both kidneys were removed and frozen.

Standard protocol: ethacrynic acid–urea diuresis. The experiments were begun with an ethacrynic acid diuresis as outlined above (dogs $U_{15}$, $U_{17}$, $U_{18}$). In addition, to serve as a control procedure for subsequent studies involving intra-arterial infusions, both renal arteries were dissected free, and in dogs $U_{18}$ and $U_{19}$ isotonic saline at a rate of 0.25 ml per minute was infused into each kidney throughout the experiment. Once a stable state of diuresis due to ethacrynic acid was achieved for several periods, an intravenous infusion of 8% urea in 75 mM saline was added. This was given at various rates in the different experiments so as to achieve different steady state values of $V$ and urinary urea concentrations ($U_{\text{urea}}$) at the end of the experiment. When $V$ was stable within 1 to 2 ml per minute for 30 to 45 minutes during the combined ethacrynic acid–urea diuresis, the urine was collected separately from each kidney for two to four collection periods, after which both kidneys were removed and frozen. In several experiments, in order to prevent marked sodium depletion and a sharp fall in glomerular filtration rate (GFR), we gave additional isotonic saline after institution of ethacrynic acid diuresis.
Standard protocol plus hemorrhage. In two additional experiments (dogs U₁, U₅), in order to study the effects of hemorrhagic hypotension and to reduce V markedly while still maintaining a reasonably high filtered load of urea, we removed arterial blood from the femoral artery during the ethacrynic acid–urea diuresis to lower mean arterial blood pressure (BP) to approximately 50 mm Hg (from previous values exceeding 100 mm Hg). BP was monitored with a mercury manometer. During stable V and BP, the kidneys were removed and frozen.

Standard protocol plus nonprotein diet. Dogs U₁₀, U₁₁, and U₁₂ received a synthetic nonprotein diet for 1 week before we performed standard ethacrynic acid–urea diuretic experiments.

Experiments involving use of inhibitors. In 12 experiments, the study was begun with an ethacrynic acid diuresis. In all of these animals, during the initial preparations, both renal arteries (before the bifurcation) were dissected free and no. 23-gauge needles inserted into each vessel, the tip pointing against the direction of blood flow. The needles were kept open with infusions of isotonic saline at a rate of 0.25 ml per minute. A saline infusion to the contralateral renal artery was maintained, and this served as the control kidney. The dosages of both cyanide and iodoacetate chosen were in the dose range already demonstrated by Fujimoto, Nash, and Kessler (15), and by Herms and Malvin (16), to have had definite unilateral effects on renal function (urinary osmolality, sodium excretion, or both) compared to the contralateral control.

Cyanide. In three such experiments (dogs U₁₁, U₁₆, U₂₆), sodium cyanide was infused into one kidney at a rate of 0.012 mg per kg per minute (approximately 2.4 × 10⁻⁵ mmole per kg per minute). Thirty minutes after initiating the cyanide infusion, the intravenous infusion of 8% urea was begun, and 60 to 70 minutes later, during a stable V, both kidneys were removed and frozen. The mean total dose of cyanide per kidney per experiment was approximately 0.4 mmole.

Iodoacetate. In three experiments (dogs U₁₆, U₁₇, U₂₆) moniodoacetate was infused into one renal artery during stable ethacrynic acid diuresis at a rate of 0.10 to 0.15 mg per kg per minute (approximately 5.3 to 7.4 × 10⁻⁶ mmole per kg per minute). Forty to 60 minutes later, the intravenous infusion of 8% urea was started, and the experiment was terminated with removal of the kidneys approximately 50 to 60 minutes later during stable V. The mean total dose of iodoacetate per animal was 1.2 mmole. Three additional experiments with iodoacetate were performed (dogs U₁₁, U₁₇, U₂₆), in which comparable doses of iodoacetate were infused into one renal artery for 80 to 100 minutes after the institution of the combined ethacrynic acid–urea diuresis. Thus, in three animals, iodoacetate was given before the urea infusion, and in three others iodoacetate was given after the urea diuresis was established.

Acetamide. In dogs U₁₆, U₁₇, and U₁₇, during the ethacrynic acid diuresis, a solution containing acetamide in quantities calculated to provide a renal arterial blood level of approximately 45 mmols per L was infused into one renal artery in place of the saline infusion. This infusion was continued for 30 minutes, after which it was replaced by a solution infused at the same rate which contained the same concentration of acetamide plus urea at a concentration equal to one-half that of acetamide. A solution containing the identical concentration of urea was also simultaneously infused into the renal artery of the control kidney. Thus, acetamide and urea were infused in 2:1 proportions into the experimental kidney, and only urea was infused into the control. In approximately 60 minutes, during stable V from both kidneys, the kidneys were removed and frozen.

Processing of renal tissue and chemical analyses. The kidneys of all experimental animals plus ten kidneys from five nondiuretic hydropenic animals were frozen and sliced in the manner described below.

Two cross-sectional slices approximately ½ inch thick were taken from each side of each frozen kidney. With a cartilage knife, slices perpendicular to the cortico-papillary axis were taken from cortex, outer papilla (i.e., peripheral portion of the inner zone adjacent to the outer zone of the medulla), and the tip of the papilla. Each slice was divided longitudinally into two pieces, each weighing 100–300 mg. We dried one in an oven at 103 to 105°C for 48 hours to estimate water content of tissue. The dried tissue, after weighing, was then digested with 5 ml of concentrated nitric acid, after which the solution was diluted with water; sodium and potassium were determined by flame photometry.

The second piece of each slice, after weighing, was transferred to a mortar and pestle for homogenization and dilution with 10 ml distilled water. One-ml samples of this homogenate were taken in duplicate for determination of urea by the Conway microdiffusion technique (17). Ammonia blanks were done in each analysis. Preliminary experiments revealed that the precision of the determination of urea concentration in tissue water in our laboratory was 1 to 2 mmole per L or better in duplicate specimens, and recovery from tissue was 100 ± 0.5%. In each experiment, sodium, potassium, urea, ammonia, and water content were determined on two slices from each kidney at a given level (cortex, outer papilla, and papillary tip); the values were averaged to yield the mean value of that level in a given kidney.

Dialysis of tissue homogenates. To assess the extent of tissue protein binding of urea, we performed two additional in vitro experiments. Each was begun by inducing an ethacrynic acid diuresis in a dog. During stable V, the kidneys were removed and the inner white medulla from each kidney was removed and homogenized in a mortar and pestle, with sufficient isotonic saline to provide no more than 1:2 to 1:3 dilution of the tissue. Isotonic saline solution was used as the dialyzing fluid. In experiment 1, sufficient urea was added to homogenate and dialyze to provide approximately equivalent concentrations of urea on both sides of the membrane before dialysis. In experiment 2, no urea was added to the predialysis dialyzing fluid. Dialyses were performed for 240 minutes with a cuprophane membrane suspended between two Lucite chambers, each with a capacity of 2.5
ml. Turbulence was maintained on each side of the membrane by bubbling air through the dialyzer and homogenate.

All blood and urine specimens were analyzed for osmolality, sodium, potassium, creatinine, and urea by methods described previously (18). Urea was determined by the Conway microdiffusion technique (17). Osmolar clearances were calculated as follows: 

\[ C_{\text{osm}} = \frac{U_{\text{osm}} \times V}{P_{\text{osm}}} \]

and resorption (T′mNO) were calculated as follows: 

\[ T′_{\text{mNO}} = C_{\text{osm}} - V \]

Uosm = urinary osmolality (milliosmoles per kilogram), Posm = plasma osmolality (milliosmoles per kilogram), and V = urine flow (milliliters per minute). Statistical calculations were done with standard techniques (19).

**Results**

Mannitol–urea experiments. Because mannitol diuresis has been a technique used in rats to produce a higher urea concentration in the renal papilla than in final urine (5, 12), three preliminary experiments with mannitol diuresis followed by urea diuresis were performed. In these experiments, during stable V from both kidneys greater than 17 ml per minute, the urine was still hypertonic (mean Uosm/Posm = 1.21), and there were still intrarenal gradients for both urea and nonurea solute from cortex to papillary tip. In two of the three dogs (U1 and U2), the urea concentration of papillary tip water was lower than final urinary urea concentration. Mean Δ[urea] (i.e., Uurea minus papillary water urea concentration) of all kidneys in these animals was +16 mmoles per L. In dog U3, Δ[urea] of the left and right kidneys was −5 and −7 mmoles per L, respectively. An uphill urea gradient was, therefore, present in one of three dogs (two of six kidneys), but the difficulty of reproducing this phenomenon in the dog made it necessary to utilize a different approach to study urea transport.

**Experiments utilizing ethacrynic acid.** When an experiment was initiated by a sustained ethacrynic acid diuresis, an uphill urinary–papillary gradient (negative Δ[urea]) was produced in every instance, except in those kidneys subjected to certain inhibitors (vide infra). Table I outlines a typical ethacrynic acid–urea experiment (standard protocol) in dog U4. During the initial ethacrynic acid diuresis Uurea fell to very low levels, but it was subsequently elevated by the urea infusion to a higher steady state level at the end of the experiment. The renal tissue and urinary analytical data of this experiment (dog U4) are contained in Table II, which summarizes the tissue and urinary data from all experiments employing ethacrynic acid without the use of inhibitors. Both kidneys from dog U4 show total obliteration of the intrarenal electrolyte gradient. In striking contrast, an intrarenal urea gradient was still present; furthermore, the papillary tip urea concentration of both kidneys was higher than the ipsilateral pelvic urinary urea concentration. For the left and right kidneys Δ[urea] was −29 and −37 mmoles per L, respectively.

From Table II, it can be seen that a downhill or positive Δ[urea] was present only in the hypodpressive controls, where an intramedullary electrolyte gradient was also present. In every other experiment, regardless of the Uurea, V, GFR, or plasma urea concentration, an uphill or negative Δ[urea] was obtained. Neither hemorrhage nor protein depletion had a strong influence on the magnitude of the uphill urea gradient, since there was no significant difference statistically in the Δ[urea] among groups C, D, and E of Table II. The grand mean Δ[urea] ± standard error for all

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**TABLE I**

A representative experiment utilizing standard protocol in dog U4*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Uosm (mOsm/kg)</th>
<th>Posm (mOsm/kg)</th>
<th>V (ml/min)</th>
<th>Uurea (mmoles/L)</th>
<th>GFR (ml/min)</th>
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<tr>
<td>0–21</td>
<td>1942</td>
<td>308</td>
<td>0.36</td>
<td>1,152</td>
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<tr>
<td>21–100</td>
<td>311</td>
<td>312</td>
<td>12.8</td>
<td>71</td>
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<tr>
<td>170–175</td>
<td>310</td>
<td>311</td>
<td>12.0</td>
<td>68</td>
<td></td>
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<tr>
<td>180–185</td>
<td>310</td>
<td>314</td>
<td>11.1</td>
<td>17</td>
<td>77</td>
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<tr>
<td>185</td>
<td>310</td>
<td>314</td>
<td>11.1</td>
<td>17</td>
<td>77</td>
</tr>
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</table>

* Standard protocol = hydropenia–ethacrynic acid–8% urea.

Abbreviations: Uosm = urinary osmolality; Posm = plasma osmolality; V = urine flow; Uurea = urinary urea concentration; GFR = glomerular filtration rate.

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* Downloaded from [http://www.jci.org](http://www.jci.org) March 31, 2017. https://doi.org/10.1172/JCI105540
### TABLE II

**Summary of experiments demonstrating uphill urinary-papillary urea gradient**

<table>
<thead>
<tr>
<th>Dog</th>
<th>Cortex [Na+K] mmol/L</th>
<th>Outer papilla [Na+K] mmol/L</th>
<th>Papillary tip [Na+K] mmol/L</th>
<th>Urine [Na+K] mmol/L</th>
<th>Δ[Urea] mg%</th>
<th>P&lt;sub&gt;urea&lt;/sub&gt; ml/min</th>
<th>U&lt;sub&gt;osm&lt;/sub&gt; P&lt;sub&gt;urea&lt;/sub&gt; ml/min</th>
<th>V ml/min</th>
<th>GFR ml/min</th>
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<tr>
<td>Mean</td>
<td>149</td>
<td>36</td>
<td>230</td>
<td>241</td>
<td>356</td>
<td>788</td>
<td>256</td>
<td>973</td>
<td>+185</td>
</tr>
<tr>
<td>SD</td>
<td>6</td>
<td>10</td>
<td>7</td>
<td>6</td>
<td>47</td>
<td>206</td>
<td>156</td>
<td>64</td>
<td>128</td>
</tr>
</tbody>
</table>

**A) Nondiuretic controls**

- D<sub>1</sub> 158 38 139 44 138 58 140 45 -13 8.9 1.08 1.9 23
- D<sub>2</sub> 155 21 147 28 147 35 149 23 -12 6.8 1.02 3.8 25
- D<sub>4</sub> 152 22 143 29 145 36 147 19 -17 6.9 1.00 6.6 31
- D<sub>15</sub> 149 25 154 34 172 38 155 20 -18 6.4 1.07 4.8 35

**B) Ethacrynic acid diuresis alone**

- U<sub>4</sub> L 147 149 126 177 128 205 105 176 -29 90 0.97 8.7 21
- R 144 152 129 183 143 225 105 188 -37 90 0.99 7.2 19
- U<sub>5</sub> L 137 166 129 202 128 226 101 203 -23 121 0.98 9.7 20
- R 141 187 120 188 131 218 97 203 -15 121 0.98 9.7 19
- U<sub>6</sub> L 144 63 129 95 127 104 110 94 -10 94 1.01 5.1 10
- R 148 63 135 89 130 124 97 109 -12 94 1.06 1.6 10
- U<sub>7</sub> L 143 57 125 71 134 77 120 71 -6 33 1.00 5.5 25
- R 144 54 131 77 135 81 120 71 -10 33 1.00 5.4 25
- U<sub>11</sub> L 146 93 123 138 132 164 113 141 -23 67 1.06 5.6 19
- R 147 123 125 163 131 191 101 171 -30 80 1.10 2.7 12
- U<sub>10</sub> L 141 94 135 106 134 137 113 119 -21 49 0.95 6.9 21
- R 146 86 146 96 135 137 113 112 -49 49 0.99 8.3 21

**C) Ethacrynic acid plus urea diuresis (standard protocol)**

- U<sub>8</sub> L 157 64 148 74 162 136 92 97 -39 32 1.04 1.7 18
- R 151 57 143 50 162 100 87 63 -32 32 1.01 0.5 8
- U<sub>4</sub> L 126 131 104 170 118 200 63 193 -7 83 1.06 1.4 9
- R 127 140 102 180 122 201 82 188 -13 83 1.03 2.7 11

**D) Standard protocol plus hemorrhage**

- U<sub>10</sub> L 132 132 121 167 124 209 92 176 -33 94 0.98 4.8 17
- R 134 134 116 59 120 224 93 182 -42 94 0.98 4.1 15
- U<sub>11</sub> L 130 114 116 128 121 182 90 167 -15 67 1.04 2.3 14
- R 130 110 119 126 120 167 90 146 -21 67 1.01 6.7 21
- U<sub>12</sub> L 136 92 110 167 121 215 60 194 -21 62 1.12 0.8 8
- R 137 92 111 168 110 209 71 186 -23 62 1.08 2.1 14

- In dog U<sub>11</sub> the left kidney was removed 1 hour before the right kidney, thus accounting for the higher plasma and cortical urea concentrations associated with the right kidney. Note, however, that the medullary urea gradients are similar in both kidneys.

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* Abbreviations: [Na + K] = sum of sodium and potassium concentrations; [urea] = urea concentration; Δ[urea] = difference in urea concentration between pelvis urine and ipsilateral papillary tip; P<sub>urea</sub> = plasma urea; remainder of abbreviations as in Table I.
† Mean Δ[Urea] ± standard error from all experiments in groups B, C, D, E = -21 ± 2.9 mmol/L per kg H<sub>2</sub>O, P < 0.001.
‡ Values in this group are means ± standard deviations of data obtained from ten kidneys of five maximally hydropeptic dogs.
§ In group B only, urine was collected from both kidneys throughout the experiment. Therefore, the tissue data represent the means from both kidneys, and Δ[Urea] = the difference between the urea concentrations of the combined urines and the mean values from papillae of both kidneys; for purposes of comparison with the other experiments, values given for V and GFR in group B represent one-half the sum of the values from both kidneys.
‖ In dog U<sub>11</sub>, the left kidney was removed 1 hour before the right kidney, thus accounting for the higher plasma and cortical urea concentrations associated with the right kidney.
the experimental kidneys was \(-21 \pm 2.9\) mmoles per L, \(p < 0.001\).

The effects of \(U_{\text{urea}}\) and of \(V\) on the magnitude of \(\Delta[\text{urea}]\) are illustrated in Figures 1 and 2, respectively. In these charts, in addition to the data from the experiments listed in Table II, the data from the control kidneys of the 12 experiments that utilized inhibitors are also plotted. In Figure 1, \(U_{\text{urea}}\) is plotted against urea concentration of papillary water. Since every point lies below the 45° line, it is apparent that an uphill urinary-papillary gradient was present in every experiment. Furthermore, it is clear that the magnitude of this gradient was not specifically related to \(U_{\text{urea}}\), which varied from 19 to 230 mmoles per L. This gradient did not appear to be altered significantly by hemorrhagic hypotension (upright triangles) or a nonprotein diet (inverted triangles). Similarly, as illustrated in Figure 2, \(\Delta[\text{urea}]\) was not closely related to urine flow, which varied from 0.5 to 11.6 ml per minute per kidney.

**Effects of sodium cyanide.** Figure 3 summarizes the renal tissue and urinary concentrations of urea in the three animals prepared according to the standard protocol which also received sodium cyanide in one renal artery (\(U_{21}\), \(U_{22}\), \(U_{23}\)). The data from dog \(U_{20}\) are also presented for comparison. This was an experiment in which both renal arteries were dissected free, and only isotonic saline was infused into them throughout the experiment. It is apparent that in

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**Fig. 1.** Relationship between urinary and papillary urea concentrations. Symbols: half circle = ethacrynic acid diuresis alone; solid circle = standard protocol (ethacrynic acid + urea diuresis); upright triangle = standard protocol + hemorrhage; inverted triangle = standard protocol + nonprotein diet; open circle = control kidneys of experiments using metabolic inhibitors (see text). Note that every point lies below the 45° line, indicating an uphill urinary-papillary gradient over a broad range of urinary and papillary urea concentrations.

**Fig. 2.** Relationship between magnitude of urea gradient (urinary urea concentration minus urea concentration of papillary tip water) and urine flow per kidney. The gradient was not affected by varying urine flow over a wide range. Symbols are the same as in Figure 1.

**Fig. 3.** Effects of sodium cyanide on intrarenal and urinary-papillary urea gradients. In each experiment the solid line and circles represent the control kidney, and the broken line and open circles represent the experimental kidney, which received cyanide (dogs \(U_{21}\), \(U_{22}\), and \(U_{23}\)). Dog \(U_{20}\), a sham experiment, was prepared exactly as the other animals, but isotonic saline was infused into both kidneys. PAP = renal papilla. Note the absence of any effects of cyanide. See text.
every cyanide experiment the intrarenal urea concentration pattern and the uphill urinary-papillary urea gradient were virtually the same in the control kidneys (solid lines) and the experimental kidneys (broken lines). Hence, doses of cyanide as great as those already used by Fujimoto and his colleagues (15) to demonstrate definite effects on renal oxygen consumption and sodium reabsorption had no demonstrable effect on the renal distribution of urea or on the uphill urinary-papillary urea gradient. The mean difference between the experimental Δ[urea] and the control Δ[urea] was −4.7, p > 0.25. In the 11 experiments in which the conditions were similar for both kidneys and no inhibitors were used, there was also no significant difference between the two kidneys of a given animal in the magnitude of the uphill urinary-papillary gradient (standard error of mean difference = 3.2, p > 0.5).

Effects of iodoacetate. Two types of experiments utilizing monooiodoacetate were performed. In one group (dogs U16, U17, U19), iodoacetate was infused into one renal artery after the institution of both the ethacrynic acid and the urea diureses. Thus, some build-up of papillary urea concentration was allowed to occur before the use of iodoacetate. The results from these experiments are presented in Table III. In dogs U16 and U17, unlike all other experiments with inhibitors, the control kidneys were removed and frozen before the infusion of iodoacetate into the experimental kidneys. This accounts for the higher plasma and cortical urea concentrations in the experimental kidneys in these studies. In dog U19, the control kidney remained in situ until the end of the entire experiment. Regardless of the different base lines, however, these data show that the intrarenal urea gradient of the experimental kidney was sharply reduced and the Δ[urea] of the experimental kidney was less than that of the control kidney by two-thirds to three-fourths, a difference that is statistically significant (p < 0.02). There were no consistent differences in V and GFR between the control and experimental kidneys that might be invoked to account for these differences.

In a second group of three experiments (dogs U21, U23, U24), which are summarized in Figure 4, iodoacetate was begun before initiation of the urea infusion. Both kidneys were removed together at the end of the experiment. Under these con-

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<tr>
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<tbody>
<tr>
<td>V (ml/min)</td>
<td>180</td>
<td>130</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>120</td>
<td>80</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Δ[urea] (mmol/L)</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
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<td>Standard error of mean difference</td>
<td>2</td>
<td>1</td>
<td>1</td>
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TABLE III

Intrarenal and urinary urea concentrations in three experiments in which iodoacetate was superimposed on an ethacrynic acid-urea diuresis.

*Abbreviations: cont. = control kidney; exp. = experimental kidney; V = renal plasma flow; GFR = glomerular filtration rate; Δ[urea] = difference in renal urea concentration between cortices and outer papilla; P = p value; mean (experimental Δ[urea]) - control Δ[urea] = difference in Δ[urea] between the two kidneys; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; V, GFR, Δ[urea] are given as mean values; standard error of mean difference (SEM) for higher cortical [urea] and P values in the experimental kidneys, despite the higher base line in the experimental kidney, the intrarenal urea gradient and Δ[urea] were qualitatively the same as the others.
FIG. 4. Effects of iodoacetate on intrarenal and urinary-papillary urea gradients. Dog U20 is a sham experiment, shown for comparison. Note the marked lowering of the intrarenal gradient and the obliteration of the uphill urinary-papillary gradient in all experimental kidneys (broken line) compared to contralateral control kidneys (solid line). In dog U26, the control kidney was inadvertently lost. See text.

Effects of acetamide. Data from three experiments (dogs U26, U27, U28) in which acetamide and urea were infused into the experimental kidney and urea alone was simultaneously administered to the control kidney are illustrated in Figure 5. It is apparent that, in the presence of acetamide, there was a sharp reduction in the medullary urea gradient; furthermore, the uphill Δ[urea] was virtually abolished in dogs U26 and U28 and sharply reduced in dog U27. The Δ[urea] of the experimental kidney, which received acetamide, was significantly different from that of the control (p < 0.001).

Thus, to summarize the results of the experiments with inhibitors, both iodoacetate and acetamide reduced the intrarenal urea gradient and the uphill urinary-papillary gradient for urea. On the other hand, no effects on these variables were observed with sodium cyanide. No innate differences between two kidneys in the same animal were observed in experiments similarly performed without the use of specific inhibitors.

In vitro dialysis experiments. These data are summarized in Table IV. They indicate no differential distribution of urea at equilibrium between the homogenate and dialyzer. If tissue protein binding of urea were a significant factor influencing our results, then a higher concentration of

<table>
<thead>
<tr>
<th>Table IV</th>
<th>Dialysis of homogenates of renal papilla</th>
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</thead>
<tbody>
<tr>
<td>Experiment no.</td>
<td>Predialysis [urea]</td>
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<tr>
<td></td>
<td>Homogenate</td>
</tr>
<tr>
<td>mmol/L</td>
<td>mmol/L</td>
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<tr>
<td>1</td>
<td>78.9</td>
</tr>
<tr>
<td>2</td>
<td>64.4</td>
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</tbody>
</table>
urea on the tissue side would be expected; such was not the case.

Discussion

Although active tubular reabsorption of urea has been inferred from studies in the elasmobranch (1), rat (5, 11-13), and sheep (20), this report represents the first study in which an uphill urinary–papillary urea gradient has been reproducibly demonstrated in the dog. This is of particular importance because, heretofore, the strongest arguments for passive urea transfer in the renal medulla have been based largely on clearance (9, 21), stop flow (22), and tissue slice studies (23, 24) performed in the dog. The critical procedure in our experiments was the administration of ethacrynic acid, a drug which inhibits sodium transport in the loop of Henle (18, 25), thereby obliterating the intramedullary electrolyte gradient as observed on tissue analysis. Hence, the osmotic gradient for water removal from the collecting duct was eliminated (U_{osm}/P_{osm} approached 1.0, T_{H_2O} approached zero). Despite the fact that net water reabsorption in the final concentrating operation was virtually nonexistent, medullary urea transport and the accumulation of urea along a rising intramedullary concentration gradient were still present.

Micropuncture (26) and microcatheterization (6) studies on urea transport in the rodent have revealed that the major direct source of medullary urea was the collecting duct (11). Although the details of the direction of urea movement in the dog kidney have not been so well worked out, studies by Clapp (12) indirectly suggest the presence of a medullary system of urea circulation in the dog kidney qualitatively similar to that in the rat, in which a major fraction of filtered urea is reabsorbed in the collecting duct. If this is true, our data indicate the presence of a steady state uphill transport gradient for urea.

Although this uphill transport gradient in itself suggests active transport of urea, other possible explanations involving passive transfers of urea must be evaluated. Solvent drag appears to be an unlikely mechanism because it cannot produce a solute concentration on the distal side of the membrane higher than in the solution of origin. Evidence for urea production by kidneys of the chicken in vivo (27) and the frog in vitro (28) has been presented. If this occurred in the renal medulla, then de novo urea synthesis could contribute to the total medullary urea concentration and theoretically, at least, might raise tissue urea concentration to values above that in final urine. There is no evidence, however, that renal synthesis of urea plays a quantitatively significant role physiologically. In the chicken, Owen and Robinson (27) could find no evidence that urea synthesis was the source of a significant fraction of excreted urea unless arginine was infused. In the rat, Truniger and Schmidt-Nielsen (11) observed that the specific activity of medullary urea 14C was no different from that in plasma and renal cortex; and Lassiter and associates (13) found that urea 14C concentration of blood in the vasa recta was greater than that in the collecting duct. These observations suggest, therefore, that the contribution of a urea–producing system in the renal medulla to the total urea concentration of tissue water or vasa recta plasma is negligible.

The binding of a large proportion of papillary urea to tissue protein could also account for an apparently higher urea concentration in tissue water than in urine in our experiments. This is because urea and water contents were determined independently, and the aqueous concentration of urea was then calculated. Under these conditions, the “true” aqueous tissue urea concentration could be lower than that of collecting duct urine, thus favoring passive downhill diffusion, whereas apparent “total” tissue urea concentration, which included bound urea, would be higher than that of final urine. No evidence has been provided, however, which indicates that mammalian tissue proteins bind urea to any measurable degree. Furthermore, dialysis experiments performed by Lassiter and co-workers on plasma proteins of the rat (13) and by us on canine medullary tissue have failed to uncover evidence for binding of sufficient magnitude to account for our results. In addition, the fact that iodoacetate reduced or obliterated the uphill urinary–papillary gradient argues strongly against simple physical or physicochemical binding. Still possible, but perhaps unlikely, would be a urea–binding system requiring energy derived from anaerobic glycolysis.

When the experiments with the various inhibitors are taken into consideration, the most likely
UPHILL TRANSPORT OF UREA

explaining for all of our observations is that urea is actively transported from the collecting duct into the medullary interstitium. Because iodoacetate, an inhibitor of anaerobic glycolysis (29), distinctly affected the uphill urea gradient and cyanide, an inhibitor of the electron transport system, had no measurable effect, it appears that, under our experimental conditions, the energy supply for medullary urea transport was derived principally from anaerobic metabolism. This conclusion is also compatible with our observation that both the intrarenal urea gradient and the uphill urinary–papillary gradient were maintained, despite the presence of hemorrhagic hypotension and renal ischemia. Schirmer (30) studied the in vitro metabolism of canine renal medullary slices after prolonged clamping of the renal artery and found a remarkable capacity of the medulla to maintain glycolysis despite severe ischemia. Kean, Adams, Winters, and Davies (31) hypothesized that most, if not all, of the chemical energy utilized in the renal medulla was derived from anaerobic metabolism. They based their conclusion on in vitro studies that revealed a high rate of glycolysis in the renal medulla and also on the principles of countercurrent exchange, which predict a low oxygen tension in the inner medulla. They speculated further that this system was the major energy source for sodium transport in the countercurrent multiplier system. Although this may be true, attention should also be directed to the possibility that medullary anaerobic glycolysis is of equal or greater importance in the transport of urea.

The effects of acetamide, a compound structurally related to urea, in reducing the intrarenal accumulation of urea and markedly lowering the uphill urinary–papillary gradient also support the concept of active urea transport. There are several examples wherein acetamide and another urea analog, methylurea, are transported similarly. In the renal medulla of the rat, where uphill urea transport appears to occur, both acetamide and methylurea accumulate in a manner qualitatively similar to urea (11); in the renal tubules of the elasmobranch, these compounds are actively absorbed in a manner similar to urea, but thiourea is not (32). Rabinowitz and Kellogg (33) have shown in the dog that both acetamide and methylurea and a few other analogues were capable of enhancing the concentrating ability of the kidney in a manner resembling the action of urea. Hence, in our own experimental system, acetamide may share the same transport or carrier sites as urea and thus serve as a competitive inhibitor of urea transport. An alternative, but less likely, explanation for the observed effects of acetamide in our studies is that this compound competes with urea for certain specific tissue protein–binding sites.

Several questions concerning the nature of the proposed urea transport system and its relationship to water reabsorption and to the effects of vasopressin remain to be answered. Our observation that the magnitude of the urinary–papillary uphill gradient was reasonably stable over a wide range of V and U\text{urea} may be pertinent. This finding could be explained if the urea transport system in the collecting duct, rather than being limited by the tubular load of urea presented to it, is, in fact, a gradient–limited system, and under the conditions of our experiments the limiting concentration gradient was unmasked. This interpretation is also compatible with the concept that the higher the tubular concentration of urea relative to the interstitial concentration, the more favorable are the conditions for urea transport. Thus, under normal hydropenic conditions, which favor a very high collecting tubular urea concentration due to the high rate of water reabsorption in the distal tubule and collecting duct, this energy dependent system is transporting urea downhill along its chemical gradient. Any condition that lowers the urea concentration of fluid entering the collecting duct, such as water diuresis or solute diuresis, would provide a transtubular gradient less favorable for optimal urea transport and reduce the rate of urea reabsorption. Likewise, any condition directly reducing the net reabsorption of water from the collecting duct, such as the absence of vasopressin or, more completely, the obliteration of the medullary electrolyte gradient, would also inhibit urea reabsorption and, in the latter case, unmask the limiting uphill transport gradient. Whether urea reaches the medullary interstitium by active transport, as suggested by our data, or by passive mechanisms, it would accumulate in the inner medulla according to the principles of countercurrent exchange involving the vasa recta (3) and increase papillary and urinary osmolality. An active transport system
could, in addition, account for the increase in urinary nonurea solute concentration observed under certain conditions after administration of urea and protein (11, 14).

The above hypothesis on urea transport in the collecting duct requires no direct effect of vasopressin on urea permeability. Rather, vasopressin, which alters water permeability of the distal nephron, might indirectly influence urea transport by changing intratubular urea concentration. Although vasopressin increases the permeability of the toad bladder to both water and urea (7) and in vitro perfusion studies of rat collecting duct have been interpreted as suggesting similar effects (34), recent direct observations indicate that vasopressin acts on the distal tubule of the rat (35) and the collecting tubule of the rabbit (8) to change their permeability to water and not to urea. These latter findings are perfectly compatible with our hypothesis outlined above.

Acknowledgments

We wish to thank Mrs. Lydia Kosolapovs and Mr. Leonids Kosolapovs for the meticulously performed laboratory analyses, Mr. Kenneth Christy for his technical assistance, and Dr. J. Russell Elkinton for his support and encouragement throughout the study. We also thank the Merck Sharpe and Dohme Research Laboratories for the generous supply of ethylic acid.

References