Renal handling of urea in Rana catesbeiana

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LONG, WILLIAM SCOTT. Renal handling of urea in Rana catesbeiana. Am J Physiol 224(2): 482-490. 1973.—The processes involved in renal urea excretion in the bullfrog (Rana catesbeiana) were studied by clearance, tissue, and micropuncture methods. According to micropuncture data with urea-14C and inulin-3H, secretion of urea against a concentration gradient from plasma to tubular fluid occurs across the proximal and probably the distal tubules. Tissue studies examining the cellular compartment separating plasma and tubular fluid show that secretion across the basal membranes of the cells is dependent on plasma urea concentrations but independent of flow along the nephron. The fact that urea secretion rates are linear with urine flow rates in clearance studies indicates that movement of urea from cellular stores into tubular fluid is passive. The collecting duct, ureter, and bladder show no significant reabsorption or secretion of urea and so insure excretion of the filtered and secreted urea loads (as well as the accuracy of the clearance studies' results.) An osmoregulatory function for different modes of urea excretion is proposed.

urea excretion; urea secretion; osmoregulation; amphibians; micropuncture; tissue studies; clearance studies

THE AMERICAN BULLFROG, Rana catesbeiana, is one of the few species whose kidney secretes urea from the plasma into the urine. The secretion of urea can be generically defined as the movement of urea across a permeability barrier against its concentration gradient; the identification of the gradient and the barrier depends on the method used to describe the secretion.

The first evidence of urea secretion in R. catesbeiana was the demonstration that the urine-to-plasma ratio of urea concentrations decreases toward unity with increasing plasma concentration and that the renal tissue concentration of urea is approximately 5 times the plasma urea concentration (14). Marshall (13) later showed that urea clearance in this species is 5-8 times greater than the filtration rate measured as xylose clearance.

Micropuncture studies of Rana pipiens (24) showed that the proximal and distal tubular fluids contain more urea than can be accounted for by glomerular filtration. The absence of a reliable index of water reabsorption in these experiments precluded the assignment of secretion to one or both of the nephron's segments. These investigators did not study the role of the collecting duct in urea excretion.

In clearance studies using creatinine (Cr) as the index of water reabsorption in frogs, Forster (6) demonstrated saturation of the urea transport mechanism in R. catesbeiana by showing that the secretion rate reaches maximal values at plasma urea levels of about 2.5 mM. He also showed that the secretion of urea can be diminished by the metabolic inhibitor, 2,4-dinitrophenol (DNP), and by competition by structurally unrelated compounds known to interfere with other forms of renal secretion. B. Schmidt-Nielsen and Shrauger (22) showed that of the urea analogs tested only thiourea, but not methyurea or acetamide, is accumulated by the kidney and excreted by tubular secretion in the bullfrog. In addition, they showed that DNP abolishes not only the renal secretion of urea, but also its accumulation in the renal tissue.

The majority of urea secreted by the kidney of R. catesbeiana is derived from the plasma; maximal values of de novo renal synthesis of urea account for less than 10% of the urinary urea (4, 22).

Although the secretion of urea in R. catesbeiana has been studied at the level of the renal cell, the nephron, and the whole kidney, no general model has been presented to explain how the phenomena already described at these various levels cooperate in the secretion of urea. The experiments presented below were performed in an effort to arrive at such a model, relating urea excretion to the major renal functions in this species, the reabsorption of salts and the excretion of water.

MATERIALS AND METHODS

Animals

Male adult R. catesbeiana (25-250 g) and R. pipiens (25-100 g) were obtained from Lemberger Supply House (Oshkosh, Wis.) during all seasons. For a week or two after arrival, they were kept in a greenhouse pond in which the animals had free choice of aqueous and dry environments. The animals were fed live crickets. Three or four days before use, the frogs were transferred to plastic containers holding 2-3 inches of tap water. Catheterized frogs spent the time between surgical insertion of the ureteral catheters and final use in this same environment and were fed.

Clearance Studies (31 frogs)

Twelve to twenty-four hours before sample collection, the frogs were injected with 1 ml/100 g body wt of a 5 g/100 ml solution of creatinine. At the time of sample collection, the plasma levels of creatinine (> 50 mg/100 ml) were relatively stable, falling 5% / hr or less. In uncatheterized animals, after an initial emptying of the bladder, a timed sample of bladder urine was collected by glass catheter and its volume measured. Small blood samples (0.1-0.5 ml) from both catheterized and uncatheterized animals were obtained by cardiac puncture. Analyses were made within 24 hr.
Creatinine concentrations were determined by the Beckman/Spinco adaptation of the alkaline picrate method; urea and ammonia concentration, by the Conway diffusion method. Sodium concentrations were measured on a Baird-Atomic flame photometer (model KY-Z) and osmotic concentrations, on a Fiske osmometer (model H-62) using the freezing-point depression. Appropriate standards were run with each analysis. Duplicate measurements were made in all cases with the exception of plasma urea concentrations in which the small sample size did not always permit duplicate determinations. Duplicates agreed within 2% by all these methods.

The second kidney was placed in an empty tared vial. Both vials were weighed to determine the tissue's wet weight. The vial containing the first kidney was placed at 2°C for a day to allow equilibration of tissue urea and water. The other vial was uncapped and placed in a drying oven at 100°C for 24 hr to determine water content, used in calculating the dilution factor for urea and ammonia determinations in the equilibration fluid of the first kidney.

The kidneys of the control frogs were 84.7% water by weight; those of the dehydrated animals were 82.5%.

Analysis of samples for urea and urinary creatinine was carried out by the techniques used in clearance studies.

The purpose of these studies was the comparison of renal, plasma, and urinary urea concentrations under conditions of normal hydration (six control animals per species) and dehydration (six experimental animals per species) in R. catesbeiana and R. pipiens.

For dehydration the animals were weighed and placed in dry containers; their bladders were emptied at regular intervals. The loss of 8–10% initial body weight required 8–9 hr for R. catesbeiana (80 100 g) and 3 4 hr for R. pipiens (25–40 g). In every animal anuria occurred before weight loss reached the desired levels, which precluded clearance studies in the final dehydrated state. Frogs were used in control clearance studies as described for uncatheterized frogs.

The animals were killed and blood samples removed. The kidneys were excised and the ureters and large vessels were trimmed from the margins; blood and urine were expressed from the tissue by gentle pressure.

The first kidney was then minced, placed in a capped, tared vial with a known volume of water, and boiled for at least 3 min to prevent possible enzymatic synthesis or degradation of urea. The second kidney was placed in an empty tared vial. Both vials were weighed to determine the tissue's wet weight. The vial containing the first kidney was placed at 2°C for a day to allow equilibration of tissue urea and water. The other vial was uncapped and placed in a drying oven at 100°C for 24 hr to determine water content, used in calculating the dilution factor for urea and ammonia determinations in the equilibration fluid of the first kidney. The kidneys of the control frogs were 84.7% water by weight; those of the dehydrated animals were 82.5%.

Analysis of samples for urea and urinary creatinine was carried out by the techniques used in clearance studies.

Micropuncture Studies (18 frogs)

Each frog (R. catesbeiana) was injected with creatinine as in clearance studies. Two hours before surgical preparation the animal was injected via the dorsal lymph sac with inulin-14C and urea-14C at ca. 200 μCi and 150 μCi/100 g, respectively. The isotopes were injected into the dorsal lymph sac in 0.5 ml isotonic saline with 5 g/100 ml carrier inulin to minimize the effects of inulin binding on the concentration of radioactive inulin. Equilibration of label was indicated by a ratio of specific activities of 1.01 ± 0.06 (SEM, n = 8) for (U/P)urea-14C/(U/P)inulin-14C and 1.08 ± 0.07 (n = 10) for (U/P)inulin-14C/(U/P)Cr. After anesthesia in a 0.2 g/100 ml solution of tricaine methanesulfonate (MS 222, Sandoz Pharmaceuticals), the animal was covered with wet towels. The right kidney was exposed by an incision along the ilium and covered with oil to prevent evaporation from the surface. Pipettes drawn from thick-walled Pyrex tubing (2 mm od, 1 mm id, tip diameters ca. 10 μ) and filled with colored oil were used to penetrate the tough collagenous covering of the kidney.

The arrangement of the nephrons in R. catesbeiana is shown in Fig. 1; the arrows indicate typical puncture sites. The glomeruli and distal tubules are found in the ventral half of the kidney; the proximal tubules constitute almost the entire tubular volume of the dorsal half of the kidney and roughly half that of the ventral half. The diameter of the proximal tubules (~35 μ) is roughly twice the diameter of the distal tubules (~20 μ). The proximal tubules have a more re-

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1. The effectiveness of this removal of extracellular fluid, i.e., plasma and tubular fluid, was tested by comparing the renal urea concentration of one kidney treated in this manner with that of the contralateral kidney from which tubular fluid and plasma were removed by inflation of the kidney through a ureteral catheter. In five frogs the ratio of renal urea concentration in uninflated/inflated kidneys was 0.93.
fractile inner border due to the brush border; the cytoplasm of the distal tubular cells is more opaque. The collecting duct lies perpendicular to the long axis of the kidney just below the most dorsal layer of the nephrons, occasionally appearing on the dorsal surface just medial to the ureter. The nephrons are held loosely in a meshwork of peritubal collagen fibrils, which penetrate the organ as well as cover its surfaces. Since these fibrils cannot be peeled off the surface as in the mammalian kidney, their extreme toughness presents the greatest obstacle to micropuncture.

Before sample removal, an oil column was injected into the tubule to prevent contamination of the sample with fluid distal to the collection site. The technique of sample removal is essentially that described by Richards and Walker (18) and does not correspond strictly to either the free-flow or stop-flow techniques used in mammalian studies. The first nanoliters of sample were collected by negative pressure applied by a 30-ml syringe; the major portion of the sample was collected with a 50-ml syringe to diminish intratubular pressure effects. The average rate of collection of both proximal and distal tubular samples (10-50 nl) was about 3 nl/min. Fluid from the kidney's surface and blood samples from large superficial renal vessels (100-200 nl) were also collected to check for evaporation; the surface fluid/plasma ratios for inulin-3H and for urea-14C were always close to unity. Injection of latex into the puncture site was attempted at the end of each experiment. Bladder urine samples were taken at the beginning and end of the experiments.

The micropuncture samples were kept under oil until analysis within 24 hr. Volumes of tubular fluid samples placed in counting vials were measured by micrometer in a 30-ml pipette made from constant-bore capillary tubing; pipetting error between duplicates was 3-4%. The counting vials contained 50 ml distilled water, 200 ml Hyamine (Packard Instrument Company, Inc.) and 15 ml of scintillation fluid consisting of 7 g PPO, 0.3 g POPOP, and 5 g Cab-O-Sil (Picker Corp.), all dissolved in 800 ml toluene and 200 ml ethanol. The radioactive compounds were counted in a Packard dual-channel liquid scintillation spectrometer. In the earliest experiments when only urea concentrations were measured, the chemical urea method of Mushli et al. (12) was used. The samples of plasma, surface fluid, and proximal tubular fluid showed average inulin-3H counts 5 times background and urea-14C counts 6 times background. The osmolalities were measured by the freezing point depression method with sample volumes of 0.1 nl or less (16).

RESULTS

Clearance Studies

The purpose of these studies was twofold: 1) quantitation of renal function in R. catesbeiana with regard to water, total solutes, sodium, ammonia, and urea; and 2) description of the renal excretion of urea in the context of the major osmoregulatory functions of the amphibian kidney, the excretion of water, and reabsorption of solutes.

According to the results presented in Table 1, 41% of the filtered water is reabsorbed along with more than 99% of the filtered sodium and 93% of the total solutes; when the last figure is corrected for the contribution of secreted urea, more than 95% of filtered solutes are shown to be reabsorbed.

Both major nitrogenous waste products in this species, ammonia and urea, are found in the urine in amounts exceeding the amount filtered, as shown by clearance ratios greater than unity. The contribution of renal synthesis of ammonia to the excreted ammonia load has not been measured in this work. Together ammonia and urea account for about one-third of the urinary osmolality. (The excretion of urea and ammonia under these conditions is quantitatively similar to their excretion by this species in its natural habitat (20).)

The ranges of urine flow rate (V), glomerular filtration rate (GFR), and urea/creatinine clearance ratios (Table 2) are similar to those reported by previous investigators and indicate the wide variation commonly observed in anurans (6, 13, 21). Variation in rates of glomerular filtration, urine flow, and of both urea excretion (E) and secretion (S) occurs not only between individuals of the species, but also within

| Table 1. Composition of plasma and urine in Rana catesbeiana (un catheterized) |
|-------------------------|-------------------------|-------------------------|-------------------------|
|                         | Plasma                  | Urine                   | (U/P)X                  | (U/P)X/(U/P)cre*         |
| Sodium, mEq/liter       | 97±8                    | 2.0±0.6                 | 0.018±0.005             | 0.007                   |
| (n=10)                  |                         |                         |                         |                        |
| Urea, mmol             | 0.77±0.12               | 12.5±2.9                | 16.2±1.9                | 7.00±0.63              |
| (n=10)                  |                         |                         |                         |                        |
| Ammonium, mmol         | <0.10                   | 0.85±0.07               | >8.5                    | >3.5                   |
| (n=10)                  |                         |                         |                         |                        |
| Total solutes, mOsm/liter | 210±3                  | 36±8                    | 0.17±0.04               | 0.07                   |
| (n=13)                  |                         |                         |                         |                        |
| Exogenous creatinine, mg/l | 2.45±0.31              |                         |                         |                        |
| (n=20)                  |                         |                         |                         |                        |

Values are averages ± se. *With the exception of the urea/creatinine clearance ratio, all values in this column are calculated by dividing the average (U/P) X by the average (U/P)cre value.

| Table 2. Clearance studies in Rana catesbeiana |
|-------------------------|-------------------------|-------------------------|
|                         | Average ± SE            | Range                   |
| V, ml/hr-kg body wt     | 14.2 ± 1.6              | 2.0-39.0                |
| GFR, ml/hr-kg           | 26.2 ± 2.6              | 3.7-69.4                |
| E, rate of urea excretion, μmoles urea/hr kg | 167 ± 22 | 27-600 |
| S, rate of urea secretion, μmoles urea/hr kg | 139 ± 18 | 14-505 |
| Urea/creatinine clearance ratio | 5.7 ± 0.4 | 1.5-14.3 |

Ureterally catheterized and uncatheterized animals.
a single frog over a period of time. Figure 2 shows the parallel fluctuation in rates of urine flow and urea excretion in the presence of a relatively constant urea/creatinine clearance ratio. Furthermore, other studies show marked differences in simultaneous urine flow rates from right and left ureters in catheterized frogs (10).

The observation of parallel fluctuations of urea excretion and urine flow rates in a single frog is extended in Figs. 3 and 4 where the results of 41 clearance periods (1.5–45 min duration) in 31 frogs are presented. Unusually high rates of urine flow were obtained only in ureterally catheterized animals; however, no efforts were made to induce diuresis in any animal.

The most striking correlations are those of urea excretion rate with urine flow rate (Fig. 3) and of urea secretion rate with urine flow rate (Fig. 4); similar correlations exist between glomerular filtration rate and the rates of urea excretion and secretion. All these correlations are significant ($P < 0.001$).

**Tissue Studies**

The purpose of these studies was to demonstrate that movement of urea from plasma into the cell and movement of urea from the cell into tubular fluid are two distinct, separable events. Dehydrating the frogs disclosed that increasing accumulation of urea by the renal cells occurred at a time of decreasing net movement of urea from the cells into tubular fluid.

Two species, *R. catesbeiana* and *R. p. pipiens*, were studied to compare the effects of dehydration on “weak” urea secretion (*R. p. pipiens*, average urea/creatinine clearance ratio of 2) and on “strong” urea secretion (*R. catesbeiana*, average urea/creatinine clearance ratio of 6).

The results are presented in Table 3; they are summarized as follows. 1) As previously shown (6, 14, 22), the kidneys in hydrated animals contain higher concentrations of urea than the plasma. 2) The increase in renal urea concentrations under conditions of dehydration in both species is significant ($P < 0.05$). 3) Although there is a rise in plasma urea levels with dehydration in *R. catesbeiana* and no significant change in *R. p. pipiens*, dehydration gives rise to an almost twofold increase in the urea concentration gradient between kidney and plasma in both species ($P < 0.05$). 4) In neither control nor experimental animals do the values of renal urea concentration or of (kidney-plasma) urea concentration gradients show significant interspecific differences ($P < 0.05$). 5) In *R. catesbeiana* the average urinary urea concentration is more than twice that of the renal tissue. In *R. p. pipiens* there is no significant difference between these concentrations.

In summary, renal accumulation of urea is similar in *R.*
catesbeiana and R. pipsiens. Despite this similarity, there are significant differences between the two species with regard to the urea/creatinine clearance ratios and the urine-kidney urea concentration gradients. To clarify the relationship of renal urea accumulation and the movement of urea from cell into tubular fluid within each nephron segment, micro-puncture studies are reported in the next section.

**Micro-puncture Studies (11)**

In these experiments the roles of the nephron’s segments have been studied with regard to water, total solutes, and urea. The handling of water and solutes is important to the renal excretion of urea because the major renal functions in an aquatic amphibian are concerned with their regulation in the body.

**Water and solutes.** Table 4 presents data describing the progressive dilution of glomerular filtrate along the nephron. In the proximal tubule, $16.7\% \left[1 - (P/TF)_{in}\right] \times 100\%$ of filtered water and $17.5\% \left[1 - (TF/P)_{osm}/(TF/P)_{in}\right] \times 100\%$ of filtered solutes are reabsorbed isotonically, as indicated by the $(TF/P)_{in}$ of unity. Water reabsorption in the distal tubules is very low in the present studies, despite a reabsorption of $23\%$ of filtered solutes. The hypertonic reabsorption of solutes in the distal tubule is responsible for dilution of the tubular fluid; the isotonic reabsorption of solutes in the collecting duct and bladder observed under these experimental conditions increases the dilution initiated in the distal tubule.

Although distribution of sample sites along the proximal and distal tubules could not be assigned, the average $TF/P$ inulin values in Table 4 are probably valid estimates of the ratio at the end of these two sections of the nephron. (The use of this approximation here is supported by the fact that in the proximal tubule of *Necturus* most of the water is removed in the first 10–20% of the tubule (2, 8). The indication of slight water reabsorption in the distal tubule of *R. catesbeiana* is not seriously affected by the site assigned by the average $TF/P$ inulin).

The calculated isotonicity of the reabsorbrates in the collecting duct and bladder may not be real and may mask a more hypotonic reabsorption. Part of the solute reabsorption included in that average occurs in the distal tubule, furthermore, water loss from collecting duct fluid and bladder urine is favored by the osmotic gradient of 120–130 mOsm/liter across those epithelia and by slow flow rate in the terminal portions of the nephron. All these processes may contribute to the apparent isotonicity of the reabsorbrates observed in the collecting duct and bladder under these experimental conditions.

**Urea.** The data from double-isotope experiments are presented in Table 5. All values of the $(TF/P)_{area}/(TF/P)_{in}$ ratio for the proximal tubule are greater than unity. Calculated from the average value of this ratio, almost 40% of urea in the proximal tubular fluid is the result of net tubular secretion. Continued secretion raises the figure to almost 80% in the distal tubular fluid. Although the reabsorption of water in the collecting duct and bladder raises the concentration of solutes in the bladder, the concentration of urea is not affected.

**Table 4. Water and solute reabsorption in nephron and bladder of *Rana catesbeiana* **

<table>
<thead>
<tr>
<th>Site</th>
<th>$(TF/P)_{in}$</th>
<th>Fraction of filtered water reabsorbed</th>
<th>Fraction of filtered solutes reabsorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal TF (n = 23)</td>
<td>1.20 ± 0.06</td>
<td>0.167</td>
<td>0.175</td>
</tr>
<tr>
<td>Distal TF (n = 11)</td>
<td>1.21 ± 0.10</td>
<td>0.175</td>
<td>0.405</td>
</tr>
<tr>
<td>Collecting duct-ureter TF-U (n = 9)</td>
<td>2.81 ± 0.44</td>
<td>0.644</td>
<td>0.876</td>
</tr>
<tr>
<td>Bladder U (n = 9)</td>
<td>3.31 ± 0.47</td>
<td>0.715</td>
<td>0.936</td>
</tr>
</tbody>
</table>

Values are averages ± se.
centrations of urea in the tubular fluid and urine, there is no indication of further secretion there.

The use of averages of the TF/P urea ratios may underestimate the true values of this ratio at the end of the proximal and distal tubules. One must consider the possibility that distal tubular secretion of urea, as estimated by the averages, may in fact be the result of proximal tubular secretion. The evidence for distal tubular secretion is found in Table 5; all values of the (TF/P)urea/(TF/P)in ratio are greater in the distal tubule than in the proximal tubule. If the proximal tubule were responsible for the apparent distal tubular secretion, one would expect to find overlapping values of this ratio.

The similarity of the average (TF/P)urea/(TF/P)in ratios in the collecting duct and distal tubules suggests that the maximal level of urea secretion has been attained in the earlier portions of the distal tubule under these experimental conditions. The similarity of the ratios could have been established in two ways: 1) the collecting duct has a very low permeability to urea so that essentially no urea is added to or reabsorbed from the tubular fluid in the collecting duct, 2) the collecting duct’s permeability is much higher than postulated in the first alternative so that the (TF/P)urea/(TF/P)in ratio remains unchanged because the passive efflux from the tubular fluid down its concentration gradient is equal to the rate of secretion into the collecting duct fluid. Studies of net fluxes across the tubular epithelium, like those reported here, are not sufficient to unmask such secretion.

An examination of the data supports the first alternative. In Fig. 5 the (TF/P)urea/(TF/P)in ratio is plotted against both the TF/P urea and the TF/P inulin ratios from samples taken from the terminal portions of the collecting duct and from the ureter. The (TF/P)urea/(TF/P)in ratio does not change with increasing values in either parameter. If the epithelium of the collecting duct were permeable to urea, this ratio should decrease with increasing TF/P urea ratios due to diffusion of urea out of the tubular fluid down its concentration gradient. Similarly the slope would decrease with increasing TF/P inulin values if there were significant entrainment of urea with water. The data plotted in Fig. 5 show that this is not the case.

The values for bladder urines from the same micropuncture experiments are plotted in Fig. 5 and lead to the extension of the same conclusion to the bladder. In fact, the same arguments are applicable to all segments of the nephron beyond the site at which maximal values of the (TF/P)urea/(TF/P)in ratio are reached; after that point at which net secretion ceases, the nephron is effectively impermeable to urea.

Effects of anesthesia and surgery. Table 6 shows that animals used in micropuncture experiments displayed three alterations of body fluid composition and renal function when compared to frogs used in clearance studies: 1) increased...
total water reabsorption (0.10 > P > 0.05), 2) increased
concentrations of nonurea solutes in the urine (P < 0.05),
and 3) decreased plasma osmolality (P < 0.05). In addition
an unquantitated decrease in urine flow was shown by
collection of bladder urine during micropuncture exper-
iments.

Decreased briskness in glomerular circulation and some
pooling of urine in the ureters were observed, both of which
contributed to the decreased bladder urine volume. Thus,
in micropuncture experiments contact time between fluid
and tubular epithelia was probably longer than in normal
clearance studies; this could account for the greater water
reabsorption and lower plasma osmolality.

Although the U/P ratios for nonurea solutes and for
creatinine were both increased under conditions of micro-
puncture, the fraction of filtered solutes reabsorbed in un-
operated frogs (0.955) was not very different from that in
operated frogs (0.937).

Urea handling was not significantly affected by anes-thesia
and surgery. The U/P urea values are similar in micropunc-
ture and clearance studies (P = 0.4). The difference in
urea/creatinine clearance ratios between operated and un-
operated frogs can be explained as the result of difference
in water reabsorption; even so, the diminution in terms of
percent secreted urea in the urine is only 5%, the difference
between 81% in operated and 86% in intact frogs.

In summary, surgery and anesthesia have affected the
handling of water and nonurea solutes more than the
handling of urea by the kidney.

DISCUSSION

Urea Excretion and Glomerular Filtration

At normal concentrations of plasma urea in R. catesbeiana,
the aspect of glomerular filtration most important for urea
excretion is not filtration of plasma urea but filtration of
plasma water into which cellular stores of urea can move.
(This statement is not true for R. pifiens due to its higher
plasma urea concentrations.)

In R. catesbeiana glomerular filtration accounts for only
15-20% of urea excreted; the remaining 80-85% is derived
from tubular cells at rates determined by rates of flow along
the tubule, as shown by the correlation of urea secretion
rate with rates of glomerular filtration and of urine flow.
The latter flow rate is to a large extent determined by
glomerular filtration (P < 0.001), previously noted (5).

Urea Movement Across Secretory Cells

The renal accumulation of plasma urea across the
peritubular border of secretory cells fulfills the definition of
urea secretion stated in the introduction.

Previous investigators characterized urea secretion by
comparison of urea/creatinine clearance ratios with urea
concentrations in plasma and renal tissue under various
conditions. In Fig. 6 comparison of urea concentrations in
the two compartments separated by the peritubular border
of secretory cells presents more direct evidence of saturation
of the transport process. The kidney/plasma urea concen-
tration ratio decreases toward unity with rising plasma levels.
This suggests that equilibration of urea concentrations
across the peritubular border is due to greater passive urea
influx relative to secretory influx, as the secretory mechanism
approaches saturation at higher plasma urea levels.

Urea accumulation across the peritubular face of secretory
cells occurs with and without glomerular filtration as shown
by the tissue studies reported, but net efflux across the
luminal border of these cells is dependent on fluid flow along
the nephron. Clearance studies, although they treat the
entire cellular layer as a single permeability barrier, directly
measure the flow-dependent movement of urea from the cell
along the luminal border into tubular fluid and only in-
directly the secretion of urea into the cell from the plasma.

Little is known about the character of efflux from cell to
lumen. The correlation of urea secretion rate with rates of
glomerular filtration and urine flow over a wide range of
values suggests that urea efflux into the tubular fluid is the
result of passive equilibration of urea concentrations in
the cells and the tubular fluid.

Further support for the passive net efflux of urea across
the luminal face is found by comparing urea concentration
of the whole kidney with the proximal and distal tubular
fluids, i.e., of the two compartments separated by the
luminal membrane. The average tubular fluid/kidney urea
correlation ratio is 0.46 for proximal tubular samples; in
five such samples from four experiments in which renal urea
concentrations were measured, the average is 0.44. The
average TF/K urea concentration ratio for the distal tubule
is 0.92; this value is less reliable, however, because distal
tubular mass contributes less to total renal weight than does
proximal tubular mass.

Urea Handling in Collecting Duct and Bladder

The near impermeability to urea of the collecting duct
and bladder insures that urea secreted in more proximal
regions of the nephron is almost entirely excreted. This con-
dition is necessary for reliable quantitation of tubular urea

FIG. 6. Kidney/plasma ratio of urea concentrations (±SEM) as a
function of plasma urea concentrations in R. catesbeiana; data from
these experiments (16 animals) and from B. Schmidt-Nielsen and
Shrauger (22) (9 animals). Plasma urea concentration ranges: 0-
0-0.49 mM (n = 7); 0.50-0.99 mM (n = 6); 1.00-1.99 mM (n = 6);
>2.00 mM (n = 4).
secretion by clearance studies. If the epithelia of the collecting duct and/or bladder were significantly permeable to urea, clearance studies would underestimate tubular secretion of urea. R. catesbeiana is the only amphibian species for which one can state that clearances studies have been shown by micropuncture studies to quantitate accurately the tubular secretion of urea; similar calculation from micropuncture and clearance studies shows that Necturus does not secrete urea (2, 8, 24).

Low urea permeability of the collecting duct and bladder is also found in intact animals used in clearance studies reported here. However, the linear correlation of U/P urea and U/P inulin which can be defined in the micropuncture studies is difficult to define in intact animals, due in part to the restricted range of U/P creatinine values in this group. (The greater range of U/P inulin values in operated frogs is probably due to urinary stasis.) Nonetheless, in animals of all three preparations (intact and catheterized frogs in clearance studies, operated frogs in micropuncture studies), maximal urea concentration gradients between plasma and tubular fluid/urine of 35–100 mM indicate low permeability of these epithelia to urea in all experimental conditions.

A Model of Urea Excretion in R. Catesbeiana

The glomerular filtrate, containing urea equivalent to 15–20% that excreted, gains additional urea from intracellular stores accumulated by a secretory mechanism at the peritubular border of the proximal and probably the distal tubular cells. Accumulated urea enters the proximal and distal tubular fluid by passive equilibration of intracellular and tubular fluid urea concentrations. As more proximal stores of urea decrease during continued filtration in a given nephron, the point of equilibration across the luminal border of the secretory cells may occur at increasingly distal sites along the nephron with little effect on the final urea/creatinine clearance ratio. (However, as glomerular filtration in a given nephron persists, even the more distal stores of urea in the nephron will be diminished. Glomerular intermittency (17) can give rise to variation of the urea/creatinine clearance ratio for any animal as filtration stops in some nephrons and begins in others whose secretory cells have replenished their intracellular stores of urea.)

Once equilibration of cellular and tubular fluid concentrations is reached, the excretion of secreted and filtered urea is determined by the low urea permeability of the epithelia distal to the site at which maximal values of the (TF/P)urea/(TF/P)in are reached. In conjunction with the equilibration of intracellular and tubular fluid concentration of urea, this low permeability maintains the relatively constant urea/creatinine clearance ratio, despite variation in flow rate (Fig 2).

This model does not require all-or-none glomerular filtration to explain the correlation of urea secretion rates with the rates of glomerular filtration. Although evidence presented does not disprove all-or-none glomerular filtration, it suggests that the correlation can be explained as the result of variable, intermediate, as well as all-or-none filtration rates for individual nephrons and flow-dependent, diffusional efflux of urea from secretory cell to tubular fluid.

Urea Excretion and Osmoregulation

The significant water permeability of the skin makes osmoregulation important to survival for all amphibians; dessication threatens terrestrial species; overhydration, the more aquatic species. The modes of renal urea excretion are related to the different osmoregulatory problems facing many anuran species; aside from nitrogen loss, the major effect of urea excretion is the maintenance of plasma urea levels favorable to those problems’ resolution.

Plasma urea levels are lowest (0.5–2.5 mM) among the aquatic anurans, e.g., R. clamitans and R. catesbeiana, in whom net urea secretion is strongest (urea/creatinine clearance ratios of 5–10). In terrestrial anurans, e.g., Bufo americanum (20) and R. arenarum (3), plasma urea concentrations may be 5–10 times higher than in preferentially aquatic species; in brackish water, R. cancrivora maintains plasma urea concentrations of 100–300 mM (23). Renal urea excretion in these species is characterized by net reabsorption (urea/creatinine clearance ratios <1). In plasma of semiaquatic species, e.g., R. pipsiens, intermediate concentrations of urea are found and periods of net urea secretion alternate with periods of net reabsorption (3).

The mode of renal urea excretion is also related to water reabsorption in semiaquatic and terrestrial anuran species. In terrestrial species from three different genera, Carlisky and co-workers (3) have found that the degree of urea reabsorption increases with water reabsorption; the same correlation is shown by R. cancrivora (23). Carlisky has also found in two semiaquatic species, R. pipsiens and Leptodactylus ocellatus, a general trend toward net urea secretion as water reabsorption decreases (3). The strong secretors show little diminution of net urea secretion with increased water reabsorption except during prolonged dehydration (22). A survey of urea excretion (unpublished observations by W. Sawyer and B. Schmidt-Nielsen) in a few specimens from several species of Rana, Hyla, Bufo, and Scaphiopus supports these generalities (10).

Several facts suggest that water and urea reabsorption are linked in the collecting duct and bladder; the degree of linked reabsorption may involve different inherent water and urea permeabilities as well as differential hormonal susceptibilities in the various species. 1) Micropuncture studies of R. pipsiens (24, 25) and R. catesbeiana show maximal osmotic and urea concentration gradients across the epithelia of the collecting duct and bladder, favoring the passive loss of urea and water. 2) Intracellular concentrations of urea in the secretory portion of the nephron increase in both R. catesbeiana and R. pipsiens during periods of dehydration; it seems unlikely that these portions of the nephron are involved in the net reabsorption of urea noted by Carlisky (3). 3) Antidiuretic hormone (ADH) increases the bladder’s permeability to water in R. catesbeiana (19) and to both water and urea in R. marinus (9). However, tubular effects of ADH on urea handling in amphibians have not been investigated by micropuncture.

In terrestrial anurans, the increase of plasma osmolality due to accumulation of urea decreases water loss across the skin and increases water uptake in aqueous environments. Further, elevated plasma urea concentrations, possibly in conjunction with urea recycling across the collecting duct...
and bladder, raise the filtered load of urea and reduce the kidney-plasma urea concentration gradient, thus reducing the urea/creatinine clearance ratio.

In preferentially aquatic species, the major effect of net urea secretion is maintenance of low plasma urea concentrations. Urea secretion in R. catesbeiana lowers the plasma’s urea concentration, and hence its osmolar concentration, by 5–10 mOsm/liter. For a frog in water a decreased osmotic gradient decreases water uptake and glomerular filtration and so diminishes expenditures of metabolic energy both for reabsorption of electrolytes and nutrients and for replacement of urinary electrolytes. On land, glomerular shutdown protects the animal against renal water loss and the resulting urea accumulation aids in combatting evaporative water loss. Thus, urea secretion represents a saving of metabolic energy which would otherwise be expended in plasma filtration and reabsorption of filtered solutes. This hypothesis offers a more reasonable explanation for urea secretion than avoidance of urea accumulation for whose toxicity no good evidence exists. On the other hand, it does not attempt to explain all relationships of urea excretion and osmoregulation found among anurans. (For example, several aquatic species, like *Xenopus laevis* (1), do not show urea secretion.) However, as Forster (7) has pointed out, such a hypothesis extends urea’s osmoregulatory role to present both the reabsptive and the secretory modes of urea excretion as adaptations to the continuum of osmoregulatory problems confronting many anuran species.

The author thanks Dr. Rodil Schmidt-Nielsen for her interest and support throughout the course of this work and Drs. Clifford Slayman and Guillermo Whittambury for their critical comments on the manuscript of this paper.

This work was supported by Public Health Service Research Grant AM-09575-04 and performed while the author was supported by Public Health Service Training Grant GM-01609-03.

Portions of this work were submitted in partial fulfillment of the requirements for the PhD degree at Case Western Reserve University and were presented in preliminary form at the International Colloquy on Urea and the Kidney, held in Sarasota, Fla., September 1968.

Received for publication 24 February 1972.

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