Renal function in domestic fowl

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Several aspects of kidney function that are already well elucidated in mammals still need to be investigated in many lower vertebrates. Among these are the variability of the glomerular filtration rate (GFR), the diluting and concentrating capacity of the kidney, and the excretion of water and electrolytes under various osmotic conditions. The mammalian kidney with its countercurrent system differs functionally as well as structurally from the lower vertebrate kidney in several important respects (14).

In mammals all the nephrons have loops of Henle, and, thus, they all are part of the countercurrent system and function together in creating a hyperosmotic environment for the collecting ducts. In lower vertebrates that do not have a renal countercurrent system, the tubules do not function together, and this may be the reason that wide variations in filtration rate can occur due to opening or closing of individual glomeruli. In mammals, the glomerular filtration rate does not change with the state of hydration (within normal limits), but in contrast to the lower vertebrates, it increases in many species following acute or chronic salt loading (14). In many lower vertebrates the GFR increases considerably with hydration and decreases after salt loading or dehydration.

In mammals the urine osmolality can vary over a wide range from about 10-20% of the blood osmolality to 10-20 times the blood osmolality. In lower vertebrates the urine osmolality can vary from being hypotonic to being isosmotic to the blood. However, in many reptiles (particularly the uricotelic terrestrial forms) the kidney tubules have no capacity for regulating the urine osmolality.

In mammals the fraction of filtered sodium excreted may increase under the influence of antidiuretic hormones but the evidence is conflicting (11, 21). In amphibians (7) and reptiles (10, 13) it clearly decreases.

The bird kidney is, in a sense, a peculiar mixture between a reptilian and mammalian kidney. Part of the kidney lobule histologically resembles the reptilian lobule with convoluted tubules without loops of Henle and with the collecting ducts running at right angles to the tubules. In addition, however, each lobule has some nephrons with loops of Henle which, together with the parallel running collecting ducts and capillaries, form a medullary cone rather similar to the mammalian renal medulla (4).

Because of the reptilian as well as mammalian characteristics of the bird kidney, it was of interest to compare the response to various states of hydration and salt loading with that of mammalian and lower vertebrate kidneys. In the present experiments small funnels were sewn over the ureteral openings in the cloaca of roosters and the urine could thus be collected directly as it appeared from the kidney.

All the data recorded in this paper are observations made on ureteral urine, without the subsequent modification that normally takes place in the cloaca. The role of the cloaca in modifying the urine will be presented in a following paper.

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1967.- Roosters were exposed to water and salt loading and to dehydration. The ureteral urine was collected through polyethylene funnels. The glomerular filtration rate (GFR) was found to increase an average of 23% from dehydration to hydration, when at the same time the urine flow increased from 20 μl/min per kg to 300 μl/min per kg (1,500%). At the same time the osmolal urine-to-plasma ratio varied from 1.58 to 0.37 and the excretion of water changed from 1 to 14% of the filtered load. From dehydration to hydration the salt output increased from approximately 1 to 2.5% of the filtered load. Salt loading (12-13 mEq/kg body wt) did not reduce the GFR. In ducks and turkeys a similar concentrating capacity was found. The renal function of the bird resembles that of mammals with respect to the very stable filtration rate and the capacity to dilute and concentrate the urine. It resembles that of reptiles and other lower vertebrates with respect to the small fraction of filtrate that is resorbed during hydration and with respect to the effect of dehydration on tubular sodium resorption.

bird renal function; glomerular filtration rate; tubular function; salt resorption; hydration; dehydration
Most experiments were performed on domestic roosters weighing 1–3 kg. They were kept in turkey cages and were fed Purina Layena chicken food.

**Experimental procedure.** The birds had free access to food prior to the experiment but water was withheld for 36 hr. The birds were tied loosely into a harness, and under local anaesthesia (5% Xylocaine gel) small polyethylene funnels were sewn over the ureteral openings in the cloaca. Catheters of suitable size were inserted into the wing vein and the leg vein under local anaesthesia (infiltration with 2% Xylocaine). This technique is similar to that employed by Skadhauge (19). Throughout the experiment the fowls were supported gently by hand but were allowed to move if they showed inclination to do so. Most of the time they preferred to sit. One of the ureteral funnels was flushed with distilled, deionized water delivered at a rate of 0.76 ml/min by a Harvard infusion pump. The conductivity of the rinsing water corresponded to less than 1 ppm NaCl.

The urine coming from the other catheter was collected under oil. Inulin concentration (for measuring GFR and electrolyte concentrations for measuring excretion ratio per unit time) were determined on the diluted urines, the volume of which could be determined accurately with dead space errors being minimized. The urine flow and the osmotic and ionic concentrations were determined on the natural urine. A priming dose of a fresh batch of inulin from Warner-Chilcott was given in the wing vein at least 45 min before the start of the urine collection, and a maintenance dose was given by continued infusion large enough to maintain a plasma concentration of 60–80 mg/l 100 ml. Blood was collected from the leg vein catheter at the midpoint of each urine collection period. The rate of inulin infusion was increased during the salt infusion to compensate for the resulting increase in extracellular space.

The dehydration resulted in a weight loss of approximately 6%. Following a minimum of three 20-min collection periods in the dehydrated bird, the bird was hydrated by oral loading with tap water corresponding to approximately 9% of the dehydrated body weight. After a steady high flow had been achieved, not less than three urine samples (10–15 min duration) were collected. Then NaCl was given by infusion into the wing vein of an 18% solution over a 10-min period; a total of 12–15 mEq/kg body wt were given. Following the salt loading, urine samples were collected for 1–2 hr.

**Analyses.** The following analyses were performed on all urine and plasma samples: 1) osmolality using Fiske osmometer model H-62; 2) chloride on Buchler-Cotlove chloridometer; and 3) Na⁺ and K⁺ on Baird Atomic flame photometer model KY-2. Inulin was analyzed with the anthrone method (6) modified to samples of 40 μl, and the colorimetric reading was performed on a Beckman DU 2 spectrophotometer at 629 μm.

**Calculations.** GFR, urine flow, plasma and urine osmolality, and electrolytes for each bird are given as the average value of about four urine collection periods in each of the diuretic states. GFR was calculated only when the inulin plasma level and the urine flow were stable. Therefore, since the inulin plasma level changed during the salt infusion, GFR could not be determined accurately until 30–30 min after the beginning of the salt infusion when the plasma concentration was again stable. During water diuresis GFR was calculated only when the urine flow was stable and near the peak. The filtered amounts of the various solutes have been calculated using the plasma concentration directly without corrections. The percent of filtered solutes excreted was calculated from the expression (Os U/P)/(In U/P) X 100%, and the percent of filtrate resorbed from (GFR-urine vol)/GFR X 100%.

**RESULTS**

The changes brought about in plasma osmolality and electrolytes by the osmotic stress are presented in Table 1. Plasma osmolality dropped on the average 29 milliosmols/kg H2O following hydration and increased 26 milliosmols/kg H2O when the hydrated birds were salt loaded. A typical experiment is shown in Fig. 1.

**Glomerular function.** When hydration was instituted following dehydration an average increase in GFR of 0.388 ml/min per kg or 29.8 ± 10.4% (sn) was observed, the increase is significantly different from zero (P < 0.01). Salt loading of the rehydrated bird did not result in any significant reduction of the GFR during the experimental period (1–2 hr) (Table 2). The variations were maximally 12.9%, and the GFR was increased in two experiments and reduced in four. In one experiment the salt load was given to the dehydrated bird without hydration, but the change in GFR was not greater than that observed in the other experiments.

**Tubular function.** The osmolar urine to plasma ratio (Os U/P) varied during dehydration and hydration from maximally 2.06 to 0.31 in any bird. The inulin U/P ratio varied from 111 to 5.96 (Fig. 2 and Table 2). The urine flow changed from less than 20 to approximately 300 μl/min per kg, while, at the same time, the GFR increased by only 29%. A water diuresis was thus brought about mainly by a decreased tubular permeability to water and only to a small extent by a larger filtration rate. It will further appear that the fraction of

<table>
<thead>
<tr>
<th>No. of Birds</th>
<th>Osmolality</th>
<th>Na⁺</th>
<th>Cl⁻</th>
<th>K⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>milliosmols/kg H2O</td>
<td>mEq/liter</td>
<td>mEq/liter</td>
<td>mEq/liter</td>
</tr>
<tr>
<td>Dehydration</td>
<td>7</td>
<td>341±7</td>
<td>183±4.7</td>
<td>130±3.2</td>
</tr>
<tr>
<td>Water loading</td>
<td>5</td>
<td>312±6</td>
<td>131±4.8</td>
<td>119±3.0</td>
</tr>
<tr>
<td>Salt loading</td>
<td>6</td>
<td>338±21</td>
<td>166±8.0</td>
<td>142±14.8</td>
</tr>
</tbody>
</table>

Values are means ± sd.
filtered water excreted during water loading was about 25% and during dehydration only about 1%. In some of the hydration experiments, the inulin U/P ratios were as low as 3 and the minimal urine osmolality was 25 milliosmols/kg H₂O, indicating that only two-thirds of the filtered water was resorbed. Such low tubular water resorption is also found in reptiles (2, 13, 17). In comparison, mammals in maximal water diuresis resorb not less than 90% of the filtered water.

Electrolyte excretion varied conspicuously with the state of diuresis. Water loading resulted in a marked decrease in the urine concentrations of Na⁺, K⁺, and Cl⁻ when compared to the dehydrated birds (Table 2), but the rate of excretion of Na⁺ and Cl⁻ per unit time increased by a factor of 2–3 (Table 3). This increase was also reflected in a doubling of the percent of filtered solute excreted (Table 2). The percent of filtered solute excreted in the urine is of the same magnitude as in mammals.

During salt loading an osmotic diuresis was apparent: the urine was slightly hyperosmotic to plasma and the urine flow was high. The excretion of Na⁺ and Cl⁻ was increased to 7–8% of the filtered load and approximately 13% of the filtered water was excreted. (The urine flow was 0.18 ml/min per kg equivalent to 11 ml/hr per kg; this is 1% of body wt/hr. Therefore the diuresis following salt loading did not cause any severe loss of body fluids.) The amount of sodium and chloride excreted in the urine during the 1st hr of the infusion varied from 10–32.6% of the infused amount. One hour after the salt infusion, the sodium concentration and rate of excretion began to decrease considerably, whereas the potassium concentration and excretion rate increased (Fig. 1). This was also observed in ducks.

![Graph showing electrolyte output and osmolality](image-url)
TABLE 2. Composition of urine during dehydration and during water and salt loading in the rooster

<table>
<thead>
<tr>
<th>Urine Osmolality, milliosmols/kg H2O</th>
<th>Urine Flow, al/min per kg</th>
<th>GFR, ml/min per kg</th>
<th>Insulin U/P</th>
<th>Na+ U/P</th>
<th>Cl- U/P</th>
<th>K+ U/P</th>
<th>% of Filtrate Resorbed</th>
<th>% of Filtered solute Excreted</th>
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<td></td>
<td></td>
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<tr>
<td>Dehydrated, 7 birds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>538±79</td>
<td>17.9±3.41</td>
<td>1.5±0.32</td>
<td>1.73±0.34</td>
<td>84.7±30.1</td>
<td>0.82±0.26</td>
<td>0.54±0.24</td>
<td>8.53±6.21</td>
<td>99.0±0.2</td>
</tr>
<tr>
<td>Water loaded, 5 birds</td>
<td></td>
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<tr>
<td>115±41</td>
<td>29.8±149.0</td>
<td>0.37±0.12</td>
<td>2.123±0.474</td>
<td>9.62±3.85</td>
<td>0.25±0.12</td>
<td>0.23±0.11</td>
<td>1.22±0.55</td>
<td>85.8±6.6</td>
</tr>
<tr>
<td>Salt loaded, 6 birds</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>36±62</td>
<td>181.4±133.0</td>
<td>1.06±0.15</td>
<td>2.66±0.55</td>
<td>19.8±9.8</td>
<td>0.97±0.15</td>
<td>0.99±0.32</td>
<td>5.41±2.80</td>
<td>87.2±11.0</td>
</tr>
</tbody>
</table>

Values are means ± SD.

FIG. 2. Osmolality of urine in the rooster. Individual corresponding values from 12 roosters including values from E. Skadhauge and B. Schmidt-Nielsen (manuscript in preparation) are reported. The osmolal urine-to-plasma ratio can vary from 0.2 to 0.8.

Water diuresis following acute salt loading. In three experiments roosters were salt loaded without previous water loading or dehydration. A transient diuresis with low osmolality and low electrolyte concentration was observed immediately after the salt infusion. The minimal osmolality was 161-191 milliosmols/kg H2O, the corresponding chloride concentration 55.5-69.2 mEq/liter. A transient increase in urine flow just after salt loading has also been observed by others (8, 16). No explanation of this phenomenon is apparent, but in these experiments the salt was infused over 2 min in contrast to the 10-min infusion period used in the other experiments. Thus the possibility remains that the more rapid expansion of extracellular volume might have caused a transient suppression of arginine vasotocin release in spite of the increase in plasma osmolality.

Observations on other birds. In an attempt to find a large bird with high concentrating ability, we salt loaded domestic ducks of the White Peking variety (weight 2-3 kg). A urine sodium concentration of 633 mEq/liter has been observed in these birds after salt loading (12). However, in our ducks salt loading (15 mEq/kg) did not result in such high concentration (Table 4). The highest urine osmolality was 537 milliosmols and the maximal urine concentration of electrolytes: Na+: 104, K+: 128, and Cl-: 259 mEq/liter. The osmolar U/P ratio rarely surpassed 1.5.

In male turkeys (weight 6-8 kg) dehydration, water, and salt loading revealed roughly the same concentrating and diluting ability as in the domestic fowl (Table 4). A similar concentrating ability was found by Vogel et al. (22).

DISCUSSION

Glomerular filtration rate. In comparing the variations in GFR of lower vertebrates with those of mammals two questions are of importance: 1) Does the filtration rate vary with hydration and dehydration? 2) If the GFR varies, is it then due to variations in the number of functioning glomeruli or is it due to variations in filtration rate in all or most of the glomeruli? In all lower vertebrates where this has been investigated, it has been found that variation in glomerular filtration rate is due to a change in the number of functioning glomeruli (2, 5, 9). In mammals this phenomenon has not been thoroughly investigated, but according to Homer Smith (20) the number of functioning glomeruli should remain constant while the filtration rate in the individual glomeruli varies.

Regarding the first question, there is a discrepancy between our findings and those of Korr (8). Korr found that the filtration rate in chickens varied with the state of hydration. During dehydration it was 0.6 ml/min per kg and during hydration it was 2.19 ml/min per kg. These variations are much greater than those observed in the present investigations where the filtration rate varied from 1.63 ml/min per kg in the dehydrated bird to 2.11 ml/min per kg in the hydrated bird. The rates of urine flow were essentially the same in the experiments of Korr and the present experiments. It is possible that the discrepancy in the results may have been due to the more accurate urine collection technique employed in the present study. Compared to other lower vertebrates these changes in filtration rate are extremely modest. In amphibians and in some reptiles (2, 10, 15) the filtration rate may increase from several hundreds to 1,000% of the normal value when the animal is hydrated and may decrease to zero at...
TABLE 3. Urinary electrolyte excretion during dehydration and during water and salt loading in the rooster

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>Cl⁻</th>
<th>K⁺</th>
<th>Na⁺ of Filtered Load</th>
<th>Cl⁻ of Filtered Load</th>
<th>K⁺ of Filtered Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrated, 7 birds</td>
<td>24.12±5.60</td>
<td>2.74±1.8</td>
<td>2.54±2.04</td>
<td>7.3±0.09</td>
<td>8.09</td>
<td>36.5</td>
</tr>
<tr>
<td>Water loaded, 5 birds</td>
<td>4.30±2.26</td>
<td>1.88±1.08</td>
<td>1.32±0.59</td>
<td>1.52</td>
<td>0.84</td>
<td>16.2</td>
</tr>
<tr>
<td>Salt loaded, 4 birds</td>
<td>24.12±5.60</td>
<td>2.74±1.8</td>
<td>2.54±2.04</td>
<td>7.3±0.09</td>
<td>8.09</td>
<td>36.5</td>
</tr>
</tbody>
</table>

Values are means ± SD.

TABLE 4. Average urinary osmotic and ionic concentration in turkeys and ducks under various osmotic conditions

<table>
<thead>
<tr>
<th>Osmotic Stress</th>
<th>No. of Birds</th>
<th>Osmolarity (milliosmols/kg H₂O)</th>
<th>Na⁺ (mEq/liter)</th>
<th>Cl⁻ (mEq/liter)</th>
<th>K⁺ (mEq/liter)</th>
<th>Na⁺ + Cl⁻ (mEq/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Turkey</em></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Dehydr.</td>
<td>3</td>
<td>402</td>
<td>81.0</td>
<td>97.0</td>
<td>105</td>
<td>283</td>
</tr>
<tr>
<td>Water load</td>
<td>1</td>
<td>47</td>
<td>5.5</td>
<td>9.5</td>
<td>9.2</td>
<td>17.2</td>
</tr>
<tr>
<td>Salt load</td>
<td>3</td>
<td>553</td>
<td>227</td>
<td>248</td>
<td>77.3</td>
<td>552</td>
</tr>
<tr>
<td><em>Ducks</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt load</td>
<td>5</td>
<td>462</td>
<td>133</td>
<td>103</td>
<td>117</td>
<td>443</td>
</tr>
</tbody>
</table>

moderate states of dehydration. In uricotelic terrestrial reptiles (13), the filtration rate appears to be more stable but still increases 100-200% as the result of water loading and decreases to half of its normal value after severe dehydration. Thus, the rooster is found to have a very stable filtration rate compared to that of most lower vertebrates, and it resembles more nearly mammals than reptiles in this respect.

The effect of salt loading on GFR is not clearcut in most animals because it involves a combination of osmotic increase in plasma concentration, and, at the same time, an expansion of extracellular fluid. In lower vertebrates as well as in mammals, an expansion of the extracellular fluid causes an increase in glomerular filtration rate, whereas in lower vertebrates an increase in osmotic concentration of the plasma causes a decrease in glomerular filtration rate. In turtles the effect of dehydration could be mimicked by an increase in the osmotic concentration of plasma because a small increase in the plasma concentration (20 milliosmols/kg H₂O) caused a complete shutdown of all glomeruli (9). In animals in which a higher osmotic concentration is required for an effect on the GFR, the effect of the salt loading becomes more difficult to interpret because the effect on the extracellular fluid volume then simultaneously becomes greater. In the present experiments where salt loading had no effect, the two factors may have worked in opposite direction. This is also indicated by the fact that rapid injection of NaCl caused an increased urine flow with hypoosmotic urine.

Dantzler (1) raised the blood osmolality of hens by infusing 6% sodium chloride into normally hydrated birds. When the blood osmolality was raised by 150 milliosmols/kg H₂O the GFR decreased by about 40%. Under these conditions the number of functioning glomeruli in the chicken varied in direct proportion to glomerular filtration rate, and, in this respect, then the bird kidney functions as the kidney of reptiles and amphibians.

**Tubular function.** In most uricotelic reptiles (with the exception of the water snake (10)) the tubules show little or no capacity for varying the osmolality of the urine. The desert tortoise (2), the crocodile (17), and the gecko (13) produced at all times, regardless of the state of hydration, a urine that was somewhat hypoosmotic to the blood. Other desert-living reptiles have shown no capacity at all for diluting the urine (13). In the bird the countercurrent system would be ineffective if the distal tubule could not establish an osmotic gradient between tubular lumen and blood. In the present experiment the osmolality of the urine of the roosters was found to vary from 40 milliosmols/kg during water loading to 538 milliosmols/kg during dehydration. This finding is in complete agreement with that reported by others (3, 8). Thus, the bird renal tubules like the mammalian tubules show the capacity for making a high osmotic gradient between tubular lumen and blood, a regulation of the permeability of the tubule to water. The findings, however, do not answer the question whether the two types of tubules (reptilian type and loops of Henle) function alike in their two respects.

From observations on the tubular handling of salt and water in the three osmotic situations it appears especially from Table 2 that the domestic fowl is able to resorb about the same amounts of filtered sodium, chloride, and water as mammals. Three differences, however, are apparent: 1) The bird is, during hydration, able to excrete a larger fraction of the filtered water than mammals. Only during osmotic diuresis will man, dog, or rat excrete more than 10% of the filtered water, whereas the fowl excretes up to 30%. 2) During dehydration to a given osmotic U/P ratio, the rooster resorbs more of the filtered water than mammals do (Fig. 2). This is obviously related to the uricotelic habit of the bird. The filtrate contains very little urea, which, therefore, takes little "osmotic space," and a larger fraction of filtered water can thus be resorbed in spite of a limited concentrating ability. 3) A larger amount of NaCl is resorbed during dehydration than during hydration. Increased resorption of sodium has also been observed as a result of dehydration in reptiles (13) and of administration of antidiuretic hormones in frogs (7). Furthermore, it has been observed that the osmolar clearance of hydrated chickens decreased during the infusion of arginine-vasotocin (unpublished calculation of data from 19). Thus, an increased resorption of ions allows a larger water resorption within the same osmotic ceiling. This is also to a small extent helped by the larger urea
resorption during dehydration (manuscript in preparation). The renal excretion of the salt load in these experiments seemed to follow a time course not grossly different from that observed in birds with a salt gland (12, 16). The Na+ K+ shift might be due to a mineralocorticoid action.

In summary, the renal tubule of the rooster shows the mammalian characteristics with respect to the osmotic variations of the urine (except that the rooster has a very limited capacity to concentrate urine). It differs from terrestrial uricotelic reptiles in which the tubule has little or no capacity to regulate the osmolality of the tubular urine (13, 17). With respect to the small fraction of filtered water that is resorbed during hydration, the bird tubule resembles the reptilian tubule. The finding that the tubular sodium resorption is increased during dehydration agrees with the findings in lower vertebrates.

REFERENCES