Kidney function of the American eel Anguilla rostrata

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Schmidt-Nielsen, Bodi, and J. Larry Renfro. Kidney function of the American eel Anguilla rostrata. Am. J. Physiol. 228(2): 420-431. 1975.—Urine formation in the eel, acclimated to freshwater (FW) and seawater (SW) was studied. SW eels maintained higher plasma and urine osmotic and ionic concentrations than FW eels. Polyethylene-1,2-glycol-14C (PEG-14C) clearance was 29% greater than methoxy-inulin 3H clearance. PEG 14C clearance was considered to be the glomerular filtration rate (GFR). The GFR of SW and FW eels did not differ. Urine flow rate in SW eels was one-third of that in FW eels. The primary urinary solutes in both groups were Na and Cl. Apparently, tubular fluid secretion occurred in FW and, to a lesser degree, in SW eels. With the assumption that water reabsorption was secondary to Na reabsorption in the kidney, the clearance data were used to evaluate all possible explanations for what appeared to be fluid secretion. The data were inconsistent with the possibility that GFR had been underestimated due to glomerular sieving of PEG or active or passive reabsorption of the marker, but consistent with tubular fluid secretion. Furosemide caused diuresis in both groups of eels apparently by inhibition of Na reabsorption in the distal tubule, but it had no effect on the apparent tubular fluid secretion. Tubular sodium secretion could not be conclusively implicated as a driving force for fluid secretion. However, the possibility of K, Ca, or Mg secretion in a proximal segment followed by reabsorption in a more distal part of the nephron was not ruled out.

Two major processes have been shown to be involved in the formation of the primary urine in teleosts. These are glomerular filtration and tubular fluid secretion. Although not completely characterized, glomerular filtration can easily be measured. Filtration is considered to be the primary process of urine formation in freshwater teleosts and participates as well in most seawater species.

Tubular fluid secretion is the mode of urine formation in aglomerular teleosts (20) and probably takes part in urine formation in glomerular marine species as well (9, 21). Tubular fluid secretion in teleosts is not well understood. In all epithelia which transport fluid, fluid movement has been found to be secondary to ion transport (5, 23, 27). If we accept the theory of solute-linked water transport (7), then we would expect ion secretion to be the driving force for fluid secretion in teleost nephrons. Because micropuncture data on teleosts are not available and because the teleost nephron also reabsorbs solutes from the urine, it has not been possible to definitively link an ion to the fluid secretory process. Circumstantial evidence has indicated that perhaps the secretion of divalent ions (1, 8) was the driving force for fluid secretion in seawater-acclimated teleosts. Evidence for this was reported by Hickman (10, 11, 13).

In the course of characterizing normal kidney function in the American eel Anguilla rostrata, we have discovered secretory urine formation, particularly in the freshwater-acclimated form. In this report, we have characterized normal kidney function in the freshwater- and seawater-acclimated eel, and we have used clearance data to evaluate and quantitate the process of fluid secretion by the freshwater-acclimated teleost. No ion transport was shown to take part in this process.

METHODS AND MATERIALS

Animals and Media

Commercially obtained American eels Anguilla rostrata, weighing 58-241 g, were maintained for 2-12 wk in either flowing seawater (934 mosmol/kg water; 434 mM Na; 8.8 mM K; 51.5 mM Mg; 9.8 mM Ca; and 486.1 mM Cl) or flowing, untreated well water (21 mosmol/kg water; 3.7 mM Na; 0.04 mM K; 0.29 mM Mg; 0.7 mM Ca; and 5.0 mM Cl). Water temperature during the time of acclimation varied from 8 to 15°C in seawater and 11 to 14°C in freshwater. The experiments were carried out during the months from July through December in two succeeding years. A group of 16 eels was used the 1st yr. During the 2nd yr, a group of eight eels was used in the furosemide study. The control values for kidney function differed quantitatively but not qualitatively from the 1st yr’s group. In the second group, each animal served as its own control. Furosemide (Lasix) was given intravenously at the beginning of each 24-h period following control periods. The average dosage was 1.75 mg/kg body wt. The fish were in a natural photoperiod and were not fed.

Experimental Procedures

To collect urine and blood samples from the eel with a minimum of handling, indwelling catheters were placed in the hemal vein and in the uropore prior to the experiment.

Catheterization of bladder. The bladder of the eel is a distal expansion of the fused ureters which lies above and caudal to the uropore. It is relatively small and thick walled; its volume in a 100-g eel is about 1 ml.

While anesthetized with tricaine methane sulfonate (Sandoz MS-222; 1:2000 wt/vol), a special retention catheter made from PE-60 tubing (Clay-Adams Intra-
medic) was inserted into the uropore and fastened with a purse-string suture. The retention catheter had a tapered bulb on the inserted end which prevented the catheter from slipping out. The end of the catheter was curved caudally. Urine entering the bladder from the ureters flowed continuously through the catheter into a vial where the urine was collected under oil. Thus, the fluid collected was ureteral urine.

To test if water might diffuse across the wall of catheters submerged in seawater or freshwater, the water permeability of the polyethylene catheters was measured by filling the catheters with a solution of PEG-14C in distilled water and submerging 33-cm-long pieces in freshwater or seawater for 72 h. No measurable change in PEG concentration was observed in either FW or SW. The radioactivity (counts/min) of the initial solution was 86,947 ± 62. After 72 h in the catheter submerged in seawater, it was 87,350 ± 288, and in freshwater it was 87,315 ± 294.

Catheterization of hemal vein. While the eel was still anesthetized, PE-10 tubing was inserted through a puncture into the hemal vein near the caudal end of the fish and threaded into the vein about 3 cm. The tubing was fastened with a silk suture.

Restriction of movement. Catheterized fish were placed inside Plexiglas chambers. These chambers were narrow enough to prevent the fish from turning around. Chambers were equipped with flowing seawater or freshwater (flow rate = ca. 150 ml/min) with a controlled temperature of 12.5 ± 1.0°C. The fish were allowed to recover from catheterization at least 24 h before further experimentation.

Extracellular space determination (ECS). ECS was measured with polyethylene-1,2-glycol-14C (PEG-14C, New England Nuclear Corp., mol wt 4,000, sp act 0.46 mCi/g) and inulin methoxy-3H (inulin-3H, New England Nuclear Corp., mol wt 5,000–5,500, sp act 103.2 mCi/g) as extracellular markers. Each animal was weighed and injected intravenously with 25 μl of either the PEG-14C or inulin-3H solution described above. After 24 h, the animals were removed from the water, and blood samples were taken. The animals were killed by decapitation and placed in a Waring blender with an exact volume of water. An aliquot of the homogenate was centrifuged, and the supernatant fluid and the plasma were counted by liquid scintillation spectrometry. The extracellular space was calculated from the following equation:

\[
\text{ECS (ml X kg}^{-1}\text{ body wt)} = \frac{\text{total body counts/min}}{\text{plasma counts/min per ml X kg body wt}}
\]

Glomerular filtration rate (GFR). GFR was determined with both PEG-14C and inulin-3H. Each animal was given 25 μl of a solution containing 250 μCi of PEG-14C per milliliter of 0.7% NaCl and 25 μl of a solution containing 1.0 mCi of inulin-3H per milliliter of 0.7% NaCl via the venous catheter. This catheter was subsequently rinsed with the animal’s blood at least 5 times and filled with heparinized saline. After 6–24 h of distribution time for the injected material, urine and blood sampling were begun. In most eels, five to seven urine samples and six to eight blood samples were collected. Each urine collection period lasted approximately 24 h. Blood samples, 100 μl in volume, were collected approximately every 24 h. Plasma was separated by centrifugation. The red cells were suspended in Ringer solution and reinjected. No appreciable change in hematocrit was discerned during the experimental period. The U/P PEG ratio was determined from the average of the plasma PEG-14C concentration at the beginning and end of each collection period.

In using both inulin and PEG as glomerular markers, we have attempted to find the most reliable measure for GFR. Investigations of the distribution of PEG-14C and inulin-3H in various tissues of eels (23) have shown that both of the compounds (or radioactive groups) accumulate in the kidneys of FW- and SW-acclimated eels. For PEG-14C, the distribution volumes 24 h after injection were 65 and 85% of the total kidney tissue water in FW and SW eels, respectively. For inulin-3H, the distribution volumes were much higher—95 and 132%, respectively. In other tissues, as well, the distribution volumes were higher for inulin than for PEG, and intracellular ion concentrations calculated from extracellular space determinations gave more realistic values with PEG than with inulin.

This was also the case in other fishes. Whether this accumulation is due to breakdown or binding cannot be determined from the distribution data. Hickman et al. (12), in incubation studies with isolated flounder tubules, showed binding with saturation kinetics of inulin-3H to the tissues, whereas no evidence for binding or saturation kinetics was found for PEG.

Another comparison of the two compounds as glomerular markers can be made from the decrease with time of PEG and inulin in the blood of the eels following injection. From a semilog plot of the plasma concentration of PEG or inulin with time, the rate constant (k) for the elimination of these compounds can be determined. If the compound were excreted only through the kidneys, and if it were distributed only in the extracellular space (ECS), then k should be related to GFR according to the equation:

\[
k = \frac{\text{GFR}}{\text{ECS}}
\]

or

\[
\text{GFR} = k \times \text{ECS}
\]

If one measures k and GFR and determines the regression line for equation 2, then the extracellular volume can be calculated (Table 1). If the compound is excreted via some other route in addition to the kidneys, then k will be higher and the calculated ECS will be erroneously low.

In Table 1, the regression lines calculated from k and GFR from the individual clearance periods are presented. In FW eels there was a good correlation between k and GFR (r = 0.89). The PEG space in FW eels has been determined independently (see Table 1) to be 166.7 ml X kg⁻¹ body wt, and this value is in excellent agreement with the value calculated from the regression line (167.0 ml X kg⁻¹). The inulin space, determined to be 235.7 ml X kg⁻¹, is also close to the value calculated from the regression line (218.1 ml X kg⁻¹). These data indicate that the two compounds were excreted primarily or ex-
TABLE 1  Comparison of PEG-14C to inulin-3H for estimation of glomerular filtration rates

<table>
<thead>
<tr>
<th></th>
<th>Seawater</th>
<th>Freshwater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression of k\textsubscript{GFR} vs. GFR = 29.1k + 1.1* GFR = 218.1k - 0.7 GFR\textsubscript{med}</td>
<td>r = 0.59</td>
<td>r = 0.89</td>
</tr>
<tr>
<td>Correlation coeff</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression of k\textsubscript{PEG} vs. GFR = 46.3k + 0.9 GFR = 167.0k - 0.5 GFR\textsubscript{med}</td>
<td>r = 0.55</td>
<td>r = 0.89</td>
</tr>
<tr>
<td>ECS\textsubscript{In}, ml X kg\textsuperscript{-1}</td>
<td>238.3 ± 8.4</td>
<td>235.7 ± 13.3</td>
</tr>
<tr>
<td>(3)</td>
<td></td>
<td>(3)</td>
</tr>
<tr>
<td>ECS\textsubscript{PEG}, ml X kg\textsuperscript{-1}</td>
<td>241.5 ± 17.0</td>
<td>166.7 ± 21.9</td>
</tr>
<tr>
<td>(3)</td>
<td></td>
<td>(3)</td>
</tr>
<tr>
<td>C\textsubscript{PEG}</td>
<td>1.28 ± 0.06</td>
<td>1.30 ± 0.06</td>
</tr>
<tr>
<td>(29)</td>
<td></td>
<td>(36)</td>
</tr>
</tbody>
</table>
| Values are means ± SE. Numbers in parentheses are numbers of observations. * Regression equation: glomerular filtration rate (GFR) (ml X kg\textsuperscript{-1} X h\textsuperscript{-1}) equals the extracellular space (ECS) (ml X kg\textsuperscript{-1}) times the rate constant (k) (fraction per h) for the disappearance of inulin or PEG from the plasma plus a constant (y intercept). For further explanation, see text.

ECS was determined on the whole body. The ratio of PEG clearance (C\textsubscript{PEG}) to inulin clearance (C\textsubscript{In}) shows that PEG was cleared faster than inulin.

RESULTS

Plasma and Urine Concentrations

Plasma osmolality was significantly higher in SW than in FW eels (Table 3). This was primarily due to higher Na and Cl concentrations in the plasma of SW eels. Plasma K and Mg concentrations were not significantly different in the two eels.

TABLE 3. Osmolality and ion concentrations in plasma and urine of SW and FW Anguilla rostrata

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Ultrafiltrate</th>
<th>Percent Filtered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmolality, mosmol/kg</td>
<td>309.7 ± 14.8</td>
<td>263.2 ± 9.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(17)</td>
<td>(93)</td>
<td>(35)</td>
<td>(40)</td>
</tr>
<tr>
<td>Na, mM</td>
<td>143.7 ± 2.8</td>
<td>133.6 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(16)</td>
<td>(19)</td>
<td>(39)</td>
<td>(39)</td>
</tr>
<tr>
<td>Cl, mM</td>
<td>7.4 ± 0.5</td>
<td>72.6 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(28)</td>
<td>(38)</td>
<td>(32)</td>
<td>(36)</td>
</tr>
<tr>
<td>K, mM</td>
<td>2.48 ± 0.06</td>
<td>2.69 ± 0.06</td>
<td>NS</td>
</tr>
<tr>
<td>(15)</td>
<td>(19)</td>
<td>(36)</td>
<td>(39)</td>
</tr>
<tr>
<td>Mg, mM</td>
<td>0.96 ± 0.11</td>
<td>0.91 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>(25)</td>
<td>(27)</td>
<td>(38)</td>
<td>(42)</td>
</tr>
<tr>
<td>Ca, mM</td>
<td>2.41 ± 0.06</td>
<td>2.63 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(25)</td>
<td>(23)</td>
<td>(37)</td>
<td>(42)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses are numbers of observations. * Student t test.

Sample Analyses

Osmolality was determined by measurement of freezing-point depression (Osmette A, Precision Systems). Na and K concentrations were measured by flame photometry (Instrumentation Laboratory model 343). Cl was determined by coulometric titration (Buchler-Cotlove chloridometer), and Ca and Mg were determined by atomic absorption spectrophotometry (Perkin-Elmer model 107). 3H- and 14C-labeled compounds were counted by liquid scintillation (Packard Tri-Garb).

Protein-bound, nonfilterable Ca was estimated from determination of Ca concentrations in plasma before and after ultrafiltration through a pressurized ultrafiltration unit (Amicon model 10 PA) equipped with a membrane specified to retain molecules larger than 50,000 mol wt. The ultrafiltrate obtained in these tests indicated that approximately 80% of the plasma Ca passed through the artificial membrane, and we assumed that this was also true of the glomerular membranes (Table 2).

Symbols Used in Equations

GFR = glomerular filtration, in ml X kg\textsuperscript{-1} body wt X h\textsuperscript{-1}

V = urine flow rate, in ml X kg\textsuperscript{-1} X h\textsuperscript{-1}
P\textsubscript{i} = plasma concentration of substance i, in mM
U\textsubscript{i} = urine concentration of substance i, in mM
Q\textsubscript{i,ex} = amount of substance i excreted per unit time, in μmol X kg\textsuperscript{-1} body wt X h\textsuperscript{-1}
Q\textsubscript{i, reab} = amount of substance i reabsorbed per unit time, in μmol X kg\textsuperscript{-1} body wt X h\textsuperscript{-1}
F\textsubscript{frac},i = (Q\textsubscript{i,ex} / (GFR X P\textsubscript{i}))\textsuperscript{-1}
Reab\textsubscript{i} = concentration of i in reabsorbed fluid, in mM
S = amount of fluid secreted per unit time, in ml X kg\textsuperscript{-1} body wt X h\textsuperscript{-1}
in the two groups, whereas plasma Ca was slightly but significantly higher in FW eels (Table 3).

Urinary osmotic and ionic concentrations were consistently higher in SW than in FW eels (Table 3). The urinary ions in greatest concentrations were Na and Cl in both groups. The Mg concentration of the urine of SW animals was 51 times higher than in FW eels.

**Giomerular Filtration Rates, Urine Flow, and Free Water Clearance**

The ratio of PEG-14C clearance ($C_{\text{PEG}}$) to inulin-3H clearance ($C_{\text{inul}}$) was greater than unity in both FW and SW eels (Table 1). PEG-14C clearance averaged about 29% higher than inulin-3H clearance. In the following calculations, we have used PEG-14C clearance rather than inulin-3H clearance as a measure of GFR.

GFR ($V \times U/P$ PEG-14C) was not significantly different in SW and FW animals (Table 4). In the group of eels used in the furosemide study, the GFR was higher and more variable than in the first group of animals, but there was no significant difference within the group between FW and SW fish (Table 4). Administration of furosemide did not significantly change GFR in either FW or SW eels (Table 4).

The $U/P$ PEG ratio was almost 3 times higher in SW than in FW eels (Table 4), and it follows that urine flow rate was about 3 times higher in FW than in SW eels (Table 4). The higher urine flow rate in the FW group was partly caused by a more than threefold increase in the free water clearance ($C_{\text{H2O}} = V(1 - (U/P)_{\text{osm}})$) (Table 4). Furosemide treatment of SW eels caused a decrease in fractional tubular water and electrolyte reabsorption as seen from a significant decrease in $U/P$ and a significant increase in osmolar $U/P$ ratio (Table 4). Urine flow rate doubled, but due to the large variations in GFR in this group of eels the difference was not significant. In FW eels, fractional water reabsorption was barely lowered by furosemide; however, decreased tubular electrolyte reabsorption was evident from the significant increase in osmolar $U/P$ ratio. Free water clearance in both groups was unaffected by the drug (Table 4).

**Solute Excretion and Fractional Reabsorption**

The quantity of each ion excreted ($Q_{i,\text{ex}} = U_iV$) was higher in SW than in FW eels (Table 5). This is reflected in the osmolar clearance which is twice as high in the SW group compared to the FW group (Table 4). The primary solutes excreted in the ureteral urine by FW as well as SW eels were Na and Cl (Table 5).

While fractional reabsorption ($\text{Frac}_{i,\text{reab}} = Q_{i,\text{reab}}/GFR \times P_i$) of filtered Na and Cl was different in FW and SW eels, other ions showed significant differences in fractional reabsorption between the two groups (Table 6). K was reabsorbed in SW and secreted in FW. Mg was secreted by both groups, but more so in SW eels. Fractional Ca reabsorption was significantly greater in FW than in SW (Table 6) and Ca was secreted in SW eels.

Furosemide treatment in SW eels caused a fourfold increase in the quantity of Na excreted, a threefold increase in Cl excretion (Table 5), and a significant decrease in fractional Na reabsorption (Table 6). In FW eels,

**Table 4. GFR, $V$, $U/P_{\text{PEG}}$, $C_{\text{H2O}}$, osmolar $U/P$, and osmolar clearance in SW- and FW-acclimated Anguilla rostrata and effects of furosemide treatment**

<table>
<thead>
<tr>
<th></th>
<th>Seawater</th>
<th>Freshwater</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (PEG), ml x kg$^{-1}$ x h$^{-1}$</td>
<td>2.09±0.22 (35)</td>
<td>2.24±0.35 (36)</td>
<td>NS</td>
</tr>
<tr>
<td>$V$, ml x kg$^{-1}$ x h$^{-1}$</td>
<td>0.81±0.19 (35)</td>
<td>1.49±0.15 (36)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$(U/P)_{\text{PEG}}$</td>
<td>3.61±0.46 (35)</td>
<td>1.30±0.15 (36)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$C_{\text{H2O}}$, ml x kg$^{-1}$ x h$^{-1}$</td>
<td>0.39±0.07 (35)</td>
<td>1.27±0.13 (36)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$(U/P)_{\text{osm}}$</td>
<td>0.59±0.03 (35)</td>
<td>0.15±0.01 (35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$C_{\text{osm}}$, ml x kg$^{-1}$ x h$^{-1}$</td>
<td>0.51±0.08 (35)</td>
<td>0.22±0.03 (35)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses are numbers of observations. In the furosemide study each animal served as its own control. After several control samples were collected, each animal received an average dose of 1.75 mg furosemide per kg body wt. * Student $t$ test.

Furosemide had no significant effect on the quantities of electrolyte excreted (Table 5). The drug, however, significantly lowered the fractional reabsorption of Na (Table 6).

**Discussion**

Plasma and urine compositions found in the present investigation were similar to those found by other investigators in SW and FW eels and indicated that acclimation to the two media was complete. The values reported here for the American eel are essentially the same as those measured in the European eel Anguilla anguilla (26). Sharratt et al. (26), as well as others (16), have reported an unexplained low Cl concentration in plasma of FW eels, and
we have observed the identical phenomenon. The low Cl level in the plasma of FW eels may be explained by the fact that FW eels cannot fully compensate for Cl loss by branchial uptake but must gain Cl in their food (17). Therefore, starved animals, as used in all the above studies, tend to lose Cl in FW.

Acclimation to FW in many fishes results in an increased urine flow and decreased urine solute concentrations. This is usually accomplished by increased GFR and, probably, decreased water permeability of distal portions of the nephron. Our finding that GFR was the same in SW and FW American eels was similar to the finding of Oide and Utida (22) on Japanese eels A. japonica. Other studies have usually indicated a reduced GFR in marine teleosts compared to FW forms, and in euryhaline fishes GFR is usually lower in SW than in FW (14, 18, 26).

Acclimation to FW in many fishes results in an increased urine flow and decreased urine solute concentrations. This is usually accomplished by increased GFR and, probably, decreased water permeability of distal portions of the nephron. Our finding that GFR was the same in SW and FW American eels was similar to the finding of Oide and Utida (22) on Japanese eels A. japonica. Other studies have usually indicated a reduced GFR in marine teleosts compared to FW forms, and in euryhaline fishes GFR is usually lower in SW than in FW (14, 18, 26).

The aglomerular toad fish Opsanus tau can, in spite of the fact that it has no glomerular filtration, increase urine flow when transferred from SW to FW (19). This compensation in FW indicates that a mechanism exists that can be used to increase or decrease urine output without the need of an increased or decreased GFR. The low water and ion permeability of the toad fish is apparently a key factor to its survival in FW (19). Similarly, the eel (at least the adult migratory form, the silver eel) has the lowest water and ion permeability of any teleost studied (17). The adult eel has a fully glomerular kidney; however, the leptocephalus larvae of at least one species of eel (Ariosoma balearicum) has an agglomerular kidney (15), and it is possible that agglomerular function is partly carried over to the adult stage.

This study, and the study by Jones et al. (16), in which all of the major cations of the ureteral urine were analyzed, indicated that Na and Cl were the primary cations in the ureteral urine of SW eels. The leptocephalus bladder has been shown to considerably alter the composition and volume of fluid stored within it (24). The final urine produced by an eel that was not catheterized might contain less Na and Cl due to reabsorption of these ions by the urinary bladder. We have made preliminary experiments in which eel bladder urine was analyzed which showed that final urine may be as high in Na and Cl as ureteral urine. The question needs further investigation, and the fact must be taken into account when one considers the overall salt and water balance of the eel.

### Table 3. Quantities of several ions excreted in urine of SW- and FW-acclimated Anguilla rostrata and effects of furosemide treatment

<table>
<thead>
<tr>
<th>Ion</th>
<th>Seawater</th>
<th>Freshwater</th>
<th>P Value</th>
<th>Control</th>
<th>Furosemide</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>48.10±7.73</td>
<td>21.40±3.36</td>
<td>&lt;0.01</td>
<td>36.2±21.1</td>
<td>92.6±16.7</td>
<td>NS</td>
</tr>
<tr>
<td>Cl</td>
<td>66.82±10.40</td>
<td>8.98±1.49</td>
<td>&lt;0.01</td>
<td>59.4±32.7</td>
<td>32.2±6.3</td>
<td>NS</td>
</tr>
<tr>
<td>K</td>
<td>3.98±0.54</td>
<td>2.50±0.46</td>
<td>&lt;0.05</td>
<td>17.1±3.83</td>
<td>0.56±0.08</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mg</td>
<td>17.12±3.83</td>
<td>0.56±0.08</td>
<td>&lt;0.01</td>
<td>3.70±0.30</td>
<td>1.77±0.30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ca</td>
<td>3.70±0.30</td>
<td>1.77±0.30</td>
<td>&lt;0.01</td>
<td>32.2±16.7</td>
<td>18.38±21.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses are numbers of observations. In the furosemide study, each eel served as its own control. After several control samples, each animal received an average dose of 1.75 mg furosemide per kg body wt. * Student t test.

Fluid secretion was indicated by the fact that urine flow rate exceeded GFR measured as either PEG clearance or inulin clearance in 15 out of 35 clearance periods in FW eels (Fig. 1). It was generally more frequent at filtration rates below 1.0 ml X kg⁻¹ X h⁻¹. In Table 1, this is shown by the PEG or inulin U/P ratios less than unity. Ratios less than 1 did not occur in SW (Fig. 2). Other studies of renal function in FW-acclimated eels have not reported tubular fluid secretion in either the American eel (3) or the European eel (16, 26). The finding of U/P ratios below 1% did not occur in SW. This was obviously not caused by the choice of PEG rather than inulin as a glomerular marker, since the PEG¹⁴C clearance gave higher estimates of the filtration rate than inulin clearance in 15 out of 35 clearance periods in FW eels (Fig. 1). Because both inulin and PEG were found to accumulate in the renal tissue of the eel (25), the possibility existed that metabolic breakdown of the labeled compounds during the time interval following the injection could cause an increase in tubular backdiffusion of the labeled group and result in an underestimation of GFR. If this were the case, we would expect the U/P ratio to decrease toward unity, but not to become less than unity. Furthermore, since the metabolic breakdown of the glomerular marker would increase with time after injection, we would expect the greatest error in GFR measurements to occur in urine samples collected the longest time after the injection of the glomerular marker. However, U/P ratios less than 1 were found only in time after injection (Table 7). In eels

Apparent Fluid Secretion and Ion Movements

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**Table 5. Quantities of several ions excreted in urine of SW- and FW-acclimated Anguilla rostrata and effects of furosemide treatment**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Seawater</th>
<th>Freshwater</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>36.2±21.1</td>
<td>92.6±16.7</td>
<td>NS</td>
</tr>
<tr>
<td>Cl</td>
<td>59.4±32.7</td>
<td>32.2±6.3</td>
<td>NS</td>
</tr>
<tr>
<td>K</td>
<td>9.60±8.34</td>
<td>18.38±4.86</td>
<td>NS</td>
</tr>
<tr>
<td>Mg</td>
<td>13.16±5.54</td>
<td>3.37±0.30</td>
<td>NS</td>
</tr>
<tr>
<td>Ca</td>
<td>4.06±2.54</td>
<td>1.77±0.30</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses are numbers of observations. In the furosemide study, each eel served as its own control. After several control samples, each animal received an average dose of 1.75 mg furosemide per kg body wt. * Student t test.
TABLE 6. Fraction of filtered water and electrolytes reabsorbed by kidneys of SW- and FW-acclimated Anguilla rostrata and effects of furosemide treatment

<table>
<thead>
<tr>
<th></th>
<th>Seawater</th>
<th>Freshwater</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H2O 0.659 ± 0.043 (29)</td>
<td>−0.580 ± 0.344 (35)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Na  0.873 ± 0.022 (28)</td>
<td>0.891 ± 0.021 (34)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Cl  0.769 ± 0.050 (22)</td>
<td>0.077 ± 0.034 (31)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>K   0.339 ± 0.064 (28)</td>
<td>−0.336 ± 0.225 (35)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Mg  −6.195 ± 1.200 (29)</td>
<td>−0.132 ± 0.375 (35)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Ca  0.327 ± 0.072 (25)</td>
<td>0.578 ± 0.062 (34)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses are numbers of observations. * Student t test.

FIG. 1. Relationship of urine flow (V) to glomerular filtration rate (GFR) in FW-acclimated Anguilla rostrata. Solid diagonal line is line of identity. In FW, tubular fluid secretion is indicated by values above solid diagonal line. Lower dashed line indicates relationship of V to GFR when no fluid secretion has occurred. Upper dashed line is relationship of V to GFR when a constant fluid secretion (S) of 1.5 ml × kg⁻¹ × h⁻¹ has occurred. Equation used to calculate V when GFR and S are known was obtained by combining equations 5 and 6 (see text) and is as follows: V = GFR (1 − (PNa × FracNa)/ReabH₂O) + S.

25 and 34, the ratios were below unity during the entire period from 18 h following the injection of the radioactive markers to 163 h. In eel 36, the U/P ratios were higher than unity during a similar period.

Possible error could have occurred if the catheter leaked during some of the urine collection periods. This is unlikely for the following reasons: first, we would expect the osmolality and ion concentrations of the urine to be lower in the samples showing lower U/P PEG ratios due to the entry of water. However, there was no correlation between U/P PEG and urine osmolality (r = 0.26), Na (r = 0.38), Cl (r = 0.37), or Mg (r = 0.27). Second, if water could enter the catheter of FW eels by leaks, we would expect it to enter the catheter of SW eels, as well, but no evidence for addition of SW to urine of SW eels was found.

If water moved across the catheter wall by diffusion, we would expect dilution of the urine of FW eels and concentration of the urine of SW eels. As shown in METHODS.
The polyethylene catheters were impermeable to water during the time studied.

Thus, we have not been able to find any technical error in the measurement of GFR and urine flow that could be responsible for the U/P PEG and inulin ratios less than unity. We were then faced with four alternative possibilities: 1) PEG-14C and inulin-3H may be incompletely filtered (sieving); 2) active reabsorption of both inulin and PEG takes place after it is filtered; 3) fluid is secreted into the renal tubules secondary to Na and Cl in approximately the same concentration as in plasma; and 4) fluid secretion is present but it is not secondary to Na and Cl secretion. These four possibilities can be evaluated mathematically and from the data: if i) GFR is measured correctly, ii) fluid enters the renal tubule by filtration only, and iii) the reabsorbate has a constant concentration ($k$), then the amount of Na reabsorbed ($Q_{Na,reb}$ = GFR $P_{Na} - U_{Na}$) must be proportional to the amount of H$_2$O reabsorbed (GFR - V)

$$Q_{Na,reb} = k(GFR - V)$$

This is illustrated by line 1 in Fig. 3, where $k = 180$ mM. If, on the other hand, GFR has been underestimated by an amount ($N$) due to either incomplete filtration (sieving) of the glomerular marker or active reabsorption of the glomerular marker, then both the amount of Na and H$_2$O reabsorbed would have been underestimated. For the underestimated values we give the symbols $Q_{Na,reb}'$ and GFR'. Then

$$Q_{Na,reb}' = Q_{Na,reb} + NP_{Na}$$

GFR - GFR' + N

Substituting into equation 3

$$Q_{Na,reb}' + NP_{Na} - k(GFR' + N - V)$$

or

$$Q_{Na,reb}' = k(GFR' - V) + N(k - P_{Na})$$

when

$$Q_{Na,reb}' = 0$$

then

$$GFR' - V = N \frac{k - P_{Na}}{k}$$

The amount $N$ by which GFR had been underestimated could be a variable if it represented a fixed fraction of GFR ($N = \alpha GFR$). This would probably be the case if the glomerular marker were being sieved out of the filtrate (possibility 1), since the sieving would be proportional to the filtration rate. If, on the other hand, the glomerular marker were freely filtrable, but actively reabsorbed by the tubule (possibility 2), one would expect either that a constant fraction of the glomerular marker was reabsorbed or, in the event of a low $Tm$ for the marker, a constant amount of the marker would be reabsorbed, giving us a constant value for $N$ (except at very low filtration rates, where it should be almost totally reabsorbed).

The case where $N$ is a fixed fraction of GFR ($N = \alpha GFR$) is complicated since equation 4 then has two independent variables (GFR' - V) and GFR. The equation describes a straight line only if GFR' - V decreases in direct proportion to GFR. In that case, the slope of the line is not $k$, but a function of $k$. The intersection with the Y axis

$$-\alpha GFR \left( \frac{k - P_{Na}}{k} \right)$$

is zero, when GFR is zero.

In the case where $N$ is a constant amount, equation 4 shows that the line would have the same slope as the line for the correct measurement of GFR but it would not go through zero when $k \neq P_{Na}$. It would intersect with the Y axis at $-N(k - P_{Na})/k$ (equation 5). This is illustrated by line 2 in Fig. 3, where $N$ has been given the arbitrary value of 2 ml/kg/h. By underestimating GFR, the points in the graph would move down and to the left, as shown by $a$, $b$, and $c$ on line 1 and their counterparts $a'$, $b'$, and $c'$ on line 2. Negative values for $Q_{Na,reb}'$ could be expected. Furthermore, it is clear from equation 4 that

![Fig. 2. Relationship of urine flow (V) to glomerular filtration rate (GFR) in SW-acclimated Anguilla rostrata. See Fig. 1.](image)

![Fig. 3. Theoretical relationship between amount of Na reabsorbed ($Q_{Na,reb}$) and amount of water reabsorbed (GFR - V). Line 1 shows $Q_{Na,reb} = k(GFR - V)$ where $k = 180$. Line 2 shows same relationship where GFR' and $Q_{Na,reb}'$ represent values when GFR has been underestimated by constant amount N ($N = 2$ ml X kg$^{-1}$ X h$^{-1}$). Line 3 shows relationship when GFR is measured correctly but a constant amount of fluid $S$ ($S = 2$ ml X kg$^{-1}$ X h$^{-1}$) has been secreted into tubules.](image)
when the concentration of Na in the reabsorbate is equal to its concentration in the plasma \( (k = P_{Na}) \), underestimating GFR would not move the line to the left, but could still result in negative values for \( Q_{Na,\text{reab}} \), and GFR’ \(- V\).

Equation 1 would also be valid if GFR were measured correctly, but tubular secretion of fluid were secondary to NaCl secretion (possibility 3), since this situation cannot be distinguished from an underestimation of GFR. In this event, \( N \) would mean the amount of fluid secreted with the same NaCl concentration as that of the plasma.

Finally, if GFR were measured correctly, and an amount of water \( (S) \) without Na and Cl were added to the tubule by secretion (possibility 4), then the amount of water reabsorbed would have been underestimated by the amount of fluid secreted \( (S) \). The real amount reabsorbed would be GFR + \( S - V \).

Substituting into equation 1, we have

\[
Q_{Na,\text{reab}} = k(GFR + \dot{S} - \dot{V})
\]

or

\[
Q_{Na,\text{reab}} = k(GFR - \dot{V}) + \dot{S}k
\]

when

\[
Q_{Na,\text{reab}} = 0
\]

then

\[
GFR - \dot{V} = -\dot{S}
\]

This is illustrated by line 3 in Fig. 3, where \( \dot{S} \) has been given the value 2 ml/kg\( \cdot \)h\( ^{-1} \). By underestimating the amount of H\( _2 \)O reabsorbed, the points in the graph \( a, b, \) and \( c \) would move to the left (\( a'', b'', \) and \( c'' \)). The estimation of \( Q_{Na,\text{reab}} \) would not be affected. Therefore, the points would remain at the same level, and no negative values for \( Q_{Na,\text{reab}} \) would be expected.

In view of these theoretical considerations, the present data were plotted in the same way as shown in Fig. 3. Thus, the apparent amount of each ion reabsorbed or secreted by the renal tubule \( (\dot{Q}_{1,\text{reab}}) \) was plotted against the apparent amount of water reabsorbed or secreted \( (GFR - \dot{V}) \).

The results of the first set of experiments are shown in Figs. 4–8, whereas the results of the chloride data from the furosemide experiments with controls are shown in Fig. 9. For each of the ions, with the exception of K and Mg, the values fall on two separate lines, so that the line for the FW eels falls to the left of the line for SW eels.

Looking first at the Na and Cl data, it is seen that in the first set of experiments (Figs. 4 and 5), the lines do not intersect with the \( X \) axis at zero. In FW eels, the intersection point with the \( X \) axis for the Na line was \(-0.86 \) ml\( \times \)kg\( ^{-1} \)\times h\( ^{-1} \); in SW eels, it was \(-0.53 \) ml\( \times \)kg\( ^{-1} \)\times h\( ^{-1} \). The intersection points for Cl were similar. No negative values for Na or Cl reabsorption were measured in either FW- or SW-acclimated eels.

In the second set of experiments, the lines for SW eels (control as well as furosemide-treated eels) intersect at zero (Fig 9). The line for FW eels is moved to the left and intersects at 3.66 ml\( \times \)kg\( ^{-1} \)\times h\( ^{-1} \). The point of intersection was not changed by furosemide treatment. In none of the eels, FW or SW acclimated, untreated or furosemide treated, did we find negative values for \( Q_{Na,\text{reab}} \).

The slopes of the lines for Na and Cl indicate the net concentration of the reabsorbate (m\( \mu \)mol/ml H\( _2 \)O reabsorbed). In FW eels in the first set of experiments, the average reabsorbate would have a Na concentration of 184 m\( \mu \)M and a Cl concentration of 143 m\( \mu \)M. The reabsorbate thus has a higher concentration than that of the plasma (see Table 3): \( \text{Reab}_{Na}/P_{Na} = 1.38 \) and \( \text{Reab}_{Cl}/P_{Cl} = 1.87 \). In SW eels, the average concentration of the reabsorbate for Na was 152.1 m\( \mu \)M and for Cl it was 125.1 m\( \mu \)M. The reabsorbate had about the same Na and Cl concentrations as the filtrate: \( \text{Reab}_{Na}/P_{Na} = 0.983 \) and \( \text{Reab}_{Cl}/P_{Cl} = 0.928 \).
In FW eels in the second set of experiments, the slope of the line was dramatically changed by furosemide treatment (Fig. 9). The average reabsorbate Cl concentration was 158 mM in control periods and fell to 63 mM after furosemide treatment. Reab\textsubscript{Cl}/P\textsubscript{Cl} fell from 2.08 mM in control periods to 0.89 mM in furosemide periods. The Na data show the same tendency, but were too scattered to permit a reasonable estimate of reabsorbate concentration.

We can now interpret these results in terms of the theoretical possibilities discussed above. In the present experiments, we used both inulin-\textsuperscript{3H} and PEG-\textsuperscript{3H}C as glomerular markers and found their renal clearances to be different. We can, therefore, by examination of the relationship of C\textsubscript{In} to C\textsubscript{PEG}, gain some insight into the effect of underestimation of GFR by using inulin-\textsuperscript{3H} rather than PEG-\textsuperscript{3H}C as a glomerular marker. Figure 10 shows the relationship of C\textsubscript{PEG} - C\textsubscript{In} to the C\textsubscript{In}. As C\textsubscript{In} increased,
the difference between $C_{\text{PEG}}$ and $C_{\text{In}}$ increased. The correlation coefficient for this relationship was $r = 0.75$. From this relationship, one can see that $C_{\text{In}}$ differs from $C_{\text{PEG}}$ not by a constant amount, but rather by a constant fraction. Specifically, $C_{\text{In}} = 22 \pm 3\% \ (\text{SE}) \ (n = 36)$ less than $C_{\text{PEG}}$. The regression line for $\dot{Q}_{\text{Na,reb}}$ vs. GFR $- V$, calculated from $C_{\text{In}}$, yields a line with a steeper slope than the same data calculated from $C_{\text{PEG}}$ (Fig. 11). Most significantly, however, the intersection of the two lines with the $X$ axis is not changed. The steeper slope for the inulin line (Fig 11) is due to the fact that the amount of Na reabsorbed is dependent primarily on the amount filtered, or the GFR, when fractional Na reabsorption is relatively constant. Because of this relationship, low values for GFR usually correspond to low values for $\dot{Q}_{\text{Na,reb}}$. Thus, as GFR approaches zero, a fractional correction of GFR would be insignificant, and for that reason the two lines in Fig. 11 have the same $X$ intercept.

The results for FW-, SW-, and furosemide-treated eels of GFR. Consequently, the U/P ratios below unity cannot have been caused by a fractional underestimation of GFR due to sieving or active reabsorption of the glomerular markers in proportion to the amount filtered.

The fact that the point of intersection is clearly to the left of zero in all of the plots leads to the next possibilities. In case we underestimated GFR by a constant amount (possibility 2) or in case tubular fluid secretion were secondary to NaCl secretion (possibility 3), equation 4 should apply. This, however, leads to a number of inconsistencies: first, the total absence of negative values for $\dot{Q}_{\text{Na,reb}}$ makes it unlikely that GFR was underestimated. This is even more true in furosemide-treated eels where fractional Na reabsorption was significantly decreased. Second, the values for the underestimation of GFR would have to be very high. Calculated from the intersection point with the $X$ axis (Fig. 4), the Na concentration of reabsorbate (Fig. 4) and $P_{\text{Na}}$ (Table 3), GFR should have been underestimated consistently by 3.06 ml X kg$^{-1}$ X h$^{-1}$ in FW eels in the first set and by 7.06 ml X kg$^{-1}$ X h$^{-1}$ in the second set of experiments (Fig 9 and Table 3). Third, the finding that in furosemide-treated eels the line did not intersect with the $X$ axis at, or near zero (Fig. 9), is totally inconsistent with equation 4 since in furosemide-treated FW eels, the $\text{Reab}_{\text{Cl}}$ was slightly less than $P_{\text{Cl}}$ and, therefore, an underestimation of GFR would not shift the line to the left of zero.

In view of these findings, the first three possibilities discussed above appear to be invalid. We are left with possibility 4: that fluid is secreted into the tubule (equations 7 and 8). Since the data plotted in Figs. 4, 5, and 9 are entirely consistent with this hypothesis, we will assume it to be correct. The amount of fluid secreted ($\bar{S}$) can be calculated from the regression lines shown in the figures. We can, however, also calculate the value $\bar{S}$ from each individual urine collection period if we assume that the reabsorbate concentration is constant. The rate of fluid reabsorption ($\dot{Q}_{\text{H$_2$O, reb}}$) can be calculated when we know the concentration of the reabsorbate ($\text{Reab}_{\text{Na}}$ or $\text{Reab}_{\text{Cl}}$) and the rate of the Na or Cl reabsorption ($\dot{Q}_{\text{Na,reb}}$, $\dot{Q}_{\text{Cl,reb}}$) in each clearance period. Thus

\[
\dot{Q}_{\text{H$_2$O, reb}} = \frac{\dot{Q}_{\text{Na,reb}}}{\text{Reab}_{\text{Na}}} \tag{9}
\]

or

\[
\dot{Q}_{\text{H$_2$O, reb}} = \frac{\dot{Q}_{\text{Cl,reb}}}{\text{Reab}_{\text{Cl}}} \tag{10}
\]

and since

\[
S - \dot{V} = \text{GFR} + \dot{Q}_{\text{H$_2$O, reb}} \tag{11}
\]

we have, by combining equations 9 and 11

\[
S - \dot{V} = \text{GFR} + \frac{\dot{Q}_{\text{Na,reb}}}{\text{Reab}_{\text{Na}}} \tag{12}
\]

or, by combining equations 10 and 11

\[
S = \dot{V} - \text{GFR} + \frac{\dot{Q}_{\text{Cl,reb}}}{\text{Reab}_{\text{Cl}}} \tag{13}
\]

The results for FW-, SW-, and furosemide-treated eels...
Fluid secretion was present in FW eels in all but two urine collection periods, even when U/P PEG ratios were greater than unity. Fluid secretions based on either Na reabsorption or Cl reabsorption data were not significantly different (Table 8).

Data obtained from eels during the 1st yr of experimentation are separated from those of 1 yr later in Table 8. It will be noted that there is a marked quantitative difference in S between these two groups in both SW and FW. However, the animals were qualitatively similar in that S was consistently higher in FW than in SW. Furosemide treatment had no effect on the quantity of fluid secreted in either medium.

Because fluid secretion appeared not to be secondary to tubular Na or Cl secretion, we examined the possibility that it might be secondary to the tubular secretion of other ions measured in this study. Theoretically, we would expect the amount of fluid secreted to be sufficient to account for the amount of fluid secreted, i.e., a solution with the concentration $Q_{i, secreted}/S$ should have an osmolality approximately equal to that of the plasma. The possibility exists, however, that an ion is secreted in one part of the tubule and reabsorbed in another. Since we cannot account for the amount reabsorbed for an ion that is both secreted and reabsorbed, this evaluation can only be tentative.

Calcium reabsorption was predominant in FW eels, but SW eels showed clear evidence of occasional secretion. It appeared that insufficient Ca was secreted in FW eels to account for the water secretion (Fig. 6). In fact, since little or no calcium was secreted in FW eels, the intersection of the line with the X axis should again indicate the average amount of water added to the tubule. This value is $0.97 \text{ ml} \times \text{kg}^{-1} \times \text{hr}^{-1}$, which agrees well with the value obtained from the Na and Cl graphs. The slope of the line was 1.87 mM. The concentration in the filtrate was $0.8 \times 2.63 \text{ mM}$ (80% of plasma Ca was filtrable) = 2.10 mM. The ratio, then, is $\text{Reab}_\text{Ca}/\text{Filtrate} - 0.89$. In SW eels, Ca was secreted; therefore, the slope of the line does not represent the concentration of the reabsorbate.

K reabsorption and secretion showed no significant relation to the amount of water secreted (Fig. 7). K secretion was found more consistently in FW than in SW eels. This secretion does not appear to be responsible for the fluid secretion, since not more than 3 mM of K were secreted per liter of water secreted.

These results provide no firm evidence that the fluid secretion is secondary to any of the ions measured and leave us with no conclusion as to a possible mechanism for fluid secretion.

Fluid reabsorption. The rate of fluid reabsorption is given by equation 9. The fraction of filtered water reabsorbed must, therefore, be

$$\frac{Q_{\text{Na, reab}}}{\text{Reab}_\text{Na} \cdot \text{GFR}} = \frac{P_{\text{Na}} \cdot \text{Frac}_{\text{Na}}}{\text{Reab}_\text{Na}}$$

From the data presented in Fig. 4, Tables 3 and 6, the average fraction of filtered water reabsorbed in FW eels can be estimated to be

$$\frac{133.6 \times 0.89}{184} = 0.65$$

and in SW eels

$$\frac{154.7 \times 0.87}{152.1} = 0.88$$

The renal tubule of the eel has a proximal and a distal tubule. If the proximal and distal segments of the fish tubule are approximately similar to those of other vertebrates, such as amphibians and mammals, we must assume that the reabsorbate from the proximal tubule is isosmotic to the plasma and the reabsorbate from the distal tubule is hyperosmotic in FW eels. If the proportions between proximal and distal reabsorption remain constant, the average concentration of the reabsorbate will remain constant, as we have assumed here. If the proportions change through inhibition of distal reabsorption, we would expect the concentration of the reabsorbate in FW eels to decrease toward the concentration of the proximal tubular fluid.

In mammals, furosemide has been shown to selectively inhibit active Cl- transport by the thick ascending limb of the loop of Henle (2). This drug is also believed to have some effect on proximal as well as distal tubular Na reabsorption (4, 7). As was pointed out above, furosemide...
was effective in the eel in reducing fractional Na reabsorption. From Fig. 9 it can be seen that, in SW eels, furosemide had no effect upon the slope, i.e., although it decreased fractional Na reabsorption, furosemide did not change the concentration of Na or Cl in the reabsorbate relative to that of the control (Fig. 9 and Table 8). This finding indicates that in SW eels the reabsorbate is isosmotic to the plasma in both proximal and distal tubules.

In FW eels, furosemide decreased the slope of the line for both Na and Cl, i.e., the concentration of the reabsorbate was decreased by furosemide from an average Cl concentration of 158 to 63 mM. This indicates that furosemide inhibited the distal hyperosmotic reabsorption, since the ratio of ReabCl/PiCl fell from 2.08 to 0.89. The Na data show the same tendency, but were too scattered to permit a reasonable estimate of reabsorbate concentration.

In conclusion, it was found that the renal clearance data from the American eel Anguilla rostrata are consistent with the hypothesis that fluid is secreted into the renal tubule.

On the other hand, the data are incompatible with the hypothesis that GFR was underestimated due to either glomerular sieving of the glomerular marker PEG or active or passive reabsorption of PEG.

The glomerular filtration rate was the same in SW and FW eels. Urine flow rate was greater in FW- than in SW-acclimated eels due to a greater volume of fluid secreted in FW eels and due to a lower fractional reabsorption of fluid. The tubular fluid secretion was found not to be secondary to Na or Cl secretion.

It was not possible to conclusively implicate secretion of other ions, Ca, Mg, or K as the driving force for the fluid secretion. This leaves us with no hypothesis for the mechanism of the fluid secretion.

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