Kidneys sans glomeruli

Klaus W. Beyenbach
Department of Biomedical Sciences, Cornell University, Ithaca, New York 14853

Beyenbach, Klaus W. Kidneys sans glomeruli. Am J Physiol Renal Physiol 286: F811–F827, 2004;10.1152/ajprenal.00351.2003.—The evolution of the vertebrate kidney records three occasions, each separated by about 50 million years, when fish have abandoned glomeruli to produce urine by tubular mechanisms. The recurring dismissal of glomeruli suggests a mechanism of aglomerular urine formation intrinsic to renal tubules. Indeed, the transepithelial secretion of organic solutes and of inorganic solutes such as sulfate, phosphate, and magnesium can all drive secretory water flow in renal proximal tubules of fish. However, the secretion of NaCl via secondary active transport of Cl is the primary mover of secretory water flow in, surprisingly, proximal tubules of both glomerular and aglomerular fish. In filtering kidneys, the tubular secretion of solute and water is overshadowed by reabsorptive transport activities, but secretion progressively comes to light as glomerular filtration decreases. Thus the difference between glomerular and agglomerular urine formation is more a difference of degree than of kind. At low rates of glomerular filtration in seawater fish, NaCl-coupled water secretion serves to increase the renal excretory capacity by increasing the luminal volume into which waste, excess, and toxic solutes can be secreted. The reabsorption of NaCl and water in the distal nephron and urinary bladder concentrates unwanted solutes for excretion while minimizing renal water loss. In agglomerular fish, NaCl-coupled water secretion across proximal tubules replaces glomerular filtration to increase renal excretory capacity. A review of the literature suggests that tubular secretion of NaCl and water is an early function of the vertebrate proximal tubule that has been retained throughout evolution. Active transepithelial Cl secretion takes place in gall bladders studied as models of the mammalian proximal tubule and in proximal tubules of amphibians and apparently also of mammals. The tubular secretion of Cl is also observed in mammalian distal tubules. The evidence consistent with and for Cl secretion in, respectively, proximal and distal tubules of the mammalian kidney calls for a reexamination of basic assumptions in renal physiology that may lead to new opportunities for managing some forms of renal disease.

agglomerular urine formation; evolution; glomerular intermittency; tubular secretion; renal secretion of Cl, Mg, S, P, and organic solutes; transport models of renal tubular solute secretion

COUNTING MORE THAN 20,000 species, fish are the most numerous vertebrate group. They are also the most diverse group, and as the oldest group of vertebrates, there is not the slightest evidence that, like their amphibian and reptilian successors, fish are declining from a period of earlier glory (73). Instead, their evolution continues today to unfold a colorful spectrum of structural and functional diversity that has no equal among vertebrates. More than any other vertebrate group, fish give a glimpse at what is possible in vertebrate evolution, as for example, the renal support of extracellular fluid homeostasis without glomerular filtration.

The adaptive radiation of fish in diverse aquatic habitats has always drawn attention to teleostean feats of osmotic and ionic regulation. Early studies of the renal physiology in fish have led to diagnostic methods that are still in use. For example, the use of inulin in the measurement of glomerular filtration rate was conceived after first noting that agglomerular fish cannot excrete ordinary glucose (54). And PAH was adopted for measurements of renal blood flow after observation of the avid renal excretion of organic acids in agglomerular fish (54, 159). What is more, the acerbic controversy over tubular secretion as a real tubular function was settled, once and for all, after Marshall and Vickers (105) had documented the urinary excretion of creatine, creatinine, and phenolsulfonphthalein without the use of glomerular filtration in the agglomerular goosefish.

With these lasting contributions to renal physiology in the early 20th century, fish seem to have paid their dues to biology and medicine. However, other lessons remain in store. The loss of glomerular filtration is considered renal failure in the typical glomerular vertebrate, but agglomerular fish are not in renal failure. They suffer no obvious disadvantage from the loss of glomeruli and from their reliance on tubular transport mechanisms. To be sure, the kidney is not the sole organ of extracellular fluid homeostasis (80). The gill assists in the excretion of nitrogenous waste and in Na and Cl balance (155). Nevertheless, the contributions agglomerular kidneys make to extracellular fluid homeostasis leave many intriguing questions unanswered. How do agglomerular kidneys handle the twin challenge of osmotic water loading and salt depletion in fresh-
water, and the reverse challenge, salt loading and water depletion, in seawater? How do aglomerular kidneys excrete excess solute, organic anions, and xenobiotics without the aid of glomerular filtration? The study of these questions promises to lead to novel renal and tubular mechanisms that may also be present in other vertebrate kidneys, including mammalian kidneys, and motivate the exploration of new strategies for treating renal disease.

In the past, the formation of urine without glomeruli was viewed as so contrary to the usual filtration concept of renal homeostasis that it has been considered a degenerative form of evolution not worthy of much attention. However, in the following review, I will make the case that the difference between glomerular and aglomerular urine formation is one of degree rather than of kind. The renal tubules of glomerular and aglomerular kidneys are remarkably alike, sharing absorptive and secretory transport mechanisms.

ENVIRONMENTAL CHALLENGES AND RENAL ADAPTATIONS IN FISH

In the strongly hyposmotic environment of freshwater, fish face the twin problem of osmotic water loading and salt depletion (Fig. 1A). To remain in steady state, NaCl is replaced by active uptake across the gill, and osmotic water loads are excreted by the kidney (120, 127, 192). Freshwater fish (and euryhaline fish capable of residing in freshwater and seawater) possess the full complement of the vertebrate nephron: glomerulus, proximal tubule, distal tubule, and collecting duct. Glomeruli, the distal nephron, and the urinary bladder serve, among other functions, the renal excretion of water. Glomerular filtration delivers large volumes of extracellular fluid to the urinary space. The subsequent reabsorption of filtered salts without water in the distal nephron and urinary bladder preserves volume for excretion while diluting it to osmotic pressures <40 mosmol/kgH₂O (45, 74). Thus glomerular filtration and the epithelial reabsorption of salt without water effectively bail water in freshwater fish.

Aglomerular fish lack not only glomeruli but also the distal tubule, which severely curtails urinary dilution. As a result, the agglomerular toadfish survives for only 3 wk when held in freshwater in the laboratory, but they survive for many months in 10% seawater (10, 11, 94). On the transferring of toadfish from seawater to 10% seawater (105 mosmol/kgH₂O), urine flow rates more than double in the effort to excrete the osmotic water loads. In parallel, the urine osmotic pressure decreases from 299 to 207 mosmol/kgH₂O, the apparent lower limit of urinary dilution. Thus to excrete osmotic water loads, the agglomerular kidney is obliged to sacrifice solutes, in particular Na, Cl, and S, in the relative stoichiometry of 5:1:3 (11). Concomitant with the increase in urinary electrolyte and water excretion, the plasma osmotic pressure drops from 318 mosmol/kgH₂O in seawater to 241 mosmol/kgH₂O in 10% seawater, but it is unclear whether this drop reflects the physiological intent to reduce osmotic water loads in dilute media or the failure of the kidney to produce dilute urine (11). To remain in steady state, the solutes that are lost with the renal excretion of water must be replaced. Branchial NaCl uptake mechanisms in the gill must be relied on, but dietary salt uptake is also an important component for surviving in dilute media (94). Thus the survival of the agglomerular toadfish in hypotonic media is a delicate balance of branchial and intestinal solute uptake and renal solute loss.

In the strongly hyperosmotic environment of the sea, the environmental challenges are opposite to those in freshwater. Here, fish lose water by osmosis and gain salt by diffusion (Fig. 1B). Body water is replaced by drinking seawater and distilling new body fluids from it. Ingested NaCl is returned to the sea via the gills (50, 186), and ingested divalent ions, Mg, Ca, and SO₄, are excreted by the kidney (74, 120). Renal tubules rather than glomeruli mediate the excretion of divalent ions, because the size and function of glomeruli (and the distal tubule) are much reduced or entirely absent in stenohaline seawater fish (74). Although collecting ducts and the urinary bladder attempt to conserve body water, the osmotic pressure of the urine is limited to that of the plasma. Thus the urine of seawater fish is generally isosmotic to plasma in glomerular as in agglomerular fish.

Environmental conditions as well as physiological adaptations diminish the role of glomerular filtration in seawater (156). The loss of body water in hyperosmotic seawater and the contributions of the gill to extracellular fluid homeostasis may have reduced the filtration demands on the kidney to such a degree to allow the dismissal of glomeruli in some fish (155). Still, it is unlikely that glomeruli could have been discarded.

**Fig. 1. Osmotic and ionic regulation in freshwater and seawater fish.** A: in freshwater, fish lose salts (NaCl) by diffusion and gain water by osmosis (open arrows). Active transport of electrolytes (filled arrows) in the gill and kidney serve to recover salt and to excrete water. B: in seawater, fish gain salts (NaCl) by diffusion and lose water by osmosis. Active transport mechanisms in the gill now secrete NaCl, and the kidney excretes Mg, SO₄, and other divalent ions. In addition to the branchial and renal mechanisms of extracellular fluid homeostasis, the tolerance of plasma osmotic pressures ranging from 280 mosmol/kgH₂O in freshwater to 350 mosmol/kgH₂O in seawater helps reduce environmental salt and water challenges.
Invited Review

AGLomerular Urine Formation

F813

without secretory "preadaptations" of renal tubules for life without glomeruli. Rather than preadaptations, the evolutionary record suggests that tubular secretion is a primordial function of the vertebrate renal tubule, glomerular or not. Tubular secretion as a primeval, intrinsic function of the vertebrate nephron explains how aglomerular fish could have evolved on three separate occasions, and why they may evolve again.

MULTIPLE EVOLUTIONARY BEGINNINGS OF AGLOMERULAR RENAL FUNCTIONS

Of the 45,000 species of vertebrates existing today, a tiny minority, no more than 30 species of presently known fish, have kidneys without glomeruli (11, 17, 21, 94). The number of aglomerular species may well be larger, but neither all aglomerular fish, nor all vertebrate species for that matter, have been counted or discovered (185). What this small group of aglomerular fish lacks in numerical significance, it makes up in the defiance of glomerular filtration as the first step of extracellular fluid homeostasis. Devoid of glomeruli, they use tubular secretion to initiate the renal turnover of extracellular fluid. Renal academics have considered them quirks of nature and not worthy of much attention.

Aglomerular fish appear independently in three lineages widely separated in time, and each from an apparently glomerular ancestor (Fig. 2). For each group that is aglomerular, there is a much larger sister group that is glomerular (47, 96). The first aglomerular fish appeared 120 million years ago in the superorder Elopomorpha. Among them is the deep sea eel; its glomerular sister is the common eel (76). The second group of aglomerular fish, the Paracanthopterygii, evolved 65 million years ago, namely, 5 million years after the first placental mammals. Goosefish in this group include the infamous toadfish and goosefish that Marshall and Grafflin (103, 104, 195) studied at the Mt. Desert Island Biological Laboratory, Salisbury Cove, ME. The well-known cod is a glomerular relative.

The youngest lineage of aglomerular fish, the perciforms, evolved only 23 million years ago, long after apes, monkeys, and whales had appeared. Perciforms include the aglomerular seahorse, pipefish, dragonet, sea poacher, scorpion fish, rockfish, pufferfish, porcupine fish, clingfish, and some notothenioid antarctic fish (8, 44, 84). However, most of the 10,000 species of perciforms have glomerular kidneys; among them are the sea bass, halibut, tuna, flounder, and the coral reef fish. One perciform, the shorthorn sculpin Myoxocephalus scorpius, defines the agglomerular condition in functional rather than anatomic terms. Here, the kidney is home to both glomerular and agglomerular renal tubules, but renal clearance gives no evidence of glomerular filtration (53).

Hickmann and Trump (74) opined that about the only generalization possible regarding aglomerular fish is that they are among the most exotic, highly specialized, and least active fish known. Some agglomerular fish are indeed inactive, bottom dwellers like the toadfish and goosefish. However, puffers and porcupine fish are rather active swimmers. Moreover, puffers are effective regulators of the plasma that supports their incursions into estuaries (129).

Homer Smith considered agglomerular fish queer animals out of the sea, specialized products of the oceans that, under continuous selection to conserve water in hypersomatic media, have reduced and even obliterated glomeruli. And those few agglomerular fish that have subsequently left the sea to return to brackish or fresh water, such as the toadfish of the Chesapeake Bay and the pipefish in the freshwater rivers of Thailand, Malaysia, the Philippines, Panama, and the West Indies, were sorry for having discarded glomeruli: "It is not in the nature of evolution that the clock can be turned back to give them glomeruli again" (158). Still, Smith must have wondered then, as we wonder today, how these agglomerular fish maintain osmotic and ionic balance in freshwater.

The evolution of agglomerular kidneys on three separate occasions suggests that purely tubular mechanisms of renal homeostasis have always been present in the kidneys of fish and perhaps all vertebrates. In agglomerular fish, these tubular mechanisms have been amplified to handle the salt and water problems in hyper- as well as hyposomatic media.

EVIDENCE FOR LARGELY TUBULAR ACTIVITIES IN THE KIDNEY

Environmental and Physiological Conditions Permissive of Agglomerular Urine Formation

Because the density of water is ~1,000 times greater than air, the orthostatic demands on skeletal and cardiovascular systems are much reduced in fish over those of terrestrial animals. Cartilage rather than bone is good enough for structural support in water, and a low-pressure circulatory system (consisting of a 2-chambered heart and branchial and systemic circulations in series) suffices to meet the circulatory needs of the animal. The capacity of water to store heat renders aquatic habitats far more thermostable than terrestrial habitats. Accordingly, the selection pressure on the development of warm-bloodedness and its associated metabolic cost and glomerular filtration is low in aquatic habitats (156). Fish have metabolic
rates 20–30 times lower than those of mammals, which reduces the demands for the renal turnover of the extracellular fluid (130, 156). Thus metabolic and cardiovascular pressures in fish do not call for high glomerular development nor for the strong arterial perfusion of the kidney. Indeed, the renal blood supply in fish is predominantly venous from the caudal vein (74).

A so-called caudal heart assists the venous perfusion of the kidney in some long-bodied fish such as the eel, shark, and hagfish, where the venous pressure of the kidney may be very low because of the long distance from the branchial heart. The caudal heart is a cardiac pump in the sense that it is a valved vascular structure that generates cyclical pressures at a frequency of 18 beats/min in the shark and up to 60 beats/min in the hagfish (142). It is driven by an independent motor center located in the terminal spinal cord. However, unlike true hearts, caudal hearts are formed of veins or sinuses in close proximity to skeletal muscles that rhythmically compress them.

Measures of the PAH/inulin clearance ratio assign a single number to the relationship between renal plasma flow and the glomerular filtration rate. In humans, the number is five, indicating that 20% of the plasma passing through glomeruli is filtered. In contrast, this number is 1,304 in the shorthorn sculpin, reflecting a filtration fraction of only 0.08% (53). The PAH/inulin clearance ratio is undefined in anatomically aglomerular fish. In the absence of glomeruli and in the presence of low filtration rates in glomerular marine fish, renal functions rely largely on the venous perfusion that delivers blood to peritubular sinuses in the kidney.

### Glomerular Intermittency

Glomeruli in mammalian kidneys are thought to filter all the time, although rates of filtration may vary with physiological regulation, nephron type, and metabolic activity of the whole animal (156, 157). In fish, glomerular filtration may cease altogether in some nephrons, causing rates of glomerular filtration and urine flow to rise and fall together (74). The phenomenon is known as glomerular intermittency, which was best illustrated in measurements of the single-nephron filtration rate in the highly active rainbow trout (26). Three functional glomerular types were identified: 1) perfused and filtering, 2) perfused but nonfiltering, and 3) nonperfused. In freshwater trout, 45% of all glomeruli were perfused and filtering, but only 5% in seawater. Perfused but nonfiltering glomeruli were surprisingly numerous (40%) in freshwater and seawater trout, indicating a large glomerular reserve in both environments. The percentage of nonperfused (and nonfiltering) glomeruli was only 13 in freshwater but 51 in seawater. Thus 91% of the glomeruli are out of commission in seawater, rendering the glomerular trout essentially aglomerular in seawater. Although the anatomic presence of glomeruli is the norm in fish, glomeruli are not obliged to filter all the time, not even in freshwater fish. Moreover, glomerular shutdown in seawater fish does not mean renal shutdown or renal failure as it does in mammals.

### Similar Volume and Composition of Urine From Glomerular and Aoglomerular Kidneys

In seawater, the reduction of the glomerular filtration rate to <10% in freshwater shifts the renal mechanisms of extracellular fluid homeostasis to the renal tubules (25, 58, 171). The switch to largely tubular renal functions is reflected in both the volume and composition of the urine. Urine flow rates are similar in seawater fish regardless of whether glomeruli are or are not present (Table 1). Furthermore, Mg, SO₄, Na, and Cl are the major urine electrolytes and osmolytes irrespective of glomeruli, pointing to largely tubular activities.

### Secretion of Fluid in Proximal Tubules Isolated From Glomerular and Aglomerular Fish

The tubular secretion of solute and water can be observed in isolated fish renal tubules in a number of ways. One way is to perfuse the lumen of the proximal tubule with light mineral oil (Fig. 3A). After the perfusion of the lumen is stopped, the transepithelial secretion of fluid produces gaps in the column of oil occupying the tubule lumen. With time these gaps grow, driving the oil from the lumen in ~1 h (14). A second way is to crimp close one end of the proximal tubule via a hairpin turn of the tubule in a holding pipette and to collect secreted fluid from the other end of the tubule (Fig. 3B). Using this method, we measured an average fluid secretion rate of 37 pl·min⁻¹·mm tubule length⁻¹ in proximal tubules of the glomerular winter flounder, similar to the secretion rates in proximal tubules of glomerular killifish, glomerular dogfish shark,

---

### Table 1. Renal functions in glomerular and aglomerular fishes in seawater

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>Kidney</th>
<th>GFR</th>
<th>Urine Flow, ml·kg⁻¹·h⁻¹</th>
<th>πᵣ, (mosmol/kgH₂O)</th>
<th>Urine Composition, mM</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Paralichthys lethostigma</em></td>
<td>Southern flounder, marine, SW</td>
<td>![Kidney Image]</td>
<td>1.69</td>
<td>0.22</td>
<td>304</td>
<td>133</td>
<td>68</td>
</tr>
<tr>
<td><em>Oncorhynchus kisutch</em></td>
<td>Coho salmon, euryhaline, SW</td>
<td>![Kidney Image]</td>
<td>1.48</td>
<td>0.41</td>
<td>80</td>
<td>56</td>
<td>133</td>
</tr>
<tr>
<td><em>Opisanus tau</em></td>
<td>Oyster toadfish, marine, SW</td>
<td>![Kidney Image]</td>
<td>0</td>
<td>0.03</td>
<td>299</td>
<td>152</td>
<td>116</td>
</tr>
<tr>
<td><em>Lophius piscatorius</em></td>
<td>Goosefish, marine, SW</td>
<td>![Kidney Image]</td>
<td>0</td>
<td>0.44</td>
<td>406</td>
<td>137</td>
<td>42</td>
</tr>
</tbody>
</table>

GFR, glomerular filtration rate; SW, seawater.

---

*AJP-Renal Physiol* • VOL 286 • MAY 2004 • www.ajprenal.org
TRANSPORT MECHANISM DRIVING FLUID SECRETION IN RENAL PROXIMAL TUBULES OF FISH

Active Transepithelial Secretion of Mg

In marine fish, Mg concentrations in bladder urine as high as 150 mM (Table 1) impress with powers of renal excretion in view of plasma Mg concentrations between 1 and 2 mM (74). However, much of the urinary concentration of Mg stems from the reabsorption of NaCl and water in the distal nephron and urinary bladder, because Mg concentrations in the tubule lumen of proximal tubules, the major site of tubular Mg secretion, are on average 22 mM (Table 2, Refs. 16, 20, 39, 133). Measures of transepithelial voltage and Mg concentration differences in isolated proximal tubules show that Mg is secreted into the tubule lumen against its electrochemical potential (16, 18). The active transport pathway for transepithelial Mg secretion must pass through epithelial cells because measured transepithelial voltages cannot support the high-Mg concentrations found in secreted fluid (Fig. 4A). The entry of Mg from the peritubular medium into the cell is energetically downhill in view of cell negative intracellular voltages and low intracellular Mg concentrations similar to those of peritubular medium (Fig. 4A). As a first hypothesis, Mg enters the cell via a membrane channel of the transient receptor potential (TRP) family, known to be permeable to Ca, Mg, and other divalent cations (89, 91, 116, 145). The active transport step at the brush-border apical membrane extrudes Mg from the cell into the tubule lumen (15). Na/Mg exchange could mediate this transport step as suggested by Natochin and Gusev (119) and Renfro and Shustock (136). An Na-dependent Mg antiport has been observed in a variety of cells, mediated perhaps by an isoform of the Na/Ca exchanger (148, 161, 172). Mg exchange with H might also be possible in view of the V-type H+/ATPase that we have detected at the apical membrane of killifish proximal tubules (Fig. 4A). An H/Mg antiport is well established in yeast and plants (3, 23, 153), and it has been proposed as the mechanism of Mg transport in mitochondria of the liver (147).

Using the mass spectrometer, a look at renal proximal tubules of killifish injected with cold isotope 26Mg revealed the punctate cytoplasmic distribution of injected 26Mg undergoing transepithelial secretion (Fig. 4C). However, it is unclear whether the punctate distribution stems from a mechanism of exclusion or one of inclusion (30). Intracellular vesicles containing high concentrations of Mg have been observed near the apical membrane of flounder proximal tubules, suggesting the incorporation of Mg into vesicles for exocytotic transport into the tubule lumen (49, 72).

Transport of Cl via Secondary Active Transport of Mg

Renal proximal tubules of the shark, which do not secrete much Mg (Table 2), have yielded the first clear evidence for active transepithelial secretion of Cl in a vertebrate proximal tubule (19). Isolated proximal tubules prepared for the study of fluid secretion as in Fig. 3B secreted an NaCl-rich fluid at a rate of 28 pl-min⁻¹-mm⁻¹. The rate of fluid secretion significantly increased by 80% in the presence of cAMP (143), in parallel with depolarization of the transepithelial and the basolateral membrane voltage and reductions in the transepithelial resi-

and agglomerular oyster toadfish (Table 2). Moreover, the ionic composition was found to be strikingly similar in fluid secreted by glomerular and agglomerular proximal tubules (Table 2). Remarkably, Na and Cl were the dominant electrolytes in secreted fluid, and not Mg and S, which renal proximal tubules of fish are known to secrete.

The concentrations of Na and Cl in fluid secreted by isolated fish proximal tubules are many-fold greater than the concentrations of Mg and S (Table 2). In contrast, the reverse is true for bladder urine, in which Mg and S concentrations exceed the concentrations of Na and Cl (Table 1). The comparison reflects the well-known transport functions of the distal nephron and the urinary bladder, which reabsorb Na, Cl, and water with the effect of concentrating Mg and SO₄ (55, 56, 75, 97).

Fig. 3. Fluid secretion in isolated renal proximal tubules of the glomerular flounder in seawater (A and B) and of killifish adapted to fresh water (C). A: flounder has a kidney with ~10,000 glomerular renal tubules (118). However, flounder proximal tubules secrete fluid in vitro, breaking up a column of oil that had been perfused into the tubule lumen (14). B: flounder proximal tubule prepared for the study of spontaneous fluid secretion. One end of the tubule is crimped closed with a hairpin turn in a holding pipette (14, 17). The other end opens into a collection pipette, where secreted fluid accumulates under light mineral oil at an average rate of 37 pl-min⁻¹-mm⁻¹ (22). The photographic focus aims to show secreted fluid accumulating in the collection pipette. C: fluid secretion in the first segment of the proximal tubule of the glomerular killifish adapted to freshwater for ~35 days (37). The tubule is crimped closed near its own glomerulus. Fluid secreted by the first part of the proximal tubule exits the open end of the tubule and accumulates in the collection pipette at an average rate of 34 pl/min⁻¹-mm⁻¹ (38).
tance and the fractional resistance of the apical membrane (19). The data indicate that cAMP increases the Cl conductance of the apical brush-border membrane, consistent with the stimulation of the secretory efflux of Cl from cell to tubule lumen (Fig. 5). Cl enters the cell on the other side, apparently via Na-K-2Cl cotransport, judging from the inhibition of fluid secretion by the loop diuretic furosemide. Thus the mechanism of Cl secretion in the shark renal proximal tubules resembles CFTR-mediated Cl secretion that is widely distributed in vertebrate epithelia (154). Since then, we have observed this mechanism of tubular Cl secretion also in proximal tubules of killifish kept in seawater and, surprisingly, freshwater (37, 38).

**Secretion of Organic Solutes**

Since the observation of phenol red secretion in isolated proximal tubules of fish, teleost renal tubules have been favorite objects for the study of renal organic anion transport (103). Our understanding of renal organic anion transport has advanced much in recent times through the use of confocal fluorescence microscopy and fluorescein, an organic anion, which is secreted by renal proximal tubules. The method for determining the kinetics of organic solute secretion using fluorescent anions was first pioneered by Sullivan et al. (164) in isolated renal tubules of rabbits and subsequently used with

---

**Table 2. Fluid secretion rates, osmotic pressures, and the ionic composition of fluid secreted in vitro by renal proximal tubules of glomerular and aglomerular fishes in seawater and freshwater**

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name/Habitat</th>
<th>Isolated Renal Proximal Tubule Fluid Secretion Rate, pL/min</th>
<th>Fluid Potential, mV</th>
<th>Ion Concentrations, mM</th>
<th>Secreted Fluid Composition, mM</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleuronectes americanus</td>
<td>Winter flounder, marine, SW</td>
<td>37</td>
<td>318</td>
<td>26 10 152 155</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>Fundulus heteroelitus</td>
<td>Killifish, euryhaline, SW</td>
<td>54</td>
<td>-</td>
<td>28 10 127 153</td>
<td></td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>34</td>
<td>-</td>
<td>15 11 147 148</td>
<td></td>
<td>38</td>
</tr>
<tr>
<td>Squalus acanthias</td>
<td>Dogfish shark, SW</td>
<td>28</td>
<td>905</td>
<td>6 1 291 272</td>
<td></td>
<td>143</td>
</tr>
<tr>
<td>Opsanus tau</td>
<td>Oyster toadfish, marine, SW</td>
<td>28</td>
<td>-</td>
<td>12 9 195 171</td>
<td></td>
<td>16</td>
</tr>
</tbody>
</table>

Ion concentrations were measured using wavelength dispersive spectroscopy, electron probe. The method detects atomic elements rather than ionic species; S measured is probably SO₄²⁻.

---

Fig. 4. Renal tubular transport of Mg in fish. A: hypothetical mechanisms for secreting Mg into the lumen of fish renal proximal tubules. Electrochemical Mg potentials favor the channel-mediated entry of Mg from the renal interstitium into the epithelial cell. Transport of Mg from cell to lumen is against the electrochemical potential, conceivably driven by Na/Mg exchange (119). H/Mg is another potential mechanism in view of the immunohistochemical evidence for V-type H⁺-ATPase at the apical membrane of killifish proximal tubules (unpublished observations). B–E: ion microscopic images of Mg transport in renal tubules of seawater-adapted killifish. ²⁶Mg that was injected into the peritoneum of the fish at time 0 was rediscovered 30 min later in epithelial cells of renal tubules, consistent with transcellular transport of Mg. B and D: images of ²⁶Mg, the natural Mg isotope normally present in epithelial cells. C and E: images of ²⁶Mg undergoing transepithelial transport. B and C: secretory Mg transport in renal proximal tubules. D and E: Mg transport in Ca, Mg-rich cells of collecting ducts, which may reabsorb Mg (38).
good effect in fish proximal tubules and other model systems (40, 68, 114, 169).

Proximal tubules of fish appear to offer only one organic anion-like transporter at the basolateral membrane (191) that mediates the entry of organic anions into the cell (Fig. 6A). The transporter is thought to be a hybrid of mammalian organic anion transporters OAT1 and OAT3 and to accept both small and large organic anions for transport (Miller D, personal communication). As in mammals, the uptake of organic anions is ultimately dependent on the inward transmembrane Na gradient generated by the Na-K-ATPase via Na-dicarboxylate (NaDC) cotransport and organic anion/dicarboxylate exchange transport (112). At the apical membrane, the multidrug resistance proteins MRP2 and MRP4 mediate the extrusion of large organic anions into the tubule lumen (107). The transport of small organic anions from cell to lumen is carrier mediated, concentrative, and independent of voltage (111), but the molecular identity of the carrier is unknown. Next to facilitated and voltage-dependent export mechanisms across the apical membrane, intracellular vesicles containing organic anions may fuse with the apical membrane, bringing about the exocytosis of organic anions into the tubule lumen (113, 114).

In mammalian proximal tubules (Fig. 6B), three transport systems for secreting organic solutes are recognized: 1) Na-dependent transport of small organic anions such as PAH and fluorescein, 2) Na-independent transport of large organic acids such as conjugated fluoresceins, and 3) transport of xenobiotics by the p-glycoprotein-like transporter (27, 132). All three systems share certain but not all characteristics. Figure 6B summarizes what is known about the best understood transport system, the secretion of endogenous organic anions and anionic drugs in mammalian renal proximal tubules (27). The two organic anion transporters, OAT1 and OAT3, belonging to the SLC22 family of organic cation/anion/wzwitterions family of transporters, mediate the uptake of organic anions across the membrane of the renal proximal tubule.

Fig. 5. Mechanism of fluid secretion driven by secondary active transport of Cl in renal proximal tubules of the dogfish shark under control conditions and in the presence of cAMP. Cl enters the cell across the basolateral membrane via loop diuretic-sensitive cotransport with Na and K. The transporter, probably an isoform of Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter, elevates intracellular Cl above electrochemical equilibrium with extracellular Cl. In the presence of apical membrane Cl channels that are activated by cAMP, Cl is free to enter the tubule lumen (19, 143). V, voltage; R, resistance; subscript a, apical membrane; subscript bl, basolateral membrane; subscript t, transepithelial.

Fig. 6. Mechanism of transepithelial secretion of organic anions across renal proximal tubules of fish and mammals. Organic anion secretion in fish (A) and mammalian (B) proximal tubules share many similarities. The uptake of organic (org.) anions into the cell across the basolateral membrane via the organic anion transporters (OAT) is "tertiary" in that it depends first on the outward α-ketoglutarate (α-KG) gradient, second on the inward Na gradient, and third on the Na-K-ATPase. ATP-driven multidrug resistance proteins (MRP) translocate large organic anions into the tubule lumen across the apical membrane. In addition, electroneutral and electrogenic OAT transporters mediate the extrusion in mammalian proximal tubules (B). So far, OAT transporters have not been identified in apical membranes of fish proximal tubules, but there is evidence for exocytotic transport (A). For a comprehensive review, in particular substrate specificities, see Ref. 27. Norepinephrine (norepi) stimulates (†) transepithelial organic anion transport by stimulating the Na/K pump. PKC inhibits (−) uptake of organic anions directly via effects on OAT and indirectly via the inhibition of the Na-K-ATPase in both fish and mammals. NaDC, Na-dicarboxylate transporter; PZA, pyrazinoate; URAT1, urate transporter-1.
basolateral membrane in exchange for α-ketoglutarate. The outward gradient for α-ketoglutarate derives from 1) its synthesis by intracellular metabolism and 2) Na-dependent uptake of α-ketoglutarate via the cotransporter NaDC3 (71). The substrate affinities of OAT1 and OAT3 overlap with an affinity of OAT3 for urate three times greater than that of OAT1 (27). OAT1 has been cloned and sequenced in a number of species including the flounder (41, 132, 191). Norepinephrine stimulates the Na-K-ATPase, thereby increasing uptake and transepithelial secretion of organic anion (70, 132). Activation of PKC inhibits transepithelial secretion of organic anions by inhibiting the Na-K-ATPase, which delivers the Na and α-keto- glutarate gradients needed for organic anion uptake across the basolateral membrane (70, 115, 132). In addition, PKC inhibits organic anion secretion by retrieving OAT1 (101, 173, 190, 194) and OAT3 from the basolateral membrane (170).

The apical membrane offers a number of transport systems that move organic anions into the tubule lumen. The multidrug resistance proteins MRP2 and MRP4 are ATP-driven pumps that transport large organic anions from cell to lumen, as in fish (98, 139, 174). In addition, the organic anion-transporting polypeptide OATP1, the kidney-specific OAT-K1, and the isoforms OAT-K2 and OAT4 mediate organic anion efflux (139). The laboratory of Endou (83) has recently discovered voltage-dependent organic anion transport that is mediated by the transport protein OATv1 located in the apical membrane of the proximal tubule.

In fish proximal tubules, the tubular secretion of other organic solutes such as taurine adds significant quantities of osmolytes to the tubule lumen, supporting the transepithelial secretion of water. The luminal taurine concentration may be as high as 25 mM when the bath taurine concentration is only 1 mM in studies of isolated flounder proximal tubules (86). Taurine is known as an intracellular osmolyte that is used to abate changes in cell volume when cells are presented with hyp- and hyperosmotic challenges. The tubular secretion of taurine at substantial rates suggests that taurine also serves extracellular volume homeostasis (13, 57, 86, 126, 196).

**Secretion of Sulfate**

As with Mg, urine/plasma ratios for sulfate can exceed 100 in fish, suggesting tubular secretion (Table 1). Indeed, isolated fish proximal tubules prepared for the observation of fluid secretion (Fig. 3) reveal that SO4 is secreted into the tubule lumen against its electrochemical potential (Table 2). The study of vesicles prepared from basolateral and apical cell membranes of flounder proximal tubules has elucidated SO4 transport mechanisms. SO4 is thought to enter the cell from the renal interstitium across the basolateral membrane via exchange transport with intracellular OH. SO4 is subsequently extruded from the cell into the tubule lumen in exchange for HCO3 (132). SO4 transport is pH dependent across both apical and basolateral membranes of the cell, but the maximal inhibition of carbonic anhydrase reduces SO4 secretion only by half. Apparently, Cl gradients can drive SO4 transport in fish kidneys (134, 135) but not in mammalian kidneys (27).

**Secretion of Phosphate**

The transport of Pi in fish kidneys may proceed in absorptive and secretory directions (132). In the seawater flounder, transepithelial Pi secretion takes place in segment II of the renal proximal tubule (Fig. 7A). Pi enters the cell across the basolateral membrane via cotransport with Na. The cotransporter NaPi-II mediates this uptake step with a coupling ratio of 3Na to 1Pi (49, 51, 52, 183, 184). Pi leaves the cell across the apical membrane, apparently as the acid anion H2PO4-. Transport here is voltage dependent and stimulated by PKC (132). The NaPi-II cotransporter found in the basolateral membrane of the proximal tubule is also found in the apical membrane of the flounder collecting duct (49), where it is thought to contribute to the reabsorption of Pi, consistent with the usual dual step of renal homeostasis: filtration/reabsorption or secretion/reabsorption.

For tubular reabsorption of Pi, the Na-Pi cotransporter is located in the apical membrane of the proximal tubule to mediate the uptake of Pi from the tubule lumen, and the voltage-dependent exit step is located at the basolateral mem-

![Fig. 7. Mechanisms of phosphate secretion (A) in renal proximal tubules of the seawater flounder and phosphate reabsorption (B) in the collecting duct of freshwater zebrafish. A: transepithelial secretion begins with the entry of inorganic phosphate (Pi) into the cell, driven by cotransport with Na via the Na-Pi II cotransporter cloned by Werner et al. (184) and localized to the basolateral membrane by Kohl et al. (88) and Elger et al. (49). The exit of Pi from the cell into the tubule lumen is voltage dependent (100) and perhaps mediated by a channel. B: in the active filtering kidney of the freshwater zebrafish, Pi is reabsorbed via the isoform NaPi-Ib located in the apical membrane of collecting tubules and ducts. The transport step across the basolateral membrane is unknown. Werner and Kinne (183) offer a comprehensive review of the evolution of Na-Pi cotransport systems.](http://ajprenal.physiology.org/)

---

AJP-Renal Physiol • VOL 286 • MAY 2004 • www.ajprenal.org
brane (60, 131, 184). However, the molecular details of this exit step are unknown. Agents that stimulate PKA increase the transepithelial reabsorption of P, (131, 132). In freshwater killifish, the NaPi-IIb-related cotransporter was found only in apical membranes of the collecting duct (Fig. 7B), but not in the P-reabsorbing proximal segment I (183).

Of the above mechanisms of tubular electrolyte secretion, the secretion of NaCl via secondary active transport of Cl is the largest mover of transepithelial water flow (Table 2). The secretion of Mg, organic solutes, sulfate, and phosphate adds additional volumes of water to the tubule lumen. For example, in the absence of peritubular Mg, isolated flounder proximal tubules secrete an NaCl-rich fluid at a rate of 18 pl/min, but rates of fluid secretion can increase to 140 pl/min at maximal transepithelial Mg secretion in the presence of the unphysiological peritubular Mg concentration of 17 mM (39). Also important are transepithelial Donnan effects due to the secretion of charged solutes such as Mg into the tubule lumen. Because the permeability of the proximal tubule to Mg is low, but high for Na and Cl, the transepithelial secretion of Mg brings Na and Cl into the tubule lumen and, consequently, water. Donnan equilibrium is not reached because renal tubules allow both radial and axial water flow. As diffusible electrolytes such as Na and Cl attempt to go to transepithelial Donnan equilibrium, the luminal osmotic pressure rises, driving secretory water flow across the tubule wall and the flow of secreted fluid downstream (21).

**TELEOSTEAN PARADOXES**

**Mg Secretion in Proximal Tubules of Freshwater Fish**

In seawater with a Mg concentration of 55 mM, >98% of the Mg in the urine arrives there by tubular secretion (Fig. 4, Table 2). Tubular secretion eliminates from the body the Mg in the urine. Using mass spectrometry and highly enriched 26 Mg, we localized Mg-rich cells in the kidney (60, 131, 184). However, the molecular details of this exit step are unknown. Agents that stimulate PKA increase the transepithelial reabsorption of P, (131, 132). In freshwater killifish, the NaPi-IIb-related cotransporter was found only in apical membranes of the collecting duct (Fig. 7B), but not in the P-reabsorbing proximal segment I (183).

Of the above mechanisms of tubular electrolyte secretion, the secretion of NaCl via secondary active transport of Cl is the largest mover of transepithelial water flow (Table 2). The secretion of Mg, organic solutes, sulfate, and phosphate adds additional volumes of water to the tubule lumen. For example, in the absence of peritubular Mg, isolated flounder proximal tubules secrete an NaCl-rich fluid at a rate of 18 pl/min, but rates of fluid secretion can increase to 140 pl/min at maximal transepithelial Mg secretion in the presence of the unphysiological peritubular Mg concentration of 17 mM (39). Also important are transepithelial Donnan effects due to the secretion of charged solutes such as Mg into the tubule lumen. Because the permeability of the proximal tubule to Mg is low, but high for Na and Cl, the transepithelial secretion of Mg brings Na and Cl into the tubule lumen and, consequently, water. Donnan equilibrium is not reached because renal tubules allow both radial and axial water flow. As diffusible electrolytes such as Na and Cl attempt to go to transepithelial Donnan equilibrium, the luminal osmotic pressure rises, driving secretory water flow across the tubule wall and the flow of secreted fluid downstream (21).

**TUBULAR TRANSPORT ACTIVITY: FAR GREATER THAN PREVIOUSLY ASSUMED**

Small as they are, the picoliter (10^-12 liter) volumes secreted by isolated proximal tubules are not trivial. The volume secreted by all renal proximal tubules of the kidney can be calculated from the urinary Mg excretion rate and the known Mg concentration in fluid secreted by proximal tubules (Tables 1 and 2). A typical marine glomerular teleost produces urine at a rate of 0.3 ml·kg^-1·h^-1 with an average bladder Mg concentration of 130 mM (Table 1) to yield a urinary Mg excretion rate of 39.0 μmol·kg^-1·h^-1 (Fig. 8A). Because the Mg concentration in the lumen of the proximal tubule is on average 22 mM, it follows that together, all proximal tubules of the kidney secrete fluid at a rate of 1.74 ml·kg^-1·h^-1 to account for the Mg voided in the urine. As shown in Fig. 8A, this rate of tubular fluid secretion is three and one-half times the glomerular filtration rate. The calculation illustrates the shortcoming of simple renal input/output analysis. If glomerular filtration is considered the only input, then the fractional water absorption is only 40% (Fig. 8A). However, in view of the secretory water flow across renal proximal tubules, the true fractional water absorption is 87%.

Simple renal input-output analyses also underestimate the renal handling of Na in marine fish. For example, Na is filtered in the kidney of the typical marine glomerular teleost at a rate of 80 μmol·kg^-1·h^-1 (Fig. 8B). Because renal proximal tu-
Fig. 8. Filtered, secreted, reabsorbed, and excreted loads in the kidney of the typical glomerular marine fish. A: volume transport. Transepithelial secretion of Mg is expected to secrete into the tubule lumen at a rate of 1.74 ml·kg⁻¹·h⁻¹; glomerular filtration delivers only 0.5 ml·kg⁻¹·h⁻¹. In view of this secreted volume, the rate of tubular fluid absorption is 1.94 ml·kg⁻¹·h⁻¹, and not 0.2 ml·kg⁻¹·h⁻¹, the difference between glomerular filtration rate and urine flow. B: Na transport. Transepithelial NaCl secretion in proximal tubules presents Na to the tubule lumen at a rate 275 μmol·kg⁻¹·h⁻¹, 3.4 times more than filtered loads. In view of the renal Na excretion of only 9 μmol·kg⁻¹·h⁻¹, the distal nephron and urinary bladder reabsorb Na at a rate of 346 μmol·kg⁻¹·h⁻¹, nearly 5 times more than the simple difference between filtered and excreted Na loads. The tubular secretion of NaCl and water increases the luminal aqueous volume beyond that provided by glomerular filtration, which increases the renal capacity for excreting solutes such as Mg, SO₄, PO₄, metabolic wastes, and xenobiotics.

bules collectively secrete fluid at a rate of 1.74 ml·kg⁻¹·h⁻¹ with an average Na concentration of 158 mM measured in isolated fluid-secreting tubules (Table 2), it follows that the rate of proximal Na secretion is 275 μmol·kg⁻¹·h⁻¹, or 3.4 times greater than the filtered Na load. Together, filtration and secretion deliver 355 μmol·kg⁻¹·h⁻¹ Na to the lumen of the proximal tubule. Because only 9 μmol·kg⁻¹·h⁻¹ of Na are voided from the bladder, 346 μmol·kg⁻¹·h⁻¹ Na are reabsorbed in the distal nephron and urinary bladder and not 71 μmol·kg⁻¹·h⁻¹, the difference between filtered and excreted Na alone. Again, when renal input is restricted to glomerular filtration, renal input-output balances yield a fractional Na absorption of 89%. However, in view of tubular secretion of Na, the fractional Na absorption is 97%, approaching the usual fractional Na absorption of the vertebrate nephron.

**NaCl-DRIVEN WATER SECRETION INCREASES RENAL EXCRETORY CAPACITY**

A physiological rationale for secreting NaCl and water into the lumen of proximal tubules via secondary active transport of Cl (Fig. 5) is gleaned by considering how the Mg concentration rises from 22 mM in the lumen of the proximal tubule to 130 mM in the urinary bladder (Fig. 8A, Tables 1 and 2). For the sake of argument, we begin with simple renal input-output analysis. A glomerular filtration rate of 0.5 ml·kg⁻¹·h⁻¹ and a urine flow rate of 0.3 ml·kg⁻¹·h⁻¹ in the typical marine fish yields, albeit incorrectly, a fractional water absorption of only 40% (see above). A fractional water absorption of only 40% increases the concentration of Mg from 22 mM in the lumen of proximal tubules to only 37 mM in the urinary bladder, and not 130 mM actually measured (Fig. 8A). Clearly, the filtered volume is too small to concentrate Mg to the concentrations measured in the urinary bladder. However, the true fractional water absorption is 87% (see above), which is sufficient to raise the luminal Mg concentration from 22 to 169 mM, higher than measured Mg concentrations of 130 mM. Thus the tubular secretion of Na, Cl, and water in proximal tubules increases the luminal volume, thereby increasing the aqueous volume into which Mg can be secreted. The subsequent reabsorption of Na, Cl, and water in the distal nephron and urinary bladder concentrates Mg for excretion while conserving body water.

Viewed from the perspective of the renal excretion of Mg, the secretion of NaCl and water across renal proximal tubules emerges as a mechanism for increasing the renal excretory capacity not only for Mg but also for SO₄, P, organic acids, xenobiotics, and other solutes. In aglomerular fish, the tubular secretion of NaCl and water is the only means for delivering water to the tubule lumen, essentially replacing glomerular filtration and providing an aqueous environment for the excretion of excess and toxic solutes.

The strategy for increasing renal concentrating and excretory capacity by secreting of NaCl and water in renal proximal tubules may be more widespread than we presently believe. It may explain why we have observed NaCl and water secretion in proximal tubules regardless of glomerular development and environmental salt and water challenges in 1) hyposmotic, glomerular, and aglomerular regulators in seawater (flounder, toadfish, and killiﬁsh); 2) isosmotic glomerular regulators in seawater (dogfish shark); and 3) hyperosmotic regulators in freshwater (killiﬁsh) (14, 16, 38, 143). Like the secretion of organic anions, the tubular secretion of Na, Cl, and water may be a fundamental property of the vertebrate proximal tubule that has not been discarded in the course of evolution.

**ON THE QUESTION OF TUBULAR SECRETION IN THE MAMMALIAN KIDNEY**

In a rare review that combines scientific and literary writing, Grantham and Wallace (67) have addressed the question of tubular secretion in the mammalian kidney. The broad-band organic anion transport system that was first described in renal proximal tubules of fish 75 years ago (104) and discussed above in some detail (Fig. 6) is present in renal tubules of vertebrates standing on the highest rungs of vertebrate evolution, i.e., mammals and birds, illustrating the retention of a primordial renal function of the proximal tubule. The primary purpose of this ancient secretory transport system is the renal excretion of diverse endogenous metabolites, xenobiotics and drugs, including nonsteroidal anti-inflammatory drugs, β-lactam antibiotics, and diuretics (27). While glomerular filtration can clear unwanted solute and water from the circulation, tubular secretion can be much more effective by removing solute from the circulation in a single passage through the
Such powerful secretory transport systems can drive transepithelial secretory transport of fluid. For example, human uremic serum containing increased levels of hippurate, a normal metabolite, reversed the net transport of fluid from absorption to secretion in isolated rabbit proximal tubules (65). In the human kidney, Grantham and Wallace (67) have estimated that as much as 1 liter of fluid/day can be secreted by hippurate-linked water transport. Although this secreted volume approximates the normal daily urine volume, it would be difficult to detect in clearance studies against the tubular reabsorption of 179 liters (63, 64). To observe hippurate-linked fluid secretion, glomerular filtration rate would have to drop to <10% of control (67).

The calculations illustrate that proximal fluid secretion can be observed in mammalian proximal tubules under conditions of diminished filtered and hence reabsorptive loads. Experimental data prove the point. In nonperfused proximal tubules (S2 segments) of rabbits, net fluid absorption reversed to net secretion when the peritubular PAH concentration was only 25 μM (66). Maximal fluid secretion rates up to 160 pl/min were measured at a peritubular PAH concentration of 1.3 mM. In perfused proximal tubules and in the intact kidney, organic anion-coupled water secretion would be expected to decrease the rate of net fluid absorption but be viewed as an inhibition of fluid absorption when in fact the secretory flux had increased.

**INHIBITION OF Na ABSorption OR StIMULATION OF CISECRETION?**

The notion that renal tubules of mammals might secrete Cl is afforded the greatest caution. It is understandable why in view of the net reabsorption of 99% of the filtered NaCl and water via largely Na-dependent transport mechanisms, Cl has been delegated the role of counterion to Na. Furthermore, any tubular secretion of Cl that might take place decreases the net reabsorption of NaCl and water. However, too often, and perhaps incorrectly, the decrease in net absorption is interpreted as an inhibition of Na absorption when it could also stem from the increase in Cl secretion.

The gall bladder has long been considered a prototype of NaCl-absorptive, leaky epithelia such as renal proximal tubules and the small intestine (137). Like renal proximal tubules, gall bladders absorb NaCl and water in isosmotic proportion. As shown in Fig. 9A, Cl is taken up across the apical membrane in exchange for HCO3, which is thought to lift intracellular Cl above electrochemical equilibrium with extracellular Cl. Cl leaves the cell across the basolateral membrane via cotransport with K and via a Cl-conductive pathway. In the presence of cAMP or dopamine, which increases intracellular cAMP concentration, transepithelial NaCl and water absorption come to a halt and may reverse to secretion (Fig. 9B, Refs. 121, 122, 137, 163, 175). cAMP is thought to inhibit Cl uptake from the tubule lumen and to activate apical membrane Cl channels via phosphorylation by protein kinases (28). The exit of Cl from the cell into the lumen of the gall bladder depolarizes the apical membrane voltage, as in cAMP-stimulated shark renal proximal tubules (Fig. 5). Thus the Cl efflux into the lumen brings about the reduction in net NaCl and fluid absorption. Although net fluid secretion is rarely observed in Necturus gall bladder, it does occur in mammalian gall bladders where cAMP stimulates transepithelial Cl secretion via CFTR-like Cl channels in the apical membrane (33–35, 46, 108, 109, 137).

As in the gall bladder, the intracellular Cl in renal proximal tubules of amphibian and mammalian proximal tubules is above electrochemical equilibrium with extracellular Cl (29, 48, 79, 92, 193). In addition, the luminal Cl concentration is significantly higher than the Cl concentration in the peritubular blood or Ringer solution. How this transepithelial Cl gradient comes about may be a matter of species difference as well as point of view (Fig. 10). The Anagnostopoulos laboratory has long maintained that in amphibian renal proximal tubules, the tubular secretion of Cl raises the luminal Cl concentration above peritubular concentrations (Fig. 10A, Refs. 4, 5, 48), a view that was confirmed and extended in the Boulpaep laboratory (1, 9). In contrast, investigators of mammalian proximal tubules maintain that Na-dependent fluid absorption elevates the luminal Cl concentration (Fig. 10B, Refs. 6, 7, 179, 181). The reality may include both mechanisms, because amphibian and mammalian proximal tubules have the molecular instruments for Cl transport in both absorptive and secretory direction (Fig. 10).

Supporting transcellular Cl absorption, apical membranes of both amphibian and mammalian proximal tubules bring Cl into the cell from the tubule lumen in exchange for organic anions such as oxalate and formate via isoforms of the SLC family of multifunctional anion exchangers (6, 7, 24, 81, 87, 152, 179, 181), and the basolateral membrane offers Cl channels that...
permit the exit of intracellular Cl into the renal interstitium (7, 149, 151). Supporting transcellular Cl secretion, same cells employ Cl/HCO₃ exchange to bring Cl into the cell from the renal interstitium (2, 90, 117, 141, 193), and the apical membrane offers Cl channels that allow the efflux of Cl into the tubule lumen (1, 31, 99, 182). Thus amphibian and mammalian proximal tubules express the transporters for absorptive as well as secretory Cl transport (Fig. 10). Whether net transcellular Cl transport proceeds in the direction of absorption or secretion depends on many variables, among them (1) the relative number and activity of transporters involved in and associated with Cl transport, 2) metabolic activity and its effects on intracellular H and HCO₃ concentrations, 3) transmembrane electrochemical potentials of all solutes participating in transepithelial Cl transport, and 4) the delivery of transported solutes to apical and basolateral surfaces of the epithelium. When glomerular filtration presents large quantities of absorbable solute (including Na and Cl) to the apical surfaces of the proximal tubule, the net transepithelial Cl gradient is absorptive (7). As glomerular delivery decreases, absorptive Cl transport decreases and secretory Cl transport may come to the fore.

Cl channels or Cl-conductive pathways are present in brush-border membrane vesicles prepared from the rabbit renal cortex (31, 182) and in a primary culture of rabbit proximal tubules (42, 43, 168). Direct studies of the effects of cAMP in rat proximal tubules microperfused in situ show that the nucleotide activates 5-nitro-2-(3-phenylpropylamino)benzoate-sensitive Cl channels in the apical membrane of mammalian proximal tubules (180). However, these Cl channels mediate transepithelial Cl absorption, together with diphenyl-2-carboxylate-sensitive Cl channels in the basolateral membrane, as long as the peritubular Cl concentration is 25 mM lower than the luminal Cl concentration, bringing about transepithelial Cl secretion (138, 160, 176, 188, 189). Isolated, non-perfused inner medullary collecting ducts respond to cAMP by increasing fluid secretion, an effect that can be inhibited with the usual inhibitors of Cl secretion, bumetanide and diphenyl-2-carboxylate (177). Cultures of the distal nephron express renal proximal tubules (59, 93). The effects combine to yield a natriuresis and diuresis (12). Dopamine also elevates intracellular cAMP concentration in proximal tubules (69, 140). In view of the effects of CAMP on Cl and fluid secretion in fish proximal tubules, gall bladders, and amphibian proximal tubules, the dopamine-induced diuresis has more to do with the CAMP stimulation of transepithelial Cl secretion than with the inhibition of Na absorption. Consistent with the mechanism of CAMP stimulation of NaCl fluid secretion in mammalian renal proximal tubules is the favorable effect aminophylline has on the outcome of acute renal failure (67). Aminophylline is a phosphodiesterase inhibitor that is expected to increase intracellular cAMP concentrations and to stimulate fluid secretion in renal proximal tubules. Furthermore, in some forms of hypertension, renal tubules have decreased responsiveness to dopamine (140). Transepithelial fluid secretion would be diminished under these conditions, bringing about fluid retention and the ensuing hypertension.

On the background of multiple Cl transport mechanisms in both apical and basolateral membranes (Fig. 10), the large transepithelial unidirectional Cl fluxes, 9.5 and 8.3 neq/cm²-s, are between three and four times greater than unidirectional Na fluxes (144). These large transepithelial Cl fluxes raise the question of whether it is appropriate to consider renal proximal tubules as primarily an Na-transporting and Na-reabsorbing epithelium.

**CI SECRETION IN THE DISTAL NEPHRON**

The distal nephron, in particular, cortical collecting tubules, and medullary collecting ducts display evidence for transepithelial Cl secretion (138, 160, 176, 188, 189). Isolated, non-perfused inner medullary collecting ducts respond to CAMP by increasing fluid secretion, an effect that can be inhibited with the usual inhibitors of Cl secretion, bumetanide and diphenyl-2-carboxylate (177). Cultures of the distal nephron express
transepithelial Cl secretion particularly well, including the mechanism of cAMP-stimulated Cl secretion via CFTR (67, 77, 78, 162, 178). Rabbit cortical collecting ducts grown in culture reveal three separate anion channels with channel conductances ranging from 30 to 267 pS (36, 95).

The Madin-Darby canine kidney (MDCK) cell line displays most clearly the building blocks of transepithelial Cl secretion: an Na-K-ATPase, K conductance, and loop diuretic-sensitive Na-K-Cl cotransporter at the basolateral membrane, and a regulated Cl conductance at the apical membrane (154). Prostaglandins and kinins capable of stimulating Cl secretion in MDCK epithelia also promote natriuresis in intact kidneys (61, 62), suggesting that the natriuresis may come about from the stimulation of Cl secretion.

When seeded in collagen gel, MDCK cells form fluid-secreting epithelial cysts akin to those observed in autosomal dominant polycystic kidney disease (ADPKD) (102). ADPKD cysts of proximal, distal, and collecting tubules express the molecular mechanisms for Cl secretion as in fish proximal tubules and in the MDCK cell line, namely, bumetanide-sensitive Na-K-2Cl cotransporter in the basolateral membrane and Cl-channels in the apical membrane (67, 165–167, 178, 187). Moreover, cAMP and agents that increase intracellular cAMP, such as AVP, PGE₂, secretin, VIP, β-adrenergic stimulation, phosphodiesterase inhibitors, and an unknown neutral lipid present in cyst fluids, all stimulate Cl and fluid secretion (67). In polycystic kidneys, the enlargement of cysts is exacerbated by the secretion of Cl and stimulated by extracellular nucleotides and nucleosides (187). Clearly, the potential for tubular Cl secretion is present in mammalian kidneys in proximal and distal segments of the nephron. However, how much Cl secretion and Cl-linked water secretion contribute to the final urine and to extracellular fluid homeostasis is a question that begs examination.

CONCLUDING THOUGHTS

It is impossible for reabsorptive rates to be greater than filtered rates if glomerular filtration is the only delivery mechanism to the tubule lumen. However, ask Grantham and Wallace (67), what if tubular secretion delivers substantial quantities to the tubule lumen in addition to glomerular filtration? Under these conditions, reabsorptive loads can exceed filter loads, as they do in the kidneys of fish. In human kidneys, the filtration of 180 l/day and a urine flow of 1 l/day suggest a tubular absorption of 179 l/day, i.e., 99.4% of the filtered load. If renal tubules secreted fluid at a rate of 10 liters/day in addition to 180 liters of ultrafiltrate, and if renal tubules reabsorbed fluid at a rate of 189 l/day, it would still be true that urine flow is 1 l/day and the fractional reabsorption of water is 99.4%, when in fact renal tubules have reabsorbed a volume equivalent to 105% of the filtered load.

The difference between 99.4 and 100 as the percentage of water reabsorption in our kidneys is minor. However, if renal tubules reabsorbed 100% of the filtered water, then any urine arriving in the urinary bladder must be the product of tubular secretion, driven by tubular secretion of organic solutes, Cl, or other solutes.

Mammalian renal tubules may indeed reabsorb more than the filtered volume because we do not know how much fluid renal tubules secrete. Input-output analyses are blind to the epithelial traffic taking place across renal tubules. In the absence of measures of the true magnitudes of absorptive and secretory fluxes across renal proximal tubules and other tubule segments, both secretory and reabsorptive powers remain underestimated in the mammalian kidney.

The secretion of organic anions in proximal tubules from vertebrates as ancient as the hagfish and as modern as the postmodern human illustrates the persistence of some renal transport systems in the course of vertebrate evolution. In addition to organic anion transport, it appears that the tubular mechanism for secreting Cl, which Frömter and I (19) have elucidated in renal proximal tubules of the shark, has also been retained in the course of vertebrate evolution. Holding on to early and highly useful transport systems may be one reason why the mammalian kidney reflects its evolutionary history to a remarkable extent (67). The mounting evidence for Cl secretion in the mammalian kidney calls for a reexamination of the renal handling of Cl (67, 154).

ACKNOWLEDGMENTS

I look back fondly on my collaboration with Eberhard Frömter at the Mt. Desert Island Biological Laboratory (19). I thank William H. Dantzler for training in vitro microperfusion of renal tubules, Sandy Helman for education in electrophysiology, and Jared Grantham and Karl Ulrich for encouraging our studies of renal secretion. I am grateful to Amy McCune for guiding me through the quagmire of fish taxonomy. I am greatly indebted to the National Science Foundation for supporting our work for many years.

REFERENCES

35. Chinet T, Fouassier L, Dray CN, Imam GM, Morel H, Mergey M, Chen PY, Illsley NP, and Verkman AS.
34. Cassola AC, Mollenhauer M, and Froemer E.
33. Beyenbach KW.
AGLOMERULAR URINE FORMATION


