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# RENAL HANDLING OF MAGNESIUM IN FISH: FROM WHOLE ANIMAL TO BRUSH BORDER MEMBRANE VESICLES

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## 1. ABSTRACT

Of all known vertebrate tissues, the kidneys of fish are the champions of Mg transport. They can switch from Mg conservation in fresh water to Mg wasting in seawater. High rates of tubular transport and the ability to alternate between Mg reabsorption and secretion make fish kidneys the model of choice investigating the mechanisms of transepithelial and membrane Mg transport and its regulation by extracellular hormones and intracellular messengers. Studies in isolated proximal tubules indicate active transepithelial Mg transport that requires metabolic energy for both tubular reabsorption and secretion. Whether active transport is primary and mediated by a Mg-pump, or secondary and mediated via cotransport or antiport, is unknown. In fresh water fish, the active transport pathway appears to include a Mg-channel located in brush-border membranes of proximal tubules. Although plasma Mg concentrations are well protected, a primary hormone controlling Mg balance has yet to be identified in any animal. Moreover, the mechanisms of intracellular Mg homeostasis, especially in epithelial cells with high Mg throughput, are unknown. New methods, including Mg imaging and genetic/molecular approaches promise to unravel Mg transport mechanisms in teleost renal tubules.

## 2. INTRODUCTION

### 2.1. Osmotic and ionic regulation in fish

Of about 42,000 species of vertebrates existing today more than half are fish. Among them bony fish of the class teleostei have been the most successful establishing themselves in aquatic habitats of diverse salt content. Inhabiting fresh water and seawater is no small measure of the physiological resourcefulness of teleost fish. They possess highly adaptive epithelial transport mechanisms in the gut, gill and kidney that solve the salt and water challenges of life in hypo- and hyperosmotic media (Figure 1).

In the hypo-osmotic environment of fresh water, fish maintain extracellular fluids at an osmotic pressure of approximately 294 mOsm via high concentrations of Na and Cl (Table 1). These two ions are largely sequestered in the plasma and interstitial fluid because cells extrude them, the kidney retains them, and the gills take them up from fresh water ponds and rivers (Figure 1, Table 1).

Preserving an extracellular fluid osmotic pressure of 294 mOsm in an environment of only 20 mOsm (and less) causes water to enter by osmosis (Figure 1, Table 1). The

**Table 1.** Osmotic and ionic gardients in fresh water and seawater fish

| | | Osmotic Pressure (mOsm/kg water) | | Na | | | Cl | | | Mg | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Medium | Plasma | Urine | Medium mM/kg | Plasma (mM) | Urine (mM) | Medium mM/kg | Plasma (mM) | Urine (mM) | Medium mM/kg | Plasma (mM) | Urine (mM) |
| Fresh water | <1[1] 20[2] | 294.0± 7.8 (23, 12) | 40.5± 5.2 (12, 9) | 0.25 2.22 | 146.2± 3.1 (36, 16) | 12.4± 1.8 (19, 12) | 0.23 2.54 | 124.6± 4.8 (36, 20) | 6.7 ± 1.7 (12, 8) | 0.04 1.67 | 1.32± 0.31 (12, 8) | 1.10 ± 0.62 (8, 7) |
| Sea-water | ~1000[3] | 375.7 ±15.1 (24, 18) | 323.0 ± 32.4 (4, 4) | 475.4 | 185.9± 2.9 (57, 49) | 22.6 ± 6.1 (8, 7) | 554.4 | 165.0 ± 3.3 (54, 52) | 101.8± 13.4 (7, 5) | 54.2 | 2.93 ±0.40 (15, 12) | 118.02 ± 16.03 (5, 7) |

mean ± SE; (number of measurements, number of species), Data are from Potts & Parry, 1963; Holmes and Donaldson, 1969; Hickman & Trump, 1969; Beyenbach, 1974; Baustian *et al.*, 1997. [1]soft water [2]hard water [3]Mediterrean seawater has an osmotic pressure in excess of 1200 mOsm. The chemical composition of seawater is not constant because of global geochemical fluxes that increase the concentrations of sodium, magnesium, sulfate, and potassium, and that decrease the concentration of calcium (de Villiers & Nelson, 1999)

fish excretes this water in urine more than 7 times as dilute as plasma (Figure 1, Table 1).

Osmotic and ionic challenges reverse in seawater where the osmotic pressure is approximately 1000 mOsm (Table 1, Figure 1). In the hyperosmotic environment of the sea, fish maintain a plasma osmotic pressure of about 376 mOsm, considerably up from 294 mOsm in fresh water (Table 1, Figure 1). Accordingly, adaptation to fresh water and seawater includes the tolerance of a wide range of plasma osmolarity, far greater than that tolerated by mammals (1, 2).

Maintaining an extracellular fluid osmotic pressure of 376 mOsm in an environment of 1000 mOsm (and higher) causes seawater fish to lose water by osmosis and to gain salt by diffusion (Figure 1). Both problems are solved in the physiological distillation of seawater: the fish drinks seawater, absorbs most of it in the intestine, excretes monovalent ions in the gills, and excretes divalent ions in the kidney (Figure 1). Thus, excretion of salt but retention of water produces new extracellular fluid from seawater. Because fish kidneys do not possess a renal medulla, the upper ceiling of renal solute excretion is thought to be limited to a urine osmotic pressure that can not exceed plasma osmotic pressure (Table 1, Figure 1). This view has been challenged (3, 4). However, the majority view considers fish kidneys incapable of generating urine more concentrated than plasma. Hence the task of distillation, of generating solute-free water for the body, falls squarely on the gills. If this is so, than the gills must be able to separate salt from water and to excrete Na and Cl without, or with a minimum of, water (Figure 1).

Whereas permanent denizens of fresh water or seawater must deal with just one set of salt and water problems, fish residing in estuaries, and fish migrating between fresh and seawater, must handle both sets. Euryhaline fish must switch between hyper-osmotic regulation in fresh water and hypo-osmotic regulation in seawater with the tides on a daily basis. In particular, epithelial transport mechanisms in the gill must be able to alternate between NaCl uptake in fresh water and NaCl extrusion in seawater (Figure 1). In the kidney, epithelial transport mechanisms must be able to switch from conserving divalent ions in fresh water to elimination in seawater (Table 1, Figure 1).

### 2.2. Challenges to renal Mg homeostasis in fresh water and seawater

Glomerular filtration is ideally suited for the excretion of the osmotic water loads in fresh water. In the rainbow trout, formerly known as *Salmo gairdneri* and recently reclassified as *Oncorhynchus mykiss*,, rates of glomerular filtration are nearly five times greater in fresh water than in seawater (Figure 2), but in fresh water only 47% of the filtered water is reabsorbed by the renal tubules (5). Thus, more than half of filtered water is voided but not before reabsorbing more than 96% of the filtered Na and Cl (Table 1, Figure 2). In contrast, only 64% of the filtered Mg is net reabsorbed reflecting the need to excrete dietary Mg. However, on balance, Mg is net reabsorbed by renal tubules because fresh water Mg concentrations are at least 42 times lower than plasma Mg concentrations, and dietary Mg can not always be counted on (Table 1).

In seawater, rates of glomerular filtration and urine flow are sharply reduced consistent with the environmental challenge of saving body fluids in hyperosmotic media (Table 1, Figs. 1, 2). Loss of body water is further reduced by allowing urine osmotic pressure to rise from 40 mOsm in fresh water to 323 mOsm in seawater (Table 1). The high osmotic pressure of urine derives from magnesium, chloride, sulfate, phosphate that are excreted as osmoregulatory byproducts rather than metabolic wastes (Table 1, Figures 1, 2; Ref. 3, 4, 6).

In summary, in fresh water fish, glomeruli serve the excretion of osmotic water loads, and the renal tubules serve the reabsorption of life-essential solutes. Large volumes of dilute urine result from this pas de deux of glomeruli and renal tubules.

In seawater, glomerular filtration serves little function in glomerular fish and no function at all in aglomerular fish (2, 7). Renal function is now dominated by tubular activities, tubular secretion in particular. Tubular secretion is not limited to those solutes that need removal from the circulation (magnesium, sulfate, phosphate, organic acids and bases and toxins), but it extends to the secretion of NaCl and water (4,8,9,10). Why NaCl and water are secreted in proximal tubules of glomerular and aglomerular fish is unclear. As a first hypothesis, tubular secretion may replace glomerular filtration and deliver Na and Cl to the apical surfaces of renal epithelial cells where these two ions can serve co- and antiport transport systems such as Na/H or Cl/$HCO_3$ exchange.

## 3. RENAL HANDLING OF MAGNESIUM IN FRESH- AND SEAWATER FISH

### 3.1. Renal handling of Mg in rainbow trout adapted to fresh water

The rainbow trout is a popular fish, also in the laboratory. Its anadromous nature - growing up in fresh

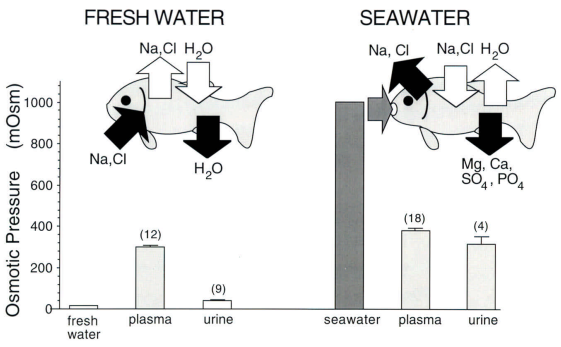


**Figure 1.** Water problems of fish in fresh water and seawater. In fresh water, fish gain water by osmosis which is excreted as urine strongly dilute to plasma. In contrast, fish lose water by osmosis in seawater. Water lost to the ocean is replaced by drinking seawater and distilling new body fluids from it via hyper-osmotic secretion of Na and Cl across the gills and nearly isosmotic excretion of divalent ions of seawater (Mg, Ca, $SO_4$ , $PO_4$) by the kidney. Mean ± SE, (number of species; data from Beyenbach (5) and Hickman and Trump (6).

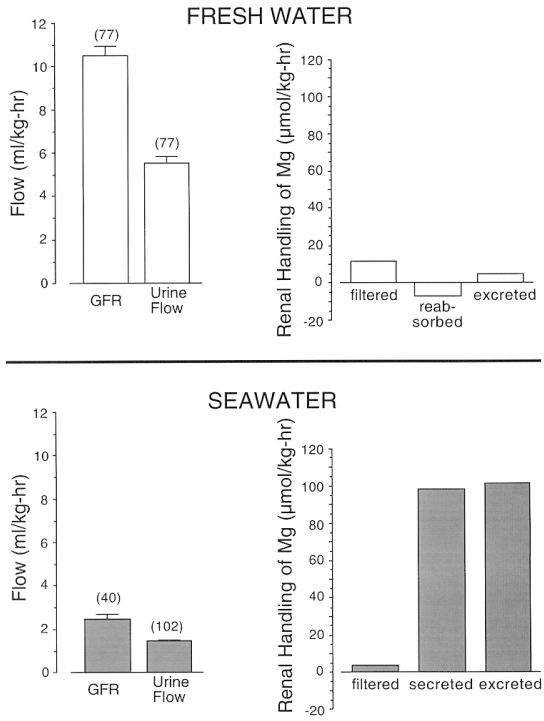


**Figure 2.** Renal handling of water and magnesium in rainbow trout, *Oncorhynchus mykiss*, adapted to fresh water and seawater; Mean ± SE; data from Beyenbach (5).

water, spending adult life in seawater, and returning to fresh water to mate - is particularly interesting to the investigator of osmotic and ionic regulation. Rainbow trout in fresh water have a glomerular filtration rate of 10.5 ml/kg-hr (Figure 2). With a plasma Mg concentration of 1.04 mM, the filtered load of Mg is 10.93 µmol/kg-hr (Figure 2). The excreted Mg load, the product of urine flow rate and urine Mg concentration, is only 3.87 µmol/kg-hr (Figure 2). The difference between filtered and excreted Mg loads is the rate of tubular Mg reabsorption, 7.1 µmol/kg-hr (Figure 2). Accordingly, renal tubules of fresh water rainbow trout conserve Mg, returning 65% of filtered Mg loads to the blood.

### 3.2. Renal handling of Mg in rainbow trout adapted to seawater

Low rates of glomerular filtration and low concentrations of Mg in the plasma render glomerular filtration inadequate for the excretion of the large loads of Mg ingested with seawater. In particular, rainbow trout adapted to seawater reduce glomerular filtration to about $1/5^{th}$ the rate in fresh water (Figure 2, Ref. 5). Even though plasma Mg concentration rises to 1.29 mM in seawater, the marked reduction in GFR lowers the filtered Mg load from 10.93 µmol/kg-hr in fresh water to 3.2 µmol/kg-hr in seawater (Figure 2). However, excreted Mg rises to an astonishing 100.8 µmol/kg-hr, the result of tubular secretion (Figure 2). So powerful is tubular secretion that it delivers to the tubule lumen 30 times the Mg filtered at the glomerulus. On a daily basis, tubular Mg secretion excretes a quantity equivalent to the entire body Mg. In particular, secretion in proximal tubules generates a tubular fluid Mg concentration of about 30 mM (8) which rises to 118 mM through the reabsorption of Na, Cl and water in the distal nephron and urinary bladder (Table 1, Figure 2, Ref. 11). Aglomerular fish that are anatomically devoid of glomeruli must rely entirely on tubular mechanisms for renal Mg homeostasis (2,7).

## 4. TUBULAR HANDLING OF MAGNESIUM

### 4.1. Tubular handling of Mg in fresh water

The renal proximal tubule is believed the likely site for reabsorbing Mg in fresh water fish - not an unreasonable hypothesis in view of the large number of transport systems serving reabsorption here (6, 8). For this reason we were surprised to observe evidence of Mg secretion in a population of proximal tubules isolated from killifish, *Fundulus heteroclitus*, adapted to fresh water (12).

Removing the kidney from the animal halts glomerular filtration for obvious reasons. But tubular reabsorption may still continue which is perhaps the reason why the tubule lumen is collapsed in about 90% of proximal tubules that we isolate from fresh water killifish (12). In the remaining proximal tubules (10%), the tubule lumen is wide open suggesting secretion of fluid. When these tubules are prepared for study as shown in Figure 3, they secrete fluid spontaneously. The average rate of fluid secretion is 34 pl/min per mm tubule length (Figure 3). The compositional analysis of secreted fluid reveals Na and Cl as the major solutes, and Mg and S at concentrations far above those in the peritubular Ringer bath (Figure 3).

The spontaneous secretion of Na, Cl, Mg, S and fluid *in vitro* was puzzling because the kidney of fresh water killifish conserves these four ions, reabsorbs and not secretes them. A solution to this paradox may be found in considerations of functional anatomy and environmental physiology.

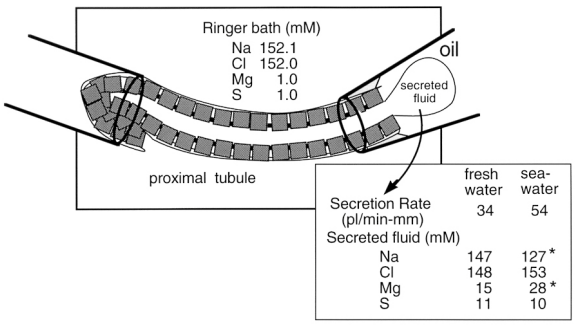


**Figure 3.** Secretion of Na, Cl, Mg, S and water in proximal tubules isolated from killifish, *Fundulus heteroclitus* adapted to fresh water and seawater. The tubule lumen is closed on one side by way of a hairpin turn in the left holding pipette; the other end of the tubule opens into the right holding pipette. Fluid secreted by the epithelial cells flows from the open end and accumulates under light paraffin oil; data from Cliff & Beyenbach (12).

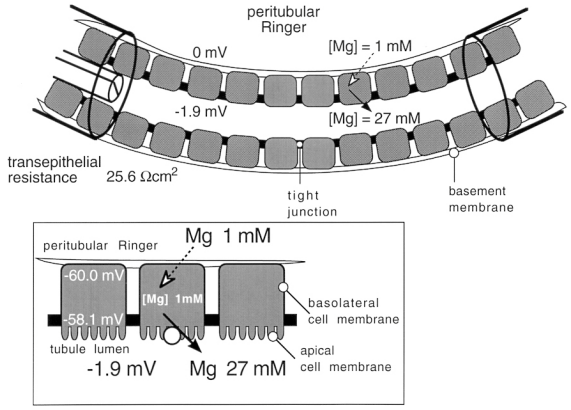


**Figure 4.** Thermodynamics of transepithelial Mg secretion in isolated proximal tubules of the seawater winter flounder, *Pleuronectes americanus*. Open arrow, transport down the electrochemical gradient; closed arrow, transport against the electrochemical gradient. The mechanism of Mg entry across the basolateral membrane and Mg extrusion across the apical membrane are unknown; data from Cliff & Beyenbach ( 8).

As to functional anatomy, Brown *et al.* (13) find three populations of nephrons in fresh water rainbow trout: 1) filtering nephrons (45%), 2) perfused nephrons that are not filtering (42 %), and 3) nephrons that are not perfused at all and consequently not filtering (13 %). Thus more than half of the renal tubules in rainbow trout are not filtering in fresh water! It is doubtful that these non-filtering tubules have also ceased tubular functions. More likely, tubular transport continues, especially secretory transport. Accordingly, secretory proximal tubules we observe in fresh water killifish may stem from non-filtering nephrons that continue their tubular activities in the absence of glomerular filtration. Fish kidneys would thus appear to have considerable reserve capacity to increase urine output by glomerular and tubular means (14). Such functional reserves could be particularly useful in euryhaline fish. For example, killifish inhabit estuaries where the salt content fluctuates widely with the tides. As the tide goes out, the estuary becomes progressively dilute in the presence of continued river input of fresh water. The process reverses when the tide comes in. Since glomeruli are poised to handle the osmotic water loads of the hypo-osmotic estuary, the large population of non-filtering glomeruli presents a functional reserve at the ready to excrete water. Similarly, proximal tubules that we observe secreting Na, Cl, Mg, S, and water *in vitro*, present a functional reserve at the ready to excrete salt loads when seawater returns with the incoming tide, or when the fish makes excursions into more saline regions of the estuary.

### 4.2. Tubular handling of Mg in seawater.

The population of killifish proximal tubules that spontaneously secrete fluid *in vitro* increases from about 10% in fresh water to up to 70% in seawater (8). Moreover, rates of fluid secretion, normalized to a 1 mm length of proximal tubule, increase from 34 pl/min in fresh water to 54 pl/min in seawater (Figure 3). In parallel, the concentration of Mg in secreted fluid nearly doubles over those in fresh water proximal tubules and Na concentration decreases (Figure 3). Overall, rates of Mg secretion increases three-fold, from 0.51 pmoles/min in fresh water to 1.51pmoles/min in seawater (Figure 3). The 3-fold increase in Mg secretion reflects the increased demands on renal Mg excretion as the seawater fish distills fresh body fluids from ingested seawater.

## 5. PATHWAYS AND MECHANISMS OF MAGNESIUM SECRETION IN PROXIMAL TUBULES OF SEAWATER FISH

### 5.1. Tubular Mg secretion via active transport.

We have determined Mg electrochemical potentials in proximal tubules of 3 species: the dogfish shark, the killifish, and the winter flounder (8, 15). Electrochemical potentials were determined by measuring transepithelial Mg concentration differences in non-perfused proximal tubules as shown in Figure 3, and by measuring transepithelial voltage in isolated perfused tubules as shown in Figure 4.

Mg is secreted from the peritubular bath into the tubule lumen against electrochemical potentials ranging from about 10 mV in the dogfish shark to 40 mV in seawater killifish and winter flounder (8, 15). For example, isolated proximal tubules of the winter flounder, generate a Mg concentration of 27 mM in the tubule lumen when the peritubular Mg concentration is only 1 mM (Figure 4). The transepithelial voltage is lumen-negative, -1.9 mV (Figure 4). Thus, transepithelial Mg secretion is against a chemical potential of about 40.8 mV and down the electrical potential of about 1.9 mV, or against a net electrochemical potential of 38.9 mV. Transport against electrochemical potentials defines active transport requiring metabolic energy. Accordingly, transepithelial Mg transport must be transcellular through epithelial cells rather than paracellular between epithelial cells.

Since the basolateral membrane voltage of proximal epithelial cells is negative inside, Mg entry from the

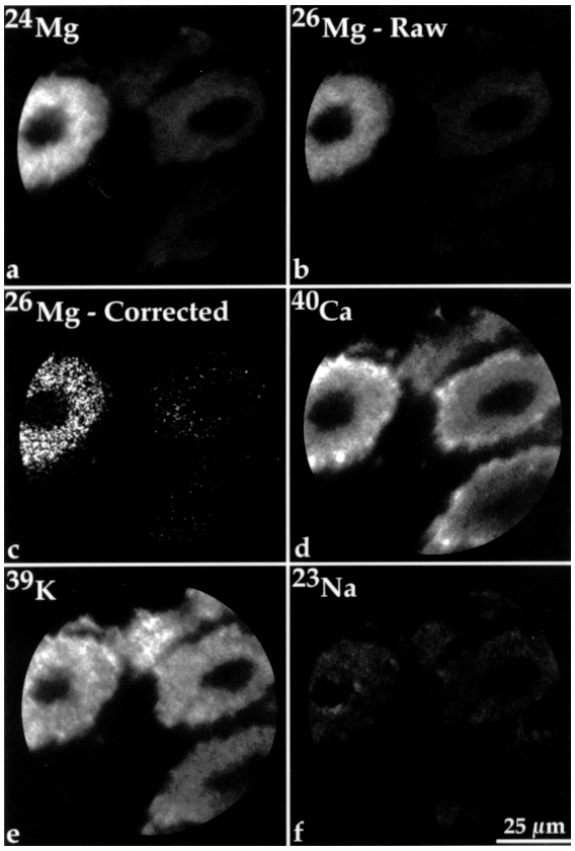


**Figure 5.** Mass spectrometry images of frozen/freeze-dried sections of a killifish kidney revealing Mg secretion across renal proximal tubules. Prior to isolating the kidney for mass spec analysis the seawater killifish had received an intraperitoneal injection of 99.5% enriched $^{26}$Mg in order to stimulate and trace the renal excretion of Mg. Mass spectrometry of freeze-dried slices of the snap-frozen kidney reveals the distribution of chemical elements in close relation to tissue morphology such that images of Mg, Ca, K and Na reflect the morphology of four cross-sections of the proximal tubule. Images of $^{24}$Mg (a) and $^{26}$Mg(b) are used to reveal the injected load of $^{26}$Mg undergoing transcellular secretion (c). Images of Ca (d), K (e) and Na (f) were produced as internal controls to document good cryo-preservation of intracellular elements; data from Chandra *et al.* (19).

peritubular bath into the cell is energetically downhill if it is assumed that the intracellular free Mg concentration of proximal epithelial cells is about 1 mM as in the usual eucaryotic cell (Figure 4 insert; Ref. 16). In contrast, extrusion from the cell into the tubule lumen is uphill against the concentration and the voltage, i.e. against an electrochemical potential of about 100 mV (Figure 4 insert). The transport of a divalent ion against this electrochemical potential is equivalent to transporting Mg from a 1 mM concentration in the cell to a concentration of 2155 mM in the tubule lumen at zero membrane voltage. Thus, the active transport step for extruding Mg across the apical membrane into the tubule lumen must be powerful.

The above thermodynamic consideration of transepithelial Mg secretion has validity if Mg crosses the epithelial cell as divalent ion, i.e. in ionized form with a charge of +2. Whether this is really the case remains to be seen. Since Mg binds to a large variety of organic molecules, the transported species may not be $Mg^{+2}$ but a monovalent or an electroneutral species. Moreover, transepithelial Mg transport may be transcytotic and include endocytosis at the basolateral membrane and exocytosis at the apical membrane. Hentschel and Zierold (17) report a zone of Mg-rich smooth clear vesicles and small vacuoles near the apical membrane of proximal epithelial cells of the dogfish shark. The small apical vacuoles contained Mg at an equivalent concentration of 229 mmol/kg water. The sequestration of Mg in vesicles and small apical vacuoles is thought to prepare Mg for exocytosis into the tubule lumen.

### 5.2. Tracing the tubular secretion of magnesium with the ion microscope

A serious impediment to the study of Mg transport is the lack of a good radioactive tracer. The half-lives of $Mg^{23}$ and $Mg^{27}$ are 11.9 sec and 9.8 minutes respectively. The longest-lived isotope, $Mg^{28}$, has a half-life of about 21.3 hours, and is produced at prohibitive costs. Fortunately, the methods of ion microscopy provide another approach of tracing the movement of magnesium (18). In brief, ion microscopy uses the mass spectrometer to detect and quantify any element (from hydrogen to uranium) in quick-frozen/freeze-dried sections of tissue. The term 'microscopy' is misleading because optics is not involved. However, with a spatial resolution of 0.5 µm, ion microscopy reveals the distribution of elements in such detail as to resemble optical images (Figure 5).

The distribution of $^{24}$Mg in epithelial cells of the proximal tubule is diffuse and punctate (Figure 5a; Ref. 19). Total Mg concentration is 38 mM. In same cells the concentrations of K and Na, measured as internal controls, are 180.6 mM and 34.4 mM respectively (Figure 5e,f).

Since mass spectrometry identifies elements on the basis of mass/charge ratio, the method can distinguish between the natural isotopes of elements. Accordingly, cold isotopes can be used like radioactive isotopes as tracers of biological transport. In the experiment shown in Figure 5, the killifish had received an intraperitoneal injection of 99.5% enriched $^{26}$Mg in order to stimulate and trace the renal excretion of magnesium. The raw-image of $^{26}$Mg (Figure 5b) includes endogenous Mg normally present in the kidney plus the injected $^{26}$Mg undergoing renal excretion. To get at injected $^{26}$Mg undergoing transepithelial secretion, image 5b must be corrected for native $^{26}$Mg already present in the kidney. The correction is done by determining native $^{26}$Mg as the product of $^{24}$Mg (Figure 5a) and the isotope ratio $^{24}$Mg/$^{26}$Mg (7.046), and by subtracting this product, pixel by pixel, from the $^{26}$Mgraw image (Figure 5b). The corrected $^{26}$Mg image (Figure 5c) reveals the punctate distribution of magnesium undergoing epithelial transport. It remains to be determined whether this punctate distribution reflects inclusion in or exclusion from vesicles and cell organelles.

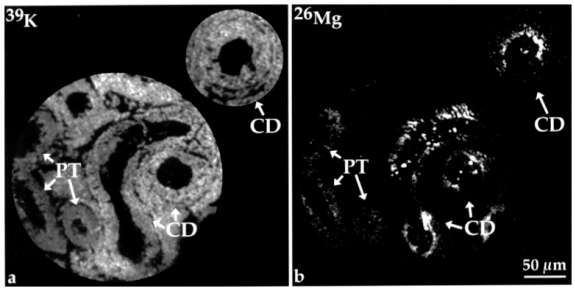


**Figure 6.** Mg/Ca-rich cells in a subpopulation of epithelial cells in collecting ducts. A section of the kidney from the same killifish of Figure 5 is shown. Correcting total $^{26}$Mg for native $^{26}$Mg normally present in the kidney reveals the renal handling of the injected $^{26}$Mg load. The correction discovers a subpopulation of epithelial cells with significant Mg transport capacity (b). The image of K (a) was produced as internal control to document good cryo-preservation of intracellular elements (PT, proximal tubule; CD, collecting duct; data from Chandra *et al.* (19).

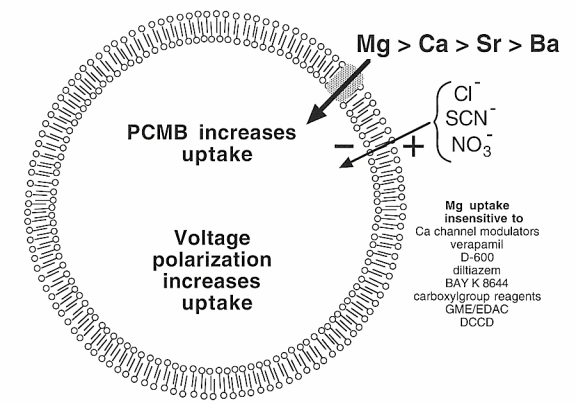


**Figure 7.** Electrodiffusive transport of Mg through brush border membrane vesicles of the proximal tubules of fresh water rainbow trout; data from Freire *et al.* (21).

Sequestration of transported Mg into vesicles would allow high rates of transcellular transport without presenting cell housekeeping functions with the challenge of rising cytoplasmic free Mg concentrations. If magnesium is indeed moved through the cytoplasm in vesicular packages, then these Mg-vesicles are diffusely distributed in cells of the proximal tubules in killifish. In shark proximal tubules Mg-vesicles are located near the luminal border (17).

Images of $^{40}$Ca reveal a heterogeneous intracellular distribution (Figure 5d). Ca content is highest near the basal side and decreases towards the apical side. It is uncertain whether this distribution reflects transepithelial Ca transport because the image of Figure 5d depicts Ca normally present in cells including 'household' Ca. Furthermore, the ion microscope measures total element leaving uncertain which Ca is free and which is bound.

### 5.3. The collecting duct, a major site of renal Mg transport?

In the course of tracing renal Mg excretion in the killifish with the use of the ion microscope we discovered a group of Mg-rich cells in the collecting duct (Figure 6). Again, the subtraction of $^{26}$Mg naturally present from the total $^{26}$Mg image uncovers $^{26}$Mg undergoing transepithelial transport. Whereas all cells of the proximal tubule seem to participate in transepithelial Mg secretion (Figure 5c), only a subpopulation of epithelial cells in large collecting ducts engage in Mg transport (Figure 6b). The direction of transport, reabsorption or secretion, can not be discerned from the images in Figure 6; timed images are needed for that. Epithelial cells enriched with $^{26}$Mg also showed enrichment with Ca (image not shown). For this reason we have named these cells Mg/Ca-rich (19).

The intracellular distribution of Mg in epithelial cells of collecting ducts differs from that in cells of the proximal tubule. Whereas the distribution of transported Mg in cells of the proximal tubule is punctate (Figure 5c), there is a transcellular Mg gradient in Mg/Ca-rich cells of collecting ducts with the highest Mg content near the apical border (Figure 6b).

## 6. PATHWAYS AND MECHANISMS OF MAGNESIUM TRANSPORT IN PROXIMAL TUBULES OF FRESH WATER FISH

### 6.1. Mg transport across brush border membranes of the proximal tubule of rainbow trout adapted to fresh water

The kidney of fresh water fish net reabsorbs Mg (Figures 1, 2). Electrochemical potentials support passive entry of filtered Mg from the tubule lumen into epithelial cells of the proximal tubule. In contrast, the extrusion of Mg from the cell into the renal interstitium is against the electrochemical potential, requiring an active transport step. We investigated the mechanism of Mg entry across the apical membrane in brush border membrane vesicles (BBMV) of the proximal tubule prepared from rainbow trout kidneys (20, 21). In brief, trout renal BBMV were isolated by the methods of differential centrifugation with sucrose (22) rather than precipitation with divalent ions (23) in order to keep background Mg concentrations low in subsequent Mg uptake experiments. At the time of these experiments radioactive $^{28}$Mg was still available in Germany which we used to good advantage. Although uptake of Mg included an appreciable binding component, most of the $^{28}$Mg flux proceeded into an osmotically active space.

### 6.2. Evidence consistent with a Mg-channel

$Mg^{28}$ uptake by renal BBMV of the rainbow trout increased in the presence of inward anion gradients (Cl, SCN, $NO_3$) that hyperpolarized membrane voltage to more negative values inside (Figure 7). Competition for uptake by Ca, Sr and Ba displayed selectivity sequence VII of alkaline earth cations (24). This specific sequence is expected of a transport pathway such as a channel with high anionic field strength capable of stripping Mg of most or all of its water shell (Figure 7). The organic mercurials, p-chloromercuribenzoic acid (PCMB) and p-chloromercuriphenylsulfonate (PCMBS), which increase Ca flux through Ca channels (25), strongly stimulated Mg uptake as well as efflux from Mg-loaded vesicles (Figure

7). These observations indicate electrodiffusive transport of Mg consistent with transport through a channel (21). Bijvelds *et al.* (26) reached similar conclusions in their study of renal brush border membranes prepared from tilapia kidneys, where a Mg-channel may be useful in the tubular reabsorption of filtered Mg. Likewise, voltage-dependent Mg entry into immortalized epithelial cells of the mouse distal convoluted tubule is consistent with a Mg channel (27).

Although data suggesting Mg channels have been observed in the ciliate protozoan *Paramecium* (28), in the CorA system of gram-negative bacteria (29), in rod outer segment of the toad (30), in cardiac myocytes (31), and in rumen epithelia (32), a true Mg channel has not been identified to this date. Recently, a divalent cation transport system named Alr, has been identified in yeast (eucaryote) with similarities to the CorA transport system in procaryotic bacteria (33). Since the CorA transporter contains a pore region (29), Alr may turn out to be the first Mg-channel identified in eucaryotic cells.

## 7. PERSPECTIVES

### 7.1. Unresolved questions of intracellular Mg homeostasis in epithelial cells transporting Mg

There is good agreement that most intracellular Mg is bound leaving free, ionized Mg in the cytoplasm at concentrations in the vicinity of 1 mM (34, 35, 36). The concentration of intracellular free Mg must be well regulated in view of the diverse roles of Mg that range from stabilizing structures such as DNA and the cytoskeleton, to activating enzymes, to modulating membrane channels and G-proteins and to antagonizing the actions of Ca (37). Rising Mg concentrations could have serious consequences. How then, do epithelial cells transport Mg from one side of the epithelium to the other without incurring the dangers of rising Mg concentrations? Secreting Mg, cells of the proximal tubule of seawater killifish pass through them 1000 times the quantity of intracellular free Mg, every minute (12). Given such a high throughput, can it be assumed that Mg passes trough the cytoplasm as free ion? Could Mg be sequestered in vesicles and isolated from the cytoplasm? Could Mg be chelated to some Mg-binding protein and be moved across the cytoplasm in inactive, bound form?

### 7.2. Unresolved questions of extracellular Mg homeostasis

How kidneys help regulate plasma Mg concentrations remains an open question. In as much as kidneys execute the tasks of extracellular fluid homeostasis, they must be told how much Mg to conserve or to excrete. Commands to kidneys are usually given by hormones that complete the feedback loop between renal activity and steady state composition of the extracellular fluid. Several hormones are known to regulate plasma Na, K, Ca, P, and Mg. In the case of Mg, as many as 6 hormones are known to affect renal Mg transport: parathyroid hormone, calcitonin, glucagon, arginine vasopressin, insulin, isoproterenol (38, 39, 40). But, to this date, a primary hormone of Mg homeostasis has not been identified in any animal. If there is such a hormone, August Krogh would have us look for one in euyhaline fish that can switch from renal Mg reabsorption in fresh water to Mg secretion in seawater: “For a large number of problems there will be some animal of choice or a few such animals in which it can be most conveniently studied” (41).

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