The Adaptations of Fish to Extremely Alkaline Environments

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ABSTRACT. The Lake Magadi Tilapia (MT; Oreochromis alcalicus grahami), the Lahontan cutthroat trout (LCT; Oncorhynchus clarki henshawii), and the tarek (Ct; Chalcalburnus tarichi) have evolved unique strategies that allow them to overcome problems associated with ammonia excretion (JAmm) and acid-base regulation in their alkaline environments. In Lake Magadi, Kenya (pH 10), the MT circumvents problems associated with JAmm by excreting virtually all (>90%) of its waste-nitrogen as urea. Base excretion appears to be facilitated by modified seawater-type gill chloride cells, through apical Cl-/HCO₃⁻ exchangers and an outwardly directed OH⁻/HCO₃⁻/CO₂⁺ excretion system. The LCT avoids potentially toxic increases in internal ammonia by permanently lowering ammonia production rates following transfer into alkaline (pH 9.4) Pyramid Lake, Nevada, from its juvenile freshwater (pH 8.4) environment. Greater apical exposure of LCT gill chloride cells, presumably the freshwater variety, probably facilitates base excretion by elevating Cl⁻/HCO₃⁻ exchange capacity. In Lake Van, Turkey (pH 9.8) high ammonia tolerance enables C. tarichi to withstand the high internal ammonia concentrations that it apparently requires for the facilitation of JAmm. It also excretes unusually high amounts of urea. We conclude that adjustments to nitrogenous waste metabolism and excretion patterns, as well as modifications to gill functional morphology, are necessary adaptations that permit these animals to thrive in environments considered unsuitable for most fishes. COMP BIOCHEM PHYSIOL 113B, 665-673, 1996

KEY WORDS. Ammonia, urea, chloride cell, acid-base balance, Lake Magadi Tilapia, rainbow trout, cutthroat trout, ion balance, high pH

INTRODUCTION

Alkaline-saline lakes are permanently alkaline and have relatively stable water pHs that usually lie between pH 9.0 and pH 10.0. These lakes are usually found in the planet's more arid regions and their extreme alkalinity is due to the presence of high dissolved concentrations of HCO₃⁻ and CO₂⁺ salts (Table 1; 44). Such lakes are also characterized by very high salinities and in the more extreme environments salinity may approach 50-60% seawater (Table 1; 8,13,32). Despite these factors, many alkaline-saline lakes still support healthy fish populations. This is probably best illustrated in Lake Magadi, Kenya, which has a pH of 10 but still supports a thriving population of alkaline resistant tilapia (Oreochromis alcalicus grahami; 40,41). In addition, several species of fish are found in Pyramid Lake, Nevada, which has a pH of 9.4 (see 6,13 for reviews), including the prized gamefish, the Lahontan Cutthroat trout (Oncorhynchus clarki henshawii), the endangered Cui ui (Chasmistes cujus), tui chub (Gila bicolor) and Tahoe sucker (Catostomus tahoensis). However, attempts to plant other salmonids, such as the rainbow trout (Oncorhynchus mykiss), kokanee (Oncorhynchus nerka) and brown trout (Salmo trutta), have been unsuccessful due to the inability of these fish to adapt to Pyramid Lakes moderately high salinity (10% sea water) and pH (6). Finally, Lake Van, Turkey, supports a population of tarek (Chalcalburnus tarichi), a Cyprinid that is the only inhabitant of that lake's highly saline (60% seawater) and alkaline (pH 9.8) environment (7,8).

In the foregoing discussion we outline the challenges that are faced by fish in permanently alkaline environments by initially examining the physiological responses of the relatively alkaline intolerant rainbow trout, to acute high pH exposure (pH 9.5 to pH 10.0). We will then describe how the Lahontan Cutthroat trout (Oncorhynchus clarki henshawii), the Lake Magadi tilapia (MT) and C. tarichi, circumvent problems that are associated with nitrogenous waste excretion and acid-base balance in alkaline water. Although these alkaline tolerant animals face similar challenges, it will be apparent that each has evolved
### Table 1. Typical Chemical Composition of a Freshwater Lake (Lake Ontario), and of Alkaline-Saline Lakes including Pyramid Lake, Nevada, Lake Magadi, Kenya, and Lake Van, Turkey.

<table>
<thead>
<tr>
<th>Lake</th>
<th>pH</th>
<th>[HCO₃⁻] (mM)</th>
<th>[CO₂⁺] (mM)</th>
<th>Titr. Alkal. (mM)</th>
<th>[Na⁺] (mM)</th>
<th>[Cl⁻] (mM)</th>
<th>Osmolality (mOsm · kg⁻¹)</th>
<th>Total salinity (g · l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ontario</td>
<td>8.1</td>
<td>2.0</td>
<td>&lt; 0.1</td>
<td>2.1</td>
<td>0.6</td>
<td>0.8</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Pyramid</td>
<td>9.4</td>
<td>13.8</td>
<td>5.0</td>
<td>23.1</td>
<td>38</td>
<td>60</td>
<td>4.4</td>
<td>4.4</td>
</tr>
<tr>
<td>Magadi</td>
<td>10.0</td>
<td>40</td>
<td>133</td>
<td>450</td>
<td>342</td>
<td>108</td>
<td>525</td>
<td>525</td>
</tr>
<tr>
<td>Van</td>
<td>9.8</td>
<td>—</td>
<td>—</td>
<td>133</td>
<td>337</td>
<td>154</td>
<td>551</td>
<td>551</td>
</tr>
</tbody>
</table>

Data taken from †Wilkie et al. (50), ‡Wood et al. (56), ‡Laurent et al. (27) and ‡Danual and Selcuk (8). †The osmolality of sea water is assumed to approximate 1000 mOsm and its salinity is typically 34–37 g · l⁻¹ (19).

The sharp increases in blood ammonia concentration at high pH (Fig. 2A, 46,47,65), in combination with increases in blood pH (see below), leads to a higher blood P_NH₃ which helps the rainbow trout to reestablish favourable blood-gill water ΔP_NH₃. As a result, J_Amm generally returns to preexposure rates after about 1–2 days at high pH (Fig. 2A; 46, 47, 48, 65). In more extreme instances, where internal NH₃ and NH₄⁺ concentrations rise too rapidly and/or approach toxic levels, elevated internal ammonia can ultimately contribute to high pH-induced mortality (49, 65). However, large reductions in plasma Na⁺ and Cl⁻ concentration might also contribute to high pH-induced mortality under these circumstances (e.g., 18, 49, 65).

### Nitrogenous Waste Excretion and Metabolism at High pH

#### The Situation in the Alkaline "Intolerant" Rainbow Trout

At circumneutral pH (pH 6–8), about 90% of the total nitrogenous waste (J_waste-N) produced by fish is excreted across the gill and ammonia excretion usually accounts for about 85% of this total (see 54 for review). Urea excretion generally makes up the remaining 10–20% of total J_waste-N. Although many researchers have argued that ammonia excretion (J_Amm) in fresh water takes place via branchial Na⁺ /NH₄⁺ exchange (25,30,37,58), recent evidence suggests that ammonia is primarily excreted in the unionized, NH₃ form (2,45,2). At present, it appears that J_Amm is dependant upon the presence of suitable NH₃ partial pressure gradients (ΔP_NH₃) between the blood and the unstirred boundary layers of the gill (also referred to as the gill water; 39). In this model (Fig. 1A), J_Amm is facilitated by the hydration of CO₂ in the gills unstirred boundary layers, which results in the production of protons that trap NH₃ as NH₄⁺, as it passively diffuses across the branchial epithelium (61). This effectively creates a “sink” that continuously favours NH₃ diffusion under circumneutral pH (about pH 6–8 in the bulk water) conditions (Fig. 1 A).

When freshwater teleosts are exposed to alkaline water there is an immediate reduction in ammonia excretion rate and a corresponding increase in plasma ammonia concentration (Figs. 2A, 3; 4,46,47,48,58,59,65). To understand how this occurs one only has to look at the NH₃ ↔ NH₄⁺ equilibrium, which is described by the following equation:

\[
\text{NH}_3 + H^+ \rightleftharpoons \text{NH}_4^+ \tag{1}
\]

Since, the pK of this relationship is approximately 9.5 (at 15°C), any increase in bulk water pH results in a corresponding elevation of gill water pH (or decreased H⁺ concentration), which shifts the NH₃ ↔ NH₄⁺ equilibrium towards NH₄⁺ formation. The resultant increase in gill water pH reduces ΔP_NH₃ and is reflected by lower ammonia excretion rates (Fig. 1B). Alkaline water may act directly upon branchial Na⁺/NH₄⁺ transporters, but in view of the dominant role that NH₃ diffusion likely plays in facilitating J_Amm in freshwater, this latter effect is probably minimal (45,47). High pH does inhibit branchial Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchange processes in rainbow trout (47), and can result in considerable drops in plasma Na⁺ and Cl⁻ concentration (18,46,65).

The role of urea excretion

Fish, such as the Lake Magadi Tilapia (MT), the Lahontan cutthroat trout (LCT) and C. tarichii have all evolved strategies that allow them to circumvent problems that are associated with ammonia excretion in their native alkaline waters. Perhaps, the most dramatic adaptation is exhibited by the MT, which excretes virtually all of its nitrogenous waste as urea, instead of ammonia (Figs. 2A,2B; 40,55,57). In fact, urea production in this unusual fish is due to the presence of a fully active complement of the ornithine urea cycle (OUC) enzymes. Although, most teleosts have a full complement of genes for the OUC, the cycles key enzymes are usually not fully active or, in some cases, are completely absent (21,35,63). Accordingly, most urea production in teleosts results from the degradation of purines via the uricolytic pathway or it occurs through the catabolism of dietary arginine (36,54).

The J_Urea patterns of the LCT are not altered following transfer into alkaline Pyramid Lake (Fig. 7B; 50). Although, the percentage contribution of J_Urea to total J_waste-N is higher in lakewater acclimated LCT, about 25–30%, and slightly higher than percentages reported for "typical" freshwater teleosts (34,62), activities of key OUC enzymes, such as carbamoyl phosphate synthetase III (CPS III), are too low to suggest the presence of a functional OUC (49,50). In fact, significant uricolytic enzyme activities suggest urea production occurs via the typical teleosts pathway of uricolyis (49,50). C. tarichii, on the other hand, excretes about 37% of its nitrogenous waste as urea, but it too has no functional OUC (7). Thus, urea production in this fish is probably via uricoly-
Fish in High pH Waters.

It is interesting that the basal levels of $J_{\text{Urea}}$ are far greater in C. tarichi, than in any of the alkaline tolerant fishes native to Pyramid Lake (Fig. 2B). McGeer et al. (34) extensively surveyed the $J_{\text{Urea}}$ patterns of a number of the fish native to Pyramid Lake, including the LCT, Cui ui, tui chub and Tahoe sucker and found that $J_{\text{Urea}}$'s were comparable to values measured in other fishes and generally accounted for about 20–30% of $J_{\text{waste-N}}$ (Fig. 2B). Perhaps the higher rates of basal $J_{\text{Urea}}$ in C. tarichi are related to the much higher pH of Lake Van (pH 9.8), and the tarek's inherently high internal ammonia levels (see below)?

Interestingly, transiently elevated rates of $J_{\text{Urea}}$ appear to be a common ammonia detoxification response of salmonids to acute elevations in environmental pH (Fig. 2B; 46, 49). Wilkie et al. (49) demonstrated that LCT, acclimated to pH 9.4, but challenged at pH 10, increased their reliance on urea excretion, presumably through enhanced rates of uricolyis. Similarly, rainbow trout, which also lack a functional OUC, doubled their urea excretion rates at pH 9.5 (Fig. 2B; 46). It should be emphasized that these elevations in $J_{\text{Urea}}$ were temporary and did not persist beyond 2 or 3 days of high pH exposure, at which time $J_{\text{Amm}}$ had been fully reestablished (Fig. 2B; 46). Recently, Wright et al. (64) reported that embryonic rainbow trout larvae increased urea excretion by six times, following acute (4 h) exposure to pH 9.5. In view of the recent discovery by Wright et al. (64) that these larval trout probably possessed a functional OUC, it would be interesting to establish if continued exposure to high pH results in the continued expression of the OUC genes and persistently elevated $J_{\text{Urea}}$ as the trout mature.

The Benefits of High Ammonia Tolerance

Clearly, another key adaptation that would benefit fish living at high pH would be unusually high tolerance to ammonia. As one might predict, based upon its ureotelic nature, the
MT has a very high ammonia tolerance. Indeed, the LC50 for NH₃ in the MT is about six-fold higher than values reported for most teleost fish (42). Much of this high ammonia tolerance is likely related to high hepatic glutamine synthetase activity in the fish's liver (40,42,55). A typical ammonia detoxification response, seen in mammals and fish, is the glutamine synthetase catalyzed formation of glutamine from NH₄⁺ and glutamate (28,36). However, high glutamine synthesis capacity is also critical for the MT because it provides the key OUC enzyme, carbamoyl phosphate synthetase III, with the glutamine it requires for the production of urea (36).

C. tarichi, also appears to be very ammonia tolerant; this is reflected by its ability to store very high levels of ammonia in its blood (1 mmol·l⁻¹) and muscle tissue (12 mmol·l⁻¹; 7). Typical teleostean values for muscle and plasma ammonia, generally range from 0.6 to 2.0 mmol·kg⁻¹ wet weight and 50 to 200 μmol·l⁻¹, respectively (Fig. 3; 43,60). However, it should be noted that the tarik were sampled by caudal puncture (also known as venipuncture), as opposed to dorsal aortic cannulation, which may result in higher plasma ammonia levels (54). The high rates of J_u, which imply high urea production rates, as well as high brain glutamine synthetase activity, might also provide C. tarichi with unusually high ammonia tolerance (7). Unfortunately, precise estimates of C. tarichi's ammonia tolerance are unavailable, but Danulat and Kempe (7) did perform experiments which suggested C. tarichi was less ammonia tolerant than the MT.

It seems unlikely that the extremely high blood ammonia levels observed in C. tarichi were an artifact associated with feeding because Danulat and Kempe (7) failed to observe the diurnal upward JAmm pulses that normally follow feeding in fish (3,12). It is likely that the 48 h acclimation period, during which time the animals were starved, was sufficient to mitigate any influences that feeding would have had on JAmm in C. tarichi (7). In the case of the MT, it is possible that prior feeding influenced blood ammonia levels because of the necessity to experiment upon these fish within 24 h of capture, due to their vulnerability to mortality in captivity (55). Feeding did not likely influence plasma ammonia levels in the rainbow trout or LCT because the animals were generally starved at least one week prior to experimentation (e.g., 46,50).

C. tarichi's high blood ammonia levels might account for its very high ammonia excretion rates, approximately 1000
Alterations in Nitrogenous Waste Excretion and Metabolism by the Lahontan Cutthroat Trout

Instead of relying upon increased urea excretion (Fig. 2B) or tolerance to extremely high blood ammonia levels (Fig. 3), the LCT permanently lowers its rates of nitrogenous waste production immediately following transfer into Pyramid Lake, from its juvenile freshwater (pH 8.4) habitat (50). This decrease is reflected in minimal, temporary increases in plasma ammonia concentration and chronically lowered $J_{\text{Amn}}$ (Fig. 2A). As a result, potentially toxic build-ups of internal ammonia are avoided following transfer into Pyramid Lake. At present, it is not clear how the Pyramid Lake LCT lower their ammonia production rates but it could be via depressions in metabolic rate and/or increased reliance upon other fuels such as carbohydrates or fats (50). Another intriguing possibility is that LCT may not be depressing N-waste metabolism at all, it may actually be excreting an alternate N-waste product. It does not appear that urea (50) or uric acid (62) are the alternate waste product(s), but the possibility that LCT excrete significant amounts of glutamine, trimethylamine oxide, creatine or some other N-waste product (see (11) and (36) for reviews), following transfer into Pyramid Lake water deserves further study.

ACID-BASE BALANCE AT HIGH pH

Acid-Base Balance in the Rainbow Trout at High pH

At circumneutral pH many freshwater lakes and streams are slightly supersaturated with unhydrated, or gaseous, CO$_2$ (44). However, when water pH rises, usually due to increased rates of photosynthesis, there is a rightward shift of the CO$_2$ equilibrium curve favouring HCO$_3^-$ and CO$_3^{2-}$ formation (44). The corresponding decreases in water P$_{CO2}$ effectively creates a "CO$_2$ vacuum" (22) which promotes CO$_2$ losses across the gill (Fig. 1B). The resultant respiratory alkalosis can result in large increases in blood pH which, if left unchecked, can increase by as much as 0.5–0.6 units (29,46,65). Since high pH waters are rich in OH$^-$ and HCO$_3^-$, and relatively deficient in H$^+$, electrochemical gradients are often created which favour base equivalent uptake and/or acidic equivalent loss across the gill epithelium (Fig. 1B; 49). Indeed, a number, of studies have reported the development of metabolic alkaloses in trout that are acutely exposed to alkaline pH (18,48).

The alkalosis, experienced by rainbow trout at pH 9.5, is partially offset by the development of a simultaneous metabolic acidosis, which is associated with acute increases in blood lactate. Elevated concentrations of white muscle lactate suggest that this blood metabolic acid load originates in the white muscle (45), where enhanced rates of glycolysis could lead to increased lactate and metabolic proton production (20). Indeed, estimates of the loss of metabolic acid from the white muscle are more than sufficient to account for the development of the plasma metabolic acidosis (45). However, this is a metabolically costly response, and it is likely that longer-term regulation of acid-base balance in alkaline water is dependant upon the independent modulation of net Na$^+$ and Cl$^-$ movements across the gill epithelium.

It is well established that manipulation of Na$^+$ and Cl$^-$ movements across the gill epithelium allows fish to effectively regulate, and correct disturbances to, acid-base balance. The basic premise of this theory is that net inward movements of Na$^+$ and Cl$^-$ must be accompanied by net outward movements of acidic (H$^+$) and basic equivalents (HCO$_3^-$ or OH$^-$), respectively (33,53). The means by which this is achieved is through the independent regulation of Cl$^-$/HCO$_3^-$ and Na$^+$/H$^+$ exchange processes, and/or by differential alteration of the rates of diffusive Na$^+$ and Cl$^-$ efflux (16). Recently, Goss and colleagues demonstrated that correction of metabolic alkalosis in freshwater fish occurred through increases in the exposed apical fractional surface area (FSA) of "freshwater-type" branchial chloride cells (CC), the purported site of Cl$^-$/HCO$_3^-$ exchange (Fig. 4; 16,17). Increased CC FSA appears to be achieved through increases in individual CC’s density, as well as through increased apical exposure of individual CC’s.

Wilkie and Wood (47) tested the hypothesis that similar changes in gill morphometry would occur on rainbow trout gills in order to counter high pH-induced increases in blood pH. In fact, they observed a four-fold increase in the exposed CC FSA of rainbow trout gills, following 3 days at pH 9.5. They suggested that increased CC FSA at high pH might have accounted for the stabilization of blood pH that was seen in earlier studies (e.g. 46).

Branchial Adaptations of the Lahontan Cutthroat Trout

The Lahontan cutthroat trout appears to rely heavily upon high branchial CC FSA’s to counter the large inwardly directed OH$^-$, HCO$_3^-$, and CO$_3^{2-}$ electrochemical gradients it faces in Pyramid Lake’s alkaline environment (Table 2). Galat et al. (14) first pointed out that these fish had chronic CC hyperplasia and Wilkie et al. (50) later suggested, in view of the CC’s purported role in Cl$^-$/HCO$_3^-$ transporter mediated base excretion (see above), that high CC FSA represents an adaptive response to Pyramid Lake’s alkaline water. Indeed, the absence of blood lactacidosis and the rapid corre-
tion of acute metabolic alkalosis, following transfer into Pyramid Lake water, suggests there is a rapid mobilization of branchial Cl-/HCO₃⁻ exchangers which likely facilitate correction and long-term maintenance of acid-base balance in the LCT. Furthermore, CC FSA's are about 10–20 fold greater in LCT acclimated to Pyramid Lake water than in "naive" LCT which are held in pH 8.4 freshwater. Due the CC's well established role in maintaining electrolyte balance (38), it is likely that these higher CC FSA's also aid in the maintenance of internal ion balance. However, the moderate salinity of Pyramid Lake ([NaCl] = 60 mmol*l⁻¹) likely mitigates potential high-pH induced disturbances to electrolyte balance considerably.

**Branchial Adaptations of the Magadi Tilapia**

Relative to the LCT, the MT must not only contend with much higher inwardly directed electrochemical gradients, favouring OH⁻, HCO₃⁻, and CO₃²⁻ uptake (Table 2), they must also maintain internal electrolyte balance in the face of outwardly and inwardly directed gradients, favouring Cl⁻ loss and Na⁺ influx, respectively. Some of the original studies on the MT, suggest the MT gill epithelium is relatively impermeable to Cl⁻ (9,31). This branchial anionic impermeability, or gill epithelial "tightness," would not only facilitate Cl⁻ retention, it might also simultaneously restrict the entry of basic equivalents (10). Regardless, blood HCO₃⁻ concentrations in the MT are still about 1.5–2.0 times higher than values reported for other teleosts (55,56) suggesting that appreciable amounts of HCO₃⁻ are still taken up by these fish. There may be some basic equivalent passage across the gill epithelium but it is also likely that the base is ingested when the fish feed or drink (56). It is necessary that the MT drinks water so that it can maintain internal fluid balance in Lake Magadi's saline waters (32). Thus, despite the relative impermeability of its gill to basic equivalents, the MT still requires a strategy which allows it to counter continuous base loading.

Laurent et al. (27) recently performed a detailed morphometrical analysis of the MT gill, and they suggested that the MT uses modified "seawater-type" chloride cells, to cope with Lake Magadi's harsh environment. These seawater-type CC's are morphologically and functionally distinct from the freshwater-type CC's, likely encountered in the rainbow trout and LCT, and are characterized by the presence of accessory cells, which are not normally found in fish living in hypotonic environments (Fig. 5A; 26).

The mechanisms by which branchial sea-water chloride cells facilitate NaCl excretion by marine fishes has been thoroughly studied and extensively reviewed over the last several years and will only be briefly reviewed below. Readers should refer to topical reviews on this subject by Kamacky (23) or Wood and Marshall (57) for more information. In most marine fishes sea-water CC's facilitate Na⁺ and Cl⁻ excretion by first transporting the ions from the plasma into the cells interior via a basolateral Na⁺/(2 Cl⁻)/K⁺ cotransporter. The Na⁺/(2 Cl⁻)/K⁺ cotransporter mediated build-up of Cl⁻ within the cell results in the creation of favourable electrochemical gradients across the CC's apical membrane which facilitates passive Cl⁻ diffusion to the water via anion selective channels that are localized on the CC's apical epithelium (Fig. 5A). This makes the environment, immediately adjacent to the gill epithelium, more negative and helps to establish electrical gradients that favour Na⁺ diffusion. Na⁺ excretion is also facilitated by the active pumping of Na⁺, via basolateral Na⁺/K⁺ ATPase's, into the paracellular channels across the gills of Lahontan Cutthroat Trout, in Fresh Water or after acclimation to Pyramid Lake Water, and the Tilapia of Lake Magadi.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Fresh water pH 8.4</th>
<th>Pyramid Lake pH 9.4</th>
<th>Magadi Tilapia pH 10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH⁻ (H⁺)</td>
<td>+13.6</td>
<td>+67.4</td>
<td>+112.0</td>
</tr>
<tr>
<td>CO₃²⁻</td>
<td>-39.4</td>
<td>+35.5</td>
<td>+66.0</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>-18.6</td>
<td>+13.6</td>
<td>+26.0</td>
</tr>
</tbody>
</table>

*Fₒₑₒ = TEP—Trans epithelial potential (in mV), where TEP is measured across Lahontan cutthroat trout gills.

**TABLE 2. Electrochemical Gradients for Acid-base Relevant Ions Across the Gills of Lahontan Cutthroat Trout, in Fresh Water or after acclimation to Pyramid Lake Water, and the Tilapia of Lake Magadi.**

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<table>
<thead>
<tr>
<th>Electrochemical Gradient (Fₒₑₒ)</th>
<th>Lahontan cutthroat trout</th>
<th>Magadi Tilapia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh water pH 8.4</td>
<td>(pH 8.4)</td>
<td>(pH 9.4)</td>
</tr>
<tr>
<td>OH⁻ (H⁺)</td>
<td>+13.6</td>
<td>+67.4</td>
</tr>
<tr>
<td>CO₃²⁻</td>
<td>-39.4</td>
<td>+35.5</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>-18.6</td>
<td>+13.6</td>
</tr>
</tbody>
</table>
that lie between the CC's and their accessory cells. This resultant build-up of paracellular Na⁺, contributes to the creation of favourable electrochemical gradients which facilitate passive Na⁺ diffusion to the water, through leaky tight junctions (Fig. 5A).

Laurent and colleagues suggest that base excretion across the MT's sea-water CC’s is likely a combination of apical Cl⁻/HCO₃⁻ exchange and an outwardly directed OH⁻/HCO₃⁻/CO₃²⁻ transport system. Thus, the Cl⁻/HCO₃⁻ antiporter, which is typical of fresh-water type CC’s (compare Fig. 4 and Fig. 5A), not only facilitates base extrusion, but it also allows the animal to maintain internal Cl⁻ balance. The OH⁻/HCO₃⁻/CO₃²⁻ transport system is likely a multi-step process that first entails OH⁻, HCO₃⁻ and CO₃²⁻ entry into the cell, from the plasma, via substitution of the basic equivalents onto the anion transport sites of the basolateral Na⁺/(2 Cl⁻)/K⁺ cotransporter (Fig. 5B). The basic equivalents then exit the CC via its apical anion channels (Fig. 5B). Since, plasma Cl⁻ concentrations are about ten times higher than the combined total of basic equivalents in MT blood, the former should out-compete substrates, such as OH⁻, HCO₃⁻ and CO₃²⁻, for cotransporter binding sites. Perhaps the cotransporter has a higher affinity for basic anions over Cl⁻? Certainly more work should be done to elucidate how these seawater-type CC's facilitate acid-base regulation and electrolyte balance in the MT’s harsh environment.

FIG. 5. (A) A simplified model depicting how typical seawater-type chloride cells (CC) facilitate Na⁺ and Cl⁻ excretion into saline environments. (B) A model illustrating how base equivalent (CO₃²⁻, HCO₃⁻, OH⁻) substitution for Cl⁻ on the basolateral Na⁺/(2 Cl⁻)/K⁺ cotransporter of seawater-type chloride cells of *Oreochromis alcalicus grahami* facilitates base excretion in alkaline (pH 10) Lake Magadi, Kenya. Asterisks denote differences from the traditional sea-water type chloride cells shown in (A). AC represents accessory cells. See text for further details. Adapted from a model presented by Laurent et al. (27).

Does Urea Production Facilitate Acid-Base Regulation in Alkaline Environments?

Recently Atkinson (1) has argued that the presence of an ornithine urea cycle in the Magadi Tilapia supports his contention that the UOC plays a major role in acid-base regulation (also see Knepper, (24) for opposing viewpoint). Wood et al. (56) recently attempted to establish if HCO₃⁻ consumption via the UOC really did influence acid-base status in the MT by manipulating the MT’s internal HCO₃⁻ concentrations, through acidification of the water or by NaHCO₃ injection, and then monitoring urea excretion rates afterwards. Ultimately, these experiments revealed that the UOC pathway in the MT is normally saturated with HCO₃⁻ and that it has a relatively high Kₘ (low affinity) for HCO₃⁻. Based upon these observations, it appears that the UOC pathway is relatively insensitive to changes in HCO₃⁻ and therefore, it is unlikely that the UOC plays any acid-base regulatory role in the MT. Indeed, it is now evident that the OUC’s primary role is ammonia detoxification in the MT, not acid-base regulation, since urea production rates are highly sensitive to changes in internal NH₄⁺ concentration (42, 55).

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