

## A10—EPITHELIAL ANION TRANSPORT—AN INTEGRATIVE PERSPECTIVE

Organised by N. Bury and M. Grosell

This session has been supported by The Society of Experimental Biology, The Osmoregulation Group, BBA

### A10.1—Control of epithelial anion transport in euryhaline teleost chloride cells

W.S. Marshall, Dept. Biol., St. Francis Xavier U., Antigonish N.S.

Euryhaline teleosts adapt rapidly to changing salinity using combined osmotic, neural and hormonal signals. This review develops a model for this rapid regulation of epithelial ion transport. Rapid stimulation of Cl secretion by teleost chloride cells occurs by beta adrenergic agonists, AVT, glucagon, VIP, urotensin I and by hypertonicity. Agonist stimulation is mediated by cAMP and protein kinase A (PKA), apparently through activation of apical membrane anion channels (Cystic Fibrosis Transmembrane Conductance Regulator, CFTR), an effect mimicked by inhibitors of serine/threonine phosphatases (EK Hoffmann et al. B.B.A 1566:129–139, 2002). In contrast to agonist action, hypertonic stress activates Na, K, 2Cl cotransport (NKCC1) at the basolateral membrane, mediated by protein kinase C (PKC). Rapid inhibition of Cl secretion occurs by alpha adrenergic agonists, muscarinic cholinergic agonists, prostaglandin E2, urotensin II, calcium ionophores, and by hypotonic stress. Norepinephrine from sympathetic nerves acts via alpha-adrenergic receptors, inositol tris phosphate (IP3) and intracellular Ca. Hypotonicity, however, inhibits Cl secretion apparently via inhibition of protein tyrosine kinase, as genistein (but not its inactive analog daidzein) mimics the hypotonic response. The shut down of Cl secretion is followed by active closure of the apical surface of the chloride cells, likely to minimize passive loss of ions in animals exposed to fresh water. In this way, Cl secretion can be augmented in hypersaline conditions or eliminated in early stages of adaptation to fresh water. Supported by NSERC Canada.

### A10.2—Na<sup>+</sup> versus Cl<sup>-</sup> exchange in killifish subjected to rapid salinity transfers

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*Fundulus heteroclitis* lives in estuaries and is powerfully euryhaline. Its opercular epithelium is rich in 'chloride cells' and has provided a fundamental in vitro model responsible for much of our current understanding of the mechanisms of Na<sup>+</sup> and Cl<sup>-</sup> transport in marine fish, and how these change during rapid salinity transfers. Most workers implicitly assume that opercular transport function duplicates gill transport function, but the evi-

dence for this is weak. Most of the few whole animal flux measurements available were made over 30 years ago. Our recent studies have revealed marked discrepancies— for example the intact killifish exhibits unusual freshwater transport physiology with vigorous Na<sup>+</sup> uptake but negligible Cl<sup>-</sup> uptake (1), but the isolated opercular epithelium takes up only a slight amount of Cl<sup>-</sup> and no Na<sup>+</sup> from apical freshwater (2). In the present study, we acclimated killifish to 10% seawater and then monitored the unidirectional influx and efflux rates of Na<sup>+</sup> and Cl<sup>-</sup> (via radiotopes) at 12 h, 72 h, and 168 h after transfer to freshwater or 100% seawater. The measurements present methodological challenges because rate constants, pool sizes, and exchangeable fractions increase upon transfer to seawater and decrease upon transfer to freshwater, though with different patterns and time courses (supported by NSERC).

1. Patrick, M.L. and Wood, C.M. (1999). Comp. Biochem. Physiol. 122A: 445–456.
2. Burgess, D.W. et al. (1998) Comp. Biochem. Physiol. A. 121: 155–164.

### A10.3—Co-expressed apical chloride channels of mitochondria-rich cells – regulation and function

E.H. Larsen, J. Amstrup, N.J. Willumsen, August Krogh Institute, University of Copenhagen

Mitochondria-rich cells (MRC) of toad (*Bufo bufo*) skin epithelium are specialized for uptake of chloride from the external environment. Judged from single channel conductance, the apical membrane harbors several chloride channel types. A large depolarization activated channel (200–300 pS) with substates was identified by noise analysis of whole cell currents and single channel patch clamping. Macroscopic flux studies have indicated the following ranking of permeabilities,  $P_{Br}:P_{Cl}:P_I = 1.3:1:0.8$ , which is different from the ranking of conductances,  $G_{Br}:G_{Cl}:G_I = 0.7:1:0.06$ . It is hypothesized that an external binding site with high Cl<sup>-</sup> affinity controls the opening of a channel with moderate halide ion selectivity. Upon activation by cellular hyperpolarization (apical membrane depolarization) these channels mediate the passive currents coupled to active inward flux of sodium. In contrast, a population of small conductance (7–10 pS) chloride channels seems coupled to basolateral beta-adrenergic receptors. The associated macroscopic conductance is activated by db-cAMP and forskolin with a selectivity,  $G_{Cl} > G_{Br} \gg G_I$ . Immunostaining with monoclonal antibodies against human

CFTR indicated selective expression of the *B. bufo* gene in MRC. With primers constructed from cloned *Xenopus* cDNA and reverse transcribed epidermal mRNA, full length *bbCFTR* was cloned. The derived protein exhibits 89% identity with *hCFTR* with well conserved NBD1, NBD2, and R-domains.

#### **A10.4—Existence and nature of the chloride pump**

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$\text{Cl}^-$  absorption by the *Aplysia californica* foregut is effected through an active  $\text{Cl}^-$  transport mechanism located in the basolateral membrane of the epithelial absorptive cells. These basolateral membranes contain both  $\text{Cl}^-$ -stimulated ATPase and ATP-dependent  $\text{Cl}^-$  transport activities which can be incorporated into liposomes via reconstitution. Utilizing the proteoliposomal preparation, it was demonstrated that the reaction sequence of the  $\text{Cl}^-$  pump required  $\text{Mg}^{2+}$  for phosphorylation and  $\text{Cl}^-$  for dephosphorylation. By thermodynamic determination, it was ascertained that the stoichiometry was one  $\text{Cl}^-$  transported per one ATP hydrolyzed per reaction cycle. It was further shown that ATP, and its subsequent hydrolysis,  $\text{Mg}^{2+}$ ,  $\text{Cl}^-$ , and a pH optimum of 7.8 were required to generate maximal intraliposomal  $\text{Cl}^-$  accumulation, electrical negativity and ATPase activity. Additionally, an inwardly directed valinomycin-induced  $\text{K}^+$  diffusion potential, making the liposome interior electrically positive, enhanced both ATP-driven  $\text{Cl}^-$  accumulation and electrical potential. Both aspects of  $\text{Cl}^-$  pump transport kinetics and its associated catalytic component kinetics were the first obtained utilizing a reconstituted transporter protein, mRNA for both  $\text{Cl}^-$  pump and P-type ATPase activities were found in the *Aplysia* gut epithelial cells. These results strongly support the hypothesis that  $\text{Cl}^-$ -ATPase actively transports  $\text{Cl}^-$  by an electrogenic process. (This study was supported by the Eppley Foundation for Research, Inc.).

#### **A10.5—Proton pump driven cutaneous chloride uptake in anuran amphibian**

N. J. Willumsen, J. Amstrup, L. J. Jensen, E. H. Larsen, August Krogh Institute, University of Copenhagen

Putative proton pump activity in skin epithelium of toad (*Bufo bufo*) was studied with stationary pH-sensitive double barreled microelectrodes, which were positioned in vertical 50  $\mu\text{m}$  steps above the surface. When slowly superfused with  $\text{Cl}^-$ -free Ringers solution, pH in the unstirred layer adjacent to the epithelial surface was  $\sim 1$  pH-unit below that of bulk solution buffered at 7.4 (0.1

mM TRIS). In a one-dimensional diffusion regime including a proton buffer this corresponds to a proton efflux of  $8.5 \pm 2.4 \text{ pmol}\cdot\text{s}^{-1}\cdot\text{cm}^{-2}$  ( $N=17$ ). Substitution of gluconate by chloride, bromide and iodide, respectively, resulted in significant reductions of the external pH-gradient indicating secretion of base equivalents in the presence of halides. Whole skin preparations studied with pH-stat technique exhibited an active proton secretion of  $16.7 \pm 1.7 \text{ pmol}\cdot\text{s}^{-1}\cdot\text{cm}^{-2}$  ( $N=10$ ). In the presence of 2.2 mM external  $\text{Cl}^-$  this preparation generated a  $\text{Cl}^-$ -influx of  $19.5 \pm 3.5 \text{ pmol}\cdot\text{s}^{-1}\cdot\text{cm}^{-2}$  ( $N=11$ ) that was DIDS-sensitive. A rabbit polyclonal antibody, raised against a synthetic peptide corresponding to a sequence from the catalytic 70 kDa A-subunit of the bovine V-type  $\text{H}^+$ -ATPase complex (Wilson et al, J. Exp. Biol. 203: 2279, 2000), specifically stained mitochondria-rich cells. These findings indicate that active uptake of chloride is mediated by an apical  $\text{Cl}^-/\text{HCO}_3^-$ -exchanger driven by a parallel proton pump of mitochondria-rich cells.

#### **A10.6—Chloride channels involved in cell volume regulation**

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Maintenance of a constant cell volume is essential for normal cell function. Following cell swelling, due to exposure to a hypotonic environment, animal cells are able to restore their original volume by activation of two conductances:  $\text{K}^+$  and  $\text{Cl}^-$ . The loss of these ions, followed passively by water, is responsible for the homeostatic response called Regulatory Volume Decrease (RVD). Several candidates have been proposed and investigated for the chloride conductance activated by cell swelling ( $I_{\text{Cl,Swell}}$ ). In particular CIC-3, a member of the CIC family, has been recently suggested as the channel implicated in cell volume regulation. In order to test this hypothesis we have created cell lines permanently expressing CIC-3. The data demonstrate that CIC-3 is not responsible for  $I_{\text{Cl,Swell}}$  and does not play a role in RVD. Monitoring of cell volume and study of activity and cellular distribution of CIC-3 will be discussed.

#### **A10.7—Genetics and Acute Regulation of Anion Exchangers: Comparative Aspects**

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Transmembrane exchange of chloride for bicarbonate serves to control cell and luminal pH, volume, and  $[\text{Cl}^-]$ , as well as to drive secondary active transport of other anions. Anion exchangers are encoded by the

SLC4 and SLC26 gene families. The SLC4 family includes the Na-independent, electroneutral anion exchangers AE1, AE2, and AE3, as well as electrogenic and electroneutral Na-bicarbonate cotransporters, and Na-dependent anion exchangers. SLC4 homologs are expressed in plants, worms, and yeast. The SLC26 family includes anion exchangers of broad specificity for monovalent and divalent anions. AE1/SLC4A1 mutations encode variant blood group antigens, and cause spherocytic anemia and distal renal tubular acidosis. DTD/SLC26A2 mutations cause chondrodysplasias, DRA/SLC26A3 mutations cause congenital chloride diarrhea, and pendrin/SLC26A4 mutations cause congenital deafness with variably penetrant goiter. SLC26 homologs are expressed in eubacteria and archaea. Carbonic anhydrase II (CA2) binds directly to members of the SLC4 family. Bound, active CA2 is absolutely required for  $\text{Cl}^-/\text{HCO}_3^-$  exchange, though dispensable for  $\text{Cl}^-/\text{Cl}^-$  exchange. CA2 bound to one subunit within an AE1 homodimer can provide the permissive function for  $\text{Cl}^-/\text{HCO}_3^-$  exchange by the other subunit of the homodimer, but unbound cytoplasmic CA2 cannot serve this function. The role of carbonic anhydrases in control of SLC26 activity is less well understood. SLC4 and SLC26 anion exchangers differ in their responses to other acute regulatory stimuli, and in their regulation by CFTR activity. The current state of studies defining regions of AE2 required for sensitivity to these acute transport regulators will be presented.

### A10.8—Seawater calcium, bicarbonate secretion and water absorption in the marine teleost intestine

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We have recently presented evidence for a novel mechanism of epithelial water transport<sup>(1)</sup> in the intestine of marine teleost fishes. The mechanism centres around the net secretion of bicarbonate ions ( $\text{HCO}_3^-$ ) by the epithelium through  $\text{Cl}^-/\text{HCO}_3^-$  exchange. This helps drive net water absorption by at least two processes: 1) the secreted  $\text{HCO}_3^-$  is derived from non-osmotically active cellular  $\text{CO}_2$ , so  $\text{Cl}^-/\text{HCO}_3^-$  exchange results in the net absorption of anions into the cell, thus promoting osmotically obliged water to follow, 2) the alkaline (pH 8–9) and bicarbonate-rich intestinal lumen causes imbibed  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  to precipitate as insoluble carbonates thus removing these dissolved ions from the intestinal lumen and maximising the osmotic gradient for transepithelial water absorption. Thus carbonate precipitation is critical to net water transport. Furthermore, the primary stimulant of intestinal  $\text{HCO}_3^-$  secretion is specifi-

cally  $\text{Ca}^{2+}$  entering the intestine<sup>(1)</sup>, and we propose that calcium sensing receptors (CaR's) are involved in controlling the rate of  $\text{HCO}_3^-$  secretion and hence water absorption. Nearing et al. (2002)<sup>(2)</sup> have proposed that CaR's may be salinity sensors in fish (based on a molecular cloning approach) but they have not provided a mechanism to support this. In this presentation we will discuss the potential role of CaR's in osmoregulation, in particular in the regulation of intestinal bicarbonate secretion, water transport and plasma osmolality of teleost fish at different environmental salinities.

1) Wilson, RW, Wilson, JM, & Grosell, M (2002) *BBA-Biomembranes* 1566: 182–193

2) Nearing, J et al. (2002) *PNAS* 99(14): 9231–9236.

### A10.9—Sodium bicarbonate co-transporters in fish gills and kidney

S.F. Perry and M. Bayaa, Biology, University of Ottawa

Sodium bicarbonate co-transporter isoform 1 (NBC1) has been cloned from tissues of several fish species including Japanese dace (*Tribolodon hakonensis*; GenBank accession BAB83084), rainbow trout (*Oncorhynchus mykiss*; GenBank accession AAN52239), zebrafish (*Danio rerio*) and eel (*Anguilla rostrata*). In rainbow trout, NBC1 is expressed at high levels in several absorptive epithelia including gill, kidney and intestine. In the gill, NBC1 is probably localised on the basolateral membrane where it plays a role in intracellular pH (pHi) regulation (Wood and Part, 2000). Using Q-PCR, levels of gill NBC1 mRNA were determined to be increased and decreased during intravascular acid- and base-infusion, respectively. Thus, the activity of branchial NBC1 appears to be transcriptionally regulated to match the requirements of gill pHi regulation rather than to match trans-epithelial  $\text{HCO}_3^-$  efflux requirements. In the kidney, NBC1 probably plays a role in the tubular reabsorption of both  $\text{Na}^+$  and  $\text{HCO}_3^-$ . During periods of respiratory acidosis, levels of renal NBC1 mRNA increase presumably as a means to increase  $\text{HCO}_3^-$  reabsorption. This strategy, when coupled with increased urinary acidification associated with increased vacuolar  $\text{H}^+$ -ATPase activity, ensures that  $\text{HCO}_3^-$  levels accumulate in the body fluids to restore pH.

Wood, C.M. and Part, P. (2000) Intracellular pH regulation and buffer capacity in  $\text{CO}_2/\text{HCO}_3^-$ -buffered media in cultured epithelial cells from rainbow trout gills. *J Comp Physiol [B]*, **170**, 175–184.

Perry, S.F., Beyers, M.L. and Johnson, D.J. (2000) Cloning and molecular characterisation of the trout (*Oncorhynchus mykiss*) vacuolar  $\text{H}^+$ -ATPase B subunit. *J.Exp.Biol.*, **203**, 459–470.

### **A10.10—The molecular biology of basolateral organic anion transport in mammalian renal tubules**

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Basolateral transport of organic anions (OAs) into mammalian renal proximal tubule cells, the rate-limiting step in transepithelial secretion, is a tertiary active transport process. The final step in this process involves movement of OA into the cells against its electrochemical gradient in exchange for  $\alpha$ -ketoglutarate ( $\alpha$ KG) moving down its electrochemical gradient. Two homologous transport proteins (OAT1 and OAT3) that function as OA/ $\alpha$ KG exchangers at the basolateral membrane have been cloned and sequenced. Studies with knockout mice have demonstrated that both of these proteins are significant for the transepithelial secretion of OAs. However, although OAT1 and OAT3 share a common energetic transport mechanism, they differ distinctly in substrate specificity and axial distribution along the proximal tubules. For example, with regard to substrate specificity, OAT1 has a high affinity for the classical renal OA transport substrate, *p*-aminohippurate (PAH), whereas OAT3 either has a modest affinity (e.g., human, rat) or no affinity (e.g., rabbit) for PAH. In contrast, OAT3 has a high affinity for estrone sulfate transport, whereas OAT1 does not transport estrone sulfate. These differences in substrate specificity correlate closely with the apparent axial distribution of the transporters relative to primary transepithelial transport: OAT1 (and PAH secretion) in the S2 segment of the proximal tubule and OAT3 in the S1, S2, and S3 segments. Basolateral PAH transport (presumably OAT1 activity) appears to be down-regulated by activation of protein kinase C and up-regulated via mitogen-activated protein kinase (MAPK) through cyclic AMP and protein kinase A activation.

### **A10.11—Inorganic and organic anion transport by insect epithelia**

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Hormonally stimulated insect Malpighian (renal) tubules secrete near iso-osmotic fluids at fantastic rates, sufficient to exchange each cell's volume every 10–15 seconds. Whereas the predominant cation (Na or K) varies in different species or conditions, chloride is the major anion. Transepithelial ion transport is driven primarily by an apical vacuolar proton pump and to a lesser extent by a basolateral Na/K ATPase. The large size of the cells facilitates ion-selective microelectrode measurements of intracellular ion activities, and these meas-

urements can be used to determine the thermodynamic feasibility of particular transport schemes. Current models propose roles for chloride channels and cation-coupled chloride cotransporters, as well as a paracellular shunt pathway. Ion transport is controlled by multiple diuretic hormones within well-studied species, and different chloride transport pathways appear to be modulated by distinct hormonal and second messenger systems. The Malpighian tubules and hindgut are also important sites for excretion of metabolic wastes and toxins. A non-invasive electrophysiological technique reveals that specific segments of the tubules as well as the ileum and rectum transport organic anions. There are separate transporters for carboxylates and sulphonates. Transport is sodium-dependent, but does not involve organic acid/ $\alpha$  keto acid exchange. Intracellular transport of organic anions involves both diffusion and vesicles. Moreover, a role for multidrug resistant proteins has also been proposed. There is a critical need for detailed molecular and physiological studies of insect organic anion transporters in order to develop control strategies which circumvent the protective function of these transporters in pest species.

### **A10.12—Is inorganic mercury accumulation by fish gills through active transport or passive diffusion?**

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Accumulation of waterborne metals by gills of freshwater fish is generally thought to occur when cationic metals (e.g.,  $\text{Cd}^{2+}$ ) are taken up inadvertently by active transport processes designed for essential cations (e.g.,  $\text{Ca}^{2+}$ ). However, accumulation of waterborne Hg has usually been assumed to be as small, neutrally charged, lipophilic species such as  $\text{HgCl}_2$  (e.g., passive diffusion through membranes). Recent work by others suggested that inorganic Hg was taken up by the bacterium *Vibrio anguillarum* through physiologically regulated mechanisms, because Hg uptake was much greater under aerobic conditions than under anaerobic conditions, and because increasing exposure Cl concentrations did not increase Hg uptake (Golding et al. 2002, *Limnol. Oceanogr.* 47, 967–975). To distinguish active from passive uptake of Hg in fish, we exposed rainbow trout (*Oncorhynchus mykiss*) to inorganic Hg (0 to 1  $\mu\text{M}$ ) and Cl (0 to 100  $\mu\text{M}$ ) in ion-poor water, and did not measure any difference in gill Hg accumulation. In contrast, complexation of Hg with organic matter (0 to 25 mg C/L) reduced Hg accumulation by trout gills. These data suggest that uptake of inorganic Hg at fish gills may be as accidental, active transport of  $\text{Hg}^{2+}$ , not just passive diffusion of  $\text{HgCl}_2$ .

### A10.13–Sodium chloride-dependent copper transport in isolated intestinal cells from the rainbow trout (*Oncorhynchus mykiss*)

J. Burke, and R. D. Handy, School of Biological Sciences, University of Plymouth

Recent evidence from catfish indicates that the intestinal absorption of copper (Cu) has both Na<sup>+</sup>-sensitive (BBA-Biomembranes, 1566, 104–115) and Cl<sup>-</sup>-dependent components (J. Exp. Biol. 203, 2365–2377) which may involve epithelial Na<sup>+</sup> channels and basolateral Cu–Cl symport respectively. The aim of the present study was to investigate NaCl-dependent Cu uptake in isolated intestinal cells from rainbow trout. Enterocytes were isolated from the mid/hind intestine of adult rainbow trout, cells were Cu-tight with viability of 94 ± 1% (mean ± SE, n = 11 isolations). Cu-uptake was explored over total external [Cu] ranging from 0–800 μmol l<sup>-1</sup>, in the presence/absence of external Na<sup>+</sup> (low Na<sup>+</sup>, 11 mM), or in the presence of Na<sup>+</sup> channel blocking agents at normal (136 mM) [Na<sup>+</sup>]<sub>o</sub>; including amiloride (2 μmol l<sup>-1</sup>), 6 chloro-3,5-diaminopyrazine-2-carboxamide (CDPC, 10 μmol l<sup>-1</sup>) and phenamil (1 μmol l<sup>-1</sup>). Cells showed saturation accumulation with Cu within 2 hours, and flux measurements were therefore conducted over 15 or 30 minute incubations with Cu. Cells accumulated up to 1 and 0.5 Cu mmol mg prot<sup>-1</sup> h<sup>-1</sup> respectively in the presence and absence of external Na<sup>+</sup> over 15 minutes, indicating that low Na<sup>+</sup> conditions only partially revealed Na<sup>+</sup>-sensitive Cu flux. The K<sub>m</sub> for Cu-uptake in normal conditions was about 29 μmol l<sup>-1</sup> external copper. Application of phenamil completely abolished Cu uptake. However, additions of amiloride or CDPC increased Cu uptake to about 9 and 6 Cu mmol mg prot<sup>-1</sup> h<sup>-1</sup> at 800 μmol l<sup>-1</sup> Cu. The latter effects may be due to elevation of tissue K<sup>+</sup> (depolarising effects) and intracellular acidification via amiloride blockade of Na<sup>+</sup>/H<sup>+</sup> exchanger, which are known to stimulate Cu-uptake via the Cu-specific carrier *CTR1*. The data suggest the involvement of both epithelial Na<sup>+</sup> channels (ENaC's) and perhaps Cu-specific *CTR1* in Cu-uptake. Current experiments explore the chloride and pH sensitivity of these processes. This work was partly funded by The Leverhulme Trust.

### A10.14–Phosphate metabolism and distribution in rainbow trout

D. Snellgrove, S.J. Davies and R.D. Handy (University of Plymouth, UK)

Abstract not supplied

### A10.15–Transport in soft water and hard water acclimated zebrafish (*Danio rerio*)

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Uptake of both Na<sup>+</sup> and Cl<sup>-</sup> exhibited Michaelis–Menten saturation kinetics in soft and hard water acclimated zebrafish (*Danio rerio*). Soft water acclimation resulted in both enhanced uptake capacity (J<sub>max</sub>) and affinity (K<sub>m</sub>) for both ions. The most remarkable finding was extremely high Cl<sup>-</sup> uptake affinity (K<sub>m</sub> = 8 ± 1 μM) in soft water compared to hard water fish. (34 ± 10 μM). Similarly, J<sub>max</sub> for Cl<sup>-</sup> was increased from 75 ± 4 nmol g<sup>-1</sup> h<sup>-1</sup> to 226 ± 6 nmol g<sup>-1</sup> h<sup>-1</sup> by softwater acclimation. Na<sup>+</sup> uptake in soft water fish was characterized by a K<sub>m</sub> of 74 ± 15 μM and a J<sub>max</sub> of 1160 ± 72 nmol g<sup>-1</sup> h<sup>-1</sup> while hard water fish exhibited K<sub>m</sub> and J<sub>max</sub> of 160 ± 30.3 μM and 525 ± 29 nmol g<sup>-1</sup> h<sup>-1</sup>, respectively. Soft water fish exposed for more than one hour to a high Na<sup>+</sup> concentration (1200 μM) displayed down-regulated Na<sup>+</sup> uptake. Amiloride and derivatives had surprisingly little effect on Na<sup>+</sup> uptake with the only statistically significant inhibition observed by amiloride (10<sup>-4</sup>M) in soft water fish. Cl<sup>-</sup> influx was inhibited by ethoxzolamide (carbonic anhydrase inhibitor) in both soft and hard water fish while it blocked Na<sup>+</sup> uptake only in the latter group. Exposure to bafilomycin A1 (H<sup>+</sup> pump inhibitor) diminished Na<sup>+</sup> (at 1 μM) and Cl<sup>-</sup> uptake (at 50 nM) in the soft water, but not hard water fish. Immunohistochemistry revealed apical H<sup>+</sup> pump localization in both groups but soft water acclimation did not lead to elevated H<sup>+</sup> pump abundance.

### A10.16–The use of milk samples for the assessment of mineral status in dairy cattle using ICP analysis

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A total of 234 cows from 15 farms were used to assess the use of ICP as a method of assessing mineral levels in milk samples. Milk samples were collected from morning and afternoon milking at 14, 100 and 250 days of lactation. ICP was found to give accurate and repeatable assessment of mineral levels in milk with milk extracted during morning (AM) and afternoon (PM) milking showing no significant difference in mineral level Ca 1014, 985 (sem 22.3), P 721, 710 (sem 27.4), Mg 107, 106 (sem 2.03), Na 436, 423 (sem 15.6), Fe 2.0, 1.9 (sem 0.13), Cu 0.2, 0.2 (sem 0.10), Mn 0.4, 0.4 (sem 0.06), Co 0.01, 0.01 (sem 0.03), Zn 4.0, 3.8 (sem 0.14), K 105, 104 (sem 1.43) ppm in AM and PM milk

samples respectively. However, the stage of lactation had a significant effect on the levels in milk samples with Ca, Mg and Zn levels being significantly lower in mid lactation (M), compared with early (E) and late lactation (L), Ca-E 1000, M 871, L 1010 (sem 25.1) ppm, Mg-E 107, M 75, L 106 (sem 2.03) ppm, Zn-E 4.0, M 3.1, L 4.1 (sem 0.14) ppm. However, P, Na, Fe, Cu, Mn, Co and K levels were not found to differ significantly during lactation. ICP was found to give accurate and

repeatable assessment of mineral levels in milk. Some minerals were found to vary in concentration in milk during mid lactation (150 days postpartum). This variation was not consistent with the metabolic dynamics of mineral mobilisation in dairy cattle and higher milk mineral concentrations in late lactation was likely to be due to changes in size and structure of the gaps and junctions of epithelium due to mammary cell apoptosis.