Our laboratories have conducted studies pertaining to calcium and magnesium transport mechanisms in fish gills and intestinal epithelium. This is a review of published studies and recent unpublished findings resulting from this work. It is not an exhaustive review; rather, data sets on tilapia research (some still incomplete) are presented to stimulate discussion.

**CALCIUM TRANSPORT**

**Gills**

In the euryhaline teleost tilapia *Oreochromis mossambicus* kept in fresh water, the gills are the major site for exchange of calcium with the water (Flik et al., '85a). In growing freshwater tilapia, influx of calcium always exceeds the efflux, resulting in a positive calcium balance to augment the total body calcium pool (Flik et al., '88a,b). Modulation of branchial calcium fluxes in response to particular environmental water conditions (e.g., variations in calcium concentrations) is therefore a prerequisite for calcium homeostasis. Consequently, the dominant calcitropic hormones in fish (stanniocalcin, prolactin, and cortisol; see below) exert major effects on the gills.

Only recently have the mechanisms underlying the unidirectional fluxes of calcium through the branchial epithelium been elucidated. Calcium taken up from the water in freshwater fish follows a transcellular, hormone-controlled pathway located in the chloride cells of the gills. In a model for transcellular Ca\(^{2+}\) transport (Fig. 1) based on our studies on trout, eel, and tilapia, stanniocalcin (STC)

### ABSTRACT

The euryhaline tilapia *Oreochromis mossambicus* kept in fresh water takes up calcium mainly from the water via the gills, like other freshwater fish. In the gills specialized mitochondria-rich cells, the chloride cells, are thought to mediate a transcellular Ca\(^{2+}\) transport. Second messenger operated calcium channels (SMOCs) in the apical membrane, regulated by the hormone stanniocalcin, allow minute-to-minute control over the entry of Ca\(^{2+}\). In the basolateral plasma membranes of these cells, an ATP-consuming Ca\(^{2+}\) transporting enzyme provides the major driving force for extrusion of Ca\(^{2+}\) into the blood; in addition, an Na\(^+\)/Ca\(^{2+}\) exchanger is present in these membranes. The kinetics of the exchanger in vitro indicate that this extrusion mechanism dominates when intracellular calcium levels reach micromolar levels. In the gills, the transport of calcium appears primarily ATPase mediated and therefore largely independent of the Na\(^+\) status of the transporting cell. In enterocytes, similar mechanisms for transcellular transport of Ca\(^{2+}\) exist. However, in the intestinal epithelium the extrusion of Ca\(^{2+}\) is primarily via the Na\(^+\)/Ca\(^{2+}\) exchanger and to a very limited extent mediated via the Ca\(^{2+}\)-ATPase. Indeed, calcium transport over the intestinal epithelium is dependent on the Na\(^+\)-status and the Na\(^+\)/K\(^+\)-ATPase activity of the epithelium. Prolactin and cortisol are endocrine factors determining the relative densities of calcium pumps in basolateral plasma membranes.

At least 80% of the magnesium required for growth and homeostasis is absorbed from the food via the intestine. Magnesium is transported transcellularly and actively via enterocytes. The movement of Mg\(^{2+}\) over the apical membrane is passive, down an electrochemical gradient. The cytosolic Mg\(^{2+}\) concentration is kept well below its equilibrium concentration. The extrusion over the basolateral plasma membrane is mediated by an ATP-consuming enzyme. The gills contribute less than 20% to magnesium uptake, but up to 50% in tilapia fed a magnesium deficient diet. Evidence is accruing that prolactin is involved in the adaptation to low magnesium diets. © 1993 Wiley-Liss, Inc.
controls the apical membrane transition of Ca²⁺ from the water or the food, may enter the cell passively, down an electrochemical gradient. Stanniocalcin (STC) regulates calcium channels in the apical membrane; STC signal transduction requires second (cAMP, DAG, IP₃) and third (Ca²⁺) messengers. Extrusion of Ca²⁺ from the cell is either via a Ca²⁺-ATPase (route 1) or via an Na⁺/Ca²⁺ exchanger (route 2). Both extrusion mechanisms are present in branchial and intestinal epithelium. In gill cells route 1 dominates, in the enterocyte route 2. In the enterocyte, very high Na⁺/K⁺-ATPase activity safeguards the operation of the exchanger by maintaining the Na⁺ gradient as a driving force for the exchanger. The calcitropic hormones cortisol and prolactin (PRL) determine the relative abundance of the ATPases, and by doing so determine the calcium transporting capacity of the epithelium.

**Ca²⁺ entry over the apical membrane**

STC is a homodimeric glycoprotein of 56 (in most fish studied) to 60 (in eel) kilodalton from the corpuscles of Stannius (Flik et al., '89b, '90a). Physiological studies have provided circumstantial evidence that STC is the major hypocalcemic hormone in fish exerting the minute-to-minute control over apical membrane Ca²⁺ transport. In a trout, isolated head preparation STC inhibits Ca²⁺ influx within 15 min (Lafeber et al., '88). STC also inhibits ⁴⁰Ca²⁺ uptake from the water into branchial epithelium (Verbost et al., '89). These observations are in line with a conclusion reached earlier, down an electrochemical gradient through voltage-independent calcium channels (Perry and Flik, '88). Removal of the corpuscles of Stannius (rapidly) evokes a patent hypercalcemia (Hanssen et al., '89) and the severity of the hypercalcemia is positively correlated with the water calcium level (Flik, '90). These observations all indicate that Ca²⁺ from the water enters the Ca²⁺ transporting cell via an STC-controlled pathway located in the apical membrane.

We postulate that the receptors for STC are localized in the basolateral plasma membrane. Subsequently, second and third messengers transduce the STC signal to the apical membrane; this is the reasoning behind the postulate of second messenger operated calcium channels (SMOCs). An extract of trout corpuscles of Stannius lowers the branchial cAMP content and inhibits adenylyl cyclase in branchial (and in renal and intestinal) plasma membranes (Flik, '90). These results suggested that cAMP is involved as a second messenger in the STC signal transduction. However, recent studies have revealed that an extract of corpuscles of Stannius contains two bioactive components, STC and a small glycoprotein which we tentatively equate with teleocalcin, first reported on by Ma and Copp ('78). It turned out that the "cAMP-effects" in an extract of the corpuscles of Stannius must be attributed to the teleocalcin activity in the extract. This effect on cAMP levels, however, is unrelated to the direct regulation of the calcium influx, as pure teleocalcin has no effect on calcium influx (P.M. Verbost, personal communication). It is our strong belief that inositol metabolites are involved in the messenger pathway of STC. The impetus for this statement was the observation that STC stimulated the production of sn-1,2-diacylglycerol (DAG) in tilapia branchial cells (Fig. 2). DAG is one of the second messengers released in a cell when the inositol cycle is stimulated. Studies on the effects of STC on the production of inositol metabolites are now being carried out. One would predict that IP₃, the other second messenger produced when inositol metabolism is stimulated, is produced in the branchial cells after stimulation with STC. It is usually found in this field of research that IP₃ levels are low and difficult to establish. In line with our prediction is the observation that in permeabilized gill cells IP₃ stim-
Fig. 2. Stanniocalcin (STC) stimulation of sn-1,2-diacyl-glycerol (DAG) production in freshwater tilapia gill cells. DAG was determined with a commercial radioenzymatic assay kit (RPN200, Amersham). Mean values ± SEM for six fish are given. The asterisk indicates significance of the difference compared to the control \( (P < 0.03; \text{Mann-Whitney U-test}) \).

ulates \( \text{Ca}^{2+} \) (third messenger) release from endoplasmic reticular stores (Fig. 3). Thus, although IP3 production per se as a response to an STC stimulus still has to be established, the presence of an IP3 receptor in the endoplasmic reticulum of gill cells seems evident.

The overall picture emerging so far is that STC evokes in its targets second and third messenger responses, which are tentatively related to the control of the apical membrane \( \text{Ca}^{2+} \) channel: the time course of the STC inhibition of the branchial \( \text{Ca}^{2+} \) influx (significant effects within 15 min) is in accordance with the rapid second messenger responses observed (effects within 10 min). Phosphorylation studies with highly purified apical, i.e., brush border, preparations of intestinal and renal tissue may ultimately give more direct evidence for \( \text{Ca}^{2+} \) mediated modulatory actions of STC on SMOCs in these cells. Branchial apical membranes are difficult to isolate, however.

\textbf{Ca}^{2+} \textbf{extrusion in the basolateral plasma membrane}

Two mechanisms for \( \text{Ca}^{2+} \) extrusion exist in the basolateral plasma membrane of branchial epithelium. The first, a high-affinity \( \text{Ca}^{2+} \)-ATPase, represents the ATP-driven calcium pump and is well documented (Flik et al., '84a, '85a,b; Perry and Flik, '88). The second is an \( \text{Na}^{+}/\text{Ca}^{2+} \) exchange mechanism (Figs. 4 and 5a). We present here for the first time evidence for its presence in branchial epithelial plasma membranes.

ATP-driven \( \text{Ca}^{2+} \) transport and \( \text{Na}^{+}/\text{Ca}^{2+} \) exchange activity were analyzed in the same plasma membrane preparation. The methodology for determining \( \text{Ca}^{2+} \)-ATPase mediated \( \text{Ca}^{2+} \) transport in plasma membrane vesicles (determined as the difference in \( ^{45}\text{Ca}^{2+} \) accumulation in the presence and absence of ATP) has been described extensively in the references given; that for \( \text{Na}^{+}/\text{Ca}^{2+} \) exchange will be briefly described below. The kinetics of the \( \text{Ca}^{2+} \) pump (for tilapia we found a \( K_{0.5} \) of 102 ± 46 nM \( \text{Ca}^{2+} \) and a \( V_{\text{max}} \) of 3.79 nmol x min\(^{-1} \) per mg protein; \( n = 6 \)) suggest that this enzyme operates in the extrusion process (Flik et al., '85a). Typically, the enzyme is stimulated in vitro at calcium levels that are found at rest in most cells (around 100 nM) and the maximum \( \text{Ca}^{2+} \) transport capacity of the gills derived from the \( V_{\text{max}} \) (Flik et al., '85a) is realistic when compared to the \( \text{Ca}^{2+} \) influx that is observed in the gills. In addition to thermodynamic arguments (Perry and Flik, '88), further evidence for a role of the ATP-driven calcium pump in transepithelial transport comes from endocrinological studies. Hypercalcemic hormones, viz. cortisol in trout (Flik and Perry, '89) and prolactin in eel (Flik et al., '84b, '89a) and tilapia (Flik et al., '86c), stimulate \( \text{Ca}^{2+} \) influx via the gills and increase the density of the calcium pumps in the basolateral plasma membranes.

Characteristic changes in branchial epithelial plasma membranes observed by us after treatment of the fish with prolactin or cortisol are a higher activity of \( \text{Ca}^{2+} \)-ATPase relative to \( \text{Na}^{+}/\text{K}^{+} \)-ATPase and an increased \( V_{\text{max}} \) of the calcium pumps in basolateral plasma membrane vesicles. Based on the rapid turnover of chloride cells in the gills (Wendelaar Bonga et al., '90), it is our strong belief that cortisol and prolactin administration for 4–8 days (the time period required to evoke hypercalcemic responses) results in the development of a new population of chloride cells that reflect the particular hormonal status.

The first indication for \( \text{Na}^{+}/\text{Ca}^{2+} \) exchange activity in branchial epithelial plasma membranes came from an experiment in which \( \text{Ca}^{2+} \) transporting vesicles (ATP-dependent transport at 1 \( \mu \text{M} \) \( \text{Ca}^{2+} \) in the medium) were challenged after 10 min of calcium loading with 70 mM NaCl or KCl (control). The inwardly directed \( \text{Na}^{+} \) gradient thus created drives \( ^{45}\text{Ca}^{2+} \) accumulated in the vesicles as a result of the ATPase activity. The loss of \( ^{45}\text{Ca}^{2+} \) seen when KCl is added to the medium is assumed to result from an osmotic effect. Significantly greater loss of \( ^{45}\text{Ca}^{2+} \) occurred at 1 and 5 min after addition of NaCl compared to KCl (Fig. 4).
Fig. 3. IP3 induced Ca$^{2+}$ release in freshwater tilapia permeabilized gill cells. Isolated gill cells were permeabilized with saponin. $^{45}$Ca$^{2+}$ uptake was assayed in a Leibowitz medium supplemented with 3 mM ATP, 667 MBq/ml $^{45}$CaCl$_2$ (Amersham), and a calculated 0.1 μM Ca$^{2+}$, using an EGTA/NTA/HEEDTA buffer system (Flik et al., '90b). After the $^{45}$Ca$^{2+}$ uptake had reached a plateau, inositol (1,4,5) trisphosphate (Calbiochem) was added to a final concentration of 10 μM. Control experiments included the omission of ATP and the addition of oxalate and vanadate in the presence of ATP (data not shown). The addition of IP3 (arrow) results in the release of Ca$^{2+}$ out of a compartment that is oxalate-permeable and equipped with a vanadate-inhibitable Ca$^{2+}$ pump with high affinity for Ca$^{2+}$ (the endoplasmic reticulum). Values ± SEM are given for 6 fish. Release of Ca$^{2+}$ occurred in all cases.

We then further investigated the calcium kinetics of this presumed exchange activity as follows. Na$^+$-dependent Ca$^{2+}$ transport across plasma membranes was assayed as the difference in $^{45}$Ca$^{2+}$ accumulation upon transfer of membrane vesicles equilibrated in 150 mM NaCl to media containing either 150 mM NaCl (blank) or 150 mM KCl. In composing the media, a 25-fold dilution of the vesicle suspension was taken into account to yield the following final concentrations: 150 mM NaCl or KCl, 20 mM HEPES/Tris (pH 7.4), 0.5 mM EGTA, 0.5 mM HEEDTA, 0.5 mM NTA, 0.8 mM free Mg$^{2+}$, and 7.5 × 10$^{-5}$ to 5 × 10$^{-2}$ mM free Ca$^{2+}$. Free Ca$^{2+}$ and Mg$^{2+}$ concentrations were calculated as described in detail recently (Schoenmakers et al., '92b). The $^{45}$Ca radioactive concentration was 0.5 to 0.8 MBq × ml$^{-1}$. A 5 μl vesicle suspension was mixed with 120 μl medium, both prewarmed to 37°C. After 5 sec of incubation, the reaction was quenched by addition of 1 ml ice-cold stopbuffer (150 mM NaCl, 20 mM HEPES/Tris at pH 7.4, 1.0 mM LaCl$_3$) to the incubate. Membrane vesicles with retained $^{45}$Ca were collected by filtration over 0.45 μm filters (Schleicher & Schull, ME 25); the filters were washed twice with 2 ml stopbuffer. The filters were dissolved in the scintillation fluid (Aqualuma', Lumac) and the radioactivity collected on the filters was determined by liquid scintillation counting. We first analyzed the Ca$^{2+}$ dependence of the exchanger to allow an evaluation of the relative activities of the ATPase and the exchanger in the membrane as a function of the (cytosolic) calcium concentration. The exchange activity showed saturation and was well described by Michaelis-Menten kinetics; the kinetic parameters derived are a V$_\text{max}$ of 19.6 ± 2.7 nmol × min$^{-1}$ per mg protein and a K$_{0.5}$ of 1.95 ± 0.45 μM (Fig. 5a). Clearly, the exchange activity in the gills is a powerful mechanism for Ca$^{2+}$ transport, especially when cytosolic calcium concentrations exceed 1 μM (see discussion below; Fig. 6a).

The regulation of the Na$^+$/Ca$^{2+}$ exchanger activity in the gills has, until now, not received attention in our research. Studies on the effects of seawater adaptation of tilapia on the exchanger and its sodium dependence are in progress; effects of prolactin(s) and growth hormone on plasma membrane Na$^+$/K$^+$-ATPase (on which the exchanger depends) will be reevaluated in this respect.

What, then, is the role of the Na$^+$/Ca$^{2+}$ exchanger in the calcium homeostasis of the branchial cells? We have plotted the activities of the two plasma membrane calcium extrusion mechanisms as a function of cytosolic calcium levels (Fig. 6a). For the exchange activity, it was assumed that the Na$^+$ gradient was not limiting its activity. At cytosolic Ca$^{2+}$ concentrations up to 1 μM, the Ca$^{2+}$-ATPase
There is limited data available on effects of calcitropic hormones on the intestine of fish. Takagi and coworkers ('85) successfully removed corpuscles of Stannius from trout and showed effect on intestinal calcium transport. Studies by Hirano ('89) have given evidence that STC in the eel controls the passive entry of calcium into the intestinal epithelium. In our lab we have shown that an extract of corpuscles of Stannius inhibits plasma membrane adenylyl cyclase in tilapia enterocytes (Flik, '90), but this effect is likely to be attributed to the teleocalcin present in this preparation. However, recently we have been able to show a consistent stimulation by STC of IP3 production in tilapia enterocytes (P.M. Verbost, personal communication). Thus the enterocyte is a likely STC target and the presence of SMOCs in this cell should be further studied. For prolactin effects on the intestine of tilapia, indirect evidence was advanced (Flik et al., '86c): ovine prolactin reduces calcium loss via intestinal (and renal) routes.

One of the aims of our present research is to fill in the model of the endocrine regulation of Ca2+ transport in the enterocyte in analogy to the chloride cell of the gills. The intestinal epithelium has some important advantages over the branchial epithelium to make it an attractive model. The tilapia intestinal epithelium is easily stripped of its submucosa and was successfully used in an Ussing chamber setup (Flik et al., '90b; Van der Velden et al., '90). Furthermore, the epithelium is rather homogeneous as it contains no crypts. In biochemical studies, the enterocyte is easily fractionated to allow the isolation of brush border membranes (STC dependent SMOCs) and basolateral membranes (vitamin D and prolactin dependent extrusion mechanisms). We analyzed the calcium transport phenomena described here for the gills (earlier) in the intestine of the tilapia (Flik et al., '90b) and found some remarkable differences between these tissues. Cell mediated calcium transport in the intestine is dependent on the sodium status of the epithelium and it is Na+/K+-ATPase-dependent, as indicated by its sensitivity to ouabain. We subsequently analyzed the basolateral plasma membranes for Ca2+-ATPase and Na+/Ca2+ exchange activities. First we found an extremely low Ca2+-ATPase mediated calcium transport in plasma membrane vesicles (K0.5 = 27 nM; Vmax = 0.63 nmol x min⁻¹ per mg protein). The low activity could not be attributed to a low quality of the membrane preparation but appears to indicate a low density of Ca2+-ATPase therein. Surprisingly, a very high Na+/K+-ATPase could be demonstrated in these
Fig. 5. Na⁺/Ca²⁺ exchange in gills and intestine. Ca²⁺ dependence of Na⁺-driven Ca²⁺ uptake into vesicles of gill epithelium (a) and intestinal epithelium (b). Initial rates of Na⁺-driven Ca²⁺ uptake (\(\text{Na}^+ / \text{K}^+\) were corrected for "nonspecific" uptake (Na⁺ = Na⁺ = 150 mM). Ca²⁺ was varied between 10⁻⁶ and 2.5 10⁻⁵ M. Uptake rates have been plotted relative to the maximum velocities observed for comparison (\(V_{\text{max}} = 12.2 \pm 3.7\) and 20.5 ± 4.4 nmol x min⁻¹ per mg protein for gills and intestine, respectively). The \(K_m\) values are given in the figures. Mean values for six fish are given. Bars indicate SE.

membranes (specific activity around 150 \(\mu\)mol P_i × h⁻¹ per mg protein versus 30 \(\mu\)mol P_i × h⁻¹ per mg protein in a comparable plasma membrane preparation of gill cells). The high Na⁺/K⁺-ATPase activity also argues against a low quality preparation. However, a powerful Na⁺/Ca²⁺ exchange activity (\(K_{0,5} = 181\) nM; \(V_{\text{max}} = 7.2\) nmol x min⁻¹ per mg protein) was present in the plasma membranes of the enterocytes (Flik et al., '90b). The maximum activity of the exchanger exceeds that of the Ca²⁺-ATPase in the same membrane more than tenfold and that of any known Na⁺/Ca²⁺ exchange activity in (mammalian) non-excitable cells. An observation not understood at that time was the curvilinear calcium dependence of the exchanger (Flik et al., '90b), but very recently we have been able to solve this problem by improving on the procedures for calculation of free Ca²⁺ concentrations in physiological solutions (Schoenmakers et al., '92a,b). The improved calculation method
Fig. 6. The activity of the Ca\textsuperscript{2+} -ATPase (dotted lines) and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (solid lines) in the basolateral plasma membrane of the gills (a) and the intestine (b) as a function of the calcium concentration in the cell. The exchanger determines for the major part the extrusion of Ca\textsuperscript{2+}. In the intestine the Ca\textsuperscript{2+} -ATPase is dominant in the export of Ca\textsuperscript{2+} from the cell. The transport activities of both extrusion mechanisms were determined on the same resealed vesicle preparations of the respective plasma membranes. Note that at the calcium concentrations given on the x-axis, the exchanger does not reach saturation. Mean values for six fish are given; bars indicate SE; in some cases the SE bars fall within the symbol used. Data were fitted by non-linear regression analysis. Yields single Michaelis-Menten kinetics for the exchanger and leads in particular to higher estimates for the V\textsubscript{max} of the exchanger.

In Figure 6b, the activities of the Ca\textsuperscript{2+} -ATPase (K\textsubscript{0.5}: 106 ± 21 nM, V\textsubscript{max}: 0.66 ± 0.15 nmol x min\textsuperscript{-1} x mg protein\textsuperscript{-1}) and the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (K\textsubscript{0.5}: 1.95 ± 0.45 μM, V\textsubscript{max}: 19.6 ± 3.6 nmol x min\textsuperscript{-1} x mg protein\textsuperscript{-1}; roughly 30 times higher than the ATPase), determined anew proceeding from the most updated calculations for free Ca\textsuperscript{2+}, are plotted as a function of cytosolic Ca\textsuperscript{2+}. The exchanger dominates the calcium extrusion in the enterocyte.
tribution of branchial Mg\(^{2+}\) uptake from the water was calculated to 13 nmol x h\(^{-1}\), the contribution of drinking Mg containing water to 16 nmol x h\(^{-1}\) maximally, and the contribution of Mg absorption from the food to 329 nmol x h\(^{-1}\) maximally. For a fish feeding on a low Mg diet (1 \(\mu\)mol x g\(^{-1}\)) these numbers are 12, 6, and 14 nmol x h\(^{-1}\), respectively (Van der Velden et al., 91a; Van der Velden, 91). Clearly the tilapia does not increase extraintestinal Mg\(^{2+}\) uptake when fed a low Mg diet. In the latter situation, however, extraintestinal Mg\(^{2+}\) uptake stands for 50% of the total uptake.

For magnesium uptake in fish, the situation is opposite to that for calcium uptake: a not fully understood, small part of the uptake occurs normally via the gills, and the major route for Mg\(^{2+}\) uptake is via the intestine.

**Intestine**

There is ample evidence for absorption of Mg\(^{2+}\) by fish intestine. Many studies have given clear but indirect evidence by providing the fish with diets varying in Mg content and subsequently analyzing tissue elemental composition, growth rate, etc. A photonuclear reaction process has made available a preparation of \(^{25}\)Mg\(^{2+}\) with very high specific activity and it allowed us to perform some studies on unidirectional Mg\(^{2+}\) fluxes in tilapia intestine (Van der Velden et al., '90). Net absorption of Mg\(^{2+}\) could be demonstrated and a sodium dependence established (Table 1). In control conditions with saline bathing the mucosal and the serosal sides of tilapia stripped epithelium, the net mucosa to serosa flux was 23 nmol x h\(^{-1}\) x cm\(^{-2}\). When sodium was replaced by the inert ion NMDG\(^{+}\) (sodium free condition), unidirectional fluxes were strongly reduced and the net flux was abolished. Ouabain, known to completely block Na\(^+/K\)-ATPase activity in this tissue (Flik et al., '90b), reduces the mucosa to serosa flux to the level found for the serosa to mucosa flux. Apparently this blocker inhibits the transcellular movement of Mg\(^{2+}\).

**TABLE 1.** Magnesium fluxes (J) ± standard deviation across stripped intestinal epithelium of freshwater tilapia under different experimental conditions

<table>
<thead>
<tr>
<th>Saline conditions</th>
<th>Unidirectional Mg(^{2+}) fluxes in tilapia intestine</th>
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<tbody>
<tr>
<td></td>
<td>(J_{m}) (nmol x h(^{-1}) x cm(^{-2}))</td>
</tr>
<tr>
<td>Control</td>
<td>39 ± 19 (9)</td>
</tr>
<tr>
<td>Sodium-free</td>
<td>6 ± 3 (7)</td>
</tr>
<tr>
<td>+ Ouabain (1 mM)</td>
<td>16 ± 2 (3)</td>
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1The number of observations are indicated between brackets. Data taken from Van der Velden et al. (90).

On the basis of analyses of the elemental composition of the epithelium and electrophysiological data (inside of the cell -60 mV) (Bakker and Groot, '88), we have concluded that intracellular Mg\(^{2+}\) (8 nmol \(\times 1^{-1}\)) is kept far below its equilibrium concentration (100 nmol \(\times 1^{-1}\); bath ringsers Mg was 1 nmol \(\times 1^{-1}\)). It follows then that transport over the apical membrane may be passive and driven by an electrochemical potential difference of at least 25 mV. This value is probably too low, because the major part of the Mg\(^{2+}\) in the cell is in a bound form. Conversely, transcellular Mg\(^{2+}\) transport requires an energized extrusion step at the basolateral side of the cell to counteract the potential difference of at least 30 mV. Either ATP or the Na\(^+\) gradient over this membrane could provide the energy for such transport. In our Ussing chamber setup, the magnesium concentrations in both hemichambers were always identical. Also there is no electrical gradient across the epithelium in this setting. It follows then that the transport of Mg\(^{2+}\) must result from net water transport (solvent drag Mg\(^{2+}\) transport), from an active transport mechanism, or a contribution of both. The sodium dependence of the Mg\(^{2+}\) transport suggests, of course, the operation of an Na\(^+\)/Mg\(^{2+}\) exchanger. The latter hypothesis was tested in studies with tilapia enterocyte basolateral plasma membrane vesicles in which Mg\(^{2+}\) transport was determined with \(^{27}\)Mg\(^{2+}\) as radiotracer (Kolar et al., '91). So far, we have been unable to determine Na\(^+\)/Mg\(^{2+}\) exchange in assays which were comparable in their setup to those for the determination of Na\(^+\)/Ca\(^{2+}\) exchange (Flik et al., '90b). However, a consistent finding so far is that ATP stimulates Mg\(^{2+}\) transport into these vesicles (Kolar et al., '91). A characterization of ATP-driven Mg\(^{2+}\) transport in plasma membrane vesicles has been carried out (Flik et al., submitted) and this finding will open a new field of research.

How magnesium transport in fish is regulated is still an enigma. STC appears not to be involved (Hirano, '89). External magnesium levels influence prolactin cell activity (Wendelaar Bonga et al., '85). Some further circumstantial evidence for a role of prolactin in magnesium homeostasis comes from diet experiments with tilapia: long-term low Mg diets result in chronically activated prolactin cells (Van der Velden et al., '91b). How prolactin affects magnesium transport is as yet unknown.

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LITERATURE CITED


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