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Prolactin cell activity and ion regulation in tilapia, *Oreochromis mossambicus* (Peters): effects of a low magnesium diet

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Freshwater tilapia feeding on a diet containing 1 mmol kg^{-1} magnesium (control diet: 30 mmol kg^{-1}) grow although at a decreased rate. The diet does not noticeably affect the blood ionic composition. Prolactin cell activity increases in these fish as judged from the enhanced rate of synthesis of ^{3}H-leucine labelled prolactins *in vitro* and the ultrastructure of the cells. Na^+ intake and Na^+ loss decreases, and chloride cell density increases, phenomena typical for enhanced prolactin cell activity in tilapia. We conclude that tilapia manage to cope with a dietary magnesium insufficiency and suggest that prolactin is involved in the acclimation to this diet.

Key words: prolactin; magnesium; sodium; freshwater teleost; acclimation.

I. INTRODUCTION

Adult tilapia *Oreochromis mossambicus* (Peters) maintain their magnesium balance when fed a low magnesium (1 mmol kg^{-1}) diet (van der Velden *et al.*, 1991*c*). Other fish species, such as common carp *Cyprinus carpio* L., channel catfish *Ictalurus punctatus* (Rafinesque) and guppy *Poecilia reticulata* Peters may eventually die on a similar diet (Ogino & Chiou, 1976; Gatlin *et al.*, 1982; Shim & Ng, 1988), the tilapia appear to acclimate more easily (van der Velden *et al.*, 1991*c*). How vertebrates regulate their magnesium balance is an essentially open question, although calcitropic hormones have been implicated in this regulation in higher vertebrates (Littledike & Goff, 1987).

Several studies have been carried out with freshwater fish kept under conditions of dietary magnesium deficiency. Dietary magnesium is an important magnesium source for freshwater fish (Shearer, 1989; van der Velden *et al.*, 1990, 1991*a*). The ambient water is of minor importance as a magnesium source for adult fish (van der Velden *et al.*, 1991*b*). The importance of dietary magnesium in fish is indicated by the occurrence of severe deficiency symptoms in fish kept on diets with too low a magnesium content. Diets containing less than 5 mmol kg^{-1} lead to changes in the ionic composition of the soft as well as the hard tissues and eventually fish may die. Among the effects of such diets on blood plasma ion composition are changes in magnesium, sodium and potassium concentrations; moreover changes in magnesium, calcium, sodium and potassium concentrations in muscle and bone (Cowey *et al.*, 1977; Gatlin *et al.*, 1982; Knox *et al.*, 1983; Shim & Ng, 1988;

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Dabrowska *et al.*, 1989; Shearer, 1989) have been reported. Thus dietary magnesium deficiency may induce severe ionoregulatory disturbances in certain fish species. Adult freshwater tilapia, however, appear to cope well with a low magnesium diet: we observed a 37% decrease in growth rate when magnesium in the diet was reduced to 1 mmol kg^{-1}. No signs of disturbances in ionocomposition of muscle, bone or scales were observed (van der Velden *et al.*, 1991*c*). This species apparently compensates for a dietary magnesium insufficiency. Potential mechanisms of compensation could be an increased uptake of magnesium from the water and a reduction of magnesium loss. However, we were unable to demonstrate any change in Mg^{2+} intake from the water in young tilapia fed a low-magnesium diet (van der Velden *et al.*, 1991*c*), and thus the control over Mg^{2+} loss seems pivotal. The question arises now whether the tilapia respond to a low magnesium diet with an endocrine response. This is investigated here by examining parameters that reflect, directly or indirectly, changes in the ionoregulation. We analysed the ionocomposition of blood plasma, opercular chloride cell density, branchial Na^+/K^+-ATPase and prolactin cell activity in tilapia.

II. MATERIALS AND METHODS

ANIMALS

Male tilapia, *O. mossambicus* (Peters), were obtained from laboratory stock. The fish were kept in all-glass aquaria with 450 l Nijmegen tap water at a maximal density of 40 g fish per litre water. The water was recirculated through an 800 l biological filter system and continually refreshed with a flow of 300 l tap water per day. The magnesium, calcium and sodium concentrations of the tap water were $0{\cdot}20\pm0{\cdot}03$, $0{\cdot}7\pm0{\cdot}1$ and $0{\cdot}6\pm0{\cdot}1$ mmol l^{-1} ($n=3$), respectively; the water pH was around 7·6 and the temperature was kept at 25° C. The light regime (12 h light : 12 h dark) was automatically controlled.

The fish received six rations of food per day in dry pellet form at a dose of 1·5% of the estimated total wet body weight of the fish population per day. Control fish received a diet containing 30 mmol Mg per kg; experimental fish received a diet containing 1 mmol Mg per kg. In the latter diet MgO and $MgSO_4$ had been replaced by glucose monohydrate. Both diets were produced in cooperation with Hope Farms (Woerden, the Netherlands) and the composition has been described in detail previously (van der Velden *et al.*, 1991*c*).

EXPERIMENTAL DESIGN

Two types of experiments were carried out. A short term experiment was performed with fish weighing around 80 g at the beginning of the experiment. The total and ultrafiltrable blood plasma magnesium and calcium concentrations were determined after 3 days and after 1, 2, 3, 7 and 9 weeks. After 3 and 9 weeks the branchial Na^+/K^+-ATPase was determined.

The long term experiment lasted 19 weeks. At the start of the experiment the mean body weight of the controls was 213 ± 45 g ($n=41$) and did not differ significantly from that of the experimental fish (199 ± 41 g $n=40$). After 19 weeks the mean weights of the groups differed significantly ($P<0{\cdot}01$) and were 383 ± 67 g ($n=19$) and 306 ± 77 g ($n=19$) for the control and experimentals, respectively. Details on this growth rate have been described earlier (van der Velden *et al.*, 1991*c*). During this experiment the haematocrit, plasma osmolarity, blood Ca^{2+} concentration, prolactin cell activity and opercular chloride cell density were determined after 8 and 19 weeks. Furthermore the Na^+ intake from the water was determined after 19 weeks and the sodium balance of the fish calculated.

BLOOD AND BLOOD PLASMA ANALYSES

Fish were anaesthetized with 0·4 mmol l^{-1} 3-aminobenzoic acid ethyl ester (MS 222; Sigma), buffered to pH 7·4 with Tris (Sigma). Blood (mixed arterial and venous) was

collected by puncture of the caudal vessels with a syringe fitted with a 23-G needle. Ca-heparin (Radiometer) was used as an anticoagulant. Next the fish were killed by spinal transection. Blood cells were separated from plasma by centrifugation (1 min, 9000 *g*) and the plasma was stored on ice until further assay. Part of the plasma (200 µl) was ultrafiltered using Millipore Ultrafree-MC filter units (UFC3 TGC00) with a molecular cut-off of 10 kDa.

The magnesium concentration of plasma and ultrafiltered plasma was determined with a magnesium kit (Sigma diagnostic kit no. 595) and that of calcium with a calcium kit (Sigma diagnostic kit no. 587). Whole blood Ca^{2+} was determined with an Ionic Calcium Analyzer (Radiometer) as described by Fogh-Anderson (1981). Haematocrit was assessed after 10 min centrifugation (9000 *g*) of heparinized blood samples in 75 µl glass capillaries. Plasma osmolarity was assessed with a Roebling osmometer.

PROLACTIN CELL ACTIVITY

Immediately after the fish had been killed the pituitary gland was removed and transferred to Hanks' balanced salt solution (HBSS; Sigma, H13387). Next the rostral pars distalis (RPD; the so-called prolactin lobe which consists almost exclusively of prolactin cells; Flik *et al.*, 1989*a*) was dissected. The RPD was bisected medially. One part was fixed and processed for electron microscopy as described in detail elsewhere (Wendelaar Bonga & van der Meij, 1989). The other part was preincubated for 60 min in HBSS at 25° C and subsequently incubated for 4 h in 50 µml HBSS to which 3·3 GBq [^{3}H]-leucine (Amersham) had been added. Next the tissue was washed in HBSS (3 × 1 min) and homogenized in 500 µl 0·05 mol l^{-1} acetic acid with a tight glass-to-glass Potter-type homogenizer device and centrifuged for 10 min at 9000 *g*; the supernatant was lyophilized and stored dry at −20° C until further assay. The newly-synthesized products released to the medium during the 4 h labelling period were collected by trichloro-acetic acid (10% final concentration) precipitation according to Laemmli (1970); 1 µg bovine serum albumin was added to improve recovery. After overnight incubation at 0° C the samples were centrifuged for 30 min at 9000 *g* and the pellet rinsed twice with diethylether and subsequently stored at −20° C until further assay.

The protein in the RPD extract and in the incubation medium samples were separated by sodium dodecyl sulphate polyacrylamide (15%) slab gel electrophoresis (SDS–PAGE) using a Biorad Mini Protean II Slab Cell, following the protocol of Laemmli (1970).

After electrophoresis the gels were fixed in 40% methanol : 10% acetic acid in water (30 min), 5% methanol : 7% acetic acid in water (30 min) and in 10% glutaraldehyde in water (30 min) and subsequently rinsed in flowing tap water for at least 1 h. For fluorography the fixed gels were impregnated with 2–5-diphenyloxazole according to the method of Bonner & Laskey (1974). Before drying (Biorad slab gel dryer), the gels were dehydrated for 8–10 h in 50% methanol in water containing 3% glycerol. Preflashed Kodak XAR-5 X-ray film was used; exposure time was 24–72 h at −80° C. Fluorographs were scanned densitometrically with a Biorad Model 1650 transmission scanning densitometer (Fig. 1). The radioactivity recovered from the 21 and 19 kDa areas after SDS–PAGE of the RPD extract (Wendelaar Bonga *et al.*, 1983) and the incubation media was taken as a measure of the synthesis rate of the prolactin cells.

SODIUM BALANCE

The accumulation rate of sodium by the fish (F_{net}) was calculated on the basis of the growth rate of the fish and its growth-related increase in total body sodium content (Flik *et al.*, 1989*b*). We have shown previously (van der Velden *et al.*, 1991*c*) that in tilapia fed the low magnesium diet for up to 19 weeks the tissue sodium concentrations had not changed compared to controls. Therefore the previously published relation between whole body weight and sodium content, $Q_f = 40{\cdot}2 W_f + 40{\cdot}6$ µmol Na (where Q_f is the whole body Na content and W_f the fish wet weight in g; Flik *et al.*, 1989*b*), may be applied to calculate sodium content and accumulaton rate, both for the control and the experimental fish. F_{net} was calculated as $\Delta Q_f / \Delta t$.

The Na^+ intake from the water (F_{in}) was assessed by a radiotracer technique and was calculated on the basis of the initial rate of $^{24}Na^+$ intake by the fish from the water (Flik

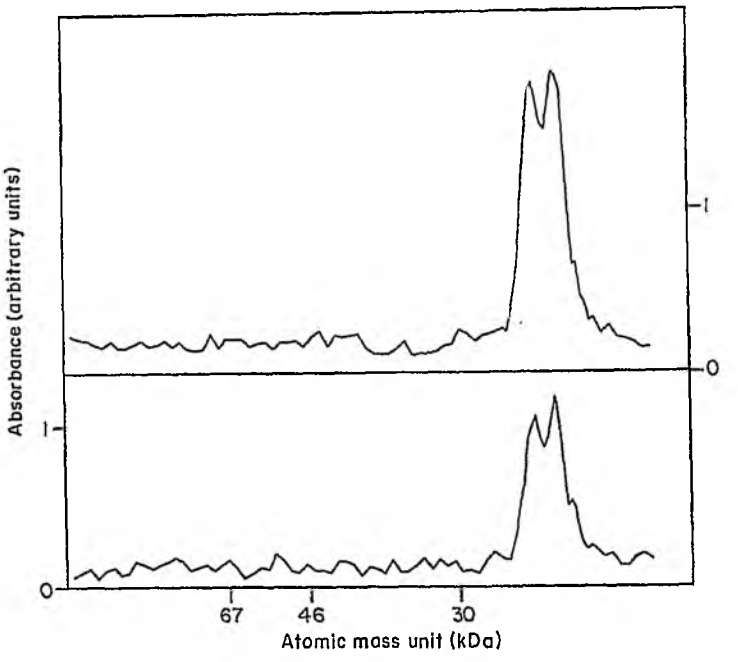

FIG. 1. Prolactin cell synthetic activity in control and low magnesium fed freshwater tilapia after 19 weeks. The prolactin synthesis rate of prolactin lobes was estimated on the basis of the quantitation of newly synthesized (^{3}H-leucine labelled) prolactins (21 and 19 kDa species) separated by SDS-gel electrophoresis and visualized by fluorography. The atomic mass of marker proteins is indicated on the X-axis; the Y-axis gives the absorbance of the fluorographs (arbitrary units). The absorbance of the 21 kDa prolactin of the controls was designated 1 (lower scan; left-hand Y-axis). In all cases the experimental fish had higher prolactin synthetic activity (upper scan).

et al., 1989*b*). Briefly, the fish were exposed to ^{24}Na (0·4 MBq l^{-1}) in a total volume of 3 l. After 2 h the fish were rinsed with tap water (2 × 1 min) and once with tap water containing 35 mmol l^{-1} NaCl. Next the fish were killed by spinal transection, microwave cooked and homogenized with water (65% of the body wet weight). Quintuple samples of approximately 5 g, weighed to the nearest 3 decimal places, were analysed for ^{24}Na γ-emission, using a LKB Ultrogamma II γ-ray spectrometer.

The rate of Na^+ loss (F_{out}) was calculated as the difference between F_{net} and F_{in}.

OPERCULAR CHLORIDE CELL DENSITY

Freshly dissected opercula were incubated for 60 min in tap water containing 20 μmol l^{-1} DASPEI (ICN, Biomedicals, Plainview, U.S.A.), a vital stain for mitochondria. Next, the opercula were rinsed in tap water and the density of DASPEI stainable cells established at anatomically fixed spots as described by Foskett *et al.* (1981). Twenty-five sites per fish with a total surface area of 5 mm^2 in a single operculum were scored. The opercular chloride cell density is expressed in the number of DASPEI-stainable cells per mm^2.

BRANCHIAL Na^+/K^+-ATPase

Soft tissue of the gills was scraped off onto an ice-cooled glass plate and collected in an isotonic buffer containing 300 mmol l^{-1} sucrose, 20 mmol l^{-1} Hepes/Tris pH 7·4, 0·1 mmol l^{-1} EDTA, 0·1 mmol l^{-1} dithiothreitol, 100 U ml^{-1} aprotinin and 50 U ml^{-1} sodium heparin. The scrapings were homogenized in this buffer using a tight glass-to-glass Potter homogenizer. Protein was determined with a protein assay (Biorad) using bovine serum albumin as a reference protein. Substrate accessibility was optimized (Flik *et al.*, 1990) by treatment with the detergent saponin (20 μg mg protein^{-1}). The Na^+/K^+-ATPase activity was determined by the method of Bonting & Caravaggio (1963).

STATISTICS

Values are expressed as mean values ± S.D., unless stated otherwise. Differences between mean values were statistically evaluated with the Mann–Whitney U-test. Significance was accepted for $P<0{\cdot}05$.

III. RESULTS

BLOOD AND BLOOD PLASMA ANALYSES

In the short term no differences were found in plasma total magnesium and calcium and plasma ultrafiltrable magnesium and calcium concentrations between control and experimental fish. The mean plasma concentrations of the six time points ($n=30$) for control and experimental fed tilapia between 0·5 and 9 weeks were for total magnesium $0{\cdot}95\pm0{\cdot}12$ and $0{\cdot}95\pm0{\cdot}13$ mmol l^{-1} and for ultrafiltrable magnesium $0{\cdot}57\pm0{\cdot}07$ and $0{\cdot}59\pm0{\cdot}06$ mmol l^{-1}, respectively. For control and experimental fish, the calcium values were $2{\cdot}74\pm0{\cdot}14$ and $2{\cdot}75\pm0{\cdot}10$ mmol l^{-1} and for ultrafiltrable calcium $1{\cdot}42\pm0{\cdot}05$ and $1{\cdot}44\pm0{\cdot}09$ mmol l^{-1}, respectively. Insufficient blood was obtained to determine ionized Ca^{2+}.

Also, in the long term, no significant differences could be demonstrated in blood plasma parameters between fish on a control diet and those on the low magnesium diet, both after 8 and 19 weeks. As no differences in the conditions with time were observed, the data were pooled. For control and experimental fish, the haematocrit (%) was $28{\cdot}1\pm3{\cdot}5$ ($n=12$) and $27{\cdot}7\pm4{\cdot}5$ ($n=13$), the plasma osmolarity (mosmol kg^{-1}) was 329 ± 7 ($n=12$) and 326 ± 7 ($n=13$) and the blood Ca^{2+} (mmol l^{-1}) was $1{\cdot}72\pm0{\cdot}03$ ($n=12$) and $1{\cdot}69\pm0{\cdot}08$ ($n=13$), respectively.

PROLACTIN CELL ACTIVITY

As judged from the ultrastructure, the prolactin cells of the experimental tilapia compared to control or untreated fish showed clear signs of stimulation (Fig. 2). In the experimental fish the rough endoplasmic reticulum and the Golgi areas were more extensive, and frequently enlarged mitochondria were observed. The rate of prolactin synthesis of the pituitary prolactin cells *in vitro* was enhanced, after 8 weeks ($20\pm11\%$; $P<0{\cdot}05$; $n=5$) as well as after 19 weeks ($70\pm23\%$; $P<0{\cdot}001$; $n=5$) on the low-magnesium diet.

SODIUM BALANCE

The mean sodium accumulation, F_{net}, was calculated as $0{\cdot}7\pm0{\cdot}21$ µmol h^{-1} 100 g^{-1} for the controls and decreased by 37% to $0{\cdot}53\pm0{\cdot}33$ µmol h^{-1} 100 g^{-1} ($P<0{\cdot}01$; $n=19$) in the experimentals. In fish feeding on the low magnesium diet, F_{in} had decreased by 35% to $5{\cdot}5\pm1{\cdot}6$ µmol h^{-1} per 100 g fish compared to the controls ($8{\cdot}5\pm1{\cdot}8$ µmol h^{-1}; $n=5$; $P<0{\cdot}025$). The calculated mean sodium loss, F_{out} ($=-\{F_{net}-F_{in}\}$), was 7·8 and 5·0 µmol h^{-1} 100 g^{-1} for controls and experimentals, respectively.

OPERCULAR CHLORIDE CELL DENSITY

Tilapia fed the low magnesium diet had significantly higher densities of chloride cells in the opercular epithelium both after 8 weeks (n $mm^{-2}\pm$S.E.M.: $52{\cdot}5\pm5{\cdot}3$ and $222{\cdot}5\pm14{\cdot}9$ for controls and experimentals, respectively; $n=5$) and after 19 weeks ($56{\cdot}1\pm4{\cdot}1$ and $174{\cdot}9\pm10{\cdot}0$ for controls and experimentals, respectively; $n=5$).

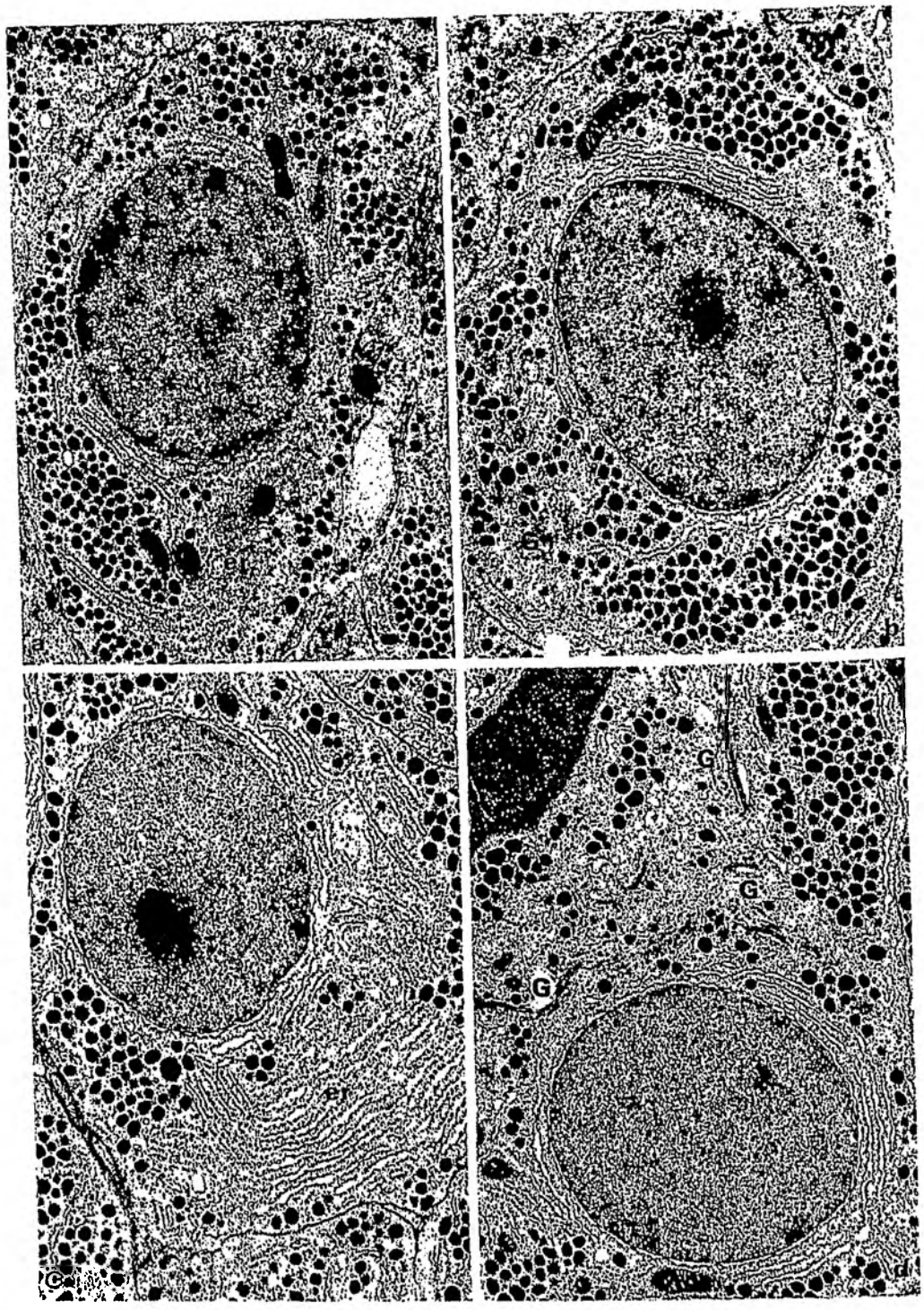

FIG. 2. Electron micrographs of prolactin cells of freshwater tilapia; er, endoplasmic reticulum; G, Golgi area; 9000×. (a) Untreated control (fish kept under standard laboratory conditions). (b) Control fish on experimental diet with normal magnesium content (30 mmol kg^{-1}). (c) and (d) Fish on the low magnesium diet; compared to the controls the cells are enlarged and the endoplasmic reticulum (c) and Golgi areas (d) are more extensive.

BRANCHIAL Na^+/K^+-ATPase

Tilapia fed the low magnesium diet for 3 weeks had a significantly higher Na^+/K^+-ATPase activity in the branchial epithelium ($22{\cdot}9 \pm 2{\cdot}3$ μmol $P_i\ h^{-1}$ mg protein^{-1}; $n=5$) than tilapia fed the control diet ($12{\cdot}4 \pm 3{\cdot}1$ μmol $P_i\ h^{-1}$ mg protein^{-1}; $n=5$; $P<0{\cdot}01$). However after 9 weeks of low magnesium feeding no significant difference was found compared to controls ($13{\cdot}2 \pm 2{\cdot}6$ and $12{\cdot}2 \pm 2{\cdot}5$ μmol $P_i\ h^{-1}$ mg protein^{-1} for experimentals and controls, respectively; $n=5$).

IV. DISCUSSION

BLOOD AND BLOOD PLASMA ANALYSES

We have determined blood ionic calcium and plasma ultrafiltrable magnesium and calcium, as the free fractions of these elements in the blood have been shown to be the most sensitive and physiologically important ones (Alvarez-Leefmans *et al.*, 1987). No acute effects on plasma total and ionic magnesium and calcium concentrations were found. The values for plasma concentrations reported here are similar to those reported earlier for this species (Wendelaar Bonga *et al.*, 1983; Flik *et al.*, 1985*a*; van der Velden *et al.*, 1989). We then tested long term effects of the diet but the low magnesium diet did not affect any of the blood parameters either after 8 or after 19 weeks. We have taken the absence of effects on plasma osmolarity to indicate that no changes occurred in plasma sodium and chloride content since these ions determine for the major part plasma osmolarity. Changes in haematocrit values may indicate ionoregulatory failure (Milligan & Wood, 1982), but we found no change in haematocrit.

The absence of an effect of the low magnesium diet on plasma magnesium levels in the tilapia contrasts with reports on other species fed comparable diets. For rainbow trout *Oncorhynchus mykiss* (Walbaum), Channel catfish and Nile tilapia *Oreochromis niloticus* (L.) significant hypomagnesaemia was reported after the fish had been fed low magnesium diets (Cowey *et al.*, 1977; Gatlin *et al.*, 1982; Knox *et al.*, 1983; Dabrowska *et al.*, 1989). In common carp fed the same diet as used in the present study, the plasma magnesium levels were reduced by more than 50% (van der Velden, 1991). We interpret the lack of an effect on plasma magnesium in the tilapia to indicate a magnesium homeostasis, even at low dietary magnesium availability.

These observations prompted us to analyse the prolactin cell activity of these fish, as it has been previously shown that in tilapia prolactin is an important ionoregulatory hormone (Foskett *et al.*, 1981; Nicoll *et al.*, 1981; Wendelaar Bonga *et al.*, 1983; Flik *et al.*, 1989*a*,*b*).

PROLACTIN CELL ACTIVITY

We found evidence for enhanced prolactin cell activity in tilapia on a low magnesium diet. First, the prolactin cells showed ultrastructural signs of enhanced secretory activity. Second, the rate of production of prolactin as estimated on the basis of the production of newly synthesized prolactin *in vitro*, had increased both after 8 and 19 weeks of feeding on a low magnesium diet. Apparently the prolactin cells become mildly but chronically activated under these conditions. In this respect, the effects of a low magnesium diet resemble the effects of long term acid

stress or of low water calcium levels on the prolactin cells in the same species (Wendelaar Bonga *et al.*, 1983; Flik *et al.*, 1989*b*).

From the data presented in this report we conclude that prolactin is implicated in the physiological acclimation to dietary magnesium deficiency of this fish. There is consensus that fish prolactin controls the integumental permeability to sodium and calcium (Mayer Gostan *et al.*, 1987; Flik *et al.*, 1986, 1989*a,b*). We suggest that the control by prolactin over the integumental permeability to ions also concerns magnesium. The mechanisms by which prolactin influences magnesium physiology need further study.

SODIUM BALANCE

Sodium intake from the water had significantly decreased in fish fed a low magnesium diet. The sodium intake in the control fish was comparable to that found by Dharmamba & Maetz (1972) in 16–24 g tilapia (15 μmol h^{-1} 100 g^{-1}) but somewhat lower than that reported by us for small tilapia (around 6 g; 45 μmol h^{-1} 100 g^{-1}; Flik *et al.*, 1989*b*). We ascribe this difference to the larger size (around 200 g) of the fish used in the experiments described here.

In low magnesium fed tilapia the sodium concentration in scales, bone and soft tissue had not changed over a 19 week period (van der Velden *et al.*, 1991*c*). We therefore may assume that the total body sodium pool of the fish is not influenced by the low magnesium diet. Hence a decreased sodium intake from the water as observed in the present experiments must have been compensated for by increased intestinal intake, by decreased losses of sodium, or both.

In our experimental set up it is difficult to determine the contribution of dietary sodium intake to the sodium balance of the fish. There is sodium present in the diet (108 mmol kg^{-1}) and indeed increases in dietary sodium have been shown to decrease branchial sodium uptake in trout (Salman, 1987). In our control and experimental conditions, however, the dietary (and water) sodium contents were the same and therefore we predict that no differences occur in intestinal sodium uptake. Moreover, the calculated loss of sodium in the fish on a low magnesium diet had decreased, and this indicates that the permeability to sodium of the gills and possibly the intestine was lower in these fish. This state of decreased integumental permeability to sodium is in accordance with an enhanced prolactin cell activity as discussed above. We have recently shown that water acidification in the long term provokes in tilapia a similar decrease in sodium turnover (lower intake and lower loss) together with an activation of prolactin cells (Flik *et al.*, 1989*b*).

OPERCULAR CHLORIDE CELL DENSITY

The opercular chloride cell density in our control fish was comparable to the value reported for the same species kept under similar water conditions (Wendelaar Bonga *et al.*, 1990). The 4·2-fold increase after 8 weeks and the 3·1-fold increase after 19 weeks indicates that the low magnesium diet chronically increased chloride cell density. This situation is strongly reminiscent of the increased densities of chloride cells seen during long term acid exposure and during low calcium exposure of this species (Wendelaar Bonga *et al.*, 1983; Wendelaar Bonga & van der Meij, 1989). It was suggested in the latter report that the number of DASPEI-stainable cells was not related to the ion transporting capacity of the gills. Indeed in tilapia exposed for 13 weeks to water of pH 4·5, an increased chloride cell number was

observed together with a decrease in sodium intake and loss, and with increased prolactin secretion. Clearly, ultrastructural studies must reveal the number of mature and functional branchial chloride cells to properly correlate chloride cell density with ion transport capacity (Wendelaar Bonga *et al.*, 1990). We tentatively conclude that the opercular chloride cell density is a useful parameter for increased prolactin cell activity in fish under low magnesium stress.

BRANCHIAL Na^+/K^+-ATPase

The Na^+/K^+-ATPase activity of the branchial epithelium found in the controls is in the same order as that found in earlier experiments with tilapia, viz. $8{\cdot}1 \pm 2{\cdot}9$ μmol P_i h^{-1} mg protein^{-1} (Flik *et al.*, 1985*b*). The transient increase of the branchial Na^+/K^+-ATPase activity after 3 weeks of low magnesium feeding points to an initially disturbed ionoregulation. After 8 weeks of low magnesium feeding differences were no longer observed.

The transient increase in Na^+/K^+-ATPase activity appeared somehow linked to the increased opercular chloride cell density observed under low magnesium feeding. However what this increase means for the ion balance of the fish during acclimation is not clear. One explanation is that the composition of the branchial epithelium changes and that a new chloride cell population with different enzyme content is recruited. It has been reported before that branchial transport enzyme content (and chloride cell density) are not directly related to the ion transport capacity, but do indicate acclimatory responses (Wendelaar Bonga & van der Meij, 1989; Flik *et al.*, 1989*b*).

Feeding freshwater tilapia a low-magnesium diet results in a changed ionoregulation of the fish. This is not reflected by the blood ionocomposition; however, changes of prolactin cell activity, sodium turnover, opercular chloride cell density and branchial Na^+/K^+-ATPase activity are evident. In the long term, tilapia manage to cope with a dietary Mg^{2+} insufficiency and prolactin appears to be involved in this acclimation.

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References

Alvarez-Leefmans, F. J., Giraldez, F. & Gamino, S. M. (1987). Intracellular free magnesium in excitable cells: its measurement and its biologic significance. *Canadian Journal of Physiology and Pharmacology* **65**, 915–925.

Bonner, W. J. & Laskey, R. A. (1974). A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. *European Journal of Biochemistry* **46**, 83–88.

Bonting, S. L. & Caravaggio, L. L. (1963). Studies on Na^+/K^+-activated ATPase. V. Correlation of enzyme activity with cation fluxes in tissues. *Archives of Biochemistry and Biophysics* **101**, 37–71.

Cowey, C. B., Knox, D., Andron, J. W., George, S. & Pirie, B. (1977). The production of renal calcinosis by magnesium deficiency in rainbow trout (*Salmo gairdneri*). *British Journal of Nutrition* **38**, 127–135.

Dabrowska, H., Meyer-Burdorff, K. & Günther, K.-D. (1989). Interaction between dietary protein and magnesium level in tilapia (*Oreochromis niloticus*). *Aquaculture* **76**, 277–291.

Dharmamba, M. & Maetz, J. (1972). Effects of hypophysectomy and prolactin on the sodium balance of *Tilapia mossambicus* in fresh water. *General and Comparative Endocrinology* **19**, 175–183.

Flik, G., Fenwick, J. C., Kolar, Z., Mayer-Gostan, N. & Wendelaar Bonga, S. E. (1985*a*). Whole body calcium flux rates in cichlid teleost fish *Oreochromis mossambicus* adapted to freshwater. *American Journal of Physiology* **249**, R432–R437.

Flik, G., van Rijs, J. H. & Wendelaar Bonga, S. E. (1985*b*). Evidence for high-affinity Ca^{2+}-ATPase activity and ATP-driven Ca^{2+}-transport in membrane preparations of the gill epithelium of the cichlid fish *Oreochromis mossambicus*. *Journal of Experimental Biology* **119**, 335–347.

Flik, G., Fenwick, J. C., Kolar, Z., Mayer-Gostan, N. & Wendelaar Bonga, S. E. (1986). Effects of ovine prolactin on calcium uptake and distribution in *Oreochromis mossambicus*. *American Journal of Physiology* **250**, R161–R166.

Flik, G., Fenwick, J. C. & Wendelaar Bonga, S. E. (1989*a*). Calcitropic actions of prolactin in freshwater North American eel (*Anguilla rostrata* LeSueur). *American Journal of Physiology* **257**, R74–R79.

Flik, G., van der Velden, J. A., Seegers, H. C. M., Kolar, Z. & Wendelaar Bonga, S. E. (1989*b*). Prolactin cell activity and sodium fluxes in tilapia (*Oreochromis mossambicus*) after long-term acclimation to acid water. *General and Comparative Endocrinology* **75**, 39–45.

Flik, G., Schoenmakers, T. J. M., Groot, J. A., van Os, C. H. & Wendelaar Bonga, S. E. (1990). Calcium absorption by fish intestine: the involvement of ATP- and sodium-dependent calcium extrusion mechanisms. *Journal of Membrane Biology* **113**, 13–22.

Fogh-Anderson, N. (1981). Ionized calcium analyzer with built-in pH correction. *Clinical Chemistry* **27**, 1264–1267.

Foskett, J. K., Logsdon, C. D., Turner, T., Machen, T. E. & Bern, H. A. (1981). Differentiation of the chloride extrusion mechanisms during seawater adaptation of a teleost fish, the cichlid *Sarotherodon mossambicus*. *Journal of Experimental Biology* **93**, 209–224.

Gatlin, D. M., Robinson, E. H., Poe, W. E. & Wilson, R. P. (1982). Magnesium requirement of fingerling channel catfish and signs of magnesium deficiency. *Journal of Nutrition* **112**, 1182–1187.

Knox, D., Cowey, C. B. & Adron, J. W. (1983). Studies on the nutrition of rainbow trout (*Salmo gairdneri*) Magnesium deficiency: the effect of feeding with a Mg-supplemented diet. *British Journal of Nutrition* **50**, 121–127.

Laemmli, U. K. (1970). Cleavage of the structural proteins during assembly of the head of bacteriophage T4. *Nature (London)* **227**, 680–685.

Littledike, E. T. & Goff, J. (1987). Hormonal control of magnesium metabolism. Parathyroid hormone and vitamin D metabolites. In *Magnesium in Cellular Processes and Medicine* (Altura, B. M., Durlach, J. & Seelig, M. S., eds), pp. 164–182. Basel: Karger.

Mayer-Gostan, N., Wendelaar Bonga, S. E. & Balm, P. H. M. (1987). Mechanisms of hormone actions on gill transport. In *Vertebrate Endocrinology: Fundamentals and Biomedical Implications*. Vol. 2. *Regulation of Water and Electrolytes* (Pang, P. K. T. & Schreibman, M. P., eds), pp. 211–238. San Diego: Academic Press.

Milligan, C. L. & Wood, C. M. (1982). Disturbances in haematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. *Journal of Experimental Biology* **99**, 397–415.

Nicoll, C. S., Wilson, W. S., Nishioka, R. S. & Bern, H. A. (1981). Blood and pituitary prolactin levels in tilapia (*Sarotherodon mossambicus*: Teleostei) from different salinities as measured by a homologous radioimmunoassay. *General and Comparative Endocrinology* **44**, 365–373.

Ogino, C. & Chiou, J. Y. (1976). Mineral requirements in fish—II. Magnesium requirement of carp. *Bulletin of the Japanese Society of Scientific Fisheries* **42**, 71–75.

Salman, N. A. (1987). Nutritional and physiological effects of dietary NaCl on rainbow trout (*Salmo gairdneri* Richardson). Ph.D. thesis, University of Dundee, U.K.

Shearer, K. D. (1989). Whole body magnesium concentration as an indicator of magnesium status in rainbow trout (*Salmo gairdneri*). *Aquaculture* **77**, 201–210.

Shim, K. F. & Ng, S. H. (1988). Magnesium requirement of the guppy (*Poecilia reticulata* Peters). *Aquaculture* **73**, 131–141.

van der Velden, J. A., Kolar, Z., Flik, G., De Goeij, J. J. M. & Wendelaar Bonga, S. E. (1989). Magnesium distribution in freshwater tilapia. *Magnesium-Bulletin* **11**, 28–33.

van der Velden, J. A., Groot, J. A., Flik, G., Polak, P. & Kolar, Z. I. (1990). Magnesium transport in fish intestine. *Journal of Experimental Biology* **152**, 587–592.

van der Velden (1991). Magnesium and its transport in tilapia and carp: a study based on nuclear methods. Ph.D. thesis, Delft University of Technology, The Netherlands.

van der Velden, J. A., Kolar, Z. I. & Flik, G. (1991*a*). Intake of magnesium from water by freshwater tilapia fed a low magnesium diet. *Comparative Biochemistry and Physiology* **99A**, 103–105.

van der Velden, J. A., Kolar, Z. I., Flik, G., Polak, P., de Goeij, J. J. M. & Wendelaar Bonga, S. E. (1991*b*). Magnesium transport between carp and water studied with $^{28}Mg^{2+}$ as radiotracer. *Applied Radiation and Isotopes* **42**, 347–352.

van der Velden, J. A., Spanings, F. A. T., Flik, G., Zegers, C., Kolar, Z. I. & Wendelaar Bonga, S. E. (1991*c*). Growth rate and tissue magnesium concentration in adult freshwater tilapia (*Oreochromis mossambicus* Peters) fed diets differing in magnesium content. *Journal of Fish Biology* **39**, 83–91.

Wendelaar Bonga, S. E., Löwik, C. J. M. & van der Meij, J. C. A. (1983). Effects of external Mg^{2+} and Ca^{2+} on branchial osmotic water permeability and prolactin secretion in the teleost fish *Sarotherodon mossambicus*. *General and Comparative Endocrinology* **52**, 222–231.

Wendelaar Bonga, S. E. & van der Meij, J. C. A. (1989). Degeneration and death, by apoptosis and necrosis, of the pavement and chloride cells in the gills of the teleost *Oreochromis mossambicus*. *Cell and Tissue Research* **255**, 235–243.

Wendelaar Bonga, S. E., Flik, G., Balm, P. H. M. & van der Meij, J. C. A. (1990). The ultrastructure of chloride cells in the gills of the teleost *Oreochromis mossambicus* during exposure to acidified water. *Cell and Tissue Research* **259**, 575–585.