Magnesium Distribution in Freshwater Tilapia

J. A. van der Velden [1, 2], Z. Kolar [1], G. Flik [2], J. J. M. de Goeij [1], S. E. Wendelaar Bonga [2]

Zusammenfassung

Der Magnesium- und Kalziumgehalt verschiedenartiger Gewebe der Tilapia (Oreochromis mossambicus) ist durch Neutronenaktivierungsanalyse oder Spektrophotometrie ermittelt worden. Die Konzentrationen des Magnesiums im Verhältnis zur trockenen Masse schwankten zwischen 18 mmol/kg^{-1} (Blutplasma) und 140 mmol/kg^{-1} (Knochen). In der wachsenden Tilapia ändern sich die Massenverhältnisse der Kompartimente relativ zur Körperschwere und deswegen soll man bei der Extrapolation der Verhältnisse zwischen dem totalen Magnesiumgehalt und der Schwere des Körpers sorgfältig vorgehen. Wir postulieren einen homöostatischen Mechanismus des Magnesiumhaushalts bei Süßwasserknochenfischen.

Summary

The magnesium and calcium quantity in several tissues of tilapia (Oreochromis mossambicus) was determined by neutron activation analysis or colorimetric methods. Magnesium concentrations on the basis of dry weight ranged from 18 mmol.kg^{-1} (blood plasma) to 140 mmol.kg^{-1} (skeletal bone). In the growing tilapia, the size of the body magnesium pools as a fraction of the total body weight changes, and care should be taken to extrapolate relations between the whole body magnesium quantity and body weight. It is suggested that a magnesium homeostatic mechanism is present in freshwater teleosts.

Résumé

On détermine la proportion de magnésium et de calcium des tissues de la tilapia *Oreochromis mossambicus)* à l'aide de l'analyse par l'activation neutronique ou par méthodes spectrophotométriques. Les concentrations de magnésium relatives à la sèche varient de 18 mmol.kg^{-1} (plasma de sange) à 140 mmol.kg^{-1} (l'ossature). Dans la tilapia croissante, les proportions des compartiments (du corps) relatifs au poids corporel varient, par conséquence il faut de la prudence quand on veut extrapoler, les relations entre l'inventoire de magnésium du corps et le poids corporel. On postule qu'il y a une méchanisme homéostatique pour la métabolisme de magnésium dans les poissons téléostéens d'eau douce.

1) Interfaculty Reactor Institute, Delft University of Technology
2) Department of Animal Physiology, University of Nijmegen

Introduction

Magnesium is an essential element and plays a pivotal role in the physiology of the cell, i. a. as an activator of a great number of enzymes. The biological significance of magnesium has been reviewed by *Ebel* and *Günther* [12] and *Aikawa* [1]. It is assumed that higher vertebrates strive for magnesium homeostasis. However, the transport of magnesium in transport epithelia such as gut, kidney and gills, which are involved in this homeostasis, is still poorly understood.

For the study of ion transport, the epithelia of freshwater fish provide excellent and well-established experimental models, viz. the gills for a tight epithelium [32] and the gut for a leaky epithelium [29].

For an understanding of magnesium metabolism and homeostasis in fish it is essential that information is available on the quantity of magnesium in different parts of the fish. So far only a few studies have evaluated the magnesium distribution in freshwater teleosts; no studies of the total magnesium inventory were available. This study reports on the magnesium concentrations in tissues and the magnesium inventory of the tilapia. A comparison was made with calcium.

Materials and Methods

Fish

Male tilapia (Oreochromis mossambicus) ranging in body weight from 25 to 52 g were obtained from laboratory stock. The fish were kept in fresh water which contained 0.2 mmol.l^{-1} $MgSO_4$, 0.2 mmol.l^{-1} $CaCl_2$, 0.5 mmol.l^{-1} NaCl and 0.06 mmol.l^{-1} KCl. It was maintained at pH 7.5 through the addition of tris-hydroxy-methyl-aminomethane (TRIS)-HCl buffer (2.5 mmol.l^{-1}). The water was constantly aerated and kept at 28°C. The photoperiod was 12 hours. The fish received six rations of tropical fish food (Tetramin; Melle, FRG) per day by means of an automated food dispenser.

Sampling

The fish were anesthetized with tricaine methane sulphate (MS-222; 0.4 mmol.l^{-1}) and weighed. Mixed arterial and venous blood was collected by puncture of the caudal vessels behind the anal fin using an ammonium heparin-rinsed tuberculin syringe. The blood was separated into plasma and cells by centrifugation at 9000 g for 3 min. Part of the blood plasma was ultrafiltered using micro-collodion bags (Sartorius SM 13202; Göttingen, FRG). Samples were stored at $-20°C$. The scales, the testis, the intestinal tract including the stomach, the kidneys, the gills and the brain were collected separately; the branchial epithelium was scraped from the gill arches. After 1 minute of pressure cooking [13] muscle was collected and the bone was divided into dermal, skeletal and head-region scalar bone. The remaining tissues were designated as "rest soft tissue". All fractionated material was weighed, lyophilized (except blood plasma) and weighed again.

Analytical Methods

The determination of magnesium and calcium in the tissues was performed by instrumental neutron activation analysis (INAA) [6]. Lyophilized samples of 5–150 mg (weight depending on the material available) were encapsulated in 0.5 ml polyethylene vials and irradiated with a thermal neutron flux of 10^{17} $m^{-2}.s^{-1}$ for 30 s. After a 20 s delay, the samples were measured in a well-type Ge(Li) semiconductor γ-ray detector connected to a computerized multi-channel analyzer. Magnesium was measured via ^{27}Mg (half-life 9.5 min, $E_\gamma =$ 844 and 1015 keV) and calcium via ^{49}Ca (half-life 8.72 min, E_γ = 3084 keV). Possible interferences due to the reaction $^{27}Al(n,p)^{27}Mg$ were checked and found to be negligible. Calibration was performed via zinc flux monitors irradiated simultaneously with the samples [6]. After every eight samples, a sample of "Standard Reference Material 1571 Orchard Leaves" was included. The average concentrations of magnesium and calcium found in this material were 259 $mmol.kg^{-1}$ (0.63 Wt.%) and 524 $mmol.kg^{-1}$ (2.1 Wt.%) respectively, while the certified values were 0.62 ± 0.02 Wt.% and 2.09 ± 0.03 Wt.% respectively [23]. Thus there was no evidence for a systematic error.
The magnesium concentrations in the blood plasma and ultrafiltered blood plasma were colorimetrically determined with a magnesium kit (Sigma diagnostic kit no 595; St Louis, USA) based on measurement of a colored complex of magnesium and calmagite (1-[1-hydroxy-4-methyl-2-phenylazo]-2-naphthol-4-sulfonic acid) at 520 nm. The calcium concentrations in blood plasma and ultrafiltered blood were also colorimetrically determined with a calcium kit (Sigma diagnostic kit no 587; St Louis, USA).

Data Handling

Data were statistically evaluated by the *Mann-Whitney* U-test (one-tailed) or by linear regression analysis (based on the least squares method), in a number of cases after natural logarithmic transformation of the data. Statistical significance was accepted at the 1 % level. The error margins indicated are standard deviations.

Results

Size of the major Parts of the Body

The sizes of the three major parts of the body (scales, bone and soft tissue) correlate with the total dry weight ($5.7\ g < W_{d,t} < 12.8\ g$) of the fish as follows:

$$W_{d,sc} = 0.275\ W_{d,t}^{0.455} \quad (1)$$
[n = 6; r = 0.976]

$$W_{d,bo} = 0.592\ W_{d,t}^{0.891} \quad (2)$$
[n = 6; r = 0.994]

$$W_{d,st} = 0.282\ W_{d,t}^{1.21} \quad (3)$$
[n = 6; r = 0.997]

where $W_{d,t}$ = total dry weight of the fish (g), $W_{d,sc}$ = dry weight of the scales (g), $W_{d,bo}$ = dry weight of the total bone (g) and $W_{d,st}$ = dry weight of the total soft tissues (g). Since the sizes of these body parts depend on the total body weight in a particular manner (cf. the exponents in equations 1, 2 and 3), the contribution of each body part to the total fish is a nonlinear function of the weight of the fish.
The mean body water content of the experimental fish was determined to be 74.7 ± 2.5 %.

Magnesium and Calcium Concentration in the issues

The concentration of magnesium and calcium in the various tissues showed no correlation with the body dry weight of the fish. For this reason, the data obtained for all fish analyzed were pooled per element and per tissue or body part. The average concentrations of magnesium and calcium are presented in Table 1 (part A) and Table 2 (part A) respectively. The magnesium concentration in blood plasma and ultrafiltered blood plasma (n=6) amounted to 1.12 ± 0.14 $mmol.l^{-1}$ (wich is equivalent to 18 $mmol.kg^{-1}$ dry material) and 0.51 ± 0.11 $mmol.l^{-1}$ respectively. The calcium concentration in blood plasma and ultrafiltered blood plasma (n=6) was 3.31 ± 0.04 and 1.86 ± 0.11 $mmol.l^{-1}$ respectively.

Magnesium and Calcium Inventory

In three fish weighing about 8 g, magnesium and calcium inventories were calculated as the product of the mean concentration and the weight of the tissues. The inventories of magnesium and calcium are given in Table 1 (part B) and in Table 2 (part B) respectively. The calcium concentration in some soft tissues samples was below the INAA detection limit; for this reason a value determined in earlier experiments [14] has been inserted here.

For a 8 gram tilapia the distribution of magnesium and calcium is as follows: Magnesium is present predominantly in the bone (62.4 %) and the soft tissues (26.9 %); a minor fraction (10.8 %) is located in the scales. The picture for calcium is different. Although the largest fraction (81.1 %) is also present in bone; the scales account for a substantial part (17.6 %), and only a minute fraction (0.3 %) of the calcium is located in the soft tissues.

Figure 1 shows the quantity of magnesium in the scales (Q_{scales}), total soft tissues ($Q_{soft\ tissue}$) and total bone (Q_{bone}) as a function of the weight. The quantities of the three body parts are calculated as the product of the calculated weight (according to equation 1, 2 and 3) and the mean magnesium concentration in these body parts.

Whole Body Magnesium Inventory

The quantity of magnesium in the whole fish, calculated from the whole body magnesium quantities of the experimental fish, can be described as follows:

$$Q_{fish} = 0.1163\ W_{d,t}^{0.896} \quad (4)$$
$$[n = 6; r = 0.961]$$

in which Q_{fish} = total magnesium quantity (mmol). This relationship is shown in Figure 1.

As can be seen from equation 4, the magnesium inventory is not linearly related to the fish weight; thus the overall magnesium concentration is a function of the fish weight. For instance, the average magnesium concentration calculated by means of equation 4 for 6 g and 12 g dry weight tilapias is 97 mmol.kg^{-1} and 90 mmol.kg^{-1} respectively.

Tab. 1: A: Magnesium concentration (± SD) in various tissues of freshwater tilapia. The number of observations are in brackets; W_d stands for dry weight (g).
B: Mean weight of tissues of 3 freshwater tilapia's with a dry weight of circa 8 gram and the magnesium quantity of these tissues.

	A Magnesium concentration (mmol.kg^{-1} W_d)		B Magnesium inventory of 8 gram tilapia: weight (g)		quantity (mmol)	
muscle	61 ± 8 (5)		2.420		0.148	
kidney	44 ± 9 (5)		0.013		0.57 10^{-3}	
liver	42 ± 3 (3)		0.052		2.18 10^{-3}	
brain	27 ± 7 (5)		0.018		0.49 10^{-3}	
testis	37 ± 12 (6)		0.021		0.78 10^{-3}	
gills	57 ± 10 (5)		0.028		1.65 10^{-3}	
gallbladder	29 ± 2 (3)		0.021		0.61 10^{-3}	
packed cells	28 ± 5 (4)		0.034		0.95 10^{-3}	
intestinal tract	45 ± 15 (6)		0.157		7.07 10^{-3}	
rest soft tissue	60 ± 11 (4)		0.671		4.03 10^{-2}	
Total soft tissue		60		3.462		0.203
dermal bone	138 ± 36 (6)		1.508		0.208	
skeletal bone	140 ± 19 (6)		1.561		0.219	
scalar bone	66 ± 11 (6)		0.714		0.047	
Total bone		122		3.785		0.474
scales	115 ± 18 (6)		0.712		0.082	
Total scales		115		0.712		0.082
Total body				7.959		0.759

Tab. 2: A: Calcium concentration (± SD) in various tissues of freshwater tilapia. The number of observations are in brackets; W_d stands for dry weight (g).
B: Mean weight of tissues of 3 freshwater tilapia's with a dry weight of circa 8 gram and the calcium quantity of these tissues.

	A Calcium concentration (mmol. kg^{-1} W_d)		B Calcium inventory of 8 gram tilapia: weight (g)		quantity (mmol)
Total soft tissue		13 *		3.462	44.8 10^{-3}
dermal bone	4 379 (6)		1.508		
skeletal bone	4 276 (6)		1.561		
scalar bone	716 (6)		0.714		
Total bone		3 600		3.785	13.78
Total scales		4 150		0.712	2.95
Total body				7.959	16.77

* Literature data of *Flik* et al. [14]

Discussion

Magnesium Concentrations in the Tissues

There are substantial differences in magnesium concentration between the various tissues.

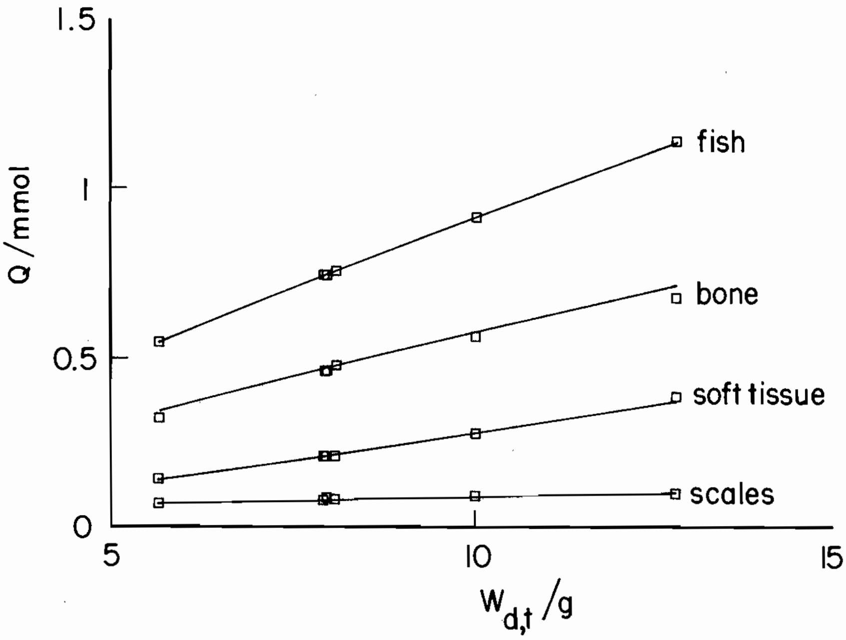

Fig. 1: Quantities of magnesium in the scales, soft tissues, total bone and whole body as function of dry weight ($W_{d,t}$ in g) of the tilapia. The magnesium quantity (µmol) of the whole fish, scales, total soft tissue and total bone is described by $Q_{fish} = 116.3\ W_{d,t}^{0.896}$, $Q_{scales} = 0.275 W_{d,t}^{0.455}$.115; $Q_{soft\ tissue} = 0.282\ Wdt^{1.21}$.60 and $Q_{bone} = 0.592\ Wdt^{0.891}$.122, respectively. The symbols indicate the actually measured inventories of the six experimental fish.

The white muscle of tilapia forms the major part of the total soft tissue magnesium pool. The muscle has a significantly higher magnesium concentration than some smaller body parts from the total soft tissue pool (plasma, packed cells, testis, brain, liver and kidney). The higher magnesium concentration in the muscle samples is in line with its higher Mg-ATP dependent myosin quantity. The magnesium concentration in the muscle may reflect the intracellular concentration. The present value for muscle magnesium concentration may be an underestimate, since no correction for extracellular space was applied. Assuming 7.54 % extracellular space as reported for the tilapia [4], we come to a magnesium quantity of 66 mmol per kg dry weight for intracellular magnesium. Magnesium can be stored intracellularly in organelles as mitochondria [12].

Like calcium, magnesium exists in blood in three fractions: *viz.* (i) free (ionic), (ii) complexed with citrate, hydrogencarbonate and phosphate, and (iii) protein-bound, non-ultrafiltrable magnesium. The former two fractions reflect the ultrafiltrable magnesium [13]. The free or ionic magnesium is thought to represent the physiologically important fraction [2].

The ultrafiltrable and the total magnesium concentrations in plasma are low, compared to the concentrations present in other tissues. However the concentration of free Mg^{2+} in plasma is of the same order as in terrestrial vertebrates. In horse plasma the free magnesium is 0.5 mmol.l^{-1} [21]. Speich et al. [31] found an ultrafiltrable magnesium concentration of 0.593 mmol.l^{-1} in human blood plasma, where 0.544 mmol.l^{-1} is ionic and 0.049 mmol.l^{-1}complexed. *Gunn* and *Burns* [15] report an ultrafiltrable magnesium concentration in human blood plasma of 0.575 mmol.l^{-1}.

The magnesium concentrations of the total bone and scales are somewhat higher than in the soft tissues. It is notable that scalar bone shows a significantly lower magnesium concentration than dermal and skeletal bone. It has been suggested that in higher vertebrates most of the skeletal magnesium is adsorbed to the mineral phase on the surfaces of apatite crystals [12, 3]. The incorporation of Mg^{2+} ions into calcium phosphates as hydroxyapatite increase the stability and forms whitelockite [11]. For fish, little is known about the chemical form of magnesium and its incorporation into bone.

Comparison with Literature Data

Literature data on magnesium concentrations in tissues of freshwater teleosts are scarce and fragmentary.

The muscle of the tilapia has a magnesium concentration of 61 mmol.kg^{-1} dry material which is equivalent to 14 mmol.kg^{-1} wet material. For the perch a wert weight muscle magnesium concentration of 15 mmol.kg^{-1} was reported [19] and for the rainbow trout *(Salmo gairdneri)* 12 and 13 mmol.kg^{-1} [17, 18].

The magnesium concentration in the kidney and liver of the tilapia is 7 and 8 mmol per kg wet material respectively. These values are similar to those reported for the kidney in rainbow trout, namely 6 and 7 mmol.kg^{-1} [10, 17, 18], and for the liver in rainbow trout 7 mmol.kg^{-1} [17] and in perch 14 mmol.kg^{-1} [19].

Houston [16] reported magnesium concentrations in the range of 5.4 to 10.4 mmol.l^{-1} cell water for packed cells in four species of teleosts. In the tilapia a concentration equivalent to 9.6 mmol.l^{-1} cell water was found.

The magnesium concentrations in blood plasma and serum from this study and from the literature, have been compared in Table 3. The magnesium concentrations for plasma found in this study are in the same range as those reported in the literature for freshwater teleosts. Since the fish compared were kept under different conditions, it appears that external conditions such as water temperature and magnesium concentration do not substantially affect plasma magnesium concentration. The blood plasma magnesium concentration seems also independent of the total body weight *Nanba et al.* [22] reported that in the carp *(Cyprinus carpio)* the magnesium concentration in the blood plasma remains constant throughout the year.

The data determined for total bone magnesium concentration are in good agreement with data by *Lutz* [20], who found a concentration of 124 $mmol.kg^{-1}$ dry material in the total bone of the perch *(Perca fluviatilis)*.

Based on the above data, we conclude that there is little variation in the concentration of magnesium in the tissues of freshwater teleosts.

Tab. 3: Data compilation of magnesium concentrations in plasma and serum in freshwater teleosts.

species	body weight (g)	n	temp (° C)	magnesium concentrtion ($mmol. l^{-1}$)			lit
				water	plasma	serum	
Anguilla anguilla		25	12	0.45		2.13±0.22	[8]
		17	10		1.25		[7]
	250–1 000	8			2.07		[9]
Carrasius auratus	30±19	67	25		0.72±0.19		[16]
			20–25			0.83	[27]
Cyprinus carpio	106±96	14	16		1.15±0.16		[16]
					0.6–1.35		[22]
Esox lucius	350–2 000		10	0.12	0.67±0.26		[33]
	240–1 410	34	7.5–16	0.06	0.77		[25]
Mugil cephalus	(length: 7.5–23.7 cm)	8	20	0.69	1.39±0.02		[24]
Oncorhynchus kisutch	10–18				0.65		[35]
tsawytsch	66	18		0.05	0.99±0.30		[30]
Oreochromis mossambicus	20	10	25	0.2	1.2		[34]
	25–52	6	28	0.2	1.12±0.14		this study
Perca fluviatilis	(length: 180–250 cm)	16	15		1.44±0.06		[19]
Salmo gairdneri	30		15			0.92	[10]
	35		15	0.15		1.06±0.06	[17]
	21		6–10	0.15	0.33		[18]
	62±2	15	8–10		0.76		[5]
	157±47	70	10		0.61±0.14		[16]
	140–190	8	11.5	0.02	0.66		[26]
Salvelinus fontinalis	420±155	24	14		0.76±0.06		[16]

Magnesium Regulation

The slight differences in magnesium concentration between the tissues of various teleosts suggest the presence of a magnesium homeostatic mechanism in teleosts. When fish indeed show magnesium homeostasis, questions arise concerning supply routes (gills, gut), magnesium bioavailability, internal storage and regulatory mechanisms.

It is probable that the external water is an important and unlimited magnesium source for fish. In higher vertebrates, the bone pool can act as a magnesium buffer [28, 3]. For calcium, fish bone contains the larger part of the whole body pool. Bone calcium may be mobilized in times of increased need for Ca^{2+} [13], although fish normally depend on the external calcium for homeostasis and growth, as has been shown for tilapia [14]. Interesting questions that require further investigation are whether fish in analogy to Ca^{2+}, extract their Mg^{2+} required for their growth and homeostasis from the external water via the gills and whether the bone can act as a magnesium reservoir.

Our forthcoming research is aimed at the delineation of magnesium transport routes in freshwater teleosts and how these contribute to homeostasis.

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For the authors: *J. A. van der Velden,* Interfaculty Reactor Institute, Delft University of Technology, Mekelweg 15, NL-2629 JB Delft, The Netherlands