Magnesium Transport between Carp and Water Studied with $^{28}\text{Mg}^{2+}$ as Radiotracer

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Neutron activation analysis and $^{28}\text{Mg}^{2+}$ as radiotracer were used to measure magnesium distribution in fish and magnesium flows between carp and water. A novel tracer kinetic approach was applied to determine magnesium flows “to fish from water” ($F_{fw}$) and “to water from plasma (fish)” ($F_{wp}$). This revealed that $F_{fw} < F_{wp}$ and thus pointing to another magnesium source than water, namely food.

Introduction

Magnesium is an essential element for all living organisms. In animal cells it is the most abundant divalent cation. In animals magnesium ions are, for example, required as an activator for many enzymatic reactions and for the initiation of DNA synthesis (Aikawa, 1981). It has been suggested that magnesium serves as a static regulator of cell function as well (Alvarez-Leefmans et al., 1987). Despite the pivotal role of magnesium in the physiology of vertebrates including fish, only a few studies (e.g. Renfro and Shustock, 1985) have dealt with the topic of transepithelial magnesium transport in fish. Recent availability of radioactive $^{28}\text{Mg}^{2+}$—an ideal tracer for magnesium ions—prompted us to study the magnesium transport between fish and water.

This investigation deals with carp. It was designed to establish the relative importance of the integument (gills and body surface) for magnesium intake by measuring the unidirectional flows of magnesium between fish and water. To our knowledge no such measurements have been reported in the literature so far. The distribution of magnesium in carp was also a subject of investigation.

Tracer Kinetic Approach

With regard to magnesium the present water–fish system may be considered as a closed multicompartment system with a number of intercompartmental connections (magnesium flows). One of the magnesium compartments represents magnesium (dissolved) in water. The other magnesium compartments are confined to the fish and represent magnesium in bones, scales, muscles, blood plasma, etc. To determine the outflow of a tracee from a compartment of such a multicompartment system, a tracer should be added to this compartment and subsequently the time curve for the tracer quantity in this compartment measured (Shipley and Clark, 1972). At zero time the tracer moves only out of this compartment while no tracer is returning from any other compartment, since none is present there. The instantaneous (down)slope of the curve for tracer quantity in the compartment to which tracer initially had been added, normalized to the tracer quantity in this compartment at zero time, is—except for the sign—equal to the rate constant of outflow of tracer. Thus for time $t = 0$,

$$-d[q(t)/q(0)]/dt = k$$

where $q(t)$ and $q(0)$ are the tracer quantities (in Bq) at time $t$ (in s) and $t = 0$, respectively, and $k$ is the rate constant (in s$^{-1}$), defined as

$$k = F/Q$$

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where $F$ is the (out)flow of tracee (in mol·s$^{-1}$) and $Q$ is the tracee quantity (in mol) in the compartment into which the tracer was added at $t = 0$. Combining equations (1) and (2) one obtains:

$$F = -Q \frac{d[q(t)/q(0)]}{dt} \tag{3}$$

or the outflow is equal to the product of the tracee quantity and the absolute value of the instantaneous (down)slope at $t = 0$.

Note that no assumption has been made with regard to the number of compartments and their connections. In this respect, the present tracer kinetic approach to ion transport in fish–water systems differs from that used by other authors (Maetz, 1956; Potts et al., 1967; Kirschner, 1970).

The outflow of magnesium from water and thus the flow of magnesium to fish from water $F_{w}$ can in principle be calculated from the magnesium quantity in water $Q_{w}$ and the slope at $t = 0$ of time curves for the tracer quantity in water $q_{w}(t)$ normalized to the tracer quantity in water at $t = 0$, $q_{w}(0)$, using equation (3). However, in practice it may be difficult to estimate the initial (down)slope of the $q_{w}(t)/q_{w}(0)$ curve due, for example, to the poor alignment of data points or inadequate steepness of the curve. In such a case one can try to measure the tracer quantity in alive fish $q_{f}(t)$ and in water at $t = 0$, $q_{w}(0)$. $F_{w}$ can then in principle be calculated from $Q_{w}$ and the (up)slope of the $q_{f}(t)/q_{w}(0)$ curves at $t = 0$ using equation (3) but omitting the negative sign. However, when the measurement of $q_{f}(t)$ is not feasible and when only the quantity of tracer in the fish at the end of an experiment $q_{f}(t_{e})$ and the tracer quantity in water at $t = 0$ can be determined, one has to use the approximation:

$$F_{w} = Q_{w}\frac{[q_{f}(t_{e})/q_{w}(0)]}{t_{e}} \tag{4}$$

where $t_{e}$ stands for the duration of the experiment. The underlying assumption for equation (4) is that $q_{f}(t_{e})/t_{e}$ is equal to $d[q_{f}(t)/q_{w}(0)]/dt$ at $t = 0$.

The outflow of magnesium from plasma and thus—by assuming that there are no other outflows than from plasma to water—the flow of magnesium across the integument to water from fish, $F_{w}$, can in principle be calculated from the magnesium quantity in the plasma, $Q_{p}$, and the (down)slope of the time curve for the tracer quantity in plasma, $q_{p}(t)$, normalized to the tracer quantity in plasma at $t = 0$, $q_{p}(0)$, using equation (3). However, none of these quantities are easily assessable. However, the tracer quantity in water, $q_{w}(t)$, and the specific activity of magnesium in plasma at the end of the experiment, $S_{p}(t_{e})$ (in Bq·mol$^{-1}$), may well be determined. $S_{p}(t_{e})$ is defined as $q_{p}(t_{e})/Q_{p}$ and equal to $C_{p}q_{w}(t_{e})/C_{p}q_{w}(t_{e})$ where $C_{p}q_{w}(t_{e})$ is the tracer concentration in plasma (in Bq·L$^{-1}$) and $C_{p}q_{w}(t_{e})$ is the magnesium concentration in plasma (in mol·L$^{-1}$) both at $t = t_{e}$. By rearranging equation (3) and assuming that at zero time $-d[q_{f}(t)/q_{w}(0)]/dt = d[q_{f}(t)/q_{w}(0)]/dt$ one obtains:

$$F_{w} = [Q_{w}/q_{w}(0)] d[q_{f}(t)/dt] \tag{5}$$

or

$$F_{w} = \frac{1}{S_{p}(0)} \frac{d[q_{f}(t)]}{dt} \tag{6}$$

where $S_{p}(0)$ is the specific activity of magnesium and $d[q_{f}(t)/dt]$ is the (up)slope of the time curve for tracer quantity in water, both at $t = 0$. In practice it may be difficult to determine $S_{p}(0)$ in fish. However, for a limited time period $S_{p}(t)$ is approximately constant and consequently $S_{p}(t_{e})$ may be used instead of $S_{p}(0)$. This yields an approximation:

$$F_{w} = \frac{1}{[S_{p}(t_{e})]} \frac{d[q_{f}(t)]}{dt}. \tag{7}$$

A determination of $F_{w}$ starts by introducing a certain quantity of tracer into the fish via intraperitoneal injection. To condition the fish for the actual experiments, the fish have to be kept in a large volume of water for an extended period of time. This procedure results in a partial transfer of tracer to water from fish and simultaneously in a distribution of tracer among the magnesium compartments of the fish, resulting in a period of a more or less steady specific activity in the plasma. An actual experiment starts ($t = 0$) by placing a thus conditioned fish into a small volume of water.

## Materials and Methods

### Experimental animals and water

Carp (Cyprinus carpio) weighing from 34.0 to 92.3 g were obtained from laboratory stock. Up to 10 fish were held in 120 L glass aquaria filled with 80 L artificial freshwater (initially) containing (mmol L$^{-1}$): 0.5 NaCl, 0.06 KCl, 0.2 MgSO$_4$ and 0.2 CaCl$_2$. The pH of water was maintained at 7.5 by means of an end-point titration system (ETS822, Radiometer, Copenhagen, Denmark) adding a sodium hydroxide solution. The water was kept at 24°C and filtered by recirculation through a nylon-wool thermofilter (2113, Eheim, Deizisau, Germany) and constantly aerated. Twice a week one third of the aquarium content was replaced with freshly prepared water. The photoperiod was 12 h. Daily the fish received six rations of dry fish food (TetraMin, Tetra, Melle, Germany) containing about 31 µmol·g$^{-1}$ magnesium. The quantity of food was adjusted to match the maintenance level (Huisman, 1976).

### Radiotracer

$^{28}$Mg$^{2+}$ was used as radiotracer for Mg$^{2+}$. It was produced either by irradiation of Li–Mg alloys with thermal neutrons in the IRI nuclear reactor (van der Velden et al., 1989b) or by irradiation of PC13 with high energy bremsstrahlung obtained from the NIKHEF 500 MeV electron linear accelerator (Polak et al., 1989). In both cases the irradiation was followed by separation of magnesium from irradiated material leading to aqueous solutions of MgCl$_2$ containing $^{28}$Mg$^{2+}$ and its decay product $^{28}$Al$^{3+}$. The specific activities of these preparations calculated from the activities as determined with Ge(Li) detectors
just before the experiments and magnesium concentrations as determined by spectrophotometric titration with EDTA or by Atomic Absorption Spectrometry, were about 0.2 and 26 GBq mol⁻¹ for the reactor and the accelerator production mode, respectively.

The radioisotope ²⁸Mg decays with the half-life of 20.93 h to ²⁸Al by emitting β⁻-particles of Eₘₐₓ = 0.418 (95%) and 0.459 (5%) MeV and γ-rays of 0.031 (95%), 0.401 (36%), 0.942 (36%), 1.342 (54%), 1.373 (5%) and 1.589 (5%) MeV. ²⁸Al decays with the half-life of 2.24 min to stable ²⁶Si by emitting β⁻-particles of Eₘₐₓ = 2.878 MeV (100%) and γ-rays of 1.779 MeV (100%) (Lederer and Shirley, 1978).

Mg²⁺ flow to fish from water

To determine F_w, a fish was weighed and placed into an all-glass vessel with 2 L artificial freshwater in which MgSO₄ was replaced by MgCl₂ containing 80 kBg ²⁸Mg. The water was constantly aerated with pre-humidified air and kept at 24°C. By means of a peristaltic pump the water was recirculated (mean residence time of water in the vessel was 520 s) through a glass spiral round about a cylindrical 3” × 3” NaI(Tl)-scintillation detector (Type 12S, Harshaw Chemie, De Meern, The Netherlands) equipped with a multichannel analyzer (Series 35, Canberra Industries, Meriden, U.S.A.). During the experiments the spectra of the γ-radiation emitted by both ²⁸Mg and ²⁸Al present in water were measured in subsequent intervals (counting time per interval 1000 or 5000 s) and stored in a personal computer. The areas of the 0.401, 0.942 and 1.342 MeV peaks—all belonging to ²⁸Mg only—were determined for all data points and then corrected for decay.

The experiments, during which the fish were not fed, lasted 21.5 up to 25.4 h (= tₑ) in all but two cases (for the fish weighing 92.3 and 54.2 g, tₑ was 5 and 14.5 h, respectively) and were stopped by adding “MS-222” to the water (final concentration 0.4 mmol L⁻¹). After 2–5 min the fish were taken out of the radioactive water, rinsed with artificial freshwater, blotted with filter paper and killed by spinal transection. Finally the fish were minced and aliquots were put into one or more 20 mL counting vials. Each vial contained 20 mL miniced fish. Its weight was determined by weighing the vials before and after filling. The γ-spectra of samples of minced fish were measured in a well-type 3” × 3” NaI(Tl)-scintillation detector (Type 12S W 12, Harshaw Chemie) connected to the before-mentioned counting equipment. To allow for the decay of ²⁸Al present in the samples at the moment of sampling and for the establishment of a ²⁸Mg-²⁸Al equilibrium all samples were measured at least 30 min after sampling. The counting time was 500 or 1000 s. The 20 mL water samples taken at the beginning of the experiment were measured in a similar way. All the pulses pertaining to 0.1–2.0 MeV region were taken into account. Their number was corrected for background and decay of ²⁸Mg and the counting rates were calculated.

During the experiments no measurable decrease of radiotracer concentration in the water recirculating around the detector was observed. Consequently, F_w (in mol h⁻¹) had to be calculated using equation (4) in which:

\[
Q_w = V_w C_w
\]

where V_w is the volume of water (in L) and C_w is the magnesium concentration in water (in mol L⁻¹);

\[
q(tₑ)/q(0) = R_w(tₑ)W_f/R_w V_w
\]

where R_w is the (mean) counting rate per unit weight of miniced fish (in counts s⁻¹ g⁻¹) measured as 20 mL sample(s), W_f is the weight of the fish (in g) and R_w is the counting rate per unit volume of water (in counts s⁻¹ L⁻¹) measured as 20 mL sample; tₑ is expressed in hours.

Mg²⁺ flow to water from plasma

To determine F_p, the fish were weighed and injected intraperitoneally with 0.1–0.2 mL saline (0.9% NaCl) with 2–4 μmol MgCl₂ and approximately 80 kBg ²⁸Mg. After the injection the fish were put into a 50 L all-glass aquarium with 30 L constantly aerated, artificial freshwater which was kept at 24°C. After 9–14 h the fish were taken out, rinsed with artificial freshwater for 2 min and the experiment was started by placing a fish into an all-glass vessel with 1.7 L artificial freshwater which was constantly aerated with pre-humidified air and kept at 24°C. Water samples of 20 mL were taken 1, 5, 10, 15, 30, 45, 60 min and then every 30 min thereafter. To each of these samples Al³⁺ (as AlCl₃ dissolved in dilute sulphuric acid) was added to serve as hold-back carrier for ²⁸Al³⁺ and thus prevent its adsorption onto the inner wall of the counting vial. The experiments,

Magnesium transport in carp studied with ²⁸Mg

The quantities of magnesium in hard tissues, soft tissues and thus in the whole carp were assessed as follows. The fish were anesthetized in 0.4 mmol L⁻¹ solution of “MS-222” (3-aminobenzoic acid ethyl ester, Methanesulfonate Salt, Sigma, St Louis, U.S.A.) for 2–5 min and weighed. Blood samples (mixed arterial and venous blood) of up to 2 mL were taken by puncture of the caudal vessels behind the anal fin using heparinized syringe. The fish were then killed by spinal transection. The blood was separated into cells and plasma by centrifugation at 9000 g for 3 min. A portion of the plasma was ultrafiltered without microcolloids (SM 13202, Sartorius, Göttingen, Germany). Each carp was divided into hard (bones and scales) and soft (muscle and organs) tissue. The tissues were weighed, lyophilized and weighed again.

The magnesium concentrations in the blood plasma and in its ultrafiltrate were determined colorimetrically (Diagnostic kit 595, Sigma) whereas the magnesium concentrations in the lyophilized tissues of carp were determined by instrumental neutron activation analysis (van der Velden et al., 1989a).

\[
R_w = \frac{Q_w}{V_w C_w}
\]

Where R_w is the counting rate per unit volume of water (in counts s⁻¹ L⁻¹) measured as 20 mL sample(s), W_f is the weight of the fish (in g) and C_w is the magnesium concentration in water (in mol L⁻¹);

\[
q(tₑ)/q(0) = R_w(tₑ)W_f/R_w V_w
\]

where R_w is the (mean) counting rate per unit weight of miniced fish (in counts s⁻¹ g⁻¹) measured as 20 mL sample(s), W_f is the weight of the fish (in g) and R_w is the counting rate per unit volume of water (in counts s⁻¹ L⁻¹) measured as 20 mL sample; tₑ is expressed in hours.

Mg²⁺ flow to water from plasma

To determine F_p, the fish were weighed and injected intraperitoneally with 0.1–0.2 mL saline (0.9% NaCl) with 2–4 μmol MgCl₂ and approximately 80 kBg ²⁸Mg. After the injection the fish were put into a 50 L all-glass aquarium with 30 L constantly aerated, artificial freshwater which was kept at 24°C. After 9–14 h the fish were taken out, rinsed with artificial freshwater for 2 min and the experiment was started by placing a fish into an all-glass vessel with 1.7 L artificial freshwater which was constantly aerated with pre-humidified air and kept at 24°C. Water samples of 20 mL were taken 1, 5, 10, 15, 30, 45, 60 min and then every 30 min thereafter. To each of these samples Al³⁺ (as AlCl₃ dissolved in dilute sulphuric acid) was added to serve as hold-back carrier for ²⁸Al³⁺ and thus prevent its adsorption onto the inner wall of the counting vial. The experiments,
during which the fish were not fed, lasted for 5 h and were stopped by transferring the fish into 2 L artificial freshwater containing 0.4 mmol·L⁻¹ "MS 222" for 2-5 min. A blood sample was taken and the fish was killed, minced and put into counting vials. See above for details of these procedures. Counting samples containing 0.250-0.863 mL blood plasma and water adding up to 20 mL were prepared.

The ²⁸Mg activity in liquid samples was determined by Cerenkov counting of β⁻-particles of (mainly)²⁸Al by means of a Liquid Scintillation Counter (Tri-carb 300c, Packard Instruments). The samples were measured at least 30 min after sampling to allow for the establishment of a ²⁸Mg-²⁸Al equilibrium. The counting data were corrected for background and decay of ²⁸Mg. The tracers quantities in water for all sampling times were calculated by taking into account the amount of tracer removed from water by subsequent sampling:

\[ q_w(i) = \left( R_w(i) \left[ V_w - (i - 1)\epsilon \right] \right) \]

where \( i \) is the sequential sample number, \( R_w(i) \) is the counting rate of \( i \)th water sample (in counts·s⁻¹·L⁻¹), \( V_w \) is the sample volume (in L) and \( \epsilon \) is the counting efficiency (in counts·s⁻¹·Bq⁻¹). In the following calculations the counting efficiency \( \epsilon \) cancels out and therefore knowledge of its actual value is not required. Note that for \( i = 1 \) \( t = 1 \) min, \( i = 2 \) \( t = 5 \) min etc. Hence for \( i = 1 \), \( R_w(i = 1) = R_w(0) = 0 \).

\( R_w(i) = \) the counting rate of water sample (in counts·s⁻¹·L⁻¹).

\( S_p(t) = \frac{[R_p(t)]}{C_p} \)

where \( R_p(t) \) is the counting rate of plasma (in counts·s⁻¹·L⁻¹) pertaining to \( t = 5 \) h and \( C_p \) the magnesium concentration in plasma (in mol·L⁻¹): a single value (determined in separate measurements as described above) for all fish instead of \( C_p(t) \) values for each individual fish was used (this because all the plasma was needed for the preparation of the counting samples).

The (up)slope at \( t = 0 \) of the \( q_w(t) \) vs time (in h) curve (in counts·s⁻¹·L⁻¹·h⁻¹), required for equation (7), was determined by fitting a straight line through data points for \( t \leq 1 \) h calculated by equation (10).

**Results and Discussion**

**Magnesium distribution**

The total magnesium concentration in blood plasma of carp, \( C_p = (0.96 ± 0.06) \times 10^{-3} \) mol·L⁻¹ (mean value ± standard deviation of \( n = 12 \) fish). This result is close to (0.93 ± 0.05) and (1.15 ± 0.16) \times 10⁻³ mol·L⁻¹, reported for carp by Hunn (1972) and Houston (1985), respectively. The magnesium concentration in plasma ultrafiltrate was (0.61 ± 0.01) \times 10⁻³ mol·L⁻¹ (\( n = 4 \)) and this indicates that about 32% of magnesium is protein bound and about 67% is in ionic and/or complexed form.

The amounts of dry hard and dry soft tissues (including blood) per fish, the magnesium concentration in these tissues and the calculated quantities of magnesium therein are given in Table 1. For carp, weighing 42.3-83.1 g, about 53% of the magnesium is confined to the soft tissue. The quantity of magnesium in these fish is approximately proportional to their weights (13.0 µmol·g⁻¹). Tacon _et al._ (1984) found in carp (mean weight 55 g) a somewhat higher value of 17.3 µmol·g⁻¹.

**Table 1. Magnesium distribution in carp**

<table>
<thead>
<tr>
<th>W_f</th>
<th>W_s</th>
<th>C_d</th>
<th>Q_h</th>
<th>Q_s</th>
<th>Q_f</th>
<th>Q_f/Q_h</th>
<th>C_f</th>
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<td>523</td>
<td>11.13</td>
<td>57.9</td>
<td>644</td>
<td>1167</td>
</tr>
</tbody>
</table>

| Mean value: | 0.53 | 13.0 |

| Standard deviation of the mean: | 0.03 | 1.0 |

W_f, W_s, and W_r are the weights of respectively dry hard and dry soft tissue (all in g); C_d, C_s, and C_f (=Q_f/W_f) are the magnesium concentrations in respectively dry hard tissue, dry soft tissue and (wet) fish (all in µmol·g⁻¹); Q_h (=Q_h/C_d), Q_s (=Q_s/C_s) and Q_f (=Q_f+C_s) are the magnesium quantities in respectively hard tissue, soft tissue and whole fish (all in µmol).
Magnesium transport in carp studied with $^{28}$Mg

As the specific activity of plasma was determined 5 h—thus not really shortly—after the start of an experiment, the question may arise whether the use of $S_p(t_e = 5$ h) instead of $S_p(0)$ does not lead to a significant overestimation of $F_{wp}$ due to the possible loss of tracer from plasma and consequently: $S_p(t_e)/S_p(0) < 1$. One may estimate this ratio by supposing a uniform specific activity of magnesium throughout the extracellular fluid and consequently equal specific activities in its two components, namely plasma and interstitial fluid ($S_i$) i.e. $S_i(t_e) = S_i(t)$ for $0 < t < t_e$. The tracer balance for extracellular fluid and water results in the relation:

$$S_p(t_e)/S_p(0) = 1/[1 + q_w(t_e)/S_p(t_e)(Q_w + Q_i)]$$

(13)

where $Q_i$ is the magnesium quantity in interstitial fluid (in mol). The tracer quantity in water at the end of the experiment $q_w(t_e)$ was calculated by means of equation (10) (for $t_e = 5$ h). Hence $Q_i = V_pC_i$, where $V_p$ is the volume of plasma in carp (in L) and $C_i$ is the magnesium concentration in the interstitial fluid (in mol L$^{-1}$). According to Thorsen (1961) in carp $V_p = 1.8 \times 10^{-4}$ Wf and $V_i = 13.7 \times 10^{-3}$ Wf. For $C_i$, the magnesium concentration in plasma ultrafiltrate was used. The foregoing estimation procedure yields $S_p(t_e)/S_p(0)$ ranging from 0.69 to 0.86 or equal to 0.78 ± 0.05 (mean value for seven fish ± standard deviation). Apparently the specific activity of magnesium in plasma of carp changes during the 5 h lasting experiments. Although the decrease of specific activity in plasma remains limited to 31% or less, the preceding $F_{wp}$ values were corrected by multiplying each of them with the corresponding $S_p(t_e)/S_p(0)$ ratio (Fig. 2). Linear regression of (new) $\ln F_{wp}$ values on $\ln W_f$ values resulted in a power function analogous to equation (13) with $a = 1.23$ and $b = 1.43$ (Fig. 2, middle curve). For this regression $r = 0.924$.

**Magnesium balance**

Growing fish constantly incorporate magnesium in their body. The uptake or net flow of magnesium into the fish, $F_{\text{net}}$, is the resultant of two oppositely directed flows: $F_{in}$ being the inflow of magnesium to fish from both water ($F_{wp}$) and food ($F_{fp}$) and $F_{out}$ being the outflow of magnesium to water from fish along both the integumental ($F_{wp}$) and excretal routes ($F_{wp}$).

The present study deals with fish fed at maintenance level thus with nongrowing fish. The whole body magnesium quantity in these fish is expected to remain constant before and during the experimentation. Obviously $F_{\text{net}} = 0$ which means that $F_{in} = F_{out}$ or:

$$F_{wp} + F_{fp} = F_{wp} + F_{wp}$$

(14)

The curves in Fig. 2 point out that for the carp under consideration $F_{wp} < F_{wp}$ for all $W_f$ values. For both the fish weighing 40 and 80 g, $F_{wp}/F_{wp}$ (corrected) = 0.16.

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**Mg$^{2+}$ flow to fish from water**

The $F_{wp}$ values, obtained for fish weighing 36.7 to 92.3 g, are shown in Fig. 2 as function of the fish weight. Linear regression of $\ln F_{wp}$ values on $\ln W_f$ values yielded a power function for which the equation is:

$$F_{wp} = a(W_f)^b$$

(12)

where $F_{wp}$ is here expressed in nmol L$^{-1}$ h$^{-1}$, $a = 0.16$ and $b = 1.48$ (Fig. 2). The correlation coefficient ($r$) for the above-mentioned linear regression was 0.880.

Magnesium dissolved in water may enter the fish through the integument (gills and body surface) and via the gut by drinking. The $F_{wp}$ values as obtained in this study may comprise both of these routes. The drinking related part of $F_{wp}$ may be estimated by using a drinking rate value of $51 \mu L\cdot h^{-1} (100\ g)^{-1}$, as obtained for Carassius auratus (Motais et al., 1969). This results in $5\ \text{nmol}\cdot h^{-1}$ magnesium for a 50 g carp which is only about 10% of $F_{wp}$.

**Mg$^{2+}$ flow to water from plasma**

The $F_{wp}$ values, obtained for fish weighing 34.0–77.6 g, are shown in Fig. 2 as function of the fish weight. Linear regression of $\ln F_{wp}$ values on $\ln W_f$ values showed that the trend of the plotted data points may be represented by a power function for which the equation is analogous to equation (12) but with $a = 1.16$ and $b = 1.51$ (Fig. 2). Here $r$ was 0.886.

The magnesium may leave the fish through the integument and by urinary and faecal excretion. The $F_{wp}$ values as obtained in this study represent the integumental route viz the flow of magnesium along the route: plasma $\rightarrow$ gills and body surface $\rightarrow$ water, only. However, this statement is subject to the condition that during the very first hour of the experiment the urinary and faecal excretion of $^{28}$Mg$^{2+}$ does not significantly contribute to the tracer quantity in water.

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[Fig. 2. Magnesium flow to fish from water, $F_{wp}$ (squares + lower curve) and to water from plasma, $F_{wp}$, both as function of the fish weight ($W_f$ in g). Given are the primary (circles + upper curve) as well as the for $S_p$ decrease corrected $F_{wp}$ values (triangles + middle curve). The curves represent the corresponding best fit power functions as given in the text.]
and thus it may be regarded as independent on $W_f$. By rearrangement of equation (14) and using the preceding $F_{fw}/F_{wp}$ value one obtains:

$$F_f = 5.3F_{fw} + F_{ef}$$ (15)

Evidently $F_f \gg F_{fw}$ and amounts to at least 84% of the magnesium inflow into the carp. Consequently $F_f$ represents the most important route for magnesium inflow to these nongrowing fish. Ogino and Chiou (1976) came to a similar conclusion, namely "that carp cannot absorb sufficient amount of magnesium from the rearing water to meet their requirement". Note that the conclusion of these authors was based on measurements of the magnesium content of growing fish subjected to diets containing graded amounts of magnesium.

The above-mentioned and our own study indicate that, in contrast to calcium (Flik et al., 1985), a significant fraction of the magnesium inflow in carp is realized along another route than the integumental one, i.e. the intestinal route.

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