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RENAL EXCRETION OF MAGNESIUM IN A FRESHWATER TELEOST, *SALMO GAIRDNERI*

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SUMMARY

Infusion of magnesium salts into the body cavity of freshwater-adapted rainbow trout led to elevated plasma magnesium concentrations and to stimulation of renal tubular secretion of magnesium. The majority of the infused load was excreted renally, no net branchial excretion being detected. Magnesium sulphate infusion led to increased tubular secretion of sulphate. Magnesium chloride infusion led to reduced tubular reabsorption of chloride. Magnesium could either be reabsorbed or secreted in control freshwater-adapted trout, apparently as a function of nutritional status. Fish could switch from reabsorption to secretion in response to magnesium loading. It is suggested that freshwater fish eliminate excess dietary magnesium renally.

INTRODUCTION

Whilst a number of studies have been carried out on the control of plasma sodium and chloride concentrations in teleosts, relatively little attention has been paid to the regulation of magnesium and sulphate levels. This is perhaps not surprising since, as stated in a recent review of magnesium transport in the mammalian kidney (Quamme & Dirks, 1980), 'little is known of the mechanisms that regulate magnesium conservation and excretion' and even less is known about sulphate. Fish plasma magnesium concentrations are maintained within the range 1–4 mmol l^{-1}, being higher in marine than in freshwater teleosts (Evans, 1979). In the euryhaline European eel, *Anguilla anguilla*, plasma levels are higher when in sea water than when in fresh water (Chester Jones, Chan & Rankin, 1969) but the difference is relatively small (4 mmol l^{-1} compared to 2 mmol l^{-1}).

Magnesium concentrations of fresh water are approx. 200 μmol l^{-1} or less; in ocean water, it is the third most abundant ion with a concentration of more than 50 mmol l^{-1} (Rankin & Davenport, 1981). In marine fish, branchial osmotic losses must be replaced by drinking, and this leads to continual intestinal absorption of

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magnesium and sulphate ions; these are thought to be eliminated exclusively by the kidneys (Hickman & Trump, 1969; Evans, 1979).

Freshwater fish must obtain magnesium and sulphate ions either by active uptake or in the diet. There is no evidence for active branchial uptake and only indirect evidence of absorption from the alimentary canal (Cowley *et al.* 1977). Dietary loads will be highly variable depending on the feeding habits of the species. Breakdown of tissues and their proteins will release large quantities of magnesium and sulphate ions, for example. In the few studies carried out on freshwater fish, ultrafiltered magnesium appears to be reabsorbed (Hickman & Trump, 1969), although experiments on starved fish would obviously not reveal the mechanisms responsible for the elimination of magnesium loads. Infusion of magnesium salts stimulates renal secretion of magnesium ions in marine southern flounder (Hickman, 1968), freshwater-adapted Japanese eels, *Anguilla japonica* (Hirano, 1979) and river lampreys, *Lampetra fluviatilis* (Rankin, Henderson & Brown, 1983).

Euryhaline fish may need to be able to initiate renal magnesium secretion rapidly. The Baltic teleost *Myoxocephalus quadricornis*, a species living and surviving only in dilute salinities, seems to possess a huge overcapacity to secrete magnesium ions in relation to the salinity range in which it is able to osmoregulate (Oikari, 1978*a*). Such a capability may be only a relict from marine ancestors or it may have some ionoregulatory function in freshwater teleosts. The responses of a freshwater-acclimated euryhaline fish, the rainbow trout *Salmo gairdneri*, to experimental magnesium loading were therefore studied.

MATERIALS AND METHODS

Animals

Rainbow trout (*Salmo gairdneri* Richardson), weighing 140–190 g, were obtained from a local hatchery (Upper Mills Trout Farm, Clwyd). After transfer to Bangor they were acclimated in well-aerated, dechlorinated, copper-free tap water (Na 0·15; Cl 0·18; Mg 0·022 mmol l^{-1}; pH 6·8; temperature 11·5 ± 1 °C) for at least 2 weeks with a 12 : 12 light : dark regime (L : 08·00 to 20·00). Fish were fed daily (except as specified later) *ad libitum* with commercial trout food (Shell Salmon Food, ∅3 mm).

Most trout were starved for the last 4–6 days before operation (= fish in postabsorptive stage). A few (= fish in absorptive stage) were fed to satiation 25–30 h before urine collections were started. These nutritional stages were also monitored as absence and presence of faecal pellets on the bottom of the test chamber. A separate batch of trout was gradually – over a 5-day period – transferred to full strength Menai Straits sea water (980 mosmol kg^{-1}) and acclimatized in it at 13 °C for 3 weeks.

Surgical techniques

Urinary catheters were made from 3- to 4-cm pieces of the perforated tips of infant feeding tubes (Argyle® Cat. no. AR–33R, size 5) fitted to 40-cm length of PP 50 (Portex) polypropylene tubing shaped into an 'S' at the proximal end. Intraperitoneal cannulae were made from 30-cm lengths of PP 50 tubing with the internal end formed into a short spiral in hot (90 °C) water. This design, with appropriate ligatures,

prevented the cannula from sliding out. In both types of tubing two or three bubbles were made by heating with a loop of tungsten wire heated by a 4·5 V d.c. current. When stitching the tubing to the body wall all sutures were made distally to these anchoring bubbles.

Trout were anaesthetized in aerated solution of MS222 (ethyl-*m*-aminobenzoate methane sulphonic acid salt, Sigma, 100 mg l^{-1}) and kept upside down in a V-shaped trough with the head immersed in an aerated solution of anaesthetic at the minimum concentration needed to maintain anaesthesia (less than 50 mg l^{-1}). The perforated tip of the catheter was pushed 10–15 mm inside the the urinary papilla, tied in with a ligature around the papilla and then anchored to the skin by three sutures. The intraperitoneal cannula was inserted, with the aid of a sharpened steel wire inside the tubing, into the body cavity – first under the skin for 10–13 mm and then through the latero-abdominal muscles – and was fixed in place with two sutures. *Post mortem* examination showed that the tip of the cannula was adjacent to connective and fatty tissues surrounding the small intestine.

Urine collections

Operated trout were allowed to recover in the experimental chamber, a partially black-painted Perspex box, sufficiently small (26 × 3·8 × 7 cm) to prevent most swimming activity and turning, with a water flow of 1·3–1·5 l min^{-1}. Within 3 h the trout had adjusted to the box, and steady urine output was obtainable for 8–12 h.

The box was connected by siphon to a 100-l recirculation tank with 95 % replacement every 10 h. Experimental water temperatures (T_w) varied during the study period (February–March) from 12·5 to 13·6 °C, but for each fish T_w was adjusted to within ± 0·3 °C or less. The urinary catheter led to a fraction collector (LKB Ultrarack) about 10 cm below the fish. Collection periods generally lasted 60 or 90 min and, when urine volumes were small, as in seawater fish, were made under water-saturated liquid paraffin to prevent evaporation. Prepared tubes were weighed to the nearest 0·5 mg, and urine flow rates ($\dot{V}$ in ml kg^{-1} h^{-1}) was calculated on a weight basis. All samples were either kept at 2–4 °C or frozen at −20 °C before analysis.

Glomerular filtration rate (GFR) was measured as inulin clearance (C_{in}). About 20 μCi of ^{3}H-inulin (> 300 mCi $mmol^{-1}$; Amersham International) in 0·5 ml distilled water was injected intraperitoneally *via* a cannula (freshwater fish) or by hypodermic needle (seawater fish) at least 12 h before each experiment. Three blood samples were collected at 12- to 16-h intervals from minimally anaesthetized (150 mg MS222 l^{-1}) fish by puncturing the caudal vein and were immediately centrifuged. Aliquots of 25 or 50 μl of plasma were counted in a liquid scintillation counter (Beckman LS 7000) with correction for quenching. Plasma radioactivity decayed in a linear manner (generally with a correlation coefficient, $r = 1{\cdot}00$) when plotted on semilogarithmic paper. The d.p.m. at the midpoint of each urine collection period was calculated and C_{in} was calculated as urine d.p.m./plasma d.p.m. × $\dot{V}$. As a first approximation, filtered ionic loads were taken as the product of C_{in} and plasma ultrafiltrate concentration, i.e. ignoring the Donnan equilibrium (see Discussion). Net reabsorption or secretion of an ion was calculated as the difference between filtered and excreted amounts.

Ionic infusions and analyses

All infusions were made at a constant rate *via* the intraperitoneal cannula using a calibrated infusion pump ('perfusor', Braun), usually for 60 min. As preliminary experiments showed that distilled water (DW) infusions from 0·8 to 10·2 ml h^{-1} per 150–160 g fish did not evoke diuresis, a rate of 1·62 ml h^{-1} per fish (approx 10·5 ml kg^{-1} h^{-1}) was adopted in most cases. This rate was found to lead to efficient uptake of an infused magnesium load into the blood plasma (cf. Fig. 1), whereas lower rates yielded slower distribution. The salts infused ($MgCl_2$, $MgSO_4$, Na_2SO_4; 50–100 mmol l^{-1}, analytical reagent quality, British Drug House) were dissolved in DW or in freshwater (FW) teleost Ringer (Rankin & Maetz, 1971) if very low doses of magnesium were needed.

Electrolyte (Mg^{2+}, SO_4^{2-}, Cl^-, Na^+) concentrations of plasma and urine were determined as described by Logan, Morris & Rankin (1980). Osmolalities were measured by freezing point depression using a Knauer Halbmikro-Osmometer. The magnesium concentration of commercial salmon food was analysed by atomic absorption spectroscopy (AAS) after extraction of duplicate 100-mg samples in 5-ml aliquots of 0·1 mol l^{-1} HCl for 5 days.

Access of dietary magnesium to the bloodstream was demonstrated as follows: dried salmon food (Shell) was soaked in 1 mol l^{-1} $MgCl_2$ solution and minced; the meal was force-fed, with the aid of flexible silicone tubing (5 mm radius), directly into the stomach of an anaesthetized trout; blood samples were taken as described above during the following 22 h and plasma magnesium concentrations were measured and compared to the pre-feeding level.

Net extrarenal magnesium flux was measured as follows: catheterized, cannulated trout were allowed to recover in the experimental chamber in the usual manner, except that the water in the chamber was also directly aerated; the inflow was then turned off and serial water samples from the well-aerated bath were collected for up to 2 h and their magnesium concentrations analysed by AAS. Net fluxes of more than 1 μmol kg^{-1} h^{-1} could be measured by this technique.

Magnesium binding in plasma

Eight rainbow trout (130–320 g, both sexes) from a Finnish Hatchery (Savon Taimen) were acclimated for 2–3 weeks in dechlorinated Helsinki tap water at 12 °C. Blood was taken from lightly anaesthetized (75 mg MS222 l^{-1}) fish and a single intraperitoneal injection of 0·25 mol l^{-1} $MgCl_2$ calculated to give a dose of 1300–2000 μmol Mg kg^{-1} was given. After 1·4 h another blood sample was taken. Plasma was immediately separated by centrifugation. A part of the plasma was ultrafiltered through dialysis tubing (A. H. Thomas Co., approximate pore size 4·8 nm) in a centrifuge (approx. 250 × ***g*** for 4 h at 12 °C; further centrifugation after 20 h gave identical results). Magnesium concentrations of the plasma and its ultrafiltrate were determined by AAS. The mean binding values obtained were used to calculate filtered loads of magnesium before and during peak excretion periods following infusions.

RESULTS

Magnesium binding in plasma

Ultrafiltration experiments revealed that an appreciable portion of the total plasma magnesium was bound to macromolecules. In control plasma samples 44 ± 10 % (mean ± S.D., $N = 8$) of the magnesium was non-dialysable; 1·4 h after magnesium chloride injection this had fallen to 31 ± 9 %. These mean values were used in all calculations of net renal secretion or reabsorption. Although the percentage bound fell in magnesium-loaded fish, the total amount bound increased, suggesting that control plasma contains more binding sites than are normally occupied.

Control urine flow rate ($\dot{V}$) and ionic composition

$\dot{V}$ varied from 2·80 to 5·61 ml kg^{-1} h^{-1} at 13 ± 1 °C (mean ± S.D. = 4·25 ± 0·90, $N = 9$). Variation between samples in individual fish was less than that between fish (standard deviations ranging from 12·7 to 20·6 % of mean, $N = 10$ fish), indicating that each trout maintained a relatively constant urine output. $\dot{V}$ was not related to the nutritional status of the fish (cf. control trout of Table 2) but was increased by even the smallest disturbances in the animal's surroundings.

Normal urine composition in trout 4–10 days after feeding is given in Table 1. Urinary magnesium concentrations of freshwater individuals were highly variable, ranging from 0·05 to 0·98 mmol l^{-1}. Sulphate concentrations were almost six times as high. Seawater fish showed much higher magnesium than sulphate concentrations. The other values in Table 1 are similar to those reported previously (Hickman & Trump, 1969; Beyenbach & Kirschner, 1975; Schmidt-Nielsen & Renfro, 1975;) for teleost fish. Plasma values are given for comparison.

Renal elimination of infused magnesium ion

Elevated plasma magnesium concentrations were attained rapidly following intraperitoneal administration and were maintained during the next hour (Fig. 1A) with a concomitant rapid increase in magnesium excretion (Figs 1B, 3) so that 15–42 % of the injected dose was eliminated within 1 h and 70–80 % within 3 h after cessation of infusion. (In the majority of experiments, plasma levels were not monitored since this involved additional periods of anaesthesia for blood sampling.) The time course of the peak excretion rate of magnesium was very similar whether magnesium sulphate of chloride was infused.

The peak magnesium excretion rate in freshwater-adapted trout during the first or second hour following a 60-min infusion was positively and linearly correlated to the dose infused up to approximately 900 μmol kg^{-1} h^{-1} (Fig. 2). Thus, no T_m (transport maximum) was observed; that is, if one does exist, it must equal or exceed 200 μmol kg^{-1} h^{-1} which is about twice the excretion rate of a seawater-adapted trout. Occasionally urine flow rate increased following magnesium loading (as in Fig. 3), but overall there was no significant increase and the increases that were observed were not related to dose administered (Table 2). Antidiuresis was never observed. During and after a 14-h continuous magnesium sulphate infusion (160–250 μmol kg^{-1} h^{-1}), magnesium excretion was maintained at between 18 and 50 times the control rate for 18 h.

Table 1. *Urine and plasma osmolality and ionic concentrations in rainbow trout in fresh water and following 3 weeks adaptation to sea water*

	Freshwater-adapted trout		Seawater-adapted trout	
	Urine	Plasma	Urine	Plasma
Osmolality (mosmol kg^{-1} water)	29·7 ± 8·7 (22)	269 ± 13 (9)	369 ± 22 (3)	373 ± 39 (3)
Magnesium (mmol l^{-1})	0·49 ± 0·30 (18)	0·66 ± 0·17 (7)	141 ± 3 (3)	2·02 ± 1·01 (3)
Sulphate (mmol l^{-1})	2·88 ± 0·40 (9)		33·2 ± 19·5 (3)	
Chloride (mmol l^{-1})	7·7 ± 3·0 (22)	124 ± 13 (10)	210 ± 34 (3)	157 ± 11 (3)
Sodium (mmol l^{-1})	6·2 ± 3·5 (9)		32 ± 21 (3)	

Means ± S.D. of mean. $N = 8$ freshwater fish except for sulphate and sodium where $N = 3$, and $N = 3$ for seawater fish. Number of urine samples analysed in parentheses.

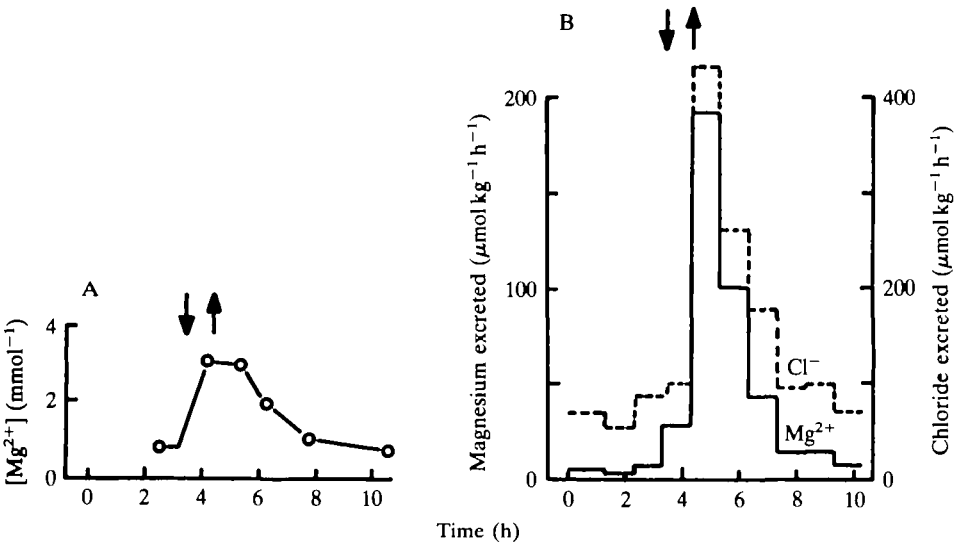

Fig. 1. Effects of infusion of magnesium chloride solution (440 μmol kg^{-1}, intraperitoneal) on (A) plasma magnesium concentration and (B) renal magnesium (solid line) and chloride (broken line) excretion rates in a rainbow trout adapted to fresh water. Arrows mark beginning and end of 1-h infusion period. Additional magnesium excretion in the 4-h period following the start of the infusion was equal to 76 % of the amount infused.

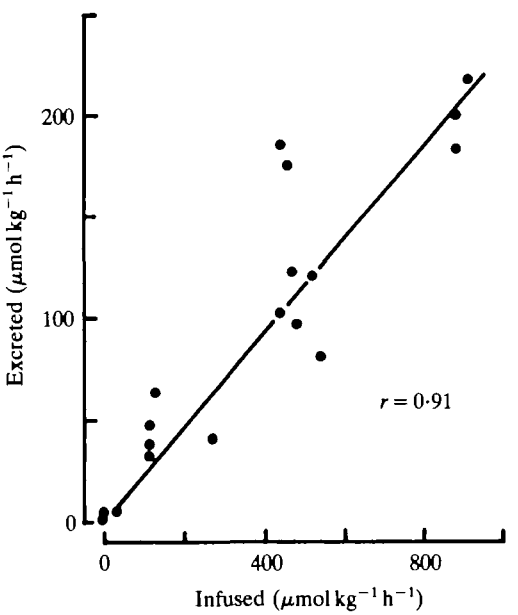

Fig. 2. Correlation between amount of intraperitoneally infused magnesium and maximum increase (over pre-infusion mean control rate) in urinary magnesium excretion rate in freshwater-adapted rainbow trout. Data are from 11 fish. All infusions and urine collection periods lasted for 1 h.

Extrarenal net flux of magnesium

It was not possible to measure any extrarenal net flux in control ($N = 7$) or magnesium-loaded (approx. 450 μmol kg^{-1} h^{-1}; $N = 6$; water temperature 14·5 °C) trout. Branchial net fluxes must therefore be less than the detection limit (1 μmol kg^{-1} h^{-1}), which is negligible (< 1 %) compared to the amounts excreted renally.

Excretion of sulphate and chloride

The peak rate of sulphate excretion during magnesium sulphate infusion closely paralled that of magnesium ($N = 4$ experiments; for example see Fig. 3). In most, but not all, cases urine chloride concentration also increased. Thus all three ions could contribute to the higher urine osmolality which invariably resulted. Sulphate excretion rate increased from 15 to 32 μmol kg^{-1} h^{-1} for 3 to 4 h following sodium sulphate infusion (275 μmol kg^{-1} h^{-1}) with no change in excretion of sodium or chloride ions. No changes in urinary sulphate excretion were observed following magnesium chloride infusion. Sulphate excretion thus can be independent of magnesium excretion and chloride can serve as an additional counterion with sulphate in the magnesium secretion process.

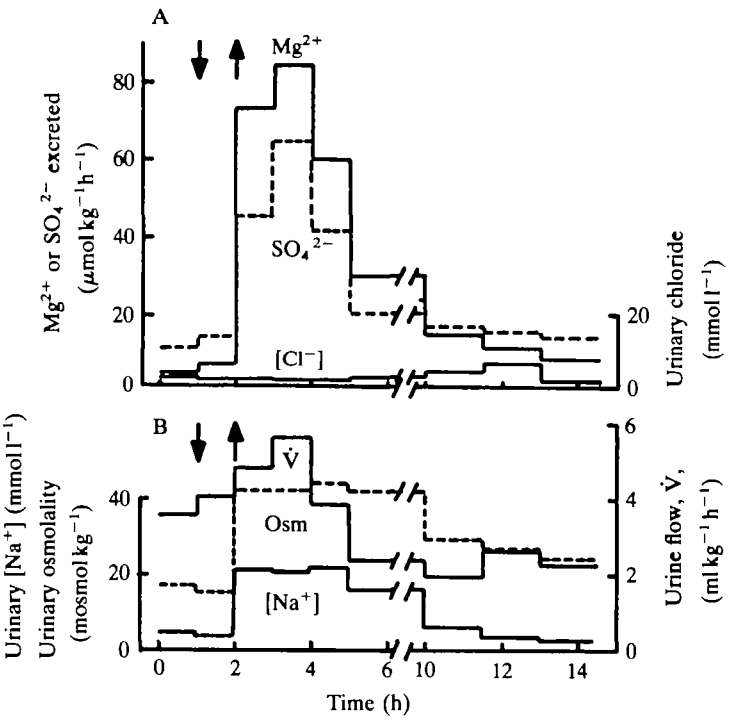

Fig. 3. Effects of infusion of 545 μmol kg^{-1} h^{-1} magnesium sulphate solution (0·8 ml h^{-1} into a 150-g trout adapted to fresh water) on (A) magnesium (solid line) and sulphate (broken line) renal excretion rates (in μmol kg^{-1} h^{-1}) and urinary chloride concentration (in mmol l^{-1}) and (B) rate of urine flow ($\dot{V}$ in ml kg^{-1} h^{-1}), urinary sodium concentration (solid line; in mmol l^{-1}) and urinary osmolality (Osm, broken line; in mosmol kg^{-1}). Arrows indicate beginning and end of infusion.

Table 2. *Effects of magnesium chloride infusions on urine flow and magnesium excretion rates in freshwater-adapted rainbow trout*

Dose of magnesium (μmol kg^{-1} h^{-1})	Urine flow rate (ml kg^{-1} h^{-1})	Inulin clearance (ml kg^{-1} h^{-1})	Peak Mg^{2+} excretion (μmol kg^{-1} h^{-1})	Mg secretion (% of amount excreted)
0 (control)	4·31 ± 1·03	10·67 ± 2·11	5·13 ± 3·73	22·8 ± 59·4†
451 ± 20	5·32 ± 1·81	13·44 ± 3·76*	139 ± 43***	90·4 ± 4·2**
898 ± 16	4·60 ± 1·23	12·52 ± 2·61	180 ± 38***	84·0 ± 5·2**
SW fish	0·77 ± 0·07***	2·00 ± 0·50***	109 ± 9***	97·7 ± 0·8**

Values for seawater-adapted (SW) fish are given for comparison.
† Three fish showed reabsorption; four showed secretion.
Means ± S.D. of mean. $N = 7$ (control), 5 (Mg-infused) or 3 (SW) fish.
Comparison with freshwater controls: * = $P < 0{\cdot}05$, ** = $P < 0{\cdot}01$, *** = $P < 0{\cdot}001$.

Infusions of magnesium chloride (0·9 and 1·8 mmol chloride $kg^{-1} h^{-1}$) did not cause significant differences in urine flow rate but did cause small increases in inulin clearance which were, however, only significant ($P < 0·05$) at the lower infusion rate (Table 2). During excretion of infused $MgCl_2$, calculated filtered loads of chloride increased but the net reabsorption rates were unchanged (Table 3). The percentage of the filtered chloride reabsorbed, however, decreased significantly ($P < 0·01$), leading to highly significant ($P < 0·001$) increases in urinary chloride concentrations at both infusion rates. (Chloride was not bound in plasma to any significant extent, so no corrections were needed in calculating filtered loads.) The chloride which acted as the counterion during magnesium secretion could therefore have come from the filtered load, rather than from any tubular Mg^{2+}/Cl^- co-transport mechanism. Whatever the site of magnesium secretion, the net result is that magnesium-chloride-loaded trout increase the molar chloride excretion to double that of magnesium (Fig 1; Tables 2, 3).

It is interesting to note that no statistically significant changes of the plasma chloride concentration occurred in magnesium-chloride-infused trout (control, 124 ± 13; high infusion rate, 122 ± 16 mmol l^{-1}, $N = 5$ fish) in contrast to the highly significant ($P < 0·0001$, $N = 10$) increase in plasma magnesium concentration.

Reabsorption and secretion of magnesium in fresh water

Control trout displayed either net reabsorption of filtered magnesium or net secretion (cf. Table 2). The renal status maintained was clearly associated with the nutritional status of the trout (Fig. 4) and we noted that it was possible to shift from net secretion to net reabsorption within 2 h of reaching the postabsorptive phase. The peak Mg^{2+} excretion following $MgCl_2$ infusion was predominantly caused by tubular secretion, the slight increase in filtered load being of negligible importance. Because GFR (C_{in}) was more than five times that of seawater-adapted trout, the filtered magnesium loads were higher, and thus the tubular secretion (as a percentage of total excretion) was lower, in freshwater trout. The results demonstrate that the freshwater trout kidney possesses active magnesium secretory mechanisms adequate to account for up to 90 % of the excreted amount.

Because plasma levels of sulphate were not measured, it was not possible to determine whether urinary sulphate of magnesium-sulphate-infused trout originated predominantly from secretion or filtration (Fig. 3). In unloaded controls, however, urinary sulphate concentrations (Table 1) were fairly high compared to salmonid plasma concentrations (0·4–2·0 mmol l^{-1}) reported by Holmes & Donaldson (1969), giving U/P ratios of between 1·5 and 7. This indicates that very little, if any, of the filtered sulphate is reabsorbed and that trout resort to net secretion to eliminate sulphate loads.

Dietary magnesium loading

Loading with magnesium chloride by stomach tube, like intraperitoneal infusion, increased plasma magnesium concentration, for example from 0·6 to 2·1 mmol l^{-1} in 30 min in response to 5 mmol kg^{-1}, remaining more than double the control value for at least 3–4 h. This demonstrated that the wall of the alimentary canal of freshwater trout is permeable to magnesium ions. In contrast to force feeding, digestion

Table 3. *Effects of magnesium chloride infusions on renal chloride excretion in freshwater-adapted rainbow trout*

Dose of chloride administered (N) (μmol kg^{-1} h^{-1})	Urinary chloride concentration (mmol l^{-1})	Peak excretion rate of chloride (μmol kg^{-1} h^{-1})	Net chloride reabsorption (μmol kg^{-1} h^{-1})	Net reabsorption of filtered chloride (%)
0 (control) (10)	8·9 ± 3·4	38 ± 16	1187 ± 178	97·0 ± 1·2
902 ± 40 (5)	55 ± 7***	291 ± 109***	1289 ± 313	81·3 ± 3·3**
1796 ± 32 (5)	72 ± 24***	348 ± 134***	1249 ± 355	78·6 ± 3·8**
SW fish (3)	210 ± 34***	164 ± 34***	147 ± 86***	45·3 ± 18·1*

Values for seawater-adapted (SW) fish are given for comparison.
For other details see Table 1.
Comparison with freshwater controls: * = $P < 0{\cdot}05$; ** = $P < 0{\cdot}01$; *** = $P < 0{\cdot}001$.

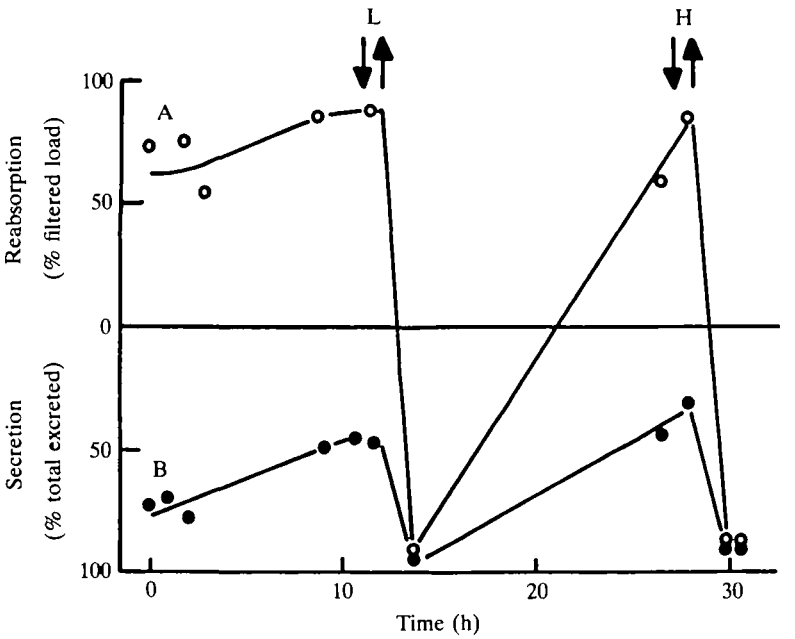

Fig. 4. Renal magnesium reabsorption/secretion in two freshwater-adapted trout loaded with low ($L = 445\ \mu mol\ kg^{-1}\ h^{-1}$) and high ($H = 890\ \mu mol\ kg^{-1}\ h^{-1}$) intraperitoneal infusions of magnesium chloride solution. Arrows indicate beginning and end of infusions. Typical examples are given from a fish in the absorptive (fish B; closed circles) and postabsorptive (fish A; open circles) phases (see Materials and Methods). Estimated filtered loads of magnesium were based on data for plasma magnesium concentrations before, during and after magnesium chloride infusions (see for example Fig. 2A) including an allowance for binding to plasma proteins.

of salmon pellets (containing $193\ \mu mol\ kg^{-1}$ magnesium) resulted in a much slower release and absorption of magnesium ions, which entered the circulation during the absorptive phase, producing a long-lasting increase in renal magnesium secretion (e.g. fish B in Fig. 4). This rate must be sufficient to prevent any significant increases in plasma magnesium concentration. Later, during the postabsorptive phase, trout kidneys switch from net secretion to net reabsorption (e.g. fish A in Fig. 4).

DISCUSSION

Methodology

Results were obtained on resting fish after recovery from the diuresis evoked by the operations. Some variations in urine output can be considerd normal (Hickman & Trump, 1969) and the highest coefficient of variation of any individual was 20·6%. Ureteral urine was siphoned continuously through the catheter, i.e. the role of the bladder (Beyenbach & Kirschner, 1975; Schmidt-Nielsen & Renfro, 1975) was absent or negligible.

Several markers, e.g. inulin, polyethylene glycol (PEG), glofil and ^{51}Cr-EDTA, have been used to measure GFR in teleosts. It seems possible that ^{3}H-inulin underestimates GFR by about 20% relative to PEG in the Americal eel (Schmidt-Nielsen & Renfro, 1975; Beyenbach & Kirschner, 1976). However, the usefulness of

these markers has not been substantiated in teleosts by micropuncture studies. In the lamprey, *Lampetra fluviatilis* (the only fish in which the reliability of any glomerular marker has been investigated using micropuncture techniques), PEG and ^{3}H-inulin clearances were identical although there was some (17 %) reabsorption of the latter (Moriarty, Logan & Rankin, 1978). Possible deviation of C_{in} from the true GFR is likely therefore to have led to underestimation of the amount of plasma magnesium filtered. Estimation of the percentage of ultrafilterable magnesium was based on the assumption that the dialysis tubing used had the same characteristics as the glomerular filtration barrier; this may have led to some error, as would the failure to correct for any possible Donnan effect. The calculations of percentage reabsorption or secretion of magnesium are therefore not exact but the changes observed (e.g. see Fig. 4) were of such magnitude that it was possible to be certain whether magnesium was being secreted or reabsorbed in spite of the above potential sources of error.

Excretion of magnesium ions in freshwater trout

It has long been realized that an important function of the marine teleost kidney is elimination of magnesium and sulphate ions absorbed from sea water drunk to maintain body water balance (Hickman & Trump, 1969; Evans, 1979), enabling the fish to maintain plasma magnesium concentrations of around 1·5–3 % of the seawater level. Micropuncture studies of lampreys have demonstrated magnesium secretion in the proximal renal tubules (Rankin, Logan & Moriarty, 1980); in teleosts, active magnesium secretion is thought to occur in the cells of the second proximal segment of the nephron (Hickman & Trump, 1969; Beyenbach, 1982). Typically, about 98 % of the magnesium excreted is secreted (Table 2); this is fairly similar to the amount (95 %) secreted by two species of Baltic sculpin living in brackish water of about 9 mmol $Mg^{2+}\,l^{-1}$ (Oikari, 1978*b*).

The present results show that freshwater trout, although normally reabsorbing magnesium, are able to switch immediately to secretion when loaded. A similar phenomenon has been observed in the freshwater lamprey (Rankin *et al.* 1983). On the other hand, it is not known whether such mechanisms are involved in the excretion of excess dietary magnesium, even in stenohaline freshwater teleosts. However, present results suggest that the inability of the kidney to secrete magnesium ions is unlikely to be a limiting factor preventing the colonization of sea water by such fish. The factor triggering the tubular secretion mechanism is not known, but a likely candidate is a direct effect on the tubular cells of an increase in plasma magnesium concentration (Babiker & Rankin, 1979). In freshwater trout there was no inverse relationship between urinary sodium and magnesium concentrations as was found in the perfused *Lophius* kidney (Babiker & Rankin, 1979); sodium reabsorption was always high as expected in a freshwater fish. Electroneutrality could be maintained either passively, by reduced reabsorption of chloride or sulphate ions (or both), or perhaps actively by secretion of sulphate ions. Active magnesium transport was not obligatorily coupled to either anion.

Biological significance of magnesium excretion in freshwater trout

The primary function of renal magnesium excretion in freshwater fish would appear to be to maintain a more or less stable plasma magnesium concentration in constantly

varying nutritional situations. Feeding is followed by diffusion of magnesium ions across the wall of the alimentary canal for a variable period, depending on the quality and quantity of food eaten, to be eliminated renally. The dietary magnesium load in a carnivorous fish can be appreciable. For example a trout digesting a fish meal of 2% of its body weight is loaded, if all the magnesium is absorbed, with 240 μmol kg^{-1} (calculation based on muscle magnesium contents given by Oikari, 1975 and Lönn & Oikari, 1982). This amount falls within the range investigated in this study (Fig. 2). In the postabsorptive stage, the trout switches to reabsorption of filtered magnesium and urinary concentrations fall to low (approx. 0·1–0·2 mmol l^{-1}) levels. Because of the high urine flow rates this results in a loss of 0·5–1·0 mmol kg^{-1} h^{-1}, which, in trout fed on a magnesium-deficient diet, eventually leads to plasma hypomagnesaemia (Cowey *et al.* 1977). The large internal pool of bound magnesium in bones and muscles (Cowey *et al.* 1977; Lönn & Oikari, 1982) delays this process and the time lapse may well be much longer than a normally fed fish would ever encounter. It would be interesting to see if renal magnesium excretion increases during severe starvation, when intracellular energy reserves have to be mobilized, in order to eliminate intracellular magnesium. On the other hand, we may suppose that the magnesium secretion mechanism, necessary for a euryhaline fish in sea water, will always be switched on when the extracellular magnesium concentration increases above a preset level. The kidneys of freshwater trout seem to function to maintain optimal extracellular fluid magnesium concentrations by switching from net reabsorption to net secretion and *vice versa* in varying nutritional situations.

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