The production of renal calcinosis by magnesium deficiency in rainbow trout (*Salmo gairdneri*)

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1. Replicate groups of rainbow trout (*Salmo gairdneri*) were given one of five experimental diets (diets 1–5) for 16 weeks. The diets contained different amounts of calcium, phosphorus and magnesium and were prepared so that there were three levels of Ca (g/kg): 14 (diet 1), 26 (diets 2 and 3) and 40 (diets 4 and 5), Ca:P being approximately 1:1 in all diets. Diets 1, 2 and 4 had basal Mg levels (not more than 0·063 g/kg) whereas diets 3 and 5 contained supplementary Mg (1·0 g/kg).

2. Weight gains of the trout given diets containing supplementary Mg were twice those of trout given diets with basal levels of Mg. At both dietary Mg concentrations weight gain was unaffected by the dietary Ca level.

3. Serum Mg levels were significantly reduced in those trout given diets without supplementary Mg. The serum Ca level in those trout given the lowest concentration of Ca in their diet (14 g/kg, diet 1) was significantly greater than in those given higher amounts of Ca in their diets. Serum P levels were not significantly different with any of the experimental diets.

4. The renal Ca concentration was increased in trout given diet 3 (26 g Ca/kg; basal Mg levels). No further increase in renal Ca concentration occurred in trout given diet 5 (40 g Ca/kg; basal Mg levels). With diets containing supplementary Mg renal Ca levels were increased at a dietary Ca level of 40 g/kg but not at a dietary Ca level of 26 g/kg. Renal Mg and P concentrations were not significantly different between any of the dietary treatments.

5. Renal calculi were demonstrated by light and electron microscopy in tubules of those trout given diets 3 and 5 (basal Mg; 26 and 40 g Ca/kg respectively). Electron-probe micro-analysis showed that these calculi contained or comprised tricalcium phosphate.

6. The skeletal muscle of Mg-deficient trout contained significantly more sodium than that of normal trout. It is suggested that this is indicative of an increase in extracellular fluid in the muscle of Mg-deficient trout.

A fundamental relationship exists between magnesium, calcium and phosphorus in the nutrition of birds and mammals (Forbes, 1963). This interdependence has been intensively studied and abnormalities arising from a reduction in the ratios, Mg:Ca and Mg:P are now well characterized. One of the most striking symptoms of Mg deficiency in birds and mammals is calcification of the kidney and other soft tissues (Britton & Stokstad, 1970; O'Dell, Morris & Regan, 1960).

Mg deficiency in carp (*Cyprinus carpio*) has been characterized by high mortality, poor growth, loss of appetite, sluggishness and convulsions, but soft tissues were not examined for any derangement of Mg, Ca or P metabolism (Ogino & Chiou, 1976). Although the kidney of freshwater fish acts primarily to conserve electrolytes and the urine concentrations of Mg and Ca are normally low (Hickman & Trump, 1969) nevertheless calculi have been observed in the kidneys of rainbow trout (*Salmo gairdneri*) under certain conditions not apparently associated with mineral imbalance (Smith, Brin & Halver, 1974; Kloppel & Post, 1975).

Many fish foodstuffs contain relatively large amounts of components (such as fish meal) that have comparatively high contents of Ca so that abnormalities arising from a high Ca intake or an imbalance of Ca, Mg and P are of practical interest. Finally, there is evidence that Mg deficiency symptoms in mammals are exacerbated by a high protein intake (Bunce, Reeves, Oba & Sauberlich, 1963) and by lowering the environmental temperature (Hegsted,

Vitale & McGrath, 1956). As both these factors feature in the life of fish these animals might appear especially prone to abnormalities resulting from Mg deficiency.

We have therefore studied the effect of Mg deficiency on the growth and kidney pathology of rainbow trout given diets containing up to 40 g/kg Ca and P.

EXPERIMENTAL

Animals

Rainbow trout of approximate mean weight 30 g were obtained from D. M. Brien, Almondbank, Perth; they had been reared on a commercial diet (Beta trout diet, Cooper Nutrition Products Ltd, Stepfield, Witham, Essex) containing (g/kg) 400 crude protein (nitrogen × 6·25), 45 oil and 45 fibre (manufacturers' specification). On analysis this diet contained (g/kg) 3·27 Mg, 50·35 Ca and 52·8 P. The trout were randomly distributed among fifteen fibreglass tanks (0·7 × 0·8 × 0·6 m deep) twenty fish/tank, three tanks/treatment and given, manually, a diet similar to diet 1 (Table 1) except that it contained 0·5 g Mg/kg. Within 3 d this diet was readily accepted by the fish.

Water from the City of Aberdeen domestic supply flowed into an elevated reservoir tank where it was heated to 15 ± 1° and vigorously aerated. Water from this reservoir was distributed to the fish tanks at the rate of 1·0 l/min per kg biomass fish; this water contained less than 0·7 μmol chlorine/l, it was not recirculated and ran to waste after leaving the fish tanks. Fish were fed to satiation four times/d (between 08.30 and 16.30 hours), food pellets being put sparingly into each tank only so long as they were actively consumed. Fish were fed 6 d/week, one person only doing the feeding. The tanks were housed in an aquarium room with an ambient temperature averaging 15°.

When the fish were completely accustomed to the experimental diet (about 10 d) initial weight measurements were made. All fish from each tank in turn were removed by hand-net into a 10 l bucket of water. Water was then poured from this bucket through a net which retained the fish. The net was allowed to drain for 10 s and the fish carefully transferred without handling to a second bucket of water which was already in position and tared on a top pan balance (Mettler P10, Gallenkamp & Co Ltd, East Kilbride, Scotland). After weighing, the fish were immediately returned to the fish tank and when all groups had been weighed replicate tanks were given the five experimental diets (see Table 1). Thereafter fish were weighed every 4 weeks throughout the experiment which lasted 16 weeks.

Diets

The composition of the experimental diets used is shown in Table 1; they were prepared in bulk as described previously (Cowey, Adron, Brown & Shanks, 1975) and made into moist pellets using an Alexanderwerk pelleting machine (Orthos (Engineering) Ltd, Market Harborough, Leicestershire). The pellets were freeze-dried then stored at −20° until required.

The actual quantities of Ca, P and Mg in the diets by analysis, are shown in Table 1. The intention was to compare the effects on growth and kidney histology of diets containing Mg at either a basal level or a level of 1·0 g/kg, together with Ca levels of 15, 25 and 40 g/kg, dietary Ca:P being 1:1. With the aquarium resources available, however, it was not possible to include a diet containing supplementary Mg at the lowest Ca and P level used. It can be seen from Table 1, that excluding the P level of diet 1, there was good agreement between the intended levels of Ca, P and Mg in the diets and the amounts found by analysis.

Table 1. *Composition (g/kg dry diet) of the experimental diets given to rainbow trout* (Salmo gairdneri)

Ingredient	Diet 1	2	3	4	5
Casein*	500	500	500	500	500
Dextrin	100	100	100	100	100
Cod-liver oil	20	20	20	20	20
Soya-bean oil	40	40	40	40	40
Mineral mixture†	34·7	34·7	34·7	34·7	34·7
Vitamin mixture‡	28	28	28	28	28
α-Cellulose	201·3	133·2	123·1	65·2	55·1
Binder§	50	50	50	50	50
Calcium lactate	26	26	26	26	26
$CaHPO_4 2H_2O$	0	50·9	50·9	101·8	101·8
NaH_2PO_4	0	17·2	17·2	34·3	34·3
$MgSO_4.7H_2O$	0	0	10·1	0	10·1
Chemical composition					
Calcium	14·16	26·47	27·36	39·79	39·85
Phosphorus	11·55	24·83	25·07	39·04	39·35
Magnesium	0·026	0·040	0·997	0·063	1·005

* Supplemented with (g/950 g casein): arginine 25, cystine 10, methionine 10, and tryptophan 5.

† Supplied (/kg dry diet): $Ca(H_2PO_4)_2.H_2O$ 24.3 g, $CaCO_3$ 5·2 g, $FeSO_4.7H_2O$ 1·0 g, KCl 2·1 g, NaCl 1·7 g, $Al_2(SO_4)_3.16H_2O$ 6·9 mg, $ZnSO_4.7H_2O$ 139 mg, $CuSO_4.5H_2O$ 34·7 mg, $MnSO_4.4H_2O$ 124·9 mg, KI 6·9 mg, $CoSO_4.7H_2O$ 34·7 mg.

‡ Supplied (/kg dry diet): riboflavin 200 mg, pyridoxine hydrochloride 40 mg, nicotinic acid 800 mg, calcium pantothenate 280 mg, *myo*-inositol 4 g, biotin 6 mg, pteroylmonoglutamic acid 15 mg, *p*-aminobenzoic acid 400 mg, choline chloride 8 g, ascorbic acid 2 g, DL-α-tocopheryl acetate 400 mg, menaphthone 40 mg, cyanocobalamin 90 μg, thiamin hydrochloride 50 mg.

§ Methofas; ICI Ltd, Ardrossan, Ayrshire.

Chemical methods

Replicate samples of each diet were ashed in a muffle furnace at 600° for 18 h. The ash was dissolved in a solution of strontium chloride hexahydrate (30·4 g $SrCl_2$ $6H_2O$/l, the Sr solution) containing also 1 M-hydrochloric acid; it was then made to volume (10 ml) with Sr solution. Portions of the sample suitably diluted with Sr solution were analysed for Ca and Mg by atomic absorption spectroscopy. Reagent blanks and standard solutions of Ca and Mg in Sr solution and containing HCl at the same concentration as the test samples, were also analysed, with the Sr preventing phosphate interference in the Ca and Mg estimations. Separate portions of the ashed sample were appropriately diluted with deionized, distilled water for P analysis (Bartlett, 1958).

Kidneys were analysed by the same procedure. Serum from blood obtained from the caudal vein of trout was deproteinized with trichloroacetic acid (50 g/l final concentration) before dilution with Sr solution. Serum Ca and Mg levels were then estimated by atomic absorption spectroscopy, aspirating the diluted serum directly into the flame of the spectrometer. Inorganic P was measured in separate portions of serum by the method of Bartlett (1958) after deproteinization with trichloroacetic acid.

Portions of skeletal muscle were treated in the same manner as were the kidneys before Ca estimation. Sodium and potassium levels in the solutions of ashed muscle were also estimated by flame emission spectroscopy.

Analyses were carried out on tissues from ten fish/treatment. Trout were killed by means of a sharp blow on the head, a blood sample taken from the caudal vein and tissues immediately removed. These were either weighed and ashed immediately, or stored in separate,

Table 2. *Total initial and final weights and food consumption of groups of rainbow trout* (Salmo gairdneri) *given diets containing different amounts of calcium and magnesium*

(Mean values with their standard errors for three tanks each with twenty fish)

	Total biomass (g/tank)				Food consumption (g/tank)		
	Initial		Final				g weight gain/g food eaten
Diet no.*	Mean	SE	Mean	SE	Mean	SE	
1	645	10	1276	20	990	30	0·64
2	658	12	1320	23	1020	45	0·65
3	626	9	1980†	29	1290†	80	1·05
4	636	10	1254	25	1030	50	0·60
5	635	9	2068†	30	1510†	105	0·95

* For details, see Table 1.
† Significantly greater than other treatments ($P < 0·01$).

sealed polythene bags at −20° until they could be analysed, or placed in fixative for histological analysis.

Histological methods

For light microscopy, small pieces of kidney from six fish/treatment were fixed in a solution containing 40 g formaldehyde/l and 0·05 M-phosphate, pH 7·0. Paraffin wax sections (thickness 4 μm) were cut and stained by the silver method of Von Kóssa (Pearse, 1972) which depends on the presence of phosphate or carbonate (although insoluble phosphates and carbonates in animal tissues are nearly always those of Ca) and by the phthalocyanin method for Ca (Pearse, 1972). Other paraffin sections were stained with haematoxylin and eosin.

For electron microscopy, samples of kidney tissues from three trout from each experimental group were fixed at 4° for 60 min in a solution containing 40 g formaldehyde/l, 50 g glutaraldehyde/l and 0·1 M-sodium cacodylate, pH 7·6. The samples were then briefly post-fixed in cacodylate-buffered osmium tetroxide (20 g/l), pH 7·6, dehydrated in graded alcohols and propylene oxide, then embedded in araldite. Ultra-thin sections were examined in an electron microscope (Philips EM 201:Pye Unicam Ltd, Cambridge) operating at 40 kV. Electron probe x-ray microanalysis was carried out with an analytical electron microscope (AEI CORA; AEI Scientific Instruments Ltd, Manchester) or a scanning electron microscope (Philips PSEM 500) fitted with transmission and X-ray detectors.

RESULTS

There was no mortality among the fish given the experimental diets; apart from a sluggishness apparent in the trout given diet 4, there were no behavioural or gross external abnormalities.

The weight gains of the trout are shown in Table 2. Fish given diets 3 and 5 gained significantly more weight than those given the other three diets. The weight gains of those fish given diets containing only basal levels of Mg (diets 1, 2 and 4) were not themselves significantly different, and during the experimental period high dietary Ca and P levels (40 g/kg; diet 4) were not themselves more deleterious in respect of weight gain than lower dietary Ca levels (14 g/kg; diet 1) under conditions of Mg deficiency.

Food consumption is also shown in Table 2. Trout given diets containing supplementary

Table 3. *Mean concentrations (μmol/l) of calcium, phosphate and magnesium in the sera of rainbow trout* (Salmo gairdneri) *given diets containing different amounts of these minerals*

(Mean values with their standard errors for ten fish/treatment)

	Ca		Phosphate		Mg	
Diet no.*	Mean	SE	Mean	SE	Mean	SE
1	5200†	163	6·1	0·3	234	12·1
2	4630	110	6·5	0·3	323	57·5
3	4280	78	6·1	0·2	905†	75·8
4	4380	80	5·8	0·3	355	26·7
5	4380	110	6·5	0·3	915†	41·7

* For details, see Table 1.
† Significantly greater than other treatments ($P < 0·01$).

Mg consumed significantly more food than trout given diets deficient in Mg. Thus Mg deficiency led to loss of appetite. In addition the ratio, weight gain: food eaten was markedly inferior in Mg-deficient trout to that in normal trout, food conversion being adversely affected by Mg-deficiency.

Mg and Ca were monitored in the aquarium water during the experiment, the mean concentrations (mmol/l) found were 0·05 Mg and 0·17 Ca. Although freshwater fish do have the capacity to take up some ions from the water through the gills or other permeable surfaces (Ichikawa & Oguri, 1961; Templeton & Brown, 1963) any such capability with respect to Mg was clearly inadequate to compensate for Mg deficiency in the diet under the prevailing conditions.

Serum Mg concentrations were 3–4-fold lower in trout given the diets deficient in Mg than in those given diets containing supplementary Mg (Table 3). Serum Ca concentration was slightly, but significantly, increased in those trout given diet 1 containing the lowest levels of Ca (14 g/kg) and Mg (26 mg/kg) employed in the experimental treatments. These trout apart, serum Ca and orthophosphate concentrations were similar in trout given the five experimental diets, so that even up to high levels of Ca and P intake serum levels of these minerals were controlled and this control was not affected by a low dietary Mg level.

The Mg content of the kidneys of trout was constant irrespective of Mg level in the experimental diet (Table 4). However, the Ca content of the kidney was greatly influenced by dietary Mg level; at a dietary Ca concentration of approximately 25 g/kg the Ca content of the kidney was 7-fold greater in trout given a diet deficient in Mg compared with those given supplementary Mg (diet 2 *v.* diet 3; Table 4). In the absence of supplementary Mg, increasing the dietary Ca concentration to 40 g/kg did not significantly increase the renal Ca levels (diet 2 *v.* diet 4), but at this dietary Ca concentration, renal Ca levels were increased even in the presence of supplementary Mg (diet 4 *v.* diet 5). At the lowest dietary Ca level (14 g/kg; diet 1) the renal Ca concentration was 0·86 g/kg. Had facilities permitted the use of a comparable diet containing supplementary Mg it is likely that the kidney Ca levels resulting would have been significantly lower, because the kidneys of hatchery-reared trout (for details of mineral levels see p. 128) contained (mean ± SE; g/kg) 0·15 ± 0·004 Mg, 0·17 ± 0·02 Ca and 2·23 ± 0·05 P.

Histological examination of the trout kidneys revealed the presence of Ca deposits in the proximal tubules of all fish given diet 4. The Ca deposits completely blocked the lumen of the tubules, the epithelial cells of which were necrotic (Plate 1 A and B). Renal calculi were also present in the trout given diet 2, but were not seen in sections from every individual examined;

Table 4. *Mean concentrations (g/kg wet weight) of calcium, phosphorus, magnesium and ash in the kidneys of rainbow trout* (Salmo gairdneri) *given diets containing different amounts of these minerals*

(Mean values with their standard errors for ten fish/treatment)

	Ca		P		Mg		Ash	
Diet no.*	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	0·86[a]	0·26	3·00[a]	0·14	0·15[a]	0·002	14·81[a]	0·57
2	2·88[b]	0·96	4·16[a]	0·42	0·15[a]	0·003	19·83[a]	2·74
3	0·40[a]	0·07	3·05[a]	0·18	0·15[a]	0·003	14·16[a]	0·32
4	2·83[b]	0·82	4·10[a]	0·45	0·15[a]	0·002	20·14[a]	2·11
5	1·59[b]	0·68	3·08[a]	0·28	0·15[a]	0·006	15·25[a]	1·52

Values in the same column with unlike superscripts were significantly different ($P < 0·01$).
* For details, see Table 1.

qualitatively their incidence was lower than in fish given diet 4. No renal calculi were present in any of the sections from individuals given diets 1, 3 and 5.

Electron microscopy of the kidneys of the experimental trout indicated that two types of deposit were present in the kidneys of abnormal individuals, these we have denoted types 1 and 2. The 'type 1' deposits (Plate 2A) were extremely electron dense and completely occluded the lumen of the renal tubules, the tubule cells showing signs of mitochondrial degeneration and necrosis. The 'type 2' deposits (Plate 2B) contained several foci and were associated with necrotic debris in the lumen of renal tubules. Both types of deposit appeared as an amorphous central region surrounded by needle-shaped crystals. These crystals often appeared as a halo in the 'type 2' deposits. The depleted zone occurring in some 'type 2' deposits between the needle-shaped crystals and amorphous central core was produced by the crystallization process and indicated that the crystals were produced during specimen preparation by dissolution and reprecipitation of the calculus, which in vivo is probably entirely amorphous.

The sections were examined in the analytical electron microscope to determine the elemental composition of the calculi. The X-ray spectrum of a 'type 1' deposit is shown in Fig. 1A. Elemental peaks were seen for P (2 keV), lead (2·4 keV also at 10·5, 12·6 and 14·8 keV) arising from the counterstain used for contrast enhancement, Ca (3·7 and 4·0 keV) and copper (8·0 and 8·9 keV) derived from the Cu specimen-support grid. Mg was not detected in the deposits. Background counts were taken from adjacent areas of the specimen. When 'type 2' deposits were analysed the same elemental peaks were obtained for the amorphous centre and needle-shaped crystals; however, neither Ca nor P was detected in the intervening clear area. The value for Ca:P of the deposits was calculated after subtraction of background counts and by comparison with a $CaHPO_4$ standard (Fig. 1B). The value thus determined was $1·43 \pm 0·12:1·0$ (n 6); the 'whole number' value for the ratio was 3:2, i.e. that for tricalcium phosphate (apatite). We were not able to demonstrate the characteristic electron diffraction pattern for apatite.

The concentrations of Ca, Na and K in the muscle of trout given four of the experimental diets are shown in Table 5. It had not been our intention to analyse skeletal muscle and therefore no samples of tissue were taken from the first batch of fish to be examined (those given diet 2). When tissues were taken the next day from the next group of fish, which were those given diet 4, the skeletal muscle was obviously more flaccid and different in texture from that of normal trout. Consequently muscle samples were taken for analysis from these fish and from those given diets 1, 3 and 5. Tissue samples were taken on subsequent days; tissue collection took place over a 5 d period.

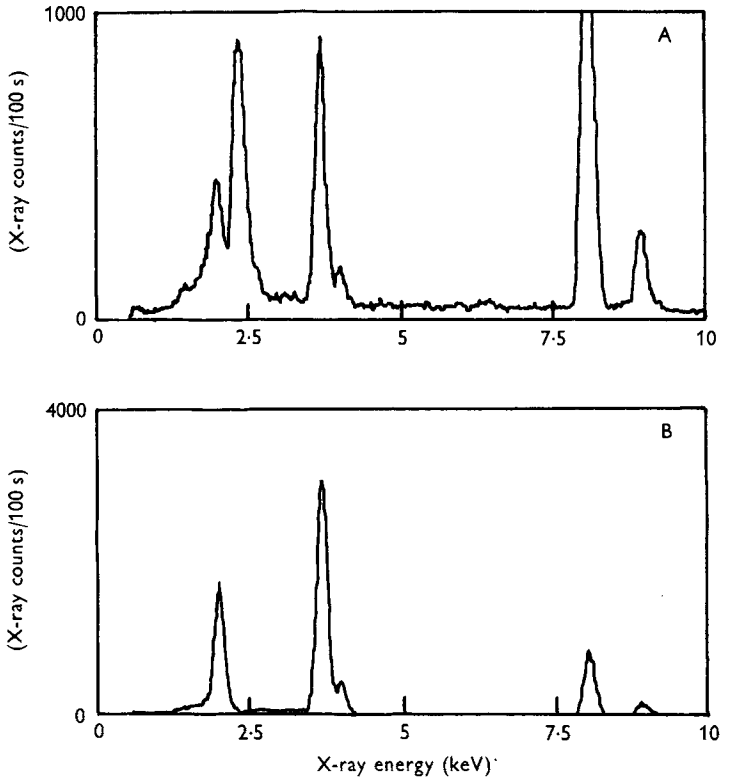


Fig. 1. Analytical electron microscopy of calcified tissue: (A) X-ray spectrum of 'type 1' deposit (see p. 132) in renal tubule of rainbow trout (*Salmo gairdneri*) given diet 4 (see Table 1); (B) X-ray spectrum of calcium phosphate standard.

Table 5. *Mean concentrations (mg/kg wet weight) of calcium, sodium and potassium in the muscle of rainbow trout* (Salmo gairdneri) *given diets containing different amounts of calcium, phosphorus and magnesium*

(Mean values with their standard errors for ten fish/treatment)

	Ca		Na		K	
Diet no.*	Mean	SE	Mean	SE	Mean	SE
1	0·66[c]	0·17	827[a]	68	5801[a]	282
2	L		L		L	
3	0·41[b]	0·05	299[b]	17	5362[a]	88
4	0·96[a]	0·17	1141[b]	127	4890[a]	164
5	0·38[b]	0·04	330[b]	38	5019[a]	121

Values in the same column with unlike superscripts are significantly different ($P < 0·01$).
L, samples lost.
* For details, see Table 1.

There were no significant differences in muscle Mg (mean ± SE 0·25 ± 0·008 g/kg) or P (mean ± SE 2·47 ± 0·08 g/kg) in the four treatments for which results were available. Levels of both Na and Ca in muscle were significantly increased in those trout given diets lacking supplementary Mg. K concentration in the muscle was not significantly changed by alterations in either the Ca (diet 3 *v.* diet 5) or Mg (diet 4 *v.* diet 5) levels in the diet.

As K is primarily an intracellular ion and Na the principal cation in extracellular fluids the observed changes in trout muscle under conditions of Mg deficiency may be indicative of an increase in extracellular fluid, the tissues becoming oedematous.

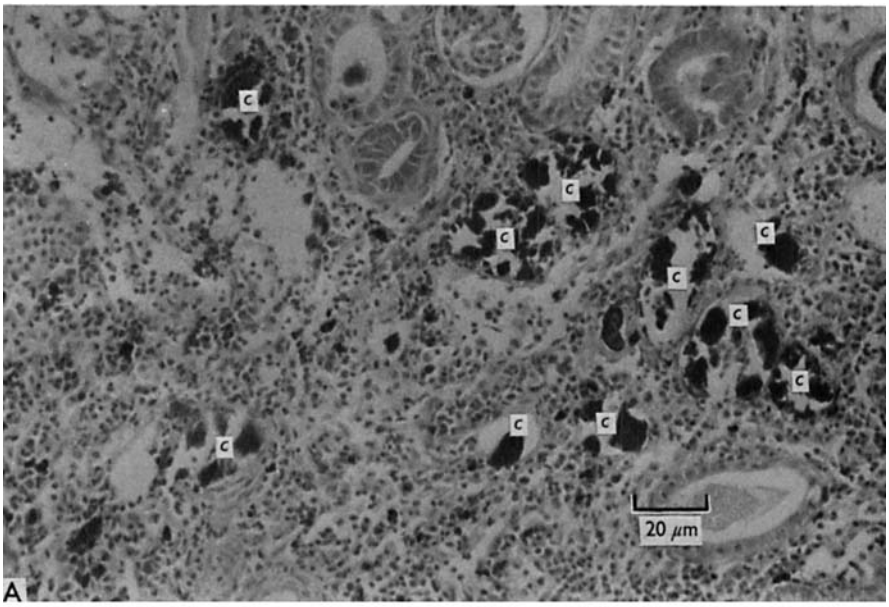
DISCUSSION

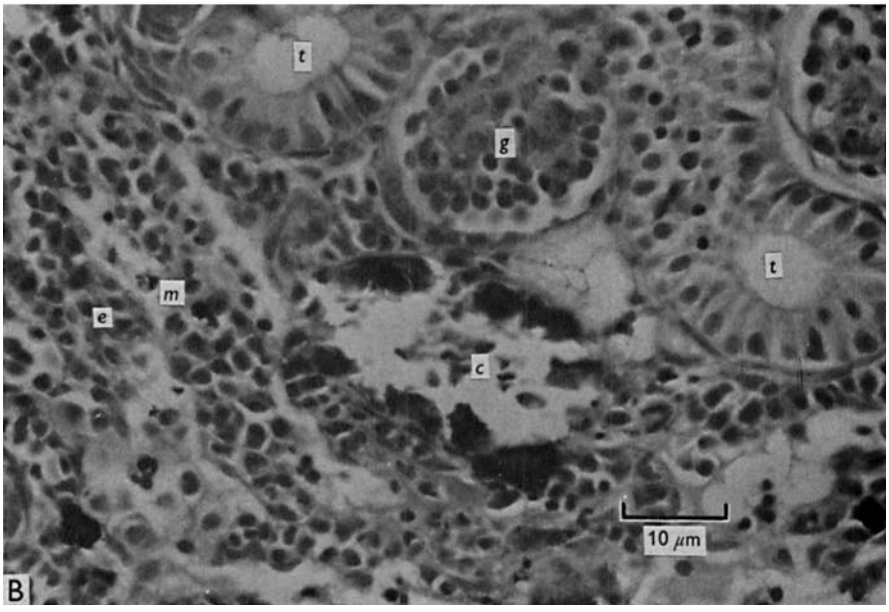
Although the tissue abnormalities that occur in trout as a result of Mg deficiency bear a marked qualitative similarity to those occurring in mammals some differences are apparent at a gross level. Thus there was no mortality in trout given diets containing as little as 26 mg Mg/kg and these fish doubled their live weight during a 16-week experimental period. By contrast Britton & Stokstad (1970) found it necessary to supplement diets containing 80 mg Mg/kg with an additional 20 mg Mg/kg in order to ensure survival of rats during a 28 d experimental period. This ability of fish to survive and grow at much lower dietary Mg levels than the rat may depend on uptake of Mg from the water. Ogino & Chiou (1976) include high mortality as a symptom of Mg deficiency in carp and indeed mortalities of up to 16 % occurred in carp given a diet containing 52 mg Mg/kg. However, in a second experiment no mortality occurred at dietary Mg concentrations of 80 mg/kg even though the requirement of young carp for Mg was estimated to be 400–500 mg/kg.

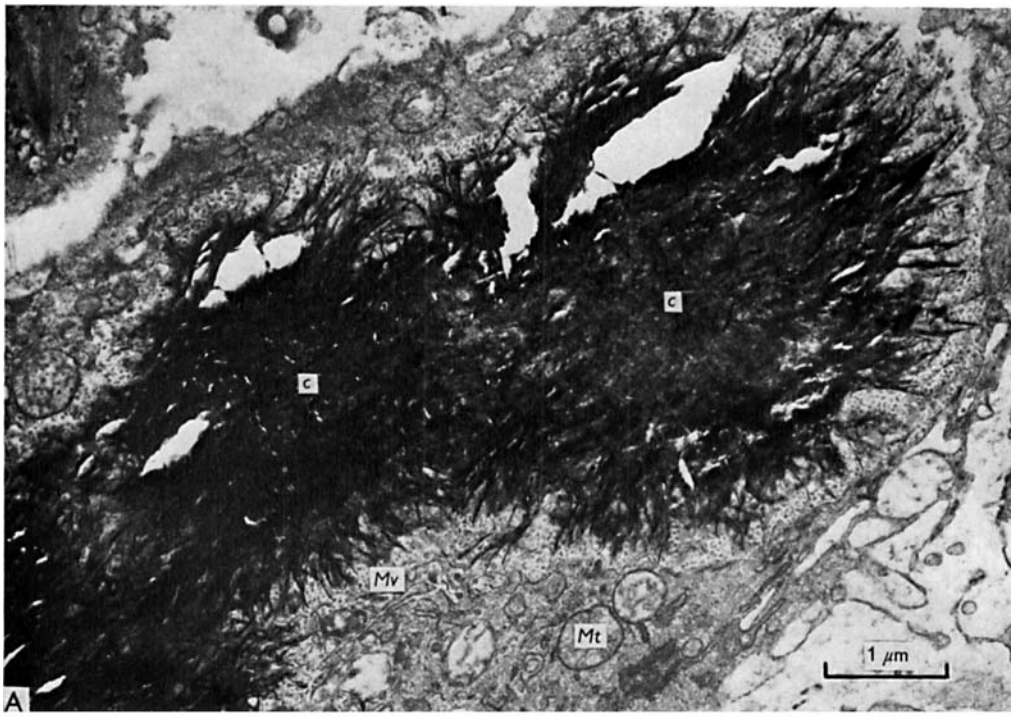
The severity of nephrocalcinosis in Mg-deficient rats appears to depend on many factors, including the age of the animal, the duration of the experiment and the basal level of Mg in the deficient diet. Thus renal Ca levels may be increased by as little as 4-fold (MacIntrye & Davidsson, 1958) or by more than 100-fold (Britton & Stokstad, 1970). The Mg concentration in the basal, Mg-deficient diet used by Britton & Stokstad (1970) was similar to that used in the present work on trout and their findings thus form an appropriate basis for comparison of nephrocalcaemia in the two animals. In their experiments renal Ca levels were as high as 8·3 g/kg after rats had been given a Mg-deficient diet (0·1 mg Mg/kg in total) for 4 weeks; this increased renal Ca level occurred when dietary Ca levels were quite low (6·2 g/kg). The increase in renal Ca concentration in Mg-deficient trout, on the other hand, was much less severe even at a dietary Ca level of 14 g/kg in an experiment of 16 weeks' duration. Possible reasons for the abnormalities being less severe in trout are the availability of some Mg in the water, and the fact that the freshwater fish normally excretes large amounts of a very hypotonic urine which may assist in removal of Ca from the kidney.

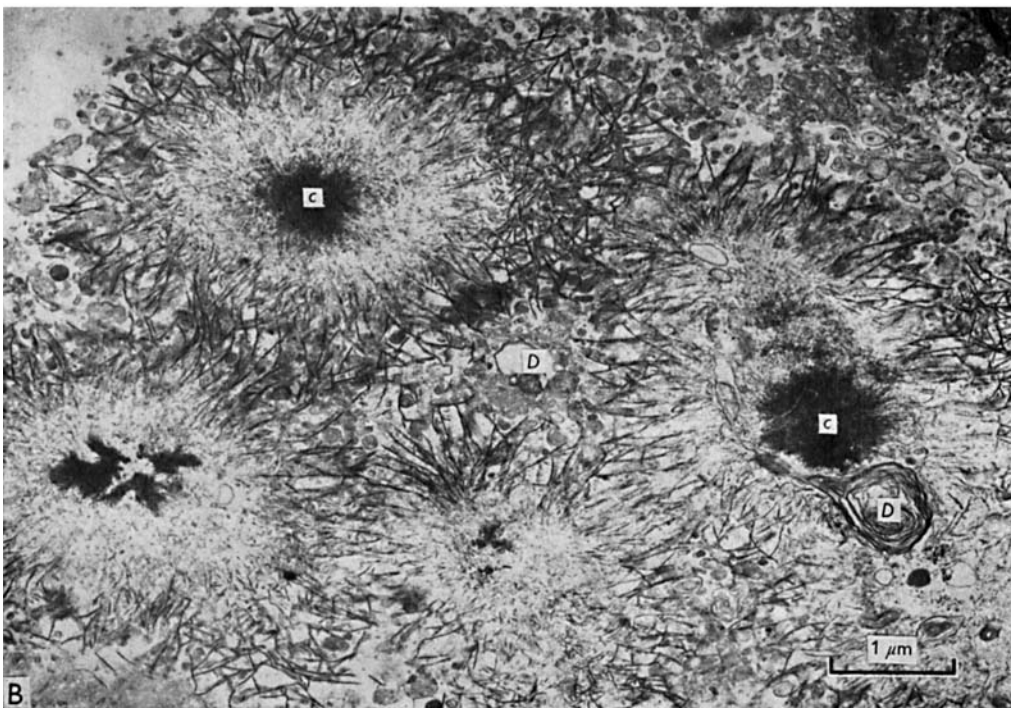
The low renal Ca concentrations (0·17 g/kg) in the hatchery-reared trout suggest that very high dietary Ca levels (50 g/kg) are not deleterious provided a proportionately high dietary level of Mg is also supplied (the Mg level in the 'hatchery' diet was 3·27 g/kg). In this context it is noteworthy that increasing Ca levels in the experimental diets from approximately 26 g/kg (diet 2) to approximately 40 g/kg (diet 4) in the absence of supplementary Mg did not result in any further increase in renal Ca concentration (Table 4), although the incidence of renal calculi in trout given diet 4 appeared qualitatively greater than in trout given diet 2.

The marked decrease in serum Mg concentration in the Mg-deficient trout, while muscle and kidney Mg levels remained unchanged, is in line with observed effects of Mg deficiency in the soft tissues of the rat (Britton & Stokstad, 1970; Elin, Armstrong & Singer, 1971). Mg is probably held in a non-diffusable state in the soft tissues, bone Mg (mainly vertebral column in fish) serving as a reservoir on which soft tissues draw under conditions of Mg deficiency. Thus, in contrast with the constancy of soft tissue Mg concentration in Mg-deficient trout, Ogino & Chiou (1976) observed that the Mg content of vertebrae of carp decreased from 2·61 to 1·56 g/kg dry matter in Mg deficiency. The skeletal reserve of Mg in fish is likely to be much less than that of mammals both because fish lack the skeletal structure of mammals and because the vertebrae of fish contain much less Mg than do typical









C. B. COWEY AND OTHERS

bones of mammals (e.g. rat femur 7·3 g Mg/kg bone ash (Elin *et al.* 1971), carp vertebrae 2·6 g Mg/kg ash (Ogino & Chiou, 1976)). Many metal-activated enzymes specifically require Mg as a co-factor and the constancy of Mg concentration in soft tissues would tend to limit metabolic derangement.

The muscles of Mg-deficient rats have increased concentrations of Na and reduced concentrations of K; there is a shift in tissue water, the interstitial fluid increasing in quantity and the intracellular fluid decreasing (Elin *et al.* 1971); the intracellular K concentration also decreases. The increase of Na concentration in muscle of Mg-deficient trout is indicative of an increase in interstitial fluid. This implies a relative reduction in cellular volume within the muscle tissue as a whole and should lead to a decrease in over-all K concentration of the muscle. The K concentration of the muscle did not, however, change significantly in the treatments used. An explanation of this question might be that the intracellular K concentration actually increases during Mg-deficiency or that K leaks into the interstitial fluid. These questions are being pursued by attempting to obtain values for volumes of fluid spaces in the muscle under different conditions and on the concentrations of ions in these fluids.

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EXPLANATION OF PLATES

Plate 1 (A). Photomicrograph of kidney from rainbow trout (*Salmo gairdneri*) given diet 4 (see Table 1) showing the incidence of calcified tubules (*c*) (stain: phthalocyanin); (B) photomicrograph of kidney from trout given diet 4 showing calcified tubule (*c*), the calcified area having shattered during sectioning, with necrotic epithelial cells. Normal tubules (*t*), glomerulus (*g*), erythropoietic tissue (*e*) interspersed with melanocytes (*m*) were also present.

Plate 2 (A). Electron micrograph of calcified kidney tubule from rainbow trout (*Salmo gairdneri*) given diet 4 (see Table 1). Transverse section of tubule showing lumen occluded by 'type 1' deposit (see p. 132) (*c*). The microvilli (*Mv*) and mitochondria (*Mt*) of the tubular cell appeared intact; (B) electron micrograph of calcified kidney tubule from fish given diet 4. Section of collecting duct showng 'type 2' deposits (see p. 132) (*c*) associated with necrotic debris (*D*).

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