The Relation between Skeletal and Extracellular-Fluid Magnesium *in vitro*

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1. Femora from control and magnesium-deficient rats were incubated in media of different magnesium concentrations. 2. Magnesium was lost at the same rate from fresh and boiled bones to a magnesium-free medium. 3. The equilibrium established was dependent on the magnesium concentrations in both the bone and the medium. 4. Parathyroid extract increased the rate of magnesium loss from bone to a magnesium-low medium containing bovine serum (50%), but had no effect in a protein-free medium.

Magnesium deficiency in the rat has been repeatedly shown to cause a lowering of the magnesium concentration in bone (e.g. Cunningham, 1936; Watchorn & McCance, 1937). Duckworth, Godden & Warnock (1940) showed that the total magnesium present in the femur of the weanling rat decreased by 30% during a 6-day period of dietary magnesium depletion. Moreover, when magnesium was restored to the diet of similar magnesium-deficient rats it was redeposited in the bone. Martindale & Heaton (1964) demonstrated a net loss of magnesium from the femur of adult magnesium-deficient rats, but the loss amounted to only 17% of the original magnesium content during a 62-day period of deficiency. These observations indicate that part of the skeletal magnesium acts as a reserve which can be mobilized during deficiency to meet the requirements of other tissues and that, like calcium, the availability of the skeletal magnesium is proportionally greater in the young than in the adult rat.

The observation of a close direct relation between the magnesium concentrations in the plasma and the femur of magnesium-deficient rats (McAleese & Forbes, 1961; Martindale & Heaton, 1964) and calves (Smith, 1959) supports the view that the skeleton provides the magnesium reserve in the body and suggests the existence of an equilibrium between the magnesium in the plasma and the bone. The present experiments, where bones from magnesium-deficient and control rats have been incubated in media of different Mg concentra-

tions, were undertaken in an attempt to elucidate further the relation between plasma and bone magnesium.

EXPERIMENTAL

Experiments were conducted with female Wistar albino rats weighing 90–110g., which were always obtained from the same breeder. The animals described under sections (1) and (3) of the Results section were maintained on stock diet 41B (Oxo Ltd.). The rats described in section (2) were given synthetic magnesium-deficient and control diets (Martindale & Heaton, 1964), but the magnesium content of the control diet was twice that employed previously.

The rats were killed by cardiac puncture under ether anaesthesia. Both femora were rapidly removed and freed from adhering tissue by scraping with a sharp scalpel. When fresh femora were required they were immediately immersed in a small volume of Mg-free incubation medium A. Other bones were killed by boiling in distilled water for 2 min. (section 1) or 10 min. (section 3).

Incubation procedure. Two types of incubation medium of pH 7.4 and 10.14, and having a mineral composition similar to normal rat serum, were employed. Medium A contained: NaCl (110 mm), NaHCO₃ (20 mm), KCl (6.4 mm), NaH₂PO₄ (1.4 mm), Na₂HPO₄ (0.3 mm), CaCl₂ (1.5 mm) and glucose (4.4 mm). Medium B consisted of equal volumes of medium A and bovine serum that had been treated with a cation-exchange resin, Zeo-Karb 225 (Na⁺ form). The resin removed 63% of the Mg and Ca from the serum, replacing them with Na, the concentration of which rose by 5 mm. Additional Ca was added to this medium to replace the Ca removed by the resin. Both media were equilibrated with CO₂ + O₂ (5:95) before use and sufficient MgCl₂ was added to give the desired Mg concentration.

Experiments with parathyroid extract used Para-Thor-Mone (Eli Lilly and Co. Ltd., Basingstoke, Hants.) of the same batch number containing 100 U.S.P. units/ml. Sufficient extract was added to give a concentration of 7 units/ml. in the incubation medium. The parathyroid

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extract contained phenol (0.2%) as a preservative and the incubation medium for control experiments was therefore treated with the same volume of aq. 0.2% phenol solution. The addition of the parathyroid extract or phenol lowered the ionic strength to 0.13 and the pH to 7.35 in all the media used for this series of experiments.

A complete femur was used for each incubation and the two bones from each rat were assigned to different treatments to facilitate comparison. Each femur was cut transversely into five pieces, the marrow was removed from the shaft and the pieces were placed in a conical flask of 50 ml. capacity containing 15 ml. of the appropriate medium. The flasks were placed in a water bath at 37°, gassed with CO₂+O₂ (5:95) and shaken mechanically for the required time. The flasks were re-gassed periodically during the incubation to ensure that no significant change in the composition of the gaseous phase occurred. The pH of the medium fell by 0.1 unit during incubation in all experiments, but there was no significant change in the ionic strength of the medium.

Analytical methods. The incubation medium was removed at the end of the experiment and the bone fragments were rinsed with a small volume of distilled water, then dried and ashed as described by Martindale & Heaton (1964). Magnesium was determined in the medium and the solution of bone ash by atomic-absorption spectrophotometry (Dawson & Heaton, 1961). The statistical significance of differences was assessed by the *t* test.

RESULTS

(1) *Loss of magnesium from fresh and boiled femora in a Mg-free medium.* Femora from 11 normal rats were incubated in Mg-free medium A for various periods up to 24 hr. One femur from each rat was incubated when fresh and the other was boiled before incubation.

Magnesium was lost rapidly and at the same rate from fresh and boiled bone to the medium during

Fig. 1. Magnesium loss from fresh (○) and boiled (●) femora incubated in Mg-free medium. Experimental details are given in the text.

the first 5 hr. of incubation (Fig. 1). The magnesium lost from boiled bone increased only very slowly after 9 hr., but the loss from fresh bones was considerably greater than from boiled femora after 14 and 24 hr. The media from fresh bones incubated for 14 and 24 hr. were, however, heavily contaminated with bacteria. When the experiment was repeated with chloramphenicol (2mg./100ml.) in the medium no difference was observed between fresh and boiled bones, confirming that the differences shown in Fig. 1 were due to bacterial growth in the media containing fresh femora.

(2) *Relation between magnesium concentrations in the bone and the medium at equilibrium.* Fresh femora from three rats given the magnesium-deficient diet for 14 days and three control rats were incubated for 10 hr. in medium A containing different Mg concentrations. The initial Mg concentrations used were 0.00, 0.75 and 1.89 mg./100 ml. The magnesium concentrations in the femora from the magnesium-deficient and control rats were 4.80 and 6.81 mg./g. of ash respectively.

The change in bone magnesium content varied with the Mg concentration in the medium (Fig. 2). The bones from both groups of rats lost magnesium to the two media of lowest Mg concentration, and gained magnesium from the medium of highest Mg concentration. The gradients of the lines relating change in bone magnesium content to Mg concentration in the medium were similar for bones from magnesium-deficient and control rats; the intercepts with the

Fig. 2. Relation between change in bone magnesium content and the final Mg concentration in the medium for femora from control (○) and magnesium-deficient (●) rats. Experimental details are given in the text.

abscissa, at zero change in bone magnesium content, indicated equilibria with Mg concentrations in the medium of 1.12 and 1.50 mg./100 ml. respectively.

In a second experiment, femora from control rats and rats given the magnesium-deficient diet for periods of 6 and 20 days were incubated for 9 hr. in medium B of initial Mg concentration 0.22 and 1.71 mg./100 ml. The relation between the change in bone magnesium content and the Mg concentration in the medium was similar to that shown in Fig. 2. The intercepts with the abscissa indicated equilibria with Mg concentrations in the medium of 1.03, 1.42 and 1.60 mg./100 ml. with the bones from severely deficient, slightly deficient and control rats respectively.

The change in bone magnesium content varied with the magnesium concentration in the femur when incubated in either medium (Fig. 3). All bones gained magnesium from the medium containing 1.71 mg. of Mg/100 ml. and lost magnesium to the medium of Mg concentration 0.22 mg./100 ml. The increase in bone magnesium content was greatest in the femora of severely deficient rats and the loss of bone magnesium was greatest from the femora of control rats.

(3) *Effect of parathyroid extract on the loss of magnesium from femora in a Mg-free medium.* Fresh femora from ten normal rats and boiled femora from 12 normal rats were incubated singly

for 5 hr. in medium B of Mg concentration 0.10 mg./100 ml. One femur from each rat was incubated in medium supplemented with 7 units of parathyroid extract/ml. and the other femur was incubated in medium treated with the same volume of aq. 0.2% phenol solution.

Magnesium was lost from the bone into the medium under all conditions (Table 1). The loss was significantly greater in the presence of parathyroid extract than in the corresponding control preparations, irrespective of whether the bones were fresh or boiled. The increase in magnesium loss produced by parathyroid extract (12.2%) was the same with fresh and boiled bones.

The effect of parathyroid extract on the loss of bone magnesium in a protein-free medium was investigated by incubating fresh femora from six normal rats for 5 hr. in Mg-free medium A treated with the same amounts of parathyroid extract or phenol solution as were used in the previous experiment. No significant difference in magnesium loss was observed between the bones treated with parathyroid extract and those treated with phenol (Table 2).

Fig. 3. Relation between change in bone magnesium content and the magnesium concentration in the bone when femora were incubated in media containing 0.22 (○) and 1.71 (●) mg. of Mg/100 ml. Experimental details are given in the text.

Table 1. *Effect of parathyroid extract on the loss of magnesium from fresh and boiled femora incubated in medium B*

Experimental details are given in the text: $n=10$ for fresh femora; $n=12$ for boiled femora. The magnesium concentration in bone is 6.32 mg./g. of ash.

	Fresh femora		Boiled femora	
	Parathyroid	Phenol	Parathyroid	Phenol
Mean fall in bone magnesium content (mg./g. of ash)	0.249	0.222	0.267	0.238
Mean difference	0.027		0.029	
S.E.M.	0.005		0.007	
P value	< 0.001		< 0.01	

Table 2. *Effect of parathyroid extract on the loss of Mg from fresh femora incubated in medium A*

Experimental details are given in the text: $n=6$. The magnesium concentration in bone is 6.32 mg./g. of ash.

	Parathyroid	Phenol
Mean fall in bone magnesium content (mg./g. of ash)	0.273	0.274
Mean difference	-0.001	
S.E.M.	0.003	
P value	> 0.7	

DISCUSSION

The relation between the magnesium concentrations in the femur and the incubation medium observed *in vitro* during the present experiments is in good agreement with the direct relation between the magnesium concentrations in the femur and the plasma reported during magnesium deficiency in intact rats (McAleese & Forbes, 1961; Martindale & Heaton, 1964). The equilibrium established after 9 hr. incubation was dependent on the magnesium concentrations in both the medium (Fig. 2) and the bone (Fig. 3). Moreover, as no difference was observed between fresh femora and bones previously inactivated by boiling (Fig. 1), this equilibrium is apparently independent of enzymic activity and must therefore be physicochemical in nature.

The dependence of the equilibrium on the magnesium concentrations in both the medium and the bone suggests that the relation between bone and extracellular-fluid magnesium is analogous to the ionization of a poorly dissociated salt, with the magnesium in bone corresponding to the undissociated salt. This contrasts with the behaviour of calcium, which appears to give a constant calcium phosphate solubility product in the medium at equilibrium when bone powder is incubated *in vitro* (MacGregor & Nordin, 1962).

The action of parathyroid extract in increasing magnesium loss from normal bone into a Mg-low medium (Table 1) is consistent with the observation that the mobilization of skeletal magnesium during a period of dietary magnesium deficiency is impaired in parathyroidectomized rats (Heaton, 1965). The effect of parathyroid extract was observed with

both fresh and boiled bone. As the metabolic activity of the fresh bone was not established, this observation does not exclude the possibility that parathyroid extract may exert a greater effect on living than on dead bone. It does, however, indicate that the extract can promote the mobilization of magnesium as well as calcium (Gordan, 1963) from dead bone under suitable conditions. The action of parathyroid extract in increasing magnesium loss from bone was observed in media containing bovine serum (Table 1) but not in protein-free media (Table 2), which is consistent with Gordan's (1963) suggestion that the physicochemical action of parathyroid preparations may involve the binding of bivalent cations by a parathyroid peptide-albumin complex.

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REFERENCES

- Cunningham, I. J. (1936). *N.Z. J. Sci. Tech.* **18**, 419.
Dawson, J. B. & Heaton, F. W. (1961). *Biochem. J.* **80**, 99.
Duckworth, J., Godden, W. & Warnock, G. M. (1940). *Biochem. J.* **34**, 97.
Gordan, G. S. (1963). *Acta endocr., Kbh.*, **44**, 481.
Heaton, F. W. (1965). *Clin. Sci.* **28**, 543.
McAleese, D. M. & Forbes, R. M. (1961). *J. Nutr.* **73**, 94.
MacGregor, J. & Nordin, B. E. C. (1962). *J. biol. Chem.* **237**, 2704.
Martindale, L. & Heaton, F. W. (1964). *Biochem. J.* **92**, 119.
Smith, R. H. (1959). *Biochem. J.* **71**, 609.
Watchorn, E. & McCance, R. A. (1937). *Biochem. J.* **31**, 1379.