Br. J. Nutr. (1975), **34**, 45

Influence of magnesium deficiency on horse foal tissue concentrations of Mg, calcium and phosphorus*

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(*Received* 1 *July* 1974 – *Accepted* 12 *September* 1974)

1. The effects of feeding with a purified magnesium-deficient diet (−Mg, 7–8 mg Mg/kg) on horse foal blood serum and tissue concentrations of Mg, calcium and phosphorus were studied, and the results compared with histopathological findings.

2. Serum concentrations of Ca and P were unaffected by feeding with the −Mg diet, whereas serum Mg concentrations decreased from a mean initial (day 0) concentration of 0·78 mmol/l to 0·53 mmol/l 7 d after foals were placed on the −Mg diet, and then continued to decrease at a slower rate.

3. Aorta concentrations of Ca and P, but not Mg, were positively correlated with the period of time foals were given the −Mg diet, verifying histopathological findings. Results for both aorta Ca and P analyses and histopathological studies indicated that mineralization of the aorta began approximately 30–35 d after foals were placed on the −Mg diet.

4. Feeding with the −Mg diet had no significant, analytically detectable effect on brain, liver, kidney, lung, spleen, skeletal or cardiac muscle concentrations of Ca, P or Mg, although microscopic evidence of mineralization was seen in some of these tissues from foals given the −Mg diet for 71–180 d.

5. A significant negative correlation was found between bone ash concentrations of Mg (rib, metacarpus and metatarsus) and the length of time foals were fed on the −Mg diet. Bone ash concentrations of Ca and P were, however, unchanged.

6. Low serum Mg values and negative correlations between the bone ash concentration of Mg and the period of time foals were fed on the −Mg diet supplemented with 390 mg Mg as MgO/kg were interpreted as suggesting that either this level of Mg supplementation is marginal for the growing foal, or that the Mg in MgO is not readily available to the growing foal.

Magnesium is assumed to be an essential mineral nutrient for the horse but information relating to the effects of a deficiency of Mg in this species is meagre. Although Green, Allcroft & Montgomerie (1935) reported that the hypocalcaemic-associated tetany syndrome described earlier for Welsh mountain ponies by Montgomerie, Savage & Dodd (1929) was also complicated by hypomagnesaemia, neither group of workers studied the diets of affected animals, and the possible relationship between this condition and an inadequate intake of Mg, remains uncertain. More recently, Harrington (1974) described degenerative changes and mineral deposition in the cardiovascular and skeletal muscle systems of horse foals given a purified diet containing 7–8 mg Mg/kg. Lesions found were similar to those reported for Mg-deficient calves (Moore, Hallman & Sholl, 1938), dogs (Vitale, Hellerstein, Nakamura & Lown, 1961; Bunce, Jenkins & Phillips, 1962), rats (Heggtveit, 1969; Britton &

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Table 1. *Composition (g/kg) of magnesium-deficient (−Mg) and Mg-supplemented (+Mg) diets given to horse foals*

Ingredient	+Mg	−Mg
Casein	160·000	160·000
Glucose	388·004	388·654
Maize starch	250·000	250·000
Cellulose*	130·000	130·000
Cottonseed oil	10·000	10·000
Vitamin mix†	10·000	10·000
Mineral mix‡	51·106	51·106
MgO	0·650	
Choline chloride§	0·240	0·240

* Solka Floc BW20; Brown Company, Boston, Mass., USA.

† Mixed with glucose and supplied (mg/kg diet): nicotinic acid 4·476, *myo*-inositol 40, *p*-aminobenzoic acid 2·2, thiamin hydrochloride 1·544, riboflavin 0·455, calcium pantothenate 0·455, pyridoxine hydrochloride 0·356, folic acid 0·207, 2-methyl-1,4-naphthoquinone 0·037, DL-α-tocopheryl acetate 5·0, retinyl palmitate 0·242, ergocalciferol 0·0014.

‡ Contained (mg/kg diet): $CaHPO_4$ 2400, NaCl 1000, $K_2CO_3.1{\cdot}5H_2O$ 1690·4, $ZnSO_4.7H_2O$ 8·8, $MnSO_4.H_2O$ 4·5, $CuSO_4$ 1·28, $CoCl_2.6H_2O$ 0·6, KI 0·5, $FeSO_4.7H_2O$ 4·5.

§ Choline chloride and cyanocobalamin (50 μg/kg diet) were dissolved in approximately 5 ml distilled water and added to the diet during mixing.

Stokstad, 1970; Merker & Günther, 1970), rabbits (Hunt & Harrington, 1974) and mice (Hamuro, Shino & Suzuoki, 1970).

The purpose of the study reported here, was to further characterize the effects of Mg deficiency in the horse, specifically the effects on the concentrations of Mg, calcium and phosphorus for various tissues, and to correlate these results with histopathological findings.

EXPERIMENTAL

Animals and treatment

Fifteen 1- to 3-week-old horse foals of mixed breeds (eight males and seven females) were obtained from a local nurse-mare farm for use in this study. On arrival at the laboratory the foals were given a commercial liquid milk-substitute (Carnation Co. Inc., Kansas City, Kansas, USA) at a rate of 15 g total milk solids/kg body-weight per d, and were allowed free access to a complete, pelleted, purified diet and, within a 1- to 2-week period, were weaned completely on to this diet. The composition of this purified diet, which was Mg-deficient (− Mg, 7–8 mg Mg/kg), is shown in Table 1. Four of the fifteen foals, two males and two females (foals C1–C4), were randomly selected as the control group and were given the −Mg diet supplemented with 390 mg Mg as MgO/kg (+Mg diet); the remaining eleven (foals D1–D11) were maintained on the −Mg diet. This level of supplementation was recently reported to be satisfactory for growth, and maintenance of normal blood levels of Mg for horse foals given purified diets (Stowe, 1969). The foals were individually housed in 12 × 12 m cement-block stalls with concrete floors. As the foals drank a large quantity of water and the laboratory's water-distilling facilities were limited when this study was done, most of the foals were given tap-water from the municipal water supply. During the

study the Mg content of the tap-water fluctuated from < 1 to 5 mg/l. Two of the foals given the −Mg diet (foals D5, D9) and one of the +Mg foals (C4) were given distilled water throughout the study. All foals were offered food and water *ad lib*. Foals were examined daily and blood for mineral analyses was collected daily for the first 7 d and then at 5–7 d intervals. Foals were killed and post-mortem examinations done after periods of between 125 and 225 d for foals C1–C4 and 15–180 d for foals D1–D11. Tissues taken for mineral analyses were: liver, kidney, lung, pancreas, spleen, brain (cerebrum), heart (left ventricle), aorta (cranial descending thoracic), skeletal muscle (rectus femoris), eighth rib, distal diaphyseal and midshaft metacarpal and metatarsal bones. All tissues were sealed in polyethylene bags and stored at −20° until analysed.

Analytical methods

Soft tissues were prepared for analysis by wet-ashing with a mixture of concentrated nitric–perchloric–sulphuric acids (40:7:1, by vol.). Bone samples were dried to constant weight at 105°, extracted for 8 h with chloroform–methanol (2:1, v/v), re-dried to constant weight and dry-ashed at 550°. Mg and Ca contents of soft tissues and bone Mg content were determined by atomic absorption spectrophotometery (Model no. 303; Perkin-Elmer Corp., Norwalk, Connecticut 06852, USA). For analysis, portions of the soft tissue digest or bone ash containing 50–75 μg Mg or 100–450 μg Ca were dissolved in 5 ml 0·62 M-hydrochloric acid containing 11·73 g lanthanum oxide and diluted to 50 ml using twice-distilled water. Serum Mg, Ca and P contents and P contents of soft tissues were determined using an automated system (Technicon AutoAnalyzer; Technicon Corp., Chauncy, New York, USA) and methods based on procedures described by Kessler & Wolfman (1964) for Ca, Fiske & Subbarow (1925) for inorganic P and Hill (1962) for Mg.

RESULTS

Clinical findings and pathological changes

Details of clinical findings and pathological changes for the foals during this study have been reported elsewhere (Harrington, 1974). However, as the results of the present report are related to the lesions already reported, a brief résumé of some of the pathological findings is appropriate.

Mineralized elastic fibres were found, by microscopy, in the thoracic aortas from all foals given the −Mg diet for 32–120 d (D2–D11). No lesions were found in the abdominal aorta. The extent and severity of the lesions were proportional to the length of time foals were fed on the −Mg diet, ranging from 1–3 mineralized elastic fibres/cross-section of the aorta for foals maintained on the −Mg diet for 32–34 d, to numerous sites of mineralization in the elastic lamellae, and necrosis and mineralization of non-elastic tissue from foals maintained on this diet for 120–180 d. Although the pulmonary artery was not analysed for its mineral content, there was mineralization of elastic fibres in this blood vessel for all foals given the −Mg diet for 71–180 d (D4–D11).

A few mineralized elastic fibres were found in the splenic trabeculae and capsule

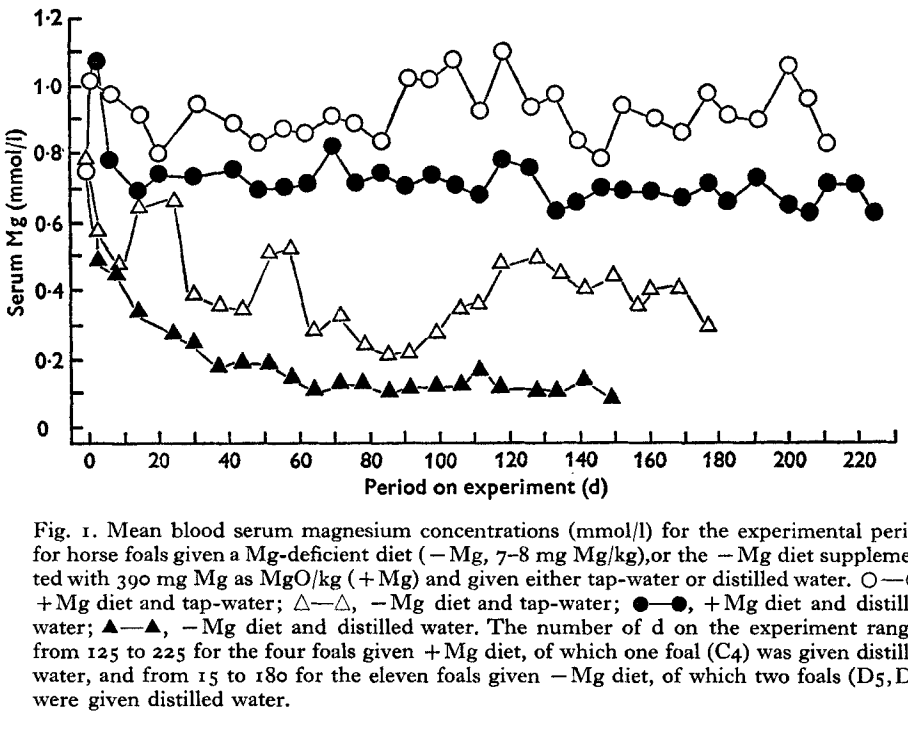

Fig. 1. Mean blood serum magnesium concentrations (mmol/l) for the experimental period for horse foals given a Mg-deficient diet (−Mg, 7–8 mg Mg/kg),or the −Mg diet supplemented with 390 mg Mg as MgO/kg (+Mg) and given either tap-water or distilled water. ○—○, +Mg diet and tap-water; △—△, −Mg diet and tap-water; ●—●, +Mg diet and distilled water; ▲—▲, −Mg diet and distilled water. The number of d on the experiment ranged from 125 to 225 for the four foals given +Mg diet, of which one foal (C4) was given distilled water, and from 15 to 180 for the eleven foals given −Mg diet, of which two foals (D5, D9) were given distilled water.

from two foals (D5, D10) and in the stroma of the pulmonary pleura from five foals (D5, D8, D9, D10, D11) fed on the −Mg diet. Scattered foci of skeletal muscle degeneration, with or without mineralization, were found in foals maintained on the −Mg diet for 71–180 d. Focal areas of elastic tissue mineralization were present in the subepi- and subendocardium of the auricles from foals given the −Mg diet for 96–180 d. Foal D9 also had mineral deposits in the Purkinje fibres of the heart, and cardiac muscle degeneration in the ventricles.

Serum Mg, Ca and P

Results for serum Mg concentrations are shown in Fig. 1. Mean initial (day 0) serum Mg concentrations (with their standard errors) for foals given the +Mg and −Mg diets were 0·74 ± 0·04 and 0·78 ± 0·04 mmol/l respectively. Serum Mg concentrations for +Mg foals receiving tap-water (C1–C3) ranged from 0·74 to 1·11 mmol/l, while foal C4, which was given access to distilled water, had lower serum Mg levels (0·62–0·82 mmol/l), after a small initial increase to approximately 1·07 mmol/l during the first week of the experiment.

The sensitivity of serum Mg to dietary Mg intake was marked. The introduction of foals to the −Mg diet was followed by a rapid decrease in serum Mg concentrations. During the first 7 d of the experiment, serum Mg concentrations for this group of foals (D1–D11) decreased from a mean initial (day 0) value of about 0·78 mmol/l to approximately 0·53 mmol/l. Foals given access to distilled water and the −Mg diet (D5, D9) showed an almost continuous but slower decrease in serum Mg concentra-

Table 2. *Blood serum concentrations (mmol/l) of calcium and phosphorus for horse foals given magnesium-deficient (−Mg) and Mg-supplemented (+Mg) diets*

(Mean values for individual foals for the period of the experiment)

Diet	Foal	Period on experiment (d)	Ca			P		
			Initial (day 0)	Final	Mean	Initial (day 0)	Final	Mean
+Mg								
	C1	125	2·97	3·34	3·22	1·6	1·8	2·3
	C2	150	3·09	3·19	3·12	2·1	1·7	1·7
	C3	190	2·99	3·04	3·04	2·1	2·1	2·3
	C4*	225	2·82	2·92	2·89	1·9	2·4	2·1
Mean			2·97	3·12		1·9	2·0	
−Mg								
	D1	15	2·84	3·12	2·89	1·7	1·9	2·1
	D2	32	2·74	2·59	2·72	1·4	2·6	2·3
	D3	34	2·74	2·50	2·67	1·7	1·9	2·1
	D4	71	2·89	3·61	3·34	2·3	2·3	2·4
	D5*	96	2·89	1·67	2·64	1·9	2·2	2·1
	D6	120	2·74	2·67	3·24	2·0	1·4	1·9
	D7	120	3·09	3·14	2·79	1·6	1·4	2·1
	D8	120	3·07	3·37	2·54	2·6	1·1	1·9
	D9*	150	3·34	3·09	3·27	1·6	1·9	1·8
	D10	160	2·97	2·87	2·79	2·3	1·1	2·0
	D11	180	3·12	3·21	3·04	2·0	2·0	2·0
Mean			2·95	2·89		1·9	1·8	

* Received distilled water during the experiment; others received tap-water.

tions for the period between days 40 and 50, leveling off between days 50 and 60, whereas foals D1–D4, D6–D8, D10, D11, given access to the −Mg diet and tap-water, maintained somewhat higher serum Mg concentrations, which fluctuated; the fluctuations corresponded with variations in the Mg content of the tap-water.

Feeding with the −Mg diet had no significant effect on serum concentrations of Ca or P (Table 2).

Mg, Ca and P contents of soft tissues

Results for Mg, Ca and P contents of the soft tissues are given in Table 3. No significant differences were found between groups of foals given +Mg and −Mg diets for Ca and P concentrations of brain, liver, kidney, lung, pancreas, spleen, skeletal and cardiac muscle. Regression analyses (linear and quadratic) indicated that there was no significant relationship between the number of d the foals were fed on the −Mg and +Mg diets and concentrations of Mg, Ca and P for the soft tissues. Mean concentrations of Mg for these tissues from foals given the −Mg diet were slightly lower than those from foals given the +Mg diet but the difference was not significant.

Although the mean concentration of Mg for the aorta from foals was not significantly changed by feeding with the −Mg diet, concentrations of Ca and P in this blood vessel were increased. The mean concentration of Ca was approximately ten times higher for the group given the −Mg diet (3·003 and 0·301 mg Ca/g wet tissue

Table 3. *Concentrations (mg/g wet weight) of magnesium, calcium and phosphorus for soft tissues from horse foals given Mg-deficient (−Mg) and Mg-supplemented (+Mg) diets*

(Mean values with their standard errors for four +Mg and eleven −Mg foals)

		Mg		Ca		P	
Tissue	Diet	Mean	SE	Mean	SE	Mean	SE
Aorta	+Mg	0·0812	0·0043	0·3013	0·0200	0·90	0·01
	−Mg	0·0768	0·0046	3·0037	0·8646	1·90	0·28
Brain	+Mg	0·1475	0·0143	0·0915	0·0236	3·55	0·20
	−Mg	0·1384	0·0025	0·0797	0·0190	3·39	0·10
Liver	+Mg	0·1995	0·0078	0·1263	0·0422	3·19	0·27
	−Mg	0·1936	0·0104	0·1001	0·0159	3·13	0·46
Kidney	+Mg	0·1735	0·0101	0·3308	0·0841	2·99	0·17
	−Mg	0·1492	0·0051	0·2941	0·0369	2·84	0·11
Skeletal muscle	+Mg	0·3000	0·0102	0·0615	0·0048	2·80	0·07
	−Mg	0·2729	0·0090	0·0680	0·0047	2·66	0·07
Cardiac muscle	+Mg	0·2573	0·0060	0·0735	0·0067	2·70	0·12
	−Mg	0·2449	0·0057	0·0656	0·0049	2·58	0·04
Lung	+Mg	0·1380	0·0121	0·1050	0·0359	2·27	0·33
	−Mg	0·1281	0·0050	0·1833	0·0494	2·28	0·23
Pancreas	+Mg	0·2520	0·0070	0·1933	0·0105	4·15	0·27
	−Mg	0·2446	0·0089	0·2258	0·0765	4·38	0·11
Spleen	+Mg	0·1937	0·0139	0·0947	0·0074	3·03	0·13
	−Mg	0·1568	0·0090	0·0992	0·0108	2·45	0·31

for foals given the −Mg and +Mg diets respectively). The variation in the Ca and P contents of the aortas from individual foals in the groups given the −Mg diet was, however, large (coefficient of variation 95 and 49 respectively). For this reason and because the results of histopathological studies indicated that the amount of mineral deposited in the aorta was related to the period of time foals were fed on the −Mg diet, regressions for the Ca, P and Mg contents of the aorta *v.* number of d the foals were fed on the +Mg and −Mg diets were calculated. The equations for these relationships are as follows:

(*a*) for foals given the −Mg diet,

$$Y_{Ca} = 0{\cdot}041X - 1{\cdot}125 \quad (r\ 0{\cdot}804,\ P < 0{\cdot}05), \tag{1}$$

$$Y_{P} = 0{\cdot}14X + 4{\cdot}6 \quad (r\ 0{\cdot}856,\ P < 0{\cdot}001), \tag{2}$$

$$Y_{Mg} = 0{\cdot}09X + 71{\cdot}8 \quad (r\ 0{\cdot}336); \tag{3}$$

(*b*) for foals given the +Mg diet,

$$Y_{Ca} = 0{\cdot}0005X + 0{\cdot}201 \quad (r\ 0{\cdot}634), \tag{4}$$

$$Y_{P} = -0{\cdot}004X + 9{\cdot}8 \quad (r\ -0{\cdot}206), \tag{5}$$

$$Y_{Mg} = 0{\cdot}19X + 43{\cdot}3 \quad (r\ 0{\cdot}923); \tag{6}$$

where Y_{Ca}, Y_{P} and Y_{Mg} are the aorta concentrations of Ca and P (mg/g wet weight) and Mg (μg/g wet weight) respectively; X is the number of d the foals were given the −Mg or +Mg diets. The plots for equations 1–6 are shown in Fig. 2.

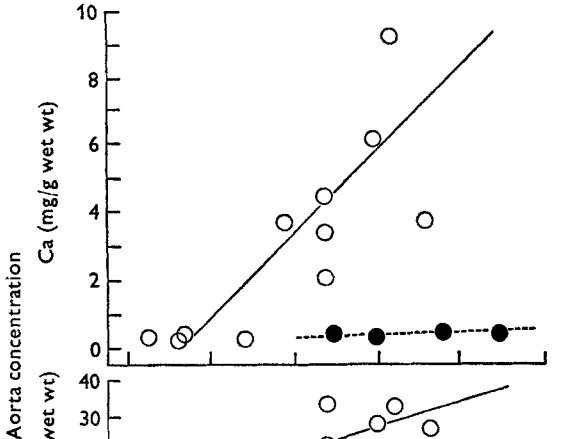

Fig. 2. Plots of regression equations showing the relationship between the period of time horse foals were given a magnesium-deficient diet (−Mg, 7–8 mg Mg/kg) or the −Mg diet supplemented with 390 mg Mg as MgO/kg (+Mg), and aorta concentrations of calcium, phosphorus and Mg. ●----●, +Mg diet; ○—○, −Mg diet. (For equations, see p. 50). The number of d on the experiment ranged from 125 to 225 for the four foals given +Mg diet and from 15 to 180 for the eleven foals given −Mg diet.

Results of the regression analyses confirmed the microscope findings and suggested a significant relationship between Ca ($P < 0{\cdot}05$) and P ($P < 0{\cdot}001$) contents of the aorta and the number of d that foals were given the −Mg diet. Moreover, values obtained for equations 1 and 4 (Ca), and 2 and 5 (P) indicated that aorta Ca and P concentrations for foals given the −Mg diet began to diverge after 32–33 d from values for foals given the +Mg diet. A similar estimate of the number of d feeding required before Ca and P concentrations in the aorta of foals fed on the −Mg diet began to increase (34 and 31 d respectively) was obtained when mean Ca (0·301 mg/g wet weight) and P (9·0 mg/g wet weight) concentrations for foals given the +Mg diet were substituted into the appropriate equations derived from results for foals fed on the −Mg diet (equation 1 for Ca and equation 2 for P).

Mg, Ca and P contents of bone ash

Rib and distal diaphyseal and midshaft metacarpal and metatarsal ash concentrations of Ca and P were unaffected by feeding with the −Mg diet (Table 4). Mean bone Ca concentrations for foals given the +Mg and −Mg diets ranged from 397 to 409 and from 401 to 413 mg/g ash respectively. Mean P concentrations ranged from

Table 4. *Concentrations (mg/g ash) of magnesium, calcium and phosphorus for various bones from horse foals given Mg-deficient (−Mg) and Mg-supplemented (+Mg) diets*

(Mean values with their standard errors for four +Mg and eleven −Mg foals)

		Mg		Ca		P	
Bone	Diet	Mean	SE	Mean	SE	Mean	SE
Eighth rib	+Mg	5·480	0·215	397·5	8·5	181·8	1·2
	−Mg	3·091	0·358	400·9	3·4	175·2	2·5
Metacarpus							
Distal	+Mg	5·605	0·201	396·3	15·7	174·3	4·0
diaphysis	−Mg	3·224	0·287	455·5	2·9	174·2	3·6
Midshaft	+Mg	5·400	0·134	403·8	7·5	178·3	2·4
diaphysis	−Mg	4·019	0·149	402·7	4·3	176·9	5·8
Metatarsus							
Distal	+Mg	5·323	0·119	408·7	8·5	183·3	2·0
diaphysis	−Mg	3·524	0·357	413·2	10·8	179·2	4·9
Midshaft	+Mg	5·515	0·213	407·5	12·6	184·2	2·2
diaphysis	−Mg	3·792	0·252	401·3	3·0	175·8	2·2

174 to 184 and from 174 to 179 mg/g ash for the +Mg and −Mg groups respectively. Regression analyses also indicated that Ca and P concentrations for bone ash were unaffected by the period of time foals were fed on the +Mg and −Mg diets.

The results for Ca and P differed markedly from the results for the bone Mg studies (Table 4). Bones from the +Mg foals consistently contained more than 5·00 mg Mg/g ash; the mean concentrations for the various bones ranged from 5·40 to 5·60 mg Mg/g ash. Mean values for bones from foals given the −Mg diet ranged from 3·09 to 4·02 mg Mg/g ash. The influence of the duration of experiment on bone Mg reserves was found to be significant when bone ash Mg concentrations for the two groups were plotted *v.* the number of d the foals were fed on the +Mg and −Mg diets (Fig. 3). The regression lines shown in Fig. 3 are given by the equations:

(*a*) for foals given the −Mg diet,

$$Y_{R} = 4{\cdot}989 - 0{\cdot}0190X \quad (r\ -0{\cdot}888,\ P < 0{\cdot}001), \tag{7}$$

$$Y_{DDMC} = 4{\cdot}586 - 0{\cdot}0130X \quad (r\ -0{\cdot}797,\ P < 0{\cdot}01), \tag{8}$$

$$Y_{MMC} = 4{\cdot}767 - 0{\cdot}0075X \quad (r\ -0{\cdot}839,\ P < 0{\cdot}001), \tag{9}$$

$$Y_{DDMT} = 5{\cdot}058 - 0{\cdot}0181X \quad (r\ -0{\cdot}846,\ P < 0{\cdot}001), \tag{10}$$

$$Y_{MMT} = 4{\cdot}915 - 0{\cdot}0095X \quad (r\ -0{\cdot}863,\ P < 0{\cdot}001); \tag{11}$$

(*b*) for foals given the +Mg diet,

$$Y_{R} = 6{\cdot}925 - 0{\cdot}0080X \quad (r\ -0{\cdot}859), \tag{12}$$

$$Y_{DDMC} = 7{\cdot}102 - 0{\cdot}0086X \quad (r\ -0{\cdot}953,\ P < 0{\cdot}05), \tag{13}$$

$$Y_{MMC} = 6{\cdot}371 - 0{\cdot}0056X \quad (r\ -0{\cdot}890), \tag{14}$$

$$Y_{DDMT} = 6{\cdot}224 - 0{\cdot}0052X \quad (r\ -0{\cdot}965,\ P < 0{\cdot}05), \tag{15}$$

$$Y_{MMT} = 6{\cdot}835 - 0{\cdot}0076X \quad (r\ -0{\cdot}792), \tag{16}$$

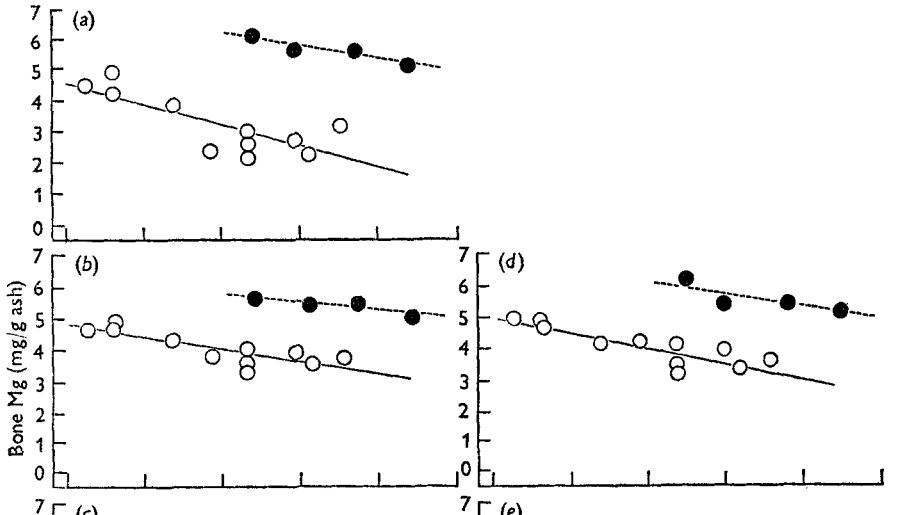

Fig. 3. Plots of regression equations showing the relationship between the period of time horse foals were given a magnesium-deficient diet (−Mg, 7–8 mg Mg/kg) or the −Mg diet supplemented with 390 mg Mg as MgO/kg (+Mg), and bone ash concentrations of Mg. ●----●, +Mg diet; ○—○, −Mg diet. (*a*) Distal metacarpus, (*b*) midshaft metacarpus, (*c*) distal metatarsus, (*d*) midshaft metatarsus and (*e*) eighth rib. (For equations, see page 52.) The number of d on the experiment ranged from 125 to 225 for the four foals given +Mg diet and from 15 to 180 for the eleven foals given −Mg diet.

where Y_R, Y_{DDMC}, Y_{MMC}, Y_{DDMT} and Y_{MMT} are the concentrations of Mg in the rib, distal diaphyseal metacarpus, midshaft metacarpus, distal diaphyseal metatarsus and midshaft metatarsus bones respectively expressed as mg Mg/g ash; X is the number of d foals were fed the −Mg and +Mg diets. Values for the slopes for plots of equations 7–11 suggest that bone Mg in foals fed on the −Mg diet decreased at a rate of 0·0075 (midshaft metacarpus, equation 9) to 0·019 (rib, equation 7) mg Mg/g bone ash per d. Plots for all equations for the +Mg foals also had negative slopes. Two of these regressions were significant: equation 13 for the distal diaphyseal metacarpus (r −0·953, $P < 0·05$) and equation 15 for the distal diaphyseal metatarsus (r −0·965, $P < 0·05$).

DISCUSSION

The results reported here indicated that analytically detectable changes in equine tissue concentrations of Mg, Ca and P resulting from Mg deficiency are limited to certain tissues. Thus our results are in agreement with those of other workers using

different species (Blaxter, Rook & MacDonald, 1954; Aikawa, Reardon & Harms, 1962; Bradbury, Kleeman, Bagdoyan & Berberian, 1968; Woodward & Reed, 1969). With the exception of the aorta, there were no significant changes in the concentrations of Mg, Ca and P for soft tissue, although evidence of degeneration was found by microscopy in some tissues analysed (lung, spleen and skeletal and cardiac muscle). The apparent incompatibility of the results of our tissue mineral analyses with those of the pathological studies may, however, be reconciled when the severity of lesions, in terms of amount of tissue mass affected, is considered. Skeletal muscle degeneration, for example, while consistently found for all foals given the −Mg diet for 71 d or longer, was never extensive. On the contrary, lesions were not only microscopic in size but typically sparse and focal in distribution: lesions in the heart and lung were similar. In the instance of the spleen, only two of the eleven foals fed on the −Mg diet were found by microscopy to have evidence of mineralization and in neither foal was this extensive. It may be concluded therefore that the amount of tissue degeneration that occurred in foals in this study, relative to the total amount of 'normal' tissue analysed, was not of sufficient magnitude to be reflected in analytically detectable changes in total soft tissue concentrations of Mg, Ca or P.

Results for Ca and P analyses of the aortas from foals fed on the −Mg diet could be correlated with those for the histopathological studies. The severity of the aortic lesions, evaluated by microscopy, in terms of the number of individual lesions and the amount of elastic tissue mineralization, were reported to be related to the period of time foals were fed on the −Mg diet (Harrington, 1974). The present study confirmed these subjective findings using quantitative analytical methods, and a functional relationship between the duration of deficiency and the amount of mineral deposited was found. In addition, results obtained from regression analyses indicated that mineral deposition in the aorta began 31–34 d after foals were placed on the −Mg diets. These results were in close agreement with those of the histopathological studies in which minimal mineral deposition was found in the aorta of foals fed on the −Mg diet for 32 (foal D2) and 34 (foal D3) d. The rate of mineral deposition was approximately 0·041 mg Ca/g wet tissue per d and 0·14 mg P/g wet tissue per d. Bunce, Jenkins & Phillips (1962), who used Ca and P concentrations for the aorta as one of their criteria for the estimation of the Mg requirement for the dog, also reported that dogs receiving −Mg diets were found to have a 10- to 40-fold increase in Ca concentrations and a 2- to 4-fold increase in P concentrations relative to values obtained for the aortas of dogs receiving adequate dietary Mg. They also reported that quantitative differences between the amount of mineralization found in two of their experiments were apparently related to the period of time dogs were fed on the −Mg diets. They also found, as we did in the present study with foals, that Mg deficiency in dog had no significant effect on the Mg content of the aorta.

Serum Mg concentrations are very sensitive to dietary intake, therefore the determination of changes in serum Mg concentrations has been used as a common method for monitoring the progress of experimental Mg depletion, and also as a criterion for the determination of dietary Mg requirements (Kunkel & Pearson, 1948*a*, *b*; Bunce, Jenkins & Phillips, 1962; Harrington, Walsh, Marroquin & White, 1973), and for the

evaluation of the availability of Mg from various dietary ingredients and mineral supplements (Gerken & Fontenot, 1967; Walsh & Harrington, 1973; Walsh, 1974). In the present study a sharp decrease in serum Mg levels was detected within 24–48 h after foals were given the −Mg diet; within the first 5–7 d, the mean reduction was approximately 0·62 mmol/l serum. The slope of the depletion curve was subsequently found to decrease. This was probably the result of the mobilization of Mg from bone, the principal site of Mg reserves (Walser, 1967). The two sharp increases found in the serum Mg concentration curve (Fig. 1) of foals receiving tap-water and the −Mg diet during the first 60 d of the experiment represent periods of time during which heavy summer rains and leaching of soil Mg into the municipal water supply occurred. The more gradual increase and plateau in values obtained for serum Mg concentrations in this group of foals during the latter part of the study was associated with late summer and autumn rains.

The results obtained for the serum Mg monitoring studies suggested that mineral deposition in the aorta, as determined by histopathological and mineral analyses, began after serum Mg concentrations had fallen below 0·4 mmol/l. Although it is tempting to speculate that this value may represent the critical serum Mg concentration below which mineralization of the aorta is to be expected, it must be recognized that calcification of elastic tissue, the principal site of initial mineral deposition in the vascular tissue (Moore *et al.* 1938; Martin, Schiffmann, Bladen & Nylen, 1963; Schiffmann, Martin & Corcoran, 1964; Merker & Günther, 1970), is a complex phenomenon dependent upon many interacting factors. Results obtained for −Mg animals suggested that mineralization could be the final result of (*a*) a derangement of the rate of synthesis or turnover of elastin itself; this was also indicated by the results of studies in rats (Britton & Stokstad, 1970), or (*b*) a change in the amino acid composition of elastin (Lansing, Alex & Rosenthal, 1950; Yu & Blumenthal, 1963). As the elastin component of blood vessels is largely formed during the growth period (Walford, Carter & Schneider, 1964; Looker & Berry, 1972), the period of development when nutrient demands are also maximal, the onset as well as the degree of mineralization are undoubtedly functions of the age at which an animal is exposed to a −Mg diet. Other nutritional factors, for example, intake of Ca, P, potassium and vitamin D (Bunce, Chiemchaisri & Phillips, 1962; Grace & O'Dell, 1970; Hamuro *et al.* 1970; Jacob & Forbes, 1970) have also been reported to affect the degree of mineralization of soft tissues in −Mg rats and dogs.

A reduction in ossified tissue concentrations of Mg is a characteristic feature of Mg deficiency (Walser, 1967). The lability of bone Mg is known to depend upon an animal's age and rate of bone growth (Duckworth & Godden, 1941; Breibart, Lee, McCoord & Forbes, 1960; Smith & Field, 1963). Differences in the sensitivity of various bones to depletion are also recognized (Smith, 1959; Smith & Field, 1963). The results of the study reported here indicated that the rate of reduction in bone Mg for foals fed on the −Mg diet varied not only between bones but also between different anatomical areas of the same bone; the value obtained for the rate of reduction for the eighth rib and for those portions of the metacarpus and metatarsus composed mostly of the cancellous type of bone (distal diaphysis) was (mg Mg/g bone

ash per d), 0·013–0·019 compared with 0·0075–0·0095 for the compact lamellar-type portions (midshaft diaphysis) of the metacarpus and metatarsus. These differences are interpreted as reflexions of differential rates in the pattern of formation and remodelling that occur in various bones during growth (Leblond, Wilkinson, Belanger & Robichon, 1950; Johnson, 1966; Vaughan, 1970).

The Mg requirements of the growing horse have not been estimated, although the results of studies reported by Stowe (1969) indicate that 390 mg Mg/kg purified diet, the level of supplementation used in the present study, is adequate for foals when 'adequacy' is evaluated in terms of serum Mg concentrations and growth rate. This level of supplementation is also similar to the values presently recommended for swine, rabbits and most other laboratory animal species ((US) National Research Council, 1966, 1968, 1972). Although the absence of pathological changes in our +Mg foals appears to confirm the findings of Stowe (1969), the negative bone Mg regression data (equations 12–16) in conjunction with the low serum Mg concentration found for foal C4, which was given distilled water, suggest that this level of supplementation may be marginal. On the other hand, similar types of response might be anticipated if the availability of Mg in MgO, the supplemental form of Mg recommended by the (US) National Research Council (1973) and used by Stowe (1969) and myself, is not as great as assumed. The results of this report suggest that additional studies of the availability of supplemental sources of Mg as well as the Mg requirement of the growing horse are needed.

This investigation was carried out in connexion with a project of the Kentucky Agricultural Experimental Station, supported by a grant made by the Grayson Foundation Inc., and is published by permission of the Director of the Agricultural Experimental Station. The author thanks Dr Carlos R. Marroquin and Mrs Virginia White for technical assistance.

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Printed in Great Britain