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Comparison of Magnesium Deficiency in the Rat and Mouse[1] (38058)

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Hypocalcemia is associated with magnesium depletion in man (1), monkeys (2, 3), sheep (4, 5), the dog (6–8), calves (9), and the pig (10). Of the species previously studied, the magnesium-depleted rat appears to be unique in developing hypercalcemia when fed diets of the same calcium content as those which produce hypocalcemia in other species (3, 11). In the study reported here, a magnesium-deficient diet produced significant differences between rats and mice in symptomatology and in plasma calcium levels.

Materials and Methods. All animals were housed in individual cages with wire mesh bottoms throughout. Three separate experiments were carried out. In experiment 1, groups of 24-day-old male CD rats (Charles River Laboratories) were either placed on magnesium-deficient diet (Diet A) or were pair-fed or fed *ad libitum* with the control diet (Diet A + Mg). They were sacrificed after 14 or 21 days. In experiment 2, male Swiss white mice (Charles River Laboratories) 30 or 43 days old were fed *ad libitum* either Diet A or Diet A + Mg. The younger mice were sacrificed after either 14 or 20 days on the respective diets and the older mice after 11 or 21 days. Experiments 1 and 2 were run concurrently using the same batches of the respective diets for both rats and mice. In experiment 3, 28-day-old Swiss white mice were fed *ad libitum* either Diet A, Diet A with 2 mg% magnesium (Mg) added (Diet A + 2 mg% Mg), Diet A with 5 mg% Mg added (Diet A + 5 mg% Mg), or control diet (Diet A + Mg). Animals were sacrificed at intervals up to 65 days as indicated in the results section.

The composition of Diet A has been published (11); its magnesium content was 1.0 mg%. Magnesium chloride was used for all supplements of magnesium. Diet A + Mg contained 40 mg of magnesium per 100 g of diet. Calcium content was 0.14%. Each batch of diet was analyzed for magnesium and calcium before use.

Animals were weighed weekly. At sacrifice, blood was collected from the dorsal aorta of ether-anesthetized animals. Serum magnesium and calcium were determined by atomic absorption (12), and inorganic phosphate by the method of Sumner (13). Plasma urea nitrogen was determined by a micro method (14). In experiments 1 and 2 the right kidney of each animal was removed, dried at 105° for 36 hr, and then weighed and ashed at 450°. The ash was dissolved in 2 ml 2*N* HCl and an aliquot of the solution used for the determination of magnesium and calcium (12). Inorganic phosphate was determined after heating a 1 in 2 dilution of the dissolved ash at 100° for 10 min to convert any inorganic pyrophosphate to orthophosphate.

Results. After 6–10 days on Diet A, the rats developed the well-known erythema of the extremities followed by increasing hyperirritability, and later many of the animals were seen to have a convulsive seizure lasting for approximately 30–90 seconds; in many cases this was fatal. 45% of the rats fed Diet A died between days 16 and 21, and animals found dead showed signs of effusions from the mouth and nose which were evidence of a probable convulsion.

Erythema did not develop in any of the mice fed Diet A. Convulsions observed in this species were different from those in the

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TABLE I. Effect of Mg Deficiency on Weight Gain and Plasma Mg, Ca and P in Rats.

Initial age (days)	Period on diet (days)	Diet[a]	Δ Wt (g)	Mg (mEq/L)	Ca (mg%)	P (mg%)
24	14	A	40	0.42 (10) ± 0.03[b,c,e]	10.62 (10) ± 0.17[f]	7.1 (10) ± 0.26[c]
		A + Mg (restricted)	45	1.73 (10) ± 0.04	9.98 (10) ± 0.09[d]	7.2 (10) ± 0.14[c]
		A + Mg (*ad lib*)	70	1.70 (10) ± 0.04	10.46 (10) ± 0.15	9.06 (10) ± 0.22
24	21	A	52	0.54 (10) ± 0.03[c,e]	11.34 (10) ± 0.16[e]	6.50 (10) ± 0.24[c,f]
		A + Mg (restricted)	75	1.78 (10) ± 0.04	10.14 (10) ± 0.11[d]	7.65 (10) ± 0.25[d]
		A + Mg (*ad lib*)	120	1.80 (9) ± 0.04	10.87 (10) ± 0.21	11.1 (9) ± 1.56

[a] Mg content of Diet A was 1.0 mg%; Mg content of Diet A + Mg was 40 mg%.
[b] Results expressed as mean ± S.E.M. (no animals).
[c] Difference from *ad lib* control significant $P < 0.001$.
[d] Difference from *ad lib* control significant $P < 0.01$.
[e] Difference from restricted control $P < .001$.
[f] Difference from restricted control $P < 0.01$.

rat. An apparently normal mouse would suddenly have a violent spasm which threw it across the cage with almost immediate death. The tonic-clonic spasms of the rat were not seen. Of many mice observed in a convulsive seizure, only one recovered. In experiment 2 there was a 50% and 20% mortality among the 30 and 43-day-old mice, respectively, between days 16 and 21. In experiment 3, mortality was inversely proportional to the magnesium content of the diet as noted below.

Hypomagnesemia was marked in all rats and mice fed Diet A (Tables I & II). The magnesium-depleted rats were hypercalcemic in relation to their pair-fed controls. Restriction of food intake in controls resulted in a relatively small but significant decrease in calcium in comparison with the *ad libitum* fed group. Serum inorganic phosphate in depleted rats at 14 days was lower than that of controls fed *ad libitum;* 21 days of depletion resulted in serum inorganic phosphate lower than that in both control groups.

TABLE II. Effect of Mg Deficiency on Weight Gain, Plasma Mg, Ca and P in Mice.

Initial age (days)	Period on diet (days)	Diet[a]	Δ Wt (g)	Mg (mEq/L)	Ca (mg%)	P (mg%)
30[b]	14	A	2.9 (10)	0.60 (10) ± 0.001[c,d]	8.1 (10) ± 0.31[e]	12.5 (10) ± 0.39[d]
		A + Mg	6.3 (10)	1.76 (9) ± 0.001	9.9 (9) ± 0.46	10.1 (7) ± 0.51
	20	A	2.6 (6)	0.72 (5) ± 0.001[d]	8.5 (5) ± 0.60[e]	9.4 (5) ± 0.57
		A + Mg	8.8 (10)	1.84 (9) ± 0.001	10.0 (9) ± 0.14	9.4 (9) ± 0.24
43[b]	11	A	0.2 (10)	0.64 (10) ± 0.001[d]	8.6 (10) ± 0.31[d]	9.4 (10) ± 1.19
		A + Mg	1.3 (9)	1.78 (10) ± 0.08	10.1 (10) ± 0.17	8.5 (10) ± 0.41
	21	A	0.3 (8)	0.55 (7) ± 0.03[d]	8.5 (7) ± 0.12[d]	10.3 (7) ± 0.67[e]
		A + Mg	4.1 (10)	1.71 (10) ± 0.05	9.8 (10) ± 0.12	7.7 (10) ± 0.27

[a] Mg content of Diet A was 1.0 mg%; Mg content of Diet A + Mg was 40 mg%.
[b] Initial mean starting weight of 30-day group = 22.9 g; of 43-day group = 31.2 g.
[c] Results expressed as mean ± S.E.M. (no. animals).
[d] Difference from mean for control group significant $P < 0.001$.
[e] Difference from mean for control group significant $P < 0.01$.

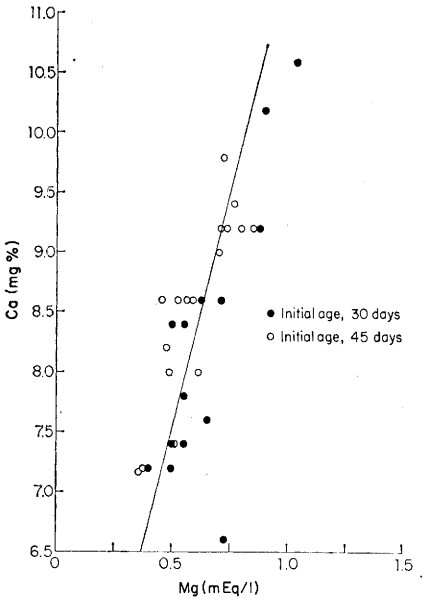

FIG. 1. Distribution and regression line for plasma magnesium vs plasma calcium for all magnesium-depleted mice in Experiment 2. $r = 0.693$; $P < 0.001$.

The mean serum urea nitrogen levels ranged from 9.6 to 12.2 for all groups of animals.

In contrast to rats all mice fed Diet A developed significant hypocalcemia (Table II). There was a highly significant correlation ($r = 0.693$; $P < 0.001$) between serum magnesium and calcium in the magnesium-depleted mice in experiment 2 (Fig. 1). Serum inorganic phosphate was significantly elevated in the younger magnesium-depleted mice at 14 days and in the older mice at 21 days (Table II). Mean serum urea nitrogen was unaffected by magnesium depletion in mice and ranged from 14.5 to 18.8 mg% in the various groups.

Figure 2 depicts the experimental design of experiment 3 and the variation in weight of mice fed various levels of magnesium. The diet containing 5 mg% magnesium permitted a growth rate which was only slightly less than that of controls; that with 2 mg% was obviously inadequate. The levels of serum calcium and magnesium in relation to dietary magnesium and duration of ingestion of diet are summarized in Fig. 3. Each of three severely depleted mice fed Diet A and sacrificed on day 23 was markedly hypomagnesemic and hypocalcemic; the three mice which were sacrificed between day 28–35 were slightly less hypomagnesemic and had appreciably higher calcium levels; two of the three were within normal limits. Mice fed Diet A + 2 mg% magnesium were hypomagnesemic, but there was a tendency for the serum magnesium to rise slightly with time; 5 of 6 animals sacrificed between days 16–36 were markedly hypocalcemic as were 3 of 4 sacrificed at day 63. All mice fed Diet A + 5 mg% magnesium and sacrificed between days 16 and 35 were hypomagnesemic and hypocalcemic while still maintaining a good growth rate; both magnesium and calcium in this group increased slightly with time. By day 63, 9 of 10 animals were normocalcemic or hypercalcemic although serum magnesium was still low in most animals. Six animals who were transferred from Diet A + 5 mg% to Diet A + 2 mg% Mg from day 35–63 were appreciably more hypomagnesemic than those with 5 mg%, and they remained hypocalcemic.

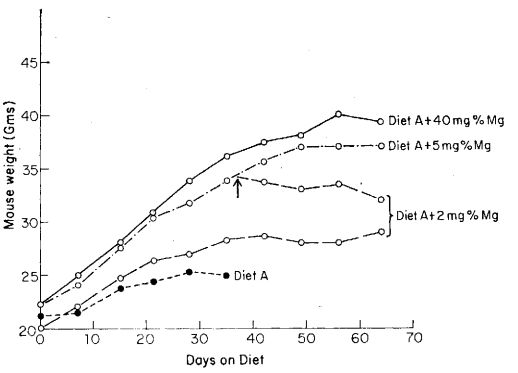

FIG. 2. Variation in growth rate of mice fed diets containing different levels of magnesium in Experiment 3. At arrow, the magnesium content of the diet was reduced from 5 to 2 mg% for a proportion of the mice.

All animals fed Diet A + 5 mg% Mg survived to the 65th day. Of ten animals transferred on day 35 from Diet A + 5 mg% Mg to Diet A + 2 mg% Mg, 2 died on days 49 and 63, respectively; 20 of 29 fed Diet A + 2 mg% Mg throughout died between days 19 and 49, and 9 of 15 fed Diet A died by day 35 when the remainder of this group were sacrificed.

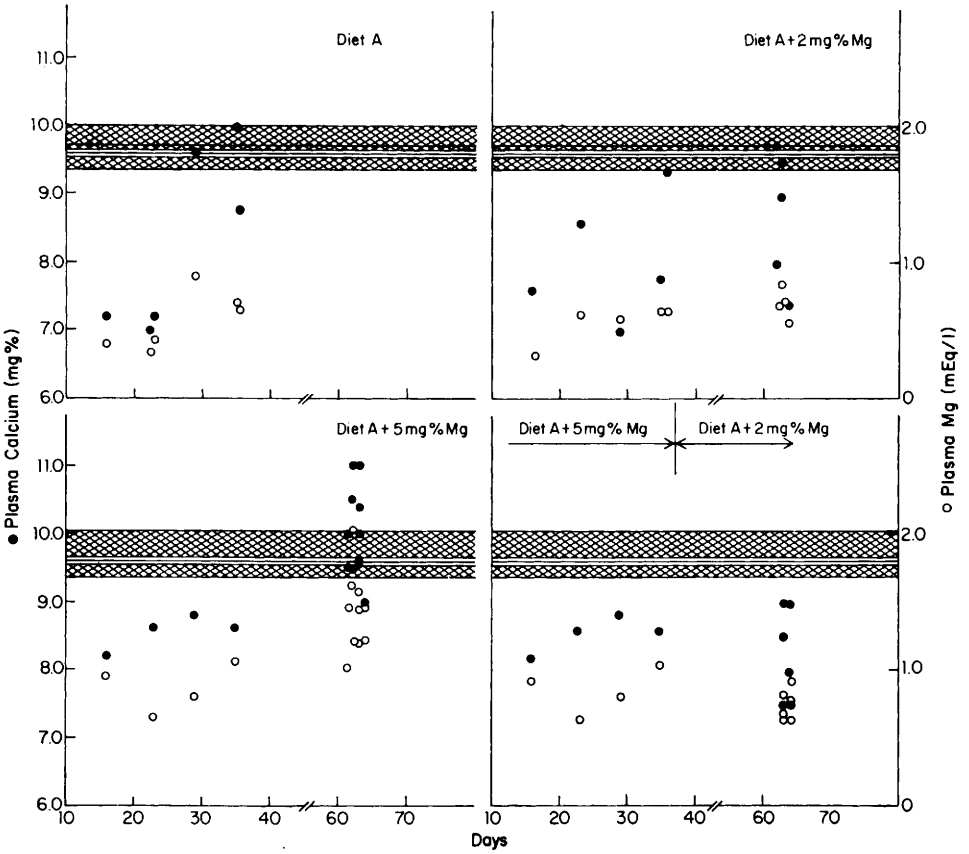

FIG. 3. Plasma magnesium and calcium in mice sacrificed between days 16 and 65 after commencing diets of different magnesium content in Experiment 3. Cross-hatched area = Mean ± S.D. of plasma calcium for control animals (Diet A + 40 mg% Mg); narrow clear zone in wider calcium band = Mean ± S.D. of plasma magnesium for control animals.

Kidney magnesium, calcium and total phosphorus were unaltered by magnesium depletion with Diet A in both rats and mice. The total kidney phosphorus in the two species was similar—mean 13.3 ± 0.3 (S.E.M.) μg/gm dry tissue and 12.8 ± 0.3 μg/gm dry tissue—for 10 control mice and nine control rats, respectively, in a typical group of each species. In contrast, kidney calcium and magnesium were consistently and significantly lower in mice than in rats. In addition, there was considerable variability in calcium in mice. The corresponding mean values for magnesium were: 72.4 ± 4.4 (S.E.M.) μEq/gm dry tissue in 9 control mice and 101.4 ± 3.64 in 10 control rats; for calcium they were: 162.6 ± 17.9 μg/gm dry tissue in 8 control mice and 256.6 ± 0.6 in 10 control rats.

Discussion. These results demonstrate a species difference between the rat and mouse strains tested in their ability to maintain plasma calcium levels during acute magnesium depletion. The hypocalcemia found in the magnesium-depleted Swiss mouse is consistent with almost all other species studied including man, monkey and the dog (1–3, 6–8). Hence, the reaction of the magnesium-depleted rat which results in normocalcemia or hypercalcemia cannot be considered to be typical of all rodents.

The plasma calcium level may be an important factor in survival since the only 3 mice on Diet A surviving to days 28 and 36 had plasma calcium levels approaching normal.

In female Swiss Webster mice fed for 15 days a diet deficient in magnesium with

1.2% calcium, the plasma calcium was reported to be unaltered (15). Hence, either sex or strain differences or differences in calcium content of the diet may account for discrepancy with our data. Rats fed diets grossly deficient in both calcium and magnesium will develop hypocalcemia (16). In this study, variation in dietary calcium concentration has been eliminated as an explanation for the difference in plasma calcium.

The correlation between serum calcium and magnesium in magnesium depletion also occurred in monkeys (2).

It is interesting that, by the time the animals on the various diets stopped growing—days 30, 40 and 60 on Diets A, A + 2 mg% Mg and A + 5 mg% Mg, respectively—many had higher serum calcium and magnesium levels than those sacrificed earlier. Possibly decreased incorporation of magnesium into tissue lead to an increase in serum magnesium with an accompanying increase in serum calcium. The group transferred from the 5 mg% Mg supplement to 2 mg%, however, had mixed results as though some had become acutely depeleted.

Neither species demonstrated nephrocalcinosis despite severe magnesium depletion on Diet A. This is in contradiction to the accepted statements that calcification of the kidney is a characteristic of magnesium deficiency in the rat. It is our present opinion that kidney calcification is dependent upon higher levels of dietary calcium. The kidney calcification observed in acutely magnesium-depleted female KK mice (17, 18) is consistent with this suggestion or may be due to sex or strain differences.

Summary. A comparative study has been made of the reactions of mice and rats subsisting on the same magnesium-deficient diet. Deficient young male rats developed the classical erythema, hyperirritability and tonic-clonic convulsions. While there was a high mortality with the convulsions, a good proportion recovered. Deficient male mice did not develop erythema or hyperirritability; they did convulse, but it was a single violent spasm wtih almost immediate death and rare survival. The deficient rats were either normocalcemic or hypercalcemic, whereas the mice were hypocalcemic. There was a positive correlation between the plasma magnesium and calcium in the deficient mice. Although growth of mice receiving 5 mg% of magnesium in their diet was close to that of controls with 40 mg%, the plasma magnesium and calcium remained low for approximately 5 weeks.

Renal glomerular dysfunction and calcification did not occur in depleted animals in either species.

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