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Dietary Calcium and Phosphorus and Seizure Susceptibility of Magnesium Deficient Rats[1]

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CHAISTITWANICH, R , A W MAHONEY, D G HENDRICKS AND D V SISSON *Dietary calcium and phosphorus and seizure susceptibility of magnesium deficient rats* PHARMACOL BIOCHEM BEHAV 27(3) 443–449, 1987 —Convulsions are characteristic of magnesium deficiency and hypocalcemia In this study, weanling rats were fed magnesium deficient diets with varying concentrations of calcium and phosphorus Diets were either normal (Mg=) or low (Mg−) in magnesium and were either low (Ca− or P−), normal (Ca= or P=) or high (Ca+ or P+) in calcium or phosphorus After consuming the diets for 17 days, the rats were tested for audiogenic seizures and blood was then drawn for serum mineral analyses Rats fed Mg−Ca=P=, Mg−Ca=P−, Mg−Ca+P= or Mg−Ca+P+ diets had high incidences of seizures Those fed Mg−Ca−P=, Mg−Ca−P−, Mg−Ca=P+, Mg−Ca−P+ or Mg−Ca+P− diets had low incidences of seizures In general, animals with low serum magnesium and calcium levels and high serum potassium levels were susceptible to audiogenic seizures In this model, serum magnesium level is the most important determinant of seizure susceptibility, followed by calcium and potassium

Diet Audiogenic seizure susceptibility Magnesium deficiency Calcium Potassium Serum Rats

MAGNESIUM deficient rats are highly susceptible to audiogenically induced seizures [3, 7–9, 11, 32] Susceptibility depends on the magnesium concentration of the cerebrospinal fluid rather than the serum [8,9] Hyperexcitability associated with magnesium deficiency in the rat is related to the cerebrospinal fluid magnesium concentration but not to concentration in the serum [8] Fasting decreases audiogenic seizure susceptibility but does not affect latency in magnesium deficient rats [32] Seizures increased in rats fed a high-fat diet [32]

The four ions directly involved in neuroexcitability are either stimulants (Na^+ and K^+) or depressants (Ca^{++} and Mg^{++}) [45] Thus, increases in extracellular ionic potassium and decreases in extracellular ionic calcium will increase neuronal excitability and support the development of seizure activity [31] However, magnesium has an antagonistic reaction with calcium on acetylcholine release from nerve endings into the synaptic space [1, 18, 23, 34, 44] DBA mice are genetically susceptible to audiogenic seizures At 21 and 110 days of age, the cerebral cortex, cerebellum, and brainstem of these mice contain less intracellular sodium and more intracellular potassium than other mice [24] Epileptiform activity is associated with increases in extracellular ionic potassium and with decreases in calcium concentration [31] However, these changes due to focal activity cannot by themselves explain either seizure initiation or termination [4, 28, 40] Phenytoin, a commonly used anticonvulsant, inhibits the voltage-dependent uptake of calcium ions into presynaptic nerve preparations and also inhibits the calcium-dependent release of norepinephrine and acetylcholine from nerve terminals [19] Other drugs with anticonvulsant activity also affect cellular movement of calcium [19,46]

Diets containing high levels of calcium increase the susceptibility of animals to seizures [6, 13, 16, 17] Low calcium and low magnesium concentrations in the cerebrospinal fluid causes hyperexcitability of the central nervous system [27,38] Intraventricular injection of calcium ions eliminated restlessness in goats previously injected with intraventricular sodium [45]

Phosphorus depletion causes hypomagnesemia, hypercalcemia and hypophosphatemia [29] Phosphorus deficiency increases magnesium excretion and decreases serum magnesium concentration High phosphorus intake is associated with hypocalcemia [41,43] Bernhard [3] showed that

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TABLE 1
CONCENTRATION OF MINERALS IN DIETS

Diet	Mg mg/g	Ca mg/g	P mg/g	Na mg/g	K mg/g
Mg=Ca=P=†	0 34±0 15‡	5 43±0 38	6 11±0 12	3 08±0 52	2 46±0 11
Mg−Ca=P=	0 06±0 01	5 51±0 30	5 82±0 26	3 05±0 26	2 60±0 21
Mg−Ca=P−	0 04±0 01	5 34±0 26	3 35±0 17	3 37±0 17	2 13±0 29
Mg−Ca−P=	0 06±0 01	0 16±0 02	4 49±0 39	2 57±0 42	2 33±0 07
Mg−Ca−P−	0 05±0 01	0 19±0 03	1 70±0 16	3 68±0 24	2 10±0 25
Mg−Ca=P+	0 05±0 01	5 51±0 29	13 43±0 08	9 25±0 36	2 68±0 38
Mg−Ca+P=	0 05±0 01	15 81±0 30	6 25±0 23	3 06±0 15	2 22±0 07
Mg−Ca−P+	0 06±0 01	0 43±0 34	13 42±0 68	10 19±0 91	3 44±0 12
Mg−Ca+P−	0 08±0 03	16 22±0 34	1 59±0 18	3 95±0 26	2 05±0 27
Mg−Ca+P+	0 07±0 02	17 35±1 77	14 93±0 69	9 80±1 27	2 60±0 11

*Requirement for normal growth (mg/g) Mg=0 40, Ca=5 60, P=4 40, Na=0 60, K=2 00
†Analyzed value − means deficient dietary level, = means normal dietary level + means high dietary level
‡ Mean±standard deviation

diets low in phosphorus reduce the incidence of magnesium-deficient seizures, however, a diet low in both phosphorus and calcium increases seizures in magnesium-deficient rats

Magnesium deficiency is associated with increased or unchanged sodium concentration in serum [6,48] Chutkow [14] reported that magnesium-deficient rats fed high levels of sodium suffered more-severe audiogenic seizures

The magnesium-potassium relationship is similar to the magnesium-calcium antagonism High levels of potassium in a magnesium-deficient diet increases susceptibility to audiogenic seizures [14,17] Moreover, high levels of calcium and potassium in a diet increase the severity of magnesium-deficient syndromes A high potassium diet reduces plasma magnesium levels in animals [33] and chronic excesses of potassium cause magnesium depletion [22] It is believed that magnesium is deposited in the tissues since urinary magnesium levels do not increase in rats fed a high-potassium diet [21] Magnesium deficiency causes hypokalemia [42] in humans It caused hyperkalemia in one rat study [10] but not another [15] A diet deficient in both magnesium and potassium prevented magnesium-deficiency symptoms [25]

In rats, magnesium deficiency can be reversed with no permanent brain damage by magnesium rehabilitation The purpose of this research was to determine how varying levels of calcium and phosphorus in magnesium-deficient diets affected susceptibility to audiogenic seizure and serum mineral concentrations in young rats

METHOD

Animal Care

Male weanling Sprague-Dawley rats (Simonsen Laboratory, Inc , Gilroy, CA) were randomly assigned to ten groups of 10 animals each On the first day of the experiment, they weighed between 59 and 91 g (mean±standard deviation, 77±6 g) They were housed individually in stainless steel cages with wire mesh fronts and bottoms Diets and demineralized water were provided ad lib The animal room temperature was kept between 23 and 28°C Lights were on automatically from 0800 to 2000 hr daily

Animals were acclimated to Purina Laboratory Chow for 48 hr before starting the experiment They were fed the following diets (1) magnesium control (normal diet, Mg=Ca=P=), (2) magnesium deficient (Mg−Ca=P=), (3) magnesium deficient, low phosphorus (Mg−Ca=P−), (4) magnesium deficient, low calcium (Mg−Ca−P=), (5) magnesium deficient, low calcium-low phosphorus (Mg−Ca−P−), (6) magnesium deficient, high phosphorus (Mg−Ca=P+), (7) magnesium deficient, high calcium (Mg−Ca+P=), (8) magnesium deficient, high calcium-low phosphorus (Mg−Ca+P−), (9) magnesium deficient, high calcium-high phosphorus (Mg−Ca+P+)

The diets contained (g/kg) casein, 200, corn oil, 50, cellulose, 50, vitamin mixture, 22, mineral mixture, 0 5, and dextrose to make 1 kg The dietary magnesium, calcium, phosphorus, sodium and potassium levels were controlled by altering the amounts of $MgCO_3$, $CaCO_3$, NaH_2PO_4, Na_2CO_3 and KCl added The vitamin mixture contained (g/kg) alpha-tocopherol, 5 0, l-ascorbic acid, 45 0, choline chloride, 75 0, D-calcium pantothenate, 3 0, I-inositol, 5 0, menadione, 2 25, niacin, 4 5, p-aminobenzoic acid, 5 0, pyridoxine-HCl, vitamin B-12, 0 0014, vitamin A acetate, 900,000 international units (IU), calciferol-D_2 100,000 IU and dextrose to 1 kg The mineral mixture contained (g/100 g) $ZnSO_4$ $6H_2O$, 13 74, $MnSO_4$ H_2O, 39 96, $CuSO_4$ $5H_2O$, 5 16, Na_2MoO_4 $2H_2O$, 0 14, KI, 046, $CoCl_2$ $6H_2O$, 046, and $FeSO_4$ $7H_2O$, 40 9 The dietary magnesium, calcium, phosphorus, sodium and potassium levels were confirmed by analysis (Table 1)

On day 17, rats were tested for audiogenic seizure susceptibility The animals fed a normal diet (Mg=Ca=P=) were not tested because they were not susceptible Seizure tests were performed by placing the rats in a metal chamber which contained two school emergency buzzers producing 115 dB for 90 sec or until tonus occurred (latency) as described by Buck *et al* [7,11] Seizure scores were rated on a scale of 0 to 5 Zero signified no seizure, 1=rapid running, 2=clonic, characterized by full body convulsions but with the animal usually on its feet, 3=tonic flexion, characterized by the animal on its side, rigid and kicking, 4=tonic extension, characterized by rigidity and extended legs, 5=lethal seizure Seizure testing and blood sample collections were

TABLE 2

SUMMARY OF WEIGHT GAIN AND PERCENT AUDIOGENIC SEIZURE AS AFFECTED BY DIETARY MAGNESIUM, CALCIUM AND PHOSPHORUS LEVELS*

Diet	Weight Gain (g)	Percent Seizure	Seizure Score	Latency (sec)
Mg=Ca=P=	65 ± 7	—	—	—
Mg−Ca=P=	36 ± 3	100	3 8 ± 0 4	5 ± 1
Mg−Ca=P−	33 ± 5	80(NS)†	3 1 ± 1 6	24 ± 35
Mg−Ca−P=	47 ± 3	30(10 8)	0 6 ± 1 2	82 ± 24
Mg−Ca−P−	53 ± 5	50 (6 7)	1 5 ± 1 7	63 ± 40
Mg−Ca=P+	1 ± 16	30(10 8)	1 1 ± 1 7	68 ± 36
Mg−Ca+P=	23 ± 3	100(NS)	4 1 ± 0 3	7 ± 2
Mg−Ca−P+	40 ± 7	0(20)	0	90
Mg−Ca+P−	14 ± 8	0(20)	0	90
Mg−Ca+P+	14 ± 6	89(NS)	3 8 ± 1 5	28 ± 42
LSD (0 05/0 01)	6/7‡	—	1 1/1 4	26/34

*Mean ± standard deviation

†The number in ( ) is Chi-square ($p<0$ 05) compared with Mg−Ca=P= NS is not significant The overall Chi-square value is 50 7

‡Mean differences must equal or exceed the least significant difference value to be statistically significant at the indicated levels of the probability

TABLE 3

SERUM MAGNESIUM, CALCIUM, PHOSPHORUS, POTASSIUM AND SODIUM CONCENTRATIONS (mg/dl)

Diet	Mg	Ca	P	K	Na
Mg=Ca=P=	1 77±0 26^{a}	7 2±0 6^{ab}	8 2±0 8^{ab}	33 0±5 4^{ab}	240±11^{a}
Mg−Ca=P=	1 01±0 27^{b}	9 8±1 0^{cd}	11 0±3 1^{b}	38 8±7 6^{a}	255±26^{abc}
Mg−Ca=P−	1 26±0 39^{bcd}	10 8±1 2^{c}	8 2±3 1^{ab}	33 1±6 6^{ab}	259±26^{abcd}
Mg−Ca−P=	1 05±0 32^{bc}	7 4±0 8^{abe}	7 5±2 0^{ab}	25 4±3 3^{cd}	268±19^{cd}
Mg−Ca−P−	1 16±0 31^{bcd}	7 9±0 8^{ae}	7 5±3 0^{ab}	27 1±6 1^{cd}	265±20^{cd}
Mg−Ca=P+	1 31±0 28^{cd}	4 8±1 7^{f}	19 8±7 2^{c}	21 4±7 5	263±23^{bcd}
Mg−Ca+P=	1 36±0 53^{d}	9 5±2 2^{cd}	13 4±4 8^{d}	37 7±7 0^{a}	242±16^{ab}
Mg−Ca−P+	1 68±0 45^{a}	6 1±0 7^{bf}	9 7±2 0^{b}	21 0±2 5^{d}	247±23^{abc}
Mg−Ca+P−	1 16±0 7^{bcd}	8 6±0 7de	5 5±0 9^{a}	30 9±3 1^{bc}	243±25^{ab}
Mg−Ca+P+	1 17±0 34^{bcd}	7 5±2 5^{ae}	14 8±4 5^{d}	37 8±9 0^{a}	278±27^{d}
LSD (0 05/0 01)	0 17/0 26	0 8/1 3	2 3/3 6	3 8/6 0	17/22

Mean±standard deviation Values with the same subscript are not significantly different ($p<0$ 01) Mean differences must equal or exceed the Least Significant Difference value to be statistically significant at the indicated levels of probability

done across treatments to minimize any time and handling effects Blood was collected approximately 5 minutes after the animal recovered from the seizure by inserting a heparinized glass capillary tube into the retro-ocular capillary bed [47] The animal then was killed by decapitation

Mineral Analyses

Diets were analyzed by ashing 1–2 g of diet for 48 hr at 550°C After cooling, 5 ml of 6N HCl was added The samples were slowly heated for 10–20 min to completely solubilize the ash They were then diluted to 100 ml with demineralized H_2O, and analyzed for magnesium, calcium, phosphorus, sodium and potassium as described below

Blood samples were allowed to clot in tubes at room temperature for approximately 2 hr They were then centrifuged at 1100 times gravity for 30 minutes Serum was transferred to new acid-washed test tubes using Pasteuer pipettes and kept refrigerated for approximately 20 hr before dilution for mineral analyses

The serum was diluted 1 100 with demineralized water For magnesium analysis 1 ml of the diluted serum was added

to 0 1 ml of 100,000 ppm lanthanum solution in a 10 ml volumetric flask and demineralized water added for a final dilution of 1 100 For calcium analysis, 0 1 ml serum was added to 0 1 ml of 100,000 ppm lanthanum solution and diluted to 10 ml with demineralized water for a final dilution of 1 100 Lanthanum solution was made by dissolving 117 20 g La_2O_3 in 220 ml HCl and diluted to 1 liter with demineralized H_2O Dietary calcium and magnesium levels were determined by atomic absorption spectrophotometry on diluted ash samples prepared with 1 percent of 100,000 ppm lanthanum solution Standard curves were prepared with blanks and calcium and magnesium standard solutions

Serum phosphorus analysis was determined by adding 1 ml of 30 percent trichloroacetic acid to 5 ml of diluted (1 100) serum, mixed with a vortex and allowed to stand at room temperature for 20 min [37] The samples were then centrifuged at 1100 times gravity for 10 min One milliliter of molybdovanadate reagent was added to 4 ml of the supernatant At least 15 minutes later, optical densities of the samples were determined at 400 nm Dietary phosphorus was determined colorimetrically with molybdovanadate reagent [37]

Serum sodium was determined directly by emission spectroscopy on serum diluted 1 100 with demineralized water using blanks and sodium standards Similarly, serum potassium was determined directly on serum diluted 1 1000 using blanks and potassium standards Dietary sodium and potassium were determined by emission spectroscopy after dilution

Statistical Analysis

Means and standard deviations were calculated Chi-square analysis was used to analyze the seizure incidence data at the 5 percent probability level Analysis of variance (ANOVA) was performed on serum mineral values and body weights, seizure scores and latencies A completely randomized design ANOVA was used to test for differences in serum mineral concentration among treatment diets A factorial arrangement of treatments (3×3) was used to analyze the relationship between low, normal and high dietary calcium and phosphorus levels on magnesium deficiency Least significant difference (LSD) values were computed for all F ratios significant at the 1 and 5 percent levels of probability

RESULTS

General Observations

The results of the analyses of susceptibility to seizures, weight gains, and serum mineral analyses are presented in Tables 2 and 3 Weight gains were not related to susceptibility to audiogenic seizures ($r=0\ 002$), but were related to dietary treatment ($p<0\ 05$) Higher than normal levels of dietary phosphorus consistently decreased weight gain (Mg−Ca=P= vs Mg−Ca=P+, Mg−Ca−P= vs Mg−Ca−P+, Mg−Ca+P= vs Mg−Ca+P+) Seizure susceptibility was also diet dependent Seizure severity scores ($r=0\ 98$) and latencies ($r=-0\ 97$) were closely related to seizure incidence

The symptoms of magnesium deficiency (erythema and edema of the ears, nose and claws, hyperexcitability, reduced weight gains) occurred as expected [30] These symptoms were not observed in animals fed the normal magnesium diet Edema and redness of the extremities were very severe on the 5th and 6th day, and then decreased in severity Animals had very poor appetites and grew slowly Animals wasted half or more of their daily food when they dug up and/or overturned the feeding cups This behavior did not happen in the control animals To minimize this behavior, anti-digging rings were installed in the food cups and animals were fed every day

Almost all magnesium-deficient animals had skin lesions, even those that did not show redness of ears, nose and paws or hyperexcitability Animals fed the Mg−Ca=P=, Mg−Ca=P−, Mg−Ca−P=, Mg−Ca−P−, Mg−Ca=P+, Mg−Ca+P= and Mg−Ca+P+ diets had the classic symptoms of magnesium deficiency [30] However, animals fed Mg−Ca−P+ diets did not have erythema and experienced significantly fewer sound-induced seizures (Table 2)

Magnesium Deficient Diet (Mg−Ca=P=)

All animals fed this magnesium-deficient control diet experienced seizures (Table 2) Serum magnesium levels decreased and potassium levels increased (Table 3) Serum calcium and phosphorus concentrations were inversely related to serum magnesium levels (Mg=Ca=P= vs Mg−Ca=P=) Serum sodium level was slightly elevated by this diet It was confirmed that a magnesium-deficient diet causes hypomagnesemia, hyperkalemia, hyperphosphatemia and hypercalcemia in rats [6, 10, 42]

Magnesium Deficient-Low Phosphorus Diet (Mg−Ca=P−)

Animals fed this diet experienced hypomagnesemia and hypercalcemia, which confirms the findings of others [29,39] that some magnesium-deficient diets cause hypercalcemia Serum potassium and sodium levels were not significantly different from levels in animals fed the control diet (Mg=Ca=P=) Seizure incidence and severity were reduced slightly and latency was increased slightly with this diet

Magnesium Deficient-Low Calcium Diet (Mg−Ca−P=)

Animals fed this diet had less-severe symptoms of magnesium deficiency Their incidence and severity of seizures were significantly reduced and their latencies were increased compared with animals on the magnesium-deficient control diet (Mg−Ca=P=) This diet did not affect serum calcium level, but it caused serum potassium level to decrease and serum sodium level to increase slightly compared with animals fed the magnesium deficient control diet (Mg−Ca=P=) Compared with animals fed the normal diet (Mg=Ca=P=), serum magnesium and potassium levels were decreased, and sodium level was increased

Magnesium Deficient-Low Phosphorus-Low Calcium Diet (Mg−Ca−P−)

These animals were less susceptible to audiogenic seizures, experienced less-severe seizures and latency periods were longer than in animals fed a magnesium-deficient diet (Mg−Ca=P=) Compared with animals fed the Mg−Ca=P= diet, serum calcium, phosphorus and potassium values were decreased Serum sodium levels did not change Compared with healthy animals (Mg=Ca=P=), serum magnesium and potassium levels were lower and sodium level was higher

Magnesium Deficient-High Phosphorus Diet (Mg−Ca=P+)

These animals were hyperexcitable and very agressive They also consumed the least and gained the least weight This diet also ameliorated physical symptoms of magnesium

TABLE 4

SUMMARY OF SERUM MINERAL CHANGES AND AUDIOGENIC SEIZURE INCIDENCE, ANTICIPATED AND OBSERVED*

Diet	Magnesium	Calcium	Potassium	Seizures Anticipated	Percentage Seizures Observed
Mg=Ca=P=	Normal	Normal	Normal	No*	0†
Mg−Ca=P=	−−	Normal	++	Yes	100
Mg−Ca=P−	−	+	Normal	No	80
Mg−Ca−P=	−−	Normal	−−	No	30
Mg−Ca−P−	−−	Normal	−−	No	50
Mg−Ca=P+	−	−−	−−	?	30
Mg−Ca+P=	−	−−	++	Yes	100
Mg−Ca−P+	Normal	−−	−−	No	0
Mg−Ca+P−	−−	+	Normal	No	0
Mg−Ca+P+	−−	−−	++	Yes	89

*It is assumed that decreased (−,−−) ionic calcium and magnesium and increased (+,++) ionic potassium extracellularly will support the development of seizure activity [31], and that minerals in the brain extracellular fluid will equilibrate at least to some extent with chronic changes in serum mineral concentrations [12]

"No" means that no seizures are anticipated "Yes" means seizures are anticipated "?" means susceptibility could not be predicted

†We did not test the animals in this group for seizures, however, it has been our experience that these animals are not susceptible to sound-induced seizures

deficiency, including reduced seizure susceptibility and severity and prolonged latency It was confirmed that high phosphorus levels in a magnesium-deficient diet cause hypocalcemia [41,42] Serum phosphorus levels were lower than animals receiving magnesium deficient (Mg−Ca=P=) or normal (Mg=Ca=P=) diets

Magnesium Deficient-High Calcium Diet (Mg−Ca+P=)

All animals fed this diet were susceptible to audiogenic seizures, and these animals had the highest average seizure severity score Some animals (6 of 9 rats) fed this diet also convulsed spontaneously when animals in other groups were tested One animal died before testing day No animals in the other groups suffered spontaneous seizures Colby and Frye [16,17] also observed that high dietary calcium levels exacerbated the symptoms of magnesium deficiency in rats This diet resulted in increased serum magnesium levels but were not significantly different from levels in animals fed Mg−Ca=P= diet Compared with rats fed the normal diet (Mg=Ca=P=), serum magnesium levels were lower and serum calcium, phosphorus, and potassium levels were higher

Magnesium Deficient-Low Calcium-High Phosphorus Diet (Mg−Ca−P+)

This diet reduced the physical symptoms of magnesium deficiency Some animals in this group had skin lesions Serum magnesium levels were normal and no animals fed this diet had seizures, even though the diet was clearly magnesium deficient (Table 1) Serum calcium and potassium levels were decreased while serum phosphorus and sodium remained unchanged compared with levels in rats fed magnesium-deficient control diet (Mg−Ca=P=), as well those fed the normal diet (Mg=Ca=P=)

Magnesium Deficient-High Calcium-Low Phosphorus Diet (Mg−Ca+P−)

The physical symptoms of magnesium deficiency did not occur in this treatment Animals in this group were aggressive, but less so than animals fed the Mg−Ca=P=, Mg−Ca=P+ and Mg−Ca+P+ diets No audiogenic seizures occurred even though serum magnesium levels were low (Tables 2 and 3) Serum phosphorus levels were very low, serum potassium levels were reduced and serum sodium levels were not significantly different from animals fed the magnesium-deficient control diet (Mg−Ca=P=) Compared with rats fed the normal diet (Mg=Ca=P=), serum magnesium, phosphorus and potassium levels were low, and serum calcium level was elevated while sodium remained unchanged

Magnesium Deficient-High Calcium-High Phosphorus Diet (Mg−Ca+P+)

Animals fed this diet had classic symptoms of magnesium deficiency and were very aggressive Chutkow [13] found that magnesium deficient diets, which were also high in calcium and sodium, increased the severity of audiogenic seizures, an observation consistent with the results of this study in which three of 10 animals in this group died during testing Compared with magnesium-deficient control diet (Mg−Ca=P=), serum calcium level was low, and serum phosphorus and sodium levels were high, while potassium level remained unchanged Compared with rats fed the normal diet (Mg=Ca=P=), serum magnesium level was low and serum phosphorus and sodium levels were high while serum calcium and potassium levels remained unchanged These effects are probably due to high phosphorus intake, which causes hyperphosphatemia and hypocalcemia [41,43]

DISCUSSION

Varying the dietary calcium and phosphorus levels in the

diets changed the appearance, behavior and audiogenic seizure susceptibility of animals Forbes [26] also observed that major changes in varying dietary calcium and phosphorus levels would eliminate the erythema of the ears and paws of magnesium-deficient rats, and increasing dietary calcium decreased the weight gains of magnesium-deficient rats

It is well-known that increases in extracellular sodium and potassium and decreases in calcium and magnesium are associated with neuronal hyperexcitability [31,45] Injecting calcium into the cerebrospinal fluid prevents seizures in magnesium-deficient rats [9], and causes eating in sated pigs [2], sheep [31] and rats [36] Calcium levels in the cerebrospinal fluid of women with eclampsia, a condition of pregnancy associated with convulsions, hypertension and edema are high [35], and large doses of magnesium sulfate are routinely given intravenously to prevent convulsions in preeclampic women [49] Intravenous magnesium supresses neuronal burst firing and interictal EEG spikes caused by penicillin-induced epileptic foci [5]

The extracellular fluid minerals of the brain are in direct equilibrium with the cerebrospinal fluid Inorganic ions enter the brain from plasma at relatively low rates [20] The fractional extraction rates of calcium and phosphate into dog brain are low [20], and the decrease in cerebrospinal fluid magnesium lags behind major decreases in serum magnesium by at least six days [8] The increase of potassium in cerebrospinal fluid of magnesium-deficient rats is small but consistent [10] In rats, calcium influx into brain and cerebrospinal fluid is linearly related to the ionized calcium concentration in the plasma [12] Thus, chronic changes in serum mineral levels caused by diet can be expected to change mineral levels in brain extracellular fluid and to affect behavior

In this study, seizure susceptibility of the magnesium-deficient rats reflects changes in serum mineral concentrations relative to animals fed normal diets (Mg=Ca=P=), which are not susceptible to sound-nduced seizures In the magnesium-deficient control group (Mg−Ca=P=), the serum magnesium level is low and the potassium level is elevated (Table 3) This condition favors susceptibility to seizures, as confirmed by several studies (Table 2 and references [8, 9, 11, 14, 30, 32]) Reducing the level of dietary phosphorus (Mg−Ca=P−) elevates the level of serum calcium, the level of serum potassium is normal, but lower than that of calcium Also, because the level of serum magnesium was not as low as in animals fed the magnesium-deficient control (Mg−Ca=P=) diet, susceptibility to seizures should have been reduced Incidence of seizures in this group was reduced slightly (Table 4) In animals fed the low calcium (Mg−Ca−P=) and low calcium-low phosphorus (Mg−Ca−P−) diets, serum potassium levels were decreased while calcium levels remained normal (Table 3), conditions that should have reduced seizure susceptibility Seizure incidence was reduced in both groups (Table 4) In animals fed the high-phosphorus diet (Mg−Ca=P+), serum magnesium level was again reduced less than expected and potassium was reduced, conditions that increase susceptibility to seizures However, the calcium level was reduced, which would increase susceptibility, thus it was impossible to predict the resulting reduced incidence of seizures (Table 4) In animals fed high calcium (Mg−Ca+P=) and high calcium-high phosphorus (Mg−Ca+P+) diets, the serum mineral levels would seemingly have increased susceptibility, and all animals did experience seizures (Table 4) In animals fed low calcium-high phosphorus diet (Mg−Ca−P+), the serum magnesium concentration was normal and no seizures would be anticipated and none were observed (Table 4) In animals fed the high calcium- low phosphorus diet (Mg−Ca+P−), serum calcium level increased and potassium remained normal, conditions that would likely prevent seizures, no seizures occurred even though a high incidence was observed in a similar situation (Mg−Ca=P−) above

The serum mineral data made it possible to accurately predict whether seizure incidence would be high or low in 7 of 9 magnesium-deficient treatments (Table 4) Thus, a chronic decrease in serum magnesium and calcium and a chronic increase in serum potassium apparently increases susceptibility to seizures Serum magnesium level is the most important determinant of seizure susceptibility, and serum calcium level is more important in the etiology of seizures than potassium level Certainly, low serum magnesium levels alone do not cause maximal seizure susceptibility

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