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Effect of various degrees and duration of magnesium deficiency on lipid peroxidation and mineral metabolism in rats

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Severe magnesium (Mg) deficiency changed mineral homeostasis, increased lipid peroxidation, and reduced Mg^{2+}/Ca^{2+} antagonism. To investigate whether the pathobiochemical effects directly correlate with the degree of Mg deficiency or whether there might be a threshold for significant alterations, diets with 70, 110, 208, 330, and 850 ppm of Mg were fed to growing Wistar rats. After feeding the diets for 0, 10, 20, and 30 days, parameters of free radical action (malondialdehyde and vitamin E content), mineral content (Mg, Ca, Fe, Zn) in various tissues (liver, spleen, heart, kidney, muscle) and plasma parameters (Mg, Ca, Fe, Zn, alanine- and aspartate-aminotransferase, tumor necrosis factor-α [TNF-α]) were analyzed. The tissue Mg content was either unchanged or only slightly reduced in severe Mg deficiency. The iron (Fe) content rose when the extracellular Mg^{2+} concentration was below 0.25 mmol/L. There was a close positive correlation between the tissue Fe and the malondialdehyde content and a negative correlation between the malondialdehyde and the vitamin E content. Below the threshold of about 0.25 mmol/L of plasma Mg^{2+} concentration, elevated zinc (Zn) concentrations were found in liver and kidney as well as in plasma increased transaminases and TNF-α. The same threshold could be observed for the increase of tissue calcium (Ca) content, except in the kidney where calcifications were found already in less severe Mg deficiency. Concerning changed mineral homeostasis with subsequent lipid peroxidation and membrane damage, plasma Mg^{2+} concentrations must be below 0.25 mmol/L; above this threshold effects of Mg deficiency alone can be compensated. (J. Nutr. Biochem. 6:681–688, 1995.)

Keywords: magnesium deficiency; malondialdehyde; iron; vitamin E; minerals; rats

Introduction

The pathobiochemical effects of magnesium deficiency have been studied intensively. Feeding a severely magnesium (Mg)-deficient diet for several weeks to growing rats leads to a reduction in growth, increased stress susceptibility, changed mineral homeostasis, and increased generation of free oxygen radicals.[1,2] Additionally the induction of lymphoma and intestinal tumors were observed.[3,4]

Compared with the situation in humans, the degree of Mg deficiency in these experiments was extreme. Serum Mg concentrations dropped to less than 20% of the corresponding control values, i.e., below 0.2 mmol/L. In humans, however, serum Mg concentrations below 0.5 mmol/L have only rarely been found; however, a mild degree of Mg deficiency might occur frequently.

To investigate whether the pathobiochemical effects directly correlate with the degree of Mg deficiency or whether there might be a threshold for significant alterations, diets with various Mg contents were fed to growing rats to induce different degrees of Mg deficiency. After 0, 10, 20, and 30 days, the parameters of free radical action (malondialdehyde [MDA] and vitamin E content) and mineral content in various tissues of these animals were analyzed.

Methods and materials

Ninety-six male Wistar rats (Interfauna, Tuttlingen, Germany) were used for the experiment. Fifteen rats were randomly assigned

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to each experimental group. Five groups received diets with different Mg content ad libitum. Diets were prepared by adding $MgCl_2$ to basal Mg-deficient diets (Ssniff, Soest, Germany; fat content: 3% soy bean oil; 140 mg/kg α-tocopherol acetate) achieving the following Mg contents: 70 ppm, 110 ppm, 208 ppm, 320 ppm, 850 ppm. Additionally, 15 rats receiving the 850 ppm diet were pair fed to the 70 ppm group. The Mg content of the diets was verified by measuring Mg by atomic absorption spectrophotometry (AAS; Philips SP9, Kassel, Germany) in ashed aliquots. All rats were housed under standard conditions (dark/light period: 12 hr/12 hr; temperature 22°C, 60% humidity) and received distilled water ad libitum. Six rats served as controls at experimental day 0. After 10, 20, and 30 days, 5 rats of each group were killed with ether anesthesia. Heparinized blood was obtained by puncture of the aorta abdominalis and centrifuged. Plasma was withdrawn, and a part of it was frozen at −20°C. The other part was stored at 4°C. Erythrocytes were washed twice with 150 mmol/L of NaCl. Liver, heart, kidney, spleen, and muscle (musc. quadriceps) were removed and washed in 250 mmol/L of sucrose. All tissue samples were frozen at −20°C until analysis.

Activity of alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) was determined in unfrozen plasma with commercial test kits (Sigma Diagnostics, München, Germany, DG159-UV, DG158-UV). Tumor necrosis factor-α (TNF-α) in plasma was determined by an ELISA kit for mouse TNF-α which cross-reacts with rat TNF-α (Genzyme, Cambridge, MA, USA).

Mg, calcium (Ca), zinc (Zn), and iron (Fe) in plasma were determined by AAS. For determination of the tissue contents of Mg, Ca, Fe, and Zn, frozen tissue was freeze-dried and powdered. The powdered tissues were ashed in the Plasma Processor 200 E (Technics, München, Germany). The ash was dissolved in 0.1 mol/L of HCl, and the minerals were determined after the appropriate dilution by AAS. To estimate the content of non–hemoglobin-bound Fe, hemoglobin was determined in spleen by means of the cyanomethemoglobin method, and hemoglobin-bound Fe was subtracted from total Fe. For measurement of vitamin E and MDA, the tissues were homogenized (20% homogenate) after thawing in 150 mmol/L of KCl. The vitamin E content of the homogenates was determined by its fluorescence in hexane extracts according to Taylor et al.[5] For calibration D,L-α-tocopherol (Serva, Heidelberg, Germany) was used.

MDA was determined by a variation of the thiobarbituric acid (TBA) method.[6,7] A 20% homogenate of the tissue in 150 mmol/L of KCl was at once diluted 1:1 with 5% trichloroacetic acid and centrifuged for 5 min at 13,000*g*. Five hundred microliters of TBA (1%, pH 7) was added to 500 μL of supernatant and heated at 95°C for 15 min. After cooling, the samples were extracted with 3 mL of 1-butanol by vortexing for 30 sec and centrifugating at 2,100*g* for 15 min. MDA in the butanol phase was measured fluorometrically (Perkin Elmer LS 50B, Überlingen, Germany; excitation: 532 nm; emission: 553 nm; slit width: 5 nm). The calibration curve was prepared with malonaldehyde tetraethylacetal (Sigma) which was treated in the same way.

Results

Body weight, plasma parameters, and erythrocyte Mg content

Feeding the diets with decreasing Mg content to growing rats led to a proportional reduction of plasma and erythrocyte Mg^{2+} concentration and to a corresponding reduction of weight gain (*Table 1*). Three hundred and thirty parts per million of Mg, however, were sufficient to achieve the same weight gain as in the 850 ppm Mg group. The Mg content of erythrocytes after 30 days was correspondingly reduced. Pair feeding resulted in reduced weight gain. However, the body weight of the pair-fed group was higher than in the 70 ppm group indicating better food utilization in the pair-fed rats. In rats receiving 850 ppm of Mg, after 20 and 30 days the plasma Mg^{2+} concentration was reduced as compared with days 0 and 10. The plasma Ca^{2+} concentration was slightly increased in Mg deficiency. There was a pronounced increase in the plasma Fe content in the 70 and 110 ppm groups after 20 and 30 days compared with the respective 850 ppm groups. Overall, however, the plasma Fe content dropped from day 0 to day 30. This may be caused by a lower Fe content of the experimental diet (180 mg/kg) compared with the normal diet (350 mg/kg) which was fed to the rats prior to the experiment. Zn in plasma was neither changed by time nor by diet (data not shown). AST and ALT were elevated at days 20 and 30 only in the group receiving the diet with the lowest Mg content. TNF-α, measured at day 30, increased in the 70 ppm group.

Liver

Mg deficiency did not reduce the total Mg content in liver (*Table 2*). There was a tendency toward increased Ca content in the 70 ppm group which reached significance only in the 20-day group. Fe accumulated significantly in the 70 and 110 ppm groups during the experiment leading to more than double the content of controls after 30 days in the group with the lowest Mg intake. The Zn content was elevated in the 70 ppm group after 20 and 30 days. Increased MDA concentrations were found already after 10 days and to a higher degree after 20 and 30 days in the 70 ppm group and to a smaller degree in the 110 ppm group. An inverse situation could be detected concerning the vitamin E content of liver; a significant reduction could be observed in rats receiving the 70 ppm diet.

Muscle

The highest muscle contents of Fe and Zn after 10, 20, and 30 days were consistently found in the group receiving the diet with the lowest Mg content without reaching significance (*Table 3*). The Ca content was elevated in the same group with a significant effect after 20 days. The MDA content was increased in the severely Mg-deficient rats with no significant effects concerning vitamin E. The higher MDA content at days 0 and 10 may be caused by a lower activity of protective enzymes (superoxide dismutase, glutathione peroxidase) which increase only slowly after birth.[8]

Heart

In heart muscle, a significant reduction of Mg content was observed (*Table 4*). An approximately 10% reduction was found in the 70 ppm group as early as 10 days after beginning the diet. A significant increase of Ca content was detected in the same tissues. After 30 days, Fe and MDA contents were also increased in the 70 ppm group whereas the vitamin E content was reduced. The Zn content was not changed.

Table 1 Body weight, contents of Mg, Ca, Fe, alanin-aminotransferase (ALT), aspartate-aminotransferase (AST), and TNF-α in plasma at day 0 and after feeding diets with different Mg content for 10, 20, and 30 days as indicated, and erythrocyte Mg content in rats after feeding the different diets for 30 days

Groups	Body weight (g)	Plasma						Erythrocytes Mg (mmol/kg of dry weight)
		Mg (mmol/L)	Ca (mmol/L)	Fe (μmol/L)	ALT (U/L)	AST (U/L)	TNF-α (pg/mL)	
Day 0	203 ± 3	0.73 ± 0.04	2.22 ± 0.05	63.8 ± 3.8	36 ± 1	91 ± 6		
Day 10								
70 ppm Mg	261 ± 5*	0.18 ± 0.03‡	2.29 ± 0.06	44.5 ± 1.4	36 ± 4	126 ± 16		
110 ppm Mg	281 ± 6	0.15 ± 0.01‡	2.11 ± 0.10	39.5 ± 1.4	33 ± 3	104 ± 12		
208 ppm Mg	284 ± 5	0.40 ± 0.03‡	2.17 ± 0.12	50.5 ± 1.2	30 ± 1	94 ± 4		
330 ppm Mg	275 ± 6	0.46 ± 0.03‡	2.13 ± 0.05	52.2 ± 4.5	29 ± 1	88 ± 2		
850 ppm Mg	282 ± 10	0.81 ± 0.04	2.27 ± 0.03	44.2 ± 2.2	31 ± 3	114 ± 5		
pair-fed	269 ± 3	0.67 ± 0.02‡	2.12 ± 0.02	33.3 ± 1.6‡	29 ± 2	90 ± 10		
Day 20								
70 ppm Mg	323 ± 7‡	0.09 ± 0.01‡	2.34 ± 0.03†	44.4 ± 2.7‡	42 ± 2‡	178 ± 18‡		
110 ppm Mg	349 ± 5*	0.12 ± 0.01‡	2.35 ± 0.05†	40.0 ± 2.8†	28 ± 2	95 ± 6		
208 ppm Mg	366 ± 15	0.32 ± 0.03‡	2.12 ± 0.09	35.3 ± 1.2	29 ± 1	87 ± 8		
330 ppm Mg	386 ± 12	0.41 ± 0.02‡	2.18 ± 0.07	37.6 ± 2.2*	30 ± 2	96 ± 6		
850 ppm Mg	386 ± 10	0.57 ± 0.02	2.12 ± 0.03	30.8 ± 1.3	28 ± 2	101 ± 7		
pair-fed	353 ± 4*	0.60 ± 0.02	2.17 ± 0.03	29.2 ± 1.8	25 ± 2	98 ± 5		
Day 30								
70 ppm Mg	343 ± 8‡	0.08 ± 0.01‡	2.28 ± 0.03	46.2 ± 2.5‡	42 ± 6†	141 ± 9‡	57 ± 13‡	2.70 ± 0.11‡
110 ppm Mg	390 ± 8‡	0.12 ± 0.02‡	2.21 ± 0.03	42.7 ± 1.4†	30 ± 1	97 ± 3	22 ± 7	3.09 ± 0.40‡
208 ppm Mg	417 ± 13*	0.31 ± 0.02‡	2.25 ± 0.05	33.0 ± 1.1	28 ± 1	89 ± 3	17 ± 4	5.82 ± 0.23†
330 ppm Mg	454 ± 12	0.50 ± 0.03†	2.29 ± 0.03	34.4 ± 0.9	29 ± 1	100 ± 3	14 ± 3	6.55 ± 0.10
850 ppm Mg	458 ± 16	0.59 ± 0.02	2.24 ± 0.04	35.4 ± 1.3	31 ± 2	106 ± 7	14 ± 4	7.07 ± 0.23
pair-fed	380 ± 8‡	0.57 ± 0.03	2.10 ± 0.04†	30.0 ± 2.3	30 ± 2	113 ± 4	18 ± 4	6.96 ± 0.17

Mean ± SEM of five rats in each group. Significant differences to the 850 ppm Mg groups by single factor analysis of variance; *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

Kidney

Mg deficiency induced significant reductions of Mg content in the kidney after 20 and 30 days in the 110 and 70 ppm Mg groups, respectively (*Table 5*). Ca content was tremendously increased by Mg deficiency leading to massive calcium deposits in some animals of the 70 ppm group and increased Ca content in the 208 ppm group and after 30 days in the 320 ppm group. The Zn content in the 70 ppm group was significantly increased after 10, 20, and 30 days. Also the Fe content was slightly elevated in this group although significance was not reached. In the same group, MDA was increased and vitamin E had a tendency toward lower values.

Spleen

The Mg, Ca, and Zn levels in the spleen were not affected by dietary Mg deficiency (Table 6). The Fe content increased with time and was particularly elevated in the 70 ppm group after 20 and 30 days. MDA was inversely correlated to vitamin E content. The MDA content in the 70 ppm group after 20 days was doubled, and after 30 days it was three times that of the 850 ppm group whereas the vitamin E content was reduced.

Discussion

As intended, feeding the different diets to growing rats resulted in graded Mg deficiency. However, even though the diet containing 850 ppm Mg is well above the reported 500 ppm Mg needed by rats,[9] this amount of Mg in the diet was not sufficient to achieve a normal plasma Mg^{2+} content in this fast-growing Wistar strain after 20 and 30 days. Significant changes compared with the 850 ppm Mg group were mainly detectable in the 70 ppm group and for some parameters in the 110 ppm groups. The most significant change was an Fe accumulation in various tissues, as has been observed in previous experiments in severe Mg deficiency.[2,10]

Weakly bound Fe is a potent inducer of free oxygen radicals.[11] The increased generation of these radicals enhanced lipid peroxidation which can be measured by the MDA content of tissues although part of MDA is metabolized and excreted.[12,13] Increased production of free radicals will also lead to an increased consumption of antioxidants. A reduction of vitamin E, therefore, is additional evidence for increased free radical action. Fe is able to catalyze the production of free radicals which induce lipid peroxidation. However, only a small fraction of Fe acts catalytically. This fraction is supposed to be bound to low molecular mass substances with rather low affinity, e.g., nucleotides, amino acids, citrate. The size of this pool is not defined. It may amount to 0.5 to 1 μM[14] or 20–30 μmol/kg wet weight.[15] Fe, bound in ferritin and hemosiderin, is not involved in lipid peroxidation. Part of this Fe pool can become active after liberation from its storage proteins, e.g., by acidification and by radicals.[16,17] Despite these considerations on the size of the active Fe pool, in spleen, liver, and muscle the total Fe content correlates very well

Table 2 Mg, Ca, Fe, Zn, malondialdehyde (MDA), and vitamin E content of liver from rats at day 0 and after feeding diets with different Mg content for 10, 20, and 30 days as indicated

Groups	Mg (mmol/kg d.w.)	Ca (mmol/kg d.w.)	Fe (mmol/kg d.w.)	Zn (mmol/kg d.w.)	MDA (μmol/kg w.w.)	Vitamin E (μmol/kg w.w.)
Day 0	31.8 ± 0.3	1.95 ± 0.07	2.86 ± 0.21	1.08 ± 0.02	1.22 ± 0.16	102.6 ± 5.8
Day 10						
70 ppm Mg	33.8 ± 0.5	2.54 ± 0.22	3.86 ± 0.29	1.22 ± 0.06*	2.51 ± 0.21†	77.1 ± 3.9
110 ppm Mg	33.5 ± 0.8	2.31 ± 0.09	3.33 ± 0.32	1.10 ± 0.05	2.41 ± 0.10†	74.8 ± 2.5*
208 ppm Mg	33.3 ± 0.6	2.15 ± 0.14	3.20 ± 0.33	1.09 ± 0.03	2.02 ± 0.25	86.2 ± 4.3
330 ppm Mg	32.8 ± 0.2	1.93 ± 0.03	3.26 ± 0.36	1.01 ± 0.03	1.69 ± 0.10	83.3 ± 4.7
850 ppm Mg	32.9 ± 0.7	2.20 ± 0.07	3.18 ± 0.36	1.04 ± 0.04	1.75 ± 0.09	88.4 ± 6.8
pair-fed	33.1 ± 0.5	1.88 ± 0.10	2.93 ± 0.28	1.02 ± 0.07	1.63 ± 0.15	100.0 ± 4.2
Day 20						
70 ppm Mg	33.1 ± 0.5	2.57 ± 0.12†	5.06 ± 0.61‡	1.17 ± 0.01*	2.87 ± 0.28‡	67.1 ± 8.6*
110 ppm Mg	33.4 ± 0.1	2.20 ± 0.09	3.97 ± 0.24	1.09 ± 0.03	1.90 ± 0.06*	82.2 ± 4.8
208 ppm Mg	33.4 ± 0.4	2.20 ± 0.16	3.91 ± 0.20	1.06 ± 0.03	1.12 ± 0.13	86.9 ± 4.7
330 ppm Mg	33.0 ± 0.2	2.04 ± 0.05	2.99 ± 0.19	1.04 ± 0.01	1.17 ± 0.20	82.7 ± 4.6
850 ppm Mg	31.8 ± 0.6	2.07 ± 0.06	3.10 ± 0.31	1.02 ± 0.04	1.27 ± 0.09	83.2 ± 6.1
pair-fed	32.6 ± 0.4	1.97 ± 0.11	3.21 ± 0.34	1.03 ± 0.05	1.44 ± 0.17	78.9 ± 7.5
Day 30						
70 ppm Mg	32.5 ± 0.5	2.54 ± 0.12	7.63 ± 1.06‡	1.19 ± 0.03*	3.04 ± 0.28‡	63.3 ± 1.0†
110 ppm Mg	32.5 ± 0.5	2.35 ± 0.12	5.14 ± 0.37*	1.10 ± 0.02*	1.48 ± 0.13*	74.4 ± 4.8
208 ppm Mg	31.6 ± 0.3	2.61 ± 0.04	4.01 ± 0.17	1.02 ± 0.04	1.22 ± 0.11	79.1 ± 5.6
330 ppm Mg	33.3 ± 0.9	2.37 ± 0.19	3.49 ± 0.60	1.03 ± 0.02	1.13 ± 0.15	84.7 ± 6.4
850 ppm Mg	32.1 ± 0.2	2.26 ± 0.12	3.12 ± 0.08	1.01 ± 0.04	0.98 ± 0.14	86.4 ± 4.4
pair-fed	32.5 ± 0.4	2.17 ± 0.11	3.40 ± 0.27	1.05 ± 0.02	1.14 ± 0.03	82.1 ± 3.1

d.w. = dry weight, w.w. = wet weight. Mean ± SEM of five rats in each group. Significant differences to the 850 ppm Mg groups by single factor analysis of variance; *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

with the MDA content (*Figure 1*). In accordance with this conclusion, the low molecular weight Fe pool correlated to MDA formation in Fe-loaded liver.[18]

It is not yet clear by which mechanism Mg deficiency increases the Fe content of tissues especially in liver and spleen. The most plausible cause might be an increased erythrocyte fragility and destruction and consequently increased tissue uptake and storage of Fe. Osmotic resistance of erythrocytes is reduced in Mg deficiency,[19] resulting in a small reduction in the number of erythrocytes. A possible reason for the reduced osmotic resistance may be the formation of plaques within the erythrocyte membrane.[20] Mg

Table 3 Mg, Ca, Fe, Zn, malondialdehyde (MDA), and vitamin E content of rat muscle

Groups	Mg (mmol/kg d.w.)	Ca (mmol/kg d.w.)	Fe (mmol/kg d.w.)	Zn (mmol/kg d.w.)	MDA (μmol/kg w.w.)	Vitamin E (μmol/kg w.w.)
Day 0	45.6 ± 3.3	3.58 ± 0.23	0.74 ± 0.25	0.67 ± 0.08	3.42 ± 0.60	40.0 ± 1.5
Day 10						
70 ppm Mg	50.5 ± 1.2	4.98 ± 1.43	0.91 ± 0.11*	0.85 ± 0.12	6.95 ± 2.36†	40.7 ± 2.2
110 ppm Mg	47.4 ± 0.8	3.74 ± 0.11	0.79 ± 0.22	0.62 ± 0.06	5.73 ± 1.06*	40.2 ± 2.6
208 ppm Mg	46.1 ± 1.3	4.55 ± 0.67	0.64 ± 0.16	0.80 ± 0.09	4.31 ± 0.90	42.6 ± 1.2
330 ppm Mg	46.0 ± 1.1	4.11 ± 0.23	0.65 ± 0.27	0.82 ± 0.11	3.24 ± 0.64	42.5 ± 1.0
850 ppm Mg	48.4 ± 1.0	3.87 ± 0.30	0.59 ± 0.14	0.73 ± 0.08	3.91 ± 0.82	42.7 ± 2.3
pair-fed	50.3 ± 2.7	4.26 ± 0.32	0.57 ± 0.14	0.76 ± 0.14	4.25 ± 0.92	42.2 ± 1.9
Day 20						
70 ppm Mg	39.2 ± 2.9	5.35 ± 0.75‡	0.94 ± 0.20*	0.80 ± 0.14	6.65 ± 0.99†	41.2 ± 3.3
110 ppm Mg	47.3 ± 1.2	3.50 ± 0.09	0.73 ± 0.21	0.80 ± 0.11	4.35 ± 2.28*	41.8 ± 2.6
208 ppm Mg	45.8 ± 3.0	3.82 ± 0.37	0.68 ± 0.18	0.86 ± 0.17	2.99 ± 0.61	40.9 ± 3.0
330 ppm Mg	43.0 ± 0.7	4.07 ± 0.15	0.58 ± 0.21	0.81 ± 0.11	1.50 ± 0.12	43.5 ± 3.0
850 ppm Mg	46.4 ± 4.3	3.49 ± 0.19	0.53 ± 0.22	0.68 ± 0.08	2.96 ± 0.74	42.7 ± 0.8
pair-fed	43.8 ± 3.1	3.58 ± 0.06	0.81 ± 0.34	0.80 ± 0.13	2.12 ± 0.13	42.8 ± 3.4
Day 30						
70 ppm Mg	43.2 ± 1.2	4.37 ± 0.25	0.93 ± 0.45	0.91 ± 0.11	3.36 ± 0.87†	45.9 ± 1.0
110 ppm Mg	47.2 ± 2.1	3.86 ± 0.20	0.87 ± 0.32	0.84 ± 0.11	2.68 ± 0.71	48.7 ± 1.7
208 ppm Mg	49.1 ± 1.0	4.00 ± 0.17	0.86 ± 0.23	0.79 ± 0.06	1.91 ± 0.53	48.4 ± 1.3
330 ppm Mg	47.8 ± 1.1	3.46 ± 0.25	0.77 ± 0.15	0.70 ± 0.07	1.84 ± 0.51	47.3 ± 1.3
850 ppm Mg	48.6 ± 0.7	3.74 ± 0.22	0.61 ± 0.19	0.74 ± 0.08	1.64 ± 0.27	48.3 ± 3.3
pair-fed	45.8 ± 1.4	3.84 ± 0.58	0.77 ± 0.16	0.73 ± 0.07	1.91 ± 0.07	44.6 ± 3.7

See legend to *Table 2*.

Table 4 Mg, Ca, Fe, Zn, malondialdehyde (MDA), and vitamin E content of rat heart

Groups	Mg (mmol/kg d.w.)	Ca (mmol/kg d.w.)	Fe (mmol/kg d.w.)	Zn (mmol/kg d.w.)	MDA (μmol/kg w.w.)	Vitamin E (μmol/kg w.w.)
Day 0	41.3 ± 1.0	2.21 ± 0.16	5.96 ± 0.66	1.03 ± 0.03		
Day 10						
70 ppm Mg	35.5 ± 1.5†	3.01 ± 0.18*	6.54 ± 0.51	0.98 ± 0.04		
110 ppm Mg	36.1 ± 0.8†	2.76 ± 0.17	6.54 ± 0.63	0.95 ± 0.02		
208 ppm Mg	38.6 ± 1.0	2.67 ± 0.11	6.06 ± 0.46	0.99 ± 0.02		
330 ppm Mg	38.0 ± 0.4	2.48 ± 0.06	5.53 ± 0.08	0.93 ± 0.03		
850 ppm Mg	40.2 ± 0.9	2.56 ± 0.09	5.69 ± 0.25	1.02 ± 0.01		
pair-fed	40.6 ± 0.8	2.31 ± 0.12	5.91 ± 0.05	1.00 ± 0.03		
Day 20						
70 ppm Mg	36.4 ± 1.4*	2.51 ± 0.27*	6.39 ± 0.26	0.90 ± 0.03		
110 ppm Mg	39.8 ± 1.6	2.03 ± 0.22	5.89 ± 0.26	0.99 ± 0.03		
208 ppm Mg	39.4 ± 1.6	1.98 ± 0.17	5.74 ± 0.32	0.93 ± 0.04		
330 ppm Mg	39.5 ± 1.6	2.08 ± 0.11	5.90 ± 0.19	0.95 ± 0.04		
850 ppm Mg	41.9 ± 1.4	1.86 ± 0.09	6.02 ± 0.09	0.99 ± 0.02		
pair-fed	41.9 ± 1.1	1.94 ± 0.20	5.88 ± 0.22	0.97 ± 0.02		
Day 30						
70 ppm Mg	35.5 ± 0.6*	2.33 ± 0.14*	7.28 ± 0.24†	0.91 ± 0.02	6.50 ± 1.85†	99.6 ± 2.1*
110 ppm Mg	37.5 ± 2.6	1.85 ± 0.07	6.01 ± 0.34	0.93 ± 0.01	3.70 ± 0.83	100.0 ± 4.2*
208 ppm Mg	39.5 ± 0.2	2.01 ± 0.08	6.04 ± 0.24	0.96 ± 0.01	3.47 ± 0.63	110.9 ± 2.0
330 ppm Mg	38.9 ± 1.1	1.90 ± 0.05	6.19 ± 0.47	0.91 ± 0.02	3.26 ± 1.06	114.3 ± 4.1
850 ppm Mg	40.5 ± 1.4	2.06 ± 0.03	5.83 ± 0.31	0.95 ± 0.04	2.48 ± 0.55	111.2 ± 3.9
pair-fed	41.5 ± 0.2	1.82 ± 0.04	6.65 ± 0.18	0.98 ± 0.01	2.37 ± 0.30	112.6 ± 4.1

See legend to *Table 2*.

content of erythrocytes decreases with decreasing plasma Mg^{2+} concentration in vivo. It is unknown whether there is an Mg loss in the whole population of erythrocytes or whether newly formed (at low extracellular Mg^{2+}) erythrocytes contain less Mg than the older ones. In vitro erythrocytes do not take up or lose Mg even when they are incubated at increased or reduced Mg^{2+} concentrations.[21] It might be that erythrocytes with a low Mg content are predominantly degraded in the spleen, possibly due to altered membrane components (e.g., phospholipids, glycolipids) or structure.

The plasma Fe concentration was increased in the 70 and 110 ppm Mg groups after 20 and 30 days, which may be indicative of increased Fe (erythrocyte) turnover. The increased plasma and tissue Fe content may be caused by increased intestinal absorption of Fe since Mg inhibited

Table 5 Mg, Ca, Fe, Zn, malondialdehyde (MDA), and vitamin E content of rat kidney

Groups	Mg (mmol/kg d.w.)	Ca (mmol/kg d.w.)	Fe (mmol/kg d.w.)	Zn (mmol/kg d.w.)	MDA (μmol/kg w.w.)	Vitamin E (μmol/kg w.w.)
Day 0	41.5 ± 0.7	3.98 ± 0.07	2.59 ± 0.14	1.31 ± 0.04	8.55 ± 0.52	60.2 ± 2.4
Day 10						
70 ppm Mg	41.1 ± 2.5	363 ± 212†	2.99 ± 0.27	1.64 ± 0.14†	18.56 ± 2.90	68.5 ± 3.1
110 ppm Mg	41.7 ± 2.6	20.3 ± 8.8	2.44 ± 0.16	1.53 ± 0.09	13.48 ± 2.77	67.4 ± 3.1
208 ppm Mg	41.0 ± 0.9	6.90 ± 2.61	2.62 ± 0.18	1.41 ± 0.04	12.96 ± 2.93	72.5 ± 2.9
330 ppm Mg	41.1 ± 0.6	3.96 ± 0.17	2.72 ± 0.12	1.30 ± 0.04	13.09 ± 1.74	67.9 ± 1.7
850 ppm Mg	39.0 ± 0.7	3.40 ± 0.21	2.91 ± 0.10	1.34 ± 0.02	13.52 ± 0.84	74.4 ± 6.0
pair-fed	42.2 ± 0.5	3.30 ± 0.11	2.37 ± 0.17*	1.40 ± 0.01	12.72 ± 2.22	76.6 ± 1.6
Day 20						
70 ppm Mg	37.6 ± 1.1	254 ± 175†	3.57 ± 0.30	1.51 ± 0.11‡	21.00 ± 2.98*	61.6 ± 3.4
110 ppm Mg	36.8 ± 0.6*	13.8 ± 8.1*	3.45 ± 0.15	1.33 ± 0.03	15.74 ± 2.70	63.0 ± 2.1
208 ppm Mg	39.5 ± 0.4	17.5 ± 6.3*	3.64 ± 0.30	1.26 ± 0.02	15.69 ± 1.57	69.0 ± 2.7
330 ppm Mg	40.5 ± 0.9	4.31 ± 0.06	3.03 ± 0.21	1.28 ± 0.03	14.21 ± 1.12	65.1 ± 2.1
850 ppm Mg	39.6 ± 0.8	4.68 ± 0.87	3.21 ± 0.11	1.23 ± 0.01	13.83 ± 1.37	67.7 ± 3.9
pair-fed	40.1 ± 0.5	4.55 ± 0.29	3.24 ± 0.18	1.27 ± 0.03	13.63 ± 1.61	67.3 ± 3.5
Day 30						
70 ppm Mg	36.9 ± 0.7*	177 ± 94†	3.60 ± 0.20	1.72 ± 0.20†	19.42 ± 1.73‡	68.1 ± 2.9
110 ppm Mg	38.2 ± 0.9	77.5 ± 53*	3.26 ± 0.15	1.60 ± 0.11	13.77 ± 1.56	67.5 ± 1.4
208 ppm Mg	37.6 ± 0.8	10.4 ± 2.5*	3.49 ± 0.06	1.36 ± 0.03	11.79 ± 0.55	69.1 ± 2.9
330 ppm Mg	37.4 ± 0.6	7.95 ± 2.94	3.43 ± 0.18	1.35 ± 0.02	12.23 ± 0.78	75.8 ± 2.4
850 ppm Mg	39.6 ± 0.3	4.51 ± 0.70	3.32 ± 0.14	1.33 ± 0.04	11.53 ± 2.44	73.5 ± 2.6
pair-fed	37.8 ± 0.7	3.62 ± 0.13	3.55 ± 0.23	1.36 ± 0.03	11.74 ± 0.99	74.1 ± 5.1

See legend to *Table 2*.

Table 6 Mg, Ca, Fe, Zn, malondialdehyde (MDA), and vitamin E content of rat spleen

Groups	Mg (mmol/kg d.w.)	Ca (mmol/kg d.w.)	Fe (mmol/kg d.w.)	Zn (mmol/kg d.w.)	MDA (μmol/kg w.w.)	Vitamin E (μmol/kg w.w.)
Day 0	44.1 ± 1.0	2.05 ± 0.15	9.29 ± 0.49	1.21 ± 0.05	11.0 ± 0.9	135.5 ± 9.6
Day 10						
70 ppm Mg	43.8 ± 1.6	2.31 ± 0.12	8.89 ± 1.34	1.33 ± 0.11	11.4 ± 1.3	133.8 ± 11.0
110 ppm Mg	42.6 ± 1.7	2.35 ± 0.16	8.12 ± 0.75	1.16 ± 0.08	10.9 ± 0.1	151.6 ± 12.3
208 ppm Mg	43.9 ± 0.3	2.03 ± 0.08	8.94 ± 0.53	1.32 ± 0.07	10.1 ± 0.2	150.9 ± 5.5
330 ppm Mg	42.6 ± 0.7	1.98 ± 0.13	9.60 ± 0.99	1.18 ± 0.06	10.0 ± 0.5	145.9 ± 8.5
850 ppm Mg	42.8 ± 0.5	2.12 ± 0.17	8.60 ± 0.60	1.22 ± 0.05	11.5 ± 1.1	150.4 ± 3.0
pair-fed	43.6 ± 0.5	2.08 ± 0.11	8.55 ± 0.37	1.17 ± 0.03	11.1 ± 1.7	156.6 ± 8.5
Day 20						
70 ppm Mg	41.0 ± 0.6	2.67 ± 0.13	34.1 ± 3.9‡	1.22 ± 0.06	17.3 ± 4.3†	119.6 ± 4.5†
110 ppm Mg	41.3 ± 0.7	2.36 ± 0.21	18.8 ± 1.1*	1.28 ± 0.07	10.6 ± 0.8	149.2 ± 9.7
208 ppm Mg	39.0 ± 0.5	2.29 ± 0.09	19.0 ± 0.6*	1.28 ± 0.08	8.4 ± 1.0	148.8 ± 11.3
330 ppm Mg	39.2 ± 1.3	2.14 ± 0.17	16.6 ± 0.5	1.18 ± 0.06	7.0 ± 1.7	148.0 ± 11.7
850 ppm Mg	40.6 ± 0.7	2.22 ± 0.15	12.9 ± 1.1	1.12 ± 0.02	8.2 ± 1.2	146.0 ± 11.5
pair-fed	38.1 ± 3.9	2.46 ± 0.35	14.0 ± 1.2	1.13 ± 0.03	7.7 ± 0.7	145.1 ± 12.8
Day 30						
70 ppm Mg	39.2 ± 1.0	2.63 ± 0.11	65.1 ± 6.8‡	1.22 ± 0.08	16.1 ± 3.6‡	135.1 ± 12.8
110 ppm Mg	38.6 ± 0.7	2.38 ± 0.11	35.0 ± 4.1*	1.17 ± 0.09	7.8 ± 1.5	136.7 ± 10.0
208 ppm Mg	39.0 ± 1.0	2.24 ± 0.09	28.7 ± 2.0	1.29 ± 0.06	7.3 ± 1.3	150.7 ± 6.6
330 ppm Mg	37.6 ± 1.2	2.22 ± 0.24	24.1 ± 1.3	1.14 ± 0.04	6.3 ± 0.7	155.6 ± 11.5
850 ppm Mg	38.1 ± 1.1	2.19 ± 0.13	23.1 ± 2.2	1.15 ± 0.09	5.2 ± 0.4	150.1 ± 3.7
pair-fed	41.0 ± 0.5	2.25 ± 0.19	20.1 ± 1.3	1.26 ± 0.09	6.0 ± 0.9	151.3 ± 4.6

See legend to *Table 2*.

^{59}Fe uptake by mouse duodenal fragments.[22] However, the intestine of Mg-deficient rats had the same capacity of Fe uptake as controls.[23]

It was reported that the increased production of free radicals in Mg deficiency is caused by elevated concentrations of TNF-α,[24] which in turn was increased by early induction of substance P.[25] Increased TNF-α concentrations, however, could only be detected in the group with the lowest Mg content in their diets, whereas increased MDA contents were already detected in the 110 ppm group. Therefore, TNF-α may contribute to the production of free radicals only in severe Mg deficiency.

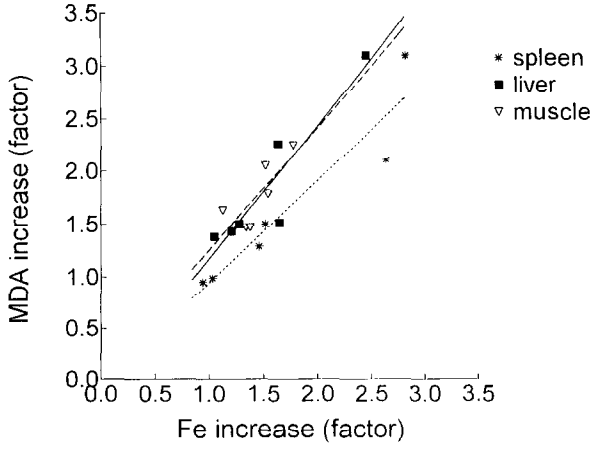

Figure 1 Correlation of MDA with Fe content by factor in spleen, liver, and muscle at days 10, 20, and 30 from rats fed 70 and 110 ppm Mg. The mean values of each group (five rats) were plotted. Correlation coefficients (r^2) were: spleen, 0.8968; liver, 0.8521; muscle, 0.6246.

Production of free radicals will not only lead to increased lipid peroxidation but also to an induction of metallothionein[26] especially in liver and kidney which will bind Zn leading to increased total Zn content. Alternatively, the increased Zn content in these tissues in the 70 ppm Mg group might be caused by an increased liberation of interleukin-1[24] or catecholamines in Mg deficiency[27] which will lead to induction of metallothionein[26] and to increased lipid peroxidation.[28] Additionally, the increased Fe content in liver may have induced the synthesis of metallothionein[29] which in turn bound Zn.

An elevated peroxidation of membrane phospholipids will ultimately result in membrane damage and Ca accumulation[30] as was found in liver, muscle, and heart. However, these effects were significant only in the group with 70 ppm Mg in the diet. In accordance with these results an increase of ALT and AST in plasma as an indicator of membrane damage in muscle and liver could also be verified in animals receiving the diet with the lowest Mg content. In the kidney, already relatively mild Mg deficiency was able to increase the Ca content. Calcification of the kidney is caused by a reduced magnesium/calcium ratio in the tubules, allowing calcium phosphate to precipitate.[31]

On the whole, the pathomechanisms in Mg deficiency mainly consist of reduced extracellular Mg^{2+}/Ca^{2+} antagonism and increased erythrocyte destruction resulting in increased Fe storage and formation of free radicals. These effects, however, become prominent only when a certain degree of Mg deficiency is achieved. It was remarkable that even the very small difference in plasma Mg content between the 70 and 110 ppm Mg group was able to reduce significantly the toxic effects of Mg deficiency. By correlating the Fe and MDA content of liver with plasma Mg^{2+} concentration (*Figure 2*) it becomes obvious that with re-

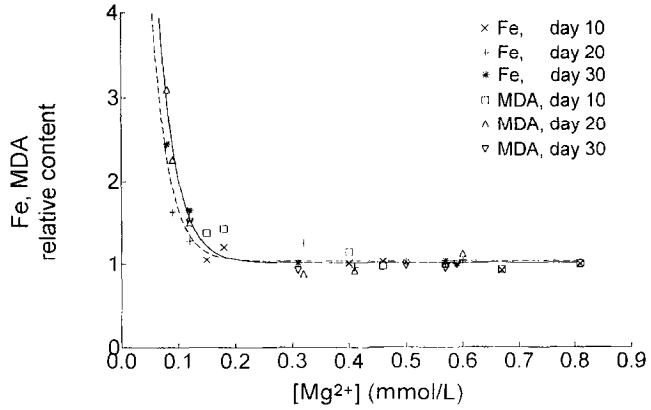

Figure 2 Relative content of MDA and Fe (by factor) in liver as a function of plasma Mg^{2+} concentration from rats fed diets with different Mg content for 10, 20, or 30 days. Mean values of each group (five rats) were plotted.

gard to Fe accumulation and lipid peroxidation a certain threshold exists at about 0.25 mmol/L Mg^{2+}. Above this threshold no effects regarding these parameters could be observed. Similar correlations at different levels of MDA formation or Fe accumulation could be obtained for other tissues (data not shown). Also the accumulation of Ca in the heart shows a threshold of extracellular Mg^{2+} concentration (*Figure 3*).

These findings suggest that above a threshold of extracellular Mg^{2+} concentration the pathobiochemical effects of Mg deficiency alone can be compensated for and do not reach significance. One possible explanation for this threshold might be a compensation of the increased Ca^{2+} influx in Mg deficiency by a higher activity of Ca^{2+}-ATPase. The capacity of this ATPase is insufficient to remove all Ca^{2+} at Mg^{2+} concentrations below 0.25 mmol/L resulting in increased intracellular concentration of free Ca^{2+} with subsequent toxic effects.[32] Therefore, a mild Mg deficiency can be compensated for and might not lead to pathological symptoms. However, mild Mg deficiency might contribute to the pathobiochemical effects if other pathogenetic factors combine with it.

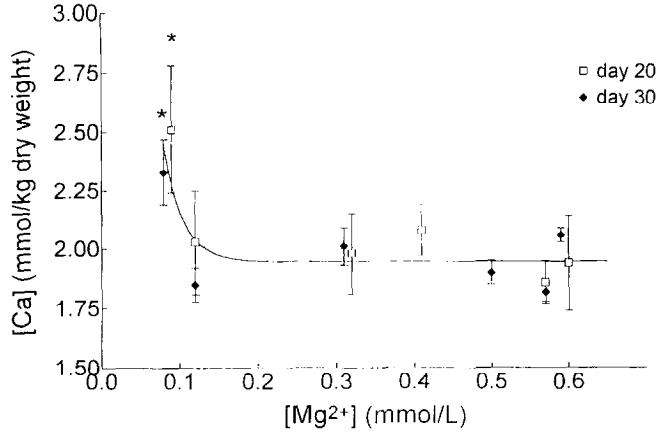

Figure 3 Ca content in hearts as a function of plasma Mg^{2+} concentration from rats fed diets with different Mg^{2+} content for 20 and 30 days. Means ± SEM. For significant differences see legend to *Table 2*.

References

1 Günther, T. (1981). Biochemistry and pathobiochemistry of magnesium. *Magn. Bull.* **3,** 91–101

2 Günther, T., Vormann, J., Höllriegl, V., Disch, G., and Classen, H.-G. (1992). Role of lipid peroxidation and vitamin E in magnesium deficiency. *Magn. Bull.* **14,** 57–66

3 Averdunk, R., Bippus, P.-H., Günther, T., and Merker, H.J. (1982). Development and properties of malignant lymphoma induced by magnesium deficiency in rats. *J. Cancer Res. Clin. Oncol.* **104,** 63–73

4 Vormann, J., Merker, H.J., Barrach, H.J., Stolpmann, H.J., Averdunk, R., and Günther, T. (1985). Induction of a tumor-like connective tissue proliferation in the intestine of magnesium-deficient rats. *Magn. Bull.* **7,** 4–10

5 Taylor, S.L., Lamden, M.P., and Tappel, A.L. (1976). Sensitive fluorometric method for tissue tocopherol analysis. *Lipids* **11,** 530–538

6 Britton, R.S., O'Neill, T., and Bacon, B.R. (1990). Hepatic mitochondrial malondialdehyde metabolism in rats with chronic iron overload. *Hepatology* **11,** 93–97

7 Gutteridge, J.M.C. and Tickner, T.R. (1978). The characterization of thiobarbituric acid reactivity in human plasma and urine. *Anal. Biochem.* **91,** 250–257

8 Günther, T., Höllriegl, V., and Vormann, J. (1993). Perinatal development of iron and antioxidant defence systems. *J. Trace Elem. Electrolytes Health Dis.* **7,** 47–52

9 Cook, D.A. (1973). Availability of magnesium: Balance studies in rats with various inorganic salts. *J. Nutr.* **103,** 1365–1370

10 Günther, T., Vormann, J., Höllriegl, V., Disch, G., and Classen, H.-G. (1992). Effects of isoproterenol and magnesium deficiency on vitamin E content, lipid peroxidation and mineral metabolism of various tissues. *Magn. Bull.* **14,** 81–87

11 Halliwell, B. and Gutteridge, J.M.C. (1990). Role of free radicals and catalytic metal ions in human disease: An overview. *Meth. Enzymol.* **186,** 1–85

12 Draper, H.H. and Hadley, M. (1990). A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde. *Xenobiotica* **20,** 901–907

13 Placer, Z., Veselkova, A., and Rath, R. (1965). Kinetik des Malondialdehyds im Organismus. *Experientia* **21,** 19–20

14 Crichton, R.R. and Ward, R.J. (1992). Iron metabolism-New perspectives in view. *Biochemistry* **31,** 11255–11264

15 Kozlov, A.V., Yegerov, D.Y., Vladimirov, Y.A., and Azizova, O.A. (1992). Intracellular free iron in liver tissue and liver homogenate: studies with electron paramagnetic resonance on the formation of paramagnetic complexes with Desferal and nitric oxide. *Free Radical Biol. Med.* **13,** 9–16

16 Ozaki, M., Kawabata, T., and Awai, M. (1988). Iron release from haemosiderin and production of iron-catalysed hydroxyl radicals in vitro. *Biochem. J.* **250,** 589–595

17 Reif, D.H., Schubert, J., and Aust, S.D. (1988). Iron release from ferritin and lipid peroxidation by radiolytically generated reducing radicals. *Arch. Biochem. Biophys.* **264,** 238–243

18 van Gelder, W., Siersema, P.D., Voogd, A., de Jeu-Jaspars, N.C.M., van Eijk, H.G., Koster, J.F., de Rooy, F.W.M., and Wilson, J.H.P. (1993). The effect of desferrioxamine on iron metabolism and lipid peroxidation in hepatocytes of C57BL/10 mice in experimental uroporphyria. *Biochem. Pharmacol.* **46,** 221–228

19 Piomelli, S.P., Jansen, V., and Dancis, J. (1973). The hemolytic anemia of magnesium deficiency in adult rats. *Blood* **41,** 451–459

20 Elin, R.J. and Tan, K.H. (1980). Formation of plaques on erythrocyte membranes from rats with magnesium deficiency. In *Magnesium in Health and Disease* (M. Cantin and M.S. Seelig, eds.), p. 125–127, Spectrum Publications, Inc., Jamaica, NY USA

21 Günther, T. and Vormann, J. (1985). Removal and reuptake of intracellular magnesium. *Magn. Bull.* **7,** 66–69

22 Raja, K.B., Simpson, R.J., and Peters, T.J. (1987). Effect of Ca^{2+} and Mg^{2+} on the uptake of Fe^{3+} by mouse intestinal mucosa. *Biochim. Biophys. Acta* **923,** 46–51

23 Schümann, K., Lebeau, A., Elsenhans, B., Hunder, G., Forth, W., and Vormann, J. (1995). Intestinal Fe transfer and Fe distribution in Mg-deficient rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **351,** R149

24 Weglicki, W.B., Phillips, T.M., Freedman, A.M., Cassidy, M.M., and Dickens, B.F. (1992). Magnesium deficiency elevates circulating levels of inflammatory cytokines and endothelin. *Mol. Cell. Biochem.* **110,** 169–173

25 Weglicki, W.B., Mak, I.T., Stafford, R.E., Dickens, B.F., Cassidy, M.M., and Phillips, T.M. (1994). Neurogenic peptides and the cardiomyopathy of magnesium-deficiency: effects of substance P-receptor inhibition. *Mol. Cell. Biochem.* **130,** 103–109

26 Kägi, J.H.R. and Schäffer, A. (1988). Biochemistry of metallothionein. *Biochemistry* **27,** 8509–8515

27 Günther, T., Ising, H., and Merker, H.J. (1978). Elektrolyt- und Kollagengehalt im Rattenherzen bei chronischem Magnesiummangel und Stress. *J. Clin. Chem. Clin. Biochem.* **16,** 293–297

28 Ceremuzynski, L., Barcikowski, B., Lewicki, Z., Wutzen, J., Gordon-Majszak, W., Famulski, K.S., Klos, J., and Herbaczynska-Cedro, K. (1991). Stress-induced injury of pig myocardium is accompanied by increased lipid peroxidation and depletion of mitochondrial ATP. *Exp. Pathol.* **43,** 213–220

29 Fleet, J.C., Andrews, G.K., and McCormick, C.C. (1990). Iron-induced metallothionein in chick liver: A rapid, route-dependent effect independent of zinc status. *J. Nutr.* **120,** 1214–1222

30 Ungemach, F.R. (1987). Pathobiochemical mechanism of hepatocellular damage following lipid peroxidation. *Chem. Phys. Lipids* **45,** 171–205

31 Bunce, G.E., Saacke, R.G., and Mullins, J. (1980). The morphology and pathogenesis of magnesium deficiency-induced nephrocalcinosis. *Exper. Mol. Pathol.* **33,** 203–210

32 Günther, T. (1990). Magnesium deficiency generally enhances cytotoxicity. *Magn. Bull.* **12,** 61–64