The humoral immune response in marginally and severely magnesium deficient rats

Marlene M. Windhauser,* Leonard C. Kappel, and Maren Hegsted*†

Department of Physiology, Pharmacology and Toxicology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA

The effects of severe, moderate, and mild magnesium deficiencies on plasma proteins, immunoglobulin M (IgM), immunoglobulin G (IgG), and specific antibody response were studied. Forty male Sprague-Dawley rats were fed diets containing 50, 160, 280, and 400 μg magnesium/gram for either three or eight weeks. Eight of the rats were fed the control diet but were pair fed with the 50 μg/gram treatment group. All rats were immunized once with sheep red blood cells. Plasma magnesium levels reflected the dietary levels of magnesium, and bone magnesium highly correlated with plasma magnesium. The severely deficient rats had significantly enlarged spleens, while all other groups were similar to controls. The severe magnesium deficiency significantly decreased total plasma proteins. Additionally, immunoglobulin M and G levels were significantly reduced in the severely deficient rats. The mean log antibody titer for the severely deficient rats was approximately half that of the controls, but was not statistically different from the other dietary groups. Conversely, total plasma proteins were not lowered by the suboptimal levels of magnesium. Immunoglobulin M levels in the marginally deficient animals correlated with their plasma magnesium concentrations, and lower immunoglobulin G levels were found with the moderate magnesium deficiencies. Antibody response was not altered by the suboptimal levels of dietary magnesium.

Keywords: magnesium deficiency; rats; globulins; antibody

Introduction

Early clinical and experimental studies of nutritional effects on immune mechanisms involved multiple nutrient deficiencies. Next, the interrelationship of single nutrients and immunocompetence received more attention.[1-5] A deficiency of magnesium was shown to affect the humoral immune system.[1,6] With the changes that occur in the lymphoid organs during magnesium depletion, and with the requirement of magnesium for protein synthesis, the effects of magnesium deficiencies on immunoglobulin synthesis and specific antibody activity were of interest.[7-9]

A marked depression and rapid recovery of serum immunoglobulin G in response to magnesium intake in rats suggested a direct role for the mineral in the synthesis or metabolism of the immunoglobulin.[7] In an analysis of the production of specific antibody, agglutinin and hemolysin titers were lower in magnesium deficient rats than controls.[9] The low antibody titer associated with magnesium deficiency might have occurred due to a lack of magnesium for cellular metabolism and antibody synthesis. Recently, maternal magnesium status was observed to influence offspring humoral immunocompetence.[10] Rat pups from magnesium deficient dams had lower plaque forming cells per spleen than those from dams fed adequate levels of magnesium.

Previous studies used diets that produced severe magnesium deficiencies, with only a few using suboptimal levels of dietary magnesium. Marginal deficiencies, however, are becoming more recognized in clinical settings, and the effects of those types of deficiencies on immunocompetence are of current in-

Address reprint requests to Dr. Leonard C. Kappel, Department of Physiology, Pharmacology and Toxicology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, USA.
*Dr. Windhauser's present address is Pennington Biomedical Research Center, 6400 Perkins Road, Louisiana State University, Baton Rouge, LA 70808-4124, USA.
†Dr. Hegsted's present address is Human Nutrition and Foods, School of Human Ecology, Louisiana State University, Baton Rouge, LA 70803, USA.
Received August 10, 1990; accepted January 25, 1991.

© 1991 Butterworth–Heinemann

terest. Therefore, for investigating the effects of marginal magnesium status on the humoral immune system, the diets in this research were designed to induce moderate and mild magnesium deficiencies. Humoral immunity was assessed by antibody response to heterologous red blood cells, immunoglobulin M (IgM) and immunoglobulin G (IgG) quantitation, and plasma electrophoresis.

Methods and materials

Animals and diets

Forty male Sprague-Dawley rats, 145 g initial weight, were fed one of five treatment diets. The purified diets were prepared to be adequate in all nutrients, except for magnesium (*Table 1*). Magnesium was added at 400, 280, 160, and 50 μg/g of the diet, to achieve an adequate level, a mild deficiency, a moderate deficiency, or a severe deficiency, respectively. The feed was provided ad libitum.

The fifth treatment group consisted of eight rats that consumed the 400 μg Mg/g diet, but were pair fed to the rats consuming the 50 μg Mg/g diet. Deionized water was provided ad libitum to all rats. The rats were housed individually in stainless-steel, wire-bottom cages. A 12/12 hour light/dark cycle was maintained throughout the experiment.

Table 1 Experimental diet formulation

Composition	Percent of total diet
Casein, purified high nitrogen*	20.0
DL-methionine	0.3
Corn starch	32.5
Sucrose	32.5
Non-nutritive fiber*	5.0
Corn oil	5.0
AIN custom mineral mix*	3.5
AIN 76A vitamin mix*	1.0
Choline bitartrate	0.2
BHT	(0.015% of oil)
Magnesium†	

Note: Based on the diet recommendations of the American Institute of Nutrition.[11]

* ICN Biochemicals, Cleveland, OH. Composition of the mineral and vitamin mixes as described by the American Institute of Nutrition.[11] The mineral mix had no magnesium.

† The basal diet without magnesium in the mineral mix contained 27.5 μg Mg/g diet. MgO (Sigma Chemical Co., St. Louis, MO) was added to the mineral mix, which then was combined with the other feed ingredients. The amount MgO/kg total feed added for each diet was: 618.4 mg (400 μg Mg/g); 419.2 mg (280 μg Mg/g); 220.0 mg (160 μg Mg/g); and 37.4 mg (50 μg Mg/g). Actual magnesium concentrations of the total feed mixtures were determined by atomic absorption spectroscopy following nitric acid digestion, and were: 392.7 μg Mg/g; 249.8 μg Mg/g; 140.8 μg Mg/g; and 58.9 μg Mg/g.

Sample collection

At the initiation of the experiment, all rats were anesthetized and blood samples were obtained by cardiac puncture in syringes with preservative-free sodium heparin, 10 U/mL blood (Sigma Chemical Co., St. Louis, MO). At week three, blood samples were collected from four rats in each treatment group. The rats then were sacrificed and the spleen and thymus were removed and weighed. The femur was removed and frozen until analyzed for bone magnesium content. The same procedures were conducted for the remaining rats at eight weeks.

Initial plasma samples obtained before the rats were fed the treatment diets provided a baseline measure of antibody titer and immunoglobulin status before antigen challenge. The rats were immunized six days prior to euthanasia with a photometrically standardized 0.5% sheep red blood cell (sRBC) suspension. A single intraperitoneal injection was given at 0.1 mL/25 g body weight.[12]

Analytical methods

Specific antibody response was measured in all plasma samples by a hemagglutination technique.[12] Undiluted prechallenge plasma and 1:5 diluted postchallenge plasma were serially diluted 2-fold. Samples were incubated with a 1% sRBC suspension at 37° C overnight. Titer was determined as the reciprocal of the greatest dilution of plasma where agglutination was observed. Antibody was expressed as the log of the reciprocal titer.

IgG and IgM were quantitated by rocket immunoelectrophoresis.[13] Goat anti-rat IgG or goat anti-rat IgM antiserum (Cappel Laboratories, CopperBiomedical, Inc., Malverin, PA) was mixed into volumes of 1% agarose gel, Type 1 low EEO, (Sigma Chemical Co., St. Louis, MO). The samples plus rat IgG standards (Pel-Freeze Biologicals, Rogers, AR) or IgM standards (Calbiochem Biochemicals, San Diego, CA) were applied. Voltage to the gel plates was maintained for three hours (BioRad, model 1400, gel electrophoresis horizontal chamber). After the precipitated proteins were fixed and stained, the areas were measured, and the concentrations of immunoglobulin in the samples were determined from a calibration curve.

Plasma alpha, beta, and gamma globulins were quantitated by cellulose acetate electrophoresis.[14] Samples, applied to cellulose acetate plates (Helena Laboratories, Beaumont, TX), were electrophoresed at 180 volts for 15 min, then were scanned in a densitometer (Quick Scan, Helena Laboratories, Beaumont, TX) at 525 nm. The concentrations of the plasma proteins were calculated from total protein determined by the Lowry procedure.[15]

Aliquots of plasma were frozen at 0° C until mineral analyses were conducted. Plasma magnesium determinations were made by atomic absorption spectroscopy with an air-acetylene flame at a wavelength of 285.2 nm (Perkin-Elmer, model 3030B, Norwalk, CT).

Bone magnesium in femur diaphyseal sections was analyzed as described by Hunt.[16] The cleaned bones were dried to a constant weight, then were ashed in a

Table 2 Plasma and bone magnesium concentrations at weeks 3 and 8 by magnesium dietary treatment (n = 4)

Mg Diet (μg)	Plasma (mmol/L)		Bone (mg/g bone, dry weight)	
	Wk 3	Wk 8	Wk 3	Wk 8
50	0.23 ± 0.02*	0.27 ± 0.02*	2.79 ± 0.05*	1.93 ± 0.05*
160	0.59 ± 0.02†	0.78 ± 0.02	4.33 ± 0.05†	3.94 ± 0.05*
280	0.71 ± 0.02	0.93 ± 0.02	4.47 ± 0.05‡	4.59 ± 0.05
400				
Pair fed	0.79 ± 0.02	0.89 ± 0.02	4.73 ± 0.05	4.64 ± 0.05
Control	0.84 ± 0.02	0.87 ± 0.02	4.82 ± 0.05	4.78 ± 0.05
ANOVA				
Diet	$P < 0.001$		$P < 0.001$	
Week	$P < 0.001$		$P < 0.001$	
Diet week	$P < 0.001$		$P < 0.001$	

Note: Values are mean ± SEM.
* † ‡ Different from control within each week, $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively, as determined by LS Means.

muffle furnace for 16 hr at 600° C. The ash was dissolved in 5 N HCl and diluted with 0.5% lanthanum chloride. Magnesium concentrations were determined by atomic absorption spectroscopy.

Statistical analyses

The data were analyzed as a completely randomized design with a factorial arrangement of treatments (diets × sampling time-periods). The pre-test data from the humoral assessments were included as covariates. When the null hypotheses were rejected at the 0.95 level of significance, the least square mean paired t tests were used to test differences between the means.[17]

Results

The rats were similar at the start of the dietary treatments in weight (158.9 ± 2.2 g) and plasma magnesium (0.90 ± 0.02 mmol/L). Clinical signs of magnesium deficiency began to appear in the rats consuming the 50 μg Mg/g diet two weeks into the study and remained. These included ulcerative lesions about the head and neck, a coarse hair coat, and skin necrosis.

Nutritional status

After three weeks on the diets, plasma magnesium levels reflected the dietary levels of magnesium. Rats in the pair-fed treatment group had mean plasma magnesium levels similar to the control rats (*Table 2*). Although the mean plasma magnesium concentration for the rats in the 280 μg Mg/g diet group was lower than the control group, it was not significantly different. By eight weeks, the plasma magnesium concentration for the rats in the 160 μg Mg/g diet group also was not significantly different from that of the control animals.

Bone magnesium was highly correlated with plasma magnesium at three weeks (r = 0.95, $P < 0.001$), and at eight weeks (r = 0.84, $P < 0.001$). The severely deficient diet significantly depleted bone magnesium stores, while the marginal diets reduced bone magnesium to a lesser degree (*Table 2*). Throughout the study bone magnesium stores were maintained by the diets containing adequate magnesium in both the ad libitum and pair-fed treatment groups.

Rats consuming the adequate diet ad lib had the highest mean weight gain (*Table 3*), followed by those fed the 280 μg Mg/g diet and the 160 μg Mg/g diet, respectively. The smallest mean weight gains were for the severely deficient rats fed the 50 μg Mg/g diet and for the pair-fed rats.

Lymphoid organs

For comparative purposes, the spleen and thymus weights were expressed as mg/g of total body weight (*Table 3*). Spleen weight negatively correlated with plasma magnesium at three weeks (r = −0.61, $P < 0.01$) and at eight weeks (r = −0.78, $P < 0.001$). The severely magnesium deficient rats had significantly enlarged spleens, while rats in all other treatment groups were similar to controls. Thymus weight was not significantly altered by dietary magnesium.

Humoral immunity

A significant decrease in total plasma proteins was observed in the severely magnesium deficient rats, but not in the marginally deficient rats (*Table 4*). At the same time, the pair-fed treatment did not affect plasma protein. The lower plasma protein content for the animals receiving the 50 μg Mg/g diet could be attributed to lower amounts of albumin and the gamma globulins.

The severely deficient rats also had significantly lower levels of IgM and IgG (*Table 5*). IgM levels in all animals correlated with their plasma magnesium concentrations at three weeks (r = 0.63, $P < 0.01$) and at eight weeks (r = 0.57, $P < 0.01$). IgG levels correlated with plasma magnesium at three weeks (r = 0.58, $P < 0.01$), but not at eight weeks (r = 0.04, $P < 0.87$).

The mean log antibody response for the severely deficient rats was 44% and 59% that of the controls at 3 weeks and 8 weeks, respectively (*Table 5*). How-

Table 3 Weight gain, spleen and thymus weights at weeks 3 and 8 by magnesium dietary treatment (n = 4)

Mg diet (μg/g)	Weight gain (g)		Spleen weight (mg/g total body wt)		Thymus weight (mg/g total body wt)	
	Wk 3	Wk 8	Wk 3	Wk 8	Wk 3	Wk 8
50	82.0 ± 5.5*	153.8 ± 5.7*	0.30 ± 0.01*	0.30 ± 0.01*	0.23 ± 0.01	0.15 ± 0.01
160	90.8 ± 5.5	201.3 ± 5.7	0.22 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	0.18 ± 0.01
280	106.8 ± 5.5	200.0 ± 5.7	0.23 ± 0.01	0.18 ± 0.01	0.25 ± 0.01	0.16 ± 0.01
400						
Pair fed	98.0 ± 5.5†	152.5 ± 5.7†	0.23 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.15 ± 0.01
Control	129.3 ± 5.5	205.3 ± 5.7	0.22 ± 0.01	0.20 ± 0.01	0.23 ± 0.01	0.15 ± 0.01
ANOVA						
Diet	$P < 0.01$		$P < 0.001$		NS	
Week	$P < 0.001$		$P < 0.01$		$P < 0.001$	
Diet week	NS		NS		NS	

Note: Values are mean ± SEM.
* † Different from control within each week, $P < 0.001$ and $P < 0.01$, respectively, as determined by LS Means.

Table 4 Plasma proteins at weeks 3 and 8 by magnesium dietary treatment (n = 4)

Mg diet (μg/g)	Total protein (g/L)		Albumin (g/L)		Gamma globulin (g/L)	
	Wk 3	Wk 8	Wk 3	Wk 8	Wk 3	Wk 8
50	72 ± 5*	79 ± 5*	33 ± 3	38 ± 3*	2.1 ± 0.4*	2.0 ± 0.4*
160	81 ± 5	111 ± 5	36 ± 3	56 ± 3	2.2 ± 0.4	3.4 ± 0.4
280	73 ± 5	94 ± 5	36 ± 3	49 ± 3	3.0 ± 0.4	3.4 ± 0.4
400						
Pair fed	83 ± 5	90 ± 5	45 ± 3*	44 ± 3	2.6 ± 0.4	4.2 ± 0.4
Control	73 ± 5	102 ± 5	36 ± 3	48 ± 3	2.4 ± 0.4	3.3 ± 0.4
ANOVA						
Diet	$P < 0.01$		$P < 0.01$		$P < 0.05$	
Week	$P < 0.001$		$P < 0.001$		$P < 0.01$	
Diet week	NS		$P < 0.05$		NS	
Covariate†	NS		NS		NS	

Note: Values are mean ± SEM.
* Different from control within each week, $P < 0.05$, as determined by LS Means.
† Initial concentration of total protein, albumin, and gamma globulin, respectively.

Table 5 Immunoglobulins and antibody titer for the magnesium dietary treatments at week 3 and week 8 (n = 4)

Mg diet (μg/g)	IgM (g/L)		IgG (g/L)		Log titer	
	Wk 3	Wk 8	Wk 3	Wk 8	Wk 3	Wk 8
50	0.96 ± 0.08*	0.78 ± 5.7*	6.62 ± 0.84*	5.72 ± 0.87*	7 ± 2	10 ± 2
160	1.30 ± 0.08	1.30 ± 5.7	9.40 ± 0.84†	5.60 ± 0.87†	12 ± 2	20 ± 2
280	1.28 ± 0.08	1.66 ± 5.7	10.86 ± 0.84	11.60 ± 0.87	16 ± 2	20 ± 2
400						
Pair fed	1.30 ± 0.08	1.48 ± 5.7	16.56 ± 0.84	9.83 ± 0.87	11 ± 2	17 ± 2
Control	1.25 ± 0.08	1.46 ± 5.7	12.88 ± 0.84	11.30 ± 0.87	16 ± 2	17 ± 2
ANOVA						
Diet	$P < 0.05$		$P < 0.01$		NS	
Week	NS		$P < 0.05$		NS	
Diet week	NS		NS		NS	
Covariate‡	NS		$P < 0.001$		NS	

Note: Values are mean ± SEM.
* † Different from control within each week, $P < 0.01$ and $P < 0.05$, respectively, as determined by LS Means.
‡ Initial concentration of IgM, IgG, and log titer, respectively.

ever, the difference was not statistically significant. Mean log titers were similar for all other dietary groups.

Discussion

Studies concerning single nutrient deficiencies have focused on the involvement of nutrients in immunity, and most of those projects used severely deficient animals. Only a few studies to date have investigated humoral immunity in marginally deficient animals. The diets in our research were designed to induce various levels of magnesium deficiencies in rats. The results confirm those from other studies of magnesium deficiency.[5,6,18,19,20]

The marginally deficient diets in our study provided sufficient amounts of magnesium for the synthesis of albumin and the globulins. Rats consuming the 50 μg Mg/g diet had significantly reduced plasma proteins. This result was not surprising due to the role of magnesium in protein synthesis.[21,22]

Part of the loss in total plasma protein in the severely deficient rats resulted from a decrease in gamma globulins. IgM and IgG, which comprise most of the gamma globulins, also were decreased. Similar changes were found by other investigators.[7-9] McCoy and Kenney[9] found a 142 μg Mg/g diet provided for normal gamma globulin synthesis and maintained adequate IgM and IgG concentrations. In our experiment, the moderately deficient rats (160 μg/g) had lower IgG levels than control rats, while mildly deficient rats (280 μg/g) were similar to the control rats.

The pair-fed rats had plasma protein levels similar to the ad libitum control rats. The restriction of total calories did not appear to depress protein synthesis. Although pair-fed rats consumed less food than the ad libitum control rats, adequate magnesium status was supported. When the total dietary magnesium intake was calculated as mg magnesium consumed/100 g body weight, both the pair-fed and the ad libitum rats consumed similar levels of magnesium (data not shown).

Based on their equal body weights, the rats fed the 50 μg Mg/g diet had consumed magnesium levels that were only 14% of the levels consumed by rats fed the 400 μg Mg/g diets (data not shown). Because the intake of all other nutrients were equal to that of the pair-fed control rats, the reduction in plasma proteins in the severely deficient rats was associated with the magnesium deficiency and not with a more generalized malnutrition.

Overall, magnesium status appeared to influence IgM and IgG levels, based on the correlation between plasma magnesium and immunoglobulin concentrations, especially early in the experiment. The level of available magnesium seemed to be important for the synthesis of the immunoglobulins.

The severely deficient rats had a low mean specific antibody titer that paralleled the reduction in plasma proteins. But due to the variability of response among the rats, the lower antibody titer was not statistically different from that of the control rats. Other investigators have found lowered specific antibody titer with concomitant reductions in immunoglobulin concentrations in severely magnesium deficient animals.[8,9] Rats studied by McCoy and Kenney[9] had lowered concentrations of gamma globulin in addition to agglutinin and hemolysin activities. The lack of dietary magnesium influenced cellular metabolism in such a way as to depress serum antibody titers.

The rats in our study were immunized once with a sheep red blood cell suspension, initiating a primary immune response. The specific antibody titer appeared to correspond with IgM levels. The severely deficient rats had the lowest IgM concentrations and mean log titer, while the marginally magnesium deficient rats did not differ from the control rats with respect to antibody titer and IgM. Elin[8] found that splenocytes from magnesium deficient mice had a reduced ability to respond to an antigenic stimulus and to synthesize IgM. The process of antigen recognition and processing with subsequent antibody synthesis was determined to be impeded with magnesium deficiency.

Lower mean specific antibody titer in the severely deficient rats could have been due to a failure to synthesize interleukins which are involved in B cell differentiation and the secretion of immunoglobulins. Formation of the antibody protein also could have been depressed with the severe magnesium deficiency.

In this study, the marginally deficient diets provided sufficient amounts of magnesium for antigen recognition and for the production of specific antibody during the primary immune response. Further assessment of the antigenic response including the determination of splenic plaque forming cells would provide more detailed information on humoral immune function. Additionally, the response to repeated antigen challenge may differ with suboptimal dietary levels of magnesium.

Spleen weight in our rats was affected only by the 50 μg Mg/g diet. This was consistent with the significantly enlarged spleens previously described with severe magnesium deficiencies.[8,9,23,24] Zieve et al.[24] found that protein synthesis was depressed in the spleens of magnesium deficient rats, based on their studies on isotope incorporation. The reduction in protein synthesis may explain the impaired immune response observed in the severely deficient animals in this and other studies. During the eight weeks of our study, thymus weights from all rats were not altered. Rats depleted of magnesium up to 12 weeks in the experiment by Alcock et al.[23] had thymuses that were enlarged with cells that resembled transformed lymphocytes.

Early in the experiment, plasma magnesium reflected the amount of magnesium in the diet. Later, rats consuming the marginal levels of dietary magnesium had plasma magnesium levels similar to controls. The high physiological demands for magnesium during growth had diminished toward the end of the experiment when the rats reached the plateau phase of growth. It was believed that by this time the plasma

magnesium pool reached the steady state, thus magnesium homeostasis was maintained. This, in part, might describe why humoral immunocompetence was not affected by suboptimal levels of dietary magnesium.

In summary, the severe dietary deficiency of magnesium, and not the marginal deficiencies, affected humoral immunity by decreasing immunoglobulin concentrations. A severe magnesium deficiency appeared to have its effect on protein synthesis. On the other hand, suboptimal levels of dietary magnesium had little influence on immunoglobulin concentrations, and were sufficient to support immunocompetence when challenged with an antigenic stimulus.

Acknowledgment

We thank Jill McClure for her advice and Susan Pourciau for her technical assistance.

References

1 Beisel, W.R. (1982). Single nutrients and immunity. *Am. J. Clin. Nutr.* **35** (Suppl. 2), 417–468

2 Beisel, W.R., Edelman, R., Nauss, K., and Suskind, R.M. (1981). Single-nutrient effects on immunologic functions. *J. Am. Med. Assoc.* **245,** 53–58

3 Chandra, R.K. and Dayton, D.H. (1982). Trace element regulation of immunity and infection. *Nutr. Res.* **2,** 721–733

4 Nauss, K.M. and Newberne, P.M. (1981). Trace elements and immunocompetence. In *Trace Element Metabolism in Man and Animals* (J.M. Gawthorne, J. McC. Howell, and C.L. White, eds.), pp. 603–610, Springer-Verlag, New York

5 Chandra, S. and Chandra, R.K. (1986). Nutrition, immune response, and outcome. *Prog. Food Nutr. Sci.* **10,** 1–65

6 Galland, L. (1988). Magnesium and immune function: an overview. *Magnesium* **7,** 290–299

7 Alcock, N.W. and Shils, M.E. (1974). Serum immunoglobulin G in the magnesium-depleted rat. *Proc. Soc. Exp. Biol. Med.* **145,** 855–858

8 Elin, R.J. (1975). The effect of magnesium deficiency in mice on serum immunoglobulin concentrations and antibody plaque-forming cells. *Proc. Soc. Exp. Biol. Med.* **148,** 620–624

9 McCoy, J.H. and Kenney, M.A. (1975). Depressed immune response in the magnesium-deficient rat. *J. Nutr.* **105,** 791–797

10 Kubena, K.S., Cohill, D.T., and McMurray, D.N. (1989). Effect of varying levels of magnesium during gestation and lactation on humoral immune response and tissue minerals in rats. *Am. Nutr. Metab.* **33,** 7–14.

11 American Institute of Nutrition (1977). Report of the AIN Ad Hoc Committee on standards for nutritional studies. *J. Nutr.* **107,** 1340–1348

12 Garvey, J.S., Cremer, N.E., and Sussdorf, D.H. (1977). *Methods in Immunology*, pp. 140–143, 209–210, W.A. Benjamin, Inc., Reading, MA

13 Barta, O., Pourciau, S.S., and Hubbert, N.L. (1984). Immunoelectrophoretic techniques. In *Laboratory Techniques of Veterinary Clinical Immunology* (O. Barta, ed.), pp. 123–137, Charles C. Thomas, Springfield, IL.

14 Barta, O. and Pourciau, S.S. (1984). Electrophoresis. In *Laboratory Techniques of Veterinary Clinical Immunology*, (O. Barta, ed.), pp. 116–122, Charles C. Thomas, Springfield, IL

15 Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275

16 Hunt, B.J. (1969). The estimation of magnesium in plasma, muscle and bone, by atomic absorption spectrophotometry. *Clin. Chem.* **15,** 979–996

17 Steel, R.G.D. and Torrie, J.H. (1980). *Principles and Procedures of Statistics*, McGraw-Hill, Inc., New York

18 McCoy, J.H. and Kenney, M.A. (1987). Magnesium and immune function: a review. In *Magnesium in Cellular Processes and Medicine*, (B.M. Altura, J. Durlach, and M.S. Seelig, eds.), pp. 196–211, Karger, Basel, Switzerland

19 Kubena, K.S., Landmann, W.A., and Carpenter, Z.L. (1983). Suboptimal intake of magnesium in rats: effects during growth and gestation. *Nutr. Res.* **3,** 385–394

20 Kubena, K.S., Landmann, W.A., Young, C.R., and Carpenter, Z.L. (1985). Influence of magnesium deficiency and soy protein on magnesium and zinc status in rats. *Nutr. Res.* **5,** 317–328

21 Aikawa, J.K. (1981). *Magnesium: Its Biologic Significance*, CRC Press, Inc., Boca Raton, FL

22 Vernon, W.B. (1988). The role of magnesium in nucleic-acid and protein metabolism. *Magnesium* **7,** 234–248

23 Alcock, N.W., Shils, M.E., Lieberman, P.H., and Erlandson, R.A. (1973). Thymic changes in the magnesium-depleted rat. *Cancer Res.* **33,** 2196–2204

24 Zieve, F.J., Freude, K.A., and Zieve, L. (1977). Effects of magnesium deficiency on protein and nucleic acid synthesis in vivo. *J. Nutr.* **107,** 2178–2188