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The Effect of Magnesium Deficiency in Mice on Serum Immunoglobulin Concentrations and Antibody Plaque-Forming Cells (38596)

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The magnesium ion is an essential cofactor for many biochemical reactions in mammalian metabolism particularly those involving transphosphorylation (1). Magnesium deficiency in experimental animals has been shown to impair several anabolic processes as manifest by diminished growth rate (2–5), decreased weight of the liver and kidneys (6), decreased synthesis of serum albumin (7, 8) and low serum protein concentration (6–8). An effect of magnesium deficiency on immunoglobulin metabolism was suggested by a significant decrease in the serum gamma globulin concentration in magnesium-deficient rats (6). Recently Alcock and Shils demonstrated decreased IgG immunoglobulin concentrations in magnesium-depleted rats (9). The immunologic implications of these observations were evaluated in mice by the plaque-forming cell (PFC) assay and by determination of serum immunoglobulin concentrations.

Materials and Methods. Experimental animal. Male random-bred Swiss white mice 7–8 wk of age (20–25 g) from the National Institutes of Health colony were used. These mice were in the growth phase of development. The mice were individually housed in a suspension cage and provided diet and deionized water *ad libitum*.

Diet. A magnesium-deficient test diet (3.5 mg magnesium/100 g) was purchased from General Biochemicals, Chagrin Falls, OH (10). The control diet consisted of the test diet with 40 mg magnesium/100 g added as magnesium sulfate. The diets were stored at 4° and used within 3 wk of preparation.

Serum collection. The mice were exsanguinated by transection of the axillary artery and vein. The blood of one mouse was collected in an acid-washed test tube, allowed to clot and the serum separated by centrifugation. The serum of each mouse was stored at −20° prior to use.

Magnesium determination. The serum magnesium concentration was determined by atomic absorption spectroscopy (11).

Measurement of immunoglobulin concentration. Quantitative determination of individual immunoglobulin components (IgG_1, IgG_2, IgA and IgM) was performed by a single-radial diffusion method using antibody-agar plates (12, 13). Antisera were raised in goats by repeated immunization with purified mouse myeloma proteins or their Fc fragments in complete Freund's adjuvant (14). The antisera were made specific by appropriate absorption with purified myeloma proteins and germ-free mouse serum.

Hemolytic plaque assay. Mice were immunized by the intravenous injection of 5×10^8 washed sheep red blood cells (SRBC) stored in Alsever's solution. Four days later the number of SRBC direct PFC in the spleens of the immunized mice was determined as previously described (15–18).

Experimental design. After 2 days of consuming either the magnesium deficient or control diet, 12 mice in each group were immunized with SRBC. At 6 days, the spleens of 10 mice in each group were weighed and the number of PFC determined. In the same manner, mice were immunized after 8 days of the respective diets and the PFC determined at 12 days. Mice were weighed at the beginning of the study and just before being sacrificed. In addition, blood was obtained from each mouse when sacrificed, and the serum magnesium concentration was determined.

The serum of 15 mice was collected at the start of the study to provide baseline immunoglobulin and magnesium concentra-

TABLE I. SERUM MAGNESIUM CONCENTRATIONS, EXPERIMENTAL BODY WEIGHT DIFFERENCES AND SERUM IMMUNOGLOBULIN CONCENTRATIONS IN MAGNESIUM-DEFICIENT AND CONTROL MICE.[a]

Time (days)	Diet	Serum magnesium (mg%)	Body weight difference[b] (g)	Serum immunoglobulin concentrations (mg/ml)			
				IgG_1	IgG_2	IgA	IgM
0	(15)[c]	2.09±0.10[a]		2.78±0.19	3.81±0.15	0.55±0.03	0.24±0.01
3	Mg deficient (15)	1.48±0.06	2.06±0.26	2.48±0.19	3.33±0.17	0.62±0.05	0.20±0.01
	Control (15)	2.17±0.18	2.32±0.21	2.76±0.14	3.72±0.29	0.56±0.03	0.24±0.02
	p Value (<)	0.001	0.5	0.3	0.3	0.3	0.1
6	Mg deficient (15)	1.14±0.03	1.08±0.39	2.13±0.15	2.87±0.14	0.37±0.03	0.19±0.01
	Control (14)	2.23±0.12	3.50±0.38	2.72±0.14	3.63±0.17	0.52±0.04	0.24±0.01
	P Value (<)	0.001	0.001	0.01	0.005	0.01	0.005
9	Mg deficient (12)	1.02±0.03	−0.09±0.22	2.11±0.26	2.84±0.12	0.42±0.04	0.16±0.01
	Control (15)	2.13±0.17	4.57±0.30	2.66±0.15	3.87±0.28	0.53±0.05	0.24±0.01
	P Value (<)	0.001	0.001	0.1	0.005	0.2	0.001
12	Mg deficient (11)	1.01±0.03	−1.09±0.26	2.70±0.20	2.92±0.11	0.43±0.03	0.16±0.01
	Control (15)	2.18±0.13	5.65±0.21	2.81±0.16	3.68±0.19	0.57±0.05	0.24±0.02
	P Value (<)	0.001	0.001	0.7	0.005	0.05	0.005

[a] All results are expressed as the mean with SEM.
[b] Body weight of the mouse when sacrificed minus the body weight at the beginning of the study.
[c] The numbers in parentheses represent the number of animals in each group.

tions. The experimental and control groups were each composed of 60 mice (15 animals for each of the four-time periods). If an animal died during the experiment, it was not replaced. After 3, 6, 9 and 12 days on the respective diets, immunoglobulin and magnesium concentrations in the mouse sera were determined.

Statistics. All results are expressed as the mean and standard error of the mean (SEM). The groups were compared by applying Student's *t* test.

Results. The concentration of four serum immunoglobulins decreased in magnesium-deficient mice compared with control animals (Table I). The serum IgG_2 concentration in magnesium-deficient mice diminished significantly ($P < 0.005$) to a plateau value of 79% of the control group by 6 days. A similar pattern was found for the serum IgM concentration except the plateau value (67% of the control value) occurred after 9 days. The serum IgA concentration in magnesium-deficient animals reached a nadir at 6 days (71% of the control value) and then rose slightly at 9 and 12 days. At 6 days the serum IgG_1 concentration in experimental animals showed a significant decrease ($P < 0.01$). However, at 9 days the IgG_1 concentration reached its lowest value which did not differ significantly from the control group ($P < 0.1$) but then rose to 96% of the control concentration at 12 days. The body weight differences between the two groups showed progressive divergence with the magnesium-deficient mice losing weight at 9 and 12 days (Table I). The serum magnesium concentration decreased significantly ($P < 0.001$) by 3 days and continued to decrease during the remainder of the study (Table I). The baseline concentrations of serum immunoglobulins and serum magnesium were similar to those in mice on the control diet over the duration of the study.

Restricted dietary magnesium for 6 and 12 days effected a significant decrease ($P < 0.001$) in the number of PFC/10^6 splenocytes and PFC/spleen compared with animals on a control diet (Table II). A significant decrease ($P < 0.001$) in the number of PFC/10^6 splenocytes and PFC/spleen in magnesium-deficient animals occurred between the sixth day and the twelfth day. The mice consuming the magnesium-deficient diet were unable to gain weight and actually lost an average of 1.79 g of body wt at 12 days while the control diet enabled mice to gain weight (Table II). However, no significant difference between the two groups was found for the splenic weights at 6 and 12 days. Serum magnesium concentrations confirmed the magnesium deficiency of the experimental mice (Table II).

Discussion. After 6 days of dietary magne-

TABLE II. SERUM MAGNESIUM CONCENTRATIONS, EXPERIMENTAL BODY WEIGHT DIFFERENCES, SPLENIC WEIGHTS AND PLAQUE-FORMING CELLS (PFC) IN MAGNESIUM DEFICIENT AND CONTROL MICE.[a]

(days)	Diet	Serum magnesium (mg%)	Body weight difference[b] (g)	Spleen weight (mg)	PFC / 10^6 Splenocytes	PFC/Spleen $\times 10^2$
6	Mg deficient (10)[c]	1.22 ± 0.04	0.31 ± 0.36	96 ± 6	205 ± 18	254 ± 24
	Control (10)	2.18 ± 0.12	2.92 ± 0.32	105 ± 10	537 ± 32	816 ± 43
	P Value (<)	0.001	0.001	0.5	0.001	0.001
12	Mg deficient (10)	0.95 ± 0.03	−1.79 ± 0.49	126 ± 14	66 ± 11	106 ± 18
	Control (10)	2.19 ± 0.14	4.91 ± 0.29	141 ± 21	498 ± 22	1071 ± 48
	P Value (<)	0.001	0.001	0.6	0.001	0.001

[a] All results are expressed as the mean with the SEM.
[b] Body weight of the mouse when sacrificed minus the body weight at the beginning of the study.
[c] The numbers in parentheses represent the number of animals in each group.

sium deficiency, there was a significant decrease in each of the four serum immunoglobulin concentrations (Table I). This observation is supported by the recent study of Alcock and Shils with rats in which a 54% reduction in the serum IgG immunoglobulin concentration occurred after 2 wk of dietary magnesium restriction (9). The changes in immunoglobulin concentrations in the present study are probably not related to large variations in the volume of the intravascular compartment since the IgM immunoglobulin concentration, the only one of the four immunoglobulins limited to the intravascular space, showed a progressive decrease in concentration thereby limiting the possible change in the intravascular space to an expansion (19). However, expansion would be unlikely due to the decreasing body weight differences of the magnesium-deficient animals (Table I). This suggests that the changing immunoglobulin concentrations were not related to expansion of the intravascular space.

After 12 days of a magnesium deficient diet, the serum IgG_1 immunoglobulin concentration rose to almost the control value and the IgG_2 and IgA concentrations were increasing (Table I). A possible explanation of this initial impairment of immunoglobulin biosynthesis (6 days) followed by a partial recovery by day 12 would be protein catabolism after 6 days providing endogenous magnesium which could be reutilized thereby effecting a temporary recovery of anabolic processes. This hypothesis is supported by the negative body weight differences at 9 and 12 days documenting catabolism (Table I). In addition, magnesium-deficient rats with depressed IgG immunoglobulin concentrations were able to increase their IgG concentrations above control values 24 hr after repletion with magnesium (9). This indicates a rapid restoration of anabolic processes with restoration of magnesium and may explain the changes in immunoglobulin concentrations reported above. Additional studies will be needed to define the effect of magnesium deficiency on immunoglobulin concentrations.

The ability of the humoral immune system within the spleen to respond to an antigenic stimulus was progressively impaired by dietary magnesium deficiency in mice. The results, expressed two different ways, were consistent indicating a significant decrease in the total number of PFC per spleen and in the ratio of PFC/10^6 splenocytes in the magnesium-deficient animals (Table II). Since the direct PFC assay employed in this study detected cells that were actively synthesizing 19S or IgM immunoglobulins specific for the sensitizing antigen, these results correlated with a concomitant progressive diminution of serum IgM concentrations in magnesium-deficient mice (Table I). Also, as reported previously in rats (6, 20), this study showed a progressive increase in the weight of the spleen with a concomitant decrease in total body weight of magnesium-deficient mice.

However, in spite of the increasing mass of the spleen, the splenocytes demonstrated a decreasing ability to respond to an antigenic stimulus. Therefore, a deficiency of magnesium somehow impedes the complex process of antigen recognition and processing with subsequent antibody synthesis.

The mechanism of the suppression of the humoral immune system in magnesium deficiency is unknown. The cell mediated immune response has been studied in protein deficient mice (21). The PFC response to SRBC in spleens of control and protein deficient mice 6 days after immunization was essentially the same in both groups. This indicates that the results of the PFC assay in the present study are not related to a possible protein deficiency in the magnesium-deficient animals. However, magnesium deficiency in rats effects a 40–60% decrease in protein biosynthesis in heart, brain, kidney, muscle and liver (22). Magnesium deficiency in growing rats significantly reduces the synthesis of liver proteins and serum albumin (7, 8). In addition, changes in the formation and stability of polysomes have been reported to occur in bacterial cultures maintained in a magnesium-deficient media (23) and smaller size polysomes have been described in the livers of magnesium-deficient guinea pigs (24). These studies indicate that an impairment of protein biosynthesis produced by a deficiency of magnesium is systemic in nature and not confined to the immune system. Therefore, some of the effects of magnesium deficiency on the immune system may be derivative and too complex to be explained on the basis of the data presented.

Summary. Magnesium-deficient mice immunized with SRBC showed a significant decrease ($P < 0.001$) in the number of PFC in their spleens compared with mice on a control diet. Serum immunoglobulin concentrations (IgG_1, IgG_2, IgA and IgM) were determined after 3, 6, 9 and 12 days on the respective diets. The serum IgG_2 and IgM concentrations of magnesium-deficient mice were decreased ($P < 0.005$) by 6 days and remained at these concentrations until 12 days. The serum IgG_1 and IgA concentrations of magnesium deficient animals also decreased ($P < 0.01$) by 6 days but returned toward control concentrations at 12 days. Serum magnesium concentrations confirmed the magnesium deficiency of the experimental animals. Therefore, magnesium deficiency has profound immunosuppressive capabilities in mice by significantly reducing the number of antibody synthesizing cells and serum immunoglobulin concentrations.

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1. Lehninger, A. L., and Wadkins, C. L., Ann. Rev. Biochem. **31,** 47 (1962).
2. Kruse, H. D., Orent, E. R., and McCollum, E. V., J. Biol. Chem. **96,** 512 (1932).
3. Martindale, L., and Heaton, F. W., Biochem. J. **92,** 119 (1964).
4. Schwartz, R., Wang, F. L., and Woodcock, N. A., J. Nutr. **97,** 185 (1969).
5. Elin, R. J., Armstrong, W. D., and Singer, L., Proc. Soc. Exp. Biol. Med. **137,** 635 (1971).
6. Elin, R. J., Armstrong, W. D., and Singer, L., Proc. Soc. Exp. Biol. Med. **134,** 542 (1970).
7. Schwartz, R., Woodcock, N. A., Blakely, J. D., Wang, F. L., and Khairallah, E. A., J. Nutr. **100,** 123 (1970).
8. Lizarralde, G., Jones, J. E., Shane, S. R., and Flink, E. B., Res. Commun. Chem. Pathol. Pharmacol. **1,** 433 (1974).
9. Alcock, N. W., and Shils, M. E., Proc. Soc. Exp. Biol. Med. **145,** 855 (1974).
10. Ko, K. W., Fellers, F. X., and Craig, J. M., Lab. Invest. **11,** 294 (1962).
11. Sunderman, F. W., Jr., and Carroll, E. A., Amer. J. Clin. Pathol. **43,** 302 (1965).
12. Mancini, G., Vaerman, J. P., Carbonara, A. O., and Heremans, J. R., *in* "XI Colloquium on Protides of the Biological Fluids" (H. Peeters, ed.) p. 370. Elsevier Publishing Co., Amsterdam (1964).
13. Fahey, J. L., and McKelvey, E. M., J. Immun. **94,** 84 (1965).
14. Biozzi, G., Asofsky, R., Lieberman, R., Stiffel, C. Mouton, D., and Benacerraf, B., J. Exp. Med. **132,** 752 (1970).
15. Jerne, N. K., and Nordin, A. A., Science **140,** 405 (1963).
16. Hage, J. S., and Cole, L. J., J. Immun. **96,** 559 (1966).
17. Fauci, A. S., and Johnson, J. S., J. Immun. **107,** 1052 (1971).
18. Fauci, A. S., and Johnson, J. S., J. Immun. **106,** 1396 (1971).

19. Waldman, T. A,, and Strober, W., Prog. Allergy **13,** 1 (1969).
20. Alcock, N. W., Shils, M. E., Lieberman, P. H., and Erlandson, R. A., Cancer Res. **33,** 2196 (1973).
21. Gautam, S. C., Aikat, B. K., and Sehgal, S., Indian J. Med. Res. **61,** 78 (1973).
22. Amarlal, S., and Murti, C. R. K., Biochem. J. **128,** 47 (1972).
23. McCarthy, B. J., Biochem. Biophys. Acta **55,** 880 (1962).
24. Grace, N. D., and O'Dell, B. L., Can. J. Biochem. **48,** 21 (1970).

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