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Serum Immunoglobulin G in the Magnesium-Depleted Rat[1] (37910)

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In a previous communication (1), thymic hyperplasia which developed in a significant number of rats depleted of magnesium for 6–12 weeks was described. The hyperplastic glands consisted almost entirely of large cells which morphologically resembled lymphocytes transformed *in vitro* by antigenic stimulation (2). This observation, together with the requirement of magnesium for protein synthesis, raised the question of the relation of magnesium to the immune status in general and to immunoglobulin synthesis and secretion in particular. The present study was carried out to assess the effect of dietary magnesium depletion on plasma or serum immunoglobulin G (IgG) levels in the rat.

Materials and Methods. Animals and diets. Male Sprague–Dawley rats (Sprague–Dawley Farms, Madison, WI), initial mean weight approximately 115 g and aged approximately 35 days, were used in all except one preliminary experiment in which Charles River C.D.[2] rats (Charles River Laboratories, Boston, MA) of the same age and sex were used. The animals were housed in individual wire-mesh cages and fed either a magnesium-deficient diet (Diet A) or a control diet (Diet A + Mg) previously described (1) either ad lib. or by paired feeding as indicated under *Results*. The duration of experiments ranged from 14 to 88 days. Some animals were repleted with magnesium after 60–77 days of depletion with either subcutaneous or intramuscular injections of 20 mg of magnesium (as 5% magnesium sulphate in physiological saline) administered over a period of 4 days in each of three experiments (Expts 1, 2, and 3). In Expt 4, two subcutaneous injections of the solution, each containing 10 mg of magnesium, were given 6 hr apart. From the time of the first injection to the termination of experiments, all of these animals were fed the same amount of Diet A + Mg as was consumed by the animals fed Diet A. At time of sacrifice, blood was collected from the dorsal aorta of ether-anesthetized animals into either heparinized syringes or in the absence of anticoagulant. In Expt 4, blood was also collected from the tip of the tail of unanesthetized animals at the commencement of and at various times during the experiment. The thymus of each animal was examined at autopsy, and in experiments of 6 or more weeks duration the gland was removed and weighed.

Analytical Methods. Plasma or serum IgG was determined by the method of Fahey and McKelvey (3) with the following modifications. The antibody-agar gel was prepared in plastic petri dishes after adding 0.5 ml of either lyophilyzed rabbit antiserum to rat IgG reconstituted in sterile distilled water (Miles Laboratories Inc., Kankakee, IL) or rabbit antiserum to rat IgG (Microbiological Associates, Bethesda, MD) to 4.5 ml of 1% special grade agarose Schwarz/Mann, Orangeburg, NY) in B-1 Beckman barbital buffer, ionic strength 0.025 (Spinco Division, Beckman Instruments, Inc., Palo Alto, CA at 56°. Cylindrical wells were made in the gelled agar with a flat-tipped 13-gauge hypodermic needle. These were quantitatively filled with appropriately diluted serum, plasma, or rat IgG standards using a capillary tube. Rat

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[2] Sprague–Dawley strain, cesarian derived.

IgG (Miles Laboratories, Inc.) was reconstituted with sterile distilled water to contain 1 g% IgG. Plasma, serum, or IgG was diluted with physiological saline so that the IgG concentration of the sample assayed was in the range 62.5–500 mg%. The diffusion dishes were incubated for 20 hr at 4°C in a humid atmosphere. The diameter of the antigen–antibody precipitate ring was measured in unstained plates and in some cases remeasured after staining with triple stain described by Crowle (4). Plasma or serum total protein was determined by the method of Lowry *et al.* (5) and calcium and magnesium by atomic absorption spectrophotometry (6).

Results. Animals fed the magnesium-deficient diet showed classical depletion symptoms of erythema and hyperirritability after 7–10 days. All were hypomagnesemic and had either slightly elevated or normal plasma or serum calcium levels. In two preliminary short-term experiments, rats of two different strains fed the magnesium-deficient diet had lower levels of serum IgG than control animals. The mean serum IgG levels for pairs of deficient Charles River rats sacrificed at 2, 4, or 6 weeks were 450, 320, and 395 mg% compared with 665, 595, and 500 mg%, respectively, for pairs of controls. Five Sprague–Dawley rats fed the deficient diet for 14 days had mean serum IgG levels of 151.6 ± 21.0 (SEM) compared with a level of 280.0 ± 71.8 mg% in 5 control animals. Sprague–Dawley rats fed the deficient diet for 68–88 days in three separate experiments (Expts 1–3) had plasma or serum levels of IgG which were 64, 41, and 48% of the respective control levels (Table I). Animals depleted of magnesium for 60–77 days and then repleted had serum IgG levels 10 days later (Expt 1) or 28 days later (Expts 2 and 3) which were similar to or greater than those of control animals (Table I).

When serial determinations were made in Expt 4, serum IgG in the rats fed the control diet increased throughout the experiment (Fig. 1a). Rats fed the magnesium-deficient diet also showed an increase in mean serum IgG at 14 days followed by a plateau over the next 3 weeks after which a significant decrease in values occurred, in contrast to those of the control rats which continued to rise. Serum IgG after 50 days on the magnesium-deficient diet was 1.7 times the initial value as compared to a rise of 3.5 times for the controls (Fig. 1a). The administration of magnesium (2 × 10 mg) to 7 depleted animals and addition of magnesium to their diet (with restriction of food intake) resulted in an increase to normal of the serum magnesium and an increase in serum IgG to a level above that of controls 24 hr later (Fig. 1a); this was followed by a marked increase in serum

TABLE I. Plasma or Serum IgG in Sprague–Dawley Rats.

Expt. No.	Diet	No. of days	No. of animals	IgG (mg%)	
1	A + Mg	70	4 (P) [a]	556.0 ± 32.7 [b]	$P < .05$
	A	68–80	9 (P)	356.4 ± 79.4	
	A	60	3 (P)	698.7 ± 128.5	
	A + Mg	10			
2	A + Mg	86	4 (S) [c]	559.0 ± 67.3	$P < .025$
	A	86	3 (S)	226.7 ± 54.8	
	A	63–77	4 (S)	472.5 ± 77.2	
	A + Mg	28			
3	A + Mg	88	9 (S)	469.9 ± 118.2	$P < .025$
	A	88	16 (S)	223.9 ± 33.3	
	A	60	33 (S)	522.3 ± 53.7	
	A + Mg	28			

[a] P = plasma.
[b] Mean ± SEM.
[c] S = serum.

IgG throughout the remaining 2 weeks of the experiment.

Serum magnesium between the 14th and 50th days of magnesium depletion showed statistically significant correlation ($P < 0.05$) with serum IgG (Fig. 2). Thymus size in magnesium-depleted rats was not correlated with the level of IgG.

The mean total serum proteins in the magnesium-depleted rats in Expts 1, 2, and 3 were 85.2, 77, and 87% of the control values, respectively. In Expt 4, serum total proteins were slightly depressed at 14–50 days of magnesium depletion (Fig. 1b). Magnesium repletion resulted in a return to normal serum total protein.

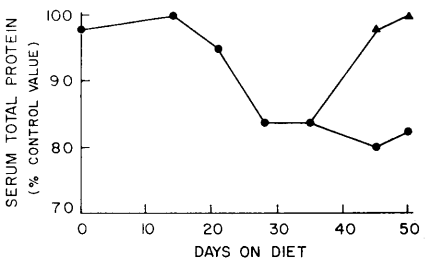

FIG. 1b. Comparison of mean serum total protein levels of rats fed magnesium-deficient Diet A (●—●) and deficient animals repleted on Day 35 (▲—▲) with those of controls taken as 100%. Numbers of animals as given in Fig. 1a.

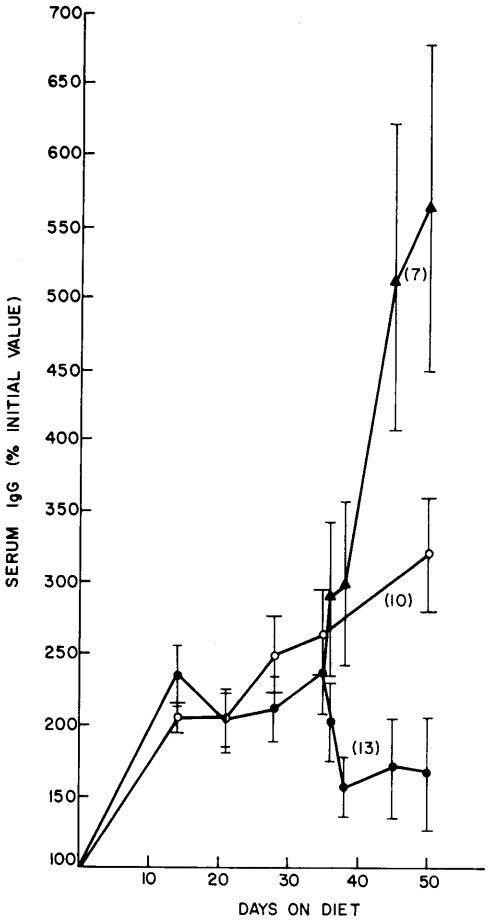

FIG. 1a. Changes in serum IgG as percentage of initial values (mean ± SEM) in a group of rats fed control Diet A + Mg (○—○) or magnesium-deficient Diet A (●—●) throughout the experiment, and in deficient rats repleted with magnesium and given Diet A + Mg beginning on Day 35 (▲—▲). Numbers of animals are given in parentheses.

Discussion. A slight decrease in serum total proteins has previously been reported in magnesium-depleted rats (7). The more marked depression of the serum IgG resulting from the deficiency and the rapid increase following the administration of magnesium suggest a direct role for magnesium either in the synthesis, release, or metabolism of immunoglobulin G. The possibility that the lymphocyte/plasma cell is specially sensitive to magnesium depletion with regard to immunoglobulin synthesis should be considered. Whether this observation holds true for other immunoglobulins remains to be investigated as specific anti-

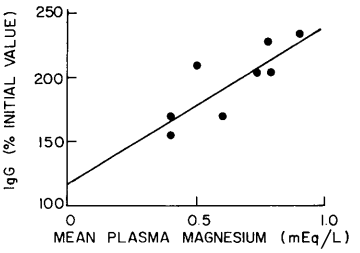

FIG. 2. Correlation between mean serum IgG (as percentage of initial mean value) and mean serum magnesium levels in rats fed the magnesium-deficient Diet A for 14–50 days. Times of sampling and numbers of animals are those shown in Fig. 1a. The correlation coefficient is $r = 0.783$; $P < 0.05$.

sera become available. An association between thymus histology and immunoglobulin synthesis has not been established, but there was no apparent relation between the magnitude of changes in the thymus in some magnesium-depleted rats and depression of their serum IgG levels. The function of both T and B lymphocytes in the magnesium-depleted rats has yet to be assessed.

Summary. Magnesium-depleted rats had decreased levels of serum or plasma IgG. Twenty-four hours after repletion with magnesium and feeding of control diet, serum IgG levels were increased above control values and continued to rise markedly during the following 14 days.

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