

## Design of Parenteral Magnesium Load Tests in Weanling and Young Adult Rats (41042)

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**Abstract.** There is great difficulty in assessing the nutritional status of magnesium (Mg) in young, living mammals. The parenteral Mg load test has been advocated as a useful indicator, but requires further study, first of all, in an animal model. The present study was conducted to (a) design an appropriate Mg load test for Mg-deficient and Mg-sufficient rats in two age groups—*weanling* and *young adults*, and (b) to learn whether or not the test can be effectively conducted by giving the parenteral load of Mg by the *intramuscular (im)* route instead of the *intraperitoneal (ip)* or *intravenous (iv)*. To design the parenteral Mg load test, all animals were fed identical purified diets, except for the addition of 150 mg of magnesium/100 g of diet in the control diets and zero magnesium in the deficient diet which contained only 0.3 mg/100 g. Plasma and urinary Mg values were followed. The final test included: (a) a fasting 6-hr preload urinary collection, (b) an 18-hr refeeding period, (c) intraperitoneal loading with 15 mg of magnesium/kg body wt for weanlings or 12 mg/kg for young adults, and (d) a fasting, 6-hr postload urinary collection. Weanling and young adult rats fed Mg-sufficient diets excreted substantial amounts of Mg pre- and postload, retaining less than a mean of 25% of the load; rats fed the Mg-poor diet excreted little Mg preload, retaining a mean of at least 85% of the load. We compared muscle Mg values from the Mg-injected anterior thigh muscle versus the contralateral noninjected muscle taken from the same animals at the end of the 6-hr load test and found no difference; apparently, Mg was completely absorbed from the muscle, even in very sick weanling rats. Mg load test results using im and ip loading showed that the two routes of Mg loading gave essentially the same Mg load test data.

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There is a serious lack of reliable diagnostic tests to assess the nutritional status of magnesium (Mg) of the human patient in a clinical setting. The shortcomings of available diagnostic tests have been reviewed (1-8). The parenteral Mg load test has been suggested as a potential useful diagnostic tool (2-6). It is reasoned (2) that estimates of retention of parenterally administered Mg can be employed to measure Mg depletion, based on the following observations that have been made in human subjects: (a) parenteral administration of Mg does not significantly alter the gastrointestinal excretion of Mg (9, 10); (b) normal individuals excrete a parenteral load of Mg almost quantitatively by way of the kidney (5, 11, 12); and (c) Mg-deficient subjects retain Mg.

To learn more about the parenteral magnesium load test, it must first be studied in animal models. We could find no animal model for this test, and, therefore, under-

took the present investigation to design an appropriate test for weanling and young adult rats. There is a question regarding the appropriateness of the intramuscular (im) route, particularly for very ill, young infants; this route has been used in testing such human patients (6, 12, 13). We, therefore, determined whether or not a parenteral load is completely absorbed from the injected muscle, even in weanling rats experiencing shock-like episodes. Finally, we compared results of im and intraperitoneal (ip) loading in weanling rats; the ip route provides the same information as the intravenous (iv) route in rats (14).

**Methods.** Male Sprague-Dawley rats purchased from Hilltop Lab Animals, Scottsdale, Pennsylvania, were studied. On the day of arrival, Day 0, weanling rats weighed 35 to 40 g and young adult rats weighed 125-145 g. Groups of 6 to 10 rats were matched for weight and were immediately fed one of two study diets and

deionized distilled water *ad libitum*. The basal diet (Table I) contained 0.3 mg of magnesium/100 g with 150 mg of added magnesium/100 g (control diet) or zero added magnesium (deficient diet). Animals were individually housed in raised-bottom stainless-steel cages in an air-conditioned room under 12-hr illumination.

*Experiment 1. The design of the parenteral Mg load test for weanling rats.* The Mg load test was designed by monitoring plasma Mg and renal retention of graded ip loads of Mg, taking as a starting point a dose employed in human infants, 6 mg of magnesium/kg body wt (0.5 meq of magnesium/kg body wt) (6, 12, 13). The overall objective was to find a loading dose of Mg that was essentially rejected by control, Mg-fed animals, and retained by Mg-deficient animals—and that did not unduly elevate the plasma Mg concentration. Collections of urine were continued for time enough to complete clearance of the load from the control plasma; 6 hr sufficed. Because the powdered diet invariably contaminated the samples of urine, the feet of

animals were rinsed in deionized water before they entered the metabolic cages, and no food was placed in the cages during the collection periods; only deionized water was provided. Therefore, pre- and postload collections were made on separate days, with an intervening recovery period for re-feeding the assigned diet. To avoid diurnal variation (1), the pre- and postload collections were made during the same hours of the day, between 9 AM and 3 PM. At 9 AM on Day 6, weanling rats were placed in stainless-steel cages that had been deionized with a 1% solution of disodium ethylenediaminetetraacetic acid (EDTA), followed by rinses of deionized water. A layer of stainless-steel wire mesh prevented fecal pellets from entering the urinary receptacle. Six hours later, the rats were returned to their assigned cages and diets, the metabolic cage floor was rinsed with a jet of deionized distilled water, and the washings were added to the urinary collection. At 9 AM the following morning, the ip load was given, the postload 6-hr urinary collection was then made, and the rats were finally anesthetized with sodium pentobarbital and bled by percutaneous cardiac puncture. (Every rat in this study that provided a blood sample was killed after bleeding; a rat could be studied once.) Minerals were analyzed using an atomic absorption spectrophotometer (Perkin-Elmer Model 303). The percentage of the magnesium load retained was calculated from the data from the preload base period, and the next excretion after the load; (for the method please see Table II).

When an appropriate loading dose was found for weanling rats, six rats from each of the two dietary groups were studied.

*Experiment 2. Mg load testing in young adult rats.* Compared with the weanling rats, these more mature rats, presumably with larger reserves of Mg, required a longer period of dietary treatment to achieve severe Mg deficiency. Preload urinary collections were made on Day 13; on the following day, the load was given and the postload urinary collection was made. The test was then conducted as described for Experiment 1, using the Mg load found appropriate for these older rats.

TABLE I. COMPOSITION OF THE BASAL DIET

	g/100 g diet
Casein (vitamin free) <sup>a</sup>	20.0
Glucose monohydrate <sup>b</sup>	68.24
Corn oil <sup>c</sup>	5.5
Salts (Mg omitted) <sup>d</sup>	4.26
Choline chloride <sup>e</sup>	0.30
L-Cystine <sup>e</sup>	0.20
Water-soluble vitamin mix <sup>f</sup>	1.00
Fat-soluble vitamin mix <sup>g</sup>	0.50

<sup>a</sup> Mogul Corp., Madison, Wis.

<sup>b</sup> Cerelose, Clinton Corn Processing Co., Clinton, Iowa.

<sup>c</sup> Mazola, Best Foods, Engelwood Cliffs, N.J.

<sup>d</sup> Provided (g/100 g diet): Mg, 0.003; Ca, 0.528; P, 0.455; Na, 0.17; K, 0.49; Cl, 0.13. In ppm: Mn, 38.7; Cu, 5.7; Al, 0.20; Co, 1.6; Zn, 100; F, 0.016; I, 1.34; and Fe, 83.2.

<sup>e</sup> Sigma Chemical Co., St. Louis, Mo.

<sup>f</sup> In glucose, provided (mg/100 g diet): thiamin-HCl, 2.5; riboflavin, 1.0; nicotinamide, 5.0; pyridoxine-HCl, 2.2; Ca pantothenate, 7.0; folic acid, 0.4; biotin, 0.10; PABA, 2.5; inositol, 25.0, and B<sub>12</sub>, 0.005.

<sup>g</sup> In corn oil, provided (per 100 g diet): Retinyl palmitate, 1000 IU; vitamin D<sub>2</sub>, ergocalciferol, 2000 IU;  $\alpha$ -tocopheryl acetate, 10 mg; and 3-phytylmenadione (Vitamin K<sub>1</sub>), 0.1 mg.

**Experiment 3. Assessing the adequacy of im Mg load testing.** Mg loads were given im or ip in groups of weanling rats fed the control and deficient diets, and Mg load tests were conducted as in Experiment 1. In rats assigned im loading, the load was given in the right anterior thigh muscle group, and at the end of the postload collection period, the rats were sedated, bled, and both the right and left anterior thigh muscle groups were removed. The muscle was dried to constant weight, digested in a mixture of two parts of 30% hydrogen peroxide and one part of 70% perchloric acid (Fisher Scientific Co.), and aliquots were diluted in 0.1% lanthanum chloride and analyzed for Mg. The concentration of Mg from the injected muscle was compared with that of the contralateral, noninjected muscle.

**Statistical analyses.** Appropriate statistical comparisons were made using two-tailed Student *t* tests and the analysis of variance (15, 16). Data were expressed as mean  $\pm$  SEM, with the number in the sample in brackets and indicated by *N*. Load tests were discarded when a sample of urine was apparently contaminated, as evidenced by urinary magnesium values that exceeded the mean value for that group by more than 2 SD.

**Results. Experiment 1. The design of the Mg load test in weanling rats.** Loading doses of 6 Mg of magnesium/kg of body wt ( $N = 2$  rats) and 7.5 mg/kg ( $N = 2$ ) were inadequate in these growing, Mg-fed rats.

Retention values ranged from 63 to 100% of the load ( $86.8 \pm 7.0\%$ ). A load of 18 mg/kg was excessive; 15 min after an ip injection of this load, the plasma Mg concentration was 4.6 mg/100 ml, more than twice the control level. The most appropriate dose found for weanling rats was 15 mg of magnesium/kg body wt (Fig. 1A); results of testing at that dose are shown in Fig. 2 and Table II. The upper panel in Fig. 2 provides a schematic representation of the Mg load test, the middle panel shows time-related plasma Mg values, and the lower panel shows time-related body weights. In our laboratory, normal weanling and young adult rats usually show plasma Mg levels of 1.6 to 2.4 mg/100 ml. The plasma levels of control rats in this study were elevated 5

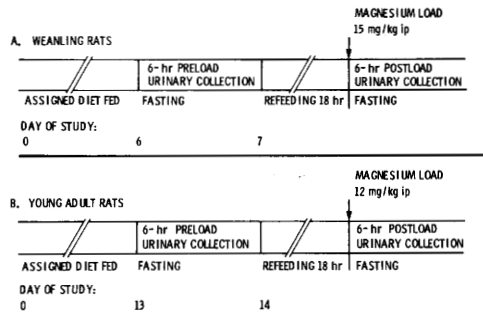


FIG. 1. Overall plan of the investigation from the day of admission, Day 0, to the end of the parenteral magnesium (Mg) load test experiment. (A) Weanling rats were studied between Days 6 and 7 of dietary treatment, and (B) young adult rats were studied between Days 13 and 14. Note that the magnesium load is 25% higher in the weanling rats, but that other features of the test are the same.

min post ip loading, but rapidly normalized, and the pre- and 6-hr postload plasma values did not differ. The plasma levels of deficient rats were about one-fifth of the control values, increased following the load, and showed a small but consistent increase 6-hr postload ( $P < 0.001$ ). Table II shows the experimental data from which the percentage retention was calculated, and indicates the method of calculation. The control animals excreted about 45 times more Mg than the deficient rats during the pre-load period ( $P < 0.001$ ), and essentially cleared the load, retaining only 15%. The rats fed the Mg-poor diet conserved Mg postload as well as preload, retaining about 90% of the load.

**Experiment 2. The design of the Mg load test in young adult rats.** Pilot studies conducted in 3 to 5 Mg-fed controls per group showed that rats loaded with 6 mg of magnesium/kg body wt or with 7.5 mg of magnesium/kg retained 45.5 to 54.5% of the load. At loads of 15 mg of magnesium/kg body wt, the mean plasma level 15-min postload was elevated, 5.1 mg of magnesium/100 ml. At 12 mg of magnesium/kg body wt (Fig. 1B), controls retained only  $21.3 \pm 6.6\%$  of the load, and control plasma Mg levels were only moderately elevated 15-min postload (upper panel, Fig. 3A). Therefore, this last dose was used for testing Mg-sufficient (A) and -deficient (B)

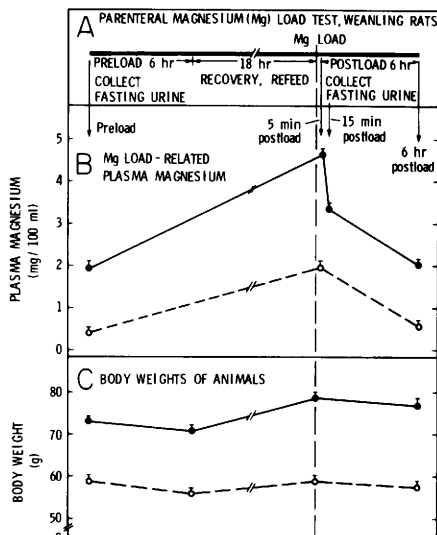


FIG. 2. Schematic representation of the parenteral Mg load test in weanling rats, showing serial changes in plasma Mg and body weights during the Mg load test period. The upper panel (A) provides a time frame. The middle panel (B) shows plasma Mg values (mean  $\pm$  SEM) in three to five control (○—○) and three to five Mg-deficient (○---○) rats studied preload and 5 min, 15 min, and 6 hr after the ip loading of 15 mg of magnesium/kg body wt. (C) shows the mean  $\pm$  SEM of body weights of 10 rats in each dietary group weighed before and after each 6-hr urinary collection period.

young adult rats. In each set, the first column shows the preload urinary excretion of Mg; the second column, the injected Mg load, and the third column, the postload urinary excretion of Mg. Compared

with the deficient rats, the controls excreted over 30 times more Mg preload, and they excreted most of the load, retaining 20% ( $N = 5$ ). The deficient rats conserved Mg, retaining 85% ( $N = 6$ ). No difference was found in plasma Mg values studied preload and 6-hr postload.

**Muscle Mg values.** The im Mg load was apparently completely absorbed from muscle of weanling rats in 6 hr; no difference was found on comparing the Mg concentration of im injected muscle and the contralateral noninjected muscle. The values in micrograms of magnesium per gram of dry weight, given as the mean  $\pm$  SEM for the Mg-sufficient rats, were  $1121 \pm 16$  (injected) and  $1160 \pm 18$  (noninjected). Corresponding values for the Mg-deficient rats were  $1082 \pm 37$  and  $1076 \pm 58$ . ( $N = 5$  or 6 for each group.)

**Parenteral Mg loading in weanling rats: im versus ip.** No significant difference was found in Mg retention values obtained by im or ip loading. The mean value for sufficient rats tested im was  $15.2 \pm 4.3\%$  ( $N = 6$ ) and ip,  $5.5 \pm 3.3\%$  ( $N = 5$ ). The im loaded deficient rats retained  $86.7 \pm 3.3\%$  ( $N = 5$ ), and the ip loaded rats retained  $90.7 \pm 2.8\%$  ( $N = 5$ ).

**Discussion.** The inadequacies of plasma and soft tissue Mg as diagnostic tissues are well known (1-8), but in numerous studies, including this one, the urinary Mg concentration accurately reflects the dietary Mg. However, urinary Mg may be a misleading

TABLE II. PARENTERAL Mg LOAD TEST DATA IN WEANLING RATS

	Preload	Postload	Net excretion <sup>a</sup>	Load	Net retention <sup>b</sup>	Retention <sup>c</sup>
	( $\mu\text{g}$ magnesium excreted/6 hr)		( $\mu\text{g}$ magnesium)			(%)
Mg sufficient ( $N = 10$ ) <sup>d</sup>	$618.5 \pm 107^e$	$1701 \pm 135$	$1083 \pm 100$	$1164 \pm 15^f$	$177 \pm 64^g$	$15.4 \pm 5.6^h$
Mg deficient ( $N = 10$ )	$13.8 \pm 3.1$	$87.8 \pm 14.9$	$76.2 \pm 14.0$	$873 \pm 11$	$797 \pm 15$	$91.3 \pm 1.5$

<sup>a</sup> Postload minus preload 6 hr urinary Mg.

<sup>b</sup> Load minus net excretion.

<sup>c</sup> Net retention divided by the load.

<sup>d</sup> Number in sample.

<sup>e</sup> Mean  $\pm$  SEM.

<sup>f</sup> Loading dose was 15 mg of magnesium/kg body wt, given ip.

<sup>g</sup> Minus values were assigned the number 0; therefore, arithmetic reflects this.

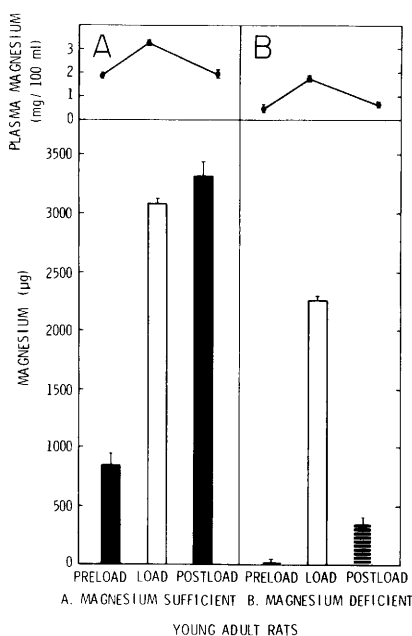


FIG. 3. Parenteral Mg load test results in young adult rats studied as shown in Fig. 1B, using an ip load of 12 mg of magnesium/kg body wt. Upper: (A) Mean plasma Mg concentration from four to six Mg-sufficient rats studied preload, 15-min postload, and 6-hr postload, and (B) from four to six Mg-deficient rats studied at the same times. Lower: (A) The three bars represent the mean  $\pm$  SEM values for the total Mg content of 6-hr base or preload urinary collections (solid bar) of the Mg load (hollow bar) and of the 6-hr postload urinary collections (solid bar). Five controls excreted a considerable amount of Mg during the base and cleared most of a parenteral load, retaining  $19.6 \pm 5.5\%$  (B) Six deficient animals showed avid renal conservation of Mg, excreting relatively little Mg during the base and postload period (striped bars). The mean retention of the load was  $85.6 \pm 2.6\%$  SEM.

diagnostic index. Even very well nourished subjects will show greatly reduced urinary Mg values after a few days of dietary Mg restriction (7, 18). Paunier *et al.* (19) reported a wide range of urinary Mg values in well nourished children and could find no direct relationship between dietary Mg and urinary Mg excretion, even after oral Mg supplementation. Many factors may account for a variable urinary excretion of Mg. In studies of intestinal absorption of radioactive Mg (20, 21) absorption values of 12 (21) and 85% (20) were reported; presumably these differences would be reflected in a wide range of urinary Mg values.

Aikawa (21) linked uniformly poor gastrointestinal absorption of Mg to uniformly low urinary excretion of Mg. Other mechanisms or factors influencing renal clearance of Mg include a series of drugs, foods, and metabolites, exercise, and diurnal variation (1, 3, 22, 23). The influences of such variations on this test can be essentially cancelled if the pre- and postload collections are made for the same length of time during the same time of day and under the same clinical conditions.

In designing this test, the dose employed was the one providing the most reasonable plasma Mg and Mg load retention data for control and deficient rats of the same age. A suitable loading dose for weanling rats was higher than the one used for older animals. In humans, larger doses in young children may be required to accommodate the more rapid growth and higher basal metabolic rate of early childhood compared with corresponding findings in older subjects (25). A current pediatric dose for parenteral Mg loading, 6 mg of magnesium/kg body wt (0.5 meq/kg) (6, 12, 13) is larger than a suggested adult dose, about 4 mg/kg body wt (20 to 24 meq of magnesium total dose) (5, 26, 27).

The rats in this study were fed diets almost devoid of Mg or providing an abundance; retention values reflected these dietary extremes. Further studies will be required to more exactly define and explore this test. In studies in man, several investigators have suggested that 40% or more of the above current doses is retained in Mg deficiency (5, 6, 12, 13, 26, 27). Wilkinson (6) warned that rapid infusion of an iv Mg load is followed by prompt renal excretion of Mg, even when there is severe depletion of the ion. Therefore, we reduced doses that resulted in high plasma levels. It would be helpful to know the renal threshold for Mg, but it is not precisely known. It may be close to the lower limit of the normal blood level in some animals (3). Massry *et al.* described a dynamic system: When Mg salts were infused alone in dogs, Mg reabsorption increased two- or threefold as the filtered load of Mg rose to  $280 \mu\text{g}/\text{min}/\text{kg}$  body wt (23).

Only plasma values from control rats were followed to assess clearance of the Mg

load; values from deficient rats were unreliable. Tremulous behavior and seizure activity increases the plasma concentration of Mg in Mg deficiency (17), and there is probably some normalization of severely depressed plasma Mg values after Mg loading.

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