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Magnesium Metabolism in the Hyperammonemic Rat (37874)

R. L. PRIOR,[1] A. ZIMBER,[2] AND W. J. VISEK
Department of Animal Science, Cornell University, Ithaca, New York 14850

Several reports prompted us to consider the role of magnesium in the response of animals to ammonia (i.e., $NH_3 + NH_4^+$).[3] Head and Rook (1) suggested that hypomagnesemia of grazing ruminants may result from inadequate intestinal absorption of magnesium associated with high ruminal ammonia concentrations. Smith and McAllan (2) concluded that abnormally high concentrations of ammonia in the ileum could reduce availability of Mg if the Ca^{2+} concentrations were very low. Kemp (3) suggested that high concentrations of N in forage decreased Mg in the blood and increased the likelihood of tetany. Hypomagnesemia has been reported in patients with liver cirrhosis (4) and a close association between low serum magnesium and deleterious effects of nitrogenous compounds in patients with liver cirrhosis has been suggested (5). Magnesium ammonium phosphate hexahydrate occurs in human and rat urinary calculi (6, 7). The formation of such a complex in plasma during acute ammonia intoxication has been suggested (8).

Increases in plasma citrate (9) and liver citrate (10) have been observed during terminal stages of acute ammonia intoxication. Based upon a stability constant of 2.8×10^{-4} (11) for the chelate of Mg^{2+} and citrate, calculations have predicted a 1.8–3.2-fold increase of the chelate in liver during hyperammonemia. Present evidence does not establish if the calculated increase in complexed Mg^{2+} can explain the metabolic and neurologic disturbances observed during hyperammonemia. The uncertainties of magnesium action are amplified because it is not known whether the ionic or bound forms of magnesium or both are biologically active (12).

Altered carbohydrate metabolism during magnesium deficiency seemed a very likely possibility because several enzymes of glycolysis and the Krebs cycle are Mg dependent. Hyperglycemia is a consistent finding in animals severely intoxicated with ammonia (9, 13, 14). Elevated serum urea and ammonia have been observed in cattle grazing pastures that often lead to hypomagnesemia (15). One such animal studied by Bartlett and coworkers exhibited convulsions and hyperglycemia (15).

Reported herein are comparisons of Mg metabolism during acute hyperammonemia induced by injection of crystalline jackbean urease (16) in groups of rats fed diets adequate or deficient in magnesium.

Materials and Methods. Three experiments were conducted with male Sprague–Dawley rats. Jackbean urease (urea amidohydrolase EC 3.5.1.5, sp act approximately 100,000 U/g, 1 unit is the quantity of enzyme activity that liberates 1 mg of NH_3–N from a urea phosphate buffer in 5 min at 20° at pH 7.0) was extracted from finely ground jackbean meal and initially crystallized according to the method of Sumner (17). Recrystallizations were

[1] U.S.D.A., A.R.S., U.S. Meat Animal Research Center, Clay Center, Nebraska 68933.

[2] Department of Anatomy and Physiology, P.O.B. 12, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel.

[3] Chow, K. W., and J. K. Loosli. Complexing of essential ions by ammonia. J. Ani. Sci. 31, 219 (Abstract) 1970.

according to Dounce (18). The process of dilution for injection and the immunologic characteristics of the crystalline enzyme prepared according to this procedure have been described (19). The advantages of urease injection as a means of producing ammonia intoxication have been discussed recently (20). Injections of 0.15 *M* NaCl or active urease were made in volumes of 1 ml/100 g body wt.

For Expt 1, 325–425 g rats were injected intraperitoneally with 0.15 *M* NaCl (controls) or 55 U of urease activity/kg body wt. These animals were used for determinations of plasma Ca, Mg, PO_4^{3-}, and NH_3–N. Rats (205–225 g) of Expt 2 were injected with saline or urease as in Expt 1 and 45 min later were given an ip injection of 3 μCi of ^{28}Mg (Brookhaven National Laboratory, Upton, NY). Animals of Expt 2 were placed in metabolism cages immediately after injection. They were killed in pairs (control and urease) at 85–175 min after ^{28}Mg injection. Urine was aspirated from the bladder and added to the excreted urine at the time of decapitation. The times for killing of the animals were determined by the signs of hyperammonemia. The toxic signs are described in *Results*. Samples of liver, kidney, brain, heart, colon, biceps femoris plus quadriceps femoris, and tibia were collected.

For Expt 3, male rats (260–280 g) were injected intraperitoneally with 0.15 *M* NaCl or crystalline urease (1 U/animal) at 8:00 AM and this was repeated 4 hr later. Ninety minutes after the second injection, 5.443×10^6 cpm of ^{28}Mg were injected into each animal via the tail vein. Blood samples were drawn from the tail vein at approximately 30-min intervals during the next several hours. The animals were killed between 9:00 and 10:00 AM on the following day and tissues were collected as in Expt 2.

Liver slices from animals in Expts 2 and 3 were incubated in a modified Ca^{2+}-free Krebs–Ringer phosphate buffer (pH 7.4) with and without Mg. The rate of release of ^{28}Mg was determined by counting 1-ml aliquots of the supernatant at 5 min, 1 hr, 2 hr, 3 hr, and 4 hr. At the end of the incubation and centrifugation at 12,000 rpm for 15 min, the tissue pellet was digested in 3 ml of concentrated HNO_3 and an aliquot was assayed for radioactivity.

Liver, kidney, and brain tissue from rats of Expts 2 and 3 were homogenized in glass distilled H_2O (1 g + 4 ml) using a glass homogenizer with Teflon pestle. One 0.5-ml aliquot of the homogenate was digested with 0.5 ml 16 *N* HNO_3 for Mg determination and another was assayed for ^{28}Mg. Heart, colon, and muscle samples were weighed and immersed in 2 ml 16 *N* HNO_3. After complete digestion, aliquots were assayed for ^{28}Mg and Mg. Radioactivity was assayed in a well-type scintillation spectrometer employing appropriate standards (Model 4218, Nuclear Chicago Corp., Des Plaines, IL).

The basal diet fed in Expt 4 contained in g/100 g: casein, 25.0; dextrose, 62.7; corn oil, 5.0; D,L-methionine, 0.3; vitamin mixture, 1.0; and mineral mixture, 6.0. Vitamins in mg/100 g of diet were supplied as follows: *p*-aminobenzoic acid, 10.0; vitamin B_{12} (0.1% in mannitol), 3.0; biotin, 0.04; Ca–Pantothenate, 4.0; choline, free base, 200.0; folic acid, 0.20; inositol, 10.0; menadione, 0.50; niacin, 4.0; pyridoxine HCl, 4.0; riboflavin, 0.80; thiamine-HCl, 0.50; (in U/100 g) vitamin A, 2,000; vitamin B_2, 200; vitamin E acetate, 10; and dextrose to make 1000 mg/100 g diet. The mineral mixture supplied the following in mg/100 g diet: $CaCO_3$, 1997; $Ca(H_2PO_4)_2 \cdot H_2O$, 499; $CaSO_4 \cdot 5H_2O$, 210; $Fe(C_6H_5O_7) \cdot 5H_2O$, 186; $MnSO_4 \cdot H_2O$, 26.6; KI, 5.3; KH_2PO_4, 2163; NaCl, 1118; $ZnCO_3$, 1.7. Following analysis, $MgSO_4 \cdot 7H_2O$ was added to adjust the Mg content at 6 and 50 mg/100 g for the Mg-deficient and control diets, respectively. Male rats (60–70 g) were randomly assigned to one of the two diets. After 1 week when rats fed the deficient diet began to show hyperemia of the extremities, 3 Mg-depleted and 3 control animals were given an ip injection of crystalline jackbean urease as in Expt 1. Three additional animals of each group received an equal volume of 0.15 *M* NaCl intra-

TABLE I. Ammonia, Ca, Mg, and PO_4^{-3} in Plasma of Hyperammonemic Rats (Expt 1).[a]

	Saline	Urease
Ca (plasma)	9.78 ± 0.17	10.15 ± 0.77
Mg (plasma)[b]	2.86 ± 0.07	3.33 ± 0.26
PO_4^{-3} (plasma)[b]	8.71 ± 0.31	4.91 ± 0.72

[a] Ca, Mg, and PO_4^{-3} concentrations as mg/100 ml. NH_3–N concentrations as μg/ml. Means ± SEM of 5 animals per treatment. Hyperammonemia was produced by the injection of 55 U urease/kg body wt 3 hr prior to killing the animals.

[b] Treatment means differ ($P < 0.05$).

peritoneally. After 3 hr, they were decapitated and serum was collected for Mg and organic acid analysis.

Calcium and magnesium were determined in the presence of lanthanum chloride by atomic absorption spectrometry. Phosphate was determined by the method of Fiske and Subbarow (21) except that $FeSO_4$ was used to reduce the phosphomolybdic acid. Organic acids were determined by partition column chromatography and indicator titration (22, 23). Data were analyzed by analysis of variance as outlined by Steele and Torrie (24). Pooled standard errors of treatment means (SEM) (Tables II, III, and VI) were calculated as the square root of the error mean square divided by 3.7 for Expt 2 or by 3 in Expt 4. A divisor of 3.7 was used in Expt 2 because of a different number of animals in each treatment group.

Results. Plasma Mg tended to increase in rats receiving urease injections (statistically significant in one experiment) (Tables I and II). Phosphate fell to about 50% of the average observed in control animals (Tables I and II). Total plasma Ca did not show significant differences between hyperammonemic animals and controls (Table I).

In Expt 2, the urease-injected animals were classified into two groups based upon clinical signs of toxicity at time of killing. Group A (U-A) showed mild signs of hyperammonemia and 2 of the 3 rats exhibited periodically a myoclonic spasm involving all the muscles of the body. The myoclonus could be stimulated by sound such as rapping on the cage or by physical contact with the rat. In contrast, animals in Group B (U-B) (Tables II and III) were semicomatose and paralyzed, and 2 of the 3 exhibited grand mal-type convulsions seconds before decapitation. The U-B animals showed signs of intoxication earlier and were killed earlier than the U-A animals. This introduced factors into interpretation of the data on ^{28}Mg metabolism which are considered in the *Discussion.*

TABLE II. Plasma Blood and Urine Metabolites in Rats Given Injections of Saline or Crystalline Jackbean Urease (Expt 2).[a]

	Saline	Urease		
		A	B	SEM[c]
Blood				
^{28}Mg (cpm/ml)	4230[c]	3696[b]	5344[d]	154
Plasma				
^{28}Mg (cpm/ml)	3642[b]	2798[b]	5386[c]	331
Mg (mg/100 ml)	2.88	3.03	3.30	0.12
PO_4^{-3} (μmoles/ml)	3.39[b]	3.45[b]	1.85[c]	0.09
Urine				
PO_4^{-3} (μmoles/135 min)	28.9	34.0	20.0	14.0
^{28}Mg (% dose/135 min)	11.3	11.9	11.9	0.9
Volume (ml/135 min)	1.05	1.28	1.83	0.24

[a] Rats were injected intraperitoneally with saline or urease (55 U/kg) and 45 min later 3 μCi of ^{28}Mg were injected intraperitoneally. The rats were sacrificed approximately 135 min later. Means of 5 control animals and 3 in each of the urease-treated groups.

[b–d] Treatment means with different superscripts differ ($P < 0.05$).

[e] Pooled standard error of means.

TABLE III. Magnesium in Tissues of Rats Given an Injection of Saline or Crystalline Jackbean Urease and ^{28}Mg (Expt 2).[a]

	Saline	Urease		Statistical significance
		A	B	
Liver				
^{28}Mg (cpm/mg Mg × 10^{-3})	62.2 ± 7.5	58.2 ± 3.2	67.8 ± 2.1	N.S.
(% dose/organ)	8.1 ± 0.8	7.8 ± 0.1	9.5 ± 0.4	N.S.
Mg (mg/100 g tissue)	38.2 ± 1.3	39.0 ± 1.5	41.7 ± 0.9	N.S.
Kidney				
^{28}Mg (cpm/mg Mg × 10^{-3})	101.9 ± 2.1[b]	92.7 ± 3.6[b]	116.1 ± 3.8[c]	$P < 0.01$
(% dose/organ)	2.5 ± 0.1	2.3 ± 0.0	2.9 ± 0.1	N.S.
Mg (mg/100 g tissue)	39.3 ± 1.2[b]	38.8 ± 0.2[b]	33.7 ± 1.1[c]	$P < 0.01$
Brain				
^{28}Mg (cpm/mg Mg × 10^{-3})	12.3 ± 0.6	13.4 ± 0.4	11.1 ± 0.6	N.S.
(% dose/organ)	0.20 ± 0.01	0.21 ± 0.01	0.19 ± 0.00	N.S.
Mg (mg/100 g tissue)	27.2 ± 1.2	26.6 ± 0.6	27.9 ± 1.0	N.S.
Bone				
^{28}Mg (cpm/g tissue × 10^{-3})	83.7 ± 0.7[b]	69.1 ± 5.4[c]	89.3 ± 1.1[b]	$P < 0.01$
(% dose/organ)	3.2 ± 0.03[b]	2.6 ± 0.20[c]	3.4 ± 0.04[b]	$P < 0.01$
Heart				
^{28}Mg (cpm/mg Mg × 10^{-3})	112.6 ± 4.5	117.9 ± 4.3	118.8 ± 6.2	N.S.
(% dose/organ)	0.92 ± 0.02	0.96 ± 0.07	0.98 ± 0.10	N.S.
Mg (mg/100 g tissue)	31.6 ± 0.7	29.8 ± 1.3	30.6 ± 1.2	N.S.
Colon				
^{28}Mg (cpm/mg Mg × 10^{-3})	62.2 ± 4.8	59.9 ± 5.3	72.8 ± 6.4	N.S.
(% dose/organ)	1.13 ± 0.08	1.02 ± 0.05	1.09 ± 0.02	N.S.
Mg (mg/100 g tissue)	32.9 ± 1.9	36.4 ± 1.8	28.4 ± 0.12	N.S.
Muscle				
^{28}Mg (cpm/mg Mg × 10^{-3})	15.0 ± 0.7	17.0 ± 1.4	13.5 ± 0.4	N.S.
(% dose/g tissue)	0.22 ± 0.01	0.25 ± 0.03	0.20 ± 0.00	N.S.
Mg (mg/100 g tissue)	38.3 ± 1.0	38.1 ± 0.4	37.8 ± 1.3	N.S.

[a] Rats were injected intraperitoneally with saline or urease (55 U/kg) and 45 min later 3 μCi of ^{28}Mg were injected intraperitoneally and the rats sacrificed approximately 135 min later.

[b,c] Treatment means with different superscripts differ significantly ($P < 0.01$).

Blood and plasma ^{28}Mg were significantly increased in the U-B animals compared to U-A or control animals. There was an inverse relationship between the percent of injected ^{28}Mg remaining in the total blood and the percentage in the urine (Table II). The regression equation of the percent of the injected ^{28}Mg remaining in the total calculated blood volume (25) on the percent appearing in the urine (X) during the first 92–175 min after injection was $Y = 3.34 - 0.113X$. The correlation coefficient (r_{xy}) was 0.848. In U-A animals killed at about 162 min after ^{28}Mg injection, a larger percentage of the injected ^{28}Mg appeared in the urine and less remained in the blood, while in U-B rats the reverse was true (Table II). When the ^{28}Mg excreted in the urine was corrected to a standard time following ^{28}Mg injection, the differences between treatments were not significant. When this correction was made apparent, differences in ^{28}Mg metabolism appeared to be due entirely to time of sampling and not to hyperammonemia per se.

The differences between treatment groups in nonradioactive and radioactive Mg in liver, brain, heart, colon, and muscle tissues were not significant (Table III). Less ^{28}Mg was present in the bones of group U-A compared to group U-B or controls. The specific activity of Mg was higher in the

TABLE IV. Release of ^{28}Mg and Phosphate into the Supernatant during *In Vitro* Incubation of Liver Slices from Control and Hyperammonemic Rats Labeled *In Vivo* with ^{28}Mg.

	Treatments			
	Without Mg		With Mg	
Time	Control (4)[c]	Urease (6)	Control (4)	Urease (6)
Experiment 2				
^{28}Mg released[a]				
0.08	11.7 ± 0.3	14.7 ± 1.8	13.5 ± 1.1	12.8 ± 1.8
1.0	27.3 ± 0.9	27.6 ± 2.1	31.9 ± 1.8	28.7 ± 2.5
2.0	40.7 ± 1.4	36.7 ± 2.3	41.9 ± 1.7	39.2 ± 2.6
3.0	45.3 ± 1.0	46.0 ± 2.7	50.4 ± 1.5	47.0 ± 3.0
4.0	52.6 ± 0.5	52.0 ± 2.4	57.6 ± 1.0	55.7 ± 2.7
P_i released[b]				
4.0	95.8 ± 3.4	102.5 ± 4.5	91.2 ± 4.7	106.0 ± 11.2
Experiment 3				
^{28}Mg released[a]				
0.08	12.9 ± 1.0	12.7 ± 1.4	13.3 ± 1.6	14.4 ± 2.3
1.0	31.4 ± 2.3	35.1 ± 1.8	32.1 ± 4.5	32.5 ± 3.3
2.0	43.8 ± 2.3	48.4 ± 1.8	47.4 ± 3.0	46.4 ± 4.3
3.0	52.6 ± 1.8	56.0 ± 1.8	55.4 ± 3.2	56.2 ± 1.5
4.0	59.2 ± 1.3	61.0 ± 0.6	60.5 ± 2.5	62.4 ± 0.7

[a] Percentage ± standard error of ^{28}Mg in the flask found in supernatant after 4 hr incubation.
[b] μg PO_4^{-3} released/g liver/4 hr.
[c] Number of observations in parenthesis.

kidneys of group U-B than in U-A or control animals (Table IV). Previous treatment of rats with urease did not alter the rate or amount of ^{28}Mg or phosphate released from liver slices incubated *in vitro* for 4 hr in either Expt 2 or in Expt 3 (Table IV). The presence or absence of Mg in the incubation media did not affect the rate of ^{28}Mg release (Table V). The difference in rate of disappearance of ^{28}Mg from the blood of saline- or urease-injected rats in Expt 3 was not significant. Regression equations for the first and second components of a two-compartment model describing these relationships are presented in Table V.

Rats fed the Mg-deficient diet exhibited hyperemia in the extremities after about 7 days. Following urease injection, signs of ammonia toxicity and their time of onset were similar in control and Mg-depleted animals. Serum Mg was reduced to less than 50% of the control levels at the time

TABLE V. Regression Equations[a] for the Disappearance of ^{28}Mg from Blood of Rats Receiving Two Injections of Saline or Crystalline Urease (Expt 3).

	First Component	Second Component
Saline	$Y = 0.5121 - 0.0997X$	$Y = 3.8770 - 0.00088X$
SD[b]	0.1018	1.2898
N[c]	39	20
Urease	$Y = 0.4605 - 0.90901X$	$Y = 3.9109 - 0.00065X$
SD[b]	0.2827	1.2298
N[c]	44	16

[a] In each case Y = log (blood ^{28}Mg as cpm/ml) and X = time (hr) after injection of ^{28}Mg (10 μCi). Rats were injected subcutaneously with either saline or urease (2 U/animals) at 315 and 90 min prior to ^{28}Mg injection.
[b] Standard deviation of Y for fixed X.
[c] Number of observations.

TABLE VI. Serum Organic Acids[a] and Blood and Serum Magnesium in Magnesium-Depleted (—Mg) and Supplemented (+Mg) Rats Given Saline or Crystalline Jackbean Urease (Expt 4).

	Treatments				
	Saline		Urease		
	+Mg	—Mg	+Mg	—Mg	SEM[b]
Organic acids[a]					
Acetate	832	622	857	987	122
Formate	141	134	113	160	15
β-OH-Butyrate	159	334	201	169	147
Lactate	5515[c]	5467[c]	8080[d]	7325[d]	508
Pyruvate	90[c]	71[c]	186[d]	161[d]	26
Citrate	772[c]	720[c]	827[c]	1101[d]	55
α-Ketoglutarate	60[c]	43[c]	99[d]	98[d]	17
Succinate	55[c]	36[d]	55[c]	39[d]	4
Malate	47[c,d]	31[c]	78[d]	35[c]	11
Magnesium					
Blood (mg/100 ml)	5.72[c]	4.25[d]	5.81[c]	4.09[d]	0.16
Serum (mg/100 ml)	2.94[c]	1.29[d]	3.21[c]	1.36[d]	0.12
Ratio (serum/blood)	0.51[c]	0.30[d]	0.55[c]	0.33[d]	0.03

[a] μEq/ml serum.
[b] Pooled standard error of mean.
[c,d] Horizontal means without a common superscript differ significantly ($P < 0.05$).

of urease injection in the Mg-deficient rats (Table VI), whereas total blood Mg was decreased less markedly (Table VI), indicating that primarily extracellular Mg was depleted by the Mg-deficient diet. Serum lactate, pyruvate, and α-ketoglutarate were increased in all urease-injected animals. Serum citrate was significantly higher in the Mg-deficient animals than in Mg-supplemented animals injected with urease or saline (Table VI). Serum malate was lowest in the Mg-deficient rats and highest in the urease treated fed 50 mg of Mg/100 g of diet.

Discussion. ^{28}Mg released into the incubation media from liver slices was unchanged by previous hyperammonemia (Table IV), indicating that hyperammonemia failed to inhibit the efflux of Mg^{2+} out of cells. If binding of free Mg was altered appreciably by hyperammonemia, plasma disappearance of ^{28}Mg should have been altered, and this was not observed (Table V).

Hyperammonemia failed to alter Mg concentration or the uptake of ^{28}Mg by the liver, brain, heart, colon, or muscle (Table IV). Since Mg in muscle is not altered markedly even during severe Mg deprivation, the results for muscle seem reasonable (26). The metabolism of ^{28}Mg by kidney and bone appears to have differed between the two urease-treated groups, which may be explained in part by differences in time of killing following ^{28}Mg injections. Animals in group A and B were killed at about 100 and 162 min after ^{28}Mg injection, respectively. The differences in time of killing were necessary because of the varied response of animals to the LD_{50} injection of urease. The increase in specific activity of kidney Mg appears to be due to the decline in Mg concentration in group U-B animals which may be a consequence of hyperammonemia and not related to the time of killing. Whether the kidneys release Mg into the plasma during hyperammonemia remains to be determined (Tables I and II).

Lizarralde and coworkers (27) reported that magnesium deficiency decreased the activity of liver ornithine transcarbamylase. Since argininosuccinate synthetase is the rate-limiting step in urea biosynthesis (28, 29), a small decrease in the activity of ornithine transcarbamylase is not likely to

impair the efficiency of the urea cycle. In these studies, ammonia did not appear to be any more or less toxic in the rats fed a Mg-deficient diet. An elevation in plasma glucose has been observed under similar conditions of hyperammonemia used in these experiments (14). A release of insulin in response to the hyperglycemia would cause the movement of phosphate and amino acid from the plasma into tissues (30, 31).

The results of these experiments do not demonstrate any significant relationship between Mg and the clinical or biochemical aberrations observed during acute hyperammonemia. However, these results fail to rule out impaired Mg absorption from the gastrointestinal tract because of an interaction with ammonia as has been suggested (1–3).

Summary. Hyperammonemia was induced by injection of crystalline urease intraperitoneally in normal and Mg-deficient male rats. In hyperammonemic rats, plasma Mg tended to increase and plasma phosphate fell to about 50% of control values. Total Mg and the uptake of ^{28}Mg by the liver, brain, heart, colon, or muscle were not changed. Mg concentrations in the kidney were decreased following acute ammonia toxicity, and release of ^{28}Mg by liver slices *in vitro* was not changed by previous hyperammonemia. Hyperammonemia did not alter the rate of disappearance of ^{28}Mg from blood. Serum Mg of rats fed a Mg-deficient diet for 7 days was 50% of control, but the biochemical and toxicological responses to hyperammonemia were not markedly altered. Serum lactate, pyruvate, and α-ketoglutarate were increased in all urease-injected animals. Serum succinate was significantly lower in Mg-deficient rats. Significant changes in Mg metabolism during hyperammonemia were not observed.

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