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Generalized Aminoaciduria in the Magnesium Deficient Rat.* (31499)

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The experimental induction of magnesium deficiency in the rat has been demonstrated by numerous investigators to cause growth failure, renal damage, hypercalcemia, phosphaturia, urinary nitrogen loss, and kaliuresis (1,2,3). These findings prompted our investigation of urinary amino acid excretion and this report documents the presence of pathologic aminoaciduria and changes in renal sodium-potassium activated Adenosine Tri Phosphatase (Na-K ATPase) in the magnesium deficient rat.‡

Materials and methods. Albino female rats (Holtzman) of initial weight 150-200 g were divided into control and deficient groups. Animals were housed in individual metabolic cages with stool-urine separators in a constant temperature room with 12-hour light cycles. Magnesium deficient diet (General Biochemicals, Chagrin Falls, Ohio), demonstrated to be nutritionally adequate with the exception of magnesium, was homogenized in deionized water and an aliquot of the homogenate representing 7.5 g of the diet was given to each rat twice daily by gavage feeding (15 g diet total daily feeding per rat). With the exception of the addition of 1 mEq magnesium acetate per feeding to control rats, diets were identical for each group. All rats were fed the magnesium-supplemented diet for the initial 6 days to establish baseline values.

Experiment 1: 24 hour urines were collected under toluene, refrigerated, and pooled in 6 day batches for each rat. Stools were collected on a 6 day schedule, weighed, and homogenized in a standard volume of deionized water and refrigerated until analysis. The duration of this experiment was 30 days.

Experiment 2: Control and deficient rats were begun with conditions identical to Exp. 1 except that the magnesium content of the diet of the deficient animals was increased to 0.065 mEq per day to allow a slower onset

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‡ Some of these results have been described in abstract form(4).

TABLE I. Analyzed Content of 15 g Daily Feeding of Magnesium Deficient Diet.

Sodium:	2.297 mEq	Calcium:	5.017 mEq
Potassium:	1.797 mEq	Phosphorus:	.316 g
Magnesium:	.0165 mEq	Nitrogen:	.560 g

TABLE II. Mean Daily Urine and Stool Nitrogen Excretion and Overall 30 Day Balance Results. Values in Grams.

	Urine	Stool	Balance
Control (9 rats)	.292 ± .072*	.039 ± .012	+.229 ± .062
Deficient (10 rats)	.353 ± .042	.033 ± .007	+.181 ± .050
p =	<.05	>.10	>.05

* ± one std deviation.

of deficiency so that a more prolonged analysis of changes in blood levels of amino acids could be obtained. No excreta were collected from the animals in this experiment. The duration of this experiment was 42 days. Each week, 5 control and 5 deficient rats from this experiment were anesthetized with ether and exsanguinated *via* the abdominal aorta using heparinized tubes for blood collection. Plasma was separated immediately and frozen. After exsanguination, the kidneys were quickly removed. The left kidney was decapsulated, weighed immediately, and homogenized in deionized water at 4°C and assayed for ATPase activity within 15 minutes. The right kidney was placed in neutral 10% formalin for histopathological study. At the completion of the study period, analyses for amino acids, sodium, potassium, urea nitrogen, calcium, and magnesium were carried out on the frozen plasmas.

Plasma sodium, potassium, calcium, magnesium, urea nitrogen, and plasma, fecal, and urine total nitrogen were measured by methods previously described(5). Urine and plasma free amino acids were determined quantitatively by a modification of the method of Moore and Stein(6). Na-K ATPase in kidney homogenates was assayed by the method of Beauchene *et al*(7). Analysis of variance, standard deviation and paired t Tests were performed according to Bailey(8).

Results. Results of the analysis of the diet are given in Table I. Exp. 1: The expected transient hyperemia of ears and paws was seen in deficient rats at days 12-15 and was followed within 5 days by coarsening of the hair and the development of ulcerated lesions about the head and neck. Sound induced convulsions appeared sporadically after day 24. Diarrhea was not seen with the concentration and volume of diet used. Benzidine tests of stools showed no blood in any specimen. Glucose oxidase paper strip tests of urine showed no glycosuria. Final body weight was significantly less in the deficient rats (210 g mean), than controls (232 g mean, $p<0.05$). Total urinary nitrogen was significantly increased in the deficient rats while no statistical differences existed in stool nitrogen between the two groups (Table II).

Mean daily excretion levels of urinary amino acids during the initial 6 day baseline periods are given in Table III.

Comparisons of the values of daily urinary amino acid excretion during each 6 day study period to the original 6 day baseline period were calculated in terms of percent change and significant separations between control and deficient rats were demonstrated (Fig. 1A through 1D). The control rats uniformly had a 50% fall of urinary amino acid levels

TABLE III. Mean Excretion Values (mg per day) of Urinary Amino Acids During Baseline Collection Periods.*

Threonine	.5338 (.0834)†	Leucine	.3991 (.0606)
Serine	.4038 (.0389)	Tyrosine	.3093 (.0433)
Glutamic acid	.9995 (.2510)	Phenylalanine	.2970 (.0423)
Proline	.6470 (.1070)	Lysine	.6240 (.0661)
Glycine	.7633 (.0785)	Histidine	.2044 (.0307)
Alanine	1.2969 (.3330)	Arginine	.2746 (.0401)
Valine	.3596 (.0586)	Ornithine	.2926 (.0466)
Methionine	.4845 (.0594)	Citrulline	.1637 (.0372)
Isoleucine	.1895 (.0298)		

* Mean values determined from 6-day urine collections in each of 12 rats during administration of control diet.

† Values in parentheses = standard error of mean.

TABLE IV. Plasma Free Amino Acid Levels in Control and Magnesium Deficient Rats in the 5th Week of Study in Experiment 2. Values given are mg per 100 ml plasma ± one standard deviation. (5 rats in each group.)

Amino acid	Control	Mg def	Amino acid	Control	Mg def
Threonine	3.53 ± .81	3.68 ± .82	Isoleucine	1.02 ± .25	1.02 ± .29
Serine	3.99 ± .28	3.78 ± .76	Leucine	1.71 ± .34	1.60 ± .32
Glutamic acid	3.34 ± .69	3.13 ± 1.1	Tyrosine	1.57 ± .11	1.49 ± .46
Citrulline	1.90 ± .30	1.51 ± .24	Phenylalanine	.98 ± .13	.89 ± .15
Glycine	2.78 ± .24	2.23 ± .16*	Ornithine	.55 ± .07	.60 ± .18
Alanine	4.39 ± 2.13	5.02 ± 1.1	Lysine	6.63 ± 3.50	7.56 ± .77
Valine	1.98 ± .47	1.88 ± .52	Histidine	1.52 ± .80	1.00 ± .31
Cystine half	.70 ± .36	.72 ± .26	Arginine	3.04 ± .23	2.73 ± .37
Methionine	.63 ± .16	.63 ± .11			

* $p < .05$.

during the first 6 days of observation, but excretion levels remained steady thereafter. The deficient rats showed minimal changes of excretion during the first 6-day period of deficiency, but during the second period, a significant increase in the excretion of glutamic acid, proline, glycine, valine, phenylalanine, lysine, ornithine, and citrulline was noted. During the third 6-day period of magnesium deficiency, significant increases in the excretion

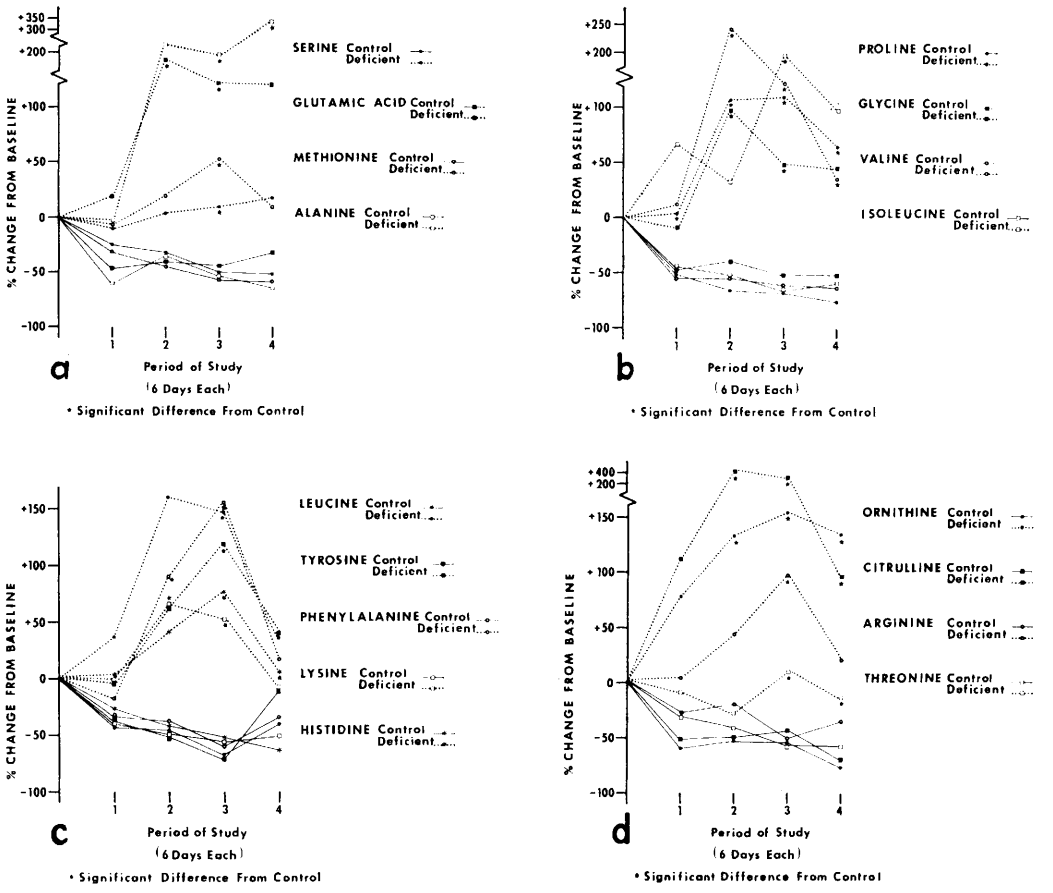

FIG. 1. (a-d) Mean excretion of urinary amino acids in 6 control and 6 magnesium deficient rats. Values are expressed as per cent change from the initial 6 day baseline values. Significant difference from control = $p < .05$.

TABLE V. Mean (± One Standard Deviation) Values for Plasma Sodium, Potassium, Magnesium, Calcium, Urea Nitrogen, and Erythrocyte Magnesium in Control (C) and Magnesium Deficient (D) Rats.

		Week of study					
		1	2	3	4	5	6
Plasma sodium, mEq/l	C	138.0 ± 4.9	146.0 ± 2.4	139.2 ± 5.4	145.6 ± 3.29	134.8 ± 10.7	140.4 ± 9.3
	D	144.0 ± 2.8	160.0 ± 15.7	153.2 ± 10.4*	148.0 ± 6.99	140.4 ± 6.99	144.4 ± 5.37
Plasma potassium, mEq/l	C	3.68 ± .64	3.12 ± .27	2.28 ± .44	2.20 ± .45	1.92 ± .34	2.60 ± 1.34
	D	2.90 ± .20*	3.40 ± .60	3.20 ± .45*	2.00 ± .00	2.40 ± .89	2.08 ± .179
Plasma magnesium, mEq/l	C	2.20 ± .21	2.06 ± .19	2.10 ± .26	1.74 ± .19	1.96 ± .09	1.98 ± .47
	D	1.64 ± .12*	1.26 ± .16*	1.01 ± .19*	1.02 ± .13*	.85 ± .11*	.74 ± .11*
Erythrocyte magnesium, mEq/l	C	—	—	5.12 ± .05	5.72 ± .39	5.38 ± .74	5.24 ± .21
	D	—	—	3.86 ± .60*	3.71 ± .21*	2.97 ± .58*	3.31 ± .52*
Plasma calcium, mg/100 ml	C	11.9 ± .96	12.74 ± .83	13.63 ± 1.26	12.12 ± .66	12.52 ± 1.04	12.21 ± 1.05
	D	12.82 ± .82	11.55 ± .74*	13.19 ± .47	13.49 ± 2.08	13.23 ± 1.18	13.84 ± .64*
Plasma urea nitrogen, mg/100 ml	C	19.8 ± 4.1	19.2 ± 2.6	18.4 ± 1.67	14.4 ± 2.61	11.6 ± 1.67	14.0 ± 1.4
	D	13.8 ± 2.1*	21.0 ± 4.5	20.4 ± 4.09	20.4 ± 2.2*	15.2 ± 3.03*	22.0 ± 5.1*

* $p < 0.05$.

of threonine, serine, alanine, methionine, isoleucine, leucine, tyrosine, histidine, and arginine were seen. The urinary excretion of all amino acids determined decreased during the last 6-day collection in the deficient group as the animals became terminally ill.

Exp. 2: Symptoms of deficiency were seen approximately 10 days later than in Exp. 1. No statistical differences in plasma free amino acid levels were noted between control and deficient rats during the 5th week of comparisons (Table IV) which would correspond to the time of maximal urinary excretion of amino acids in the deficient rats in Exp. 1.

Plasma and erythrocyte magnesium, plasma calcium, sodium, potassium, and urea nitrogen values appear in Table V. Plasma magnesium levels in animals receiving the magnesium deficient diet declined during the first week and plasma and erythrocyte magnesium values fell gradually throughout the remainder of the experiment. Plasma urea nitrogen and calcium levels rose slightly in the deficient rats during the later stages of magnesium deficiency. No differences in plasma sodium or potassium were observed between control and deficient rats. Histological examination of the kidneys after the third week of deficiency revealed scattered lamellar calcific deposits in the proximal tubules at the corticomedullary junction, and degenerative changes in the tubular epithelium. There were no evident morphological changes in the glomeruli, papillae, or interstitiuum.

Results of renal Na-K ATPase activity are given in Table VI. An increase in activity of Na-K ATPase was noted in deficient animals, but no change in the total ATPase activity was seen.

Discussion. Since dietary magnesium deficiency was the sole difference in handling of the rats, it is evident that the low magnesium intake was the starting point for the observed abnormalities. The absence of differences in plasma amino acid levels mitigates against "overflow" aminoaciduria. The observed decline in urinary amino acid excretion during the first 6 days in control rats is an adaptive result to the change to a new diet. After the first 6 day collection, urinary amino acid excretion values remained at a relatively con-

stant level. The generalized aminoaciduria in magnesium deficient rats might be explained on the basis of damage to the renal tubules by the luminal calcification, however, the process is much more subtle than this. Firstly, the studies of Hess *et al*(9) demonstrated changes in the subcellular particles of the renal tubular cells of the magnesium deficient rat as early as the third day of deficiency and 6 days before the luminal calcium deposits became evident. Secondly, the tubular calcifications are present in a small number with only 10-20 calculi seen in the 100-200 tubules visible in a given microscopic section. This is a small fraction of the available reabsorptive surface. Thus, a basic alteration in tubular transport mechanism is suggested.

TABLE VI. Renal Na-K ATPase and Total ATPase in Control and Magnesium Deficient Rats.

	Na-K ATPase			Total ATPase		
	Control (9 rats)	Mg def (9 rats)	p	Control (10 rats)	Mg def (10 rats)	p
μMole/hr/mg protein	.886± .553*	1.777± .935	<.05	13.83± 1.17	14.29± 2.18	>.10
μMole/hr/total kidney	90.6 ± 79.0	208.9 ±114.0	<.05	1657 ±244	1753 ±157	>.10
μMole/hr/g kidney	170.3 ±157	356.7 ±193	<.05	2815 ±292	2925 ±263	>.10
μMole/hr/100 g body wt	91.4 ± 85.2	194.9 ±105.4	<.05	864 ±127	948 ± 69	>.05

* ± One standard deviation.

The reported association of Na-K ATPase with cation transport(10) and the significant correlation found between the activity of this enzyme and the accumulation by kidney slices of para amino hippuric acid and alpha-amino isobutyric acid(7), stimulated us to study such enzymatic systems in the magnesium deficient rat. The finding of a significant increase in the Na-K activated ATPase activity in kidney homogenates of magnesium deficient rats in the presence of several manifestations of tubular dysfunction, may be the result of alterations in all or any of the reactions leading to energy production, storage, and utilization. The reported morphological abnormalities in tubular cell mitochondria of magnesium deficient rats tends to support this concept(9). Further investigation will be needed to identify the mechanism by which this enzyme is activated and to determine the significance that this change may bear in relation to the abnormal renal physiology of the magnesium deficient rat.

Summary. The excretion of urinary amino acids, levels of plasma amino acids, and activity of renal ATPase were determined in control and experimentally induced magnesium deficient rats. Urinary amino acid excretion increased significantly during the progression of magnesium deficiency. This increase involved all amino acids studied. No significant changes were noted in plasma amino acid levels between control and deficient animals. Renal Na-K ATPase was significantly greater in magnesium deficient rats than in control rats. This study suggests that the aminoaciduria seen in the magnesium deficient rat is a result of renal tubular dysfunction which may have as its basic etiology a disturbance of energy metabolism in renal tubular cells.

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Effect of 8-Azaguanine on Encephalomyocarditis Virus Multiplication.* (31500)

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The purine analog 8-azaguanine (azaG) inhibits the growth of mammalian cells and of several microorganisms. It has been suggested that the inhibitory effect of this analog is due to its conversion to 8-azaguanilic acid by inosine monophosphate (IMP) and/or guanosine monophosphate (GMP) pyrophosphorylases(1) and to its subsequent incorporation into RNA(1-3). When azaG-sensitive (azaG-S) mouse L cells are grown in the presence of the analog for varying periods of time, resistance may be established(4,5). Since resistance is associated with a loss of IMP-GMP pyrophosphorylases(5,6), it could be anticipated that in azaG-resistant (azaG-R) cells, azaG would not be converted to azaguanilic acid and would not, therefore, inhibit the growth of intracellular parasites. However, the analog has an inhibitory effect on psittacosis agent(4), vaccinia, and encephalomyocarditis (EMC) virus(7) in both azaG-S and azaG-R cells. The present study was carried out to investigate some aspects of the inhibitory effect of azaG on the multiplication of EMC virus.

Materials and methods. Cell cultures. Earle's strain L of mouse cells, clone 929, was obtained from Dr. H. Eagle, and grown in 12-ounce bottles with Eagle's Minimal Essential Medium (MEM)(8) containing 10% inactivated horse serum, 100 units of penicillin and 100 μg of streptomycin/ml. Analog-resistant cells were obtained by exposing monolayer cultures in logarithmic phase of growth to increasing levels of azaG.† Eventually, cells were obtained which were resistant to 100 μg/ml of the analog. However, no toxic effect on the cells, that is no change of cell morphology or rate of cell growth, was apparent once a level of resistance to 20 μg/ml had been attained.

Virus. The strain of EMC virus used in this study was originally obtained from Dr. K. K. Takemoto and has been grown in L cells. Most of the experiments were carried out with a purified small plaque variant(9) which allowed higher plaque counts per plate.

Virus assay. All virus assays were carried out on L cells using the plaque method as described by Takemoto and Liebhaber(9).

Cell growth assays. Falcon plastic Petri dishes, 60 $\times$ 15 mm, were seeded with 5 $\times$ 10^4 cells. After 1 day, azaG was added at a final concentration of 100 μg/ml to half of the plates. The cells in a measured area at the center of the plates were counted daily for a period of 4-5 days.

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† Nutritional Biochemicals.