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Response of Growing Rats to Diets Varying in Magnesium, Potassium and Protein Content. (26757)

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Hypomagnesemia accompanied by convulsions is a disturbance which occurs sporadically in ruminants grazing on young, rapidly growing forage(1). A relationship has been observed in the field between nitrogen content of the grass on which cattle graze and incidence of tetany(2). High potassium diets(3) and diets high in protein and potassium(4) reduce serum magnesium and increase fecal excretion of magnesium in sheep. There is retention of calcium in the viscera and kidneys of rats during magnesium deficiency(5,6) and in the yellow elastic fiber of the endocardium, aorta and jugular vein in calves fed low-magnesium diets(7). Colby and Frye(8,9) reported an increase in severity of magnesium deficiency in rats fed high calcium, high potassium or high protein (50%) diets. It was desired to study the effects in rats of high potassium and/or high protein diets on growth, blood electrolytes and pathological anatomy of low or normal dietary magnesium.

Methods. Weanling albino rats of the Cornell strain were used in a factorial arrangement of treatments. Rats were kept in individual wire-mesh cages. Diets (Table I) were provided *ad libitum* and distilled water was available at all times. Feed consumption and weight gains were recorded weekly. One-half of the rats from each group were sacrificed after 28 days and the remainder after 56 days on test by ether anesthesia. Blood was collected by heart puncture into heparinized tubes for serum calcium and magnesium and whole blood potassium determination. Whole blood potassium was determined in duplicate after some modifications of the method of Mosher(10). Five tenths of one ml of whole blood were diluted 1:100 with distilled water for analysis. Serum calcium and magnesium were determined by using a modification of the method of Walser(11). The following organs and structures from each rat were prepared for histological examination: proximal epiphysis of tibia, parietal bone, brain (transverse section through thalamus and cerebellum), thyroid, parathyroid, adrenals, esophagus, trachea, stomach (esophageal and fundic sections), duodenum, jejunum, colon, liver, pancreas, spleen, bone marrow, lymph glands, kidneys, ureter, urinary bladder, skin, myocardium, skeletal muscle, lungs; cross section through hilus, including lungs, bronchi, aorta, mediastinum and thymus. Bones were fixed in 10% formaldehyde and soft tissues in Bouin's fluid. Decalcification was with Perenyi (5 parts 100% HNO_3 and 95 parts 80% ethyl alcohol). Samples were paraffin-embedded, sectioned at 6 micra and stained with H and E stain.

Results and discussion. The data for growth, feed consumption and efficiency of

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TABLE I. Composition of Control Diet (Diet 1).*

Component	%
Casein	12
Corn oil	5
Corn sugar (cerelose)	72
Cellulose (Solka-floc)	1.25
Vitamin premix†	5.00
Mineral premix‡	4.75

* Diets 1, 3, 5 and 7 contained 12% casein (10% protein); diets 2, 4, 6 and 8 contained 24% casein (20% protein). Diets 1, 2, 5 and 6 contained 240 mg magnesium/kg; diets 3, 4, 7 and 8 contained 24 mg/kg. Diets 1, 2, 3 and 4 contained 2033 mg potassium/kg; diets 5, 6, 7 and 8 contained 8132 mg/kg.

† Mixed with cerelose so that 50.0 g were required to supply the following vitamins for 1 kg of diet: vit. A, 4200 I.U.; vit. D, 135 I.U.; vit. E, 120 mg; vit. K, 0.2 mg; thiamine, 2.5 mg; riboflavin, 5.0 mg; pyridoxine, 2.4 mg; niacin, 30.0 mg; calcium pantothenate, 16.0 mg; choline chloride, 1500 mg; vit. B_{12}, 10 μg.

‡ Composition (units/kg of diet): Ca $(H_2PO_4)_2 \cdot H_2O$, 22 g; KH_2PO_4, 7 g; $CaCO_3$, 12 g; NaCl, 1.5 g; MgO, 0.4 g; $MnSO_4 \cdot H_2O$, 0.3 mg; $FeSO_4$, 0.25 g; $CuSO_4 \cdot 5H_2O$, 0.30 g; $ZnCO_3$, 0.045 mg; KI, 0.10 mg; Na selenite, 0.40 μg.

feed utilization are summarized in Table II. Low dietary magnesium (24 mg/kg) resulted in a highly significant ($P<0.01$) decrease in daily gain, feed consumption, efficiency of feed utilization. High dietary potassium (8132 mg/kg) had no effect on daily gain and feed consumption during the first 28 days but resulted in a highly significant reduction ($P<0.01$) in both after 56 days. There was no effect of level of dietary potassium on efficiency of feed utilization nor were there any potassium × protein interactions on any of these 3 criteria. High dietary protein (20%) resulted in a highly significant increase ($P<0.01$) in daily gain and efficiency of feed utilization after 28 days. This effect on daily gain but not on feed utilization was maintained after 56 days. There was a significant ($P<0.05$) magnesium × protein interaction in terms of daily gain. Mean daily gain of rats fed the low magnesium-low protein (10%) diets (3 and 7) was 0.98 g for the first 28 days and 0.76 g after 56 days. The corresponding values for those fed the low magnesium-high protein diets (4 and 8) were 1.15 and 0.65 g. However, a positive response to high protein occurred when dietary magnesium was adequate (2.30 *vs.* 1.62 g daily gain after 28 days and 1.97 *vs.* 1.52 g daily gain after 56 days) demonstrating that inadequate dietary magnesium prevents maximal utilization of protein for growth.

Blood values are shown in Table III. Low dietary magnesium decreased blood serum magnesium and calcium ($P<0.01$). Blood serum magnesium was affected by a highly significant ($P<0.01$) magnesium × protein interaction manifested in the rats sacrificed after 28 and 56 days on test. There was no

TABLE II. Effect of Dietary Magnesium, Protein and Potassium Level on Performance of Growing Rats.

Diet		Daily gain (g)§		Daily feed (g)‖		Feed/g gain (g)¶	
No.	Designation*	28†	56‡	28†	56‡	28†	56‡
1	HMg-LP-LK	1.72	1.53	9.9	10.7	5.9	7.1
2	HMg-HP-LK	2.25	2.08	9.7	10.4	4.5	5.0
3	LMg-LP-LK	1.07	.90	8.3	9.1	8.0	10.7
4	LMg-HP-LK	1.23	1.09	7.8	8.6	7.5	8.4
5	HMg-LP-HK	1.52	1.50	9.0	9.6	6.2	6.5
6	HMg-HP-HK	2.35	1.85	9.8	10.2	4.3	5.6
7	LMg-LP-HK	.88	.61	8.0	7.8	9.8	14.2
8	LMg-HP-HK	1.06	.61	7.5	7.5	7.8	16.3

* HMg = 240 mg Mg/kg diet; LMg = 24 mg Mg/kg diet; LK = 2033 mg K/kg diet; HK = 8132 mg K/kg diet; LP = 10% protein; HP = 20% protein.

† Each value represents mean of 8 rats fed 28 days.

‡ Each value represents mean of 4 rats fed 56 days.

§ Significantly increased by high protein ($P<0.01$); significantly decreased by low magnesium ($P<0.01$) and significant magnesium × protein interaction ($P<0.05$) at 28 days. At 56 days same, plus significantly decreased by high potassium ($P<0.05$).

‖ Significantly decreased by low magnesium ($P<0.01$) at 28 days. At 56 days same, plus significantly decreased by high potassium ($P<0.05$).

¶ Significantly increased by low magnesium ($P<0.01$) at 28 days. At 56 days same, plus significantly decreased by high protein ($P<0.01$).

TABLE III. Effect of Dietary Magnesium, Protein and Potassium Level on Serum Calcium and Magnesium and Whole Blood Potassium of Growing Rats.

Diet		Serum Ca (mg %)§		Serum Mg (mg %)‖		Whole blood K (mg %)¶	
No.	Designation*	28†	56‡	28†	56‡	28†	56‡
1	HMg-LP-LK	8.2	9.7	3.2	2.6	206	211
2	HMg-HP-LK	8.4	9.5	2.8	2.5	197	219
3	LMg-LP-LK	6.7	6.9	1.0	.9	224	195
4	LMg-HP-LK	7.2	6.7	1.0	.9	221	220
5	HMg-LP-HK	8.2	9.9	2.5	2.6	220	217
6	HMg-HP-HK	8.2	9.3	2.6	2.6	224	222
7	LMg-LP-HK	7.0	7.4	1.6	1.8	211	190
8	LMg-HP-HK	7.1	7.2	1.6	1.6	217	195

* HMg = 240 mg Mg/kg diet; LMg = 24 mg Mg/kg diet; LK = 2033 mg K/kg diet; HK = 8132 mg K/kg diet; LP = 10% protein; HP = 20% protein.

† Each value represents mean of 4 rats fed 28 days.

‡ Each value represents mean of 4 rats fed 56 days.

§ Significantly reduced by low magnesium ($P < 0.01$) at 28 and 56 days.

‖ Significantly decreased by low magnesium ($P < 0.01$) and significant magnesium × potassium interaction ($P < 0.01$) at 28 days. Same plus significantly increased by high potassium at 56 days.

¶ Significant magnesium × potassium interaction ($P < 0.05$).

effect of high potassium diets on serum magnesium when dietary magnesium was adequate. However, mean serum magnesium of rats fed low magnesium-normal potassium diets (3 and 4) was 1.0 mg% after 28 days and 0.9 mg% after 56 days. The corresponding values for those fed low magnesium-high potassium diets (7 and 8) were 1.6 and 1.7 mg% indicating an inhibition by potassium of the reduction in serum magnesium associated with consumption of a magnesium-deficient diet. Typical magnesium deficiency symptoms were observed, however, in all 4 treatment groups (diets 3, 4, 7 and 8). Symptoms included reddening of the ears and paws and convulsions lasting 1-5 minutes culminating in death in 5 rats.

Histological examination revealed slight to severe nephrocalcinosis to be present in rats representing all groups as early as 28 days. This condition has been previously reported to occur in rats fed adequate diets. Otherwise no lesions were found. It is of interest that no tissue alterations associated with treatment appeared even after 56 days on test despite the fact that gross magnesium deficiency symptoms appeared as early as the first week.

Summary. Dietary magnesium, potassium and protein interactions were studied in weanling rats. Growth rate, efficiency of feed utilization, and blood serum magnesium and calcium levels were significantly lower in rats fed a magnesium-deficient diet than in those fed a similar magnesium-adequate diet. A diet containing 4 times as much potassium (8132 mg/kg) increased the serum magnesium level 60% after 28 days (1.0 *vs.* 1.6 mg%) and 90% after 56 days (0.9 *vs* 1.7 mg%) indicating an inhibition by potassium of the reduction in serum magnesium associated with consumption of magnesium-deficient diet. Typical gross magnesium deficiency symptoms, including reddened ears and paws and convulsions lasting 1-5 minutes were observed, however, in all groups fed low magnesium diets regardless of potassium level. Diets containing 20% protein produced significantly greater growth rate than those containing 10% in the presence of adequate magnesium but not during magnesium deficiency. No histological lesions associated with treatment occurred even after 56 days on test despite the fact that gross magnesium deficiency symptoms appeared as early as one week on test.

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Detection of Agents which Interfere with the Immune Response. (26758)

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Interference with antibody production by cytotoxic drugs, notably antagonists of nucleic acid constituents, has been reported by several investigators(1-8). Substances which exhibit this effect are of interest not only for the insight which they may provide into the immune process, but also for their possible applications to transplantation problems(9,10) and to autoimmune diseases(11).

Screening tests for such materials recently have been devised by Berenbaum(5) and by Sterzl(12) using antigens of bacterial origin. The method to be described herein is based on the agglutinin response in mice to administered sheep cells, and may therefore be regarded as a modification of the procedure used by Makinodan for investigation of radiation effects(13). As will be shown, the modifications which have been made appear to be essential for detection of the effects of drugs. The method as presently employed is simple, economical and capable of providing reproducible results.

Materials and methods. Mice. Specific-Pathogen Free (SPF) Swiss Bagg male mice* weighing 19-21 g were used.

Antigen. Sterile, washed sheep cells† were exposed for 10 minutes at 37°C to an equal volume of 1:10,000 tannic acid in buffered saline solution‡ collected by centrifugation, washed with buffered saline‡(7) and resuspended in the same medium.

Procedure. The initial inoculum of antigen consisted of 0.25 ml of a 30% suspension of cells per mouse, administered intravenously to groups of 10 mice. In the experiments reported herein a second dose of 1.0 ml of a 15% suspension of cells was administered intraperitoneally on the seventh day. The effects of this second dose have been found to be minimal, and in subsequent work it will be omitted. On day 13, the mice were decapitated and the pooled blood was allowed to clot. The serum was used for determination of hemagglutinins.

Hemagglutination. The hemagglutinin titer was determined by serial 2-fold dilution essentially by the method of Stavitsky(14) using 0.1 ml of a 1% suspension of tanned sheep cells per ml of saline-diluted serum. After preparation, the tubes were shaken and then allowed to stand for 18 hours at 5°. Agglutination was then scored 0, 1+ to 4+ described by Stavitsky(14).

Index. The score for each tube was multiplied by the appropriate exponent of the 2-fold dilution series, *i.e.*, a tube showing a 4+ agglutination at a dilution of 2^6 would have a value of 24. These values were then summed for each series. The index of drug effect was obtained as the ratio of these sums for the treated to the untreated control:

$$\text{Antibody Index (A.I.)} = \frac{\Sigma\,(S_1 + 2S_2 + 3S_3 + \ldots . nS_n)\,T}{\Sigma\,(S_1 + 2S_2 + 3S_3 + \ldots . nS_n)\,C}$$

where n refers to the exponent of the dilution

* Darrow Breeding Laboratories.

† Colorado Serum Co.

‡ Saline (0.85% NaCl) is mixed with an equal volume of buffer (29.9 ml M/15 KH_2PO_4 + 76.0 ml M/15 Na_2HPO_4).