Adrenal Gland Factors in Magnesium-Deficient Rats[1] (34832)

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Some manifestations of magnesium deficiency in animals are suggestive of hypocorticosteronism. These signs include anemia, inability to gain weight, erythema, lethargy, and hypercalcemia (1). Richer, Veilleux, and Bois (2) observed decreased plasma corticosterone levels in magnesium-deficient animals. This study was designed to determine whether glucocorticoid or ACTH administration prevents or reduces any of the alterations of magnesium deficiency.

Materials and Methods. One hundred Holtzman strain male rats each weighing about 100 g were used. They were individually caged and provided diet and deionized water *ad libitum*. The magnesium concentration of the deficient diet (3) was 0.11 ± 0.01 m*M*. A control diet was prepared by adding magnesium sulfate to the basal diet to increase the magnesium content to 16.5 m*M*. The rats were divided into five groups of 20. The animals in Group A received the magnesium-deficient diet and a daily intramuscular injection of two units of ACTH. Group B animals were given the magnesium deficient diet and a daily intraperitoneal injection of 1 mg of corticosterone (Compound B). The animals in Group C were given the control diet. Group D animals received the magnesium-deficient diet. Group F animals were provided with the magnesium deficient diet and given daily ip injections of 1 mg of cortisol (Compound F).

Ten animals from each group were sacrificed by decapitation after 20 days and the remaining animals were sacrificed after 35 days on the experimental regimens. Food was removed from the cages 12 hr before the animals were sacrificed, and the animals were not handled during this period. Blood was collected in a beaker containing 0.2 ml of heparin solution. Immediately after sacrifice of the animals, the adrenal glands, liver, spleen, kidneys, and testes were removed and weighed.

Plasma and erythrocytes were separated by centrifugation. Plasma alkaline phosphatase activities (4) were assayed with and without addition of 10 m*M* Mg^{2+} to the incubation media; the assay media without added magnesium contained only the magnesium present in 0.1 ml plasma. The assays for alkaline phosphatase with magnesium-enriched media were performed in order to assure an adequate concentration of this cofactor for full enzymatic action (5). Determinations were made of plasma magnesium (6), plasma corticosterone (7), plasma calcium [35 days only] (6), serum protein [35 days only] (8), and erythrocyte magnesium (6) concentrations. Erythrocyte pyrophosphatase activity assays were made by the methods of Bailey (9). In addition, serum protein electrophoretic patterns were prepared with selected specimens from the animals sacrificed at 20 days.

Results. All results are expressed as means with values of the SEM. The numbers in parentheses in the tables given the number of animals, or the number of determinations when this was a lesser number than the number of animals.

The results from the protein electrophoretic patterns of the sera are shown in Table I. In Table II are displayed the mean body weight increments of the animals during 20 days and the weights of the adrenals, kidneys, spleen, liver, and testes at the time of sacrifice. Similar data obtained after 35 days

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TABLE I. Serum Protein Concentrations of Rats at 20 Days of Magnesium Deficiency (g/100 ml).

		Total serum protein	Albumin	Alpha 1 globulin	Alpha 2 globulin	Beta globulin	Gamma globulin
Untreated magnesium deficient	(D)	4.49 ±0.08 (10)	1.83 ±0.04	0.79 ±0.03	0.52 ±0.04	1.14 ±0.03	0.18 ±0.01
Magnesium-deficient treated with corticosterone	(B)	4.95 ±0.13 (4)	2.23 ±0.08	0.88 ±0.06	0.58 ±0.06	1.13 ±0.08	0.15 ±0.03
Control (Mg fed)	(C)	6.05 ±0.15 (4)	2.48 ±0.08	1.10 ±0.06	0.60 ±0.00	1.43 ±0.06	0.40 ±0.04
p Value D-C		0.001	0.001	0.001	0.30	0.001	0.001
p Value B-C		0.001	0.05	0.025	0.70	0.01	0.001

TABLE II. Body Weight Increment and Organ Weights of Rats in Magnesium Deficiency and With Hormone Administration (20 Days on Experimental Regimens).

		Organ weights				
	Body weight growth (g)	Adrenals (mg)	Kidneys (g)	Spleen (g)	Liver (g)	Testes (g)
Group A (10) (ACTH)	50.60 ± 2.56	40.41 ± 1.52	1.55 ± 0.06	0.65 ± 0.05	6.29 ± 0.15	2.67 ± 0.06
Group B (10) (Cpd B)	55.30 ± 2.02	35.05 ± 1.34	1.57 ± 0.04	0.84 ± 0.05	6.62 ± 0.20	2.72 ± 0.08
Group C (10) (control; Mg-fed)	137.90 ± 4.19	25.73 ± 1.34	2.16 ± 0.08	0.75 ± 0.04	9.67 ± 0.46	2.85 ± 0.04
Group D (10) (untreated, Mg-deficient)	49.40 ± 4.12	32.69 ± 2.75	1.56 ± 0.07	0.86 ± 0.07	6.75 ± 0.30	2.72 ± 0.07
Group F (10) (Cpd F)	53.90 ± 2.58	35.01 ± 1.12	1.60 ± 0.04	0.74 ± 0.05	6.49 ± 0.20	2.68 ± 0.04

TABLE III. Body Weight Increments and Organ Weights in Magnesium Deficiency and With Hormone Administration (35 Days on Experimental Regimens).

		Organ weights				
	Body weight growth (g)	Adrenals (mg)	Kidneys (g)	Spleen (g)	Liver (g)	Testes (g)
Group A (6) (ACTH)	78.00 ± 3.42	66.37 ± 2.35	1.76 ± 0.03	0.74 ± 0.03	6.62 ± 0.16	3.09 ± 0.06
Group B (6) (Cpd B)	88.33 ± 4.72	42.47 ± 1.93	1.85 ± 0.10	1.13 ± 0.07	7.50 ± 0.43	3.09 ± 0.23
Group C (10) (control; Mg-fed)	227.80 ± 7.22	30.82 ± 1.60	2.58 ± 0.08	0.81 ± 0.02	11.54 ± 0.29	3.37 ± 0.06
Group D (9) (untreated, Mg-deficient)	92.56 ± 2.36	41.90 ± 2.35	1.93 ± 0.03	1.12 ± 0.04	7.79 ± 0.15	3.61 ± 0.13
Group F (7) (Cpd F)	91.00 ± 5.89	39.91 ± 1.41	1.71 ± 0.13	0.88 ± 0.10	7.26 ± 0.37	2.79 ± 0.35

TABLE IV. Enzyme, Corticosterone, and Electrolyte Changes in Magnesium Deficiency and Hormone Supplementation[a] (20 Days on Experimental Regimens).

	Plasma alkaline phosphatase (*p*-nitrophenol units)[a]		Erythrocyte pyrop'ase (mg P/g Hb)	Plasma corticosterone (μg/100 ml)	Plasma Mg (mg/100 ml)	Erythrocyte Mg (mg/100 ml)
	With Mg[a]	Without Mg[a]				
Group A (10) (ACTH)	39.33 ± 2.04	31.23 ± 1.39	82.20 ± 3.36	34.54 ± 5.19 (9)	0.41 ± 0.03	3.25 ± 0.30
Group B (10) (Cpd B)	39.64 ± 2.20	32.00 ± 1.70	72.60 ± 2.28	12.51 ± 1.64 (8)	0.33 ± 0.01	3.31 ± 0.20
Group C (10) (control; Mg-fed)	91.00 ± 3.55	74.44 ± 2.96	125.00 ± 3.27	17.11 ± 1.07 (10)	1.95 ± 0.03	6.08 ± 0.11
Group D (10) (untreated, Mg-deficient)	38.88 ± 2.85	30.57 ± 2.11	87.00 ± 3.21	27.95 ± 2.81 (8)	0.47 ± 0.04	3.16 ± 0.23
Group F (10) (Cpd F)	40.55 ± 1.61	32.48 ± 1.24	75.40 ± 2.65	16.87 ± 2.15 (6)	0.31 ± 0.01	2.79 ± 0.11

[a] The units of plasma alkaline phosphatase activity are milligrams of *p*-nitrophenol liberated per 100 ml of plasma; the results are those obtained with added Mg and without added Mg to the incubation media (see text).

of experimentation are presented in Table III. The results for plasma and erythrocyte enzyme activities, plasma corticosterone contents, and plasma and erythrocyte magnesium concentrations of animals on the experimental regimens for 20 days are given in Table IV and the same results after 35 days of experimentation are shown in Table V. Total serum protein contents and plasma calcium concentrations are also given in Table V.

Discussion. The increments of body weight (Tables II and III) indicated that glucocorticoid supplementation of magnesium-deficient animals did not result in any significant differences in body weight gain when compared with those of the magnesium-deficient animals not treated with steroid hormones. These observations demonstrate that deficiencies in glucocorticoid synthesis in magnesium deprivation could not have been the sole cause of impaired ability of the magnesium-deficient animals to gain weight at the normal rate. A significant decrease in rate of body weight gain of the animals of Group A, compared to those of Group D, was observed at 35 days (Table III) but not at 20 days (Table II). This result is probably related to the marked depression of steroidal synthesis in the animals of Group A by 35 days as indicated by the low plasma corticosterone levels (Table V) at this time.

In previous studies by Hungerford and Karson (10) and Richer *et al.* (2) no differences in adrenal gland weights between magnesium-deficient and control rats were found at 21 or 20 days. In our investigation, however, significant increases of adrenal weights were found in all groups of animals which received the magnesium-deficient diet for 20 and 35 days (Tables II and III). The adrenal hypertrophy was particularly marked in the animals given ACTH and the magnesium-deficient diet. Since ACTH is the only compound known to effect increases of adrenal weights (11), it is suggested that the magnesium-deficient state stimulates ACTH production and/or the liberation of ACTH. In support of this hypothesis are the reports that substantial increments of histamine appear in the plasma and urine of magnesium-

TABLE V. Enzyme, Corticosterone, and Electrolyte Changes in Magnesium Deficiency and Hormone Supplementation (35 Days on Experimental Regimens).

	Plasma alkaline phosphatase (*p*-nitrophenol units)[a]		Erythrocyte pyrop'ase (mg P/g Hb)	Plasma corticosterone (μg/100 ml)	Plasma Mg (mg/100 ml)	Erythrocyte Mg (mg/100 ml)	Plasma Ca (mg/100 ml)	Serum protein (g/100 ml)
	With Mg[a]	Without Mg[a]						
Group A (6) (ACTH)	35.83 ± 0.95	23.24 ± 0.67	87.17 ± 2.95	10.23 ± 0.43 (4)	0.33 ± 0.02	4.02 ± 0.19	9.45 ± 0.24	4.40 ± 0.10
Group B (6) (Cpd B)	38.25 ± 2.35	29.09 ± 2.41	102.67 ± 1.52	18.99 ± 2.82 (5)	0.36 ± 0.01	4.89 ± 0.10	10.00 ± 0.32	4.87 ± 0.12
Group C (10) (control; Mg-fed)	70.26 ± 2.11	55.98 ± 1.85	112.00 ± 1.69	26.92 ± 5.57 (6)	1.88 ± 0.03	5.84 ± 0.07	9.74 ± 0.26	6.17 ± 0.14
Group D (9) (untreated, Mg-deficient)	29.62 ± 1.30	22.23 ± 0.96	78.78 ± 3.65	20.68 ± 2.94 (6)	0.38 ± 0.01	4.47 ± 0.10	9.90 ± 0.21	4.36 ± 0.11
Group F (7) (Cpd F)	42.56 ± 2.08	32.65 ± 1.76	98.29 ± 1.91	19.54 ± 3.96 (4)	0.38 ± 0.01	4.53 ± 0.23	10.14 ± 0.35	5.44 ± 0.27

[a] See footnote to Table IV.

deficient rats (12), and the well-known influence of histamine in promoting ACTH secretion (13). Since the animals in Groups B, D, and F had approximately the same adrenal gland weights at 20 and at 35 days, it would appear that the magnesium-deficiency state produced a relatively uniform stimulation of ACTH production which was independent of exogenous glucocorticoid administration. Parenteral administration of ACTH to magnesium-deficient rats, however, produced a significant increase in the weights of the adrenal glands when compared with the other magnesium-deficient animals at 35 days (Table III). This result suggests that the endogenous and exogenous ACTH were cumulative in stimulation of adrenal glands of magnesium-deficient animals.

The relatively uniform lower weights of the livers and kidneys of the magnesium-deficient animals, compared to those of the Group C animals, reflect the changes in body weight gain and indicated no significant glucocorticoid effect on the weights of these organs (Tables II and III). The findings with respect to splenic and testicular weights suggest that these organs were not affected in a major way by magnesium deficiency.

A generalized curtailment of protein synthesis in magnesium deficiency was indicated by the lowered values for serum protein concentration, and of plasma alkaline phosphatase and erythrocyte pyrophosphatase activities in magnesium-deficient animals (Tables I, IV, and V). Electrophoretic patterns of the serum proteins showed a significantly lower concentration of all serum protein fractions, with the exception of alpha-2-globulins, in the magnesium-deficient state at 20 days (Table I). The maintenance of normal levels of alpha-2-globulins in sera of magnesium-deficient animals is possibly related to increased synthesis of these proteins in disease states associated with tissue destruction and debility (14).

There is evidence that parenteral administration of glucocorticoids over 35 days to magnesium-deficient animals affected the balance in turnover of some proteins. The total serum protein concentration and the plasma alkaline phosphatase and erythrocyte pyro-

phosphatase activities (Table V) were significantly elevated in the animals of Groups B and F when compared with magnesium-deficient animals not receiving glucocorticoids (Group D). However, the serum protein concentration and enzyme activities in the animals of Groups B and F were lower than the values for the control animals (Group C). These observations are consistent with the findings of Feigelson *et al.* (15) that exogenous glucocorticoids stimulate protein synthesis in the liver of rats.

In contrast to the results of Richer *et al.* (2), a significant elevation of the serum corticosterone concentrations in magnesium-deficient animals (Group D) was present after 20 days on the regimens compared with control animals (Table IV). The largest elevation in plasma corticosterone levels at 20 days was found in the ACTH-treated group, indicating a response of the adrenal glands to ACTH at 20 days. However, after 35 days of experimentation the plasma corticosterone concentrations of animals of all magnesium-deficient groups, including those treated with ACTH, were below the results found with the control animals, and the lowest plasma corticosterone concentrations were seen in the animals treated with ACTH (Group A, Table V). These observations suggest that the adrenal glands of the magnesium-deficient animals given ACTH suffered some degree of exhaustion in ability to synthesize corticosterone by 35 days, while the deficient animals (Group D) not given ACTH were still able to produce corticosterone although at reduced levels.

The plasma magnesium concentrations of all animals on the magnesium-deficient diets decreased to at least 25% of control values. At 20 days the animals of Groups B and F showed significantly greater reductions of plasma magnesium contents than those of Groups A and D. This can possibly be explained by the observation of Aikawa, Harms, and Reardon (16) that glucocorticoids potentiate the renal loss of magnesium.

The erythrocyte magnesium contents of the magnesium-deficient animals were reduced by 20 days and, at 35 days, were at levels about half those of the control animals. It is suggested that the reductions of red-cell magnesium content were due to cells formed during the period of magnesium deficiency rather than to depletion of existing cells. Plasma calcium concentration was not significantly affected by magnesium deprivation.

The results for plasma alkaline phosphatase assays, with and without added magnesium, confirm those of Heaton (5) that the magnesium content of the plasma of rats given magnesium-deficient diets is not sufficient to allow full exhibition of the enzyme activity *in vitro*.

Summary. Parenteral administration of glucocorticoids did not prevent the signs and biochemical changes of magnesium deficiency in the rat. The adrenal glands increased in weight in magnesium deficiency, probably secondary to an increase in production and/or liberation of endogenous ACTH. The increase in adrenal weights was not prevented by parenteral glucocorticoid administration. Magnesium-deficient rats given daily parenteral ACTH showed an increase in adrenal weights, a decrease in plasma corticosterone levels, and a more advanced state of debility compared with magnesium-deficient animals not receiving ACTH at 35 days. Exogenous glucocorticoids stimulated some protein syntheses in chronically magnesium-deficient animals.

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