# Effect of Magnesium Deficiency in Guinea Pigs on Kidney Function and Plasma Ultrafiltrable Ions[1] (34159)

E. R. MORRIS[2] AND B. L. O'DELL

*Department of Agricultural Chemistry, University of Missouri, Columbia, Missouri 65201*

Magnesium deficiency in the guinea pig results in hypomagnesemia, elevated serum inorganic phosphorus, metastatic calcification, depressed growth rate, and tooth abnormalities (1, 2). This species is somewhat unique as regards vulnerability to soft tissue calcification and anemia when subjected to magnesium deprivation (3). Phosphorus, as well as calcium, accumulates in the soft tissues of magnesium-deficient guinea pigs, and the excess phosphorous is present almost entirely as inorganic phosphate (4).

A physiological antagonism between magnesium and calcium was demonstrated by Mendel and Benedict (5, 6) early in this century, and this relationship has been used to explain the fact that excess dietary calcium increases magnesium requirement. Excess dietary phosphorus also increases magnesium requirement (2), but there is no evidence for a specific physiologic antogonism between magnesium and phosphorus. Clark (7) reported that high magnesium intake increases phosphorus retention in rats when the level of dietary phosphorus is adequate.

Magnesium deficiency in the rat is accompanied by hypercalcemia and hypophosphatemia (8, 9) but parathyroidectomized, magnesium-deficient rats do not develop the hypercalcemia and hypophosphatemia which characterize the intact rat (10). Meyer and Forbes (11) found that mineral accumulation in the kidneys of the magnesium-deficient rat depends upon the presence of the parathyroid glands. Other recent observations have shown that hypermagnesemia inhibits parathyroid gland activity in the rat (12). These results suggest that magnesium deficiency induces a hyperparathyroid state, at least in the rat.

One explanation for the severe metastatic calcification observed in magnesium deficient guinea pigs relates to the fact that the level of blood phosphate is elevated while calcium remains constant. This condition would not be expected in hyperparathyroidism but might arise from impaired kidney function. Kidney dysfunction including polyuria has been observed in magnesium-deficient rats and cats (13, 14). The initiation of metastatic calcification is dependent upon the concentration of calcium and phosphate ions in tissue fluids. Only the unbound calcium and phosphate ions would be directly involved in the ion product and only ionic magnesium would participate directly in membrane and transport phenomena. Thus, the concentration of free ions in plasma and interstitial fluids is an important consideration, but there is little information as to the proportion of ultrafiltrable magnesium in deficient animals.

The present communication is concerned with the effect of magnesium deficiency on (a) renal function, and (b) concentration of ultrafiltrable ions in plasma.

*Materials and Methods.* The experimental conditions were essentially the same as previously described (2) except that weanling female guinea pigs weighing 100–130 g were used for the ultrafiltration studies. For the kidney function studies, animals were fed

[1] Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 5622. Supported in part by National Science Foundation Grant, GB-5877.

[2] Present address: Human Nutrition Research Division, Agricultural Research Service, Agricultural Research Center, Beltsville, Md. 20705.

diet A,[3] composed of : acid washed casein, 30; cellulose,[4] 15; soybean oil, 4; salts,[5] 4; potassium acetate, 2.7; $NaH_2PO_4 \cdot H_2O$, 0.75; sucrose, 43.45, choline·Cl, 0.1; vitamin and antibiotic supplements.[6] This diet was calculated to contain 0.6% phosphorus and 0.9% calcium and by analysis contained 0.01% magnesium. Diet B, which was based on EDTA-washed soybean protein and contained a slightly modified salt mix,[7] was used in the ultrafiltration studies. The magnesium content was adjusted to 75 ppm so that most animals would survive at least 4 weeks. All control diets were supplemented with MgO to supply 0.3% magnesium.

For kidney clearance studies, animals fed the respective diets for 8 weeks were anesthetized with sodium pentobarbital. A dose of 3.9 mg/100 gm of body weight usually gave satisfactory anesthesia. Water, 3 ml/100 gm of body weight, was administered by stomach tube to insure urine formation. Twenty min after administration of water, the bladder was catheterized. This procedure required care because on occasion the catheter would enter the seminal vesicles. A syringe was attached to the catheter by means of a 22-gauge hypodermic needle and the bladder was rinsed several times with 3 ml of physiological saline. A clean syringe was attached and urine was collected at 15-min intervals for 1 hr. Near the end of the collection period the bladder was again rinsed with saline; the last rinse corresponded to the end of the period. The urine and rinses were combined in a 25-ml volumetric flask. Two drops of concentrated sulfuric acid were added, except that when creatinine was to be determined, acid was omitted until the aliquot for the creatinine determination had been removed.

In the first clearance trials, two consecutive 1-hr collections were made. Blood was withdrawn by cardiac puncture at the midpoint of each collection and the plasma was separated promptly. When both creatinine and phosphorus clearance were determined, only a 1-hr urine collection was made and a large blood sample was withdrawn at the end of the period because of the amount of plasma needed for creatinine determination.

For determination of ultrafiltrable ions, blood was collected by cardiac puncture, transferred immediately to chilled, heparinized centrifuge tubes, and the plasma was separated by centrifugation in the cold. The concentrations of total calcium, magnesium and phosphorus were determined on a protein-free filtrate prepared by dilution of the plasma with 10% trichloroacetic acid (1:10). An ultrafiltrate was prepared by passing plasma through a gel membrane which retained solutes of molecular weights greater than 10,000.[8] All filtration procedures were performed at room temperature and in air. The filters were reused but to eliminate contamination they were first rinsed with distilled water, allowed to stand in 0.1 *M* EDTA solution for 20 min and finally rinsed with doubly distilled water.

Plasma, calcium, and magnesium were determined by atomic absorption spectrometry (15), and urine calcium by permanganate titration (16). Standard methods were used for phosphorus (17) and creatinine (18).

*Results.* Table I summarizes the renal clearance data. The plasma concentrations of creatinine and phosphorus were significantly elevated in the magnesium-deficient animals.

[3] The authors gratefully acknowledge gifts of vitamins and diet supplements from Merck and Co., Rahway, N. J.; American Cyanamid Co., Princeton, N. J.; Hoffmann-LaRoche, Nutley, N. J.; Distillation Products Industries, Rochester, N. Y.; and Dawe's Laboratory, Chicago, Ill.

[4] Solka Floc, Brown Co., Berlin, N. H.

[5] Diet A. salts mix supplied as percentage of the diet: $CaCO_3$, 2.05; $KH_2PO_4$, 0.832; KCl, 0.440; NaCl, 0.432; $FePO_4$-soluble, 0.161; $MnSO_4 \cdot H_2O$, 0.0744; $CuSO_4 \cdot 5H_2O$, 0.00588; $AlK(SO_4)_2 \cdot 12H_2O$, 0.000664; KI, 0.00294; $CoCl_2 \cdot 6H_2O$, 0.000294; $ZnSO_4 \cdot 7H_2O$, 0.00243; and NaF, 0.00392.

[6] Grace, N. D. and O'Dell, B. L., J. Nutr. 94, 166 (1968).

[7] Diet B salts mix supplied as percentage of the diet: $CaHPO_4$, 0.877; $CaCO_3$, 1.85; $Na_2CO_3$, 0.691; KCl, 0.763; Fe–citrate, 0.036; $MnSO_4 \cdot H_2O$, 0.062; $CuSO_4$, 0.0017; $KIO_3$, 0.0013; and $ZnCO_3$, 0.015.

[8] Diaflo syringe ultrafiltration system and UM-1 Diaflo membrane, Amicon Corporation, Cambridge, Mass.

TABLE I. Effect of Magnesium Deficiency on Renal Clearance of Creatinine and Inorganic Phosphorus.

| | Diet A | A + 0.3% Mg |
|---|---|---|
| Plasma conc: | | |
| Creatinine (mg/100 ml)[a] | 0.53 ± 0.02[b] | 0.32 ± 0.04[d] |
| Inorganic P (mg/100 ml)[c] | 9.3 ± 0.5 | 7.4 ± 0.4[d] |
| Renal clearance: | | |
| Creatinine (ml/hr/kg of body wt)[a] | 145 ± 13 | 310 ± 29[d] |
| Inorganic P (ml/hr/kg of body wt)[c] | 35 ± 4 | 64 ± 5[d] |
| Tubular reabsorption of P (%)[a] | 78.2 | 78.6 |

[a] Four animals per dietary treatment.
[b] Mean ± standard error of the mean.
[c] Ten animals fed Mg-deficient and 9 animals the Mg-supplemented diet.
[d] Significantly different from value for Mg-deficient group at $p < .01$.

Both plasma constituents were cleared by the deficient animals at about 50% of the control rate. Based on the assumption that creatinine clearance equals glomerular filtration rate, the percentage of tubular reabsorption of phosphorus was the same for animals fed the two diets. Thus, renal function appears to be impaired in magnesium deficiency primarily by reduction of the glomerular filtration rate. This impairment may be the cause of the elevated blood phosphorus commonly observed in magnesium-deficient guinea pigs.

Whether the kidney dysfunction results from known morphological changes or from a biochemical defect is not known. Unpublished observations had shown that the elevated blood phosphorus falls to near normal within 1–2 weeks after magnesium supplementation. This suggested that magnesium may exert a biochemical function in excretion of phosphorus. To test this hypothesis, magnesium-deficient and control guines pigs were fasted overnight, then placed in metabolism cages and given a 5% glucose solution *ad libitum*. The animals were injected with either sodium sulfate or sodium chloride and urine was collected for calcium and phosphorus analysis. The preexperiment treatment, used to establish control values, was followed 2 days later by injection of magnesium sulfate. Urine was collected over the 0–10-hr and 10–24-hr periods.

The two sodium salts were used during the control period to compare the effects of the sulfate and chloride ions and since the anions had little or no effect on the excretion of calcium and phosphorus, all values were averaged to establish the control values (Table II). Magnesium sulfate treatment stimulated phosphorus excretion in both groups during the first 10-hr period, but only in the deficient group was the difference statistically significant ($p < 0.025$). The excretion of phosphorus during the 10–24-hr period was not influenced by the magnesium sulfate injection. As expected from earlier work (6), calcium excretion was stimulated by magnesium sulfate, the effect being most pronounced in the case of the deficient animals. In the deficient animals, stimulation of calcium excretion continued over the 24-hr period.

As shown in Table II, during the control period the urine volume of the deficient animals was more than twice that of those supplemented with magnesium. Furthermore, magnesium sulfate treatment had no effect on urine volume of the control animals, but it doubled that of the deficient animals.

The concentrations and ultrafiltrable fractions of calcium, magnesium and phosphorus in plasma are shown in Table III. The deficient animals had a significantly lower level of plasma calcium and a significantly higher concentration of total inorganic phosphorus than controls, but there was no effect on the proportion that was ultrafiltrable. Magnesium deficiency caused a marked reduction in the total plasma magnesium and a slight increase in the ultrafiltrable fraction. These results

TABLE II. Effect of Magnesium Injection upon the Urinary Excretion of Calcium and Phosphorus by Magnesium-Deficient and Supplemented Guinea Pigs.

| Diet | 24-hr urine volumes (ml) | Treatment[a] | Urinary calcium (mg/kg of body wt) 0–10 hr | Urinary calcium (mg/kg of body wt) 10–24 hr | Urinary phosphorus (mg/kg of body wt) 0–10 hr | Urinary phosphorus (mg/kg of body wt) 10–24 hr |
|---|---|---|---|---|---|---|
| A (—Mg) | 71 | Av, control | 17.7 ± 2[b] (9)[c] | 9.9 ± 3 (6) | 38.6 ± 4 (9) | 56.1 ± 9 (6) |
| | 139 | $MgSO_4$, exptl. | 32.9 ± 4[d] (9) | 30.3 ± 4[d] (7) | 52.8 ± 3[d] (9) | 56.3 ± 4 (7) |
| A + 0.3% Mg | 35 | Av, control | 11.3 ± 1 (6) | 6.2 ± 1 (5) | 26.9 ± 5 (6) | 60.3 ± 7 (5) |
| | 37 | $MgSO_4$, exptl. | 18.4 ± 3[e] (5) | 7.0 ± 2 (6) | 34.9 ± 4 (5) | 57.2 ± 8 (6) |

[a] $MgSO_4$ given as 0.15 *M* solution to provide 50 mg of $Mg^{2+}$ per kg of body wt; NaCl and $Na_2SO_4$ were also given as 0.15 *M* solutions to provide anions equivalent to $Mg^{2+}$.

[b] Standard error of the mean.

[c] Number of animals shown in parentheses.

[d] Significantly different from the average control period, $p < 0.025$.

[e] Significantly different from the average control period, $p < 0.05$.

are in agreement with those obtained by Hoobler *et al.* (19), who found an increase, although not significant, in the proportion of ultrafiltrable magnesium in serum of deficient dogs.

The plasma samples used for determination of ultrafiltrable ions were prepared by centrifugation in the cold to retard hydrolysis of phosphate esters but were not maintained anaerobic. Prasad and Flink (20) reported that ultrafiltrable calcium values of human sera did not differ when measured under anaerobic and aerobic conditions. However, equilibration of the plasma with a mixture of 5% $CO_2$ and 95% $O_2$ lowered the pH and significantly increased the ultrafiltrable calcium. In this investigation, the average pH values of 6 freshly drawn blood samples maintained under anaerobic conditions were 7.45 and 7.37 for deficient and control guinea pigs, respectively. Plasma, prepared from these samples in the same manner as for the ultrafiltration, had pH values of 7.73 and 7.68. The slightly higher pH of the deficient plasma would, if anything, tend to decrease the percentage of ultrafiltrable calcium and magnesium.

TABLE III. Effect of Magnesium Deficiency on the Concentrations and Proportions of Ultrafiltrable Calcium, Phosphorus, and Magnesium in Plasma.

| Plasma ion | Total (mg/100 ml) | Ultrafiltrable (%) |
|---|---|---|
| Diet B (—Mg)[a] | | |
| Calcium | 10.8 ± 0.5[b] | 58.1 ± 1.6 |
| Phosphorus | 8.7 ± 0.4 | 79.7 ± 1.6 |
| Magnesium | 0.38 ± 0.04 | 79.9 ± 3.0 |
| Diet B + 0.3% Mg[c] | | |
| Calcium | 12.5 ± 0.2[d] | 61.2 ± 1.8 |
| Phosphorus | 6.8 ± 0.3[d] | 78.1 ± 1.4 |
| Magnesium | 2.9 ± 0.16[d] | 71.6 ± 1.2[e] |

[a] Seven animals.

[b] Mean ± standard error of the mean.

[c] Ten animals.

[d] Significantly different from deficient group, $p < .01$.

[e] Significantly different from deficient group, $p < .05 > .02$.

*Discussion.* Providing there is no change in renal blood flow and the rate of intestinal absorption, a lowered renal clearance of phosphorus would cause an elevation of the blood level. The impaired phosphorus clearance observed in this study was associated with a decreased glomerular filtration rate. Reduction in glomerular filtration might result from renal pathology involving structural change but this is not the only factor involved. Magnesium injections produced an immediate and significant increase in excretion of urinary phosphorus in deficient animals, but not a significant increase in the controls. The results are compatible with the concept that

magnesium has a specific biochemical role in the renal clearance of phosphorus.

The effect of magnesium on urinary calcium excretion by the control animals is in agreement with the observations made in other species (6, 19, 21), but the effect on the magnesium-deficient guinea pigs was more dramatic and sustained than in the controls. Clark and Geoffroy (21) demonstrated that a portion of the calcium excreted by rats following magnesium sulfate injection is released from the bones. In view of the metastatic calcification observed in magnesium deficiency, the increased and continued excretion of calcium by the deficient animals may have been due to mobilization of calcium from mineral deposits in the soft tissues.

The assumption that creatinine clearance closely approximates the glomerular filtration rate is supported by the findings of Oyen and Boylan (22). The clearance rate reported here, 310 ml/hr/kg of body weight, for anesthetized guinea pigs is about 50% greater than they found in unanesthetized animals.

In contrast to the rat, magnesium-deficient guinea pigs develop hyperphosphatemia with normal or slightly low serum calcium. This relationship argues against the concept that a state of hyperparathyroidism exists in the magnesium-deficient guinea pig. Although tissue levels of ionic calcium were not determined, it seems unlikely that any pool of ionic calcium would be sufficiently elevated to offer an explanation for the extensive metastatic calcification observed in magnesium deficient guinea pigs. One might expect metastatic calcification when the concentrations of ionic calcium and phosphate exceed the solubility product. The Ca $\times$ P (mg/100 ml) products in the plasma ultrafiltrates, 43.4 and 40.5 for deficient and control animals, respectively, were not sufficiently different to explain the commonly observed extraosseous calcification.

*Summary.* Renal function and ion binding in plasma were studied in magnesium-deficient and control guinea pigs. Renal clearance of creatinine and phosphate in magnesium-deficient guinea pigs was one-half the control rate. Magnesium sulfate, administered intraperitoneally, stimulated phosphate excretion significantly in deficient animals, but not in controls. Impaired renal function may well contribute to the elevated blood phosphorus levels in magnesium-deficient guinea pigs, and magnesium appears to exert a direct biochemical role in phosphate clearance. The percentages of ultrafiltrable calcium and phosphate in plasma were not altered by magnesium deficiency, but because of hypocalcemia and hyperphosphatemia, the concentration of ultrafiltrable calcium was less and of ultrafiltrable phosphate greater in the deficient animals. The concentration of plasma magnesium was markedly reduced but the ultrafiltrable proportion was slightly increased.

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Received March 14, 1969, P.S.E.B.M., 1969, Vol. 132.