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OBSERVATIONS IN EXPERIMENTAL MAGNESIUM DEPLETION *

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Studies of experimental magnesium depletion have been reported in the past (1–8). This report describes a re-evaluation of this problem in which some of the previous observations have been confirmed and extended. In particular these studies were concerned with the interrelationships between magnesium deficiency and the metabolism of potassium, calcium, and phosphorus. In addition, the pathologic alterations of the kidneys were examined. These latter observations will be alluded to here, although they have been reported in an abstract (9), and will be the subject of a more detailed discussion in another publication.

METHODS AND EXPERIMENTAL DESIGN

Female Sprague-Dawley rats were used throughout. The animals were pair-fed by groups a synthetic diet free of sodium, potassium, chloride, phosphate, and magnesium. The composition of this diet is listed in Table I. In additon, each animal received daily by gavage an appropriate electrolyte solution which will be described in specific terms later. Demineralized water was allowed at will.

At the end of each experiment the animals were anesthetized with hexobarbital sodium administered intraperitoneally, and then exsanguinated from the abdominal aorta. Thigh, leg, and lumbar muscles were taken for chemical analysis, and the other tissues obtained for histologic examination. In some experiments total carcass was also analyzed. The preparation of erythrocytes for analysis involved an initial centrifugation in a plastic tube, followed by the removal of plasma and buffy coat. The red cell mass was then recentrifuged at 20,000 *g* for 15 minutes. At the end of this time the tube was frozen, and then a cut was made just below the top of the erythrocyte mass. In this fashion a quantity of relatively pure red cells was obtained.

The chemical methods were as follows: urea nitrogen with the autoanalyzer utilizing the colorimetric reaction with diacetyl monoxime (10); sodium and potassium, with a conventional internal standard flame photometer; chloride by the Cotlove chloridometer (11); total CO_2 content by the method of van Slyke and Neill (12); phosphorus, by the method of Fiske and Subbarow (13). In addition, the concentrations of calcium and magnesium in serum were initially estimated with the titration method of Walser (14). Later, the serum and all of the tissue analyses for calcium and magnesium were made by flame photometry, using the Zeiss spectrophotometer with double monochromator, according to the method of MacIntyre (15). Other methods are described in a previous report from this laboratory (16).

Experiment I. In this experiment two groups of animals were studied for 31 days while they ingested the electrolyte-free diet (EFD). The control and experimental groups received the electrolyte solution referred to in Table II. At the end of 31 days half of each group was killed and the other half was permitted to live, subsisting on an ordinary complete laboratory chow diet for 5 months, at which time they, too, were killed and similar observations were made.

Experiment II. This experiment differed from Experiment I only in that both the control and experimental animals received 4 mmoles of potassium per day instead of 2 mmoles per day. The specific composition of the gavage is presented in Table II.

TABLE I

Constituents of the basal diet

Substance	Per 100 g diet	Substance	Per 100 g diet
Na^+	0.35 *mEq**	Thiamin HCl	1.0 *mg*
K^+	0.76 *mEq**	Riboflavin	1.0 *mg*
Cl^-	2.0 *mEq**	Pyridoxine	1.0 *mg*
Ca^{++}	20.0 *mEq**	Nicotinic acid	10.0 *mg*
Mg^{++}	0.29 *mEq**	Ca pantothenate	10.0 *mg*
Corn oil	14.2 *g*	Biotin	0.1 *mg*
Sucrose	19.0 *g*	Folic acid	0.1 *mg*
Dextrin	40.0 *g*	Inositol	20.0 *mg*
Casein	25.0 *g*	PABA	20.0 *mg*
Choline	0.1 *g*	Vitamin D_2	0.01 *mg*
Cystine	0.2 *g*	Vitamin K	0.50 *mg*
$CaCO_3$	1.0 *g*	Alpha tocopheral acetate	10.0 *mg*
$Fe(C_6H_8O_7)\cdot 6H_2O$	0.1 *g*	Vitamin A	3,000 IU
$MnSO_4\cdot 4H_2O$	0.090 *g*		
$CuSO_4\cdot 5H_2O$	0.012 *g*		
$ZnCl_2$	0.001 *g*		
$CoCl_2\cdot 6H_2O$	0.001 *g*		

* Determined by analysis as described in Methods. All other values are those specified to the manufacturer of the diet but were not ascertained by analysis.

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TABLE II

Composition of electrolyte gavage solution

Experiment	Group	Na	K	Cl	P	Mg
		millimoles per 5 ml				
I, IV	Control	1.25	2.0	2.5	0.75	0.7
	Experimental	1.25	2.0	2.5	0.75	0
II	Control	1.25	4.0	4.5	0.75	0.7
	Experimental	1.25	4.0	4.5	0.75	0
III‡	Control	1.25	0	0.5	0.75	0.7
	Experimental	1.25	0	0.5	0.75	0

Experiment III. This study ran concurrently with Experiment II and included two groups of animals, an experimental and a control with respect to the absence or presence of magnesium in the gavage solution, but in which neither group received potassium. (*See* Table II.) This variation in design was made to permit an evaluation of the question of whether magnesium-deficient rats excrete more potassium in the urine. It was felt that only by eliminating potassium from the dietary regimen would it be possible to detect the small differences that might obtain in urinary excretion.

Experiment IV. In this study the period of depletion was prolonged to 60 days, and the electrolyte content of erythrocytes was examined, along with the other analyses, which included total carcass. (*See* Table II).

Experiment V. The observations in this study were obtained from analyses of intact rat diaphragms that were bathed in solutions which alternatively did and did not contain magnesium. Sprague-Dawley rats weighing 30 to 60 g were used, and the diaphragms were obtained in a manner described by Relman, Gorham, and Levinsky (17). The bath unit was the tank of the Travenol twin coil kidney with a capacity of 100 L. The large size of the bath insured virtually no alteration in its composition over an eight-hour period without having to change the fluid. A Lucite apparatus was fabricated to suspend 24 diaphragms in the center well of the unit. Circulation of the bathing fluid was accomplished by employment of both the circulating and drain pumps. The fluid was gassed with a mixture of 95% oxygen and 5% CO_2 at a rate of flow of 5 L per minute. The temperature of the bath was maintained at 37° C, and the pH and calculated pCO_2 ranged between 7.38 to 7.40 and 39 to 41 mm Hg, respectively. The composition of the bath is described in Table VII. In addition, the bath contained glucose at a concentration of 100 mg per 100 ml and phosphorus at 3.7 mg per 100 ml; bicarbonate made up the anion not otherwise described in Table VII. The bath also contained chloramphenicol, penicillin, and streptomycin in the same concentrations used by Relman and associates (17). Bacterial growth at the end of eight hours was negligible as confirmed by culture.[1]

[1] The authors are indebted to Parke, Davis & Company, Detroit, Mich., and Merck Sharp & Dohme, Philadelphia, Pa., for their contributions of the antibiotics.

TABLE III

*Mean serum values**

Experiment	Group	Na	K	Cl	CO_2	Mg	Ca	P	Urea N
		mEq/L							*mg/100 ml*
I	Control [9]	144 ±1.9	3.8 ±0.4	106 ±2.7	21 ±2.5	1.85 ±.16†	5.0 ±.35†		25 ±3.5†
	Experimental [7]	148 ±2.4	3.8 ±0.3	106 ±1.8	21 ±2.5	0.64 ±.19	5.4 ±.27		33 ±4.8
II	Control [10]	142 ±1.9	4.3 ±.36	107 ±2.6	22 ±4.0	1.99 ±.30†	4.9 ±.13†		14 ±2.6†
	Experimental [10]	142 ±2.3	4.4 ±.44	105 ±2.4	22 ±3.9	0.60 ±.39	5.3 ±.20		22 ±2.4
III	Control [7]	141 ±2.7†	2.4 ±.29	90 ±4.4	32 ±3.7	2.04 ±.27†	4.6 ±.19‡		23 ±4.3
	Experimental [7]	146 ±1.7	2.6 ±.34	90 ±2.9	37 ±3.6	0.82 ±.31	5.0 ±.40		28 ±5.9
IV	Control [6]	145 ±2.1	3.2 ±.44	105 ±1.9	21 ±2.9	1.63 ±.12†	4.9 ±.16†	4.9 ±.65	
	Experimental [8]	145 ±1.9	3.7 ±.64	104 ±2.6	22 ±2.8	0.34 ±.07	5.4 ±.22	4.8 ±.64	

* All data given as mean ± SD.
† Significantly different at $p < .01$.
‡ Significantly different at $p < .02$.

TABLE IV

Muscle data

Experiment	Group	Na	K	Cl	Mg	Ca	Nai	Total water	Chloride space
		mEq/100 g fat-free dry solids						*g/100 g FFDS*	
I	Control [9]	8.6 ±.68*	43.7 ±1.3*	4.6 ±.21*	10.14 ±.31	1.52 ±.24	2.9 ±.97	310 ±4.5	39 ±2.1*
	Experimental [7]	10.1 ±.95	41.0 ±.48	5.8 ±.63	10.0 ±.75	2.10 ±.97	2.8 ±.94	317 ±8.2	48 ±5.5
II	Control [10]	7.7 ±.33	44.4 ±1.2*	4.9 ±.23	9.9 ±.26*	1.48 ±.28	1.9 ±.56‡	325 ±4.5	41 ±2.2
	Experimental [10]	9.1 ±1.1	42.0 ±2.0	5.3 ±.81	9.6 ±.20	1.43 ±.23	2.7 ±.77	323 ±6.3	45 ±5.9
III	Control [7]	18.1 ±2.4	29.5 ±2.7*	4.2 ±.16	10.2 ±.29†	1.58 ±.73	12.1 ±2.51	300 ±6.5	42 ±1.8‡
	Experimental [7]	21.2 ±3.3	24.0 ±2.5	4.5 ±.29	9.7 ±.47	1.84 ±.85	14.4 ±3.31	296 ±5.2	45 ±3.5
IV	Control [12]	9.4 ±.67*	41.3 ±1.7*	4.8 ±.36*	9.6 ±.59*	1.23 ±.54	3.3 ±.99	311 ±8.9	40.8 ±2.5*
	Experimental [11]	12.6 ±.87	36.7 ±1.1	7.7 ±.90	8.3 ±.50	1.50 ±.31	2.9 ±.76	333 ±7.2	66 ±4.1

* Significantly different at $p < .01$.
† Significantly different at $p < .02$.
‡ Significantly different at $p < .05$.

After equilibration of the bath fluid, the intact diaphragms were rapidly prepared, rinsed in normal saline, and then suspended in the bathing fluid. The exposure to the bath was terminated after eight hours, at which time the diaphragms were rinsed with 5% dextrose in water, dissected, blotted, and placed in tared weighing vessels. Three pools of eight diaphragms each were made to permit all the chemical analyses with ease. Eight diaphragms were prepared in a similar manner, but were placed in a tared weighing vessel without prior exposure to the bath fluid to provide data concerning the initial composition of the diaphragm muscle. The tissues were dried for 72 hours at 100° C, the tissue was ground, and an approximately 20-mg sample was turned to ash in a platinum crucible at 400° C for 24 hours for determination of magnesium. The remainder was extracted in 0.75 normal HNO_3 for three days, and the supernate employed for the determination of sodium, potassium, and chloride.

RESULTS

Peripheral vasodilatation and hyperemia of the ears were noted regularly in the magnesium-deficient animals. These changes appeared between days 6 and 10 of the regimen of magnesium depletion and persisted for varying periods of one to four days. Some of the animals manifested evidences of neuromuscular irritability, which included convulsive movements. There were no differences in the weight gained between the experimental and control groups of any paired study.

Experiment I. The results of the chemical analyses of serum are presented in Table III. It will be noted that whereas the concentrations of sodium, potassium, chloride, and total CO_2 content are the same in the two groups, there is a significant hypomagnesemia, hypercalcemia, and modest azotemia in the magnesium-depleted group. Examination of the muscle data in Table IV reveals a small but statistically significant muscle potassium depletion and an expanded "chloride space" in the magnesium-deficient group. There was no increase in calculated intracellular sodium in the magnesium-depleted group. The urinary excretion of phosphorus was markedly augmented in the magnesium-depleted group, as may be seen in Figure 1.

The chemical data from both serum and muscle in the control and experimental groups were normal after five months of the repletion regimen.

Experiment II. The serum and muscle data

TABLE V

Total carcass analyses

Experiment	Group	Na	K	Cl	Mg	Ca	Total water
		mEq/100 g fat-free dry solids					*g/100 g FFDS*
III	Control [8]	25.8 ±.75	21.2 ±.88	10.9 ±.55	18.2 ±2.3*	158 ±13	282 ±3
	Experimental [6]	28.5 ±2.8	21.4 ±.95	11.4 ±1.3	10.7 ±.85	150 ±29	295 ±16
IV	Control [3]	19.0 ±1.0*	30.1 ±1.6*	12.5 ±.45	11.6 ±.61*	129 ±37	257 ±8
	Experimental [6]	22.6 ±.83	25.3 ±1.3	13.4 ±.85	7.5 ±.69	191 ±47	250 ±5

* Statistically significant at $p < .01$.

are presented in Tables III and IV, respectively. Here again are noted the same similarities and differences in the serum values; and, once again, there is a small but statistically significant muscle depletion of potassium in the magnesium-depleted group. It should be emphasized that these animals received 4 mmoles of potassium per day in their gavage. On this occasion there was a small difference in the calculated intracellular sodium. The cumulative excretions of nitrogen in the urine were calculated. The control group excreted an average of 6.497 g of nitrogen, compared with 7.124 g for the experimental group. This difference was not statistically significant.

Experiment III. In this study, where neither the control nor magnesium-deficient group received potassium, hypomagnesemia and hypercalcemia are noted in the magnesium-depleted group. (*See* Tables III and IV.) Hypokalemia and an increased total CO_2 content are noted in each group, but these changes are not significantly different from each other. There is also a modest azotemia in each group. There is evidence of muscle potassium depletion in both groups, and although the degree of hypokalemia was the same in each, the intensity of muscle potassium depletion is clearly greater in the magnesium deficient animals.

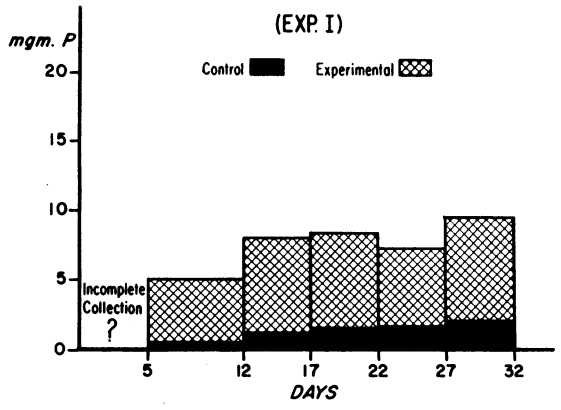

FIG. 1. MEAN DAILY URINARY EXCRETION OF PHOSPHORUS IN THE CONTROL GROUP COMPARED WITH THE EXPERIMENTAL GROUP. The urines were collected in five-day batches. The first five-day period was unavoidably incomplete. The excretion of phosphorus was unequivocally higher in the magnesium-depleted group.

The analyses of the total carcass are presented in Table V, and here no difference in potassium is noted, but there is a highly significant difference in magnesium. Although these animals had hypercalcemia, the total carcass calcium was not increased.

The daily excretion of potassium was estimated in this experiment, and these data are presented in Figure 2, where the cumulative excretion of potassium is plotted against time for each group. The augmented excretion of potassium in the magnesium-deficient animals was evident beginning with day 7 of the deficient regimen; and it was significantly different at a $p < .01$ throughout the remainder of the study, except for days 12 and 16. Furthermore, the cumulative excretion of potassium was greater in the magnesium deficient group at a $p < .01$ level by the end of twenty-one days.

The mean daily urinary excretion of phosphorus is depicted in Figure 3, where it may be noted that the magnesium-deficient animals excreted

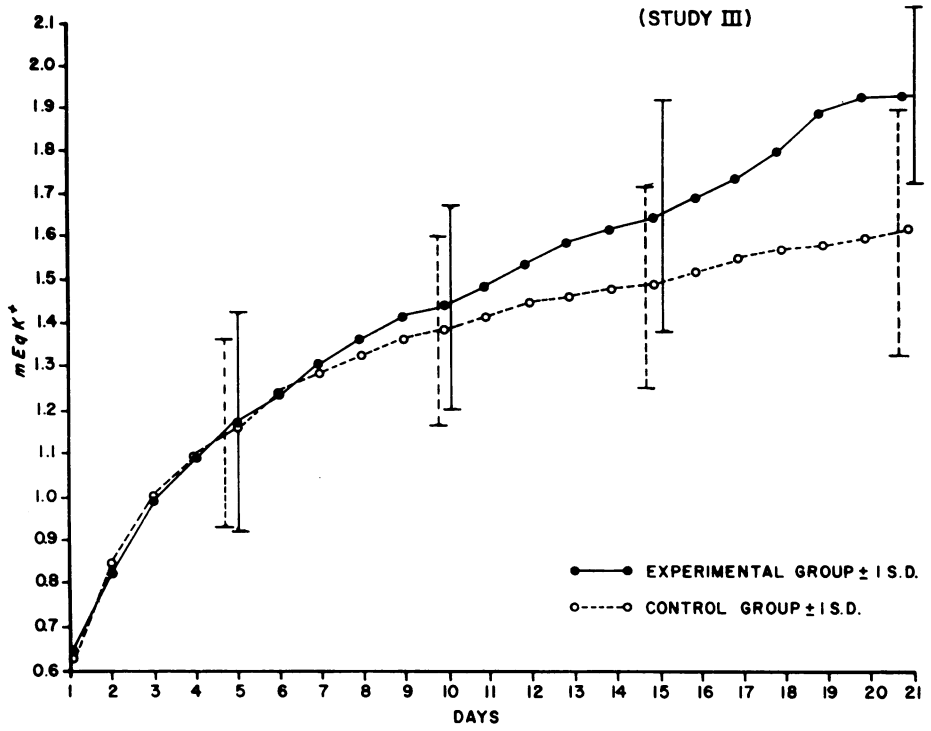

FIG. 2. CUMULATIVE URINARY EXCRETION OF POTASSIUM IN THE CONTROL AND EXPERIMENTAL GROUPS WHEN NEITHER GROUP RECEIVED POTASSIUM. The excretion of potassium was unequivocally higher in the magnesium-depleted group and at a level of $p < .01$ at day 21.

much more phosphorus than their controls. The magnitude of the difference in the urinary excretion of phosphorus was suggestively greater in Experiment III when compared with Experiment I. Such a difference could be ascribed to some influence of the simultaneous major potassium depletion which was induced in Experiment III. For this reason, the urinary excretion of phosphorus in Experiments II and III were compared, and these data are shown in Figure 4. In both experiments the absence of potassium in the gavage regimen per se promoted a greater cumulative loss of phosphorus into the urine. These differences are denoted by the shaded areas and are of statistical significance (i.e., $p < .02$ in the groups without magnesium, and $p < .05$ in the groups with magnesium.)

Experiment IV. Tables III, IV, V, and VI describe the data from this study. The same qualitative changes are again noted in the serum and muscle analyses. The serum phosphorus levels were estimated in this study and were found not to be different in the two groups. In this experiment the analyses of whole carcass (Table V) revealed a significant difference in potassium as well as magnesium, and again, there was no difference in total carcass calcium.

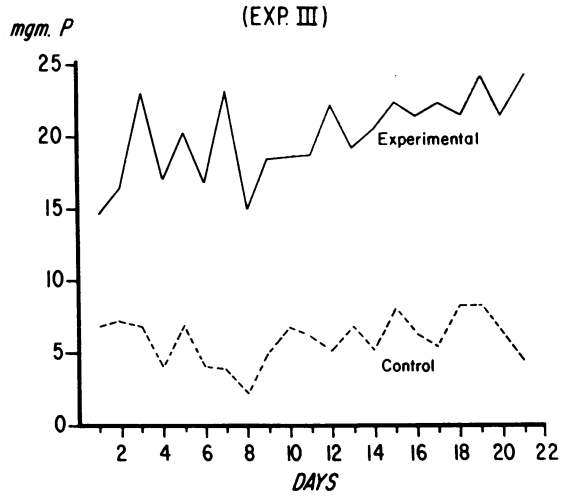

FIG. 3. MEAN DAILY URINARY EXCRETION OF PHOSPHORUS IN THE CONTROL AND EXPERIMENTAL GROUPS WHEN NEITHER GROUP RECEIVED POTASSIUM. Not only did the magnesium-depleted group excrete more phosphorus, but both groups appeared to excrete more when compared with the data of Experiment I (*see* Figure 1).

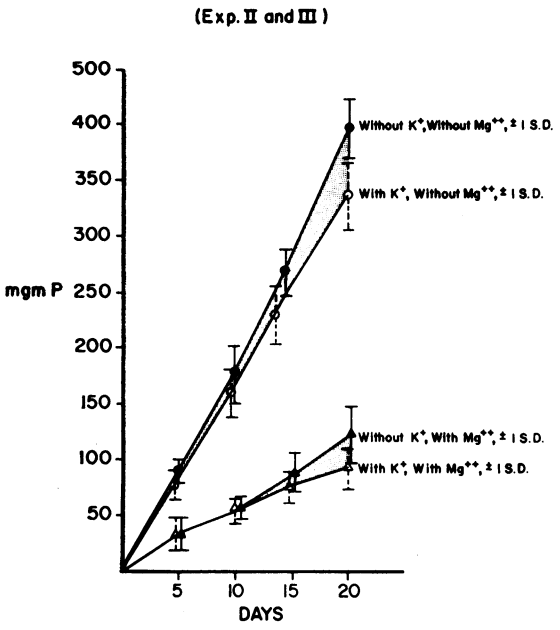

FIG. 4. CUMULATIVE URINARY EXCRETION OF PHOSPHORUS IN GROUPS WITH AND WITHOUT MAGNESIUM AND WITH AND WITHOUT POTASSIUM. It appears that a deficiency of both potassium as well as magnesium promotes an augmented urinary excretion of phosphorus. When the two magnesium-deficient groups are compared, the cumulative difference is at a level of $p < .02$; when the two groups which received magnesium are compared, the cumulative difference is at a level of $p < .05$; when the groups with magnesium are compared with those without, the cumulative difference is at a level of $p < .01$.

The composition of erythrocytes is described in Table VI. There are only two differences noted between the control and experimental groups. There was less magnesium and more sodium in the red cells of the magnesium-deficient animals. There was no alteration in the concentration of red cell potassium, whether this is expressed in terms of dry weight or cell water.

Experiment V. Table VII presents the data from this experiment. The analyses of the diaphragms that were not exposed to the bath fluid agree well with those of Relman and associates (17), except that the chloride values are higher than theirs. The muscle potassium content of these control diaphragms also agrees with Relman and co-workers (17) and is not significantly different from the values of the control diaphragms of Experiment V B, which provides some assurance that the *in vitro* technique permitted the cells to retain potassium under appropriate circumstances of bath fluid composition.

In Experiment V A both sets of diaphragms lost potassium and gained sodium, as would be anticipated with no potassium in the bath fluid (17). Nevertheless, the group with the magnesium-free bath had a significantly lower potassium than that bathed in the presence of magnesium. Since this experiment involved some rather drastic alterations, a second set was done, utilizing a bath with a 1 mM concentration of potassium with and without magnesium. Relman and co-workers (17) had previously reported that at this bath concentration these preparations of diaphragms maintain potassium levels quite well. This is confirmed in this study when the bath contained magnesium. In its absence, however, the diaphragms had a lower quantity of potassium. Furthermore, the calculated intracellular sodium was significantly increased in the potassium-depleted diaphragms. Exception must be taken to the complete validity of this calculation, since it is based on an estimate of the chloride space and this latter datum is then used to calculate the quantity of sodium in the extracellular phase. Using this *in vitro* design, Relman and co-workers (17) demonstrated that chloride enters cells when they em-

TABLE VI

Erythrocyte analyses

Experiment	Group	K	Na	Mg	Water	K	Na	Mg
		mEq/100 g red cell solids			*g/100 g RCS*	*mEq/L red cell water*		
IV	Control [9]	27.2 ±1.1	1.87 ±.15*	1.05 ±.09*	172 ±5.0	158 ±4.0	10.9 ±.88*	6.1 ±.42*
	Experimental [15]	27.2 ±1.5	2.46 ±.18	0.55 ±.11	169 ±11.5	161 ±5.4	14.6 ±1.4	3.2 ±.52

* Statistically significant at $p = < .01$.

TABLE VII

Diaphragm muscle data

Experiment	Group	Composition of bath					Diaphragm muscle				
		Na	K	Cl	Mg	Ca	Na	K	Cl	Mg	Nai
		mEq/L					*mEq/100 g FFDS*				
V A	Control [9]	140	0	114	2	2.6	53.7 ±3.3	5.8 ±.81*	19.8 ±2.9	8.58 ±.84*	29.5 ±0.9
	Experimental [9]	140	0	117	0	2.6	56.3 ±4.1	4.1 ±.88	22.5 ±2.2	6.69 ±1.31	29.1 ±2.6
V B	Control [6]	141	1	118	2	2.6	17.1 ±0.4	39.1 ±1.4†	10.3 ±0.7	9.08 ± .28*	4.86 ±.54‡
	Experimental [6]	141	1	117	0	2.6	20.3 ±1.4	36.6 ±1.3	12.1 ±1.1	8.00 ± .32	5.70 ±.57
	Fresh intact diaphragm muscle [10]						12.1 ±1.2	39.0 ±1.6	8.3 ±1.2	8.57 ±0.47	

* Statistically significant at p = < .01.
† Statistically significant at p = < .02.
‡ Statistically significant at p = < .05.

ployed inulin to estimate the size of the extracellular space. The significance of the calculated increase in intracellular sodium is supported by this observation, since to the extent that chloride penetrates cells, and is used to estimate extracellular volume, it tends to diminish and mask a calculated increase in intracellular sodium. Hence, this calculation is a minimal value—unless, of course, the penetration of chloride into cells is less when magnesium is absent from the bath.

In each part of this experiment there was an unequivocal loss of magnesium when the bath was free of this cation.

Pathologic alterations. There was nephrocalcinosis in the magnesium-depleted groups which appeared to be confined initially to the intraluminal portion of the ascending limb of Henle's loop (9). The intensity of the nephrocalcinosis was greatest in those animals receiving no potassium. The lesions persisted in the repleted group of Experiment I, but the intensity of the calcium deposit appeared to be diminishing.

DISCUSSION

The potassium depletion which accompanies magnesium depletion is unique in that it persists despite large intakes of potassium; it is unassociated with hypokalemia and increased total CO_2 content, and only rarely associated with an increase in the calculated intracellular sodium of muscle cells. Since there is an anatomic renal lesion and clear evidence of an augmented urinary excretion of potassium, it is tempting to ascribe the depletion to a defect in the renal conservation of potassium. This hypothesis appears less attractive, however, when one recalls that in conditions of potassium depletion that are a consequence of a primary renal loss such as excessive mineralocorticoid (18), or large sodium loads (18), or intrinsic renal tubular defects (19), the sequence of events is usually characterized by an extracellular deficit (reflected by some degree of hypokalemia) which is followed by a loss from cellular stores. The consistent lack of difference in the level of potassium in the serum between the control and magnesium-depleted groups suggested the alternative hypothesis that magnesium deficiency may have an influence on the ability of the cells to maintain an appropriate potassium gradient. The consequence of such a disability would be the loss of potassium from the cells, and the kidneys would excrete this in accordance with those mechanisms that maintain the concentration of potassium in extracellular fluid at a normal level. This hypothesis has other attractive features in view of the demonstration in recent years that membrane ATPase may be intimately involved in the linked transport of sodium and potassium and the fact that the activity of this enzyme is dependent on magnesium (20–23).

It was the considerations alluded to above concerning the possibility that magnesium deficiency might influence potassium loss by an effect on the cells' ability to maintain a potassium gradient that led to the design of Experiment V. The loss of

diaphragm muscle potassium when magnesium was absent from the bathing medium was significant. In addition, in Experiment V B there may have been an increase in intracellular sodium as well. These data are consistent with the hypothesis that the alteration in potassium levels in muscle in magnesium deficiency is a consequence of some effect at the tissue level rather than a defect in renal conservation.

Ginn and Cade (24) have suggested that the loss of potassium is a consequence of an increased secretion of aldosterone in magnesium deficiency. A primary influence of this mineralocorticoid seems unlikely in view of the discussion above. It may be, however, that the release of potassium from cells serving as an extracellular "load" of potassium might be responsible for an increased secretion of aldosterone (25) which, in turn, might participate in those mechanisms responsible for the urinary excretion of the "load" of potassium.

One other possibility is that the augmented potassium excretion is related to the hypercalcemia rather than to magnesium depletion. This must be suggested in view of the observations by Ferris, Levitin, Phillips, and Epstein (26) that the administration of vitamin D promotes an increased excretion of potassium. This alteration was accompanied by a muscle deficit of potassium, but there was no difference between the control and experimental group in the level of potassium in the serum. The force of this argument is diminished somewhat by another observation (27), that vitamin D also promotes hypomagnesemia, although Ferris and associates (26) looked for but did not observe this alteration.

The mechanism responsible for the hypercalcemia associated with magnesium depletion is elusive. Alcock and MacIntyre (28) suggest that magnesium and calcium compete for a common absorptive mechanism in both the gastrointestinal tract and in the renal tubule. In this fashion, the less magnesium available for reabsorption, the more calcium will be reabsorbed, thereby promoting hypercalcemia. Alternative hypotheses suggest that this may be due to a redistribution of calcium between bone and extracellular fluid. The failure to demonstrate an increased quantity of calcium in the total carcass certainly provides no support for the suggestion that there is an increased absorption from both the gastrointestinal tract and the renal tubular lumen.

Since this hypercalcemia is accompanied by a striking phosphaturia, the possibility must at least be considered that there may be a state of hyperparathyroidism. The lack of hypophosphatemia argues against this. Furthermore, it is apparently not due to a major catabolic influence, as testified by the lack of difference in the urinary excretion of nitrogen in the magnesium-depleted and control animals of Experiment II. It is possible that the loss of phosphorus simply reflects a diminution in the quantity of those phosphates that are ordinarily associated intracellularly (or in bone) with magnesium (and potassium). In this fashion phosphorus would leave cells (or bone) and find its way into the urine by the renal mechanisms responsible for maintaining a normal level of phosphorus in serum.

The azotemia is most likely a reflection of altered renal function, specifically a depressed glomerular filtration rate. This parameter has not been measured, however, and it is possible that there was an increased production of urea. This latter seems unlikely in view of the lack of difference in nitrogen excretion or body weight gain.

The mechanisms responsible for this unique renal lesion are also unknown and will be discussed in more detail in a subsequent publication. The lesion persisted following repletion, although there are data to suggest that the deposit of calcium diminished.

SUMMARY AND CONCLUSIONS

1. The characteristics of magnesium depletion as observed in these experiments are: *a*) hypomagnesemia and a diminution in erythrocyte, muscle, and total carcass magnesium; *b*) a consistent hypercalcemia; *c*) a consistent depletion of muscle potassium with no difference in serum levels of potassium or total CO_2 content and only rarely an increase in calculated intracellular sodium—in prolonged magnesium deficiency, evidence of potassium depletion by analysis of total carcass as well; *d*) an increased urinary excretion of potassium; *e*) an increased urinary excretion of phosphorus with no alteration in the serum level of phosphorus; *f*) no increases in urinary excretion of nitrogen with augmented urinary excretion

of either potassium or phosphorus; *g*) azotemia in association with a renal lesion characterized by intraluminal deposits of calcium.

2. The possible mechanisms for these alterations have been discussed, and reasons have been presented for considering the potassium loss as a consequence of an influence of magnesium at the tissue level, rather than as due to a renal tubular defect in conserving potassium. This hypothesis is supported by the fact that intact diaphragms that are bathed in a solution to which no magnesium has been added lose potassium, in contrast to their controls.

REFERENCES

1. Kruse, H. D., E. R. Orent, and E. V. McCollum. Studies of magnesium deficiency in animals. I. Symptomatology resulting from magnesium deprivation. J. biol. Chem. 1932, **96**, 519.
2. Tufts, E. V., and D. M. Greenberg. I. The biochemistry of magnesium deficiency. I. Chemical changes resulting from magnesium deprivation. J. biol. Chem. 1937–38, **122**, 693.
3. Greenberg, D. M., S. P. Lucia, and E. V. Tufts. The effect of magnesium deprivation on renal function. Amer. J. Physiol. 1938, **121**, 424.
4. Barron, G. P., S. O. Brown, and P. B. Pearson. Histological manifestations of a magnesium deficiency in the rat and rabbit. Proc. Soc. exp. Biol. (N. Y.) 1949, **70**, 220.
5. Lowenhaupt, E., M. P. Schulman, and D. M. Greenberg. Basic histologic lesions of magnesium deficiency in the rat. Amer. J. Physiol. 1950, **49**, 427.
6. Cotlove, E., M. A. Holliday, R. A. Schwartz, and W. M. Wallace. Effects of electrolyte depletion and acid-base disturbance on muscle cations. Amer. J. Physiol. 1951, **167**, 665.
7. MacIntyre, I., and D. Davidsson. The production of secondary potassium depletion, sodium retention, nephrocalcinosis, and hypercalcemia by magnesium deficiency. Biochem. J. 1958, **70**, 456.
8. O'Dell, B. L. Magnesium requirement and its relation to other dietary constituents. Fed. Proc. 1960, **19**, 648.
9. Whang, R., J. Oliver, M. MacDowell, and L. G. Welt. The renal lesion of magnesium depletion. Clin. Res. 1962, **10**, 257.
10. Marsh, W. H., B. Fingerhut, and E. Kirsch. Determination of urea nitrogen with the diacetyl method and an automatic dialyzing apparatus. Amer. J. clin. Path. 1957, **28**, 681.
11. Cotlove, E., H. V. Trantham, and R. L. Bowman. An instrument and method for automatic, rapid, accurate, and sensitive titration of chloride in biologic samples. J. Lab. clin. Med. 1958, **51**, 461.
12. Van Slyke, D. D., and J. M. Neill. The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I. J. biol. Chem. 1924, **61**, 523.
13. Fiske, C. H., and Y. Subbarow. The colorimetric determination of phosphorus. J. biol. Chem. 1925, **66**, 375.
14. Walser, M. Determination of free magnesium ions in body fluids. Improved methods for free calcium ions, total calcium, and total magnesium. Analyt. Chem. 1960, **32**, 711.
15. MacIntyre, I. Flame photometry. Advanc. clin. Chem. 1961, **4**, 1.
16. Hollander, W., Jr., R. W. Winters, T. F. Williams, J. Bradley, J. Oliver, and L. G. Welt. Defect in the renal tubular reabsorption of water associated with potassium depletion in rats. Amer. J. Physiol. 1957, **189**, 557.
17. Relman, A. S., G. W. Gorham, and N. G. Levinsky. The relation between external potassium concentration and the electrolyte content of isolated rat muscle in the steady state. J. clin. Invest. 1961, **40**, 386.
18. Seldin, D. W., L. G. Welt, and J. H. Cort. The role of sodium salts and adrenal steroids in the production of hypokalemic alkalosis. Yale J. Biol. Med. 1956, **29**, 229.
19. Reynolds, T. B. Observations on the pathogenesis of renal tubular acidosis. Amer. J. Med. 1958, **25**, 503.
20. Skou, J. C. The influence of some cations on an adenosine triphosphatase from peripheral nerves. Biochim. biophys. Acta (Amst.) 1957, **23**, 394.
21. Skou, J. C. Further investigations on a Mg^{++} and Na^{+}-activated adenosintriphosphatase, possibly related to the active, linked transport of Na^{+} and K^{+} across the nerve membrane. Biochim. Biophys. Acta (Amst.) 1960, **42**, 6.
22. Post, R. L., C. R. Merritt, C. R. Kinsolving, and C. D. Albright. Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte. J. biol. Chem. 1960, **235**, 1796.
23. Dunham, E. T., and I. M. Glynn. Adenosinetriphosphatase activity and the active movements of alkali metal ions. J. Physiol. 1961, **156**, 274.
24. Ginn, H. E., and R. Cade. Aldosterone in magnesium deficient rats. Physiologist 1961, **4**, 40.
25. Davis, J. O. The control of aldosterone secretion. Physiologist 1962, **5**, 65.
26. Ferris, T. F., H. Levitin, E. T. Phillips, and F. H. Epstein. Renal potassium-wasting induced by vitamin D. J. clin. Invest. 1962, **41**, 1222.
27. Richardson, J. A., W. D. Huffines, and L. G. Welt. The effect of coincident hypercalcemia and potassium depletion on the rat kidney. Submitted for publication.
28. Alcock, N., and I. MacIntyre. Interrelation of calcium and magnesium absorption. Biochem. J. 1960, **76**, 19 p.