MINERAL METABOLISM ON A CALCIUM DEFICIENT DIET

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DEFICIENT DIET

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INTRODUCTION

Calcium was conceivably the first element recognized by man as being associated with living matter. The association of skeletal remains and the presence of calx in these remains was probably performed early in the beginning of inductive reasoning. In spite of the early recognition of calcium and its importance in living systems, there exists a large void of specific knowledge and understanding regarding its biological functions. Perhaps of more importance is the void of knowledge in the nutritional factors surrounding calcium metabolism in maintenance of the physiologically mature animal. The ultimate aim of the nutritionist is to provide nutrients at dietary levels whereby their essential functions are performed without undue stress to the organism.

In nutrition, calcium deficiency is a term that is usually carefully avoided. Rather, it is referred to as insufficient intakes of calcium and even though these are commonly reported, calcium deficiency *per se* has yet to be described.

Nutrition research is handicapped by a lack of reliable estimates for the metabolic sufficiency or insufficiency of a nutrient. This is particularly true regarding the mineral status of a physiologically mature animal. The purpose of

this investigation was to study the effect of a calcium-deficient diet upon the status of other minerals in the blood, excreta, and bodies of rats.

LITERATURE REVIEW

Calcium is present in the diet in large quantities. Since it is available as limestone, which is easily mined from shallow deposits, it provides an economical component of the diet. Because of these factors most investigations have considered the effect of excessive quantities of calcium upon the absorption and assimilation of other minerals. The amount of calcium in the diet is quite variable between and within a species and is affected by the existence of factors which influence absorption and utilization of calcium by the organism. Factors which affect the absorption and utilization of calcium include: direct and indirect relationships between calcium and other mineral nutrients; hydrogen ion concentration of the lumen of the gastrointestinal tract; specific mechanisms involved in absorption; ingredients in the diet, other than minerals, that may accelerate or inhibit absorption; and age of the animal.

The subject of mineral interrelationships is one of great complexity. It has been shown that a high P:Ca ratio, if the intake of calcium is low, reduces calcium retention (Hansard and Plumlee, 1954). On the other hand, increasing the ratio of Ca:P in the diet impairs utilization of phytate phosphorus (Mitchell, 1947). However, wide Ca:P ratios have been used if there were adequate levels of phorphorus

and vitamin D (Schohl and Farber, 1951; Mitchell, 1955; Cramer et al., 1956; Arrington and Davis, 1955; Lewis et al., 1951). Swine on high calcium diets (1.5%) were observed to develop typical zinc-deficiency symptoms of parakeratosis (Tucker and Salmon, 1955). Numerous investigations have indicated that high levels of calcium in the diet interfere with zinc utilization (Hoekstra et al., 1956; Lewis et al., 1957; Forbes, 1960).

Calcium absorption is augmented by vitamin D (Nicolaysen, 1953), increased acidity (McCance, 1953), certain amino acids, and perhaps citrate (Steggerda, 1946). Calcium in milk is more efficiently absorbed presumably due to the enhancing effect of lactose (Lengeman et al., 1957). Age has been shown to be an important factor upon influencing the intestinal absorption of calcium (Hansard et al., 1954).

The exact calcium requirements of man are not known and extensive studies of various types such as basic studies of calcium metabolism and physiology of bone, feeding trials, epidemiological studies, etc. are recommended in an attempt to obtain some definitive answer (Hegsted, 1962). Duncan (1958), in a literature survey, compared metabolism data of Ca and P to that obtained by complete chemical analysis of bodies of cattle and sheep and found discrepency. The author suggested that to estimate the requirement of these minerals one needs analysis of representative bodies at different stages, from which increments or losses can be measured.

Perhaps the first purified diet containing a low level (0.003%) of calcium was fed by Martin (1937) and the following calcium deficiency symptoms were observed: widespread hemorrhages, prolongation of coagulation time, inflamation of the gastrointestinal tract, and osteoporosis. In a later experiment, Boetler and Greenberg (1941) confirmed the results of the above experiment and in addition observed the following symptoms: retarded growth after 4 to 5 weeks and after 7 to 10 weeks the animals exhibited a decreased sensitivity and reactivity. During this period serum calcium fell to 5 mg/100 ml without any occurrence of tetany. At autopsy, extravasation of blood was prominent in the nervous tissues. Rats maintained on a calcium-deficient diet have been shown to exhibit a higher fasting catabolism than calcium-supplied controls (Kleiber et al., 1940).

The effects of calcium depletion on the chemical composition of bone in the laying hen have been studied (Taylor and Moore, 1956). Mineral depletion was associated with decreases in bone calcium, carbonate, and citrate; the changes being most intent in medullary bone. They found no correlation between the degree of mineral depletion and the ash content of individual bones, and concluded that loss of minerals from skeleton is affected by bone resorption in contrast to bone demineralization which is not accompanied by resorption of the organic phase.

The approaches to studying calcium depletion in the diet have to the present time neither assessed nor defined

calcium deficiency. This is especially true regarding the physiologically mature animal. Many investigators have studied the effect of age and sex upon mineral composition of total animal bodies. The calcium content of the body in relation to age, growth, and food was studied (Sherman and MacLeod, 1925). For the normal rat, the percentage calcium content of live animal weight was found to increase from 0.25 per cent at birth to 1.2 per cent in the adult animal. Virgin females showed higher percentages of calcium than did males of the same age. The increase in weight and percentage of calcium was found to be rapid up to 90 days with a slower, steady rate from 90 days to 8 months. Animals which received low levels of calcium in their diets lost calcium from their bodies. Some animals continued to grow on the low calcium diets.

Another investigation was made on the influence of different levels of calcium upon the normal increase of calcium in the growing body (Lanford et al., 1941) at a given age. Increasing the calcium content of the diet from 0.35 to 0.48 or 0.64 per cent resulted in successive increases in percentage of body calcium at a given age. Rats on the 0.64 per cent calcium diet had a body percentage calcium at 2 months which was not achieved until 50 days later in rats on a 0.35 per cent diet. In a similar study animals maintained on a diet containing 0.16 per cent calcium grew at practically the same rate as animals on diets containing 0.50 per cent calcium (Sherman and Booher, 1931). Those receiving the low

calcium diet appeared to be as well nourished as those reared on the richest calcium diet, but chemical analysis revealed that their bodies were calcium-poor.

Studies of the effect of growth and development of the composition of mammals have provided valuable data regarding the mineral composition of the total body of various species (Spray and Widdowson, 1950).

These data indicate that it is possible for an animal to exhibit normal growth patterns and appearance of good health with the coexistence of a calcium-poor condition in the body. The fast rate of growth of experimental and farm animals and the cultural advances in human food technology and its effect upon growth rates of children makes the study of calcium metabolism of considerable importance. Unfortunately, the approach of total animal body mineral measurements have not been used extensively to study the effects of different diets upon the mineral status of an animal. Such an approach should certainly establish the nature and extent of any deficiency of mineral in relation to total body composition.

The objective of the investigation reported herein was to study the effect of depleting calcium in the diet upon the mineral metabolism of rats.

EXPERIMENTAL

Ninety rats, divided equally by sex, averaging 50 days of age and 120 grams, were allotted within sex to five groups of 18 rats per group. Each group was then subdivided by sex into 6 subgroups of three rats per group, with each of these being treated as an experimental unit for feeding, blood, carcass and excreta analysis. All animals were then fed the diet shown in Table I, with the exception that 1.5% $CaCO_3$ replaced an equal amount of starch during a 7-day adjustment period. At the end of this period 18 rats were sacrificed and their bodies and blood were analyzed for calcium, phosphorus, magnesium, sodium, potassium, iron, zinc and copper. This procedure was used to obtain initial levels of these elements.

The remaining rats were fed the calcium-deficient diet (Table I) until sacrificed; six subgroups being randomly selected and then sacrificed at the end of each subsequent 7-day period. Thus 18 rats were sacrificed at 0, 7, 14, 21 and 28 days after the experiment was initiated. Animals had free access to feed and water. Feces and urine were collected from each subgroup only during the week prior to their being sacrificed.

Blood, obtained by exsanguination of each rat, was heparinized and then pooled by subgroup for per cent packed

TABLE I

PERCENTAGE COMPOSITION OF DIET

Component	Percent
Soybean protein	24.70
Corn oil	10.00
Starch	32.40
Dextrose	25.00
Cellulose	5.20
B-vitamins	0.05
Choline chloride	0.20
Vitamins A and D	0.05
Minerals[a]	2.40
	100.00

[a]Mineral Mixture	Percent
Magnesium phosphate	6.050
Magnesium sulfate	6.540
Potassium chloride	21.450
Dibasic potassium phosphate	40.900
Sodium phosphate	8.810
Sodium chloride	13.260
Cupric sulfate	0.014
Ferric ammonium citrate	2.630
Manganese sulfate	0.034
Ammonium alum	0.015
Potassium iodide	0.007
Sodium fluoride	0.088
Molybdic acid	0.003
Zinc chloride	0.034
Cobalt chloride	0.014
	100.000

cells determination using the micro-hematocrit, total hemoglobin using cyanomethemaglobin reagent, and red blood cells were counted using a hemocytometer. Mean corpuscular volume (M.C.V.), mean corpuscular hemoglobin (M.C.H.), and mean corpuscular hemoglobin concentration (M.C.H.C.) were determined by the following methods as described by Wintrobe (1961):

$$\text{M.C.V.} = \frac{\text{vol. packed red cells, ml per 1000 ml}}{\text{red cell count, millions per c.mm.}}$$

$$\text{M.C.H.} = \frac{\text{hemoglobin, Gm per 1000 ml}}{\text{red cell count, millions per c.mm.}}$$

$$\text{M.C.H.C.} = \frac{\text{hemoglobin, Gm per 100 ml x 100}}{\text{vol. packed red cells, ml per 100 ml}}$$

Plasma was collected for mineral analysis. Pooled carcasses were dried, slowly charred over a Fisher burner and ashed at 600° C, with the ashes being dissolved in 4 N HCl. Cations were analyzed using a Perkin-Elmer Atomic Absorption Spectrophotometer by methods set forth by the manufacturer. Phosphorus was analyzed by the method of Fiske and Subbarow (1925). All data were subjected to an analysis of variance.

RESULTS AND DISCUSSION

Growth and hematological data are shown in Table II. The rats did not exhibit any outward appearance of poor health or listlessness; however, the rate of growth, after two weeks on the diet, appeared to be decreased. Calcium deficiency symptoms appearing in the literature were obtained primarily on young rats, weighing in the range of 50 grams. The rats used in this experiment were considerably older and apparently had sufficient calcium stores to maintain them for the four-week period without outward signs of stress.

Red blood cells increased with time ($P < .01$) and the hematocrit changed ($P < .01$) in a manner which is described by quadratic expression. Values obtained for mean corpuscular volume (M.C.V.), mean corpuscular hemoglobin (M.C.H.) and mean corpuscular hemoglobin concentration (M.C.H.C.) initially, weeks I, II, III, and IV, respectively, are as follows: M.C.V., 65.2, 62.2, 62.6, 54.6, and 47.6 microns; M.C.H., 23.9, 20.9, 23.5, 18.9, and 16.7 micromicrograms; M.C.H.C., 35.2, 33.6, 36.9, 34.5, and 34.5%. The decreases in mean corpuscular volume and mean corpuscular hemoglobin indicate that, at the end of the experiment, the animals exhibited a hypochromic and microcytic anemia. Possible causes of hypochromic microcytic (Wintrobe, 1961) anemia are

TABLE II

GROWTH AND HEMATOLOGICAL RESPONSE TO CALCIUM-DEFICIENT DIET

Period-Days	Body weight gm.		Hematocrit Packed cells, %		RBC X 10^6/mm^3		Hemoglobin gm./100 ml.	
	Males	Females	Males	Females	Males	Females	Males	Females
0 - 0[a]	89	102	46.5	47.8	7.2	7.4	16.8	16.4
	95[b]		47.2		7.3		16.6	
I - 7	88	101	44.3	44.6	7.4	6.9	14.8	15.0
	95		44.5		7.2		14.9	
II - 14	100	115	44.7	41.7	7.8	6.5	15.9	15.8
	107		43.2		7.1		15.9	
III - 21	100	122	45.2	45.6	7.9	8.8	15.8	15.5
	111		45.4		8.4		15.7	
IV - 28	108	129	45.2	44.3	8.8	9.9	15.8	15.1
	118		44.8		9.4		15.5	

[a]Animals sacrificed when experiment was initiated and then at 7-day intervals.

[b]Mean of sexes pooled.

as follows: iron deficiency associated with sprue, idiopathic steatorrhea, celiac disease and chronic diarrhea; pyridoxine requirement in pyridoxine-responsive anemia; deficiency of copper in hypocupremic syndrome of infants; genetic anomaly; and unknown factors. The diet fed in this experiment contained adequate levels of copper, iron, or pyridoxine, and no genetic anomalies are suspected.

Possible causes of iron deficiency (Wintrobe, 1961) are as follows: dietary deficiency, defective absorption, continued loss of blood through chronic alimentary or genitourinary tract bleeding, and excessive demands for iron as in repeated pregnancy.

The most plausible explanation for the anemia observed in this experiment concerns the loss of blood due to chronic bleeding. Martin (1937) and Boelter and Greenberg (1941) both observed widespread hemorrhages in rats maintained on low-calcium diets. Although the animals in the present experiment were not examined for hemorrhagic tissues, their feces were black at the end of the experiment in contrast to a white feces observed at the beginning of the experiment. The source of pigment in the feces could be related to hemorrhage in alimentary tract in these animals.

Table III shows response of plasma minerals to the calcium-deficient diet. Plasma calcium exhibits the most dramatic change by a linear decrease ($P < .01$) for the first three weeks; however, between the third and fourth weeks the value returned to its initial level. Other investigators

TABLE III

RESPONSE OF PLASMA MINERALS TO CALCIUM-DEFICIENT DIET

Period-Days	Calcium mg./100 ml.		Phosphorus mg./100 ml.		Magnesium mg./100 ml.		Sodium meq./l.		Potassium meq./l.	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
0 - 0[a]	12.26	13.52	7.52	7.21	2.23	2.25	156	162	6.86	6.68
	12.89[b]		7.36		2.24		159		6.77	
I - 7	12.03	11.74	7.94	7.08	2.22	2.25	179	157	11.12	10.84
	11.88		7.51		2.23		168		10.98	
II - 14	10.33	9.85	9.23	7.28	2.41	3.14	175	164	9.26	9.39
	10.09		8.61		2.78		170		9.32	
III - 21	9.35	9.25	8.43	7.33	2.46	2.47	182	163	8.56	7.75
	9.27		7.88		2.47		172		8.15	
IV - 28	11.31	11.23	8.18	6.46	2.53	2.52	148	141	9.10	8.12
	11.27		7.32		2.53		145		8.61	
Standard Error[c]	0.11		1.05		0.075		6.89		0.92	

[a]Animals sacrificed when experiment was initiated and then at 7-day intervals.

[b]Mean of sexes pooled.

[c]Standard error of mean of sexes pooled.

using younger rats on extremely calcium deprived diets observed lowered plasma calcium levels which persisted as long as 12 weeks (Boelter and Greenberg, 1940). After eight weeks on the severe diet these investigators observed an average low value of 5 mg per 100 ml plasma as compared to 10 mg per 100 ml for control rats. The return of plasma calcium to normal levels, which were observed at the end of four weeks on the deficient diet in the present experiment, might be explained by the age of animal and the function of parathyroid hormone in maintenance of plasma calcium homeostasis (Sherwood et al., 1966). If an animal is capable of maintaining normal plasma calcium levels in the face of calcium deprivation, then plasma calcium is not an appropriate assessor of the calcium status of that animal.

Plasma phosphorus did not change significantly in response to the deficient diet. There was a difference in response between sexes ($P < .01$); males increased plasma phosphorus while females remained constant.

Potassium levels in plasma increased ($P < .01$) on the diet to unusually high values. One possible explanation for this is the existence of hemolysis which was prevalent in all samples.

Plasma copper ($P < .01$) and zinc ($P < .01$) levels increased with time and possibly reflect the trend for the carcass to increase the amounts of these minerals during the trial (Table IV).

Neither plasma sodium nor magnesium changed during the

TABLE IV

CHANGES IN CARCASS COMPOSITION ON CALCIUM-DEFICIENT DIET

Period-Days	Mineral, grams									
	Calcium		Phosphorus		Magnesium		Sodium		Potassium	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
0 - 0[a]	3.96	5.39	2.31	2.78	0.091	0.114	0.446	0.521	0.814	0.969
	4.68[b]		2.54		0.103		0.483		0.891	
I - 7	4.44	4.42	2.00	2.32	0.151	0.201	0.458	0.511	0.847	0.884
	4.43		2.22		0.176		0.484		0.866	
II - 14	3.54	5.55	2.10	2.35	0.092	0.214	0.437	0.432	0.867	1.010
	4.54		2.22		0.153		0.430		0.938	
III - 21	3.66	3.63	2.90	2.79	0.126	0.142	0.363	0.452	0.912	1.112
	3.65		2.87		0.134		0.408		1.012	
IV - 28	4.18	3.74	2.46	2.60	0.155	0.135	0.366	0.358	1.063	1.171
	3.96		2.53		0.145		0.362		1.117	
Standard Error[c]	0.015		0.061		0.009		0.014		0.026	

[a]Animals sacrificed when experiment was initiated and then at 7-day intervals.

[b]Mean of sexes combined; values represent 3 carcasses pooled and must be divided by 3 to obtain individual values.

[c]Standard error of mean of sexes combined.

trial and both exhibited normal values.

The responses of carcass minerals to the calcium-deficient diet are shown in Tables IV and V. Total carcass minerals did not change significantly during the four weeks. After one week on the diet, when the animals exhibited growth again, there was a definite trend for the carcass mineral content to increase. This trend has significance in that it indicates an animal can increase its total inorganic mass in spite of the feeding of a severe calcium-deficient diet. As discussed later in this report, the total quantity of certain minerals in the animal body have more interpretive value when expressed as a fraction of inorganic mass.

Total carcass calcium did not change significantly after four weeks on the deficient diet. Previous investigators have shown that rats at 28 days of age placed on a diet of flour and sodium chloride survived without appreciable growth to an age of 80 days and showed no increase in body calcium, but rather a slight decrease (Sherman and MacLeod, 1925). Using a different diet, in which calcium was the only variable, it was observed that rats receiving lower calcium intakes had identical growth patterns to rats receiving normal calcium levels, but chemical analysis of the bodies showed them to be calcium-poor (Sherman and Booher, 1931). It is possible that the time interval was too short in the present experiment for carcass calcium to change. Total carcass calcium in the acute calcium depletion status does not adequately assess the animal's condition.

TABLE V

CHANGES IN CARCASS COMPOSITION ON CALCIUM-DEFICIENT DIET

Period-Days	Zinc mg		Iron mg		Copper mg		Ash gm	
	Males	Females	Males	Females	Males	Females	Males	Females
0 - 0[a]	23.9	32.4	8.6	11.2	1.33	1.53	7.65	9.82
	28.2[b]		9.9		1.43		8.74	
I - 7	23.0	25.7	10.1	10.9	1.07	1.10	7.93	8.38
	24.4		10.5		1.08		8.15	
II - 14	20.3	27.9	10.4	12.7	0.90	1.30	7.06	9.59
	24.1		11.5		1.10		8.32	
III - 21	18.7	22.3	12.8	13.1	1.20	1.60	8.24	8.16
	20.5		13.0		1.40		8.20	
IV - 28	25.2	28.8	13.8	13.8	1.10	1.37	8.79	8.45
	27.0		13.8		1.23		8.63	
Standard Error[c]	0.001		0.032		0.003		0.20	

[a] Animals sacrificed when experiment was initiated and then at 7-day intervals.

[b] Mean of sexes combined; values represent 3 carcasses pooled and must be divided by 3 to obtain individual values.

[c] Standard error of mean of sexes combined.

Carcass calcium, expressed as a per cent of the live animal weight or as a per cent of the total body ash, changed ($P < .01$). The results of this trial and those of previous investigations have shown conclusively that an animal can increase body weight without increasing total body calcium. Under these circumstances the expression of total body calcium as a per cent of body weight would assess the calcium status of an animal.

As the total carcass minerals did not change significantly, it is interesting that carcass calcium as a per cent of the total carcass minerals changed significantly. The level of calcium in the diet has been shown to be a limiting factor regarding both the storage of calcium and phosphorus in the body (Sherman and Quinn, 1926). The mineral increments of the rat fed a normal diet have been shown to cease at the following ages: calcium, 120 days; phosphorus, 90 days; magnesium, 40 days; sodium, 120 days; potassium, 40 days; and iron, 200 days (Spray and Widdowson, 1950). As the rats were about 80 days of age upon completion of the present trial, it is apparent that the maximum mineral growth potential was not attained and that calcium was the limiting factor.

Magnesium in the body of these rats showed no change when expressed either on a total or percentage basis. According to the magnesium calendar, these animals would be expected to have reached their physiological maturity regarding magnesium. Calcium deprivation had no apparent

effect on changing the quantity of carcass magnesium.

Carcass phosphorus increased on the diet and exhibited increments consistent with the phosphorus growth pattern of rats fed normal diets. These data are not consistent with the concept that phosphorus in the body of an animal is limited by the amount of calcium in the diet (Sherman and Quinn, 1926). There appears to be no relationship between the quantities of calcium and phosphorus in the bodies of rats fed the calcium-deficient diet ($r = -0.17$). When each is expressed as a per cent of the total inorganic mass of the body a close relationship does exist ($r = -0.89$, $P < .01$). The total mineral of an animal's body appears to have more interpretive value if it is expressed as a function of inorganic growth.

Total carcass sodium decreased during the trial ($P < .05$) but showed no change when expressed as a per cent of the total inorganic mass. The decrease parallels that found in the maturing rat which occurs from birth to 120 days of age.

Potassium content of the body increased totally ($P < .01$) and as a per cent of total inorganic mass ($P < .05$). These data are contrary to the potassium calendar of the normal rat in which there is an increase from birth to 40 days of age and thereafter a slight decrease to about 300 days. The significance of this is not known.

Iron in the bodies of these rats increased totally ($P < .01$) and as a percentage ($P < .01$) and was consistent

with that found in the normally growing rat. Copper and zinc showed no changes in the bodies of these rats.

Rats maintained on a severely low-calcium diet in this study exhibited increased calcium retention ($P < .01$) as shown in Table VI. As urinary calcium losses were minimal and did not significantly change, fecal calcium primarily reflected the animal's response to a low-calcium diet. Fecal calcium excretion decreased ($P < .05$) with time on diet. This could result from increased absorption and/or decreased endogenous losses of calcium occurring in adjustment to the calcium-poor diet. Other investigators have shown that both conservative forces are active in rats maintained on low-calcium diets (Hansard and Plumlee, 1957). These authors observed that rats on low-calcium diets (0.013% calcium) absorbed 98% of the dietary calcium as compared to 45% for those maintained on high (1.0% calcium) calcium diets, and that endogenous losses of calcium became smaller with decreasing levels of calcium in the diet. In addition, they obtained evidence that the current intake of calcium has less importance than does the calcium status of the animal at the time of measurement. As 98% of the fecal calcium is of endogenous origin on a 0.01% calcium diet, fecal calcium primarily reflects the endogenous losses.

The maintenance requirements of rats for calcium have also been shown to be a function of the animal's calcium status at the time of measurement. Thus, rats reared to 85 days of age on rations furnishing 24 and 100 mg of calcium

TABLE VI

EFFECT OF CALCIUM-DEFICIENT DIET UPON CALCIUM, PHOSPHORUS, AND MAGNESIUM BALANCE

Element		Calcium				Phosphorus				Magnesium			
Period, days		I 0-7	II 7-14	III 14-21	IV 21-28	I 0-7	II 7-14	III 14-21	IV 21-28	I 0-7	II 7-14	III 14-21	IV 21-28
Feces, mg.	Males	26.8	13.1	11.7	10.6	62.8	71.7	46.9	71.5	10.07	15.32	15.43	21.71
	Females	43.7	23.9	14.6	21.5	126.5	82.8	58.1	75.0	20.31	19.59	19.49	27.22
	Average	35.3	18.5	13.2	16.0	94.6	77.3	52.5	73.3	15.19	17.45	17.46	24.47
Urine, mg.	Males	0.22	0.34	0.46	0.63	236	233	393	483	5.38	5.98	12.60	18.56
	Females	0.24	0.39	0.46	0.57	237	380	447	581	5.02	14.15	9.46	22.89
	Average	0.23	0.37	0.46	0.60	237	306	420	532	5.20	10.02	11.03	20.72
Intake, mg.	Males	53.6	54.9	62.8	68.3	670	686	785	853	53.6	54.9	62.8	68.3
	Females	60.1	67.7	77.9	77.7	751	846	973	971	60.1	67.7	77.9	77.7
	Average	56.9	61.3	70.3	73.0	710	766	879	912	56.9	61.3	70.3	73.0
Retention, mg.	Males	26.5	41.5	50.7	57.0	371	382	345	298	38.2	33.6	34.8	28.0
	Females	16.2	43.5	62.8	55.7	388	384	468	315	34.8	34.0	48.9	27.6
	Average	21.4	42.5	56.7	56.4	380	383	406	307	36.5	33.8	41.8	27.8

per day required 5 and 21 mg of calcium per day, respectively, to maintain calcium status (Hansard and Plumlee, 1954). Such data imply that endogenous losses of calcium could assess the status of the animal. Using total body calcium as a per cent of total body weight or as a per cent of total body inorganic mass, to express calcium status of the rats in this experiment, changes in fecal excretion of calcium are observed to decrease with the calcium status of the animal.

The retention of phosphorus observed in Table VI is consistent with the phosphorus increment observed in the bodies of these animals. Fecal phosphorus did not change significantly with time but there appeared to be a trend to decrease levels. Urinary phosphorus increased linearly with time ($P < .01$) and a difference was observed between sexes ($P < .05$), females demonstrating the greatest increase. In studying the influence of low-calcium diets upon the metabolism of phosphorus, other investigators have observed a reduction in phosphorus absorption and retention (Hansard and Plumlee, 1954). However, these authors found that dietary phosphorus was absorbed through the intestinal tract but was rapidly excreted into the urine and thereby decreasing retention. Their ration contained 0.4% phosphorus compared to 0.5% used in the present study. The higher retention observed in Table VI might possibly be explained by the higher phosphorus containing diet used in this trial.

Earlier in this report plasma phosphorus response to a

calcium-deficient diet was discussed. It may be noted here that males exhibited increased plasma phosphorus with a lag in the time of increasing urinary phosphorus (21-28 days) as compared to females whose plasma phosphorus remained constant while there was an early increase in urinary phosphorus (14-21 days). It appears that there is a sex difference in the degree of adjustment for lowered calcium status by the urinary excretion of phosphorus. The classical function of parathyroid hormone in the inhibition of renal tubule reabsorption of phosphorus appears to play an important role not only in maintaining plasma calcium, but also in the excretion of phosphorus in order to compensate for the calcium inadequacy of the body (Greenwald and Gross, 1925).

There was no significant change in the retention of magnesium or of fecal magnesium as shown in Table VII. Urine magnesium increased linearly ($P < .05$), with the last period being 4 times the amount excreted during the first period. Although the exact relationship is not understood, it might be postulated that magnesium ions are being mutually excreted with phosphorus in an attempt to maintain ionic balance. Although the increased urinary excretion of magnesium was not large enough to produce a significant difference in retention, there was a trend toward magnesium retention to decrease.

Sodium and potassium retention were not altered significantly by the calcium-deficient diet (Table VII). Both

TABLE VII

EFFECT OF CALCIUM-DEFICIENT DIET UPON SODIUM AND POTASSIUM BALANCE

Element		Sodium				Potassium			
Period, days		I 0-7	II 7-14	III 14-21	IV 21-28	I 0-7	II 7-14	III 14-21	IV 21-28
Feces, mg.	Males	34.6	52.9	28.0	53.7	67.1	90.5	46.6	87.3
	Females	67.5	49.1	39.4	55.3	119.3	72.3	62.0	81.3
	Average	51.0	51.0	33.7	54.5	93.2	81.4	54.3	84.3
Urine, mg.	Males	266	203	265	322	258	318	397	497
	Females	244	253	284	402	265	446	470	651
	Average	255	228	275	362	261	382	433	574
Intake, mg.	Males	308	315	361	392	844	865	989	1075
	Females	346	389	448	447	947	1066	1226	1224
	Average	327	353	404	419	895	965	1108	1150
Retention, mg.	Males	7.6	56.0	67.4	16.8	519	457	545	490
	Females	33.9	86.7	124.0	-11.0	563	548	694	492
	Average	20.8	73.3	95.7	2.9	541	502	619	491

minerals increased in the urine with time on the diet ($P < .05$) but the relationship of these increments to calcium deficient status is not understood.

Because of unique conservative mechanisms for body minerals these animals were able to thrive and even grow on the calcium-deficient diet. Assessment of the calcium status of the body does not appear simple to achieve. The logical approach to assessment appears to involve the physiological responses to calcium-deficient diets such as parathyroid hormone function on intestinal absorption, bone calcium mobilization and renal excretion and reabsorption.

Thus it appears that the logical approach might involve studying the relationship of a calcium-deficiency and parathyroid hormone production, and then studying on the calcium-deficient animal, the effect of parathyroid hormone upon intestinal absorption, bone mobilization, and renal excretion and reabsorption.

SUMMARY

Ninety rats, divided equally be sex, were allotted within sex to five groups of 18 rats per group. Each group was then subdivided by sex into 6 subgroups, with each of these being treated as an experimental unit for feeding, blood, carcass, and excreta analysis. Prior to, and at weekly intervals after being placed on the calcium-deficient diet, 18 rats were sacrificed and their bodies, blood, and excreta were analyzed for calcium, phosphorus, magnesium, sodium, potassium, iron, zinc, and copper.

Plasma calcium decreased linearly for three weeks on the calcium-deficient diet and returned to initial levels between the third and fourth weeks. No significant change was observed in plasma phosphorus but a sex difference was observed and was confirmed by a sex difference in urinary phosphorus. Plasma copper, zinc, and potassium increased with time on the diet while no changes were observed in plasma sodium or magnesium.

Carcass minerals which increased on the diet were: phosphorus, potassium, and iron, while sodium decreased. No significant changes were observed with carcass calcium, magnesium, copper and zinc. Total mineral content of the carcasses did not significantly change, but there was a definite trend toward increasing. When expressed as a

function of live weight or total body inorganic mass, the calcium in the bodies of these animals decreased significantly, showing the ability of an animal to increase its mass without a corresponding increase in calcium.

Calcium retention increased even on the calcium-poor diet, reflecting an increase in apparent digestibility. Phosphorus balance was positive throughout the trial; fecal phosphorus did not change, and urinary phosphorus increased with time. No significant changes were observed in the retention of sodium, potassium and magnesium; fecal excretion of each element decreased while urinary excretion increased with time.

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