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Title INTERRELATIONSHIPS OF MAGNESIUM, POTASSIUM AND NITROGEN IN HYPOMAGNESEMIA

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The objective of this study was to investigate the influence of dietary magnesium, potassium and nitrogen on hypomagnesemia. Rats and dairy cattle were used as experimental animals. In the rat experiments, 270 albino rats, Mus norvegicus albinus, were used on 27 synthetic diets consisting of three levels each of casein (18, 24 and 30 percent of the diet, as nitrogen source), magnesium (400, 200 and 50 mg/kg of the diet) and potassium (1.8, 3.6 and 7.2 g/kg of the diet). The rats weighed 50-60 g at the start of the experiment. The feeding periods were four and eight weeks. The study with dairy cattle was a grazing survey to investigate the relationships between certain constituents of pasture forages and serum magnesium in early pasturing season which is a critical time for hypomagnesemic tetany. Sixty Holstein and Jersey cows were involved in the study. An atomic absorption spectrophotometer was used for the determinations of minerals in serum, tissue and forage samples.

Skin lesions were observed in the rats fed 50 mg/kg magnesium diets and increased in severity with the increase in dietary nitrogen and with the extension of the experimental period from four to eight weeks.

Increasing levels of dietary nitrogen and potassium and decreasing levels of magnesium highly significantly ($P < 0.01$) decreased the magnesium concentration of serum. The mean serum magnesium values were found to be 2.51, 2.18 and 0.95 mg/100 ml with dietary magnesium values of 400, 200 and 50 mg/kg, respectively. The interactions between and among periods, nitrogen and magnesium in hypomagnesemia were highly significant ($P < 0.01$). The dietary potassium, on the other hand, showed a significant ($P < 0.05$) interaction only with magnesium. Serum calcium concentration was increased by the increase in dietary nitrogen and by the decrease in dietary magnesium; however, such increase was within what might be called a physiologically "normal" range. It was unaffected by the increase of dietary potassium. The change in the dietary magnesium did not affect the serum potassium concentration.

The magnesium content of bone was markedly decreased by the increase of dietary nitrogen and potassium and by the decrease of dietary magnesium. A high correlation coefficient was found ($r = 0.978$) between bone and serum magnesium concentrations, indicating a close association between the two. The hypomagnesemia,

on the other hand, was not accompanied by a significant reduction in muscle magnesium.

In the grazing experiment, after five weeks of pasturing, the mean serum concentrations of cows was decreased and serum calcium and potassium values were increased significantly.

It may be concluded that the hypomagnesemic condition in animals is not a simple deficiency or a simple interference of another factor, but a complex one. With this consideration, it may be suggested that a variety of preventive measures should be taken against the various potential factors which could induce hypomagnesemia when cattle are turned into pasture in the early spring.

INTERRELATIONSHIPS OF MAGNESIUM, POTASSIUM AND NITROGEN IN HYPOMAGNESEMIA

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Typed by Gwendolyn Hansen

Dedicated to My Wife

ENGIN

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INTRODUCTION

Hypomagnesemic tetany, commonly known as grass tetany in cattle, has been a serious problem in many countries of the world, particularly in the western European countries. In the United States, the disease has been reported to be a serious problem in Texas, West Virginia, South Dakota and in California. A recent report (23) indicated that an estimated 4,000-6,000 head of beef cattle were lost in California during the winter of 1963-1964. The mortality rate in some herds reached 20 percent.

At present, no statistics are available on the losses from grass tetany in Oregon. The presence of the disease was first reported in Oregon by Muth and Haag (40) in 1945. It mainly occurred in the coastal regions of the state during the late winter. Since that time no reports from Oregon have appeared in scientific periodicals; however, sporadic disorders have apparently continued. Four and five such cases occurred in the Oregon State University dairy herd in the spring months of 1965 and 1966, respectively. Complaints from various counties of Oregon indicate that hypomagnesemic tetany is a problem of both dairy and beef farmers and suggests a closer look at this disease problem is necessary.

Cattle are generally affected in the spring when animals are placed on lush grass pastures. The attack of tetany may either be acute or in a less severe chronic form. Due to the rapidity of the acute form the affected cows may be found dead on the pasture or in the barn.

Irrigation and fertilization of pastures increases the incidence of hypomagnesemic tetany. Since these management practices have been getting more attention it may be expected that they will accentuate this problem. Finding a practical and effective means of prevention for the disease will make these two management practices more profitable.

In order to find a sound method for prevention of this disease the principal cause or causes have to be known. Although an extensive literature on hypomagnesemia has been assembled, no complete description is available yet on the etiology. This thesis is an attempt to reconcile scientific data on the disease through status of mineral interrelationships involved.

The financial and practical difficulties in experimenting with large animals suggests the use of laboratory animals to obtain basic information keeping bona fide species differences always in mind. With these considerations the major part of the studies reported in this thesis was conducted with the white rat, Mus norvegicus albinus. Twenty-seven different diets were fed to the rats to study the effects

of potassium, nitrogen and magnesium in hypomagnesemia. The second part of the studies was a field survey with dairy cows in the spring pasturing season of 1966. With these cows the trend of blood magnesium concentration was studied relative to the constituents of pasture forages.

LITERATURE REVIEW

Information pertaining to the presence of magnesium in living organisms is not new. Reports on the essentiality of this element for normal animal growth first appeared in scientific journals in 1926 (31). Since then numerous investigations have been completed to study its physiological and nutritional functions. Many aspects associated with the role of the element are still not completely understood, particularly the interactions of magnesium with other ions to induce deficiencies of magnesium in animals. At present, it is generally accepted that magnesium is one of the macro elements essential for life of both plants and animals. It has been demonstrated that magnesium activates many important enzymes which are required for splitting off and transferring phosphate groups and decarboxylating α-keto acids (58, p. 491).

The term "grass tetany" has long been familiar to animal scientists and farmers. First reports dealing with this anomaly came from the Netherlands about 100 years ago in which it was referred to as "grass paralysis" (39). However, detailed reports on the disorder have come from many countries around the world, especially from England and Western European countries, after Sjollema's conclusive publication in 1932 (49). In the past, the disease has also been referred to as lactation tetany, grass

paralysis, grass staggers, spring tetany, and atypical milk fever. Incidence can occur in both lactating cows and pregnant heifers; generally on grass, but occasionally stall-fed. The more correct name for the disorder would be "hypomagnesemic tetany" as first suggested by Stewart in Scotland (53), since hypomagnesemia is common to all cases.

Magnesium Deficiency Symptoms in Animals

The essentiality of magnesium for normal animal growth was first reported by Leroy (31) in young growing mice. The growth of mice on a magnesium deficient diet stopped after 9 to 13 days and continuation of the diet resulted in death at 24 to 35 days.

Rats

Kruse et al. (28) experimentally induced magnesium deficiency in rats by feeding magnesium-deficient diets. The rats weighed 35 to 45 g at the start of the experiment and within 3 to 5 days a marked vasodilation and hyperemia appeared in all the more exposed skin areas. During the hyperemic period, the animals were extremely nervous and hyperirritable. At this stage of development any sudden excitement threw the affected animal into convulsions. The excited animal usually raced at rapid speed in a wide circle and finally fell on its side in a rigid position with its head stretched

back, limbs extended outward from the body. In those animals that survived for 4 to 6 weeks, trophic skin lesions formed throughout the body. These lesions were circumscribed and erythematic in nature and subsequently were desquamated. An extensive loss of hair occurred around the eyes, on the ears, under the surfaces of the jaws and around the neck.

These experiments have been extended in detail and confirmed by many workers (43, 47, 61). It has been reported (37) that in addition to the skin lesions and hyperirritability, magnesium-deficient rats fail to synthesize protein, resulting in poor weight gain.

A number of workers (47, 60) have reported pathological changes in magnesium-deficient rats. Tissues affected were heart, vascular tissue, kidneys, liver, and brain. The serum magnesium concentrations of deficient rats fell from a normal of 2 to 3 mg per 100 ml to less than 1 mg per 100 ml (10). McIntyre and Davidson (32) reported that as the deficiency developed, magnesium and potassium content of skeletal muscle slowly fell to about 80 percent of normal. On the other hand, the magnesium and potassium content of brain, liver, and kidneys of the experimental animals did not differ from the controls.

Chicks

Bird (2) reported that baby chicks raised on sub-optimal levels of dietary magnesium showed symptoms of incoordination and convulsions. Clinically, magnesium deficiency in chicks is characterized by poor growth, poor feathering, decreased muscle tone, squatting, ataxia, fine palpable tremors, progressive incoordination of movements, convulsions and death. Neuropathologic degenerations in the cerebellar purkinje cells were found to be also characteristic in the deficiency.

Guinea Pigs

Characteristic symptoms of magnesium deficiency in guinea pigs were reported as hyperirritability and metastatic calcifications (39, 42). However, in contrast to other species, the guinea pig is fairly resistant to nervous symptoms and seldom dies in a convulsive state. The magnesium content in kidney, muscle, heart, and bone was decreased by the deficiency while the levels of ash, calcium and phosphorus were increased in the soft tissues and remained unchanged in bone (39).

Sheep

Grass tetany has been reported in sheep grazing cereal forages. The majority of cases appeared during the early stages of

lactation (22, 29, 53). Watt (59) reported that 12 percent of the acute deaths of sheep in Scotland were due to hypomagnesemia. The hypomagnesemia is usually combined with a mild hypocalcemia. In many cases the animals had lower blood magnesium concentrations for a long period of time without signs of clinical tetany (22, 29).

Cattle

Hypomagnesemic tetany appears more commonly in cattle than other species. Magnesium deficiency symptoms occur both in calves on a milk diet and in cows on forage diets.

The milk-type deficiency was first described by McCandlish (36) in 1923. However, he was unaware of the precise cause, at the time. When he raised two bull calves on a diet consisting exclusively of whole milk, they failed to grow, developed skin lesions, neuromuscular hyperirritability, and died of convulsions. In 1935, Duncan et al. (14) fed 20 calves with whole milk supplemented with cod liver oil, manganese, silicon, aluminum, iron and copper. The animals developed tetany and some of them died in convulsions. The serum magnesium level in these animals was markedly low, but calcium and phosphorus levels were normal. The deficiency symptoms did not occur until the serum concentration of magnesium fell from a normal of 2-3 mg per 100 ml to 0.7 mg per 100 ml.

There were calcified lesions in the endocardium, aorta, large arteries, muscles, diaphragm, spleen and kidney tubules.

Blaxter et al. (3) reported experimentally-induced magnesium deficiency in calves. Using whole milk as the only source of magnesium, hyperirritability and tetany appeared at levels below 1 mg magnesium per 100 ml of blood serum. The magnesium content of the bone in the affected animals decreased from 0.6 to 0.4 percent. There was no detectable loss of magnesium from the soft tissues of the body and the calcium metabolism was not affected.

Grass tetany in cattle is very similar to the milk syndrome in calves with low blood serum magnesium being common to both. Because of its heavy damage to the livestock industry in some countries, many reports have appeared on hypomagnesemia and grass tetany in both dairy and beef cattle (1, 15, 24, 30, 40).

In general, the symptoms of hypomagnesemic tetany display great variations, depending on the severity of the attack. Paresis andmotor irritation are the most severe among the nervous symptoms. In the mild form, dullness, lack of appetite, refusal to graze with other animals and un-coordinative walking are usually observed. Milk production of the cows drops gradually and experienced farmers may recognize the approaching disorder at this stage (13).

In the more severe form, the initial symptoms are

nervousness, restlessness, muscle twitching, unsteady walking, spreading of the hind limbs and grinding of the teeth. These symptoms are followed by violent tetanic contractions of the limbs and tail. The animal also displays wild expressions and shows erected ears and frequent urination. These symptoms are usually accompanied by other types of cramps, rolling of the eye balls, contractions of the masticatory and the neck muscles. In addition, frothing of the mouth due to contractions of swallowing muscles and abundant salivation are frequently observed. After the animal has fallen, tetanic convulsions start, and the animal strikes heavily with all four feet until it is exhausted or dies (13, 38).

One characteristic of grass tetany is that any excitement during the attack usually aggravates the conditions and causes the animal to become convulsive, sometimes resulting in death. Due to the rapid development of the disease, the affected animals may die before they are discovered. The temperature of the diseased animal may be normal or sub-normal. The ears are cold, muzzle is dry and conjunctiva is hyperemic. If the attacks are repeated, the animal will be in a state of excitement between the attacks and periods with paresis are often recorded (1,12, 15).

Milk fever, which is associated with low blood calcium, exhibits somewhat similar symptoms to those of grass tetany. Sometimes external symptoms make it difficult to distinguish between

the two diseases. However, the interval between calving and the appearance of the disorder may be helpful in distinguishing between the two. Milk fever usually occurs within the first few days after calving while grass tetany may appear anytime between the fifth month of pregnancy and six or seven months following calving (12). Excessive irritability associated with grass tetany is not evident with milk fever. Blood calcium and magnesium determinations will differentiate between the two since in most cases blood calcium is not affected by grass tetany, but is markedly affected in milk fever.

Etiology of Hypomagnesemia

First reports on a hypomagnesemic condition in cows, commonly known as grass tetany, was published by Sjollema (49) in 1932. He pointed out that a marked hypomagnesemia combined with a moderate hypocalcemia were responsible for the incidence of grass tetany. Since the protein, potassium and nitrate content of the pastures on which grazing animals manifested tetany were found to be consistently high the author suggested that the high levels of these were the cause of tetany. At that time, no particular attention was given to magnesium, since pastures causing tetany had a normal level of magnesium. Several reports (1, 6, 7, 15) have indicated that the magnesium content of pasture grass was sufficient to meet the animals' requirement and that no evidence pointed to the

association between the composition of the pasture and the incidence or severity of the hypomagnesemia. Ender et al. (15) reported that the intake of magnesium in normal cows during high milking periods with barn feeding is often lower than the calculated intake during grazing on pasture. Consequently, the occurrance of the tetany should not be attributed to the low intake of magnesium on pasture. This finding supports the theory that tetany is generally not caused by a lack of magnesium in the diet, but results from a conditioned magnesium deficiency. This is brought about by an inhibitor which reduced the absorption of magnesium or otherwise interferes with the matabolism of magnesium in the animal's body. In this connection, recently, Burau and Stout (5) reported that the concentration of trans-aconitic acid, an inhibitor of the tricarboxylic acid cycle, is higher in early season grasses than that of later stages. The authors suggested that the high content of trans-aconitic acid in early spring grasses might be related to hypomagnesemia in cattle and sheep.

Other reports (30, 6, 25), however, have indicated that tetany-prone pastures were significantly lower in magnesium than that of normal pastures. These reports support the theory that when cows are turned to pasture in the spring, they may not consume enough dry matter due to high moisture in the fresh grass resulting in a low intake of magnesium.

It has been shown that hypomagnesemia is not a result of a simple deficiency, but is an anomaly of a complex nature. A number of interacting factors have been shown to be responsible for the disorder. Protein or nitrogen level in the ration is one of these factors, as mentioned previously by Sjollema (49). Kemp et al. (27) studied magnesium intake and utilization from herbage by lactating cows and reported that the availability of magnesium increased as the herbage matured. The crude protein content of forages were 26, 18, and 14 percent for the first, second, and third cutting, respectively. The availability of magnesium from the forage was 10, 16, and 20 percent, respectively, for the three cuttings, indicating a negative relationship between protein and utilization of magnesium. The authors suggested that mobilization of bone magnesium was not sufficient to prevent hypomagnesemia and further suggested that hypomagnesemia in cattle results from a shortage of magnesium due to the insufficient supply of "available" magnesium.

Colby and Frye (11) fed different levels of protein to rats and noted a definite interrelationship between protein, calcium and magnesium levels in inducing hypomagnesemia. The high protein level increased the severity of the magnesium deficiency. With normal protein level (supplied by 24 percent casein) a combination of high calcium and high potassium aggravated hypomagnesemic conditions. However, if the protein level is high, high calcium did not

increase the deficiency syndrome. Menaker (37) used protein depleted rats to study the influence of protein intake on magnesium requirement during protein synthesis. His results indicated that deleterious effects of magnesium deficiency on growth increased as the protein level in the ration increased from 7 to 14 percent. A survey (30) on 187 farms in France revealed that high nitrogen levels from pastures after nitrogen fertilization increased the frequency of grass tetany in cattle.

Potassium levels of grass have been reported as one of the contributing factors of grass tetany in Holland (26), Scandinavia (24), England (6), France (30), and in the United States (29). The deleterious effect of high potassium on hypomagnesemia have been demonstrated with rats (10, 17, 60).

Kemp and Hart (26) reported that the mineral composition of herbage is an important factor in the incidence of grass tetany. The effect of potassium was shown to be more evident when it was related to the calcium and magnesium content. This relationship was expressed by the ratio of $\frac{K}{Ca + Mg}$ and the frequency of incidence of grass tetany increased when the above ratio exceeded 2.2. The ratio averaged 1.67 for non-tetany pastures and 2.37 for tetany pastures. When the potassium content of the pasture was high an increase in protein content of pastures as a consequence of nitrogen fertilization also increased the frequency of incidence. Butler (6)

conducted the same type of survey in Scotland over a period of two years. He suggested that pastures associated with grass tetany had a higher potassium and lower magnesium content than the non-tetany pastures. The incidence of tetany was 1.1 percent among cows where the $\frac{K}{Ca + Mg}$ ratio was 1.3 as compared to 10 percent in regions where the above ratio was in the range of 2.24 to 3.09. These results are in agreement with that of Kemp and Hart (26).

On the other hand, Ender et al. (15) reported that sulphate and phosphate salts of potassium, sodium and ammonium interfere with magnesium metabolism, bringing about a sudden decrease in the serum magnesium values in sheep. Dosing with other potassium and sodium salts did not have a significant effect on hypomagnesemia. As a result, the authors eliminated the tetanigenic effect of high levels of potassium in the ration. However, Kunkel et al. (29) included 5 percent of potassium as a bicarbonate salt in the ration for sheep and obtained a significant lowering of the serum magnesium. They also studied the limitations of water and of sodium chloride along with 5 percent potassium, but found no significant relationship of these to hypomagnesemia. The inclusion of potassium bicarbonate in the ration also decreased feed intake and gain.

Colby and Frye (10) studied the effect of feeding various levels of calcium, potassium, and magnesium to rats as an indirect means of finding the principal cause of grass tetany in ruminants. They

reported that high levels of calcium or potassium either separate or together enhanced the onset of hypomagnesemic symptoms by three to four days. The growth of the rats was depressed while the mortality was increased and blood magnesium level was markedly decreased from 2.1 ppm to 1.03 ppm. Forbes (17) and Welt (60) also studied the role of potassium in experimental magnesium depletion with rats. The depletion resulted with a marked hypomagnesemia, a mild hypercalcemia and a modest azotemia along with a significant muscle magnesium and potassium depletion.

Hemingway and Ritchie (22) suggested that hypocalcemia played an important role in inducing hypomagnesemic tetany symptoms in cattle, sheep, and milk-fed calves. In many circumstances, these animals may be hypomagnesemic for a long time, but not show clinical symptoms. However, the clinical signs of tetany in such animals appear after a rapid fall in plasma calcium concentration. At this stage of development, magnesium supplementation increases both plasma magnesium and calcium while the supplementation of calcium affected neither one. They concluded that severe hypomagnesemia interferes with the calcium metabolism of plasma.

Since plasma calcium was reported to be involved in clinical tetany, Vitamin D has been of interest to the scientists. Richardson and Welt (46) studied the role of vitamin D in the onset of hypomagnesemia. They fed magnesium-deficient diets to two groups of

rats. One of these groups was then injected with vitamin D_2. The hypomagnesemic condition developed in the vitamin D_2 injected group. In spite of hypomagnesemia, they did not detect any change in fecal and urinary excretion of magnesium. The magnesium levels in the muscle and total carcass were also unchanged. These findings indicated that since no loss of magnesium was involved a redistribution of magnesium from extracellular fluid to some other phase must have taken place. However, Smith (50) working with milk-fed calves found that when feeding 70,000 I. U. vitamin D_3 per day or irradiation of the calves with u.v. light, the mean fecal excretion of magnesium increased from 32 to 86 percent of the dietary magnesium in about thirteen weeks, after which it did not change greatly. On the other hand, some workers (24, 45) have used vitamin D for the correction of hypomagnesemia in cattle and man, but have not obtained any improvement in the magnesium balance.

Thus far a number of possible dietary factors affecting hypomagnesemia have been described. However, the demonstration of clinical symptoms, mainly tetany, appears to be associated with stress condition. It has been reported (7, 26) that clinical signs of tetany occurs most frequently under severe environmental conditions such as cold, wet, stormy and fluctuating temperature. McAleese and Forbes (35), however, while working with rats, reported that

the requirement for magnesium, measured by blood magnesium level, was not increased by the decrease in temperature, nor by the time on experiment. They obtained higher values for blood magnesium at 10° than at 23° C and reported that the maintenance requirement for magnesium at 10 and 23° C were 221 and 354 ppm, respectively.

Distribution of Magnesium in the Body

The distribution of magnesium in the body may be discussed in three general areas: (a) bone, (b) soft tissue, and (c) extracellular fluid. Hypomagnesemia deals with blood serum or plasma. It has been reported (48, p. 162) that 60-70 percent of the body magnesium is in the skeleton, 30 percent in the soft tissues, and only about 1 percent in the extracellular fluid.

Bone Magnesium

Bone is the major area for magnesium deposition in the body which accounts for 0.5 to 0.7 percent of bone ash. It has been reported in rats (17), calves (3, 51), dogs (4), and guinea pigs (39) that the bone magnesium in the magnesium depleted animals dropped about 30 to 60 percent. These findings suggested that bone is the reservoir of magnesium and is highly labile. In cases of normal or excess magnesium in the diet, the bone plays very little or no

role in the regulation of plasma magnesium. But in cases of deficiency, the bone becomes the supply depot for plasma magnesium. However, the mobility of bone magnesium depends on various factors, such as stage of growth, renal function, acid-base balance and the state of magnesium storage in the body. On the other hand, Field (16) found that the exchange of magnesium between plasma and bone was very slow and suggested that bone was not a sufficient reserve for maintaining plasma magnesium.

Magnesium in Soft Tissues

The concentration of magnesium in soft tissues varies according to the level of water, fat, or fibrous material in the tissue. However, the concentration of the intracellular fluid is about 36 mg per 100 ml (65). Since the extracellular fluid concentration is approximately 2.5 mg per 100 ml, it is easily understood that there is a great concentration gradient across the cell wall.

McIntyre and Davidson (32) reported the magnesium concentrations of liver, kidney, and skeletal muscle of the rat as 81.7, 82.7, and 102.2 mg per 100 g of fat free dry matter, respectively. The concentration of magnesium in soft tissue may be reported in different terms, e.g., in terms of fresh material, dry matter, or fat free dry matter. However, the fat-free dry matter is more commonly used since it gives more directly comparable results.

The magnesium concentration of fat-free kidney, heart and skeletal muscle of guinea pigs were reported to be 91.9, 105.0, and 121.0 mg per 100 g, respectively, by Morris and O'Dell (39).

Experiments with ^{28}Mg have indicated that the rate of exchange of magnesium between tissues and plasma is highest in heart, kidney, liver and is slower in ovaries, thyroid, skeletal muscle and adrenal glands in that order (4). After the intravenous injection of radioactive magnesium, the concentration of ^{28}Mg in heart muscle was found to be much higher than skeletal muscle. To study the effect of contraction on the magnesium content of muscle, skeletal muscle was stimulated to contract at the same rate as the heart muscle, but the concentration of ^{28}Mg was still markedly lower than in heart muscle. Also there was no difference in the ^{28}Mg concentrations between stimulated and resting skeletal muscles. Care's (8) experiments with ^{28}Mg suggest that there are two components of magnesium in the soft tissues. One component is rapidly exchanged while the other is rather stable. The concentration of labile magnesium in the intracellular water is about the same as that in extracellular fluid. This may be considered as an indication that labile magnesium in the soft tissue acts as a useful reserve for the extracellular magnesium.

Magnesium in Extracellular Fluid

Plasma or serum represents the extracellular fluid of the body for the study of magnesium. The concentration of magnesium in serum or plasma shows considerable variation, not only among the species but also among the individuals within a species. However, the normal levels of magnesium in serum or plasma for all species is in the range of 1.7 to 3.0 mg per 100 ml or 1.4 to 2.5 meq per liter. In cases of hypomagnesemic tetany generally the level falls below 1 mg per 100 ml (3, 14, 54). The magnesium in plasma is found both as free ions and bound to plasma proteins. The ionic magnesium is the physiologically active form in the plasma and is ultrafiltrable. This form constitutes about 70 percent of the total plasma magnesium. Wilson (65) reported that ionic magnesium in plasma ranged from 60 to 80 percent with an average value of 67 percent.

Absorption of Magnesium

Reports have indicated that magnesium is absorbed primarily in the small intestines (65) and secondarily in the fore stomach and large intestine (57). The source of magnesium absorbed by the intestine may be of two origins: (a) alimentary and (b) endogenous. The latter is found in the digestive secretions. This may mean that

any interference with the absorption of alimentary magnesium results in a loss of magnesium from the body fluids. Due to different methods, feeds, and species of animals there have been contradictory reports in the estimation of magnesium absorption. According to Garner (18), 65 to 86 percent of the magnesium of fresh grass was retained by the guinea pig and retention was not dependent on the magnesium content or the calcium-phosphorus ratio or the crude fiber content of the feed. On the other hand, it has been reported in Holland (27) that the availabilities of magnesium were 10, 16, and 20 percent at the three stages; namely, early growth, pre-bloom, and after bloom, of growth on the same pasture. Magnesium retentions for the respective stages were -1.0, -0.4, and -0.1 g per day.

Van't Klooster (57) estimated minerals in the gut content of sheep by suspending cellophane bags into the small and large intestine. A shift of ration from hay to grass resulted in a decrease in soluble calcium and magnesium concentrations in large intestine from 24 to 7.4 percent for calcium and 43 to 24 percent of magnesium.

After finding a positive relationship between alimentary intake and urinary excretion of magnesium, Storry and Rook (54) used urinary excretion of magnesium in cows as a measure of absorption. The authors estimated the availability of magnesium of various salts

and found that oxide, nitrate, acetate and lactate gave similar availability values while sulfate and silicate were lower.

The Role of Magnesium in Biochemical Processes

It was previously reported in this chapter that magnesium activates many important enzymes in the body and is involved in protein synthesis. All the enzymes which are required for transferring phosphate group(s) from ATP to a receptor, or from a phosphorylated compound to ADP are activated by magnesium (58, pp. 490-492). This property of magnesium makes the element necessary for the normal processes of glycolysis and aerobic metabolism. Magnesium is also required for the synthesis of DNA and RNA in the body.

Excretion of Magnesium

Magnesium is excreted from the body in urine, feces, and milk. Fecal magnesium constitutes about 83 percent of the dietary magnesium and is mainly unabsorbed magnesium (27). Endogenous magnesium is also present in the feces and increases with the increase in the endogenous secretion into the intestines or with the decrease in the absorption of magnesium (52).

It is generally accepted that kidneys regulate the magnesium content of the body (27, 52, 54). Storry and Rook (54) showed that

withdrawal of dietary supplement of magnesium from the ration resulted in a rapid fall in the urinary magnesium, from a value of 1-2 g per day to zero within four days. The decrease of serum magnesium from 2.7 mg per 100 ml down to 1.0 to 1.5 mg per 100 ml coinsided with the urinary fall but at a slower rate. Magnesium supplementation in the diet resulted in a rapid increase in the urinary excretion of magnesium.

Wilson (65) suggested that excretion of magnesium is principally a filtration-resorption mechanism and that interactions with other ions may influence this mechanism. Such interactions have been reported between magnesium and calcium and magnesium and potassium (45, 66). Experiments with men and dogs indicated that the infusion of magnesium resulted in an increased urinary excretion of magnesium which also coincided with an increased tubular resorption of magnesium, increased urinary excretion of calcium and a decreased urinary excretion of potassium (66).

Endocrine Glands and Magnesium

Reports on the interactions between magnesium metabolism and endocrine secretions are conflicting and do not have a clear explanation. Hanna et al. (21) reported a negative magnesium balance in hyperparathyroidism and a positive balance following removal of the parathyroids in human patients. MacIntryre et al.

(33) suggested a regulating effect of parathyroid hormone on plasma magnesium in rats but the explanation was opposite to that of Hanna et al. (21). They administered purified bovine parathyroid hormone to rats and found an increased urinary retention of magnesium indicating a linear relationship between the dosing of hormone and the amount of magnesium retained. They concluded that there was a check and balance mechanism between parathyroid hormone and plasma magnesium concentration through renal retention of magnesium.

The effect of thyroid hormone on the regulation of plasma magnesium concentration is not clear. Swan and Jamieson (55) fed iodinated protein to cows and found a drop in plasma magnesium concentration. Hanna (19), while working with rats, confirmed these findings and reported that hyperthyroidism results in a decrease in plasma magnesium concentration while the hypothyroidism results in the opposite. However, the results have not been conformed yet.

Adrenal hormones also influence the plasma magnesium concentration. Hanna and MacIntyre (20) reported that aldosterone administration to adrenolectomized rats resulted in an increased renal excretion of magnesium. However, in later work, Care and MacDonald (9) suggested that the effect of aldosterone on magnesium balance was an indirect action of aldosterone through sodium.

In summary, parathyroid, thyroid, and adrenal hormones may exert an effect on magnesium metabolism and plasma magnesium concentrations, but there is no evidence that these effects involve a check and balance mechanism.

MATERIALS AND METHODS

Part A. Experiments With Rats

The first part of the investigations for this thesis was designed to study the effects and interactions of dietary magnesium, potassium and nitrogen in hypomagnesemia using male rats as experimental animals. The design of this experiment was a 3 X 3 X 3 factorial as outlined in Table 1. The three levels of potassium were fed to the experimental animals imposing the three levels of magnesium and the three levels of nitrogen. Thus 27 different treatments allow detailed statistical comparisons among the different dietary regimes and their possible interactions.

Table 1. The over-all design of the experiments with rats.

Nitrogen	Magnesium (mg/kg diet)	Potassium (g/kg diet)		
		1.8	3.6	7.2
2.2	400	10 rats	10 rats	10 rats
	200	10 rats	10 rats	10 rats
	50	10 rats	10 rats	10 rats
3.0	400	10 rats	10 rats	10 rats
	200	10 rats	10 rats	10 rats
	50	10 rats	10 rats	10 rats
3.8	400	10 rats	10 rats	10 rats
	200	10 rats	10 rats	10 rats
	50	10 rats	10 rats	10 rats

The lowest level of potassium, 1.8 g/kg of the diet, in this experiment is recommended by the National Research Council (41) as a normal level for growing rats. The 3.6 and 7.2 g potassium per kg of diet are two and four times the recommended level, respectively. An increase in the protein level has been reported to decrease blood magnesium concentration (11). To check the validity of this theory and investigate the possible interactions with potassium and magnesium, levels of 18, 24 and 30 percent purified high nitrogen casein were fed in addition to the various dietary treatments with potassium and magnesium. The 18 percent casein level (2.2 percent nitrogen) is used as protein supplement for growing rats at the Oregon State University Small Animals Laboratory.[1] The other two levels of casein supplied 3.0 and 3.8 percent nitrogen in the rations.

The 400 mg magnesium per kg diet was based on the normal requirement of growing rat (41). The second and third levels were 200 and 50 mg per kg, respectively. It has been reported (56) that 50 mg magnesium per kg of the diet is the minimum safe level for growing rats.

Two hundred and seventy white male rats, Mus norvegicus albinus, Sprague-Dawley strain, weighing 50 to 60 g were purchased

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[1] Oldfield, J. E., R. C. Bull and W. W. Ellis. Laboratory demonstrations in animal nutrition. Mimeographed Lab Manual. Corvallis, Oregon. Oregon State University, Department of Animal Science.

from Northwest Rodent Company, Pullman, Washington and housed in the Oregon State University Small Animals Laboratory. Ten rats were randomly allotted to each treatment. Each rat was placed in an individual galvanized iron cage and was fed and watered separately. The diet was provided ad lib. in a feeder consisting of a stainless steel cup with screen and cover to reduce feed wastage. Weekly weight gains and feed consumption data were recorded. The water was provided in glass bottles fitted with a glass nipple.

The investigation with rats was conducted in three experiments.

Experiment I. 2.2 percent nitrogen

Experiment II. 3.0 percent nitrogen

Experiment III. 3.8 percent nitrogen

Each experiment consisted of nine treatments according to the three levels of potassium and magnesium.

Preparation of the Diets

The compositions of the diets and mineral mixtures are shown in Table 2 and Table 3, respectively.

The basic mineral mixture was prepared separately and the variable minerals were added according to the levels designated for each treatment. The diet for each treatment was mixed separately in a Hobart mixer for 30 minutes and stored in a plastic bag in a refrigerated room. A similar ration was used for Experiments II

and III with the exception of the level of nitrogen which was increased from 2.2 to 3.0 percent for Experiment II and to 3.8 percent for Experiment III at the expense of corn starch in the ration.

Table 2. Experimental rat diet No. I

Ingredient	g/kg
Starch[a]	643.0
Casein[b]	180.0
Cottonseed oil[c]	100.0
Glucose	5.9
Salts	40.0
Vitamin mixture[d]	11.1
Alphacel[e]	20.0

[a]Buffalo Corn Starch, Corn Products Co., New York, N. Y.
[b]Casein (purified, high nitrogen), Nutritional Biochemicals Corporation, Cleveland 28, Ohio.
[c]Kraft Oil, Kraft Foods, Chicago 90, Illinois.
[d]Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corporation, Cleveland 28, Ohio.
[e]Alphacel, Non nutritive bulk, Nutritional Biochemicals Corp.

Table 3. Salts for the rat rations[a]

Ingredient	g/kg
Common salts:	
$Ca_3(PO_4)_2$	15.4700
KH_2PO_4	6.2648
NaCl	5.2930
$FeSO_4 \cdot 7H_2O$	1.0001
$MnSO_4 \cdot H_2O$	0.1520
$ZnCl_2$	0.0098
$CuSO_4 \cdot 5H_2O$	0.0180
$CoCL \cdot 6H_2O$	0.0008

[a]The above named salts were weighed out, variables magnesium and potassium (in form of $MgSO_4 \cdot 7H_2O$ and K_2SO_4) were added for each treatment and made up to 40 g with glucose.

The skin lesions of hypomagnesemic condition were classified for each rat as to the severity of the symptoms every other day. A relative classification scheme from one to five was used. Rank one was assigned to the rat which had a limited erythematous condition while rank five was assigned to the one that had extensive hematomatous and pustulous skin lesions and extensive hair loss.

Each treatment was divided into experimental feeding periods of (a) four weeks and (b) eight weeks. At the end of the fourth week of the experiment the first five rats were removed from the experiment. Blood and tissue samples were collected from each animal.

The rats were anesthesized by dry ice and blood was drawn by heart puncture using a syringe with a 20 gauge hypodermic needle. Some difficulties were encountered during this sampling procedure and in some cases only 2-3 ml of blood could be obtained. The blood was slowly transferred from the syringe into a centrifuge tube and kept at room temperature for 30 minutes and then placed in a refrigerator. The blood samples were centrifuged at 681 X g for five minutes and the serum removed by pipette and stored in a freezer for chemical analyses.

After the animal was killed approximately 4 g each of the hind leg muscles and heart were removed. The blood residue in the heart was washed out with physiological saline solution. The right femur

was removed and the soft tissue was scraped off. All tissue samples from each animal were placed in a plastic bag and frozen for later chemical analyses.

At the end of the eight week feeding period the remaining five rats in each treatment were sampled. Same procedures were followed as previously described except that ether was used to anesthetize the animals and the jugular vein was incised to collect the blood sample.

Chemical Analyses

An atomic absorption spectrophotometer[2] was used for the determination of magnesium, calcium and potassium in serum, bone and soft tissue samples. Atomic absorption spectroscopy is based on the theory that atoms of an element absorb light at a certain wave length which coincides with the spectral beam of that particular element. A schematic diagram of the atomic absorption spectrophotometer used in this experiment is presented in Figure 1. The sample to be analyzed is aspirated through a capillary tube into the atomizer and mixed with air and acetylene in the chamber and subsequently activated in the flame. The pressures of air and acetylene

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[2] A combination of Techtron Model AA-3 atomic absorption instrument and Carl-Zeiss Model M4 Q III spectrophotometer.

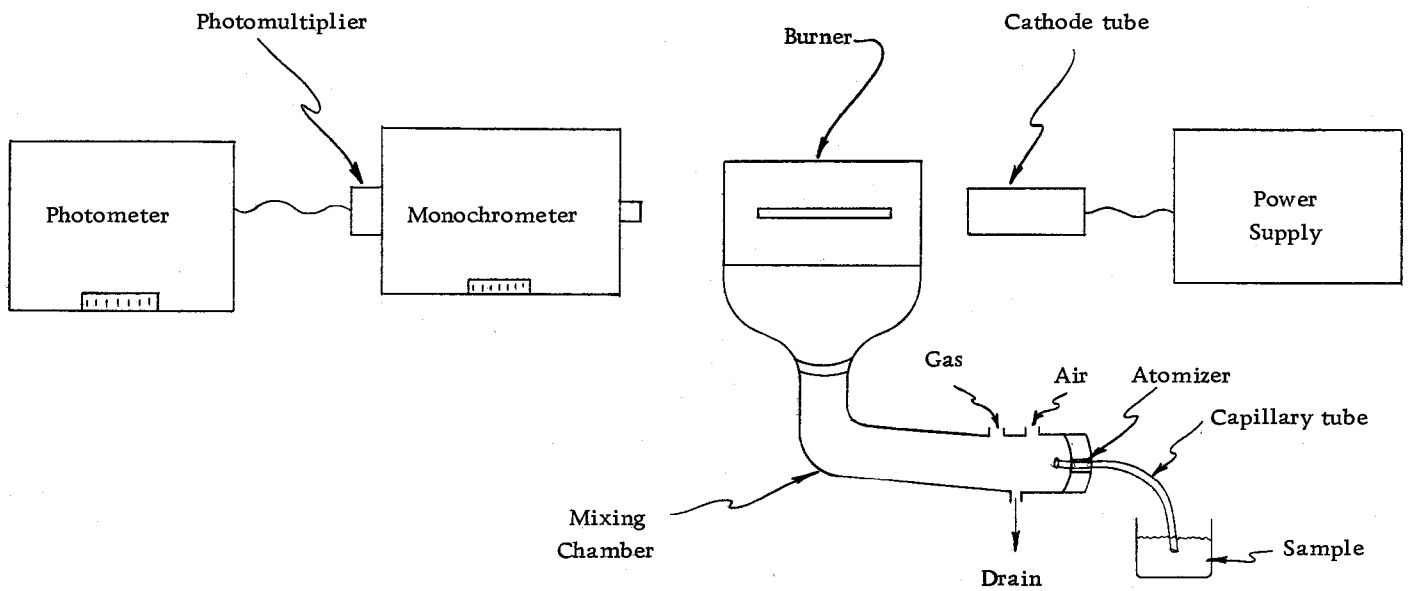

Figure 1. Schematic diagram of the atomic absorption spectrophotometer.

introduced into the spray chamber for the flame were 25 and 4 pounds per square inch, respectively. The activated particles of the element absorb some of the light beam passing through the flame. The intensity of the remaining light beam was measured by the spectrophotometer (62).

Blood Serum Analyses

Calcium and magnesium: The method developed by Willis (62, 63) was used for the determination of calcium and magnesium concentrations in the serum. A sample of 0.5 ml of serum was pipetted into a test tube and was diluted with 9.5 ml of 10,000 ppm solution of the disodium salt of ethylenediaminetetraacetic acid (EDTA. Na_2). The serum and EDTA. Na_2 solutions were mixed thoroughly by shaking. The concentrations of magnesium and calcium were then measured relative to standard calcium and magnesium solutions containing the same concentration of EDTA. Na_2. Willis (62) reported that proteins and phosphorus in the serum interfere with calcium and magnesium measurements by depressing their activation in the flame. The use of EDTA. Na_2 controls these interferences. The particular wave lengths for magnesium and calcium determinations were 2854 Å and 4227 Å, respectively. The instrument was standardized with known concentrations of each element and rechecked after every fifth unknown sample.

Potassium: For the determination of potassium the same solution as used in the determination of magnesium and calcium was used (64). A 1 ml sample was pipetted into a test tube and diluted to 10 ml with a 10,000 ppm EDTA. Na_2 solution. It was then read with the atomic absorption apparatus at 7669 Å wave length against the standard potassium solutions.

Tissue Analyses

Magnesium concentrations in bone, heart and skeletal muscle samples were determined on a fat-free dry matter basis. Moisture from the samples was removed by a Thermovac freeze-dry apparatus. Frozen tissue samples were cut into small pieces and placed in 2 inches diameter aluminum foil pans for the drying process. Dry tissue samples were then extracted with ether for 48 hours using Soxhlet apparatus. After the extraction, the tissue samples were dried at 80° C for 24 hours to evaporate the ether. The fat-free dry samples were stored in a dessicator for further chemical analyses.

The magnesium content of bone, heart and skeletal muscle were determined by a modified method of Parker (44). A sufficient amount of each sample was weighed in a 30 ml Kimax beaker. The beakers were heated in a muffle furnace at 250° C until samples stopped smoking. The temperature was then increased to 500° C and

the samples were ashed for six hours. The beakers were removed from the furnace with final cooling in a dessicator and weights were recorded for the determination of ash content.

Ten ml of a 1:4 dilution of HCl solution was added to each beaker containing the ashed sample and baked to dryness on a hot plate. Then 10 ml of 0.1 N HCl were added and warmed. The solution was filtered through a S & S American No. 589 black ribbon filter paper into a 100 ml volumetric flask. The beaker and residue on the filter paper were washed thoroughly and the filtrate was made to volume with distilled water. An aliquot sample was taken and $SrCl_2$ was added to give 2500 ppm strontium in the final solution (44). The strontium chloride was added to prevent interference from phosphate. The final solution was measured relative to the standard magnesium solutions which contained the same amount of strontium.

Data from the rat experiment were recorded on IBM data sheets and then transferred to IBM punchcards. The calculations and the statistical analyses were made by the Oregon State University Computer Center according to the programs provided by the Department of Statistics and using a CDC 3300 computer.

Part B. Survey with Grazing Cows

The objective of this survey was to study the relationships between the certain constituents of forage grasses and the

concentrations of serum magnesium of cows in the early pasturing season. Fourty Holsteins and 20 Jersey cows of the Oregon State University Dairy Herd were used for the survey. At the time the animals were placed on the pasture located at the Oregon State University Dairy Farm they were in the first six months of their lactation. The experiment started on April 18, 1966 and lasted for five weeks with two weeks and three weeks experimental periods. A rotational grazing system was practiced on five plots of nitrogen fertilized and irrigated pasture so that each plot was grazed about one week. The botanical composition of the pastures was determined using the dry weight rank method (34). It was found that the pasture contained 60 percent orchard grass, 20 percent meadow foxtail, 12 percent tall fescue and 8 percent Ladino clover.

One day before the animals were placed on the pasture a 5 ml blood sample was drawn from each cow through the tail vein and prepared for analyses of magnesium, calcium and potassium as discussed in Part A. On the day of blood sampling a representative forage sample was collected from the pasture. The dry matter, protein, magnesium and potassium were analyzed on the forage samples.

At the end of the second week a second sampling of blood and forage was performed. During this first two weeks of the experiment the cows were kept on pasture from 8 a.m. to 3:30 p.m. At

night they remained in the barn and loafing area. Each animal was given an average amount of 7 pounds of alfalfa hay, 24 pounds of grass silage and 15-20 pounds of grain daily. In the final three week period the animals remained on the pasture except for the milking and the feeding time. During this period each cow received about 7 pounds of hay, 7 pounds of silage and 15-20 pounds of grain according to the level of production per day. At the end of this period the blood and forage samples were collected as before.

Standard statistical techniques were used to analyze the data obtained from the chemical analyses of blood and forage samples.

RESULTS AND DISCUSSION

Part A. Experiments With Rats

Growth and Feed Conversion

The growth of rats was analyzed statistically in terms of weekly weight gains and feed conversions. The means of the weekly weight gains and feed conversion efficiencies are given in Table 4, with the statistical analysis in Table 5. An increase of dietary nitrogen from 2.2 to 3.0 percent resulted in an increase in average weekly weight gain from 24 to 40 g for the four week- and 19 to 32.4 g for the eight-week period. The related feed conversion efficiencies (g feed/g gain) also increased with this change of dietary nitrogen. Further increase of dietary nitrogen from 3.0 to 3.8 percent did not significantly increase the weekly weight gain nor a further increase in the feed conversion efficiency. In the over-all analysis of the data the differences in weight gain and feed conversion efficiencies for both feeding periods were found to be highly significant ($P < 0.01$) among the levels of nitrogen in the ration.

The mean weekly weight gains and feed conversion efficiencies were not significantly different for the 400 and 200 mg/kg magnesium groups. The highly significant differences ($P < 0.01$) for the weekly

weight gains and feed conversions in magnesium resulted from the decrease of dietary magnesium from 200 to 50 mg level. The weekly weight gains were markedly decreased from the first to second period along with a decrease in feed conversion efficiency, indicating the decrease of feed utilization with age. McAleese and Forbes (35) reported that daily weight gains of rats were markedly increased by elevating the dietary magnesium up to 118 ppm. Further increase of magnesium in the diet did not change the daily gain. The findings of the present investigation are in agreement with this report.

Table 4. The mean weekly weight gains and feed conversions of rats with different treatments

			Four Week[1]		Eight Week[2]	
		Level	Weekly weight gain g	Feed Conversion g feed/g gain	Weekly weight gain g	Feed Conversion g feed/g gain
Diet	Nitrogen %	2.2	24.0^{a}	3.5^{a}	19.0^{a}	4.7^{a}
		3.0	40.0^{b}	2.4^{b}	32.4^{b}	3.3^{b}
		3.8	40.5^{b}	2.6^{b}	33.8^{c}	3.2^{c}
	Magnesium mg/kg	400	38.8^{a}	2.6^{a}	31.0^{a}	3.6^{a}
		200	38.6^{a}	2.7^{a}	30.5^{a}	3.6^{a}
		50	27.1^{b}	3.2^{b}	23.7^{b}	4.0^{b}
	Potassium g/kg	1.8	33.7^{a}	3.0	28.0	3.6^{a}
		3.6	35.0^{b}	2.8	28.7	3.7^{b}
		7.2	35.9^{b}	2.8	28.5	3.9^{c}

[1] Each mean value represents 360 observations on 90 rats.

[2] Each mean value represents 360 observations on 45 rats.

[a, b, c] The mean values with unlike superscripts within each group are significantly different ($P < 0.05$)

Table 5. Analysis of variance for the weight gain and feed conversions of rats in different periods

Source of Variation	4 Week D. F.	4 Week Weight gain Mean square and F test	4 Week Feed Conv. Mean Square and F test	8 Week D. F.	8 Week Weight gain Mean square and F test	8 Week Feed Conv. Mean Square and F test
Nitrogen	2	31462**	116.4**	2	23907**	232.87**
Magnesium	2	16175**	37.4**	2	6091**	17.01**
Potassium	2	448**	4.1	2	42	5.61**
Nitrogen X Magnesium	4	2472**	17.8*	4	1277**	1.18*
Nitrogen X Potassium	4	131	11.8	4	82	0.28
Magnesium X Potas.	4	76	9.8	4	55	0.16
Nit. X Mag. X. Potas.	8	173*	9.8	8	86	1.70**
Error a	243	71	5.4	108	71	0.46
Weeks	3	508**	134.3**	7	8676**	11.28**
Treatment X Weeks	78	285**	4.3	182	147**	0.64
Error b	972	43	3.4	864	44	0.29
Total	1322			1187		

*Significant ($P < 0.05$)

**Highly significant ($P < 0.01$)

The effect of dietary potassium levels on the weekly weight gains were found to be highly significant ($P < 0.01$) for the four week period, but nonsignificant for the eight week period. The increase of potassium in the diet from 1.8 to 3.6 g/kg level resulted in a significant increase in the rate of gain for the four week period. Further increase in potassium from 3.6 to 7.2 g/kg level did not increase the weekly gain significantly. The increase of weekly weight gain due to potassium was not accompanied by a significant

increase in feed efficiency for the four week period. In the eight week period the feed conversion efficiencies were highly significantly ($P < 0.01$) decreased with the three increasing levels of potassium in the diet but the weekly weight gains were not significantly changed. This indicates that in the longer feeding period the increased levels of potassium in the diet decreases the utilization of feed by rats. It has been reported that high levels of potassium in the diet decreased the weight gain of sheep (29) and rat (10, 17). However, much higher concentrations of potassium were used by the authors cited than in the present study.

The curves of the weekly weight gains of the experimental animals with nine different dietary variables are given in Figure 2. The 2.2 percent nitrogen group had a much lower rate of gain than 3.0 and 3.8 percent groups. The rats on 3.8 percent nitrogen diet gained slightly more than those on 3.0 percent level until the sixth week. At the sixth week 3.0 percent nitrogen group showed a sharp decline on the growth curve while 3.8 percent group had a rather smooth curve. The curves joined again at the eighth week.

The growth curves for magnesium treatments point to a similarity between the weekly weight gains of rats with 400 and 200 mg magnesium levels. The growth rate at 50 mg was much lower than at the other two levels of magnesium. The three curves coincided at the seventh week. However, at the eighth week the rate

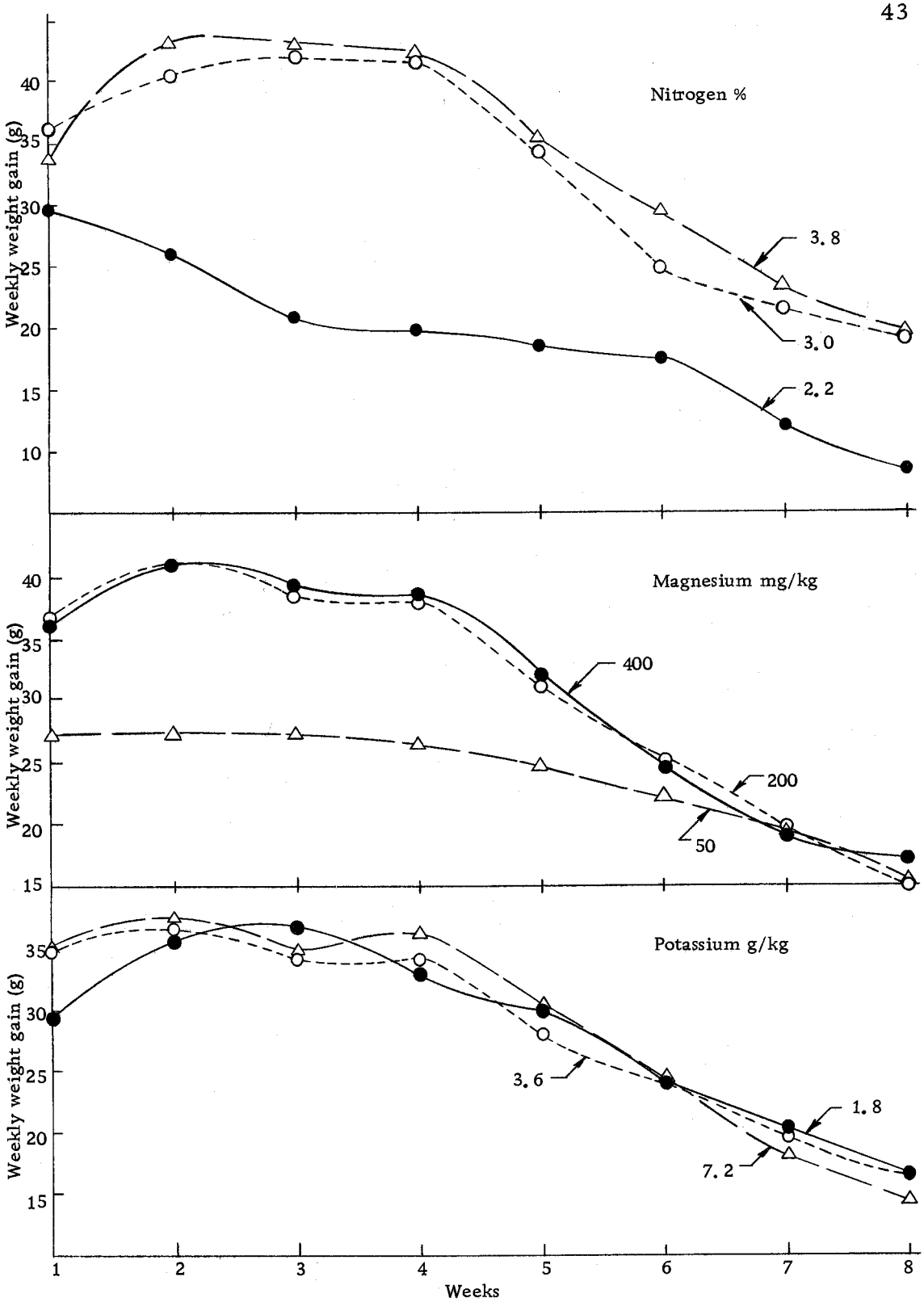

Figure 2. The weekly weight gain of rats with different dietary treatments[a]

[a]The first four weeks portion of each curve represents 90 and the rest of it represents 45 rats.

of gain on 400 mg magnesium level was 17 g while in the other two groups it was 15 g. The beneficial effect of feeding 400 mg level magnesium may have been shown by an extended period of feeding.

The three groups of rats on the three levels of dietary potassium had similar growth curves as seen in Figure 2. Statistical analysis showed highly significant ($P < 0.01$) differences for the growth rates of rats on different levels of potassium for the first four week period. These differences probably are not meaningful since the smaller rate of gain on the 1.8 g level during the first week contributed heavily to them.

One consistent point for all these growth curves is the slight decline at the third week. This decline is particularly evident on the magnesium and potassium curves. The animals showed some symptoms of respiratory disorders in the third week of the experiments and were treated with terramycin in their drinking water. Although the rats on the 3.6 and 7.2 g levels of potassium showed the most marked decline in this week, the 1.8 g group was apparently not affected. It could not be detected whether this decline was due to disease situation or interaction between the terramycin treatment and the higher levels of potassium and magnesium in the ration.

Dermal Symptoms of Magnesium Deficiency

Skin lesions were observed in rats fed the 50 mg/kg level of

magnesium. Vasodilation and hyperemia in the vascular system of skin became visible in two weeks by rats on the 2.2 percent nitrogen diet and in nine days on the 3.0 and 3.8 percent nitrogen levels. Reddish spots became more intensified within the following two to three days resulting in skin lesions as tiny oval scabs. The hair of the rat became coarse and matted all around the body. During this period, the animals were extremely nervous and hyperexcitable. The skin lesions enlarged and several would fuse forming open sores. These formations were observed around the neck, around the ears, on the ear lobes and on the nose. Figure 3 shows the general appearance of a rat affected with skin lesions in comparison with an unaffected one. The magnesium defficient rat was from the treatment of 7.2 g/kg potassium, 3.8 percent nitrogen and 50 mg/kg magnesium while the other rat was from 3.6 g/kg potassium, 3.8 percent nitrogen and 400 mg/kg magnesium diet. As it is seen in the figure the size of the deficient rat was much smaller than the other one. The more exposed skin areas had more extensive skin lesions. Other parts of the body were also affected including the skin areas of back and abdomen.

Hair loss around the affected areas later accompanied the skin lesion development. Hair loss was considerably greater around the eyes, on and around the ears, under the jaws and around the neck. Within two to three weeks the skin lesions became circumscribed,

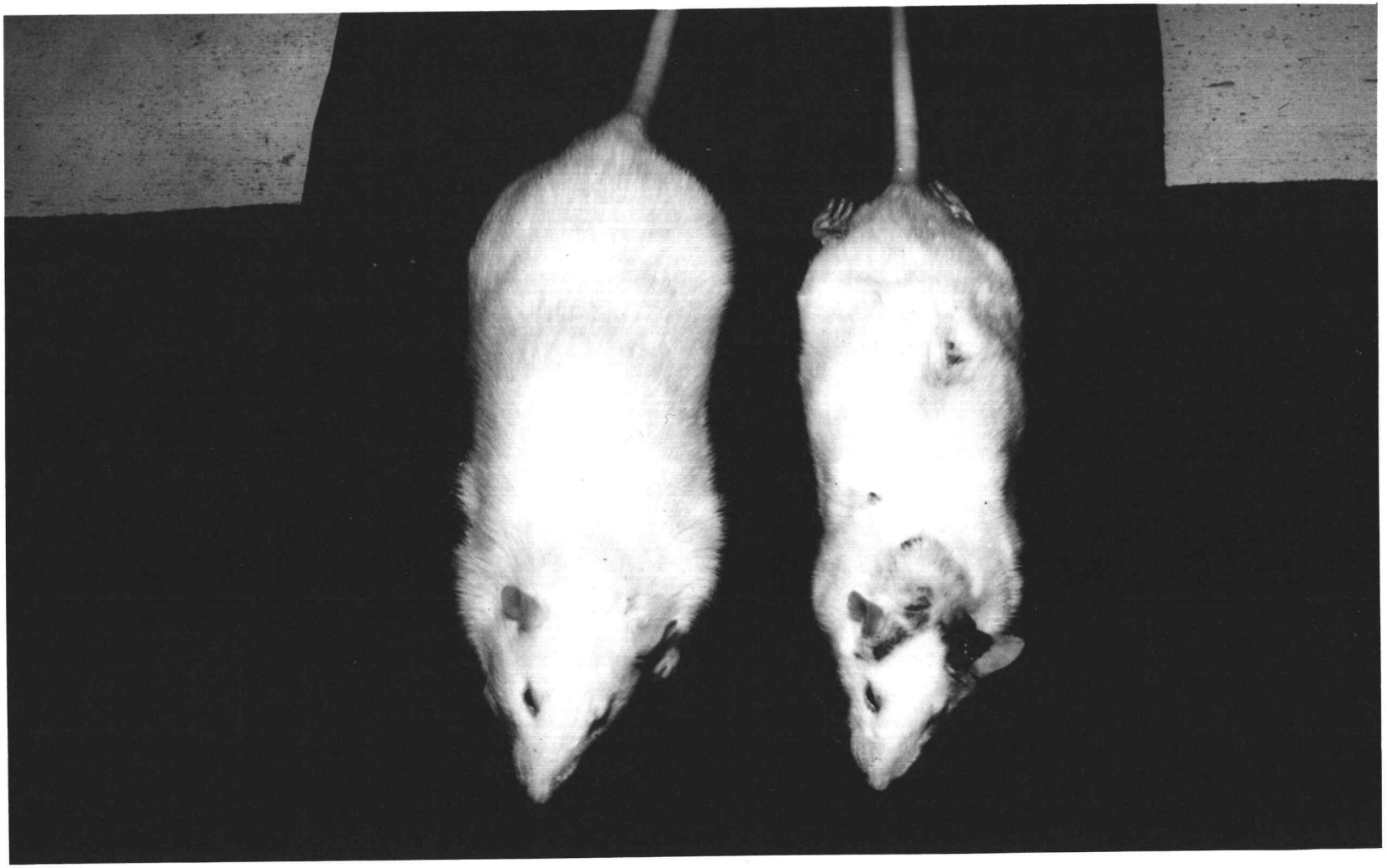

Figure 3. Skin lesions of magnesium deficiency (right) as compared to a normal rat (left).

dried and then desquamated leaving a pale appearance on the skin. The scores of the severity of the skin lesions (classified using a scale from one to five, with five representing the most serious cases) were added together for each animal and the final value was used in statistical analysis. The mean values for the treatments are given in Table 6. There were no statistically significant differences among the levels of potassium and nitrogen for the first four week period. However, in this period the 3.8 percent nitrogen group had a mean value of 102.0 while the 2.2 percent level animals had a value of 88.3. In the eight week period the severity of skin lesions showed significant increase ($P < 0.05$) with the increasing levels of nitrogen in the ration. The values for the animals on the 3.6 g/kg potassium level were lower than the other two levels in both four and eight week periods. These lower values may indicate a favorable effect of potassium at this level to prevent skin lesions. These differences were not statistically significant. Forbes (17) fed 0.1, 0.62 and 1.40 percent potassium levels with 40 ppm magnesium diet to the albino rats and observed skin lesions on about the twelfth day in all animals except than those on the 0.10 percent potassium diet. These latter animals showed neither skin lesions nor convulsions. The present study, however, did not show a beneficial effect for the skin lesions at the 0.18 percent dietary potassium level.

Table 6. Mean values of the individual cumulative scores[a] of the skin lesions for each treatment

			Potassiom (g/kg)			
			1.8	3.6	7.2	Raw Mean
4 week period	Nitrogen %	2.2	91	62	112	88.3
		3.0	118	73	77	89.3
		3.8	100	95	111	102.0
	Column mean		103.0	76.7	100.0	
8 week period	Nitrogen* %	2.2	139	130	183	150.7
		3.0	168	129	204	167.0
		3.8	233	209	247	229.7
	Column mean		180.0	156.0	211.3	

[a]A scoring system from one to 5 was used, one being the least and 5 being the most severe condition.

*Significant ($P < 0.05$).

Serum Analyses

Concentrations of magnesium, calcium and potassium in the serum were determined for each rat. The mean values of these analyses and the results of analysis of variance of the data are shown in Tables 7 and 8, respectively. The mean concentration of serum magnesium was 2.04 mg/100 ml for the four week period and it dropped to 1.73 mg/100 ml for the eight week period. This difference was found to be highly significant ($P < 0.01$). It may be interpreted either as the effect of aging of the animals (42) or prolonged feeding

of low levels of magnesium. The mean serum magnesium values at the 400 mg/kg dietary magnesium level which is recommended by National Research Council (41) as the normal requirement of growing rat were 2.64 mg/100 ml for the first period and 2.38 mg/100 ml for the second period. Lower serum magnesium concentration for the eight week period suggests that serum magnesium value of rats decreases with the age. However, the highly significant interaction ($P < 0.01$) between period and dietary magnesium indicates that age, along with longer feeding period of low levels of magnesium, accentuates the hypomagnesemic condition.

Table 7. Mean values of blood serum analysis for different periods and treatments

		Period or Level	Number of Rats	Serum			
				Magnesium mg/100 ml	Calcium mg/100 ml	Potassium No. of rats	Potassium mg/100 ml
	Period	4 weeks	135	2.04[a]	10.8[a]	90	24.2[a]
		8 weeks	135	1.73[b]	10.7[a]	90	22.6[b]
Diet	Nitrogen %	2.2	90	2.14[a]	10.5[a]		
		3.0	90	1.84[b]	10.8[a, b]	90	24.6[a]
		3.8	90	1.66[b]	11.1[b]	90	22.2[b]
	Magnesium mg/kg	400	90	2.51[a]	10.8[a]	60	23.1
		200	90	2.18[b]	10.4[b]	60	24.1
		50	90	0.95[c]	11.1[a]	60	23.1
	Potassium g/kg	1.8	90	2.06[a]	10.7	60	22.6[a]
		3.6	90	1.83[b]	10.9	60	22.9[a]
		7.2	90	1.75[b]	10.7	60	23.7[b]

[a, b, c] Mean values with unlike superscripts within each group significantly different ($P < 0.05$)

Table 8. Analysis of variance for the serum concentrations of magnesium, calcium and potassium

Source of Variation	Degrees of freedom	Magnesium Mean Square and F test	Calcium Mean Square and F test	Potassium Degrees of freedom	Potassium Mean Square and F test
Period	1	6.43**	0.48	1	118.7**
Nitrogen	2	5.41**	8.16**	1	253.9**
Magnesium	2	61.25**	10.38**	2	11.4
Potassium	2	2.22**	1.21	2	18.9*
Period X Nitrogen	2	3.71**	3.90	1	0.6
Period X Magnesium	2	0.78**	0.05	2	41.1**
Period X Potassium	2	0.05	1.92	2	0.5
Nitrogen X Magnesium	4	0.46**	11.38**	2	47.5**
Nitrogen X Potassium	4	0.04	1.87	2	1.7
Magnesium X Potassium	4	0.19*	0.48	4	6.3
Per. X Nit. X Mag.	4	0.60**	9.79**	2	49.9**
Per. X Nit. X Pot.	4	0.02	0.79	2	1.2
Per. X Mag. X Pot.	4	0.12	1.63	4	16.1**
Nit. X Mag. X Pot.	8	0.08	1.42	4	8.4
Per. X Nit. X Mag. X Pot.	8	0.02	0.97	4	14.6**
Error	216	0.07	1.41	144	4.1
Total	269	0.65	1.78	179	8.4

*Significant ($P < 0.05$)

**Highly significant ($P < 0.01$)

The serum magnesium content decreased from 2.14 to 1.84 and 1.66 mg/100 ml with the increase of dietary nitrogen from 2.2 to 3.0 and 3.8 percent, respectively. The over-all differences were highly significant ($P < 0.01$), but no significant difference was found between 3.0 and 3.8 percent nitrogen groups. These results are in

agreement with the previous reports with rats (10, 60, 61) and cows (27, 30, 49). The interactions between period and nitrogen and also nitrogen and magnesium were found to be highly significant ($P < 0.01$) for the lowering of blood magnesium concentration. However, the interaction between nitrogen and potassium was not statistically significant.

A very close association was found between the levels of magnesium in feed and in serum. The concentration of magnesium in serum decreased from 2.51 to 2.18 mg/100 ml with the decrease of magnesium level in feed from 400 to 200 mg/kg. When the feed magnesium was reduced to 50 mg level, the serum magnesium concentration sharply decreased to 0.95 mg/100 ml. The differences between and among the levels of dietary magnesium on the serum magnesium contents were found to be highly significant ($P < 0.01$).

As the level of potassium in the feed was increased from 1.8 to 3.6 g/kg the mean serum magnesium value highly significantly ($P < 0.01$) decreased from 2.06 to 1.83 mg/100 ml. Further increase in potassium in the diet from 3.6 to 7.2 g level did not affect the serum magnesium significantly. However, the analysis of variance showed highly significant difference ($P < 0.01$) among the dietary potassium treatments. The hypomagnesemic effect of increased potassium intake has been reported in rats (10, 17), sheep (29) and cows (25). Ender et al. (15), however, found that fertilization of

pastures with potassium sulfate resulted in a more pronounced hypomagnesemia in sheep than the pastures top-dressed with sulfur-free nitrogen and potassium fertilizers.

The interaction of potassium and magnesium on serum magnesium was significant ($P < 0.05$) but potassium period interaction was not statistically significant. The multiple interaction among period, nitrogen and magnesium on the serum magnesium concentration was found to be highly significant ($P < 0.01$). In fact all interactions among the variables other than potassium were highly significant. When potassium was included in the interaction the significances were no longer present. This indicates that potassium affects the serum magnesium concentration independently. The only significant interaction ($P < 0.05$) involving potassium was with magnesium.

The magnesium balance experiments with cattle (27) and guinea pigs (18) have indicated that the availability of magnesium in the diet is an important factor in hypomagnesemia. Since the increasing levels of nitrogen and potassium in the ration decrease the serum magnesium, the interference for magnesium utilization may have taken place in the digestive tract. The onset of hypomagnesemia on pastures which have adequate levels of magnesium support this assumption (1, 6, 7). Van't Klooster (57) reported that the solubilities of calcium and magnesium in the large intestine of

sheep were 24 and 43 percent from hay, but only 7.4 and 24 percent from grass, respectively. In this present study the higher levels of protein or nitrogen and potassium in the ration may have decreased the absorption of magnesium either by mass action or through lowering the solubility of magnesium. The important point here is that no significant triple interaction was detected among nitrogen, magnesium and potassium on hypomagnesemia.

The calcium content of serum did not change significantly with the extention of feeding period. The increased level of nitrogen in the ration from 2.2 to 3.0 and 3.8 percent resulted in an increase in the serum calcium concentrations from 10.5 to 10.8 and 11.1 mg/100 ml, respectively. However, the only significant difference ($P < 0.01$) was found between the dietary treatments of 2.2 and 3.8 percent nitrogen. The over-all statistical analysis of the data showed highly significant difference ($P < 0.01$) among the three levels of nitrogen on decreasing the serum calcium level. The decrease of dietary magnesium from 200 to 50 mg level resulted in a significant ($P < 0.01$) increase of serum calcium from 10.4 to 11.1 mg/100 ml. The finding that hypomagnesemia was accompanied by hypocalcemia was in agreement with Colby and Frye (11) and Welt (60). This association of calcium and magnesium was also reported by Hemingway and Ritchie (22) in cattle and sheep. The interactions between nitrogen and magnesium and also among period, nitrogen

and magnesium were found to be highly significant ($P < 0.01$). Potassium content in the feed did not have any significant effect on serum calcium concentration. When the blood samples were collected at the end of the four week period more than half of the 45 samples from 2.2 percent nitrogen group were hemolized at varying degrees. For this reason the serum potassium determinations from these samples were too high and 2.2 percent nitrogen group were not included in the statistical analysis of the data. Therefore the total degrees of freedom of the analysis of variance for serum potassium were 179 with one degree of freedom for nitrogen. The effect of the period and the level of nitrogen on the concentration of potassium were found to be highly significant ($P < 0.01$). The level of serum potassium decreased with the longer period of feeding and with a higher nitrogen level in the ration. The concentration of serum potassium was not changed significantly when the dietary level of potassium increased from 1.8 to 3.6 g. The further increase from 3.6 to 7.2 g level resulted in a significant difference ($P < 0.05$). This may be interpreted that the slight excesses of dietary potassium do not significantly affect the serum potassium concentration. Forbes (17) reported that moderate excesses of potassium carbonate but not of potassium chloride increased serum potassium. The interactions among period X magnesium X potassium, period X nitrogen X magnesium and period X nitrogen X magnesium X potassium were

found to be highly significant ($P < 0.01$). These results suggest that the combined effect of the variables on serum potassium level is higher than that of separate effects.

A more detailed information for serum magnesium concentrations in respect to the different treatments are presented in Table 9. The first column in the table gives the values for different variables with the 400 mg/kg level of magnesium. Since it was difficult to present the data in a three dimentional form showing the effects of three different variables on serum magnesium, the levels of dietary potassium were extended under the three levels of nitrogen. The mean serum magnesium decreased from 2.88 to 2.39 and 2.28 mg/100 ml with the increase in nitrogen level in the ration from 2.2 to 3.0 and 3.8 percent, respectively. This trend of decreasing serum magnesium values with the increasing levels of dietary nitrogen was also true for the 200 and 50 mg/kg levels of magnesium.

Table 9. Mean serum magnesium values (mg/100 ml) of rats for the different cross treatments[a]

Diet	Level	Magnesium mg/kg diet			Potassium g/kg diet		
		400	200	50	1.8	3.6	7.2
Nitrogen %	2.2	2.88	2.46	1.09	2.36	2.07	2.00
	3.0	2.39	2.22	0.91	2.02	1.78	1.72
	3.8	2.28	1.86	0.84	1.79	1.64	1.54
Potassium g/kg	1.8	2.76	2.38	1.03			
	3.6	2.45	2.14	0.90			
	7.2	2.33	2.02	0.91			

[a]Each mean value represents 30 serum samples obtained from 30 rats.

The mean serum magnesium concentration for 400 mg magnesium and 1.8 g potassium treatments was 2.76 mg/100 ml. These dietary treatments of magnesium and potassium were the recommended allowances for growing rats (41). Within this recommended level of dietary magnesium the mean serum magnesium value decreased from 2.76 to 2.45 and 2.33 mg/100 ml with the increase of feed potassium from 1.8 to 3.6 and 7.2 g/kg, respectively. As the magnesium level in the ration decreased from 400 to 200 and 50 mg levels the serum magnesium concentrations showed linear decreases within each level of potassium.

As the variables of potassium and nitrogen increased in the ration the mean serum concentrations of magnesium decreased linearly. The effects of all the three variables on serum magnesium concentrations were found to be highly significant ($P < 0.01$). The analysis of variance of the data were presented previously in Table 8.

Bone Analyses

The determination of ash and of the percent of magnesium in the ash and bone were determined on fat-free dry matter of femur obtained from each rat. The mean values of the different analyses of bone within each treatment and the analysis of variance of the data are given in Tables 10 and 11, respectively. The mean ash content of bone increased from 51.2 to 56.5 percent as the feeding period

Table 10. Mean values for fat-free dry matter analyses of bone with different periods and treatments

		Period or level	Number of rats	Bone		
				Ash %	Magnesium mg/100 g	Magnesium % of Ash
	Period	4 weeks	135	51.2[a]	281.5[a]	0.55[a]
		8 weeks	135	56.5[b]	274.1[b]	0.49[b]
Diet	Nitrogen	2.2	90	53.0[a]	291.2[a]	0.55[a]
		3.0	90	53.1[a]	276.7[b]	0.52[b]
		3.8	90	55.5[b]	265.5[c]	0.48[c]
	Magnesium mg/kg	400	90	53.8[a]	373.1[a]	0.69[a]
		200	90	54.7[b]	336.9[b]	0.62[b]
		50	90	53.1[c]	123.4[c]	0.25[c]
	Potassium g/kg	1.8	90	53.4[a]	292.8[a]	0.55[a]
		3.6	90	54.1[b]	275.8[b]	0.51[b]
		7.2	90	54.1[b]	265.0[c]	0.49[c]

[a, b, c]Mean values with unlike superscripts within each group are significantly different ($P < 0.05$).

Table 11. Analysis of variance for the magnesium and ash levels of the fat-free dry bone, heart and skeletal muscle samples

Source of Variation	Degrees of freedom	Bone			Heart		Skeletal Muscle	
		% Ash M. S. and F test	Magnesium mg/100 g M. S. and F test	% Magnesium of Ash M. S. and F test	% Ash M. S. and F test	Magnesium mg/100 g M. S. and F test	% Ash M. S. and F test	Magnesium mg/100 g M. S. and F test
Period	1	1869.7**	3718**	0.2394**	0.867**	557.5**	0.065	433.2**
Nitrogen	2	174.3**	14929**	0.0977**	1.314**	416.9**	1.974**	233.1**
Magnesium	2	59.4**	1638191**	5.1658**	1.100**	25.7	0.090	1202.7**
Potassium	2	15.9*	17679**	0.0966**	0.599**	74.4*	0.210*	11.1
Period x Nitrogen	2	71.4**	3110**	0.0057**	0.414**	1891.2**	0.786**	47.8
Period x Magnesium	2	15.2*	638	0.0263**	0.734**	505.4**	0.175*	66.0*
Period x Potassium	2	2.4	527	0.0006	0.384**	28.1	0.325**	47.2
Nitrogen x Magnesium	4	51.8**	7555**	0.0140**	1.150**	431.6**	0.555**	116.3**
Nitrogen x Potassium	4	2.0	1251**	0.0054**	0.316**	167.3**	0.317**	163.6**
Magnesium x Potassium	4	3.1	743*	0.0012	2.165**	52.3*	0.341**	9.8
Per. x Nit. x Mag.	4	13.7**	1184**	0.0055**	2.012**	13.5	0.262**	75.8**
Per. x Nit. x Pot.	4	3.8	485	0.0008	0.979**	11.8	0.008	37.1
Per. x Mag. x Pot.	4	2.4	50	0.0001	0.755**	57.5*	0.396**	30.2
Nit. x Mag. x Pot.	8	2.9	1358**	0.0027**	0.992**	124.8**	0.123*	155.2**
Per. x Nit. x Mag. x Pot.	8	6.1	389	0.0010	0.776**	118.0**	0.247*	42.8*
Error	216	3.4	239	0.0006	0.045	19.4	0.052	20.8
Total	269	13.6	12879	0.0420	0.235	57.7	0.107	42.6

*Significant ($P < 0.05$)
**Highly significant ($P < 0.01$)

extended from four to eight weeks. Within these time periods the concentrations of magnesium in bone and bone ash were decreased. All these differences between the two periods were found to be highly significant ($P < 0.01$). Since these rats were in the growing stage the more deposition of minerals in bones, calcium and phosphorus in particular, would certainly increase the percentage of ash. Also the higher deposition of calcium and phosphorus could decrease the percent magnesium in bone. The mean ash content of bone showed a highly significant increase ($P < 0.01$) with the increasing level of nitrogen in the ration. On the other hand, the magnesium content of bone and bone ash decreased linearly with these changes in the nitrogen levels. The magnesium content of bone ash decreased ($P < 0.01$) from 0.55 to 0.48 percent with 2.2 and 3.8 percent nitrogen levels, respectively, a decline of 12.7 percent. The increase of bone ash along with a decrease in bone magnesium due to the increasing dietary nitrogen suggests that higher levels of protein stimulates bone formation by increasing the mineral deposition but decreases the magnesium deposition in the bone.

The effect of dietary magnesium in bone ash was highly significant ($P < 0.01$) but was not linear. The mean ash content of bone increased from 53.8 to 54.7 with the decrease of magnesium from 400 to 200 mg/kg of the ration. When the dietary magnesium was further reduced to 50 mg level the bone ash was decreased to

53.1 percent. On the other hand, the decrease of magnesium in the bone and bone ash was very marked with the decrease of magnesium in the diet. The mean magnesium contents of bone were 373 and 123 mg/100 g of the fat-free dry bone by rats on the dietary levels of magnesium of 400 and 50 mg, respectively, a decrease of 67 percent. This reduction of bone magnesium was also reported previously in rats, guinea pigs and calves (17, 39, 51).

McAleese and Forbes (35) found that the magnesium content of the bone ash in rats decreased from 0.56 to 0.15 percent with the change of dietary magnesium from 400 to 80 ppm. The present study along with those of previous reports suggests that the mobility of bone magnesium is an important factor in magnesium nutrition. If the mobilized bone magnesium is directly used to compensate the serum magnesium deficiency it may prevent the onset of hypomagnesemic condition for a considerable time. However, magnesium balance experiments with cows (27) indicated that the rate of mobilization of stored bone magnesium was insufficient to prevent hypomagnesemia in mature cows. It was also concluded that the dietary supply of available magnesium plays the major role in magnesium nutrition and hypomagnesemia. In the present study the correlation coefficient between the percent magnesium of bone ash and serum magnesium concentration was found to be $r = 0.978$ which was highly significant ($P < 0.01$).

During the dissecting of femurs from the rats on the 50 mg magnesium level it was noticed that the long bones were brittle. In fact, two rats in this group and one rat in the 200 mg group had broken femurs during the feeding period.

The ash content of bone increased from 53.4 to 54.1 percent with the increase of dietary potassium from 1.8 to 3.6 g level. Further increase of dietary potassium did not significantly change the percent of bone ash. The mean concentrations of bone magnesium were found to be 292.8, 275.8 and 265 mg/100 g of fat-free dry bone with the 1.8, 3.6 and 7.2 g/kg of dietary potassium, respectively. The differences between the treatments of potassium were highly significant ($P < 0.01$). In contrast to the hypomagnesemia situation the interactions between and among dietary nitrogen, magnesium and potassium on the magnesium content of bone and bone ash were all found to be highly significant ($P < 0.01$). The higher requirement of magnesium in bone may have made these interactions significant.

Heart and Skeletal Muscle Analyses

The determinations of ash and magnesium content on heart and skeletal muscles were made on a fat-free dry matter basis. The mean values of the determinations for the different variables are given in Table 12 and statistical analysis of these data are given in

Table 11. The effects of all the variables and their interactions were found to be highly significant ($P < 0.01$) for the ash content of heart muscle. These differences in all the means may suggest that the mineral exchange in the heart muscle is very high and largely dependent on the dietary variables involved. Brandt et al. (4) reported that after intravenous administration of ^{28}Mg into dogs the highest tissue concentrations and differential absorption ratios of ^{28}Mg was found in heart muscle. This report also suggests higher rate of mineral exchange in heart muscle.

Table 12. Mean ash and magnesium values for fat-free dry matter analyses of heart and skeletal muscle

		Period or level	Number of rats	Heart		Skeletal Muscle	
				Ash %	Magnesium mg/100 g	Ash %	Magnesium mg/100 g
Diet	Period	4 weeks	135	4.76[a]	100.8[a]	4.82	114.9[a]
		8 weeks	135	4.88[b]	98.0[b]	4.79	112.3[b]
	Nitrogen %	2.2	90	4.96[a]	101.8[a]	4.64[a]	114.9[a]
		3.0	90	4.74[b]	97.8[b]	4.85[b]	111.8[b]
		3.8	90	4.76[b]	98.6[b]	4.92[c]	114.1[a]
	Magnesium mg/kg	400	90	4.69[a]	99.8	4.83	115.2[a]
		200	90	4.87[b]	99.6	4.77	116.2[a]
		50	90	4.90[b]	98.8	4.81	109.4[b]
	Potassium g/kg	1.8	90	4.86[a]	99.8[a]	4.76[a]	114.0
		3.6	90	4.87[a]	100.0[a]	4.86[b]	113.3
		7.2	90	4.72[b]	98.3[b]	4.79[a]	113.6

[a, b, c]The means with unlike superscripts within each group are significantly different ($P<0.05$)

The mean magnesium values of heart and skeletal muscle decreased with the extension of feeding period from four to eight weeks. The average magnesium content for skeletal and heart muscle of all the rats were found to be 113.6 and 99.4 mg/100 g of the fat-free dry matter samples, respectively. Reports on the magnesium content of skeletal muscle have shown a range from 100 to 125 mg/100 g (17, 32, 39). It has also been reported that heart magnesium was about 15 to 20 mg lower than the skeletal muscle magnesium. The findings of the present study are in agreement with those of the earlier works.

The differences in the magnesium concentrations of heart and skeletal muscle with the change of nitrogen in the diet was found to be highly significant ($P < 0.01$), but not linear. In case of skeletal muscle the magnesium concentration decreased from 114.9 to 111.8 mg with the increase of dietary nitrogen from 2.2 to 3.0 percent. Then the muscle magnesium increased to 114.1 mg with the further increase of nitrogen to 3.8 percent.

The decrease of dietary magnesium resulted in an increase of heart muscle ash but it had no significant change in heart muscle magnesium. The situation was reversed in skeletal muscle, that is the magnesium content of muscle significantly ($P < 0.01$) decreased but ash content was not changed. These findings do not fully confirm the previous reports (32, 39) that magnesium deficiency results

in an increase of ash in soft tissue.

The contents of heart ash and magnesium were decreased by the increase of potassium in the ration. However, the effect of potassium treatment on skeletal muscle magnesium was not significant and the effect on skeletal muscle ash was significant but not linear. The mean ash content of skeletal muscle increased with the increase of dietary potassium from 1.8 to 3.6 g level. Then further increase of potassium to 7.2 g resulted in a decrease of muscle ash. The correlation coefficient between the magnesium contents of serum and skeletal muscle was found to be $r = 0.25$. The low, non-significant correlation coefficient indicates that the decrease of serum magnesium is not accompanied by a decrease of muscle magnesium.

Part B. Survey With Grazing Cows

This survey was designed to study the serum concentrations of magnesium calcium and potassium in cattle as influenced by the grazing on pasture forages in the spring. Table 13 shows the results of chemical analyses of serum collected from 40 Holstein and 20 Jersey cows at the three different grazing periods. There were no significant differences for the serum concentrations between the first and second blood sampling. The reason for this may be partially explained by the experimental treatment during the time period with

the cows remaining on the pasture only from 8 a.m. to 3:30 p.m. In this period the major part of the animals' roughage consumption was provided by 7 pounds of alfalfa hay, 24 pounds of grass silage and 15-20 pounds of grain which were supplied in the barn. Thus the inadequate consumption of green forage from the pasture did not significantly alter the concentrations of serum magnesium, calcium and potassium. In the second period of the experiment the cows were kept on pasture day and night except the time of milking and feeding. The amount of silage fed to the cows was also reduced to 7 pounds.

Table 13. Mean serum values (mg/100 ml) of magnesium, calcium and potassium

Breed	Serum constituent	Time of blood sampling		
		April 18	May 3	May 24
Holstein	Magnesium**	2.74 ± 0.04	2.77 ± 0.05	2.42 ± 0.04
	Calcium**	10.5 ± 0.05	10.4 ± 0.21	11.4 ± 0.21
	Potassium**	20.1 ± 0.20	19.4 ± 0.28	21.0 ± 0.32
Jersey	Magnesium*	2.88 ± 0.06	2.79 ± 0.07	2.55 ± 0.06
	Calcium**	10.1 ± 0.14	9.9 ± 0.22	11.2 ± 0.22
	Potassium**	19.1 ± 0.44	18.2 ± 0.45	22.1 ± 0.42

*Significant ($P < 0.05$)

**Highly significant ($P < 0.01$)

The analysis of variance for the three different blood sampling times indicated a significant difference ($P < 0.05$) for the serum magnesium in Jerseys and highly significant differences ($P < 0.01$) in the rest of the minerals for the two breeds. The concentrations of the three minerals at the final blood sampling on May 24 contributed to all these highly significant differences. The blood magnesium concentrations were decreased from 2.77 and 2.79 to 2.42 and 2.55 mg/100 ml from the second to third blood sampling for Holsteins and Jerseys, respectively. These lower concentrations of serum magnesium on pasture are in agreement with previous reports (6, 15, 25). The concentrations of calcium and potassium increased in the blood at the third sampling.

On the third day of the second experimental period (May 6) two Jersey cows in the morning and one Jersey and two Holsteins in the afternoon developed signs of hypomagnesemic tetany. Three of the five cows were found lying in the pasture. They showed occasional fierce attempts to get up, but were unable to do so and after exhaustion they lay down unconscious. The other two cows stopped grazing, showed uncoordinated walk and had no interest in the other cows of the herd. The two Jersey cows in the morning were treated with a calcium, magnesium and phosphorus preparation (CGP-Reinforced[3]) before blood samples could be drawn. The results of the chemical

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[3]CGP-Reinforced, Haver-Lockhart Laboratories, Shawnee, Kansas.

analyses on the blood samples collected from the other three cows are given in Table 14. As it is seen in this table the concentrations of all three minerals were considerably lower than the mean values of the previous blood sampling.

Table 14. Serum values (mg/100 ml) of magnesium, calcium and potassium in the three cows with tetany symptoms

Cow Number	Magnesium	Calcium	Potassium
J401	1.80	3.4	14.1
H755	1.77	6.6	17.9
H805	1.87	7.4	18.7

The critical serum magnesium concentrations of cows with the symptoms of hypomagnesemic tetany have been reported to be about or lower than 1 mg/100 ml of serum (6, 15, 24, 26, 53). In these three cases the serum magnesium concentrations were much lower than the mean values of the herd, but they were considerably higher than those reported in the literature. The serum calcium values were lower for H755 and H805 and very low for J401. At this point the presence of milk fever may be suspected. However, J401 and H755 were in the fifth and H805 was in the eighth month of lactation. Originally, H805 was not among the experimental animals due to her long lactation. These long periods of milking may suggest the elimination of milk fever from the discussion (12). Hemingway and

Ritchie (22) reported that hypocalcemia plays an important role in the development of the hypomagnesemic tetany. The authors further suggested that the clinical signs of hypomagnesemic tetany may not appear until after a rapid fall in plasma calcium concentration. The situation in the present study may be explained in the light of the above suggestion. However, the hypocalcemia was more pronounced than the hypomagnesemia in these three cases. The serum potassium levels of these cows were also markedly decreased along with magnesium and calcium. The concentrations of serum magnesium, calcium and potassium for the two experimental cows with the tetany symptoms were around the herd means at the previous blood sampling time.

After the blood samples were collected J401 was treated with CGP-Reinforced. Since the other two animals showed only mild tetany symptoms they were removed from the pasture and fed dry roughages, but they were not given injections. The animals recovered from the signs of tetany after these treatments. However, it took them six to eight days to reach their previous levels of milk production.

The botanical composition of the pasture was 92 percent grass (60 percent orchard grass) and eight percent legumes. The results of the chemical analyses on the forage samples obtained at the three different times of the grazing experiment are shown in Table 15. The

percent dry matter of the forage increased along with a decrease in protein content as the season progressed. The ash and potassium contents were slightly lower on May 3 than on April 18. However, these were considerably higher on May 24 than the two previous samplings. The magnesium contents were the same for the first two samplings and then lower at the final sampling.

Table 15. Composition of forage samples expressed as percent of dry matter.

Forage Constituent	Sampling time			Alfalfa hay
	April 18	May 3	May 24	
Dry-matter %	18.9	24.0	24.7	76.9
Protein	17.8	16.1	14.4	14.2
Ash	8.7	8.4	10.4	8.0
Magnesium	0.18	0.18	0.14	0.3
Potassium	3.4	3.3	3.7	4.0

The blood magnesium levels were lower at the third sampling as was the forage magnesium content. However, it was difficult to determine from these data a relationship between low magnesium values of forage and serum. As it was pointed out earlier the cows remained on the pasture for a short time each day in the first two week period which resulted in an inadequate consumption of green forage to affect the blood magnesium level. Kemp et al. (27) reported a higher negative magnesium balance with cows grazing on

early growth of grass than during the bloom and after bloom stages. These authors also found that despite the decrease in magnesium content of forage, its availability to the cows was increased with time. In the second period of the present study the higher consumption of pasture forage rather than lower forage magnesium may have reduced serum magnesium values.

SUMMARY AND CONCLUSIONS

Experiments with rats and dairy cattle were carried out to study the various interrelationships involved in hypomagnesemia. In the rat experiment, 270 albino rats were used on 27 synthetic dietary treatments consisting of three levels each of casein (as nitrogen source), magnesium and potassium. The survey with dairy cattle involved blood studies of 60 Holstein and Jersey cows in the early pasturing season. The results are summarized as following:

1. Skin lesions were observed within 9 to 15 days in rats fed 50 mg level of magnesium. The severity of the skin lesions was increased with the increasing levels of dietary nitrogen.

2. The decrease in magnesium and increases in nitrogen and potassium in the diet resulted in significant decrease in serum magnesium concentrations. The decrease of dietary magnesium from 400 to 200 and 50 mg per kg reduced serum magnesium significantly ($P < 0.01$) from 2.51 to 2.18 and 0.95 mg/100 ml, respectively.

3. The interactions among period, nitrogen and magnesium were found to be highly significant ($P < 0.01$), indicating that the hypomagnesemic effects of these variables were increased when they were acting together.

4. The hypomagnesemia resulting from dietary treatments was accompanied by a mild hypercalcemia.

5. The increase in dietary nitrogen and potassium and the decrease in dietary magnesium resulted in linear decreases in bone magnesium. Decreasing dietary magnesium from 400 to 50 mg per kg reduced bone magnesium by 67 percent, indicating that the bone magnesium was highly mobile.

6. The hypomagnesemia was not accompanied by a significant reduction in muscle magnesium.

7. In the grazing survey with cows, a significant ($P < 0.01$) decrease in the serum magnesium and significant increases ($P < 0.01$) in the serum calcium and potassium concentrations were found after five weeks of pasturing.

8. As the spring progressed the concentrations of forage dry matter increased, nitrogen decreased but magnesium and potassium were not changed significantly.

It may be concluded that the hypomagnesemic condition in animals is not a simple deficiency or a simple interference of another factor, but a complex one. The experiments in this thesis showed that increasing dietary nitrogen and potassium as well as decreasing dietary magnesium concentrations have a harmful effect on magnesium levels in the body. The interferences of nitrogen and potassium in magnesium metabolism might have taken place during the absorption in the digestive system and also at the cellular level. Since the magnesium ion is involved in numerous biological

processes in the body it may be expected that any disruption in these functions in turn may affect the magnesium metabolism. This conclusion is confirmed by a recent report that trans-aconitic acid, an inhibitor of the tricarboxylic acid cycle, was suggested to be partially responsible for hypomagnesemia in cattle in early spring. With these considerations, it may be suggested that a variety of preventive measures should be taken against the various potential factors which could induce hypomagnesemia when the cattle are turned into pasture in the early spring.

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