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Effects of temperature and diets on magnesium, calcium and potassium metabolism in sheep and rats

Mohan Chandra Sanwal

To the Graduate Council:

I am submitting herewith a dissertation written by Mohan Chandra Sanwal entitled "Effects of temperature and diets on magnesium, calcium and potassium metabolism in sheep and rats." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

Sam L. Hansard, Major Professor

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August 14, 1974

To the Graduate Council:

I am submitting herewith a dissertation written by Mohan Chandra Sanwal entitled "Effects of Temperature and Diets on Magnesium, Calcium and Potassium Metabolism in Sheep and Rats." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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EFFECTS OF TEMPERATURE AND DIETS ON MAGNESIUM, CALCIUM AND POTASSIUM METABOLISM IN SHEEP AND RATS

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

Mohan Chandra Sanwal

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ABSTRACT

Several biological and environmental interactions appear to be involved in the etiology of hypomagnesemic tetany. Effects of ambient temperature and diet on magnesium status of six ewe lambs and 72 adult male rats were studied in this investigation. Percentage absorption and fecal endogenous magnesium excretion were higher and urinary magnesium was lower in sheep fed a low magnesium diet than those fed normal magnesium diets. Effects of low dietary magnesium on fecal endogenous and urinary magnesium were increased by cold. Magnesium absorption was elevated by high dietary protein in sheep fed low magnesium, especially in cold, but with low magnesium intake, total radiomagnesium excretion by sheep fed the high protein level was 80% higher than when normal protein was fed. In general, magnesium concentrations in bone epiphysis, heart, muscle, liver, spleen, and certain other tissues were higher in cold exposed sheep than in those at room temperature. Tissue turnover rate of magnesium in sheep was higher in cardiac muscle and lower in skeletal muscle. Feed efficiency was decreased and feed consumption increased ($P < .001$) in rats by cold exposure. Total magnesium excretion by rats was increased by cold except when certain high potassium diets were fed but high potassium alone increased urinary magnesium. Urinary magnesium was also increased by high dietary iodine.

High dietary protein appeared to counteract high magnesium excretion. Rats fed diets high in both potassium and protein had lower ($P < .05 - < .001$) plasma magnesium levels in cold than at room temperature. Bone magnesium levels were reduced ($P < .05 - < .01$) by high dietary protein, and increased by cold exposure in rats fed the low iodine control diet. Cold exposure also increased magnesium deposition in liver ($P < .01$) and heart ($P < .05$) but did not change skeletal muscle magnesium levels of rats.

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CHAPTER I

INTRODUCTION

A metabolic disorder of ruminants called "grass tetany" or hypomagnesemic tetany has been known for a long time. It principally affects cows grazing lush, rapidly growing spring pasture. Symptoms include hyperexcitability, muscular twitching, opisthotonus, convulsions, and in acute cases death within a half hour after the appearance of the clinical symptoms (Todd, 1969).

The etiology of hypomagnesemic tetany is still not clear after extensive research concerning magnesium. The progress has been slow because of an apparent involvement of several biological interactions and the lack of a predictable method for producing the tetany syndrome (Rumsey and Putnam, 1972).

Several possible contributing factors to the disease have been investigated, and some examples are: soil density (Todd, 1969), high dietary potassium (Newton et al., 1972; Suttle and Field, 1969), dietary organic acids (Rumsey and Putnam, 1972; Stout et al., 1967; Bohman et al., 1969), endocrine factors (Todd, 1969; Agna and Goldsmith, 1958; Vitale et al., 1957; Green, 1948, Grunes et al., 1970; Allcroft and Burns, 1968), nitrogen in the diet (Moore et al., 1972; Metson et al., 1966), weather conditions (Allcroft, 1947; Green, 1948; Grunes et al., 1970), ammonium ion

(Wacker and Vallee, 1961), and many others.

It has been postulated that physiological malfunction of neural or endocrine mechanisms, together with environmental stresses might very well predispose the animal to the disease (Green, 1948).

Among hormones, thyroxine (Sellers and You, 1950; Szelenyi et al., 1968; Vitale et al., 1957), parathormone (Agna and Goldsmith, 1958; Clark and Rivera-Cordero, 1971; Berthaux et al., 1960; Heaton, 1965), posterior pituitary hormone (Moodie, 1968), thyrocalcitonin (Gudmundsson et al., 1966), aldosterone (Ginn et al., 1967; Richer et al., 1968; Scott and Dobson, 1965; Vitale et al., 1959; Grunes et al., 1970) have been studied with respect to magnesium metabolism, but no clear relationships have yet been found. However, indications are that thyroxine status of the animal is involved. Plasma thyroxine is increased during cold weather and plasma magnesium levels are lowered in high thryoxine animals (Dempsey and Astwood, 1943; Hanna, 1961; Leech and Bailey, 1953; Swan and Jamieson, 1956), but no concrete experimental evidence has been presented. Very little information is available on the combined effects of lower ambient temperatures, high humidity, and wind velocity on magnesium metabolism.

Magnesium is an essential element for animals and plants, suggesting that the dietary inadequacy of this ion may readily result in a deficiency disease (Wacker and

Vallee, 1961). It represents about 0.05% of the animal body of which only about 1% is in the extracellular fluid (Todd, 1969). Normal plasma levels of magnesium range from 1.8 to 3.8 mg/100 ml. Danger of tetany increases as blood plasma levels of magnesium fall below 1.0 mg/100 ml.

The exact causes of the disease are not known, however, one or more factors are involved in binding the magnesium in the diet (exogenous), or in the animal body itself (endogenous), and the stress of lactation, environment and/or imbalance of certain minerals under field conditions appear to cause the hypomagnesemia which subsequently triggers tetany in ruminants (Hansard, 1973). Several counties in Tennessee have an increasing problem with both beef and dairy cattle, and a closer look at the problem has been suggested (Ibid., 1973).

In this study an attempt has been made to determine the effects of low ambient temperatures on magnesium status in rats and sheep relative to dietary factors. Calcium and potassium have also been studied simultaneously with magnesium.

CHAPTER II

LITERATURE REVIEW

Interest in the disorders related to hypomagnesemia have increased in recent years because of the diverse or widespread geographical area involved. Although grass tetany was diagnosed in West Virginia in 1942 (Horvath, 1959), the classical symptoms of the hypomagnesemia were described by Crookshank and Sims (1955), and referred to as "wheat pasture poisoning" in the Texas-Oklahoma panhandle area. It has not been a problem in the midwest until recently (Sanderson, 1972; Pfander, 1973). However, several states now report this condition with an increasing frequency.

Importance of Magnesium in Animal Body

Magnesium was first shown to be essential for normal growth of animals in 1926 (Wacker and Vallee, 1961). It is essential in the composition of bone and teeth, and plays an important part in the metabolism of phosphorus, and the starches and sugars (Kruse et al., 1932). It is necessary for life; for if the animal is deprived of the element, it becomes increasingly irritable, heart action is not controlled, there is kidney damage and if the deficiency is carried to extremes the animal dies in convulsions (Ibid., 1932).

The magnesium ion has been shown to be an essential

component of a large number of metabolically active enzyme systems (Lowenhaupt et al., 1950), and has been found to be an activator for many enzymes in vitro. Among these are the enzymes catalyzing reactions involving ATP, which split or transfer phosphate groups. Therefore the action of magnesium is extended to all the major anabolic and catabolic processes. In addition this ion, together with thiamin pyrophosphate, serves as a cofactor in oxidative decarboxylation, and certain peptidases require the metal for activity.

Wacker and Vallee (1961) state that despite the lack of conclusive evidence for a physiological role of magnesium in enzymatic activation, inferencial reasoning strongly suggests a significant role of this ion for intracellular catalysis. In but a few instances--oxidative phosphorylation for example--has the in vitro effect of magnesium been shown to have a counterpart in vivo (Ibid., 1961).

Symptoms of Hypomagnesemic Tetany

Tetany symptoms begin with undue excitement, incoordination, and loss of appetite. As the condition progresses, viciousness, staggering, and falling are observed. Nervousness becomes more apparent with muscular twitching. The animal exhibits an anxious expression and may grind its teeth and salivate profusely. The third eyelid protrudes or flickers. General tetanic contractions of

the muscles follow until the animal nears a state of prostration. Sudden noises or touch cause a reflex response. Thereafter labored breathing and a pounding heart occur, followed by a comatose condition. If left untreated, convulsions with periods of relaxation will be seen which terminate in death. After the symptoms appear, it usually takes six to ten hours until the animal becomes comatosed. If treatment is not initiated before coma, there is little chance of recovery. The blood from such hypomagnesemic cows has been found to have lowered levels of magnesium, diffusible calcium, and inorganic phosphate, a lowered albumin-globulin ratio, with increased levels of total serum protein, globulin, and possibly potassium as compared to the blood of normal cows (Nelson, 1973). In some acute cases of hypomagnesemic tetany, death may result within a half hour of the appearance of clinical signs (Todd, 1969).

Many factors related to hypomagnesemic tetany are considered separately.

Season and Gross Dietary Factors

The incidence of hypomagnesemic tetany varies greatly from year to year and in different seasons of the year (Green, 1939). The condition has been reported in many parts of the world (Grunes et al., 1970). It has become a problem in West Virginia, Maryland, Kentucky, Georgia, Texas, California, Oklahoma, Nevada, Idaho, Utah (Ibid., 1970), and also Tennessee (Hansard, 1973).

Hypomagnesemic tetany occurs primarily in areas with temperate grasslands, and it seems doubtful if authenticated cases have occurred in tropical or subtropical areas (Todd, 1969). It principally affects cows grazing lush, rapidly growing spring pasture (Ibid., 1969). It generally occurs during early spring, but may also occur in autumn (Grunes et al., 1970). Most cases in the Netherlands occurred at ambient temperatures between eight and 14 degrees centigrade (46-57 degrees farenheit) under conditions of ample moisture supply. Spring tetany was less frequent on either very dry or very wet pastures (Grunes et al., 1970) but autumn tetany occurred only in wet seasons. Stewart (1954) has defined three types of tetany in Scotland: (1) spring type; (2) winter type, occurring in stall fed animals and characterized by sudden death; and (3) outwinter type. Custer (1959) has described the winter tetany in Maryland and West Virginia. The syndrome occurred after pasture growth had stopped.

Several dietary factors have been reported to influence magnesium availability in various ways (Peeler, 1972). Some of these factors are: composition of diet; magnesium status of the animal; various dietary ions such as calcium, phosphorus, sodium, manganese, and citrate; phytic acid and chelates in natural feedstuffs; abrupt changes in feeding systems; age and genetic differences in animals; dietary fat and vitamin D levels; nitrogen level, and season of the

year for forages, and numerous other factors (Ibid., 1972). Many of these factors appear to have a decreasing effect on magnesium absorption.

Magnesium availability is also affected by stage of maturity of grass (Peeler, 1972). Magnesium availability values to cows were 10, 16, and 20 percent, respectively, for early growth, prebloom and afterbloom grass stages (Kemp and t'Hart, 1961). However, according to Peeler (1972) the criterion chosen for measuring magnesium availability from different feeds and salt is important as variations have been observed by using different indexes.

Marshak (1959) and Ender et al. (1957) stressed the role of quantitative and qualitative malnutrition and undernutrition as factors in the etiology of tetany.

Various Specific Deitary Factors Affecting Magnesium Utilization

According to Aikawa (1965) factors controlling the gastro-intestinal absorption of magnesium are poorly understood. Several studies, however, have suggested that the absorption is influenced, in a nonlinear fashion, by the load presented to the intestinal mucosa--a higher percentage of radiomagnesium was absorbed on a low magnesium diet than on a high magnesium diet.

Nitrogen. Conflicting effects of dietary nitrogen on magnesium utilization have been reported. There is

evidence that the ability of an animal to mineralize its skeleton is very dependent on its protein intake (Sykes and Field, 1972). High nitrogen fertilization of forage increased its crude protein content and had generally adverse effects on magnesium utilization in cattle and lambs grazing the forage (Moore et al., 1972). It has been reported that fertilizers containing the sulfate radicle produce pastures with greater hypomagnesemic potential than those containing other anions (Todd, 1969). High dietary protein levels also increased the severity of magnesium deficiency syndrome in rats (Colby and Frye, 1951). In contrast, Stillings et al. (1964) found much lower availability of magnesium (10% to 16%) in wethers consuming low nitrogen forages compared to those eating high nitrogen forages (18% to 24%).

Although high ruminal fluid ammonia levels did not interfere with magnesium absorption in lambs, urinary magnesium excretion was increased resulting in lower retention ($P < .01$) for animals consuming the high nitrogen rations (Moore et al., 1972). Form of nitrogen fed (as urea or no-urea concentrate) did not significantly affect magnesium absorption and retention but absorption of phosphorus and potassium were increased ($P < .01$) in lambs fed high nitrogen rations. No significant changes in serum magnesium, calcium, phosphorus or potassium were observed between treatments. In earlier studies, however, Head and Rook (1955) observed decreased serum magnesium in rumen fistulated cows

given 1,250 gm ammonium acetate and ammonium carbonate.

Absorption and retention of magnesium were influenced only by magnesium level in the diet in studies by Garces and Evans (1971). There was no effect of nitrogen source, age of animal, or ingested calcium. L'Estrange et al. (1961) found no association between serum magnesium concentration and high rate of nitrogen fertilization, milk yield, or energy intake of lactating ewes. Moore et al. (1972) considered level of potassium in the ration of greater importance than nitrogen level in magnesium absorption.

Following the report of high positive correlations between crude protein and higher fatty acid content of pasture, it was suggested that the hypomagnesemic effect of high nitrogenous fertilizer treatments may be explained by an increased fat content of the forage (Todd, 1969). Kemp et al. (1966) have reported that concentration of long chain fatty acids increases with the nitrogen concentration in the forage, and that additions of such acids to winter rations of cattle may decrease the availability of magnesium to the animal.

Potassium. Several investigators have reported decreased availability and utilization of magnesium in the presence of high dietary potassium (Blaxter et al., 1960; Hendricks, 1962; Kunkel et al., 1953; Newton et al., 1972; Odell et al., 1952; Sanwal and Hansard, 1972; Suttle and

Field, 1969).

Both potassium and magnesium are intracellular ions suggesting a close relationship between the two (Eichelberger, 1942). Applications of nitrogenous and potassic fertilizers, especially when used together, have resulted in lowered serum magnesium levels and increased incidence of tetany in dairy cows grazing the resulting pasture. There were indications that although the magnesium content of the pasture was not markedly altered, there was an unfavorable effect on its apparent availability (Todd, 1969). Suttle and Field (1969) have indicated that the sudden increase in potassium intake which occurs when ruminants first graze spring grass may contribute to the development of hypomagnesemic tetany. These workers had previously observed (1967) that the apparent availability of magnesium to sheep was markedly reduced when the potassium content of a hay and concentrate diet was raised to a level found in "tetany inducing" pastures.

The lack of an effect of potassium on serum magnesium concentrations led Blaxter and McGill (1956) to conclude that potassium had no direct effect upon magnesium metabolism in ruminants, but the concept is still unclear. A recent study (House and Van Campen, 1971) in which wethers were fed a high potassium ration showed that magnesium absorption was significantly depressed, urinary magnesium excretion was reduced, and endogenous fecal magnesium excretion was

lowered, but total fecal output of magnesium was elevated. These authors suggested that inhibition of alimentary absorption and increased retention by body cells may possibly induce hypomagnesemia and the altered partition of magnesium between urine and feces of ruminants fed high levels of potassium. Fontenot et al. (1973) have confirmed that high dietary levels of potassium result in lower magnesium absorption. High levels of dietary nitrogen in addition to high dietary potassium did not appear to adversely affect magnesium utilization to a greater extent than high levels of potassium alone in ruminants.

Metson et al. (1966) indicated that New Zealand pastures which caused outbreaks of grass tetany in cattle had potassium concentrations which averaged 3.29%, while nitrogen concentrations averaged 5.28%. The magnesium concentration in these forages averaged 0.19%. They suggested that with the high concentrations of potassium, the amount of magnesium to prevent grass tetany might well need to be above 0.20%. These authors consider a "safe" level of magnesium in the forage may be as high as 0.25% magnesium, or even higher. Pastures should likewise not be too low in potassium, since the growth of the grass begins to suffer if the level of potassium is below 2.1% (Grunes et al., 1970).

It appears that when the ratio of potassium/(calcium + magnesium) in forage becomes greater than 2.2, there are

increased chances of tetany (Grunes et al., 1970). In a New Zealand pasture study with a 10% incidence of tetany, the ratio averaged 2.45. This type of hypotheses does not have much practical evidence, however. The fall observed in plasma magnesium by Suttle and Field (1969) was probably due in part to a reduction in the intake of magnesium from the gut. These investigators induced two cases of hypomagnesemic tetany in sheep at the lowest magnesium intake and supplementary potassium.

Johnson et al. (1972) found that diets ranging from 0.29% to 0.39% potassium did not significantly affect blood serum or muscle potassium in steers because of animal variation. They suggested that the primary influence of dietary potassium was on potassium concentration of gastrointestinal contents. Little work on the comparative biological availability of potassium compounds has been reported (Peeler, 1972).

Organic acids. Certain Krebs cycle organic acids have been mentioned as factors in the development of hypomagnesemia and the grass tetany syndrome (Todd, 1969; Grunes et al., 1970). More than 1% transaconitic acid (dry basis) has been reported to be present in early season forage grasses during seasonal outbreaks of grass tetany (Stout et al., 1967), and evidence from Nevada has shown that tetany can be produced by feeding excess

potassium, and citric acid or transaconitic acid (Maynard and Loosli, 1969). Bohman and coworkers (1969) produced symptoms similar to grass tetany in cows by introduction of about 500 gm of potassium chloride and a similar amount of either citric acid or transaconitic acid directly to the rumen of cows. The clinical effects were not produced by either acid alone, nor were consistent effects produced by the potassium salts of the acids.

Some evidence indicates that the concentration of organic acids in forage increases and decreases with potassium content (Grunes et al., 1970). Burt and Thomas (1961) found decreased plasma magnesium concentration in calves fed an equivalent of 1% citric acid (in dry matter) as sodium citrate.

It is thought that aconitic and citric acids may contribute to the tetany syndrome by alteration of magnesium and calcium metabolism through formation of chelates. However, House and Van Campen (1971) found little effect of high dietary citric acid (30 gm/day) on magnesium metabolism in sheep, and Kennedy (1968) indicated that magnesium levels in plasma and urine of sheep were not substantially changed by feeding either of these acids.

Magnesium requirement. Duncan and associates (1935) estimated that about 2,000 ppm magnesium (30-40 mg/Kg BW) were necessary to maintain normal plasma magnesium in calves

when the supplement was given as magnesium salts. Only 12-15 mg/Kg body weight from natural feedstuffs were sufficient. O'Kelley and Fontenot (1969) calculated by regression the dietary magnesium required to maintain serum magnesium concentrations of lactating or pregnant beef cows at 2.0 mg/100 ml. The requirements for early, mid, and late lactation, respectively, were 20.9, 22.1 and 18.0 gm of dietary magnesium daily. These correspond to approximately 0.18%, 0.19%, and 0.16% magnesium in the ration at the three stages of lactation. Requirements during gestation were calculated at 8.5, 7.0, and 9.0 gm/day (0.12%, 0.10%, and 0.13% of the ration) at 145, 200, and 255 days of pregnancy, respectively. Blaxter and McGill (1956) estimated the net requirement of an adult cow for maintenance to be about 1.8 gm magnesium/day, and for production 0.5 to 0.6 gm/10 lb milk secreted. Assuming an average availability of dietary magnesium of 33%, this amounts to a minimum daily requirement of 9 to 11 gm for cows producing 20 to 30 lb milk daily.

Chicco et al. (1972) calculated a minimum daily magnesium requirement of 4.03 mg/Kg to replace endogenous losses, and the renal threshold value of 1.63 mg/100 ml plasma for sheep fed magnesium oxide in a purified ration. The requirement of magnesium has been estimated to be 200 ppm for rats, 800 ppm for guinea pigs, and 2,000 ppm for

calves (Odell, 1960). McAlesse and Forbes (1961), however, found that for average blood magnesium levels in rats about 365 ppm magnesium were required. The value for maximum weight gain was 115 ppm magnesium. Field et al. (1958) considered 0.1% magnesium in dry matter as marginal, and Kemp's (1960) data have established 0.20% magnesium as the "safe" level of magnesium in forage. Metson et al. (1966) also suggest a value above 0.20% to prevent grass tetany, or maybe as high as 0.25% or even higher. Ender et al. (1957) have stressed the role of under-nutrition as a factor in the etiology of tetany. Green plants generally contain abundant magnesium, but there is much speculation on the matter of availability (Wacker and Vallee, 1958). Although the incidence of hypomagnesemia is related to low magnesium concentrations in the forage, grass tetany is not always observed even when magnesium concentrations in the forage are low (Grunes et al., 1970). The amount of magnesium retained by cattle has not been found to be dependent upon the dietary magnesium content, calcium/phosphorus ratio, or the crude fibre content (Garner, 1950). Availability aspects of magnesium are discussed later in this review.

Calcium. Many workers have shown the common occurrence of hypomagnesemic plus hypocalcemic tetany (Marshak, 1958; Udall, 1947), and in cattle no clinical state has been proven to be the result of hypomagnesemia

alone. Viosin (1963) has indicated that the magnesium requirement is increased as dietary calcium alone or dietary calcium and environmental temperature are increased. Keeping both calcium and phosphorus, as opposed to either, high in the diet, was found to be more effective in accentuating magnesium deficiency in lambs (Packett and Hauschild, 1963). Sykes and Field (1972) working with ewes suggested that the true availability of calcium at very low calcium intakes may approach 100%, and that it is increased by low protein intakes.

Carbohydrates. The protective role of readily available carbohydrate in hypomagnesemia, and perhaps hypocalcemia, may be partly due to providing essential energy and feed requirements for fatty acid and protein synthesis (Metson et al., 1966). Moreover, carbohydrates maintain a sufficiently low pH in the digestive apparatus to allow calcium and magnesium to persist in readily absorbable forms.

Forage species. Tetany occurs most frequently in animals feeding on those grasses accomplishing most of their growth during cool weather, such as observed during the spring (Grunes et al., 1970). In our southern states, tetany is often observed in ruminants being maintained on wheat (Triticum aestivum), rye (Secale cereale), and oats (Avena sativa).

Magnesium Availability and Stress

Grunes et al. (1970) state that decreased magnesium intake and/or low forage intake, coupled with comparatively high magnesium requirements are the most likely causes of grass tetany, and believe that the tetany manifestation probably results from ionic imbalances within body tissues that influence the enzymatic activities of ion transport mechanisms and muscle response.

High magnesium demand during pregnancy and lactation may be a possible factor in causing hypomagnesemic tetany (Allcroft and Burns, 1968). Grace (1972), however, found no significant effect of pregnancy or lactation on plasma magnesium levels of ewes or young dairy cattle. Pregnancy caused a significant increase in plasma magnesium level of mature cows until about 20 to 35 days before parturition. Lactation caused a significant decrease in plasma magnesium levels three to four weeks after parturition and a significant increase during early lactation. The author suggested that the lactational demand for magnesium may be a factor that could induce the hypomagnesemic condition in some cows that are more succeptible to hypomagnesemia. Blaxter and McGill (1956) found the greatest incidence of hypomagnesemic tetany in cows which had delivered their third or fourth calf.

Few estimates of the true availability of magnesium in feedstuffs have been made because of the difficulty in

estimating endogenous excretion (Todd, 1969). In most studies, apparent availability defined as the proportion of dietary magnesium not excreted in the feces has been evaluated. Apparent availability of magnesium to adult ruminants ranged from 10 to 40% in diets consisting of roughage and concentrate and from 5 to 33% in roughage plus succulant feeds (Ibid., 1969). Rook and Campling (1962) reported the availability of dietary magnesium within the range of 5 to 30% for mature nonpregnant dairy cows. The lower values were usually obtained from animals fed grasses cut at an early stage of growth and the higher values were from animals fed mature grasses. Rook et al. (1964) found that when the total magnesium intake was reduced in lactating cows from 13.9 to 3 gm/day, the magnesium availability in the diet increased from 24 to 37.5%.

Magnesium Absorption and Excretion

Smith (1969) stated in his recent review on magnesium absorption that up to about one month of age calves absorb magnesium in the large as well as small intestine, giving efficient overall net absorption (70-90% of intake). This ability is lowered with increasing age and at three to four months of age the small intestine is the only important site. This also appeared to be the main absorption site in sheep, man, and rabbits. Kemp et al. (1973) found no

significant differences in net absorption of magnesium from the upper and lower duodenum of cows when magnesium intake was normal. At high magnesium intakes net magnesium absorption occurred mainly at the proximal end. These investigators, however, could not draw a reliable conclusion on the sites of net magnesium absorption. Other studies suggested absorption to occur from the upper portion of the small intestine (Aikawa, 1965; Todd, 1969). Absorption from the large intestine was negligible.

Factors controlling the gastrointestinal absorption of magnesium have been poorly understood (Aikawa, 1965). If it is accepted that magnesium moves passively across the wall of the small intestine, then it would appear that the only factors (apart from electric potential) which are likely to be responsible for major differences in the efficiency of magnesium absorption are the concentration of available magnesium in the digesta and the time of contact between the digesta and the absorbing surface (Smith, 1969).

Experiments to examine the possibility of competition between magnesium and calcium for absorption in the gut, which if established might provide evidence of active magnesium absorption, have given contradictory results (Smith, 1969). Smith suggested that the low magnesium content of spring grass, lower dry matter intake by the animal, plus poor magnesium absorption observed in the ruminants may develop into clinical hypomagnesemia.

Metabolic activity may be concerned in the absorption of magnesium since the normal intracellular accumulation of magnesium appears to be dependent, partly, on energy derived from the oxidation of glucose (Aikawa, 1965).

According to Todd (1969) the main excretory pathway for magnesium is via the feces which represents the sum of the unabsorbed portions of dietary magnesium, and of magnesium re-entering the gut in digestive secretions. A large loss of magnesium in the feces (usually 70-95% of intake) appeared to be characteristic of adult cattle and sheep, and most of this fecal loss appeared to consist of unabsorbed food magnesium (Smith, 1969). The contribution of endogenous magnesium to the high fecal magnesium output of cattle and sheep receiving roughage or succulant diets appears to be uncertain (Allsop and Rook, 1970).

The major excretory pathway for absorbed magnesium is the kidney (Aikawa, 1965). Urinary magnesium represents the excess of absorption over requirements and is reflected in a very low urinary output in hypomagnesemic animals. Urinary excretion of magnesium appears to take place by a filtration-reabsorption mechanism with a kidney threshold of about 2 mg/100 ml plasma (Todd, 1969). The mechanism for renal excretion of magnesium has not been determined, but evidence has indicated magnesium to be secreted by the distal tubule (Ginn et al., 1959). The diffusible magnesium in plasma is filtered in the glomeruli and absorbed by the

renal tubules (Aikawa, 1965). Storry and Rook (1963) demonstrated the renal threshold for magnesium in dairy cows to be 1.46 to 1.76 mg/100 ml serum, and the value calculated by Chicco et al. (1972) in sheep fed a purified diet was 1.63 mg/100 ml plasma.

Magnesium and Mineral Interrelationships

There are over 70 known mineral interrelationships in which an additional dietary quantity of one mineral element will influence absorption or utilization of another mineral element (Jacobson et al., 1972). The umbrella concept of Schutte (1964) illustrates most of the reported dietary interrelationships.

The availability of stored minerals in bone decreases with age (Garces and Evans, 1971; Hansard et al., 1954). Excessive rather than too little dietary calcium during the dry period prior to calving likely contributes to an increased incidence of milk fever, and too little magnesium may cause problems that are not well understood, or widely appreciated (Jacobson et al., 1972). These authors suggest that all mineral elements should be considered in ration formulation for high performance and a reduction in mineral nutrition anomalies.

The bone serves as a reservoir for calcium, magnesium, and phosphorus (Lazzara et al., 1963). These three elements and potassium are all closely related to many metabolic

events of the body, and a deficiency in any of these leads to reduced voluntary feed consumption and subsequent reduced milk production (Jacobson et al., 1972). Borderline deficiencies in any one or any combination of these elements are extremely difficult to diagnose.

Nutritional interrelationships of calcium, magnesium, and phosphorus were investigated by Chicco et al. (1973) in wethers. The results indicated that high dietary calcium decreased magnesium in plasma and bone, increased calcium in plasma, and had a tendency to reduce plasma phosphorus. High dietary magnesium increased plasma and bone magnesium, reduced plasma calcium, and slightly reduced plasma phosphorus. Dietary phosphorus had little effect on magnesium utilization.

Most workers using low magnesium diets have observed soft tissue calcification (Duckworth, 1938; Odell, 1960). Calves fed milk diet alone for long periods of time may also be deficient in vitamins A, D, and E, as well as iron, copper, or manganese (Odell, 1960). Bone depleted of magnesium may or may not have an increased calcium content (Jacobson et al., 1972).

Apparent absorption of magnesium by rats was reduced by increasing dietary calcium from 0.34 to 0.68%, or phosphorus from 0.39 to 0.79% and was further reduced by increasing both. Also, increased calcium loss from the body, by high magnesium intake was prevented by high levels of

dietary phosphorus or potassium (Odell, 1960).

The magnesium-potassium interrelationships and magnesium-calcium relationships have been previously discussed under the heading of dietary factors. In rats, some evidence suggests a better utilization and retention of dietary magnesium in the presence of high levels of iodine in the ration (Sanwal and Hansard, 1972).

Low Environmental Temperature Effects on Magnesium Physiology

Grass tetany generally occurs during early spring or late winter, but may also occur in autumn (Grunes et al., 1970). There seems to exist a high correlation between inclement weather and acute hypomagnesemia (Allcroft, 1947), and in countries where the incidence of tetany is greatest the triggering of acute tetanic episodes often occurs on cold and wet mornings (Rogers, 1965). The condition is associated with a changeover from winter rations to rapidly growing pastures in the spring.

The onset of normal hibernation in animals is accompanied by a rise of serum magnesium which persists during hibernation, however, a non-hibernating mammal maintaining its body temperature in cold does not show any rise of serum magnesium (Riedesel, 1957). Thus the statement by Platner and Shield (1969) that serum magnesium is elevated in cold cannot be generalized. A rapid uptake or excretion

of excess extracellular magnesium may occur, but this leads to a consideration of the lack of lability of soft tissue magnesium (Rogers, 1965).

Acute cold exposure gave rise to diuresis, and also an increased excretion of magnesium, and a lowering of blood and plasma volume, but there is no evidence that these changes occur with prolonged cold exposure (Bass and Henchel, 1956).

Kemp and t'Hart (1957) observed two types of fluctuation in the morbidity of grass tetany for cattle in Netherlands. First was the seasonal fluctuation, when the mean daily temperature was 14 degrees centigrade (57 degrees farenheit), in the spring and autumn. Second were short term fluctuations. Five days after the mean daily temperature was higher than 14 degrees centigrade, there was greater incidence of grass tetany, but the morbidity decreased within five days after the fall in temperature. During cold, wet years, tetany occurred the whole summer in the Netherlands (t'Hart, 1960), and 95% of the cases occurred when mean ambient temperatures were between eight and 14 degrees centigrade (46-57 degrees farenheit), as has been observed in other parts of the world. t'Hart pointed out that hypomagnesemia occurred mainly when the temperature was rather low, and other conditions were favorable for the grass. Large numbers of cases of autumn tetany occurred only in wet seasons. In Scotland, according to Stewart

(1954), the winter type tetany occurs in November or December, in stall fed animals, consuming mainly grass silage or dry grass hay.

In England and Ireland, the magnesium concentration in mixed pasture grass has been reported to be lower in spring than in summer (Voisin, 1963). Similar results have been reported from other parts of the world where the disease incidence is great (Grunes et al., 1970).

It has been suggested (Grunes et al., 1970) that grass tetany occurs more frequently in cool weather because the concentrations of magnesium in the forage are often lower then. The disease may occur when a period of cool weather is followed by warm weather because the potassium concentration, as well as the ratio potassium/(calcium + magnesium), is increased.

In general, protein and mineral utilizations are reduced with growth rate by exposure to cold (Sorensen and Moustgaard, 1965).

Hormone Relationships

It is considered likely that endocrine factors are involved to a minor extent in magnesium control (Care, 1969). Care and associates (1967) suggested that much of the wide individual variation in the reaction of different animals to the factors causing hypomagnesemia may be ascribed to endocrine influences. An animal's response to

cold appears to be mediated by the pituitary (Tyslowitz and Artwood, 1942), the adrenals (Sellers et al., 1951), and the thyroids (LeBlond and Gross, 1943). Some of the hormonal effects are herein briefly discussed.

Thyroid hormones. The increased incidence of grass tetany during cold, wet, and windy weather may be associated with changes in thyroid activity (Grunes et al., 1970). Vitale et al. (1957; 1959) showed that thyroxine injection caused a 70% decrease in serum magnesium in cold exposed rats fed a normal ration. Moreover, the magnesium requirements of the rats appeared to be increased after thyroxine administration.

Intramuscular injection of tri-iodothyronine in thyroidectomized sheep caused a fall in serum magnesium levels, but only when the parathyroids were intact (Care, 1969). Thyroxine and tri-iodothyronine also increased the tissue turnover rate of magnesium and also reduced magnesium abosrption from the intestine of sheep (Care, 1969).

The activity of the thyroid gland, which is of special interest as a part of the body temperature regulating mechanism,is increased at lower environmental temperature (Sorensen and Moustgaard, 1965). It has been shown that in thyrotoxicosis (increased thyroid activity) plasma magnesium is lowered (Hanna, 1961; Jones and Fourman, 1966). Moreover, the symptoms of hyperexcitability and nervousness are

common to both the hyperthyroid state and hypomagnesemia (Todd, 1969).

However, Todd and Thompson (1962) found that plasma protein bound iodine (PBI) levels were not elevated either in hypomagnesemic cows or in clinically affected cases of tetany. Utilization of dietary magnesium, indicated by greater serum and tissue concentrations, was increased in rats fed high dietary iodine (Sanwal and Hansard, 1972). However, the mechanism by which this occurred is not known. Moreover, since the dietary iodine is an important factor which may affect thyroid function, it is essential to control this factor (Monique et al., 1969).

Parathyroids and magnesium. Conflicting reports on parathyroid hormone (PTH) effects on magnesium were found. Care (1969) suggested that PTH raises plasma magnesium and calcium levels by stimulating bone resorption, by renal tubular reabsorption and by absorption from the small intestine. However, with the advent of a specific radio-immuno-assay for PTH and the development of a technique for the controlled perfusion of a parathyroid gland isolated in situ in an anesthetized sheep or goat, it has been possible to obtain unequivocal evidence for an inverse relationship between PTH secretion rate and the plasma magnesium concentration (Buckel et al., 1968).

It is well known that significant amounts of magnesium

are found in bone and that parathyroid hormone has profound effects on bone metabolism (Roberts et al., 1954). MacManus et al. (1971) concluded from their in vitro and in vivo experiments with rats that magnesium deficiency inhibits the action of PTH on its target organs (e.g. bone) in spite of the fact that hypomagnesemia causes an increased secretion of PTH (Buckle et al., 1968). Heaton (1965) suggested that the parathyroids regulate plasma magnesium by mobilization from bone, and that deprivation of magnesium increases their activity. Gitelman et al. (1968) supports this point of view.

Effects of PTH on renal transport of magnesium are less well defined (Walser, 1969). It increased the magnesium excretion in man, dogs, and rats (Heaton, 1965), but decreased magnesium excretion has also been reported (Anast et al., 1967; Todd et al., 1962).

Early response to parathyroid ablation appears to be increased urinary excretion of magnesium (Berthaux et al., 1960; Heaton, 1965; Roberts et al., 1954). According to MacIntyre et al. (1963) PTH causes marked conservation of urinary magnesium and calcium. However, hyperparathyroidism is usually associated with hypermagnesuria (Heaton and Pyrah, 1963; MacIntyre et al., 1961). Thyroparathyroidectomy in dogs impairs the ability of the kidney to conserve magnesium in response to a magnesium free diet, independent of any changes in plasma magnesium (Walser, 1969). Parathyroidectomy

in normal dogs has little effect on magnesium balance, except for a slight decrease in serum magnesium levels (Roberts, et al., 1954).

It has been shown (Care et al., 1966) that although thyroparathyroidectomy of sheep reduced intestinal absorption of magnesium (and calcium), the intravenous administration of a large dose of bovine PTH resulted in an initial hypomagnesemic (and hypocalcemic) response which preceded the expected hypermagnesemia (and hypercalcemia). Care (1969) suggested the possibility that two substances might exist in the PTH preparations used, which might have opposite effects on the absorption of magnesium (and calcium) from the ovine small intestine.

However, the recent balance studies of Clark and Rivera-Cordero (1971) in rats have shown that PTH had little effect on the quantitative aspects of the absorption and urinary excretion of magnesium (and calcium).

Copp (1970) also observed that PTH secretion was stimulated by low plasma magnesium and was inhibited when plasma magnesium was high. It was reported that the synthesis and/or secretion of PTH might be impaired in the magnesium deficient state, and that magnesium administration would rapidly restore the ability of the parathyroid gland to respond appropriately to the level of ionized blood calcium. Another possibility is that magnesium deficiency caused increased destruction of PTH; however, little is

known concerning the metabolic fate of this hormone (Anast et al., 1972).

Targovnik et al. (1971) observed in their in vitro experiments that the release of PTH from bovine parathyroid glands increased as the magnesium concentration in the media was lowered to a level of 0.72 mg/100 ml. In a similar experiment Hamilton et al. (1971), however, did not find such a change.

Anast et al. (1972) suggest that further study is needed to establish a possible inverse relationship between the extracellular magnesium concentration and the secretion and synthesis of PTH.

Adrenal hormones. No negative feedback of magnesium (or calcium) concentration on the secretion rate of cortisol and aldosterone by sheep adrenal gland has been demonstrated (Kynes and Care, 1967; Blair-West et al., 1968). However, in magnesium deficiency adrenal glands reportedly increase in weight (Elin et al., 1970). Hypomagnesemia (and hypocalcemia) has been produced by aldosterone administration to thyroparathyroidectomized and adrenalectomized sheep maintained on injections of cortisone and desoxycortisone acetate.

Hypersecretion of aldosterone may adversely affect magnesium balance and plasma magnesium level (McAleese and Forbes, 1959; Scott and Dobson, 1965). In a study on

adrenalectomized rats (Hanna and MacIntyre, 1960) aldosterone increased both urinary and fecal magnesium, and reduced the intracellular magnesium content of muscle.

Hypomagnesemia seen in primary aldosteronism has been suggested to be secondary to potassium deficiency and kidney damage (Vitale et al., 1959), but others suggest a direct effect of aldosterone, not secondary to renal damage (Hanna and MacIntyre, 1960).

Wilson (1964) reported that aldosterone blocked the absorption of magnesium into the cells of the small intestine, and that aldosterone production was increased in response to a high potassium and low sodium intake. Thus a high potassium and low sodium intake may reduce magnesium absorption. Moreover, other investigators (Allcroft and Burns, 1968; Dobson et al., 1966) indicated that the secretion of aldosterone by the adrenal gland may decrease the availability of dietary magnesium. According to Dobson et al. (1966) increased aldosterone has only a minor role in the development of hypomagnesemia.

Later Care (1969) suggested that since high potassium and low sodium content of lush grass cause an increased secretion of aldosterone, hypomagnesemia may result from hyperaldosteronism. Increased secretion of aldosterone may cause reduced absorption of magnesium from the gastro-intestinal tract and hypomagnesemia. This theory, however, was disproved by finding that plasma magnesium level of

adrenalectomized sheep, maintained on adrenal corticoids decreased when these sheep were fed lush grass.

The effect of glucocorticoids on urinary magnesium are less well known (Walser, 1969). In normal subjects it may increase, decrease, or may cause no change in magnesium excretion.

Miscellaneous hormone effects. Thyrocalcitonin, although markedly affecting serum calcium levels, has not been shown to have a significant effect on serum magnesium levels in mammals (Gudmundsson et al., 1966). However, an increased magnesium excretion by this hormone occurred in thyroparathyroidectomized rats receiving PTH, probably due to its diminishing action on hypercalcemic response (Anast et al., 1967; Carrell and Pechet, 1967).

The influence of steroid hormones on magnesium metabolism is not known (Grunes et al., 1970). Posterior pituitary hormone in sheep had no observed effect on magnesium metabolism (Moodie, 1968). Growth hormone, glucagon, and angiotensin increase urinary excretion of magnesium, and in case of growth hormone an additional fall in plasma magnesium may occur (Walser, 1969). Vitamin D may increase magnesium excretion without a change in plasma magnesium (Ibid., 1969). However, vitamin D increased the apparent availability and retention of magnesium (and calcium) in ruminants consuming low nitrogen containing orchard grass

(Stillings et al., 1964). In contrast, vitamin D supplementation of the ration fed to cows before they were turned out to grass had no effect on subsequent serum magnesium levels (Hvidsten et al., 1959).

Magnesium and Other Selected Mineral Distribution in Body Tissues

The animal body contains about 0.041% magnesium, and about 70% of which is found in the skeleton (Maynard and Loosli, 1969). About one-third of the skeletal magnesium is labile. Blood serum normally contains 2 to 5 mg magnesium/100 ml, so the total amount of extracellular fluid magnesium is quantitatively insignificant. According to Widdowson et al. (1951) skeletal muscle contains 16 to 20 meq magnesium/Kg wet weight. This is consistent with the low magnesium content observed in adipose tissue. Chemical nature of bone magnesium was not well understood (Duckworth and Godden, 1941).

According to Rogers (1965) the intracellular magnesium concentration of soft tissues bears a 10:1 ratio to the extracellular fluid concentration. Thus distribution of magnesium resembles that of calcium in the skeleton.

Wilson (1964) reported that the magnesium in plasma exists in ultrafiltrable and nonfiltrable forms. Of the total plasma magnesium concentration, about 67% is in the ultrafiltrable fraction, the remaining being protein bound.

In cattle mean serum calcium is 12.9 mg/100 ml, whereas the diffusible portion is 4.9; inorganic phosphorus is 4.9 mg/100 ml, but total blood phosphorus is perhaps four times, and red cell phosphorus about ten times this figure (Lane et al., 1968; Scott, 1970).

In man whole blood magnesium is 4.6 mg/100 ml, red blood cell 6.6, plasma 2.7, serum 2.5, and diffusible serum magnesium 1.9 mg/100 ml (Ibid.).

The data of Dancis et al. (1972) indicated that in rats fetal depletion of magnesium can occur in the presence of adequate maternal tissue levels, and it has been suggested from experiments in dogs (Field, 1960) that bone may not be a sufficient reservoir for maintaining plasma magnesium.

Magnesium concentrations of liver, kidney and skeletal muscle of the rat are about 81.7, 82.7, and 102.2 mg/100 gm, respectively, of fat free dry matter (MacIntyre and Davidson, 1958). The values for guinea pigs for kidney, heart and skeletal muscle are 91.9, 105.0, and 121.0 mg/100 gm dry matter, respectively, according to Morris and O'Dell (1961). Ammerman et al. (1974) have given the mineral concentrations for various bovine tissues.

Exchange between intracellular and extracellular magnesium was found to be rapid in heart, kidney, and liver of rats, dogs, and rabbits (Brant et al., 1958; Rogers, 1965), and slower in skeletal muscle, thyroids, adrenals, and ovaries (Brant et al., 1958).

Two components of magnesium in soft tissues have been suggested (Care, 1960). One is rapidly exchanged while the other is rather stable. The concentration of labile magnesium in the intracellular fluid about equaled that in extracellular fluid, a possible indication that soft tissue magnesium acts as a reserve for the extracellular magnesium.

Genetic and Individual Variation

The evidence suggests that genetic variation exists both in the incidence of some disorders associated with mineral metabolism and in the concentrations of minerals in animal tissues and fluids (Wiener, 1971). This aspect needs more studies with more mineral elements, however, a large part of the variation among individual animals should not be attributed wholly to chance (Ibid.).

Wiener suggested further that if genetic variation in the requirements could be established, the nutritionist and veterinarian would have more accurate means of assessing and of meeting requirements under those circumstances where the environment could be easily manipulated. Where environment cannot be altered, it may be more appropriate to change the genotype. An important question remains: Is genetic variation in mineral status equally manifested under different environmental conditions and levels of nutrition? No direct experimental evidence exists for this, and the indirect evidence is scant and equivocal. The implications

of genetic variation may well differ for different minerals.

It is generally assumed that some cows are more succeptible to hypomagnesemia than others, although differences in magnesium intake and bone mobilization may play a role (Grunes et al., 1970). Grunes et al. point out the conclusion of Kemp (1958) that serum magnesium contents of two animals over an entire season rise and fall fairly simultaneously but on a different level.

Miscellaneous

Several other factors could be related to the occurrence of hypomagnesemic tetany. Grunes et al. (1970) state in their review that unusually high concentrations of manganese in grass (540-1,320 ppm in dry matter) have been found to be associated with certain cases of lactation tetany in cattle. It was also shown that manganese administered by stomach tube to cattle, sheep and rabbits depressed the magnesium levels in blood.

McWard (1969) observed that addition of 4% phytic acid to an isolated soy protein-glucose diet increased the chick requirements for magnesium. Addition of 500 ppm of EDTA with or without phytic acid to the diet had no effect upon the availability of magnesium to the chick. Dutton and Fontenot (1967), working with ruminants, however, found that the form of phosphorus had no significant effect on absorption and retention of magnesium and calcium, nor upon the absorption of phosphorus.

Kempt et al. (1966) reported that the addition of animal fat to winter rations for milking cows increased the percentage of magnesium in feces, thus decreasing its apparent availability to the animal.

The possibility of the effect of histamine on inducing neuromuscular excitability (such as grass tetany), especially when forage magnesium and calcium are low has been suggested (Grunes et al., 1970).

Additional conditions in which a low serum magnesium concentration has been reported include diabetic acidosis after treatment, chronic renal disease, osteolytic bone disease, hyperthyroidism, cardiac failure, pancreatitis, and diarrhoea (Agna and Goldsmith, 1958).

To test the theory that bacterial absorption of magnesium reduced the amount available for absorption by the sheep, Care et al. (1967) administered chloramphenicol orally, greatly reducing the bacterial population in the mid ileum, but this did not prevent acute hypomagnesemia.

CHAPTER III

EXPERIMENTAL PROCEDURE

The present study was conducted in two phases. The first phase of the investigation was designed to evaluate the effects of low ambient temperature on magnesium metabolism of sheep in relation to the protein content of the ration. The second phase involved studies with 72 rats in an effort to analyze the effects of low temperature on magnesium status and with different dietary combinations of protein, iodine, and potassium.

Procedure with Sheep

Six young ewe lambs of identical age, weight, breed, and nutritional background were randomly divided into three treatment groups.

The first group was fed a control ration containing 0.24% magnesium and 12% protein (Control). The second group was fed the magnesium deficient (0.04% magnesium) diet containing 12% protein (Low-Mg) and the third group was fed a low magnesium diet (0.05% magnesium) with 16.1% protein (Low-Mg, Hi-Protein). The composition and analysis of the diets are presented in Tables I and II, respectively.

The three groups of sheep were placed in separate wooden pens, and were given their respective feeds and deionized water ad libitum. The sheep were maintained under

TABLE I

SHEEP RATION COMPOSITION[a]

Ingredient	Percent	
	Control Ration	High Protein Ration
Corn cobs	50	50
Corn gluten meal	17	25
Cerelose	26	18
Corn oil	5	5
Salt (NaCl)	1	1
Dicalcium phosphate	0.7	0.7
Vitamin A, D, E concentrate	+ +	+ +
Magnesium oxide	0.418	--

[a]The magnesium deficient diet with normal protein content was similar to the control diet in composition, except that no MgO was added.

TABLE II

MINERAL AND PROTEIN ANALYSIS

OF SHEEP DIETS

Ingredient	Ration		
	Control	Low-Mg	Low-Mg, Hi-Prot
Magnesium (percent)	0.24	0.04	0.05
Calcium (percent)	0.32	0.28	0.39
Potassium (percent)	0.40	0.45	0.47
Protein (percent)	12.0	12.0	16.1

such conditions for 30 days to adapt to the experimental diets and environment. Periodic samples of blood were taken from each sheep during this period for plasma mineral and thyroxine determinations. Thereafter, three sheep, one from each group, were transferred to an environment with a temperature of 7.3 ± 0.5°C; while the other three sheep were maintained at room temperature of 20.3 ± 2.2°C. The design explaining the sheep experiment is presented in Table III. During the second period of the study (exposure period) all sheep were individually housed in metabolism crates for balance studies. After the sheep had been adapted for about a 20-hour period to the metabolism crates, a six-day balance study for magnesium, calcium, and potassium was conducted. During this period, the feed intake, total fecal excretion, and total urinary excretion were measured for a 24-hour interval for each sheep. Samples were collected of feed, urine and feces for mineral determination. Blood samples were also taken at the same time each day for plasma mineral measurements.

At the end of the six-day balance period each sheep was intravenously dosed with 92 uCi of ^{28}Mg with a specific activity of 1 mCi/mg of total magnesium. Blood samples were collected at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 16, 22, and 30 hours after dosing for radiomagnesium counting and total mineral determinations. Feces was weighed and sampled at 2, 4, 6, 8, 10, 16, 22, and 30 hours after dosing. Urine was measured and sampled at 1, 2, 3, 4, 6, 10, 16, 22, and 30 hours after dosing. Each sample was processed further

TABLE III

DESIGN OF SHEEP EXPERIMENT

Ration Treatment	Total Number of Sheep	Number of Sheep After 30 days Feeding Period	
		At 20.3°C	At 7.3°C
Control	2	1	1
Low-Mg	2	1	1
Low-Mg, Hi-Prot	2	1	1

for radiomagnesium counts and stable mineral measurements.

At the end of the balance period, all the animals were sacrificed by stunning and exsanguination. Upon sacrifice, representative tissues and contents were collected for stable mineral determination and radioactive measurements as follows: blood, skeletal muscle, cardiac muscle, liver, spleen, brain, thyroids, femur epiphysis, femur shaft, bone marrow, kidneys, rumen tissue, rumen contents, abomasal tissue, abomasal contents, duodenal tissue, duodenal contents, large intestinal tissue, large intestinal contents, and uterus. All samples, and in some cases the whole organs, and contents of the gastrointestinal tract were weighed and processed for stable and radiomagnesium determination.

All samples were weighed or measured volumetrically on a fresh basis at the spot of collection. Samples for stable mineral analysis were transferred to crucibles, dried, and ashed at about 600°C for 18 to 24 hours. The ashed samples were dissolved in 6N hydrochloric acid and diluted further as required for analysis. Each dilution contained 1% lanthanum trioxide (La_2O_3) to minimize phosphorus interference. Stable analysis of magnesium, calcium and potassium were made by atomic absorption spectrophotometry.[1] Calcium

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[1]Perkin-Elmer Manual. 1968. Analytical Methods for Atomic Absorption Spectrophotometry. Perkin-Elmer, Norwalk, Connecticut.

and potassium were determined in addition to magnesium because of their close relationship to hypomagnesemia and grass tetany. Sheep samples containing radiomagnesium were also weighed or pipetted in duplicate into counting tubes for radioactive measurement. Samples were counted with known dilutions of the dosing solution in an automatic well-type gamma spectrometer[2] and results were expressed as a percentage of the administered dose. Thyroxine was measured in blood plasma by a competitive binding procedure using materials and methods in a commercial preparation.[3]

Procedure with Rats

A total of 72 adult male albino rats of the Sprague-Dawley strain, uniform in age and body weight were equally divided into six dietary and two temperature groups in a factorial arrangement of treatments (Table IV). The six diets were: (1) Control (low iodine ration); (2) High iodine (Hi-I); (3) High potassium (Hi-K); (4) High protein (Hi-Prot); (5) High protein, high potassium (Hi-Prot, Hi-K); and (6) High protein, high potassium, high iodine (Hi-Prot, Hi-K, Hi-I) diets.

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[2]Nuclear Instrument and Chemical Corporation, Chicago, Illinois.

[3]Tetrasorb-125 (T-4 diagnostic kit). Abbot Laboratories. Radio-pharmaceutical Products Division, North Chicago, Illinois.

TABLE IV

EXPERIMENTAL DESIGN FOR RATS

Ration Treatment	Number of Rats	
	26.2±1.8°C	4.8±0.3°C
Control (low iodine)[a]	6	6
High iodine (100x)	6	6
High potassium (15x)	6	6
High protein (15% Casein)[b]	6	6
High protein, high potassium[b]	6	6
High protein, high potassium high iodine[b]	6	6

[a]Control diet was the low iodine synthetic diet supplied by Nutritional Biochemicals Company.

[b]The diet was supplemented with vitamins and minerals because of higher protein level.

The control diet, a low iodine synthetic diet purchased from Nutritional Biochemical Company,[4] served as the basal ration. The high iodine diet contained 100 times the NRC recommendation of 0.015 mg iodine/100 gm diet added as potassium iodide (KI). Similarly, the high potassium diet was made by the addition ot potassium chloride (KCl) to the basal diet to increase its potassium content to 15 times the NRC recommendation of 180 mg potassium/100 gm diet. The high protein diet consisted of the control diet plus 15% added casein. A chemical analysis of the diets fed is presented in Table V. All diets contained 0.04% magnesium.

Rats in each of the dietary groups were divided into four cages of three rats each and fed their respective diets and de-ioneized water ad libitum for a period of 21 days at room temperature. After the 21-day adaptation period, two of the four cages of rats fed each diet were transferred to low ambient temperature (4.8 ± 0.30°C) for 11 days. The remaining rats continued to be housed at room temperature (26.2 ± 1.8°C).

Feed consumption by all rats for the next nine days of exposure was measured as group average (three rats) basis. Body weights of the rats were measured several times at

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[4]Nutritional Biochemicals Company, Cleveland, Ohio 44128.

TABLE V

MINERAL AND PROTEIN ANALYSIS OF RAT DIETS

Ingredient	Diets					
	Control	Hi-I	Hi-K	Hi-Prot	Hi-Prot, Hi-K	Hi-Prot, Hi-K, Hi-I
Magnesium (percent)	0.04	0.04	0.04	0.04	0.04	0.04
Calcium (percent)	0.77	0.67	0.72	0.80	0.93	0.66
Potassium (percent)	0.44	0.49	2.53	0.41	2.53	2.30
Protein (percent)	23.70	23.70	23.70	32.54	32.54	32.54

pre-determined intervals throughout the experimental period.

On day nine of exposure, one rat from each group (at both temperatures) was placed in a metabolism cage for a 24-hour balance study. At the end of 24 hours, feed consumption, fecal excretion, and urinary excretion from all the 12 rats were measured, and sampled for the analysis of magnesium, calcium, and potassium.

At the end of the eleventh day of the exposure period all 72 rats were sacrificed individually by stunning. A blood sample was collected from each rat just before sacrifice by heart puncture. Liver, heart, skeletal muscle, and bone (femur) samples were also collected from each rat for mineral determination.

The mineral determination in all material collected was performed in the same manner as described previously for sheep. Thyroxine in blood plasma was determined using the T-4 diagnostic kit, as previously described. All the blood plasma of three rats representing one-half dietary treatment at a particular temperature was pooled for thyroxine measurement.

All statistical analysis of rat data was performed by simple analysis of variance and comparison of the means by student's t-test (Snedecor and Cochran, 1971).

CHAPTER IV

RESULTS AND DISCUSSION

Results with Sheep

Changes in plasma magnesium. Blood plasma magnesium concentrations in sheep fed magnesium deficient diets were not reduced as much as expected, even after 30 days (Table VI). Plasma magnesium declined during the second and third weeks of the feeding period but returned to normal levels. Although the ration fed contained only a very low percentage of magnesium (0.04-0.05% magnesium), it appeared that the plasma magnesium levels were maintained at normal levels partly because of undereating, and partly because the levels appeared to be maintained by magnesium controlling mechanisms in the body.

At this stage the sheep were divided into two groups as has been mentioned before for temperature studies. Plasma magnesium concentration determined at a more frequent interval during this second phase of metabolic study and temperature exposure are given in Table VII. By day seven plasma magnesium in sheep fed magnesium deficient rations averaged 30% lower than in the control animals. Sheep exposed to low ambient temperature appeared to show a sharper decrease than those exposed at room temperature in spite of the fact that plasma volume has been shown to

TABLE VI

PLASMA MAGNESIUM LEVELS IN SHEEP DURING THIRTY DAYS FEEDING PERIOD

Treatment	Sheep Number	Day of Experimental Feeding				
		2	9	16	23	30
		(mg/100 ml plasma)				
Control	1	1.96	1.65	1.80	2.12	2.07
	2	2.11	1.74	1.75	1.98	2.36
Low-Mg	1	1.82	1.49	1.69	1.96	2.00
	2	1.95	1.51	1.57	1.94	1.81
Low-Mg, Hi-Prot	1	1.91	1.56	1.62	1.51	1.95
	2	1.76	1.73	1.42	1.69	2.01

TABLE VII

PLASMA MAGNESIUM LEVELS OF SHEEP EXPOSED TO TWO TEMPERATURES

Dietary Treatment	Sheep Number	Exposed to °C	Day of Exposure 1	2	3	4	5	6	7
			(mg/100 ml plasma)						
Control	1	20.3	2.83	2.32	2.15	2.43	2.25	2.26	2.04
	2	7.3	2.57	2.49	2.36	2.32	2.44	2.45	1.87
Low-Mg	1	20.3	1.86	1.92	1.67	1.67	1.61	--	1.39
	2	7.3	1.54	1.60	1.70	1.67	1.38	1.45	1.02
Low-Mg, Hi-Prot	1	20.3	1.90	1.87	1.88	1.84	1.62	1.98	1.72
	2	7.3	2.32	2.26	2.00	1.85	1.80	1.57	1.32

decrease significantly in cold (Bass and Henchel, 1956).

Plasma magnesium values for different sheep at two temperatures during different times of the day are presented in Table VIII. Samples were collected a day before the last day of the experiment (sixth day of temperature exposure). Plasma magnesium levels tended to be lowest between 6 a.m. and 11 a.m. and highest between 1 p.m. and 1 a.m. No reports on diurnal variation in the magnesium content of the blood plasma could be found in the literature.

Magnesium balance in sheep. Stable magnesium balance data obtained during a seven-day period are given in Table IX. Obviously erroneous data obtained for sheep fed the low magnesium-normal protein ration (because the urinary catheter was accidently removed out of place) at room temperature were omitted from the table.

All animals fed a normal protein ration were in negative magnesium balance. Those fed the high protein diet, low in magnesium, especially the one exposed to cold, retained more magnesium than all other sheep. About 70-95% of ingested magnesium is normally excreted in the feces of adult sheep and cattle, most of which is unabsorbed dietary magnesium (Smith, 1969). Sheep fed the high protein ration had the lowest fecal excretion and highest true digestibility of magnesium. This, together with lower urinary magnesium excretion, resulted in a higher magnesium

TABLE VIII

VARIATION IN BLOOD PLASMA MAGNESIUM OF SHEEP EXPOSED TO TWO TEMPERATURES WITH TIME OF DAY

Treatment	°C Temperature	Time of Day					
		6 a.m.	9 a.m.	11 a.m.	1 p.m.	7 p.m.	1 a.m.
		(mg/100 ml plasma)					
Control	20.3	1.85	2.08	1.92	2.26	2.47	2.96
	7.3	2.05	1.97	2.03	2.12	2.45	2.30
Low-Mg	20.3	1.56	1.63	1.46	--	1.72	1.99
	7.3	1.42	1.45	1.55	1.45	1.50	1.57
Low-Mg, Hi-Prot	20.3	1.70	1.80	1.92	1.98	1.96	1.77
	7.3	1.67	1.67	1.56	1.57	1.54	1.90

TABLE IX

MAGNESIUM BALANCE IN SHEEP DETERMINED
AS PERCENT OF INTAKE

Dietary Treatment	°C Temperature	Excretion		Balance	Percent Fecal Endogenous Mg[a]	Percent True Digestibility
		Feces	Urine			
Control	20.3	88.28	29.14	-17.42	16.43	28.15
Control	7.3	95.50	37.72	-36.23	16.56	21.06
Low-Mg	20.3	--	--	--	--	--
Low-Mg	7.3	92.18	17.19	- 9.38	59.40	67.22
Low-Mg, Hi-Prot	20.3	58.57	28.57	+12.86	49.30	90.73
Low-Mg, Hi-Prot	7.3	64.05	19.76	+16.67	53.60	89.55

[a] Endogenous fecal Mg (gm) = $\frac{\text{Specific activity (plasma)}}{\text{Specific activity (feces)}}$ X daily Mg intake (gm)

retention than the controls. These findings, however, do not agree fully with that of Moore et al. (1972) who concluded that high levels of dietary nitrogen did not affect magnesium absorption, nor elevate the output of urinary magnesium in lambs.

The higher percentage absorption of magnesium in animals fed a low magnesium diet reported by Aikawa (1965) is confirmed by data in Table IX. Both true digestibility and endogenous loss of magnesium were higher when low magnesium diets, especially when the diet high in protein, were fed. Temperature did not appear to affect fecal endogenous magnesium although true digestibility was lower in the control sheep exposed to cold compared to the control at room temperature.

Acute cold exposure increases magnesium excretion in the urine (Bass and Henchel, 1956). Although cold exposure increased urinary magnesium for sheep fed the control (sufficient magnesium) diet, the value was lower in sheep in the cold than in sheep at room temperature fed the low magnesium-high protein diet.

Urinary and fecal excretion of ^{28}Mg during 30 hours after intravenous administration is given in Table X. The major pathway of radiomagnesium excretion was urinary in all sheep. Control sheep eliminated about 44% of the radiomagnesium in the urine during 30 hours after intravenous dosing compared to only 4.4 to 11.5% by magnesium

TABLE X

URINARY AND FECAL EXCRETION OF 28-MAGNESIUM DURING THIRTY HOURS AFTER INTRAVENOUS ADMINISTRATION
(as percent of dose)

	Control Diet		Low-Mg Diet		Low-Mg, Hi-Prot Diet	
Excretion	at 20.3°	at 7.3°	at 20.3°	at 7.3°	at 20.3°	at 7.3°
Urine	43.5	43.8	4.4	4.4	7.2	11.5
Feces	5.2	3.5	1.9	1.5	1.9	1.7

deficient sheep. McAleese et al. (1961) reported only 3.6 to 5% urinary excretion in 35 hours by control lambs given ^{28}Mg intravenously whereas controls in the present experiment excreted almost half of the ^{28}Mg by that time. Their control diet was, however, supplemented with only 0.06% additional magnesium. Low temperature did not affect urinary radiomagnesium excretion by sheep fed adequate magnesium or the magnesium deficient diet with normal protein content. However, urinary radiomagnesium excretion by sheep fed the low magnesium-high protein diet was 64% higher at room temperature and 161% higher in cold than corresponding excretions by sheep fed the low magnesium-normal protein diet. This is in partial agreement with findings by Moore et al. (1972). There was less effect of diet or temperature on fecal ^{28}Mg but fecal excretion appeared to be somewhat lower in cold than at room temperature.

Magnesium disappearance rate from sheep blood plasma. Clearance of blood plasma ^{28}Mg after intravenous administration to sheep is shown in Figure 1. Environmental temperature did not appear to have a marked effect on radiomagnesium disappearance from blood plasma. However, magnesium deficient sheep retained more ^{28}Mg in blood plasma than the control sheep which had cleared almost all of the radiomagnesium from the blood plasma in 30 hours. These

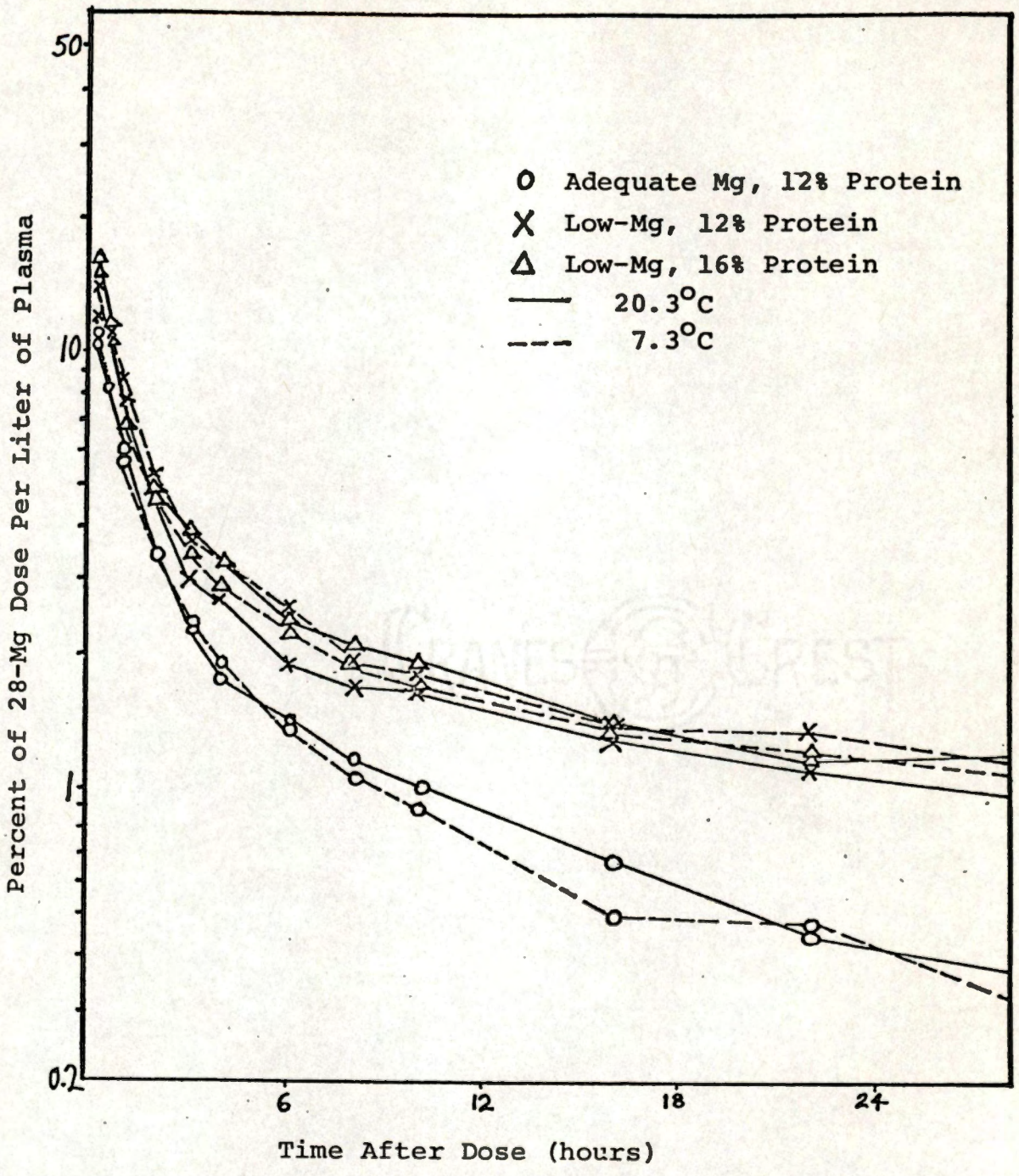

FIGURE I

EFFECT OF DIETARY MAGNESIUM, PROTEIN LEVELS AND ENVIRONMENTAL TEMPERATURE ON CLEARANCE OF BLOOD PLASMA 28-Mg AFTER INTRAVENOUS ADMINISTRATION TO SHEEP

curves do not agree with those of McAleese et al. (1961) where less plasma ^{28}Mg was retained by magnesium deficient sheep than controls, apparently because plasma ^{28}Mg was deposited in the tissues of the deficient lambs.

Magnesium and radiomagnesium in tissues. Percentage retention of radiomagnesium in tissues and gastrointestinal tract 30 hours after intravenous dosing with ^{28}Mg are presented in Table XI. In general, sheep fed the magnesium deficient diets retained more radiomagnesium than controls in almost all the tissues. This agrees with the results of McAleese et al. (1961), as opposed to their plasma data. More ^{28}Mg per unit of fresh tissue weight was retained in the femur shaft of magnesium deficient sheep in this experiment, while McAleese et al. (1961) report a higher ^{28}Mg dose retention (on a dry tissue weight basis) in control sheep than in magnesium deficient sheep. However, as observed here, they found less variation in percent dose figures for bone epiphysis. Sheep fed magnesium deficient diets and housed in the cold appeared to retain a higher percent of the dose in some of the tissues in cold than sheep housed at room temperature, for example: in femur epiphysis, heart, liver, spleen, kidneys, intestine, uterus, and skeletal muscle. The percent dose retention, in general, was higher in bone, liver, cardiac muscle, spleen, kidneys, intestine, abomasum, uterus and thyroids, was lower in

TABLE XI

PERCENT OF TOTAL DOSE OF 28-MAGNESIUM IN TISSUES AND DIGESTIVE TRACT CONTENTS AT SLAUGHTER THIRTY HOURS AFTER INTRAVENOUS DOSING[a]

	Control		Low-Mg		Low-Mg, Hi-Prot	
Tissues and Contents	20.3°C	7.3°C	20.3°C	7.3°C	20.3°C	7.3°C
Femur epiphysis	9.8	8.3	8.6	11.9	13.2	13.2
Femur shaft	6.8	5.7	10.7	11.2	14.5	10.8
Heart	4.6	4.5	11.8	16.3	12.1	13.3
Liver	4.6	3.8	11.5	14.3	11.7	12.3
Spleen	4.7	3.8	9.7	11.8	8.8	11.2
Kidneys	4.0	3.0	9.0	10.9	9.1	11.2
Uterus	2.5	2.0	3.1	7.2	2.8	4.8
Thyroids	2.1	1.3	3.7	2.6	3.6	1.9
Skeletal muscle	0.8	1.2	1.5	2.2	1.0	1.5
Brain	0.7	0.8	3.1	2.8	2.8	2.7

TABLE XI (Continued)

Tissues and Contents	Control		Low-Mg		Low-Mg, Hi-Prot	
	20.3°C	7.3°C	20.3°C	7.3°C	20.3°C	7.3°C
Bone marrow	1.3	0.7	0.6	0.6	0.7	0.7
Ruminal tissue	1.1	0.7	4.3	3.2	2.8	2.9
Ruminal contents	0.2	0.1	0.7	0.6	0.4	0.4
Abomasal tissue	1.7	1.9	4.1	5.5	4.6	4.5
Abomasal contents	0.3	0.3	1.5	2.5	1.2	1.4
Small intestinal tissue	2.5	2.2	7.5	8.7	6.8	7.2
Small intestinal contents	1.4	1.3	4.6	5.8	4.3	5.0
Large intestinal tissue	2.9	1.7	7.0	8.6	7.4	8.8
Large intestinal contents	1.6	1.3	8.5	8.2	6.8	8.8

[a]All values represent percent of total dose of 28-Mg/Kg of fresh tissue.

rumen, brain, skeletal muscle, and bone marrow.

Concentration of stable magnesium in various sheep tissues is shown in Table XII. Values were close to those found in the literature (Ammerman et al., 1974).

Cold exposed sheep showed slightly but consistently higher magnesium concentration in kidneys, small intestine, and to some extent in the uterus and skeletal muscle, when compared to those maintained at room temperature, regardless of ration composition. This may be an indication of higher absorption, deposition, and excretion of magnesium in cold. While the femur epiphysis and shaft of control sheep appeared to have lower concentrations of magnesium in cold than at room temperature, magnesium content of femur in magnesium deficient-normal protein fed sheep was slightly higher at the low temperature than room termperature. It would appear that magnesium deficient animals in cold mobilize more magnesium to bone.

Both the large intestine and its contents showed a markedly lower magnesium concentration for control sheep housed in the cold than at room temperature. Values for magnesium deficient animals were quite low despite high fecal endogenous magnesium suggesting a high magnesium absorption. Small intestinal tissue, however, showed a higher magnesium concentration in cold exposed sheep than

TABLE XII

MAGNESIUM CONCENTRATION IN VARIOUS SHEEP TISSUES AND DIGESTIVE TRACT CONTENTS[a]

Tissues and Contents	Control		Low-Mg		Low-Mg, Hi-Prot	
	20.3°C	7.3°C	20.3°C	7.3°C	20.3°C	7.3°C
Femur epiphysis	248.0	207.8	207.8	216.0	171.3	207.6
Femur shaft	411.4	366.5	396.6	309.8	342.9	355.8
Heart	14.8	19.0	14.0	19.6	17.2	17.2
Liver	18.1	16.8	17.7	21.3	14.9	16.9
Spleen	17.4	17.3	14.3	16.0	15.6	19.0
Kidneys	14.4	15.4	14.3	15.6	14.4	15.4
Uterus	8.9	9.5	5.8	10.0	11.1	11.3
Thyroids	13.9	14.4	16.7	12.0	14.7	13.6
Skeletal muscle	22.3	24.5	21.6	24.1	22.0	23.2
Brain	12.6	12.0	12.0	12.8	13.7	15.8

TABLE XII (Continued)

Tissues and Contents	Control		Low-Mg		Low-Mg, Hi-Prot	
	20.3°C	7.3°C	20.3°C	7.3°C	20.3°C	7.3°C
Bone marrow	7.4	19.0	12.5	9.3	17.9	17.3
Ruminal tissue	19.0	14.7	9.9	7.1	8.8	7.7
Ruminal contents	42.5	26.3	5.0	3.2	4.2	3.9
Abomasal tissue	20.2	18.0	8.8	--	10.6	8.7
Abomasal contents	31.6	47.6	7.1	8.9	6.8	5.0
Small intestinal tissue	14.1	16.4	9.5	11.4	13.5	19.4
Small intestinal contents	49.6	25.4	9.1	9.7	10.2	10.1
Large intestinal tissue	43.5	19.6	13.1	--	12.7	20.8
Large intestinal contents	36.7	98.7	19.6	23.6	32.1	--

[a]All values are mg Mg/100 gm of fresh material and are the average of two determinations each.

those maintained at room temperature, the difference was most apparent for sheep fed the low magnesium-high protein diet.

Magnesium deficiency appeared to increase absorption and tissue deposition of magnesium. Those fed the high protein diet, especially in cold, appeared to have the highest intestinal magnesium absorption (Table IX). The intestinal contents did not show a similar pattern as the intestinal tissue. Magnesium concentration in ruminal tissue and contents of cold exposed sheep were lower than in those at room temperature.

Skeletal muscle and cardiac muscle concentrations of magnesium in sheep appeared to be higher in cold than at room temperature, with the exception of the cardiac muscle of sheep fed the low magnesium-high protein ration. In sheep fed low magnesium-normal protein ration the uterus showed a markedly higher magnesium content in cold than at room temperature. When sheep were maintained at room temperature, especially in those fed high dietary protein, magnesium content of femur epiphysis and shaft, liver, spleen, ruminal tissue and contents, large intestinal tissue, and abomasal contents was consistently lower in those fed low magnesium diets than in controls.

Tissue turnover rate of 28-magnesium as indicated by specific activity values. Specific activity values for tissues and digestive contents 30 hours after intravenous dosing with ^{28}Mg are presented in Table XIII. Radio-magnesium turnover rates in almost all tissues and digestive tract contents collected were higher in magnesium deficient than in control sheep and were further increased in many tissues by cold exposure. Turnover rates were higher in liver, heart, spleen, kidneys, small intestinal tissue, uterus, and thyroids than in femur epiphysis, femur shaft, skeletal muscle, gastrointestinal tract and contents, brain, bone marrow, and blood plasma.

Femur epiphysis where most of the mineral mobility is known to occur showed a slightly higher specific activity in animals fed low magnesium-normal protein diet and maintained in cold than at room temperature. For the high protein ration treatment, specific activity value was actually lower in cold than at room temperature. Blood plasma reflected a higher ^{28}Mg turnover rate in magnesium deficient animals than controls, especially in cold exposed animals. Skeletal muscle appeared to show a much lower turnover rate value in all sheep as compared to their cardiac muscle figures. Specific activities of kidney, spleen, uterus, ruminal tissue and contents, bone marrow, and abomasal contents of control sheep were lower in cold than at room temperature. In contrast, specific activities

TABLE XIII

SPECIFIC ACTIVITY VALUES FOR SHEEP TISSUES AND DIGESTIVE TRACT CONTENTS THIRTY HOURS AFTER INTRAVENOUS DOSING OF 28-MAGNESIUM[a]

	Control		Low-Mg		Low-Mg, Hi-Prot	
Tissues and Contents	20.3°C	7.3°C	20.3°C	7.3°C	20.3°C	7.3°C
Femur epiphysis	3.95	3.99	4.14	5.51	7.71	6.36
Femur shaft	1.65	1.56	2.70	3.62	4.23	3.04
Heart	31.08	23.68	84.29	83.16	70.35	77.33
Liver	25.41	22.62	64.97	67.14	78.52	72.78
Spleen	27.01	21.97	67.83	73.75	56.41	58.95
Kidneys	27.78	19.48	62.94	69.87	63.19	72.73
Uterus	28.09	21.05	53.45	72.00	25.23	42.48
Thyroids	15.11	9.03	22.16	21.67	24.49	13.97
Skeletal muscle	3.59	4.90	6.94	9.13	4.55	6.47
Brain	5.56	6.67	25.83	21.88	20.44	17.09

TABLE XIII (Continued)

Tissues and Contents	Control		Low-Mg		Low-Mg, Hi-Prot	
	20.3°C	7.3°C	20.3°C	7.3°C	20.3°C	7.3°C
Bone marrow	17.56	3.68	4.80	6.45	3.91	4.05
Ruminal tissue	5.79	4.76	43.43	45.07	31.82	37.66
Ruminal contents	0.47	0.38	14.00	18.75	9.52	10.30
Abomasal tissue	8.42	10.56	46.59	49.55	43.40	51.72
Abomasal contents	0.95	0.63	21.13	28.09	17.65	28.00
Small intestinal tissue	17.73	13.41	78.95	76.32	50.37	37.11
Small intestinal contents	2.82	5.12	50.55	59.79	42.16	49.50
Large intestinal tissue	6.67	8.67	53.44	39.98	58.27	42.31
Large intestinal contents	1.17	1.32	43.37	34.75	21.18	20.22

[a]Specific activity (calculated) = $\frac{\text{Percent of 28-Mg dose/Kg of fresh tissue}}{\text{mg of Mg/gm of fresh tissue}}$

of the same tissues in magnesium deficient animals were higher at low temperature. In other tissues viz. large intestinal tissue and its contents, and brain, cold exposure resulted in higher specific activity in controls but lower specific activity in magnesium deficient sheep.

Small intestinal tissue and thyroids of sheep showed consistently lower radiomagnesium turnover rate in cold than at room temperature, irrespective of the ration treatments. In contrast, specific activities of sheep abomasal tissue, skeletal muscle, and small intestinal contents were higher in cold than at room temperature.

Total percent 28-magnesium dose retained in blood plasma and packed red cell volume (PCV), and total excretion in urine and feces of individual sheep. Thirty-hour radiomagnesium retention values in blood plasma and packed red cell volume, and radiomagnesium excretion figures are shown in Table XIV. Fifteen minutes after dosing the percent radiomagnesium retained in blood of control sheep was higher in cold exposed control sheep than at room temperature; the difference was greater for PCV than plasma. The blood activity disappeared fast, especially in cold exposed sheep until about two hours after dosing when the values became about equal in both control sheep. Blood retention of radiomagnesium was higher in magnesium deficient sheep than the controls throughout the collection period; the difference

TABLE XIV

RADIOMAGNESIUM DISAPPEARANCE FROM BLOOD PLASMA AND PACKED RED CELLS (PCV), AND CUMULATIVE PERCENT DOSE EXCRETION VALUES IN URINE AND FECES OF SHEEP DURING THIRTY HOURS AFTER INTRAVENOUS DOSING WITH 28-MAGNESIUM

Treatment and Temperature	Material	Hours After Dosing											
		0.25	0.05	1	2	3	4	6	8	10	16	22	30
Control at 20.3°C	Plasma[a]	13.02	10.74	7.46	4.32	2.96	2.24	1.77	1.43	1.24	0.85	0.58	0.43
	PCV[a]	0.55	0.23	0.42	0.42	0.33	0.32	0.14	0.31	0.21	0.26	0.22	0.24
	Urine[b]	--	--	8.99	17.95	22.30	25.04	27.32	28.46	30.10	34.50	39.72	43.56
	Feces[b]	--	--	--	0.02	--	0.11	0.20	0.29	0.49	2.49	3.62	5.20
Control at 7.3°C	Plasma	13.71	10.15	6.88	4.23	2.97	2.39	1.67	1.31	1.11	0.63	0.60	0.37
	PCV	2.55	1.24	0.37	0.38	0.31	0.43	0.16	0.27	0.25	0.31	0.25	0.25
	Urine	--	--	14.73	20.80	24.51	26.80	29.03	31.41	34.03	37.29	38.54	43.83
	Feces	--	--	--	0.01	--	0.04	0.08	0.13	0.22	1.40	2.23	3.48
Low-Mg at 20.3°C	Plasma	15.38	13.30	10.06	5.74	3.86	3.51	2.48	2.23	2.17	1.68	1.51	1.18
	PCV	2.65	0.81	1.35	1.17	0.99	0.59	0.41	0.38	0.61	0.72	0.74	0.79
	Urine	--	--	0.23	1.30	1.72	2.53	3.06	3.54	3.88	4.36	4.37	4.40
	Feces	--	--	--	0.00	--	0.04	--	--	0.08	0.26	0.50	1.83
Low-Mg at 7.3°C	Plasma	16.38	13.65	10.60	6.17	4.49	4.04	3.09	2.37	2.21	1.65	1.70	1.32
	PCV	1.03	0.85	1.55	0.79	0.28	0.80	0.52	0.64	0.51	0.55	0.52	0.58
	Urine	--	--	1.36	1.85	2.12	2.36	2.72	3.02	3.27	3.84	4.13	4.38
	Feces	--	--	--	0.01	--	0.03	0.05	0.09	0.15	0.50	0.82	1.47
Low-Mg, Hi-Prot at 20.3°C	Plasma	17.34	12.35	8.88	5.30	4.48	3.85	2.74	2.38	2.17	1.59	1.27	1.18
	PCV	1.92	1.81	1.42	0.72	0.37	0.61	0.31	0.25	0.62	0.41	0.42	0.61
	Urine	--	--	1.08	1.64	2.58	3.15	3.80	4.19	4.56	6.06	6.80	7.19
	Feces	--	--	--	0.01	--	0.04	0.07	--	0.14	0.64	1.48	1.92
Low-Mg, Hi-Prot at 7.3°C	Plasma	14.87	11.43	7.03	4.93	3.60	3.01	2.29	1.96	1.74	1.42	1.23	1.02
	PCV	2.43	1.68	0.48	0.97	0.55	0.59	0.46	0.52	0.57	0.64	0.59	0.63
	Urine	--	--	2.44	3.34	3.98	4.58	5.13	5.76	6.27	8.57	10.10	10.84
	Feces	--	--	--	--	--	--	0.03	--	0.09	0.15	0.38	1.70

[a]Total cumulative percent dose of 28-Mg/sheep (calculated for total plasma or PCV in one sheep).

[b]Total cumulative percent dose of 28-Mg excreted in feces or urine of one sheep.

being more apparent for plasma than for PCV. PCV-radiomagnesium showed very little variation in percent retention in all sheep, especially after three hours after dosing, perhaps indicating the lack of mobility of intracellular magnesium. In general, PCV-radiomagnesium retention appeared to be lowest during the six hours after dosing and then increased slowly until 30 hours.

Urinary radiomagnesium was a higher percentage of total excretion (urine + feces) in all cold exposed sheep than at room temperature. The urinary to fecal excretion ratio of 28-magnesium was higher in magnesium deficient sheep than in controls because of a high fecal endogenous magnesium value in magnesium deficient animals. In contrast, total percent fecal radiomagnesium excretion in sheep was lower in cold than at room temperature. Among magnesium deficient sheep, those fed the high protein diet had about 80% higher total radiomagnesium excretion than normal protein fed sheep (the difference was 46% at room temperature and 114% in cold).

Plasma thyroxine measurements in sheep. Plasma thyroxine values of sheep during different periods of the experiment are present in Table XV. Initial values, although variable, were higher in all sheep than those at the end of the exposure period, perhaps because of the higher metabolic rate when sheep were placed into the crates. Toward the end of the exposure period plasma thyroxine values

TABLE XV

EFFECT OF DIET AND TEMPERATURE ON THYROXINE VALUES IN SHEEP PLASMA (as ugms of total thyroxine/100 ml)[a]

Treatment	Day of the Experiment: Feeding Period 8	Feeding Period 22	Feeding Period 29	Temperature	Exposure Period 1	Exposure Period 2	Exposure Period 4	Exposure Period 5	Exposure Period 7
Control	6.96	6.58	5.57	Room[b]	7.72	7.34	4.81	4.18	5.44
Control	7.85	5.82	6.84	Cold[c]	8.73	6.83	5.70	5.70	5.57
Low-Mg	10.86	5.57	4.81	Room	6.46	5.70	4.05	3.29	2.91
Low-Mg	5.19	5.82	4.43	Cold	6.08	7.47	6.20	5.70	5.19
Low-Mg, Hi-Prot	4.68	7.21	5.57	Room	6.83	5.70	4.68	4.05	3.67
Low-Mg, Hi-Prot	5.32	5.32	5.19	Cold	8.48	4.18	5.32	4.56	6.46

[a]Total plasma thyroxine was determined by Tetrasorb-kit (Abbott Labs.), and the figures presented have been corrected for extraction efficiency.

[b]Room = Room temperature (20.3±2.2°C).

[c]Cold = Cold temperature (7.3±0.5°C).

were higher in sheep housed in the cold than at room temperature, but differences appeared more apparent in magnesium deficient animals than in the controls.

Calcium and potassium balance in sheep. Six-day balance for calcium and potassium were also calculated and results are presented in Table XVI as percent of intake. Data were not available for the sheep fed the low magnesium-normal protein ration at room temperature because of loss of the urinary catheter as mentioned previously.

Calcium excretion primarily occurred via feces in all sheep. Lower fecal and urinary calcium excretion in the cold exposed control sheep resulted in a 29.3% retention. A very high retention of calcium (41.3%) was seen in sheep housed at room temperature and fed the low magnesium-high protein diet, and was still higher (53.2%) for the cold exposed sheep, suggesting that high dietary protein levels may increase absorption and retention of calcium; dietary magnesium deficiency may also accentuate such action, as some selective absorption pattern exists between calcium and magnesium in the gut (Smith, 1969).

Potassium excretion was mostly urinary. The urinary potassium excretion for low magnesium-high protein diet fed sheep was lower in cold than at room temperature; in contrast, fecal potassium excretion was higher in cold exposed sheep than those maintained at room temperature.

TABLE XVI

CALCIUM AND POTASSIUM BALANCE IN SHEEP
AS PERCENT OF INTAKE

Treatment	°C Temperature	Calcium Excretion			Potassium Excretion		
		Fecal	Urinary	Balance	Fecal	Urinary	Balance
Control	20.3	85.68	7.18	+7.13	17.50	30.49	+52.01
Control	7.3	67.53	3.06	+29.41	17.79	52.02	+30.19
Low-Mg	20.3	--	--	--	--	--	--
Low-Mg	7.3	79.43	8.33	+12.24	35.49	50.83	+13.68
Low-Mg, Hi-Prot	20.3	53.13	5.54	+41.33	13.89	53.56	+32.55
Low-Mg, Hi-Prot	7.3	43.62	3.15	+53.23	26.13	37.96	+35.90

For the control sheep fecal potassium excretion did not appear affected by temperature, but urinary potassium excretion was much higher in sheep exposed to cold than at room temperature. The highest percent potassium retention (52%) among all sheep occurred in the control sheep maintained at room temperature.

Changes in plasma calcium and potassium of sheep. Blood plasma calcium and potassium levels in sheep during different periods are presented in Table XVII. Plasma calcium levels of all sheep remained relatively constant at both temperatures during the experiment, except a slight drop toward the end of the last day. Plasma calcium was slightly higher in magnesium deficient animals than in controls. Similar results were also observed by Colby and Frye (1951) in rats, and McAleese et al. (1961) in lambs. Plasma potassium showed variations from which no conclusions could be drawn.

Calcium and potassium in sheep tissues. No definite conclusions were drawn from the concentrations of calcium and potassium in sheep tissues and digestive tract contents (Tables XVIII and XIX).

Results with Rats

Feed consumption in rats. The feed consumption of rats, calculated as a percentage of body weight for days 1, 3, 5, 7, and 9 of the temperature exposure period are

TABLE XVII

CALCIUM AND POTASSIUM CONCENTRATIONS IN SHEEP BLOOD PLASMA[a]

Dietary Treatment	Temperature	Mineral	Value Before Exposure	Day of Exposure 1	2	3	4	5	6	7
Control	Room[b]	Ca	9.04	9.4	9.1	9.8	10.3	9.3	9.2	8.4
	Cold[c]	Ca	9.68	10.6	10.08	10.0	10.6	10.4	10.3	8.6
	Room	K	28.22	32.9	15.7	27.6	16.3	14.6	18.7	39.1
	Cold	K	27.46	28.7	21.8	27.1	17.8	25.0	24.0	25.0
Low-Mg	Room	Ca	8.41	9.6	9.6	9.5	9.4	9.2	9.9	9.0
	Cold	Ca	10.59	9.4	9.8	10.4	9.8	9.4	9.3	6.3
	Room	K	23.58	18.6	16.7	16.0	14.7	15.6	19.7	19.3
	Cold	K	19.91	20.3	16.7	19.3	18.7	16.7	18.6	22.4
Low-Mg, Hi-Prot	Room	Ca	9.71	9.5	9.8	9.6	10.0	9.4	10.2	9.3
	Cold	Ca	9.29	9.6	9.6	9.4	8.5	9.9	9.4	7.9
	Room	K	22.13	25.7	16.1	19.3	21.8	18.8	15.9	38.3
	Cold	K	19.91	17.4	23.3	18.7	15.2	17.8	15.7	19.3

[a]All values expressed as mg Ca or K/100 ml of blood plasma.

[b]Room = Room temperature (20.3±2.2°C).

[c]Cold = Cold temperature (7.3±0.5°C).

TABLE XVIII

CALCIUM CONCENTRATION IN VARIOUS SHEEP TISSUES AND DIGESTIVE CONTENTS[a]

Tissues and Contents	Control		Low-Mg		Low-Mg, Hi-Prot	
	20.3°C	7.3°C	20.3°C	7.3°C	20.3°C	7.3°C
Femur epiphysis	13.02	12.05	10.64	14.45	14.89	11.94
Femur shaft	23.11	23.41	22.25	19.00	20.01	24.86
Heart	5.7	9.0	4.1	5.4	7.8	4.2
Liver	8.1	6.6	8.2	6.5	8.6	8.9
Spleen	28.3	51.5	8.2	7.9	5.4	9.2
Kidneys	20.4	12.5	28.2	9.7	16.5	9.9
Uterus	9.9	8.5	21.5	8.4	12.6	8.6
Thyroids	8.8	21.3	12.6	14.1	18.4	14.6
Skeletal muscle	3.7	6.2	19.9	10.2	3.7	4.0
Brain	10.6	7.5	22.1	7.3	28.2	28.1

TABLE XVIII (Continued)

Tissues and Contents	Control		Low-Mg		Low-Mg, Hi-Prot	
	20.3°C	7.3°C	20.3°C	7.3°C	20.3°C	7.3°C
Bone marrow	0.3	0.7	0.5	0.2	0.9	0.3
Ruminal tissue	42.5	82.4	34.8	7.5	8.8	22.4
Ruminal contents	31.6	26.4	24.8	17.7	17.8	39.2
Abomasal tissue	29.5	25.1	43.1	--	13.1	13.3
Abomasal contents	45.8	107.5	45.9	31.4	33.8	31.5
Samll intestinal tissue	10.1	14.0	28.5	27.3	8.2	9.8
Small intestinal contents	42.3	23.0	35.4	16.1	21.0	25.0
Large intestinal tissue	30.8	54.4	39.2	--	22.4	203.9
Large intestinal contents	37.1	106.6	142.7	145.9	147.7	--

[a]All values are expressed as mg of Ca/100 gm of fresh tissue, except for femur epiphysis, femur shaft, and bone marrow which are mentioned as percent of the fresh weight of the tissue.

TABLE XIX

POTASSIUM CONCENTRATION IN VARIOUS SHEEP TISSUES AND DIGESTIVE CONTENTS[a]

Tissue and Contents	Control		Low-Mg		Low-Mg, Hi-Prot	
	20.3°C	7.3°C	20.3°C	7.3°C	20.3°C	7.3°C
Femur epiphysis	14.4	11.6	8.0	8.2	5.9	11.3
Femur shaft	3.9	9.3	7.5	8.3	9.0	9.4
Heart	18.8	38.9	27.6	37.4	30.1	31.5
Liver	31.8	27.5	36.7	34.9	40.7	30.5
Spleen	47.3	36.6	32.3	41.7	28.7	45.2
Kidneys	21.8	29.7	20.0	35.0	30.8	29.9
Uterus	24.0	23.7	8.8	32.5	8.3	31.9
Thyroids	13.9	36.7	18.6	6.8	40.5	23.3
Skeletal muscle	341.	42.3	31.5	42.5	39.3	35.8
Brain	36.3	34.5	33.1	37.8	1.5	28.0

TABLE XIX (Continued)

Tissue and Contents	Control		Low-Mg		Low-Mg, Hi-Prot	
	20.3°C	7.3°C	20.3°C	7.3°C	20.3°C	7.3°C
Bone marrow	6.9	6.6	3.2	1.3	1.5	4.0
Ruminal tissue	25.4	22.2	14.7	18.9	28.3	18.4
Ruminal contents	14.7	11.0	14.4	12.2	9.4	9.2
Abomasal tissue	15.2	12.1	11.0	--	15.9	11.5
Abomasal contents	11.4	16.3	13.5	17.2	17.2	14.0
Small intestinal tissue	33.2	20.6	18.5	19.2	20.8	17.5
Small intestinal contents	15.5	17.4	16.6	19.9	17.0	15.6
Large intestinal tissue	22.4	16.4	9.8	--	14.1	12.7
Large intestinal contents	15.1	10.9	11.2	14.2	15.8	--

[a]All values are expressed as mg of K/10 gms of fresh tissue.

presented in Table XX. Feed consumption of cold exposed rats was almost twice ($P<.001$) that of rats at room temperature. This agrees with other investigations (Treadwell et al., 1957; Sanwal, 1972). Dietary effects were greater on days 1, and 9 ($P<.01$) than during the rest of the exposure period. Differences in feed consumption of the various diets at either temperature were more apparent at the beginning of exposure period but became closer as the experiment progressed, suggesting an adaptation process.

Differences in body weight of rats. The actual (total) rat weights for days 1, and 9 are presented in Table XXI. Average body weights of rats fed the various diets did not differ ($P>.1$) before the exposure period. Weight losses of cold exposed rats averaged 7% ($P<.001$) during the nine days whereas rats maintained at room temperature gained 1%. This happened despite a significantly higher feed consumption by rats maintained in cold than at room temperature. The greatest decline in weight occurred in the rats fed the high protein and the high protein-high potassium diets. This agrees with a previous report (Sanwal, 1972).

Magnesium balance in rats. Fecal and urinary magnesium excretion, and balance data for 12 rats are presented in Table XXII. Magnesium excretion was higher in cold exposed rats than in those housed at room temperature for all rats except for rats fed the high potassium and the high protein-high potassium diets. The effect of cold exposure on

TABLE XX

FEED CONSUMPTION BY RATS CALCULATED AS GRAMS OF FEED PER DAY PER 100 GRAM BODY WEIGHT[a]

Dietary Treatment	Day of Exposure at 26.2°C					Day of Exposure at 4.8°C				
	1	3	5	7	9	1	3	5	7	9
Control (low-I)	5.5±.4	4.4±.3	3.8±.2	3.6±.2	3.4±.2	6.7±.8	5.2±.6	5.8±.8	6.4±.8	7.3±.0
Hi-I	3.8±.2	3.6±.2	3.4±.2	3.3±.2	3.3±.2	6.3±.7	5.0±.5	5.8±.6	6.3±.6	6.8±.8
Hi-K	4.1±.2	4.1±.2	4.0±.2	3.5±.4	3.6±.2	8.0±.4	6.2±.4	6.8±.3	6.8±.4	7.7±.5
Hi-Prot	4.9±.2	4.2±.3	3.9±.2	3.6±.2	3.8±.1	7.3±.8	5.2±.3	5.5±.6	6.3±.5	7.2±.0
Hi-Prot, Hi-K	3.8±.4	3.7±.3	3.6±.2	3.4±.3	3.4±.3	6.7±.6	5.2±.9	5.6±.1	5.8±.4	5.7±.0
Hi-Prot, Hi-K, Hi-I	4.7±.8	4.4±.4	4.0±.4	4.0±.3	3.9±.4	8.3±.1	6.1±.5	6.5±.4	6.4±.4	6.9±.4

[a]Each figure represents the average for six rats ± standard deviation.

TABLE XXI

EFFECT OF DIET AND TEMPERATURE ON BODY WEIGHT OF RATS (as grams)[a]

Dietary Treatment	Day of Exposure at 26.2°C		Day of Exposure at 4.8°C	
	1	9	1	9
Control (low-I)	395±21	401±24	406±33	383±24
Hi-I	420±28	423±28	395±47	373±35
Hi-K	392±24	395±25	397±18	371±21
Hi-Prot	408±20	414±20	405±31	374±23
Hi-Prot, Hi-K	373±32	379±30	400±29	364±36
Hi-Prot, Hi-K, Hi-I	389±27	395±27	398±20	380±27

[a]Each figure represents the average for six rats ± standard deviation.

TABLE XXII

TWENTY-FOUR HOUR MAGNESIUM BALANCE IN RATS CALCULATED AS PERCENT OF MAGNESIUM INTAKE[a]

Dietary Treatment	At 26.2°C			At 4.8°C		
	Excretion			Excretion		
	Fecal	Urinary	Balance	Fecal	Urinary	Balance
Control (low-I)	73.2	26.8	0.0	78.0	34.5	-12.5
Hi-I	63.8	36.1	+0.1	152.7	63.8	-116.5
Hi-K	103.9	81.3	-85.2	105.7	50.4	-56.1
Hi-prot	46.2	50.3	+3.5	51.8	47.7	-0.5
Hi-prot, Hi-K	39.2	45.9	+14.9	43.0	38.5	+18.5
Hi-prot, Hi-K, Hi-I	47.7	37.8	+14.5	64.5	39.5	-4.0

[a]Number of rats = one rat per subgroup.

magnesium excretion was especially pronounced when rats were fed high dietary iodine as such or in combination with high levels of potassium and protein. This agrees with a previous study in rats (Sanwal, 1972). Cold exposure reduced the effect of high dietary potassium in increasing magnesium excretion. These observations suggest that in the cold, high dietary iodine in the diet may have an increasing effect on magnesium excretion, and high dietary potassium may aggravate the problem by reducing magnesium absorption when both factors are combined. This lowering effect on magnesium retention was also reflected by reduced magnesium levels in bones. Indications from these data suggest a high magnesium retention effect of high dietary protein in rats, especially when ambient temperatures are not low.

Addition of protein to the diet apparently counteracted the elevated magnesium excretion caused by high dietary iodine. The high potassium diet caused very high magnesium excretion in both feces and urine, but the urinary loss was higher in animals maintained at room temperature than in the cold. Part of the potassium effect on magnesium, especially the increased urinary excretion effect, does not agree with results of House and VanCampen (1971) in wethers.

Magnesium requirements of cold exposed rats appeared to be increased unless the diet was high in protein. According to McAleese and Forbes (1973) increased magnesium loss in cold, as was also observed to some extent in the present

study, increased the dietary magnesium requirements considerably to maintain bone magnesium.

Although apparent magnesium absorption was higher in rats fed the high protein diet, they also appeared to excrete higher magnesium in urine than the controls. Moore et al. (1972) have observed that high levels of nitrogen in ration of ruminants increased urinary output of magnesium, but they did not consider it to interfere with magnesium absorption. Stillings et al. (1964) found a higher availability to wethers of magnesium in high nitrogen forages. Our results with rats indicate that high dietary protein favors magnesium absorption from the gut, however, species differences may exist.

Calcium and potassium balance data. Values in Tables XXIII and XXIV show the balance data of calcium and potassium, respectively, for 12 rats. Rats fed the high iodine diet and exposed to cold excreted more calcium than room temperature rats as was also the case with magnesium. Feces was the major route of calcium excretion, and represented mainly the unabsorbed mineral. Percent urinary excretion of calcium was consistently higher in cold than at room temperature regardless of dietary treatment, however, this did not result in significant differences for plasma calcium levels, as will be discussed later. Rats fed the high-potassium diet showed a negative calcium balance

TABLE XXIII

TWENTY-FOUR HOUR CALCIUM BALANCE IN RATS CALCULATED AS PERCENT OF CALCIUM INTAKE[a]

Dietary Treatment	At 26.2°C			At 4.8°C		
	Excretion			Excretion		
	Fecal	Urinary	Balance	Fecal	Urinary	Balance
Control (Low-I)	98.0	1.4	+0.6	90.9	2.8	+6.3
Hi-I	99.4	2.1	-1.5	166.5	4.9	-71.4
Hi-K	165.0	0.7	-65.7	122.7	4.8	-27.5
Hi-Prot	62.3	1.8	+35.9	61.1	5.3	+33.6
Hi-Prot, Hi-K	100.3	0.7	-1.0	47.8	4.4	+47.8
Hi-Prot, Hi-K, Hi-I	56.7	1.9	+41.4	69.2	5.1	+25.7

[a]Number of rats = one rat per subgroup.

TABLE XXIV

TWENTY-FOUR HOUR POTASSIUM BALANCE IN RATS CALCULATED AS PERCENT OF POTASSIUM INTAKE[a]

Dietary Treatment	At 26.2°C			At 4.8°C		
	Excretion			Excretion		
	Fecal	Urinary	Balance	Fecal	Urinary	Balance
Control (low-I)	7.7	58.1	+34.2	4.4	39.0	+56.6
Hi-I	4.2	52.6	+43.2	6.8	82.6	+10.6
Hi-K	2.1	160.8	-62.9	1.4	92.9	+5.7
Hi-Prot	2.8	108.2	-11.0	1.0	88.9	+10.1
Hi-Prot, Hi-K	7.9	115.1	-23.0	3.5	47.5	+49.0
Hi-Prot, Hi-K, Hi-I	2.5	97.9	-0.4	0.9 0.9	91.4	+7.7

[a]Number of rats = one rat per subgroup.

at both temperatures, but to a lesser degree if exposed to cold than room temperature. Calcium retention was greatly increased by addition of protein to the high potassium diet. Calcium retention by rats fed the high protein-high potassium diet was 47.8% in the cold exposed rats despite a much higher urinary calcium excretion by rats exposed to cold than to room temperature. Temperature did not appear to alter calcium balance in rats fed the high protein diet, but the percent calcium excretion in urine was higher for cold exposed rats than room temperature rats. When high potassium was added to the high protein diet the rats excreted about twice as much calcium at room temperature than in cold.

Calcium balance (availability) was higher in rats fed high protein rations than rats fed the normal protein control ration. This agrees with the work of Sykes and Field (1972) with ewes. Potassium retention was higher in cold exposed rats than in rats at room temperature with the exception of rats fed the high iodine diet.

Plasma magnesium in rats. Table XXV shows plasma concentrations of magnesium calcium and potassium in rats. Temperature effect alone did not alter plasma magnesium levels, but in combination with diets a significant change ($P < .01$) in plasma magnesium levels was observed. Dietary treatments had a significant effect ($P < .001$) on plasma

TABLE XXV

MAGNESIUM, CALCIUM, AND POTASSIUM CONCENTRATIONS IN RAT BLOOD PLASMA (as mg of Mg, Ca, or K/100 ml plasma)[a]

Dietary Treatment	Magnesium		Calcium		Potassium	
	Room[b]	Cold[c]	Room	Cold	Room	Cold
Control (low-I)	2.35±.22	2.84±.20	10.14±.87	11.26±.79	46.9±8.8	48.4±5.7
Hi-I	1.91±.25	2.31±.31	10.00±.55	10.56±.74	49.7±7.2	45.1±2.0
Hi-K	2.03±.42	1.93±.38	10.38±.68	10.14±.28	48.1±7.5	46.7±4.5
Hi-Prot	1.76±.22	1.78±.29	10.62±.38	10.27±.18	46.8±3.8	46.1±5.6
Hi-Prot, Hi-K	2.20±.23	1.86±.28	10.74±.25	10.44±.45	46.6±6.1	41.9±1.1
Hi-Prot, Hi-K, Hi-I	2.27±.24	1.58±.09	10.44±.40	9.89±.30	45.6±6.5	43.1±1.7

[a]Values presented represent the mean for six adult male rats ± standard deviation.

[b]Room temperature = 26.2 ± 1.8°C.

[c]Cold temperature = 4.8 ± 0.3°C.

magnesium concentration.

Plasma magnesium levels were significantly higher in rats exposed to cold than to room temperature in the high-iodine fed rats ($P < .05$) as well as in the control rats ($P < .01$). This finding agrees with that of McAleese and Forbes (1961), who reported blood magnesium to be higher ($P = .02$) at $10^{\circ}C$ than at $23^{\circ}C$ in animals fed a normal diet. Conversely, high protein-high potassium fed rats in this study showed a lower plasma magnesium concentration when exposed to cold ($P < .05$) than to room temperature; and this difference was found to be highly significant ($P < .001$) when high iodine was also added to this ration.

Plasma magnesium levels of rats fed the high iodine diet ($P < .01$) and the high protein diet at room temperature were both lower than controls. Colby and Frye (1951) had also observed lower blood magnesium in high protein fed rats than in controls. Rats maintained at a cold temperature showed a lower magnesium concentration for all dietary treatments ($P < .01 - < .001$) as compared to controls. This was especially noticeable in rats fed a ration high in protein, potassium, and iodine.

Changes in plasma calcium and potassium. Concentrations of plasma calcium and potassium are presented in Table XXV. Temperature itself did not affect plasma

calcium levels in rats; this agrees with the findings of McAleese and Forbes (1961), however, the interaction of diets and ambient temperature had a significant effect on plasma calcium concentration ($P < .01$). No significant differences in plasma calcium for rats fed the various diets were observed at room temperature, but at the cold termperature the high potassium diet ($P < .01$), the high protein diet ($P < .05$), and high dietary levels of protein, potassium, and iodine ($P < .01$) lowered plasma calcium values compared to the control rats. At room temperature, the high protein containing diets slightly raised plasma calcium values compared to the control, in agreement with the data of Colby and Frye (1951).

No significant differences in plasma potassium concentration were observed between dietary treatments, temperatures, or their interactions.

Bone magnesium in rats. Concentrations of magnesium, calcium, and potassium in rat femurs are presented in Table XXVI. Neither dietary treatment nor temperature alone had a significant effect on femur-magnesium concentration, a finding in agreement with those of McAleese and Forbes (1961). However, in this study, the interaction of diets and temperature influenced bone magnesium levels. Control rats exposed to cold had a higher ($P < .01$) femur magnesium concentration than rats maintained at room temperature. In

TABLE XXVI

MAGNESIUM, CALCIUM AND POTASSIUM COMPOSITION OF RAT BONE[a]

Dietary Treatment	Magnesium		Calcium		Potassium	
	Room[b]	Cold[c]	Room	Cold	Room	Cold
Control (low-I)	2.16±.11	2.50±.21	140.5±13.7	146.6±13.6	1.69±.27	1.89±.21
Hi-I	2.29±.11	2.38±.24	170.8±24.3	137.0±13.0	1.30±.15	1.87±.21
Hi-K	2.35±.19	2.46±.18	156.6±20.1	149.3± 6.4	1.44±.16	2.05±.14
Hi-Prot	2.41±.24	2.31±.12	153.9±15.2	149.3±20.7	1.83±.11	1.95±.21
Hi-Prot, Hi-K	2.41±.10	2.34±.19	166.2±29.3	146.3±20.7	1.98±.28	1.77±.21
Hi-Prot, Hi-K, Hi-I	2.48±.17	2.28±.16	147.3± 9.5	144.1±13.6	1.92±.22	1.64±.20

[a]All values are presented as mgs of Mg, Ca, or K/gm of fresh femur, and represent the mean ± standard deviation for six adult male rats.

[b]Room temperature = 26.2 ± 1.80°C.

[c]Cold temperature = 4.8 ± 0.3°C.

rats at room temperature, femur magnesium levels were higher in rats fed the high protein ($P<.05$), high protein-high potassium ($P<.01$), and high protein-high potassium-high iodine ($P<.01$) diets than in the control rats. The rats fed the diet high in protein, potassium, and iodine at low ambient temperature had lower femur magnesium than controls at the same temperature ($P<.1$), or than rats fed the same ration at room temperature. This trend corresponded with plasma levels in rats.

Femur calcium changes. Temperature alone or in combination with various dietary treatments had a significant ($P<.01$) effect on bone calcium levels (Table XXVI). Dietary treatment alone did not show much change. Usually values were lower in cold exposed rats when compared to those maintained at room temperature, except for animals fed the control diet. McAleese and Forbes (1961) found no temperature effect on calcium concentration in rat bone ash.

The high iodine fed rats maintained at room temperature showed a much higher femur calcium concentration than either the control rats at room temperature or the cold exposed high iodine fed rats ($P<.05$). High protein diets did not significantly affect skeletal calcium concentration, in agreement with the results of Sykes and Field (1971) who compared the effects of high and low protein diets on skeletal calcium of ewes.

Potassium in rat femurs. Femur potassium data for rats are presented also in Table XXVI. High iodine diet fed to rats maintained at room temperature caused lower potassium levels than either the control rats at room temperature ($P<.01$), or the high iodine fed rats in the cold ($P<.001$). Potassium in rat femur appeared to show an opposite trend than shown by femur calcium. The high potassium fed rats exposed to cold had greater femur potassium levels than rats at room temperature ($P<.001$). Rats fed the ration high in protein, potassium, and iodine, however, had lower femur potassium concentration in those rats exposed to the cold than to room temperature ($P<.05$).

Magnesium concentration in rat tissues. Magnesium levels in liver, heart, and skeletal muscle of rats are presented in Table XXVII. Cold exposure as a whole increased the magnesium concentrations in liver ($P<.01$) and heart ($P<.05$) but not in skeletal muscle. Although dietary treatments influenced liver magnesium concentration, there was no effect on cardiac or skeletal muscle.

Livers had higher ($P<.05$) magnesium levels in cold exposed rats fed the high potassium and high potassium-high protein diets than in those at room temperature. In cold exposed animals, these diets, especially the high protein-high potassium diet ($P<.05$) also resulted in higher liver magnesium concentrations than the control rats at that

TABLE XXVII

TISSUE MAGNESIUM CONCENTRATION IN RATS[a]
(as mg of Mg/100 gm of fresh tissue)

Dietary Treatment	Tissue	26.2°C	4.8°C
Control (low-I)	Liver	19.06±0.51	19.03±1.73
	Heart	18.52±0.94	18.95±0.88
	Muscle	25.54±1.22	25.33±1.04
Hi-I	Liver	18.88±0.71	18.65±1.00
	Heart	18.79±0.72	18.47±0.63
	Muscle	26.46±1.63	26.23±2.91
Hi-K	Liver	19.29±0.96	20.94±1.48
	Heart	18.87±1.06	19.33±1.84
	Muscle	25.91±1.53	26.86±1.80
Hi-Prot	Liver	18.98±1.10	20.53±2.18
	Heart	18.54±1.06	19.58±0.58
	Muscle	26.34±1.90	25.53±2.31
Hi-Prot, Hi-K	Liver	19.77±0.77	20.76±0.71
	Heart	18.31±0.75	19.08±1.11
	Muscle	25.47±1.63	26.24±1.48
Hi-Prot, Hi-K, Hi-I	Liver	19.39±1.12	20.59±2.24
	Heart	18.06±1.01	19.33±0.78
	Muscle	24.62±2.13	24.94±2.95

[a]Values presented are the mean for six adult male rats ± standard deviation.

temperature.

Soft tissue magnesium concentrations in rats were not significantly changed by the high protein diet alone as was observed in ewes by Sykes and Field (1971). Cardiac muscle magnesium concentrations of rats fed the ration with high protein, potassium and iodine were higher ($P<.05$) in cold than at room temperature. In general, cold exposure of rats fed high potassium diets produced higher magnesium deposition in liver, cardiac muscle, and skeletal muscle than exposure at room temperature, although the differences were not always statistically significant. This increase in tissue magnesium deposition may have been a part of the reason for the decrease in serum magnesium observed by some workers (Suttle and Field, 1961; Kunkel et al., 1953).

Calcium concentration in liver, cardiac muscle, and skeletal muscle of rats. Tissue calcium concentration data for rats are presented in Table XXVIII. In general, exposure to cold had no effect on tissue calcium levels either in cardiac or skeletal muscle of rats. But, temperature itself ($P<.05$), and in combination with some diets ($P<.001$) affected the liver calcium values. High iodine diet fed to cold exposed rats decreased liver calcium levels ($P<.05$) whereas feeding the high potassium diet ($P<.001$), and the high protein diet ($P<.05$) increased the values.

Diets high in both protein and potassium, however, did

TABLE XXVIII

TISSUE CALCIUM CONCENTRATION IN RATS[a]
(as mg of ca/100 gms of fresh tissue)

	Tissue	26.2°C	4.8°C
Control (low-I)	Liver	5.84±2.28	5.69±0.39
	Heart	6.07±0.18	5.84±0.98
	Muscle	7.02±0.94	6.42±1.11
Hi-I	Liver	6.16±0.79	5.12±0.75
	Heart	6.45±0.87	6.09±1.11
	Muscle	6.33±1.10	6.34±0.49
Hi-K	Liver	4.53±0.40	7.56±1.56
	Heart	6.73±0.82	7.60±0.60
	Muscle	5.91±0.92	6.29±0.56
Hi-Prot	Liver	4.78±0.96	6.49±1.52
	Heart	6.47±0.66	5.71±2.57
	Muscle	6.37±1.81	4.95±0.82
Hi-Prot, Hi-K	Liver	5.95±0.82	5.77±1.14
	Heart	6.11±0.98	5.93±1.68
	Muscle	6.46±1.02	6.78±0.38
Hi-Prot, Hi-K, H-I	Liver	6.13±1.11	6.23±0.57
	Heart	6.39±1.19	7.42±1.27
	Muscle	6.64±1.04	6.41±0.30

[a]Values presented are the mean for six adult male rats ± standard deviation.

not appear to show such a change. Cold exposed rats fed the high potassium ration had higher ($P < .05$) liver calcium levels than the controls at the same temperature. Within cold exposed rats, cardiac calcium content was higher than control when diets high in potassium ($P < .01$) or protein, potassium and iodine ($P < .05$) were fed. Skeletal muscle calcium level of cold exposed rats was also increased ($P < .05$) over control by the high protein-high potassium ration.

Potassium concentration in rat tissues. Rat tissue potassium concentrations are presented in Table XXIX. Liver potassium concentration was lower ($P < .01$) in cold than at room temperature for rats fed either the control diet or the diet high in protein and potassium.

No marked effect of temperature on skeletal muscle potassium was noticed, but there were differences within each temperature group. Within room temperature, muscle potassium levels were higher in rats fed the high protein-high potassium ration ($P < .01$), and also in rats fed the ration with high protein, potassium and iodine ($P < .05$) than the control diet fed rats. Within low temperature, the high iodine ($P < .05$), the high potassium ($P < .05$), and the high protein-high potassium-high iodine ($P < .05$) diets produced significantly higher skeletal muscle potassium than in control rats. No marked differences due to diets or

TABLE XXIX

TISSUE POTASSIUM CONCENTRATION IN RATS[a]
(as mg of K/gm of fresh tissue)

Dietary Treatment	Tissue	26.2°C	4.8°C
Control (low-I)	Liver	3.65±0.13	3.36±0.15
	Heart	3.28±0.25	3.03±0.32
	Muscle	4.51±0.26	4.11±0.67
Hi-I	Liver	3.47±0.13	3.43±0.10
	Heart	3.26±0.18	3.07±0.28
	Muscle	4.81±0.40	5.33±0.88
Hi-K	Liver	3.58±0.28	3.75±0.25
	Heart	3.14±0.35	2.96±0.38
	Muscle	5.07±0.88	5.69±1.02
Hi-Prot	Liver	3.62±0.28	3.42±0.39
	Heart	3.17±0.24	3.10±0.09
	Muscle	5.11±0.73	4.97±0.67
Hi-Prot, Hi-K	Liver	3.84±0.17	3.49±0.21
	Heart	3.29±0.38	3.10±0.22
	Muscle	5.42±0.51	4.76±0.50
Hi-Prot, Hi-K, Hi-I	Liver	3.75±0.26	3.57±0.31
	Heart	3.15±0.42	2.84±0.12
	Muscle	5.28±0.65	5.28±0.64

[a]Values presented are the mean for six rats ± standard deviation.

temperatures were observed for the cardiac potassium concentration. Soft tissue potassium levels in rats were not increased by high protein diets, as has been reported by Sykes and Field (1971) in ewes.

Plasma thyroxine determination in rats. Average plasma thyroxine measured in two pooled samples of three rats from each dietary group at two temperatures is presented in Table XXX. For the control diet (low-iodine) fed rats, the plasma thyroxine value appeared to be higher for the rats housed in cold than at room temperature. In contrast, the values appeared to be consistently lower in cold exposed rats than for room temperature for all the rest of the dietary treatments. Perhaps this is due to a faster rate of iodine utilization, metabolism and excretion in cold exposed rats, as compared to a relatively low iodine availability or thyroxine synthesis. The exact cause or mechanism of this action has not been understood. At room temperature, the highest thyroxine values were obtained in rats fed high levels of protein, potassium, and iodine combined; this was not seen in cold exposed rats.

TABLE XXX

TOTAL BLOOD PLASMA THYROXINE CONCENTRATIONS IN RATS[a]
(as ugms of total thyroxine/100 ml plasma)

Dietary Treatment	Total Plasma Thyroxine	
	26.2°C	4.8°C
Control (low-I)	6.11[b]	6.42
Hi-I	5.78	4.63
Hi-K	6.30	4.07
Hi-Prot	5.80	4.02
Hi-Prot, Hi-K	6.43	4.30
Hi-Prot, Hi-K, Hi-I	6.73	4.60

[a]Values presented are average of two pooled plasma samples for three adult male rats each.

[b]Values presented are means of two determinations for each pooled sample.

CHAPTER V

SUMMARY

The etiology of hypomagnesemic tetany is still not clear because of several biological interactions involved. Several dietary, bodily and environmental stresses together appear to contribute to the disease. Several of these factors have been investigated, but much is yet to be studied. In the present investigation the effects of low ambient temperatures on magnesium status have been studied in relation to some dietary factors using six ewe lambs and 72 male rats as experimental animals. The sheep divided into a 3 x 2 factorial arrangement of treatments were fed one of the following rations: (1) Control; (2) Low magnesium ration; (3) Low magnesium-high protein ration. After 30 days of feeding they were maintained for seven days at temperatures of either 20.3°C or 7.3°C. Each sheep was dosed intravenously with radiomagnesium the day before the end of the experiment. In the case of rats, 72 adult male rats were divided into 6 x 2 factorial arrangement of treatments and fed one of the following six diets: (1) Control (low-iodine); (2) High iodine; (3) High potassium; (4) High protein; (5) High potassium-high protein; (6) High potassium-high protein-high iodine, and after four weeks of dietary adaptation were maintained at either 26.2°C or at 4.8°C ambient temperature for 11 days. All animals were sacrificed

at the end of experimental period. Analysis of magnesium, calcium and potassium in rat materials, and also of radiomagnesium in case of sheep materials was conducted for balance and tissue ditribution studies of the minerals.

Results with sheep indicated that percentage absorption of magnesium was higher in low magnesium fed sheep than normal magnesium fed controls. High dietary protein (16%) in addition to low magnesium in the ration further increased true magnesium digestibility over normal protein (12%) fed sheep. The percentage of magnesium retained was higher for sheep fed the high protein ration and exposed to cold than sheep housed at room temperature. A higher percent endogenous fecal magnesium excretion and lower urinary magnesium excretion appeared to occur in low magnesium diet fed sheep in comparison to those fed normal levels of magnesium, especially at the low ambient temperatures. While stable magnesium balance figures showed a lower percent urinary magnesium excretion in high protein fed sheep in cold than at room temperature, the radiomagnesium excretion was higher in cold in these sheep than at room temperature. Total radiomagnesium excretion was 80% higher in low magnesium,-16% protein fed sheep than for low magnesium, -12% protein fed sheep. Blood and tissues of magnesium deficient sheep appeared to retain more radiomagnesium 30 hours after intravenous dosing than controls, and in general, retention was

higher in animals exposed to cold than at room temperature in femur epiphysis, cardiac muscle, skeletal muscle, liver, spleen and some other tissues. There were some indications that a higher magnesium absorption, muscle deposition, and urinary excretion occurred in animals exposed to the cold than those at room temperature; but bone magnesium concentrations appeared to be lowered in cold. For a large number of sheep tissues the turnover rate of magnesium was higher in magnesium deficient sheep than controls. Generally, the turnover rate of magnesium was higher in cardiac muscle, liver, heart, spleen, kidneys, small intestine, uterus and thyroids, and was lower in bone, skeletal muscle, brain, bone marrow and blood plasma. Very high retention of calcium (41.3%) by sheep was noticed when ration was high in protein, and the figure was still higher (53.2%) when ambient temperatures were also low.

Rats lost about 7% of the body weight in cold compared to about 1% gain by room temperature animals, even though rats in cold had consumed twice as much ($P < .001$) feed than at room temperature, indicating a decreased feed efficiency in cold. With the exception of some high potassium diets, rats excreted more magnesium in cold than at room temperature; the urinary magnesium excretion was accelerated by high dietary iodine. High dietary protein counteracted the high magnesium excretion in rats, especially when the temperatures were not low. High potassium alone

in the diet increased urinary magnesium excretion.

Rats fed diets high in potassium and protein both showed significantly lower ($P < .05 - < .001$) plasma magnesium levels in cold than at room temperature. Bone magnesium levels were higher ($P < .01$) in cold exposed animals than those at room temperature fed the control diets, while a lower bone magnesium concentration ($P < .05 - < .01$) was found in animals fed a high protein diet in comparison with those fed normal protein diet. More magnesium appeared to be deposited as a whole in cold in liver ($P < .01$) and heart ($P < .05$) but no change in skeletal muscle magnesium concentrations was observed. In general, a higher magnesium deposition was seen in liver, heart, and muscle of rats fed high potassium diets in cold than at room temperature (although not always significant).

Magnesium requirements of rats appeared to be increased in cold unless the diet was high in protein. Calcium availability in rats was also found to be higher for animals consuming high protein containing diets than control rations. Several other less important changes in magnesium status by low temperature and particular dietary combinations were observed in both rats and sheep.

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