THE RELATIONSHIP OF MAGNESIUM AMMONIUM PHOSPHATE TO FROTH PRODUCTION IN RUMINANT BLOAT

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THE RELATIONSHIP OF MAGNESIUM AMMONIUM PHOSPHATE TO FROTH PRODUCTION IN RUMINANT BLOAT

By

RICHARD A. PHELPS

AN ABSTRACT

Submitted to the College of Agriculture of Michigan
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A colorless, crystalline material spontaneously formed and artificially precipitated from rumen fluid was identified by physical and chemical analyses as mainly hexahydrate magnesium ammonium phosphate.

The hexahydrate magnesium ammonium phosphate-containing precipitate was quantitatively determined in rumen ingesta obtained from nine rumen-fistulated dairy cows or steers fed a total of 13 nonfroth- or froth-producing rations. The amount of precipitate formed was found to be influenced by the rumen fluid hydrogen ion concentration. The quantity of precipitate formed per unit of hydrogen ions varied with the rations fed. The magnesium and phosphorus intakes of the animals appeared to influence the amount of magnesium ammonium phosphate formed only when a specific concentration of hydrogen ions was present in rumen fluid.

Frothy rumen ingesta exhibited an average of three times more magnesium ammonium phosphate-containing precipitate and one and four-tenths times more hydrogen ions than nonfrothy ingesta.

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THE RELATIONSHIP OF MAGNESIUM AMMONIUM PHOSPHATE TO FROTH PRODUCTION IN RUMINANT BLOAT

INTRODUCTION

The riddle of ruminant bloat has plagued husbandrymen for hundreds of years. Until recent times, the incidence of this malady has apparently surpassed research on the etiology of bloat.

Recently, however, several practices have been introduced which at least temporarily prevent some forms of bloat. Moreover, additional similarities between the various types of bloat have been conclusively demonstrated. Important in this regard is the indisputable evidence that froth production is an associated factor in cases of both pasture and feed-lot types of ruminant bloat. While this is a significant contribution, the basic cause or causes of this malady have not been infallibly elucidated.

The vast amount of research on other cattle disorders may well contribute to an understanding of ruminant bloat. Thus, it is particularly important to study results obtained from research on "grass tetany", or hypomagnesemia. This malady may occur under conditions of relatively low magnesium intake combined with high ruminal ammonia production. Conversely, ruminant bloat may occur under conditions of relatively high magnesium intake and variable rumen ammonia production.

Preliminary data has indicated the possible relationship of an unidentified crystalline material in rumen fluid to froth production. The purpose of this study was to (1) identify the crystalline material, (2) determine the relationship of the material to the hydrogen ion concentration of the rumen, and (3) measure the relative concentration of the crystalline material in the rumen ingesta of cattle fed froth- and nonfroth-producing rations.

REVIEW OF LITERATURE

Introduction

The role of froth in ruminant bloat has been reviewed or noted by several workers. There has been no previous report of magnesium ammonium phosphate in rumen ingesta, but it has been found in association with other bacterial fermentations. This review is therefore concerned with: (1) literature pertaining directly to magnesium ammonium phosphate and (2) studies bearing upon magnesium, ammonia, phosphate, and pH in bloated and nonbloated ruminants.

Magnesium Ammonium Phosphate

General Properties. Magnesium ammonium phosphate usually exists in the hydrous form with the hexahydrate compound predominating over the monohydrate (Dana, 1884). The hexahydrate form was designated struvite in honor of V. Struve, a Russian statesman, and guanite by Teschemacher (Mellor, 1923). It is a colorless to yellow crystalline compound and may be microscopically identified by immersion in xylene or toluene (Purcell, 1922). Struvite rapidly loses water and ammonia upon heating (Mellor, 1923) and exhibits such properties as triboluminescence (Tomozi, 1939), pyroelectricity and piezoelectricity (Palache, 1945). It exhibits a specific infrared spectrum (Corbridge, 1954).

Magnesium ammonium phosphate is formed whenever a neutral magnesium salt solution is brought in contact with a solution containing ammonia and phosphoric acid (Mellor, 1923). The compound is soluble in acids and very slightly soluble in water (Dana, 1884). Ammonium oxalate and molybdate exert a greater solubilizing effect than the ammonium salts of hydrochloric, nitric and sulfuric acids or the sodium and potassium salts of hydrochloric and sulfuric acids. However, small quantities of ammonium hydroxide greatly reduce the solubility of struvite (Malyarov, 1934; Uncles, 1946).

Magnesium ammonium phosphate inhibits the decomposition of hydrogen peroxide except when a specific level of cobalt ions is maintained (Krause, 1955). The mechanism of inhibition was not reported.

Natural Occurrence of Struvite. Hexahydrate magnesium ammonium phosphate was first found in nature in a deposit of cattle manure in Germany (Dana, 1884). It has also been found in a bed of peat underlying deposits of organic matter (Palache, 1945), in bird and bat guano (Mellor, 1923), in urine (Rodillon, 1914; Cavazzani, 1919; Mellor, 1923; Schwartze, 1923), in extracts from fossil plants (Mellor, 1923), in intestinal concretions (Mellor, 1923; Hutton, 1945), in prostate glands (Frondel, 1946), in licorice extracts (Jermstad, 1927), with newberyite in the tooth of a Yukon mammoth (Palache, 1945), and in human lungs (Hutton, 1945). Struvite has been numerously reported in urinary calculi of several species including rats, mink and sheep (Milks, 1935; Johnson, 1940; Benjamin, 1945; Sompolinsky, 1950; Vermeulen, 1951;

Leoschke, 1952; and Leoschke, 1954) and in human salivary calculi (Bartels, 1950). The urinary calculi in mink appear to be more numerous in the females in the spring and in the males in the summer. The calculi are also more numerous among mink in the North Central States than in the Pacific Coast States and may be related to diet. Where the incidence of calculi is high a large proportion of the mink diet consists of horsemeat (Leoschke, 1952). There is a reported lower incidence of urinary calculi on mink ranches feeding high levels of fish (Leoschke, 1954). The formation of magnesium ammonium phosphate urinary calculi in mink can be prevented by the addition to the mink's diet of one gram of ammonium chloride per animal per day (*Ibid.*)

The formation of struvite has also been a problem in the canned fish industry (Purcell, 1922; Merwin, 1942; Hollett, 1942; Kreidl, 1951; Dreosti, 1949).

Hexahydrate magnesium ammonium phosphate is associated with many organisms. It has been formed around diplococci in urine (Rodillon, 1914); in colonies of *Actinomyces* (Bartels, 1950; Bartels, 1952) and various unspecified bacteria (Sierakowski, 1940; Chistyakov, 1952; Barmenkov, 1953); in cultures of *Pseudomonas* (Fredericq, 1946a; Fredericq, 1946b), *Brucella sp.* (Huddleson, 1927), and *Staphylococcus sp.* (Milks, 1935; Kalina, 1952); and associated with staphylococcal or proteus infections (Sompolinsky, 1950). The compound has also been found in cultures of typhoid, paratyphoid and Flexner's dysentery bacillus (Kalina, 1952); and in *Bacillus alcaligenes*, *Neisseria catarrhalis* and *Corynebacterium diphtheriae* cultures (Scudder, 1928).

Magnesium ammonium phosphate has also been reported in samples of tuberculin and erysipelas antitoxin where formation of the crystals was traced to contaminating ammonia-forming organisms (Babich, 1954). It has also been reported in psychrophile cultures from Lvov soils; the authors state that the parallelism in seasonal variations of soil phosphate and psychrophile cell counts in the soil are evidence that the observed crystallization is part of the nitrogen, phosphorus, and magnesium cycles in plant nutrition (Chistyakov, 1952).

Various workers have reported that magnesium ammonium phosphate is the best source of nitrogen tested for the production of soil organisms (Bierema, 1909; Wilson, 1937).

The compound has been reported to be effective in complexing ferrous ions to prevent ferrous ion inhibition in the production of penicillin (Ida, 1950).

Hypomagnesemia and Grass Tetany

The lack of reports concerning magnesium ammonium phosphate in the rumina of cattle can be attributed to the difficulty in detecting this compound, which would be soluble, and presumably ionic, in the pH range of most rumina. However, many studies of magnesium-ammonia interactions, with or without phosphorus, have been reported with cattle. These studies involve for the most part a hypomagnesemic condition, as in "grass tetany", "grass staggers" or "wheat pasture poisoning".

Occurrence of Grass Tetany and Similar Disorders. Sjollema (1930) reported a high incidence of grass staggers in sandy soil districts where intensive cattle farming had been practiced. Attacks of the disorder occurred directly after the cows had left the barn, thus excluding grass as the predisposing factor. In 1936, Mulhearn reported that grass tetany cases usually occurred within a month after calving when cows were in heavy milk production. The attacks were reported to coincide, usually, with heat periods and were associated with more or less continuous feeding on rapidly growing roughage. The animals had usually been grazing pasture for two or four weeks before the first cases occurred. It was emphasized that cattle in poor condition are rarely affected. Nolan and Hull (1941) reported several cases of grass tetany when the hypomagnesemic condition occurred during the winter in cattle fed mainly poor-quality hay. Allcroft (1947) reported that only one of 144 cases of hypomagnesemia was associated with turning out to pasture. Kemp and 't Hart (1957) reported that the number of grass tetany cases decreased as the weather became warmer. In general there was little or no further occurrence of grass tetany when the mean 24-hour temperature per decade in the spring had risen to about 14°C. When the temperature fell below this level in the autumn, there was a further outbreak of the disease. The authors concluded that, consequently, the longer it takes for the temperature to rise above a level of about 14°C, the longer is the duration of the spring tetany period. They also noted that a high degree of precipitation in the summer and autumn is accompanied by a greater number of tetany cases in these periods.

Hypomagnesemia With Tetany. The reports relating low blood levels of magnesium to the symptoms of tetany have not always been in agreement. Sjollema (1930) reported that both serum and urinary magnesium of cattle were depressed in cases of grass staggers. Nolan and Hull (1941) reported 12 cases of hypomagnesemia in cattle accompanied by clinical symptoms of grass tetany. The depression of magnesium was accompanied by a drop in serum inorganic phosphorus. Bartlett *et al*.(1954) reported that all cattle showing tetany in their experiment had low serum magnesium values; however, one cow displayed an abnormally low level of 0.5 to 0.6 mg.% for more than two weeks without displaying tetany. A similar depression of serum magnesium without evidence of tetany has been reported by Allcroft and Green (1938) and Allcroft (1947).

Seasonal Trend of Serum Magnesium. The prevalence of tetany in the spring, and occasionally in other seasons of the year, necessitated a study of the seasonal trend of serum magnesium. Allcroft and Green (1938) reported high values for serum magnesium in the summer and low values in the spring and winter. The trend was not always evident and clinical symptoms of tetany were more likely to appear when the fall of blood magnesium was sudden. Eveleth *et al*. (1940) noted peak values of blood serum magnesium in dairy cattle during the month of July when the animals had been grazing dry pasture grass. The serum magnesium fell when increased rainfall caused a regrowth of the pasture. The occurrence of hypomagnesemic tetany in the winter when cattle were receiving winter rations of poor-quality hay has been reported (Nolan and Hull, 1941). Allcroft (1947) reported that the lowest serum magnesium in cattle occurred from December to April and the highest level in July, but the finding was not valid with all cattle. The high magnesium values coincided with periods of low rainfall and little wind while the low values coincided with periods of adverse weather and low temperature. Thus the highest incidence of grass tetany and the lowest values of serum magnesium are apparently coincidental with periods of low temperature and adverse weather (Kemp and 't Hart, 1957). Head and Rook (1955) stated that the highest incidence of hypomagnesemia in dairy cattle, as well as decreased urinary magnesium values, occurred in the first few weeks of the spring grazing which followed the winter stall feeding period. Stewart and Reith (1956a) also reported low magnesium values for cattle in April and progressively higher values from June to July. Bartlett et al. (1957b) reported that some cattle showed little or no change in serum magnesium when changed from winter feeding to grazing, irrespective of fertilizer treatment of the swards grazed. In the majority of cattle, however, there was a distinct fall in the serum magnesium, sometimes within 24 hours of the beginning of grazing. If the serum magnesium fall was not acute and the cattle were kept on pasture, there was usually a spontaneous recovery of the magnesium level after two to three weeks of grazing. The authors concluded that a high serum magnesium level during the period of winter feeding is indicative of a reserve of magnesium in the body which can for a few days prevent the onset of hypomagnesemia. Bartlett and Balch (1957a) reported depressed serum magnesium values during spring grazing, with some isolated cases of tetany.

Effect of Ration on Hypomagnesemia and Tetany. Since not all cases of hypomagnesemia are associated with grazing young herbage, it is important to review alterations of the serum magnesium level by other feeds. Sjollema (1930) reported that hypomagnesemia could result from feeding high-protein rations during winter months. Eveleth et al.(1940) reported that attempts to raise low levels of serum magnesium with dry feed and hay were unsuccessful. Noland and Hull (1941), as previously reported, observed grass tetany when cattle were fed a winter ration consisting mainly of poor-quality hay. Allcroft (1947) reported that the feeding of magnesium oxide alleviated the severe fall of serum magnesium which usually occurred when cattle were grazed on spring pasture. He also reported that the usual winter depression of serum magnesium in cattle was prevented by feeding 15 to 20 pounds of hay and 60 to 90 pounds of cabbage. Allcroft considered the cabbage to be the more important factor since calves which did not receive cabbage did not show the same rise in serum magnesium. Cabbage has been reported to produce cattle bloat (Johns, 1956). Head and Rook (1955) stated that the magnesium intake during periods of hypomagnesemia associated with spring grazing is adequate, and that it is generally concluded hypomagnesemia does not arise from an inadequate intake of magnesium. Bartlett et al. (1957b) reported that supplementation with feeds containing magnesium failed to prevent the onset or reduce the severity of hypomagnesemia. Kemp and 't Hart (1957) reported

that the magnesium content of pastures with tetany cases was about ten percent lower than in pastures without cases of tetany. Line (1957) reported that a mixture of two parts linseed cake and one part dredge corn, a mixture of barley, oats, and wheat, prevented the fall of serum magnesium. Rook *et al.*(1957) observed that young leafy herbage produced a higher incidence of hypomagnesemia than more mature herbage even though the magnesium content was similar. The authors attributed the decreased hypomagnesemia to a greater consumption of the mature herbage. They further observed that the addition of starch to the rumen reduced the severity of hypomagnesemia. Head and Rook (1957) reported a reduction of rumen liquor ammonia following starch addition. Brouwer (1952) noted that grass from tetany-producing pastures was very high in potassium. Kunkel *et al.* (1953) reported a decrease of serum magnesium in sheep within 12 days by the inclusion of five percent potassium in the diet. Serum magnesium was also reduced when milo gluten meal replaced soybean meal in the diet. Bartlett *et al*. (1954) reported that cows grazed on a nitrogen fertilized plot and supplemented with concentrates did not display the severe fall in serum magnesium shown by cows not receiving supplementation. None of the concentrate supplemented animals displayed clinical symptoms whereas five of eight cows in the nonsupplemented group showed a very rapid fall in serum magnesium, and four of them displayed typical tetany symptoms. The fifth animal showed no tetany even though the serum magnesium remained at a low level for more than two weeks.

Effect of Pasture Fertilization on Hypomagnesemia and Tetany. Stewart and Reith (1956) observed that fertilization with ground limestone or ground magnesium limestone increased the magnesium content of the herbage and decreased the incidence of hypomagnesemia. However, they stated, "Thus whilst the magnesium content of herbage was still low there was some degree of recovery to a normal level of blood magnesium. This is further proof that it is not primarily the magnesium content of the diet which causes the hypomagnesemia or the upset of magnesium metabolism." Bartlett *et al.* (1957b) concluded that hypomagnesemia in cattle grazing pasture was not dependent on a specific fertilizer application to the pasture. However, they observed the most severe depression of serum magnesium in cattle which had grazed plots dressed with ammonium sulfate. They observed that invariably there was a rise in blood ammonia concentration in cattle which had grazed nitrogen fertilized plots. The extent of the rise was related to the severity of hypomagnesemia except from plots fertilized with magnesite. Bartlett and Balch (1957a) reported that over a period from October, 1956, to September, 1957, cattle grazing a grassland area to which magnesium fertilizer had been applied had a mean blood serum magnesium level of less than 2 mg.% much of the time. The authors concluded, "Thus, there is as yet no indication that the light dressing of magnesium in the fertilizer has been beneficial in the prevention of hypomagnesaemia." Bartlett *et al.* (1954) reported that three of four cows on a plot fertilized with a commercial grade of magnesium oxide plus ammonium sulfate showed no hypomagnesemia up to 20 days after the beginning of grazing. The animal that did show low blood magnesium was in no way abnormal in appearance and behavior even though the serum magnesium level decreased to 0.5 mg.%. The serum level was also low in cows grazing a nitrogen fertilized plot; however, the depression was partially prevented if the animals received supplementary concentrates.

The lack of tetany in many cases of hypomagnesemia may be partially explained by the studies of Salt (1950). He observed that dairy cattle were the only species of six studied, including the rabbit, chicken, swine, sheep and human, that yielded higher values of cell than blood serum magnesium. He found a steady fall in corpuscular values following the fall in serum magnesium. The author concluded that this behavior can be best explained by assuming that the level of magnesium in the cell is determined by that of the plasma at the time the erythrocyte is produced in the bone marrow, but that once the cell is cast into the circulation it maintains its mature value unchanged throughout the remainder of its life. In the case of grass tetany the serum magnesium falls rapidly, but if the decline occurs over a long period of time the cell magnesium will fall also. The author concluded that the serum and cell magnesium act independently.

The Relationship of Rumen Ammonia to Hypomagnesemia. It has been repeatedly observed that cattle display hypomagnesemia after ingesting large amounts of protein (Sjollema, 1930; Mulhearn, 1936; Head and Rook, 1955). Therefore it became important to determine rumen ammonia levels at the time the magnesium disorders occurred. Head and Rook (1955) found that the excretion of magnesium in the urine of the cow is markedly

and rapidly reduced with the change to spring grass. The authors postulated that if such a fall in the urinary excretion of magnesium reflects a reduced intestinal absorption it would indicate that for ruminants the magnesium of spring grass has an unusually low availability. Head and Rook observed that the level of ammonia in the rumen increased from about 15 mg.% to 50 mg.% when cattle were changed from winter stall feeding to pasture. The high ammonia production from grass was also reflected by the presence of ammonia in the jugular blood, which indicated that the capacity of the liver for conversion of portal blood ammonia had been exceeded. An attempt was made to influence the absorption of magnesium in cattle receiving hay and a concentrate ration by the addition to the rumen, via a fistula, of ammonium acetate or ammonium carbonate. Ruminal ammonia levels comparable to those observed on grass were produced and a similar reduction of urinary and serum magnesium was observed. Rook _et al._ (1957) later reported that the addition of the ammonium salts decreased urinary excretion of magnesium and increased the excretion of the element in the feces. Hypomagnesemia could also be produced by adding potassium chloride to the rumen. The authors stated, "These results show that a high concentration of ammonia or potassium within the rumen leads to an interference with magnesium absorption, and that, when the intake of magnesium is low, this interference leads to the development of hypomagnesemia. Moreover, the change in the pattern of magnesium metabolism following the additions is identical with that observed on changing from winter feed to cut grass."

Concentration of Other Elements in Blood During Outbreaks of Hypomagnesemia. The levels of blood constituents other than magnesium may be altered in outbreaks of hypomagnesemia. Sjollema (1930)

reported that serum potassium levels were normal but that the calcium: phosphate ratio was altered. The author stated that the latter abnormality may have been due to the high phosphorus content of the protein-rich feeds. He pointed out that a much higher content of urinary phosphoric acid had been found in cattle suffering from tetany. Nolan and Hull (1941) reported a terminal drop in the serum inorganic phosphorus from 5.5 mg.% to 3.4 mg.% in 11 cases of hypomagnesemic grass tetany. Allcroft (1947) reported that more than 75% of 144 cases of hypomagnesemia were associated with hypocalcemia. Bartlett et al. (1957b) reported that the most significant change in the composition of the nitrogenous fraction of the blood, following the commencement of grazing, was the rise in serum nonprotein nitrogen and urea nitrogen, the latter wholly accounting for the rise of the former. The development of hypomagnesemia resulted in no significant changes in serum concentrations of amino nitrogen, uric acid, creatinine, protein, calcium, sodium, potassium, glucose or serum pH.

Herbage Levels of Various Elements During Outbreaks of Hypomagnesemia. Kemp and 't Hart (1957) reported that herbage from grass tetany producing pastures had a lower proportion of magnesium, sodium and calcium, and a higher mean content of potassium and phosphorus, than herbage from pastures where no tetany was observed. The ratio of potassium to the other cations was appreciably higher in pastures where tetany resulted. Statistical analyses revealed that a high potassium and low calcium content of the pastures favored the occurrence of grass tetany. No significant correlation was found with the contents of crude protein, magnesium, phosphorus, chlorine and sulfur. However, the magnesium content of pastures with tetany cases was about ten percent lower than in pastures without cases of tetany. The authors reported a sharp rise of tetany cases as the potassium to calcium plus magnesium ratio (K:Ca+Mg) increased. Since Rook et al. (1957) have reported a higher incidence of hypomagnesemia with cattle grazing young rather than more mature herbage from the same plot, it is important to note the magnesium content of the forage. They found that the content of this element in the forage, the dry matter percentage, and the availability of the element were similar at both stages of growth. However, the voluntary consumption of the cut mature herbage was some 25% greater, in terms of dry matter. The authors commented, "The spontaneous and progressive recovery in serum magnesium levels which follows the initial fall at the commencement of grazing may thus be dependent to some extent on an increase in the voluntary consumption of herbage dry matter, and consequently of magnesium, as herbage matures. Any change in the availability of herbage magnesium due to the fall in nitrogen and potassium content of herbage as it matures would also play its part. From results of experiments now completed, it has been concluded that intake of magnesium as determined by the magnesium content and the consumption of herbage, is an important factor in the production of hypomagnesaemia." Sjollema (1952) attributed grass tetany to deficiencies of sodium and excessive intakes of potassium and nitrogen by cattle. He also attributed tetany to a disturbed metabolism of phosphorus and cited Norwegian experiments that supported his

conclusion. Brouwer (1952) observed that grass from tetany-producing pastures was very high in potassium. He noted that two heavy dressings of a potassium salt not only increased the potassium content of the grass but also decreased the contents of calcium and magnesium. This condition, according to the author, is conducive to the outbreak of grass tetany.

Magnesium Content of Forage and Rumen Fluid and the Possible Relationship to Bloat

Garton (1951) reported the level of soluble magnesium in the rumen of living fistulated sheep to be 7 to 13 mg.% when fed chopped meadow hay, and 11 to 20 mg.% when fed a ration of chopped meadow hay, linseed oil cake, and oats. The rumen fluid samples were obtained 2 to 12 hours after feeding. It was observed that the soluble magnesium in the rumen decreased with an increase in time after feeding. The level in the rumina of slaughtered sheep which had grazed rye grass and clover pastured averaged 6 to 12 mg.%. The soluble magnesium level decreased to 2 to 5 mg.% in slaughtered sheep which had been fed a ration of oats, chopped oat straw, ground corn, and blood meal. Slaughtered sheep which had been fed chopped meadow hay had a soluble magnesium level of 10 mg.% in the rumen liquor. Garton believed that since the soluble magnesium values found in the ruminal liquor of hay-fed sheep are higher than those reported for saliva, the increase can be accounted for by the simple solution of these ions from the food. He demonstrated this solution of ions with an *in vitro* experiment. The addition of sheep gastric juice to rumen contents evoked a slight liberation of magnesium from rumen contents.

Garner (1949) concluded from *in vitro* experiments that ruminal organisms play a significant part in the liberation of magnesium from plant cells. In a later report (1950) he stated that 63 to 75% of the magnesium retained is apparently not dependent upon the magnesium content, the calcium to phosphorus ratio or the crude fiber content of the feed.

Lakke Gowda *et al*. (1956) reported that with ruminants the amount of magnesium in the ration is directly proportional to the percent hydrolysis of phytate phosphorus. They concluded that the amount of phytate phosphorus in the ration is the deciding factor in the absorption of magnesium, calcium and phosphorus. Stewart and Moodie (1956b) experimented on anethetized sheep to discover in what parts of the alimentary tract absorption of magnesium takes place. It was shown that after heavy doses of magnesium sulfate under certain conditions, absorption of magnesium may take place from the rumen, abomasum, small intestine and caecum. The authors concluded that the principal site of magnesium absorption is probably the duodenum and the remainder of the small intestine. After large doses of magnesium salts the phosphate content of the blood was also increased but the calcium content was slightly decreased. It was shown also that the magnesium was much more quickly and efficiently absorbed after oral doses of magnesium nitrate than after similar amounts of magnesium sulfate.

Legumes, which have been incriminated as an important factor in the production of bloat, generally have a higher content of magnesium than the grasses (Bender and Eisenmenger, 1941; Morrison, 1956). Svanberg and Ekman (1946) reported that a marked deficiency of magne-

sium was rarely found on peaty soil, a type of soil reported by Weiss (1953) to yield bloat-producing alfalfa. Svanberg and Ekman (op.cit.) noted that in general forages of low magnesium content were found on acid soils. Similar data was obtained by Bender and Eisenmenger (op. cit.) who reported that legumes, with the exception of sweet clover, contained a higher percentage of magnesium when grown on a basic soil, compared with the same plants grown on a more acid soil. Truog et al. (1947) studied the magnesium-phosphorus relationship of peas. They found that the seeds of this legume revealed an appreciable and consistent increase in phosphorus content with increasing supplies of available magnesium. In fact increasing supplies of available magnesium increased the phosphorus content of the peas much more than did increasing supplies of available phosphorus.

The reduction of rumen fluid pH during outbreaks of ruminant bloat has been reported by Johns (1956, 1957). Thus it is significant that Carr and Woods (1955) reported bovine albumin bound more than nine times as much ionic magnesium at pH 7.5 than at pH 6.0. When the pH was reduced to 5.5 there was essentially no binding of the ion. Bovine hemoglobin and globulin bound less magnesium than albumin. It has also been reported that magnesium ions were freed from magnesium oleate complexes as the pH was reduced to pH 5.7 (Hartsuch, 1938). Albert (1950) studied the binding of amino acids with magnesium ions and found that sometimes the ions gave no evidence of complexing with amino acids. Where positive results were obtained relatively small amounts of magnesium ions were bound.

The production of slime or capsular material by rumen microorganisms has been suggested as one factor in the production of frothy bloat (Hungate, 1955; Jacobson, 1957a). Thus it is significant that Stacey (1953) observed the production of large quantities of fructosan and capsules by streptococci grown on a sucrose agar medium containing magnesium. The same organism grown without sucrose and magnesium produced no fructosan and no capsules.

Jackson et al. (1957) reported an increase in plasma cholesterol in cattle grazing bloat-producing alfalfa pastures. Moore et al. (1957) reported the production of severe bloating in sheep after administering a cholesterol-alfalfa juice drench. It is thus noteworthy that Kruse et al. (1933) found a marked increase in total blood cholesterol as serum magnesium decreased in dogs fed a low-magnesium ration. There was a commensurate decrease in fatty acids so that total fat remained constant. Total cholesterol in ester form was very high. There was a "terminal rise" in blood nonprotein nitrogen which was explained on the basis of an augmented protein metabolism following failure of the normal fat metabolic cycle.

Rumen Fluid Phosphate and Possible Relationship to Bloat

Garton (1951) reported a level of 27 to 41 mg.% inorganic phosphorus in the rumen of fistulated sheep fed a ration of chopped meadow hay. Slaughtered sheep which had been grazed on rye grass and clover pasture had a level of 42 to 68 mg.% inorganic phosphorus. The feeding of a ration of chopped meadow hay resulted in a rumen fluid inorganic phosphorus level of 10 mg.% whereas sheep fed a ration of oats, chopped

oat straw, ground corn and blood meal had a very high level of 117 to 164 mg.% inorganic phosphorus. The author pointed out that in vitro experiments revealed a partial precipitation of phosphate with an increase in pH from 6.85 to 7.7. Garton remarked that this might happen in vivo if rumen contents ever became alkaline. Scarisbrick and Ewer (1951) commented on the high concentration of phosphorus in the rumen. The authors believe that phosphorus-rich saliva influences the movement of phosphorus from the blood to the rumen. They anesthetized sheep, exposed the ruminal vein and carotid artery, and injected into the rumen radioactive phosphorus. The results showed that inorganic phosphate was absorbed from the rumen in widely varying amounts. On several occasions inorganic phosphate appeared to be recycled from the blood to the rumen. The authors concluded that the net absorption of inorganic phosphate from the rumen over a long period of time seems to be at most only small in amount. However, at any particular instant there may be a substantial movement of inorganic phosphate into or out of the blood traversing the ruminal wall. Clark (1953) reported that ruminants actively secreted water-soluble phosphate in their saliva. The water-soluble phosphorus in the rumen of sheep fed a phosphorus-deficient diet was greater than the level in sheep fed a normal diet, even though the opposite was true for the levels in the blood and saliva of the two groups. There was no significant difference between the rumen water-soluble phosphate of cattle grazed on phosphorus-deficient land and those supplemented with bone meal. However, the inorganic phosphorus levels in the blood and saliva were lower in the cattle grazing the phosphorus-deficient land. There was no correlation between blood phosphorus figures and the phosphorus content of the rumen.

A magnesium-phosphorus interaction in ruminants, observed by Lakke Gowda and co-workers (1955), has been reported previously. They concluded that the percent hydrolysis of phytate phosphorus seems to be directly proportional to the amount of magnesium present in the ration, and that the amount of phytate phosphorus in the ration is a deciding factor in the absorption of magnesium as well as calcium and phosphorus.

The possible relationship of phosphorus to bloat has been advanced in several reports. Troughton (1955) stated that most pasture bloat from white clover was produced on soils poor in soluble phosphorus pentoxide and potassium oxide. The difference in bloat potential of clover blocks was not attributed to herbage production. The relationship did not hold true when plots within a block were compared. The author pointed out that this may have been due to overshadowing by differences due to mixtures. Cooper and Hall (1956) reported that bloat was especially common in areas where soils were deficient in available phosphorus. The authors stated, "In bloat, a rich nitrogen or protein ration seems to be one of the significant predisposing factors. Only rarely does bloat occur from the grazing of leguminous crops produced on soils which have received liberal applications of phosphorus, or on soils where there is a geological accumulation of available phosphorus in the soil." The authors thought that if the nitrogen to phosphorus ratio changes from about 11 to 1 to approximately 15 to 1 there is a potential bloat hazard. They state that where there is a relatively large excess of nitrogen in plants, unstable azo, azoxy and diazo organic groupings may become significant factors in determining the accumulation of gases in the rumen. Cooper and Woodle (1957) claimed that fatal bloat cases were reduced five to eight times by increased phosphorus fertilization on land grazed by several hundred head of cattle.

Since various workers have implicated bacterial slime as a factor in bloat (Hungate, 1955; Jacobson, 1957a), the findings of Rorem (1955) appear noteworthy. This worker reported that cells associated with a slime polysaccharide covering had an uptake of phosphate ions 2 to 20 times that of the same number of cells without slime or capsules. The author stated that the results of this study seemed compatible with the hypothesis of polysaccharides being an important factor in ion binding by bacterial and higher plant cells.

Rumen Fluid Ammonia and Possible Relationship to Bloat

The reduction of magnesium in the blood and urine of cattle following the ingestion of high-protein rations has prompted workers to study the possible role of increased rumen ammonia in this reduction. Lewis et al. (1957) showed that there was a correlation between rumen and portal blood ammonia over a wide range of rumen ammonia concentrations. Johns (1955) observed high rumen ammonia levels in sheep after they had grazed high-protein herbage. The range of concentration was from 35 to 130 mg.% of ammonia nitrogen. The peak levels did not coincide with the peak protein levels in the herbage, nor did the low concentrations necessarily result from the ingestion of low protein herbage. The pH of the rumen fluid was never above 6.5. It has been reported previously that Head and Rook (1955) observed higher rumen ammonia values for grazing cattle than for cattle fed a dry ration.

Briggs *et al*. (1957) reported that the volatile fatty acid levels in the rumen are adequate for the neutralization of large amounts of ammonia. However, they state, "Nevertheless it appears certain that the pH-volatile fatty acid relationship is modified to some considerable degree by high ammonia nitrogen levels." They found that the rumen ammonia levels were highest at high levels of volatile fatty acids.

The possible relationship of high levels of ammonia to ruminant bloat has been suggested by Hale and King (1955). They believed that the conditions in the rumen following urea-induced bloat are similar to those observed when ruminants suffer from legume pasture bloat. Brown *et al*. (1957) reported a positive correlation between bloat severity and ammonia content of rumen ingesta, but reduction of bloat by oils or an antibiotic did not reduce rumen ammonia values. Johns (1955) found that high ammonia levels can be maintained in the rumen of sheep fed winter grass without the appearance of any signs of bloat or toxicity. Jamieson and Loftus (1958) reported the sporadic presence of a long filamentous bacterium of the *Beggiatocaceae* family in the rumina of sheep on pasture. They observed that the presence of them was indicative of a sustained level of ammonia nitrogen lower than the 11 to 18 mg.% usually found in rumen liquor of pastured cattle.

Rumen Fluid pH and the Possible Relationship to Bloat

Johns (1956) has recently shown that the hydrogen ion concentration in the rumen may differ between bloated and nonbloated cattle. He noted that the pH of the rumen was always below 6.5 when cattle bloated

on pasture. Johns et al.(1957) cited unpublished work of Reid which confirmed that the rumen fluid was in the region of pH 6.3 at the onset of bloat, and as rumen fermentation proceeded the pH dropped below 6.0. Johns (1956) cited unpublished work of Mangan who found that in the presence of salts red clover cytoplasmic proteins at a level of 20 mg.% protein nitrogen formed a very stable foam, with a maximum stability at pH 6.0. Johns et al. (1957) observed that the maximum foam strength of the rumen liquid occurred when the liquid was in the region of pH 6 with a salt concentration of o.15 to 0.2 molar. A determination of the optimum pH for maximum foam stability for red clover saponins and cytoplasmic protein extracts revealed that while the former is about pH 5, that of the latter is approximately the same as the rumen contents, or pH 6. The authors concluded that with this evidence it appeared that at the pH of the rumen contents at which bloat occurs protein is likely to be more important as a foaming agent than the saponins. They further pointed out that plant protein was rapidly released in the rumen. The level of protein necessary to produce a stable foam accumulated within 30 minutes after the animals had been placed on pasture, the length of time in which animals have been observed to bloat.

The interaction of bovine proteins, pH, and magnesium, reported by Carr and Woods (1955), was previously mentioned. They found that bovine serum albumin bound more than nine times as much ionic magnesium at pH 7.5 than at pH 6. When the pH was reduced to 5.5 no magnesium was bound by the bovine albumin. It was found that bovine globulin bound less than albumin. These observations may partially explain the influence of pH and salt concentration on foam stability reported by Johns (1956).

Since cattle have been reported to exhibit stable froth formation after ingesting immature alfalfa or Ladino clover plants (Weiss, 1953; Boda et al., 1956; Nichols, 1956; Johns, 1958) as well as with feed lot bloat (Smith et al., 1953; Phelps, 1956; Jacobson, 1957b) it is noteworthy that Ammerman and Thomas (1952) reported that juice from nonbloating blue grass forage was more highly buffered than were juices from such bloat-producing forages as Ladino clover and alfalfa. They also found that juices from more mature Ladino and alfalfa appeared to be more highly buffered than juices from the same forages when young and succulent. This is a significant finding when one considers that cattle appear to bloat more on young succulent legumes than on either mature legume or grass pastures. The authors further reported that the rumen ingesta pH of lambs grazing alfalfa or Ladino clover averaged approximately pH 6.24. When the lambs were grazing bluegrass pasture the pH rose to 6.56. Following prefeeding of hay, a practice sometimes utilized to prevent bloat, the rumen ingesta pH increased to 6.7 with legume pasture grazing and 6.9 with grass pasture grazing.

Monroe and Perkins (1939) reported a somewhat reversed observation. They observed a rumen ingesta pH of 6.47 when the cattle were grazing bluegrass pasture and a pH of 6.66 when the animals grazed alfalfa. Cason and others (1954) fed various legume and grass roughages and found that the highest rumen fluid pH coincided with the least weight of wet material in the rumen, the lowest percentage of dry matter, and the highest ash content. A high degree of relationship was found to exist between the ash content of the ingesta and the pH of the rumen.

The authors concluded that the ash moved out of the rumen more slowly than the other constituents of the ingesta and exerted a buffering action on the rumen fluid, thus influencing rumen fluid pH.

EXPERIMENTAL PROCEDURE

This study consisted of two parts with Part I composed of isolation and identification of hexahydrate magnesium ammonium phosphate. Part II involved quantitative measurements of the mineral complex and hydrogen ion concentration in rumen fluid.

Part I. Isolation and Identification of Hexahydrate Magnesium Ammonium Phosphate

Rumen ingesta was obtained from mature, rumen-fistulated dairy steers or cows which had been fed a froth-producing ration developed by Smith et al. (1953). The ingesta was strained through four layers of cheesecloth and the fluid portion centrifuged at a relative centrifugal force of 30,000 for 15 minutes in a Servall SS-1 centrifuge. Aliquots of the supernatant obtained were titrated to each 0.5 of a pH unit between pH 7 and 13 with 10% NH_4OH. Titration to a value of pH 9.0 was found to yield the largest amount of pure precipitate. The precipitate was permitted to stand for several hours and then redissolved in dilute HCl. The resulting solution was filtered through Whatman No. 1 filter paper and the filtrate obtained titrated to pH 9.0 with 10% NH_4OH. Crystallization was permitted for several hours after which the material was separated by decantation. The crystals were air-dried and then vacuum-dried for 29 hours over concentrated H_2SO_4. Qualitative analyses were carried out spectrographically. Elements shown to be present by these analyses, as well as ash and nitrogen, were determined quantitatively.

Part II. Quantitative Measurement of Magnesium Ammonium Phosphate and Hydrogen Ions

Rumen ingesta for magnesium ammonium phosphate determinations was obtained from nine rumen-fistulated dairy cows or steers ranging in age from one to seven years. A total of 13 nonfroth and froth-producing rations was fed. Rumen fluid pH determinations were carried out with eight animals fed a total of six nonfroth and froth-producing rations.

Rumen ingesta was obtained ventro-medial to the fistula and immediately prior to 8 A.M. feeding except when hourly samples were obtained. The ingesta was strained through four layers of cheesecloth and hydrogen ion determinations were immediately performed on the strained rumen fluid by means of a Beckman Model H-2 potentiometer equipped with calomel and glass electrodes. The strained fluid was then removed to the laboratory and centrifuged at a relative centrifugal force of 700 for five minutes in an International centrifuge. The supernatant obtained was then centrifuged at a relative centrifugal force of 30,000 for ten minutes in a Servall SS-1 centrifuge. The clear fluid obtained with divided into 25- or 50-ml. aliquots and adjusted to pH 9.0 with 10% NH_4OH. The base was added from a burette, and a Beckman H-2 potentiometer was employed to measure pH values. The rumen fluid was immediately poured into 50-ml. volumetric flasks with care being taken to transfer all of the formed precipitate. A 10-ml. calibrated Kolmer Pyrex centrifuge tube was then placed over the neck of the flask and the flask inverted, causing the material in the flask to move to the tip of the tube. The vacuum created inside the inverted volumetric flask prevented any loss of rumen fluid. The quantity of rumen fluid precipitate was recorded as soon as precipitation was complete.

RESULTS AND DISCUSSION

Part I. Isolation and Identification of Hexahydrate Magnesium Ammonium Phosphate

The spectrographic analysis of rumen fluid crystals revealed the presence of large amounts of phosphorus and magnesium plus a trace of calcium. The results of the ash and elemental analyses of the crystals and the calculated values for hexahydrate magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$) are presented in Table 1.

Table 1. Comparison of Rumen Fluid Crystals with $MgNH_4PO_4 \cdot 6H_2O$

Compound	Percentage				Ratio of N:Mg:P
	Ash	N	Mg	P	
Crystals from Rumen Fluid	47.14	5.90	10.07	12.77	1:1:1
$MgNH_4PO_4 \cdot 6H_2O$	45.35	5.71	9.91	12.63	1:1:1

Confirmation of the presence of $MgNH_4PO_4 \cdot 6H_2O$ was obtained by infrared spectroscopy.

The presence of $MgNH_4PO_4 \cdot 6H_2O$ crystals spontaneously formed in centrifuged rumen fluid was confirmed by the microscopic method of Wherry (cited by Purcell and Hickey, 1922). This method involved the microscopic observation of $MgNH_4PO_4 \cdot 6H_2O$ crystals suspended in toluene, a liquid having the same refractive index as the crystals. As $MgNH_4PO_4 \cdot 6H_2O$ crystals are brought into and out of focus, the bands of light along the edges of the crystals alternate between yellow and blue.

Several precautions must be observed in analyzing for rumen fluid $MgNH_4PO_4 \cdot 6H_2O$. Carbon dioxide is continually released from the ingesta with a resulting pH increase. Centrifugation of the ingesta must be completed before the pH has risen to approximately pH 7 or spontaneous crystallization will occur with the resulting loss of the product.

The optimum pH for precipitation of $MgNH_4PO_4.6H_2O$ from centrifuged rumen fluid is pH 9.0. If higher pH values are employed, rumen fluid pigments will be precipitated with the mineral complex. Precipitation may be carried out below pH 9.0, but the product yield will be lowered.

Acidification is commonly employed in the determination of $MgNH_4PO_4.6H_2O$ by precipitation. During the development of an analytical procedure for this mineral complex, it was found that the addition of HCl to rumen fluid would usually increase the amount of precipitated $MgNH_4PO_4.6H_2O$. However, it was important to measure only the $MgNH_4PO_4.6H_2O$ that would form from the addition of NH_4OH to material soluble in the rumen fluid. If acidification were employed, a further release of complexing material from previously unavailable sources would be expected to occur. Garner (1949) has demonstrated by an in vitro technique that more ultrafilterable Mg appeared as simulated ruminal contents underwent increased acidification.

Precaution must be observed in drying the $MgNH_4PO_4.6H_2O$ crystals. All water of hydration is lost if the drying temperature is allowed to reach 100 degrees centigrade. High temperatures also result in loss of ammonia. It was found that drying over sulfuric acid in a vacuum jar gave satisfactory results.

Water of hydration greatly influences the infrared spectrum of $MgNH_4PO_4.6H_2O$. Therefore, it is necessary to treat the known sample of the mineral complex in exactly the same manner as the unknown. It was found that infrared spectra of the known and unknown compounds could be matched by dissolving the compounds in dilute HCl, precipitating the material by addition of 10% NH_4OH to pH 9.0 and drying the obtained precipitate over concentrated H_2SO_4 for 29 hours.

Part II. Quantitative Measurement of Magnesium Ammonium Phosphate and Hydrogen Ions

Identification of the rumen fluid crystals from frothy rumen ingesta was followed by a study of the comparative amounts in frothy and nonfrothy rumen ingesta obtained from animals fed a variety of rations. It was known that the method for measuring $MgNH_4PO_4$ was accurate for different aliquots of the same rumen fluid sample; in the second phase, the study included a comparison of the accuracy of the method between rumen fluid samples.

The values for $MgNH_4PO_4$-containing precipitate, hereafter designated $MgNH_4PO_4$, formed from rumen fluid of dairy cattle fed a variety of rations are shown in Figure I. The values are expressed as the volume per 100 ml. of rumen fluid and per ml. of added base; thus, the quantity of added ammonium ions is equalized. The rations employed in the study are shown in Table 2 together with the range of values obtained for $MgNH_4PO_4$.

The data presented in Figure I reveal that more precipitate was obtained from those animals displaying frothy rumen ingesta than those with nonfrothy except for one animal, No. 24. The relatively large amount of $MgNH_4PO_4$ obtained from this animal displaying nonfrothy ingesta occurred when the animal was "off feed" and exhibiting very acidic rumen fluid. The effect of this highly acidic ingesta on the yield of

$MgNH_4PO_4$ is discussed below. It is important to point out that frothy rumen ingesta yielded an average of three times more $MgNH_4PO_4$ than non-frothy ingesta.

It is significant that identical twin animals, 25 and 26, fed identical rations, did not respond similarly. Twin 25 had previously been fed a high-roughage ration for several weeks before receiving the daily ration of 10 lbs. of concentrate No. 24 and 10 lbs. of alfalfa hay. This animal did not display frothy rumen ingesta. The twin of this animal, 26, although fed an identical ration, did display frothy ingesta. However, this animal had previously been receiving a froth-producing, high-concentrate ration for several weeks. It is note-worthy that the animal displaying the frothy ingesta also yielded rumen fluid with a greater concentration of both $MgNH_4PO_4$ and hydrogen ions.

It is significant that cattle fed rations containing a large percentage of roughage produced rumen fluid containing low levels of $MgNH_4PO_4$. The rumen fluid samples from these animals usually exhibited a much higher pH value than rumen fluid obtained from cattle fed a ration containing a large percentage of concentrates.

Statistical analyses of 115 rumen fluid samples revealed a highly significant correlation between the amount of $MgNH_4PO_4$ formed and the amount of base added to rumen fluid obtained from nine animals fed a total of 11 rations. However, the correlation between the same two factors was not significant with data obtained from animals fed high levels of roughage. This lack of correlation was believed due to the inaccuracy of the method when measuring extremely small amounts of $MgNH_4PO_4$ in rumen fluid obtained from roughage-fed cattle.

In Figure II, it can be seen that the hydrogen ion concentration of the rumen fluid, as measured at the moment of sampling, does not appear to be correlated with the amount of $MgNH_4PO_4$ obtained from the rumen fluid. It must be emphasized that a comparison is being made between eight different animals fed six different rations, and thus there may be a correlation between the amount of $MgNH_4PO_4$ formed from and hydrogen ion concentration of rumen fluid obtained from single animals fed single rations. This correlation will be discussed below.

Figure II also illustrates that a given ration may result in a relatively constant amount of $MgNH_4PO_4$, even though measured in different animals, if the rumen pH values are similar. Thus, rumen fluid from animals 25 and 26 yielded the same high amount of the mineral complex when the animals were fed ration G, a mixture of alfalfa hay and corn-soybean oil meal concentrate. Likewise, animals 23 and 139 yielded similar low amounts of $MgNH_4PO_4$ when fed identical rations of timothy hay, ration M. Similarly, data presented in Figure I shows that animals 3 and 25, fed approximately equal amounts of alfalfa hay, ration K, yielded almost equal amounts of $MgNH_4PO_4$. Thus, the amount of $MgNH_4PO_4$ obtained from rumen fluid appears to be influenced by the composition of the ration fed. It has not been ascertained whether the ration components exert a direct effect on the $MgNH_4PO_4$ formed or whether the effect results indirectly from altered rumen metabolism. However, the latter explanation appears more probable as will be discussed below.

In addition to the data shown in Figure II, Table 3 contains data obtained from hourly sampling of rumen fluid from four cattle fed four different rations. The data illustrate the difference in rate of formation of the mineral complex when different rations are fed. The results also show the parallelism between the hydrogen ion concentration of rumen fluid and the amount of $MgNH_4PO_4$ formed from rumen fluid when comparisons are made on the basis of individual rations. Since the greatest amount of $MgNH_4PO_4$ was usually observed in rumen fluid obtained five hours after feeding, it appeared that the amount of the complex formed may be related to the production of one or more of the volatile fatty acids. Moir and Somers (1957) reported that the total volatile fatty acid production reached a peak approximately four hours after feeding. Similar data have been reported by Reid et al. (1957) who also noted that the production of propionic acid paralleled the production of total volatile fatty acids. Butyric and acetic acid did not usually follow the same trend as propionic acid. Thus, it appears that the production of one or more volatile fatty acids may influence the amount of $MgNH_4PO_4$ formed.

Since only one of the rumen fluid samples in Table 3 was from frothy ingesta, no comparison can be made between froth production and resulting $MgNH_4PO_4$ concentration of rumen ingesta. It is noteworthy, however, that the average amount of $MgNH_4PO_4$ formed over the seven-hour period was directly proportional to the calculated magnesium and phosphorus intakes of the animals.

Additional data illustrating the relationship between rumen fluid hydrogen ion concentration and $MgNH_4PO_4$ are shown in Figures III and IV. Again, the parallelism between the concentrations of hydrogen ions and $MgNH_4PO_4$ is evident in almost all cases. Statistical analyses of the data revealed this correlation to be significant to the one percent level of probability with animals 25 and 26, to the two percent level with animal 2, and to the five percent level with animals 24 and 130. Data from the three remaining animals showed less statistical correlation of the above two factors as shown in Table 5. These animals were the only ones of the group receiving an all-roughage ration. The amount of $MgNH_4PO_4$ formed when these rations were fed was extremely low, and thus lack of statistical significance can be explained by the inability of the method to accurately measure small amounts of $MgNH_4PO_4$.

A lack of correlation between rumen fluid hydrogen ion concentration and $MgNH_4PO_4$ was pointed out in the discussion of Figure II, whereas in the discussion of Figures III and IV and Table 3 a correlation was shown to exist for five of the eight animals. The results are not conflicting. The correlations in Figure II are based on comparisons between different rations. However, all correlations between rumen fluid hydrogen ion concentration and $MgNH_4PO_4$ in Table 3 and Figures III and IV are based on data obtained from the feeding of a single ration. Thus, the comparison in the latter case is not between vastly different rations as in the former case. Therefore, it appears that the rumen fluid hydrogen ion concentration, as well as the composition of the ration fed, greatly influences the amount of $MgNH_4PO_4$ formed in rumen fluid.

The specific reason why hydrogen ion concentration should effect the yield of $MgNH_4PO_4$ obtained from rumen fluid is unknown. However, the previously cited work by Garner (1949, 1950) illustrated the increase in magnesium ions as rumen ingesta was acidified. Similarly,

Carr and Woods (1955) observed a decreased binding of Mg ions by bovine proteins with increasing concentration of hydrogen ions. A similar observation was made by Hartsuch (1938) who found that Mg was released from oleate complexes when the pH was decreased. If the release of both Mg and PO_4 ions increases with greater acidification of rumen fluid, a partial explanation for the increased amounts of $MgNH_4PO_4$ in rumen fluid treated with NH_4OH is offered. The amount of mineral ions released in rumen fluid of a constant pH will be different for different rations; thus, a feasible explanation is offered for the experimentally determined differences in $MgNH_4PO_4$ obtained from rumen fluid of cattle fed different rations.

An attempt to correlate the combined effect of the Mg and P intake plus rumen fluid hydrogen ion concentration with the determined amount of $MgNH_4PO_4$ is shown in Figure V and Table 4. The values obtained for the Mg and P content of the ration components were taken from the mineral analyses of feeds published by Morrison (1956). The hydrogen ion concentration was obtained directly from the rumen as reported in the experimental procedure. Figure V illustrates the results of the comparison and the formula employed to obtain the values. It can be readily seen that there is a direct relationship between the combined factors of Mg and P; or Mg, P, and H ions; versus the determined amount of $MgNH_4PO_4$-containing precipitate in only half of the comparisons. Even though the method of comparison is arbitrary, the values are still relative since all comparisons were made by means of the same formula.

Previously, it was shown that data presented in Table 3 indicated a direct relationship between calculated Mg and P intake and $MgNH_4PO_4$ formation. Again, there is no conflict of data. The comparison of results shown in Table 3 is between the average amount of $MgNH_4PO_4$ obtained over a seven-hour period versus the calculated intake of Mg and P. The data illustrated in Figure V compare the same calculated intake of Mg and P with average concentrations of $MgNH_4PO_4$ obtained prior to feeding. In the former comparison, $MgNH_4PO_4$ values were obtained when considerable rumen volatile fatty acid production was occurring. The effect of this acid production and the resulting rumen pH depression can have a profound effect upon the mineral ions present in the rumen as was discussed above. Thus, it appears that the amount of $MgNH_4PO_4$ formed in rumen fluid may be influenced by the Mg and P intake of the animal if the rumen ingesta becomes sufficiently acid.

In conclusion, it must be emphasized that the amount of $MgNH_4PO_4$ obtained from rumen fluid appears to be dependent both on the rumen fluid hydrogen ion concentration and on the ration fed. The average amount of $MgNH_4PO_4$ obtained from frothy rumen ingesta was three times more than that from nonfrothy ingesta. However, it must be pointed out that cattle fed rations capable of producing low rumen pH values may produce nonfrothy rumen ingesta yielding high $MgNH_4PO_4$ values. Lower rumen pH values, however, do appear to be associated with both froth production, as shown in this study when identical twin cattle were fed identical rations, and pasture bloat as reported by Johns (1956) and Johns et al. (1957).

Figure I. $MgNH_4PO_4$-containing Precipitate Obtained from Rumen Fluid

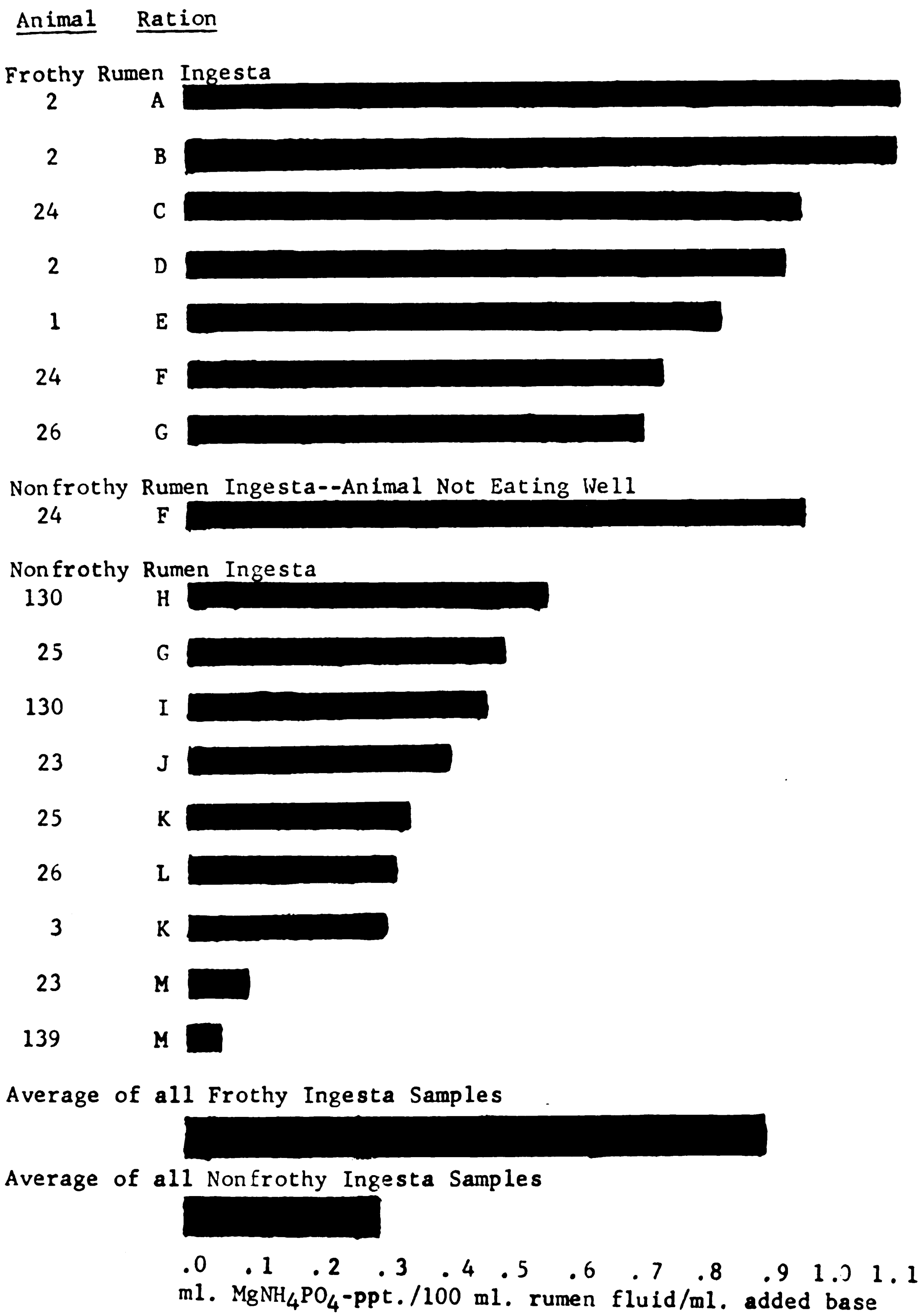


Table 2. Ration Components Fed Daily (lbs.) and $MgNH_4PO_4$ Values
(All Rations Include 50 g. NaCl Daily)
[Data corresponds to ration and animal designation shown on opposite page]

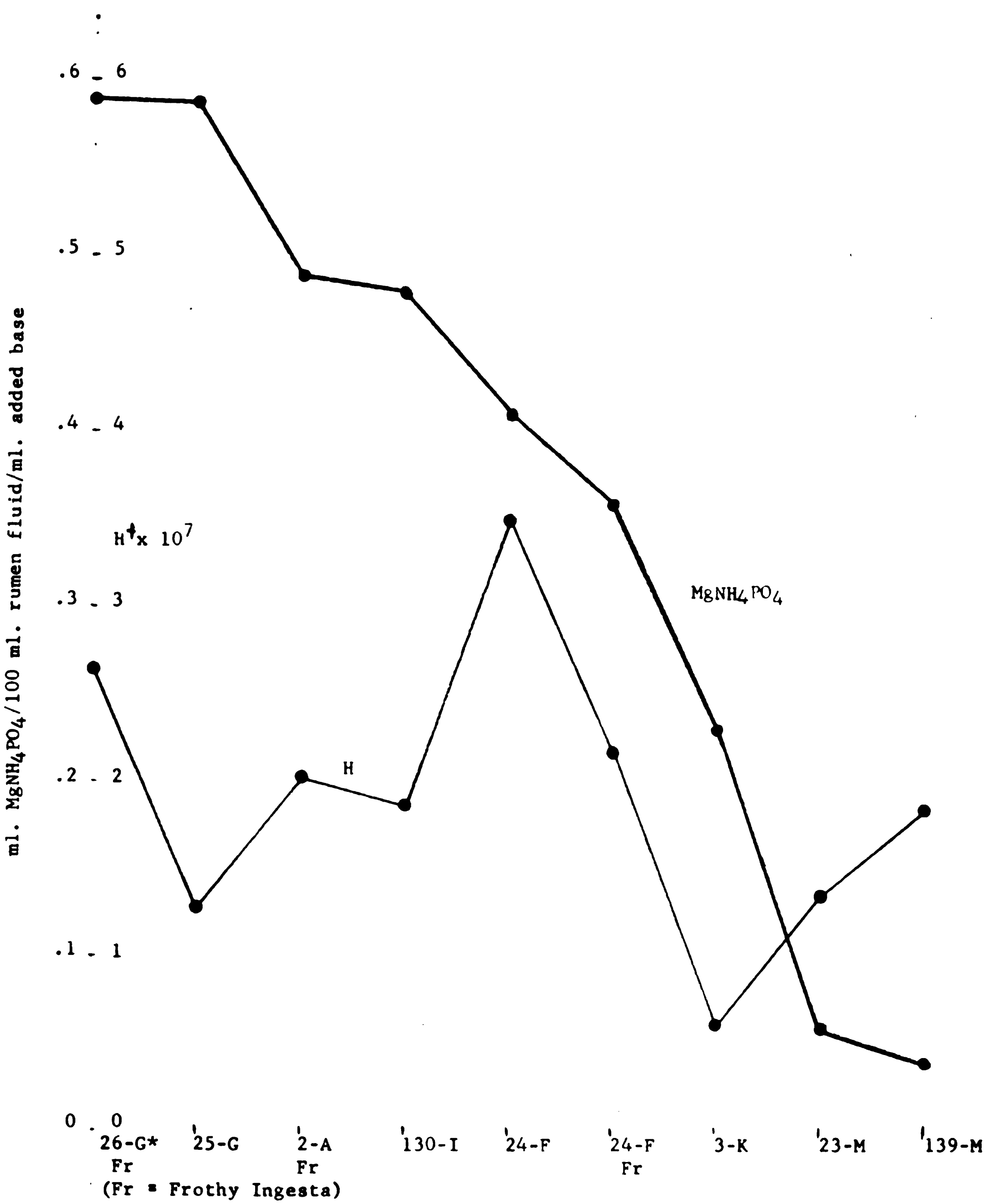
Alf. Hay	Conc. #24*	Conc. #25**	Conc. #52***	Tim. Hay	Other Components	$MgNH_4PO_4$ No. Samples	$MgNH_4PO_4$ Range of Values (ml./100 ml. rumen fluid)	$MgNH_4PO_4$ Mean (ml./100 ml. rumen fluid)
6	8					11	.33-3.04	1.12
6		8			800 ml.95%EtOH	4	.75-1.64	1.11
4	12-14					4	.42-1.53	.97
Ration B minus alcohol						5	.31-1.38	.85
Ration A plus 30 ml. Citroflex						7	.22-1.11	.83
4	0-7		0-14			9	.30-1.52	.74
10	10					14	.43-1.41	.71
Same as Ration F above						4	.43-1.94	.95
12					2 lb.No. 2 corn, ground	9	.14-1.51	.56
Same as Ration G above						15	.17-1.10	.49
Ration H plus 100 mg. Procaine Penicillin						16	.27-0.76	.42
4			8-10			6	.18-0.60	.41
20-30						10	.13-0.63	.34
Ration K plus 400 ml. 95% Ethanol						8	.08-0.63	.32
Same as Ration K above						23	.07-0.58	.30
				14-20		17	0-0.27	.09
Same as Ration M above						16	0-0.26	.05
						52	.22-3.04	.90
						105	0-1.94	.31

Concentrate Rations

% Ingredient	Corn, Ground	Corn and Cob Meal	SOM	Corn Starch	Marcol B-75	Urea	$CaCO_3$	$CaHPO_4$	Trace Salt	Vit. A#	Vit. D#
Ration 24	77.0		20.0				1.0	1.0	0.5	5×10^6	4.5×10^5
Ration 25	69.5		20.0		8.0		1.0	1.0	0.5	5×10^6	4.5×10^5
Ration 52		77.7		19.4		2.9					

#I.U.

Fig. II. Relationship of Rumen Fluid $MgNH_4PO_4$-containing Precipitate to Rumen Fluid Hydrogen Ion Concentration (Multiple Ration Comparisons)



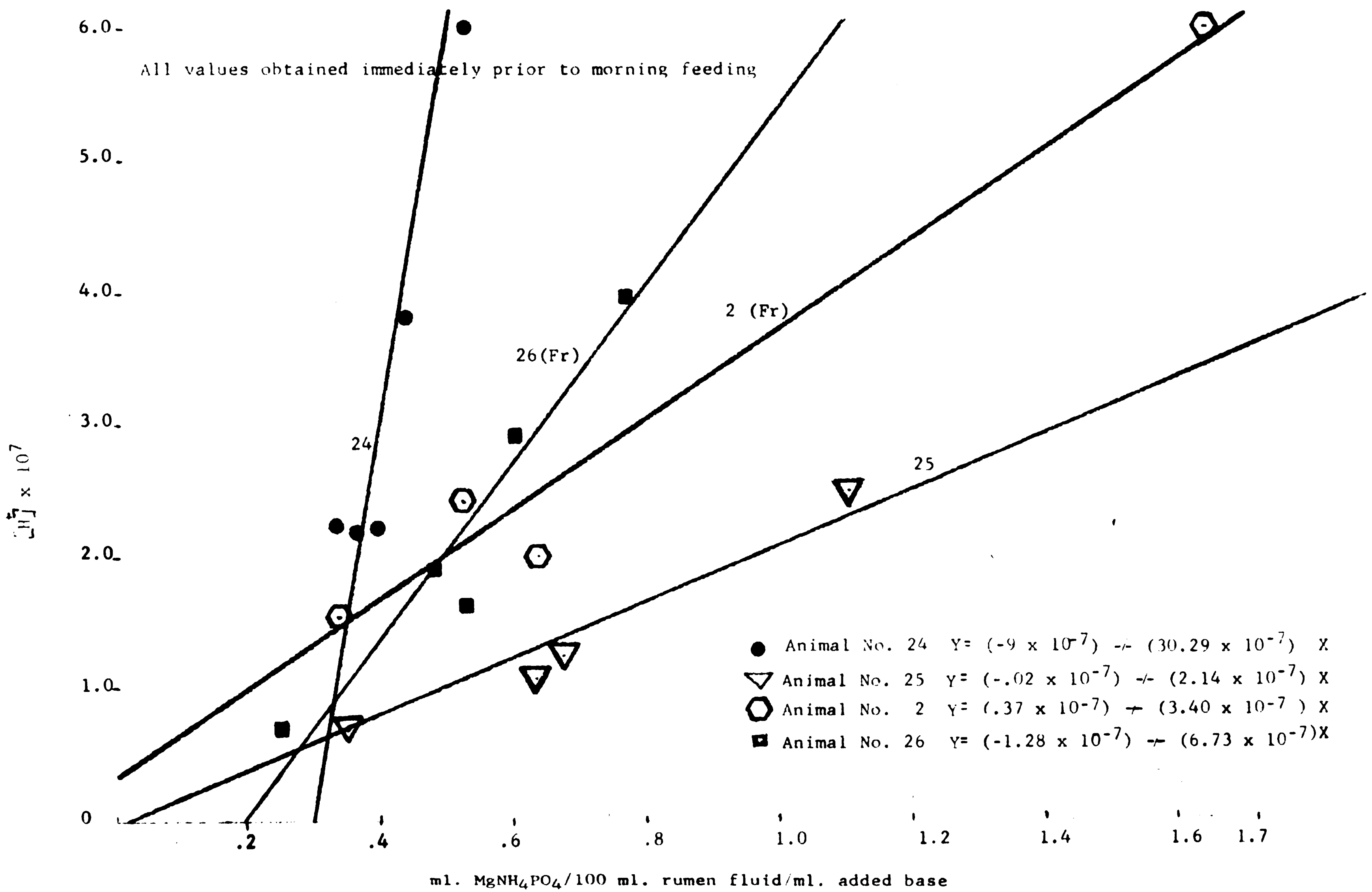
*Refer to Table 2 for ration composition

Table 3. Rumen Fluid pH and $MgNH_4PO_4$ of Cattle Fed Froth and Nonfroth-producing Rations

Animal	Ration	Sampling Time*	No. Samples	Rumen Fluid pH Range	Rumen Fluid pH Mean	Mean H x10^{-7}	ml. $MgNH_4PO_4$/ 100ml. rumen fluid/ml. added base
26**	G	0	4	6.4-7.1	6.6	2.6	0.59
25	G	0	5	6.6-7.2	6.9	1.3	0.59
2**	A	0	3	6.6-6.8	6.7	2.0	0.49
130	I	0	5	6.5-6.9	6.7	1.9	0.48
24	F	0	4	6.2-6.6	6.5	3.5	0.41
24**	F	0	1	6.7	6.7	2.2	0.36
3	K	0	5	7.1-7.3	7.2	0.6	0.23
23	M	0	5	6.6-7.2	6.9	1.4	0.06
139	M	0	5	6.5-6.8	6.7	1.8	0.04
***							
2	A	0	2	6.4-6.5	6.5	3.5	0.88
2	A	2	2	6.1-6.2	6.1	7.5	3.26
2	A	5	2	5.2-5.8	5.4	35.5	6.37
2	A	7	2	5.8	5.8	15.9	5.44
3	K	0	2	7.1	7.1	0.8	.32
3	K	2	2	6.5-6.7	6.6	2.6	1.33
3	K	5	2	5.9-6.0	5.9	11.6	7.58
3	K	7	2	5.9-6.1	6.0	10.5	8.11
23	J	0	2	6.5-6.9	6.6	2.3	.35
23	J	2	2	6.3-6.7	6.5	3.3	.54
23	J	5	2	5.6-6.0	5.7	19.1	1.15
23	J	7	2	5.6-6.0	5.8	17.1	.96
24	F	0	2	6.4-6.6	6.5	3.0	.58
24	F	2	2	5.6-6.0	5.7	19.6	2.19
24	F	5	2	5.2	5.2	61.0	1.76
24	F	7	2	5.3-5.4	5.4	44.9	--
Av. of all frothy ingesta samples			52	5.8-7.2	6.5	3.4	.90
Av. of all nonfrothy ingesta samples			105	5.7-7.6	6.6	2.5	.31

*Hrs. after feeding **Frothy Rumen Ingesta ***Portable potentiometer used on series samples

Fig. III Rumen fluid $MgNH_4PO_4$ vs. rumen fluid hydrogen ion concentration
(Single Ration Comparisons)



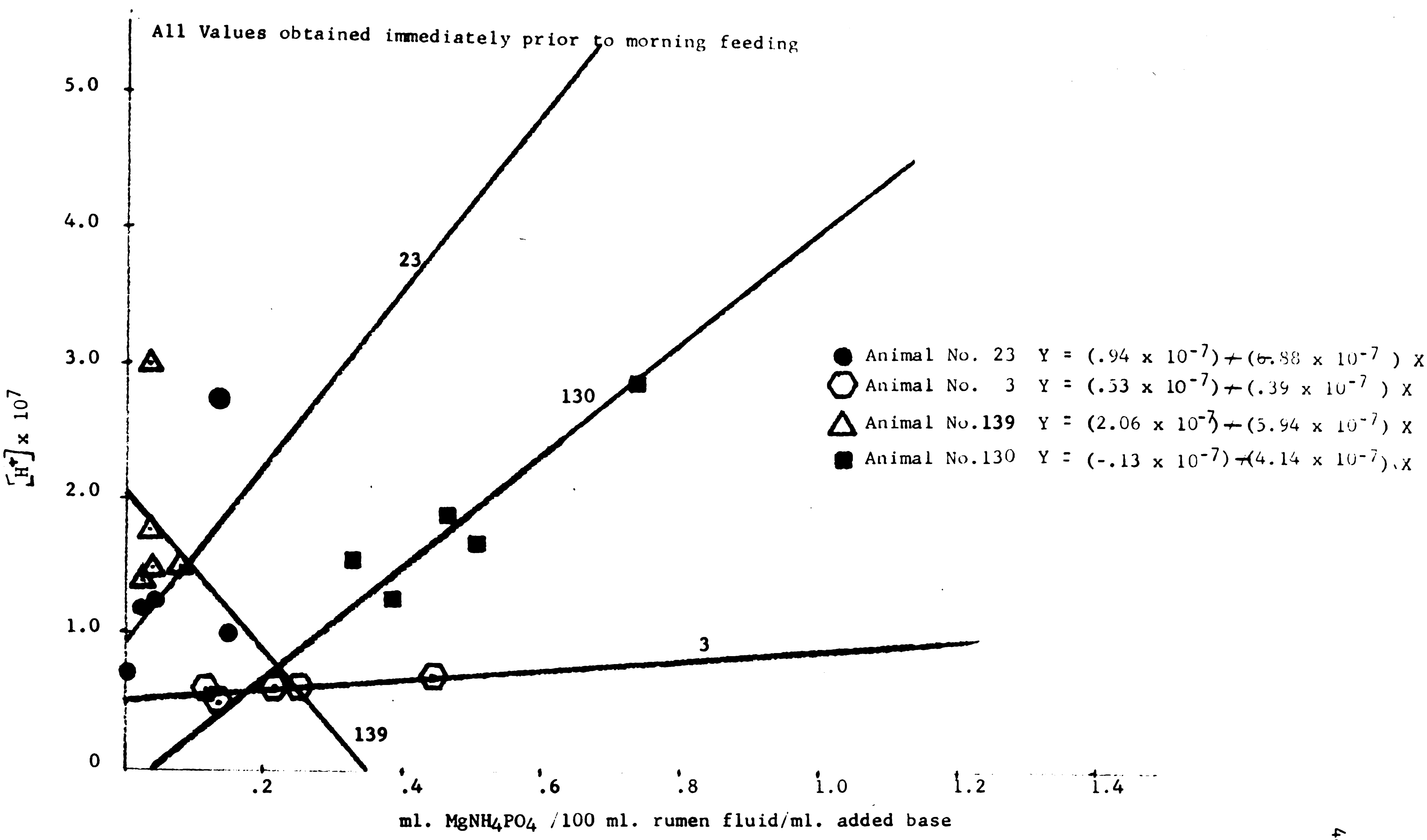


Fig. IV Rumen fluid $MgNH_4PO_4$ vs. rumen fluid hydrogen ion concentration
(Single Ration Comparisons)

Fig. V. Rumen Fluid $MgNH_4PO_4$ vs. Calculated Mg, P, and H in Rumen Ingesta

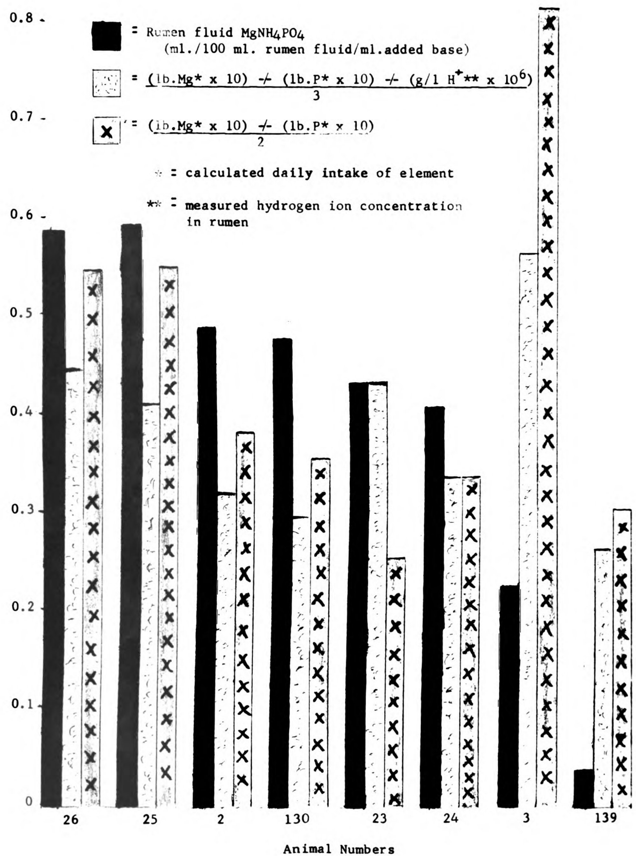


Table 4. Calculated Mg and P Intake, Rumen Fluid Hydrogen Ion Concentration, and Observed Rumen Fluid Magnesium Ammonium Phosphate-containing Precipitate

Ingredient	Values used in Calculations	
	% Mg	% P
Corn & Cob meal	0.085+	0.22
SOM	0.27	0.64
Alfalfa hay	0.31	0.24
Timothy hay	0.17	0.14
Ration 24*	0.13	0.34
Ground Corn	0.10	0.27
Corn starch	0.10+	0.27+
Ration 52*	0.085	0.22
Dicalcium phosphate	0	0.18

+value estimated from whole grain value

Animal	Frothy Ingesta	Ration	Rumen Fluid $[H^+]$g/l x 10^{-7}	Daily Intake(lb.) Mg	P	x**	y***	ml. $MgNH_4PO_4$/100ml. rumen fluid/ml. added base
25	+	G	2.62	0.044	0.058	0.43	0.51	0.59
25	—	G	1.25	0.044	0.058	0.38	0.51	0.59
2	+	A	2.02	0.029	0.042	0.30	0.35	0.49
130	—	I	1.86	0.039	0.034	0.30	0.36	0.48
23	—	J	8.14	0.020	0.032	0.44	0.26	0.44
24	—	F	3.50	0.024	0.040	0.33	0.32	0.41
3	—	K	0.62	0.093	0.072	0.57	0.82	0.23
139	—	M	1.85	0.034	0.028	0.27	0.31	0.04

*Composition given in Table 3

$$**x = \frac{(\text{lb.Mg} \times 10) + (\text{lb.P} \times 10) + (\text{g/l } [H^+] \times 10^6)}{3}$$

$$***y = \frac{(\text{lb.Mg} \times 10) + (\text{lb.P} \times 10)}{2}$$

Table 5. Statistical

Animal	Ration*	No. Samples	X**	Y***	r[a]	t[b]	p[c]
$MgNH_4PO_4$-ppt. (X) vs. Hydrogen Ion Concentration (Y); Animal & Ration Comparisons							
2	A	4	.78	3.02	+.987	+8.70	$<.02$
25	G	5	.59	1.25	+.968	+6.65	$<.01$
26	G	5	.52	2.24	+.963	+6.19	$<.01$
130	I	5	.48	1.86	+.907	+3.73	$<.05$
24	F	5	.41	3.30	+.879	+3.19	$<.05$
3	K	5	.23	0.63	+.686	+1.64	$>.05$
23	M	5	.06	1.38	+.558	+1.16	$>.05$
139	M	5	.04	1.85	−.183	− .32	$>.05$
$MgNH_4PO_4$-ppt. (X) vs. ml. of added 10% NH_4OH (Y); Animal Comparisons							
1	E	5	.73	.73	+.987	+10.82	$<.01$
2	A,B,D	14	.62	.70	+.570	+2.40	$<.05$
3	K	15	.12	.41	0	0	$>.05$
23	K,M	16	.05	.39	−.243	− .94	$>.05$
24	C,F,K	12	.27	.59	+.253	+ .83	$>.05$
25	G,K	15	.17	.41	+.184	+ .67	$>.05$
26	G,K,L	14	.21	.54	+.249	+ .89	$>.05$
130	H,I	16	.21	.49	−.302	−1.19	$>.05$
139	K,M	8	.04	.40	0	0	$>.05$

Analyses of Results

Ration*	Animal	No. Samples	X**	Y***	r^a	t^b	p^c
$MgNH_4PO_4$-ppt. (X) vs. ml. of added 10% NH_4OH (Y); Ration Comparisons							
A	2	3	.34	.69	0	0	$>.05$
B	2	4	.84	.78	−.646	−1.19	$>.05$
C	24	5	.65	.67	+.906	+3.70	$<.05$
D	2	7	.61	.66	+.856	+3.70	$<.02$
E	1	5	.73	.73	+.987	+10.82	$<.01$
F	24	7	.29	.65	+.462	+1.17	$>.05$
G	25,26	11	.29	.49	+.293	+ .92	$>.05$
H	130	9	.20	.52	−.430	−1.26	$>.05$
I	130	7	.22	.44	0	0	$>.05$
K	3,25,26	24	.13	.43	+.179	+ .85	$>.05$
L	26	7	.13	.48	+.161	+ .37	$>.05$
M	23,139	21	.03	.40	−.186	− .82	$>.05$
All Samples		115	.24	.50	+.634	+8.69	$<.01$

* Refer to Table 2

** ml. $MgNH_4PO_4$/100 ml. Rumen Fluid/ml. added base

*** g. $H^+ \times 10^7$/l. Rumen Fluid

a = Coefficient of Correlation = $\sqrt{1 - \frac{s^2y.x}{s^2y}}$

b = t = $\frac{r\sqrt{N-2}}{\sqrt{1-r^2}}$

c = P = Probability or Level of significance

SUMMARY

The isolation and identification of a colorless, crystalline compound spontaneously formed and experimentally precipitated from rumen fluid was found to be mainly hexahydrate magnesium ammonium phosphate.

The amount of this material precipitated from rumen fluid was influenced by the hydrogen ion concentration of the rumen fluid.

The quantity of material formed was related to the type of ration fed. However, animals fed identical rations yielded amounts of magnesium ammonium phosphate proportional to rumen fluid hydrogen ion concentration.

The magnesium and phosphorus intakes of the animals appeared to influence the amount of magnesium ammonium phosphate formed only when a specific concentration of hydrogen ions was present in rumen fluid.

The amount of magnesium ammonium phosphate-containing precipitate isolated from rumen-fistulated animals displaying frothy ingesta was approximately three times that of animals exhibiting nonfrothy ingesta.

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