A STUDY OF GRASS TETANY

by

EUGENE DONALD GRIM

B. S., Kansas State College, 1945

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1946

TABLE OF CONTENTS

INTRODUCTION AND PURPOSE

Grass tetany, lactation tetany, grass staggers, and wheat poisoning are all terms used to denote a metabolic disorder occurring in cattle, apparently, as a result of the use of grass pasturage. Since grass pasturage is of considerable importance in the nutrition of cattle, particularly in the case of the cattle-raiser of the Middle-Western United States where wheat grass is abundant throughout the fall, winter, and spring grazing season, any disease associated with its use is of notable consequence.

The disease, although virtually unknown fifty years ago, has increased its incidence greatly in recent years. The mortality associated with the disorder is comparatively high. Since death follows the first appearance of symptoms with haste (in many cases the animal may succumb within an hour after the onset of the initial signs), it is impossible to avoid some fatalities, even with the most apt therapy.

These facts and the knowledge that so little is known with regard to the prevention of the disease have, naturally, aroused a growing concern in the farmer. Further study, particularly in the etiology of the condition, is, then, of prime importance.

An imbalance in the blood mineral picture involving an increase in the ratio of monovalent mineral ions to divalent mineral ions is generally attendant to the disease. This has suggested the possibility of the influence of minerals in the nutrition of the animal, particularly with respect to pasture grasses. Investigations concerning this possibility should aid materially in the solution of the causes of the disorder.

REVIEW OF THE LITERATURE

The first mention of grass tetany in the literature appeared in 1928 in an article by Dayus (1) which included several statements regarding the outbreak of a "new, mysterious disease" as reported by several farmers.

A short time later, Sjollema, whose investigations have provided a basis for most of the present-day work on the disorder, described the gross symptoms of the disease (2). He pointed out that the disorder had been observed for thirty or forty years in Holland, but had become important in recent years only, during which time the number of cases had increased materially.

The initial symptoms became apparent shortly after the cows were moved into spring pasturage following the winter stall feeding. Although the symptoms varied from one case to the next, many signs were typical of all the afflicted animals. Restlessness and hyperexcitability indicated the initial phases of an attack. If the attack came on slowly, anorexia was observed. The animal was subject to muscular twitching and short, convulsive movements of the legs. It stood with hanging head, gnashing its teeth, and lowing continually. The eyes rolled wildly and salivation increased. As the condition progressed certain muscles, particularly those of the neck and tail showed tetanic contractions. The gait became awkward; and the animal staggered, butting into fence posts, outbuildings, and other obstructions. As the staggering grew worse the animal alternately fell and rose until, finally, its legs could not maintain the body weight in an upright position. As the animal lay, the muscles were observed to be in clonic-tonic convulsions, producing violent movements of the body. Soon the cow passed into a comatose condition with all the muscles in tetanic contraction. When the attack progressed to this stage, a fatality was almost a certainty.

In addition to this, he also reported a marked decrease in the serum calcium and magnesium in cattle showing the symptoms of grass tetany. He listed average values of 6.65 mgm per 100 cc of serum and 0.46 mgm per 100 cc for calcium and magnesium, respectively, in 52 cows tested. These values were compared with those obtained from 12 normal cows, where calcium and magnesium were reported as 9.35 mgm and 1.66 mgm, respectively. Furthermore, he suggested that the ratio $\frac{K-Na}{Mg-Ca}$ might be important since it changed from 30 to 46 in normal cows which became afflicted with the disorder.

Sjollema and Seekles (3,4) reported further on the condition by pointing out that the disease occurred most frequently in those dairy cattle, who were the best milk producers during their lactation period. In addition, the incidence was highest in the first days of spring pasturage. They also pointed out the therapeutic value of intravenous injections of calcium chloride in cases of grass tetany and milk fever. Supplementary determinations on the serum calcium and magnesium of afflicted animals produced average values of 6.0 mgm and 0.40 mgm for calcium and magnesium, respectively. Autopsies were made in several cases where fatalities had occurred; but these revealed no specific evidence. The death struggle seemed to be responsible for the few affectations in the muscles and the lungs.

Warringholz (5) concurred with these investigators in reiterating the fact that the disease occurred most frequently in the early spring pasturing period in good milkers, four to seven years of age, during the lactation period.

In 1932, Sjollema (6) published the first comprehensive review of grass tetany, including most of the knowledge which had been collected concerning the disorder prior to that year. Hare (7) discussed the symptoms of the

disease and reported several post mortem examinations which failed to provide any conclusive data. A published speech by Dryerre (8) told of the speaker's experience with grass tetany. He reported consistently low values for serum magnesium, but found little change in the calcium content of the blood. These findings agreed with his theory that magnesium inhibited nervous action and calcium stimulated it, thus indicating the importance of the $\frac{Ca}{Mg}$ ratio. He also reported finding the serum potassium increased in some cases. During this same year, Dehecq (9) described the appearance of a "pseudo milk fever" characterized by tetany occurring three to six weeks after parturition in various pasture areas in France. He demonstrated the merit of intravenous injections of calcium gluconate as an efficient therapeutic.

Sjollema (10) in a consideration of the causes of grass tetany indicated the use of ill-balanced winter rations containing an excess of cereals and very little hay as a pre-disposing factor. The sudden transition from this type of feeding to pasture grasses containing large amounts of protein, potassium nitrate, and low calcium and sodium was considered as precipitating, if not direct, causal, factors. He failed to agree with Dehecq in the use of calcium gluconate as a therapeutic agent. He stated that it was too slow in action and harmful in its effect on the heart.

Sjollema and Seekles (11, 12, 13) then conducted a series of experiments in an attempt to produce grass tetany artificially. Since young improved pastures may contain as high as two per cent potassium nitrate on a dry basis; and, further, since a cow in full lactation may ingest 300 gms of this salt in one day from such a source, it was considered a wise step to investigate the possibility of a correlation between nitrate poisoning and lactation tetany. A year old animal was given 200 gms of potassium nitrate

suddenly by introduction of the material into the rumen through a fistula. Typical symptoms of nitrate poisoning appeared; labored heart, diarrhea, anorexia, and a loss of body weight. About one-tenth of the nitrate fed was reduced to nitrite which in turn produced a methemoglobinemia by the partial oxidation of hemoglobin. The blood calcium and magnesium fell to about 80 per cent of its normal value, but no symptoms of grass tetany were apparent.

Since young pasture grass is very high (25-30 per cent on a dry basis) in protein content, a cow may ingest as much as four or five kgm of protein per day. Again by introducing large amounts of high protein grain meal into the rumen through a fistula, an attempt was made to produce the disease. The animal died with no appearance of signs suggestive of grass tetany. Its blood picture was virtually normal with respect to the minerals and the subsequent autopsy proved inconclusive.

The next experiment was carried out under conditions of greater similarity to natural ones. A young cow was fed pasture grass containing 2.9 per cent potassium nitrate on a dry basis. Although no signs of methemoglobinemia appeared, the serum calcium and magnesium fell from 11 mgm to 7.7 mgm and 2.0 mgm to 1.2 mgm, respectively. This fall in blood minerals, however, was not attended with any gross symptoms of grass tetany.

Broersma (14) suggested the possibility that the disorder might be connected with use of high-nitrogenous substances in the manuring of pastures. Kuipers (15) concurred by submitting the same proposal. However, Johnstone (16) failed to get any conclusive results in determining the effect of feeding grass from pastures which had been fertilized with rich top-dressing. He also pointed out that, although the serum magnesium was low in all cases of lactation tetany, the serum calcium fluctuated between

a low value and the normal value.

Sjollema (17) investigated the possibility of the aid of A. T. 10, an irradiated ergosterol product, in the prevention and cure of grass tetany with no conclusive results.

Blakemore and Stewart (18) reported on several outbreaks of lactation tetany in Great Britain, in which the serum calcium and magnesium decreased considerably. Particular emphasis was placed on the hypomagnesemia attendant to the disease. They pointed out considerable variation in the normal level of serum magnesium, by showing a 2.1 mgm average in one group and a 2.97 mgm average in another. In still another, the lowest value obtained was 2.8 mgm. They stated that the serum calcium returned to normal soon after the disappearance of gross symptoms while some evidences of hypomagnesemia remained for as long as a month. The injection of a magnesium salt could produce a spectacular recovery of the serum magnesium in less than 24 hours, however.

Allcroft and Greene (19) reviewed the literature concerning lactation tetany to date. In addition, they reported the serum calcium and magnesium as determined on 139 normal cows. The calcium varied between 8.65 to 11.65 mgm with 92 per cent of the values in the range of 9-11.5 mgm. An average magnesium value was given as 2.0 mgm with a variation of 1.85 to 3.17 mgm. In 18 cases of lactation tetany, pronounced hypomagnesemia (0.3-1.16 mgm) and general hypocalcemia (4.8-10.8 mgm) was reported. In several cases the serum calcium was apparently normal.

Hopkirk and co-workers (20) gave pasture and blood analysis in several cases of grass tetany. They found magnesium and calcium low in a number of cases; and found the serum potassium normal in six cases. Attempts were made to produce tetany artificially by feeding potassium nitrate, sodium

oxalate, high-protein meals, and diets low in calcium and magnesium. All attempts were unsuccessful. They concluded that a bacterial toxin was a strong possibility. Carlstrom (21) theorized that hypocalcemia altered the capillary and cell permeability such that metabolic toxins which are normally present could enter the cell, thus producing tetany.

A short time later Hopkirk (22) published a review from the clinical standpoint on rye-grass staggers, paspaulum staggers, and grass staggers. He found that some cases of hypomagnesemia might be connected with the use of excess superphosphate in fertilizing. He cured the condition by drenching or injecting Epsom salts. He considered the $\frac{Ca}{Mg}$ ratio to be of great importance since he supposed that the calcium produced hyperirritability.

The New Zealand Department of Agriculture (23) demonstrated the fact that dietary magnesium is not a factor in the disease. It could not be produced artificially by feeding a magnesium-deficient diet to pregnant cows for the three months preceding the calving period.

Several investigators have reported the occurrence of a disorder similar to grass tetany in other animals. Wright (24) reported a high mortality in ewes which had been shipped by rail after being removed from sparse pasture where they had lambed 14-35 days previously. The symptoms included tetanic convulsions, and the disorder was alleviated by injection of a mixture of calcium chloride and magnesium sulfate. De Gier (25) described a "transit tetany" in lactating ponies in which the serum calcium was decreased; however, the serum magnesium remained at a nearly normal level or increased slightly. Injected calcium and magnesium salts promulgated recovery in one hour in most cases. Blumer and co-workers (26) gave a short discussion on grass tetany in sheep, pointing out that it occurred in lactating ewes and was accompanied by hypocalcemia and hypermagnesemia.

Duncan and co-workers (27) observed a tetany produced in calves which had been fed on a pure milk diet. In most cases, hypomagnesemia with normal serum calcium was noted. Sjollema (32), in a further study of tetany in calves, pointed out that the disorder observed by Duncan was probably primarily the result of protein and not magnesium.

Allcroft and Green (28) found a seasonal variation in the serum magnesium of cattle where some determinations showed the magnesium to decrease to a value as low as 0.5 mgm in the period beginning in August and ending in December. There were no evidences of hyperirritability or tetany. Dietary magnesium had no apparent effect on this phenomenom. It was thought that this hypomagnesemia occurring in the winter might be a pre-disposing factor to the grass tetany which would appear in the spring.

Allcroft and Godden (29) in observing the changes in the mineral blood picture of the cow during the parturition period felt that grass tetany might be an exaggeration of the hypocalcemia attendant to calving. It had already been noted in a number of cases that the disorder appeared most frequently in dairy cows immediately post-partum. They found the average value for serum calcium to be 7.95 mgm, and also found a rise in the serum magnesium, reporting an average value of 2.7 mgm. Robinson and Huffman (30) and Hibbs and co-workers (31) have reported a hypocalcemia, but normal magnesium values.

Duncan and Huffman (33) reported the results of 2286 determinations on normal cattle plasma for magnesium. They found a range of 1.62 to 3.83 mgm with the norm at $2.414 \pm .005$ mgm per cent.

Several investigators in recent times have more or less substantiated the results obtained by other research workers in the field of grass tetany in the past. Nicholson and Shearer (34) determined calcium, magnesium,

phosphorous, sodium, potassium, chloride, non-protein nitrogen, protein, and hemoglobin in the blood of animals afflicted with grass tetany in Ireland with no evidences of changes in anything except magnesium and calcium. Nolan and Hull (35) reported the serum magnesium and calcium in 12 cases of grass tetany in Kentucky. The average values were 0.9 mgm and 6.6 mgm, respectively. McMillen and Langham (36) considered growing wheat to be an excellent source of calcium, magnesium, and phosphorous. In several cases of grass tetany they found a decrease in serum calcium and phosphorous and an increase in serum magnesium. They could find no uniformity in mineral ratio; i.e., the $\frac{Ca}{Mg}$ ratio. This suggested the disease to be a complex nervous or glandular disorder rather than a simple mineral deficiency.

A most recent review on the action of mineral elements on nerve irritability including a theory to explain the etiology of grass tetany by Caldwell and Hughes (37) contained the first attempt to uncover the basic factors in any hypo- or hyperirritability involving a disturbance in tissue minerals. They offered fundamental explanations for the profound physiological reactions of mineral elements in the animal body, including the antagonistic actions of some of them. Several, apparently unrelated, facts in physical chemistry and physiology were viewed together to produce the theory.

As Clowes (38) had pointed out, one fact of importance in the field of physical chemistry, was that soaps of monovalent ions (potassium and sodium) produce an emulsion of the "oil in water" type, while soaps of the divalent ions (calcium and magnesium) produced emulsions of the "water in oil" type. The former, since water is the continuous phase, would permit the free passage of electrically charged ions; but, the latter, with a lipid continuous phase, would not. In a mixture of the two types of soaps, the ratio

of the monovalent ions to the divalent ions, then, determined the electrical conductivity of the mixture.

In the physiology of the nervous system, these authors pointed to the well-known fact that nervous tissue contains a large amount of lipid material which would be capable of becoming one phase of an emulsion. Hence, in this "natural" emulsion, one could expect the mineral balance to play an important role. This was considered to be particularly true, if the membrane theory of nervous conduction, for which a great deal of experimental evidence had been amassed by Lillie (39), was considered plausible. In this theory, as discussed by Starling (40) and Best and Taylor (41), conduction was considered to be a surface phenomenom. The nerve fiber is surrounded by a semi-permeable membrane which is polarized when the nerve is at rest; that is, a layer of anions is contained on the inner side of the surface film, while a layer of cations is on the outer side. These layers are redistributed and the membrane depolarized when a stimulus is applied to the nerve, as a result of an increase in the permeability of the membrane. This depolarization causes a potential difference between the "activated" and adjacent "inactive" portions of the nerve. The net result is a flow of the "action current".

Caldwell and Hughes pointed out that an increase of potassium in the nerve permitted this depolarization to occur more readily, since the membrane became an emulsion, predominantly of the "oil in water" type. Thus nervous irritability was increased. The opposite effect was produced by an increase of the divalent ions.

A theory based on these statements, offered, in the authors' opinions, an explanation for the typical hyperirritability of cattle stricken with grass tetany, where the calcium or magnesium were lowered in the tissues.

Furthermore, the same theory applied to cases of familial periodic paralysis as reported in a review by Schoenthal (42). In this disease, a paralysis and hypoirritability were the outstanding symptoms. It was overcome by injections of potassium citrate as reported by Herrington (43). A tetany in cattle developed when the animals are driven or being loaded into cars for shipment could be explained by an increase in serum potassium, since Fenn (44) pointed to several references in which an increase in serum potassium as a result of muscular activity was reported.

Fenn (44) further pointed out that potassium is the most important of the monovalent elements in nervous transmission, since it is found in relatively larger amounts in the tissues than the others.

Zwemer and Truszkowski (45) produced an anorexia and muscular asthenia by increasing the blood potassium of cats. Further increase resulted in convulsions and subsequent death. They stated that a 50 per cent increase in the potassium level would return to normal in six minutes.

Spiegal and co-workers (46) found an increase in the potassium of the cerebro-spinal fluid as a result of tonic-clonic convulsions in 30 dogs which had been induced by an electric current.

Caldwell and Hughes (37) considered the high potassium content of wheat grass in the early spring (as much as five per cent on a dry weight basis as reported by Miller (47)) to be a factor in the increase of tissue potassium; and, hence, a factor in grass tetany. Miller found a seasonal fluctuation in the potassium content of wheat grass, with the two peaks occurring in the spring and late fall, coincident with the appearance of the majority of cases of grass tetany.

METHODS

Preparation of Materials

Wheat grasses were analyzed for potassium content; and the whole blood and plasma (or serum, in some cases) of cattle were analyzed for the content of potassium, sodium, calcium, and magnesium.

The samples of wheat grasses were obtained, for the most part, at a time when the plant was in the "leaf stage" and had not yet begun to branch. Each specimen was placed in a hot air oven at a temperature of 100° - 105° C and dried until crisp and brittle; this generally required 24 hours. This material was, in turn, ground in a Wiley mill to the fineness of a 100-mesh sieve, and stored in glass jars. The chemical analysis was carried on from this point.

All blood samples collected at the experiment station barn were drawn by means of venous puncture in the jugular with a hypodermic syringe needle. The blood was permitted to flow freely into citrated (10 mgm of sodium citrate per 30 cc of whole blood) 50 cc glass centrifuge tubes. The aliquots used in the determination of sodium, however, were not treated. In the latter case, a ten cc sample of blood was permitted to clot and the resulting serum collected. For whole blood sodium, an aliquot was pipetted, immediately following collection, into distilled water, as noted in the method for chemical analysis, to prevent clotting.

The tube containing the citrated blood was shaken vigorously to mix the contents well; and a 15 cc aliquot was poured into a 15 cc graduated centrifuge tube. A cover was placed on the tube; and it was centrifuged for 45 minutes at 3500 r.p.m. The volume of cells was noted and the per cent cell volume was calculated. The supernatant plasma was siphoned off with

suction and placed in a small medicine bottle. All serum and plasma samples were stored at a temperature of -22° C to prevent bacterial or mold growth. The whole blood samples were kept at 4° C and analyzed immediately.

Potassium Determination

The method used was patterned after that of Harris (57) which in turn is a modification of the original methods of Adie and Wood (48) and Kramer (49, 50). Several changes have been made, particularly in the adaptation of the method to the analysis of plant material.

With regard to serum or plasma, 1.0 ml of the material to be analyzed was added to 7.0 ml of distilled water in a conical-tipped 15 cc centrifuge tube. Then, with thorough mixing after each addition, 0.5 ml of 2/3 N sulfuric acid, 0.5 ml of ten per cent sodium tungstate, and 1.0 ml of 1.80 per cent silver nitrate were added. The tube was permitted to stand for 15 minutes and then centrifuged for ten minutes at 2500 r.p.m. A 3.0 ml aliquot, containing approximately 0.06 mgm of the potassium ion was used in the analytical procedure.

In the case of whole blood, 1.0 ml of the material was added to 16.0 ml of distilled water in a 50 cc glass centrifuge tube. Then, with thorough mixing after each addition, 1.0 ml of 2/3 N sulfuric acid, 1.0 ml of ten per cent sodium tungstate, and 1.0 ml of 1.80 per cent silver nitrate were added. The tube was permitted to stand for 15 minutes and then centrifuged for ten minutes at 2500 r.p.m. A 3.0 ml aliquot, containing approximately 0.06 mgm of the potassium ion was used in the analytical procedure.

In the analysis of plant material, a 0.100 gram sample of the dried material was accurately weighed into a porcelain crucible. A few drops of 2 N sulfuric acid were added and the mixture stirred with a platinum wire to

moisten all of the material thoroughly. The crucible and its contents were heated at 70° C until almost dry; and, then, transferred to a cold muffle calibrated to reach a temperature of 500° C. The material was ashed for 16 hours, removed, and allowed to cool.

The ash was dissolved in 0.2 ml of 2/3 N sulfuric acid, and transferred to a 100 cc volumetric flask with approximately 70 ml of distilled water. 0.2 ml of ten per cent sodium tungstate was added and the solution made up to 100 cc. The contents of the flask were thoroughly shaken and filtered. An aliquot containing 0.06 to 0.12 mgm of the potassium ion was used in the analytical procedure.

Analytical Procedure. An aliquot of the filtrate containing 0.06 to 0.12 mgm of the potassium ion (usually 3.0 ml) was pipetted into a conical tipped 15 cc graduated centrifuge tube. After the addition of 1.0 ml of 95 per cent ethanol, the mixture was stirred with a bent platinum stirring rod. An equal amount of the standard was treated in like manner.

The tubes were placed in a constant temperature water bath, maintained at 20° C for five minutes. Without removing the tubes, 2.0 ml of the precipitating reagent was added to each one. The contents were thoroughly mixed, and the tubes left in the bath for an additional 30 minutes.

At the end of this time, they were removed and centrifuged immediately for ten minutes of 2800 r.p.m. The supernatant liquid was removed to approximately 0.2 ml with suction by means of a capillary tube. Then, approximately seven ml of wash solution was blown onto the precipitate from a pipette. The precipitate and the reagent were thoroughly mixed. The tubes were again centrifuged and the supernatant liquid removed this time by decantation. The tubes were drained on coarse, dry filter paper for about five minutes; and, after the lips of the tubes were wiped clean, the washing

was repeated.

After the final centrifugation and draining, 10 ml of 0.2 N sodium hydroxide was blown onto the precipitate from a pipette. The precipitate and reagent were mixed completely. The tubes were than placed in a boiling water bath for ten minutes, after which they were removed and cooled to room temperature in running water. The contents were made up to exactly ten ml, mixed, and centrifuged for five minutes at 2800 r.p.m.

A 2.0 ml aliquot from each tube was pipetted into 50 cc graduated cylinders and mixed with a further addition of 15 ml of ten per cent acetic acid. Then, 1.0 ml of 0.5 per cent α-naptholamine and 2.0 ml of 0.5 per cent sulfanilic acid were added, the contents mixed, and the color permitted to develop for exactly six minutes. At the end of this time, the solution was made up to 50 ml with ten per cent acetic acid and mixed by inversion. The sample was compared with the standard in an Evelyn photoelectric colorimeter using a 440 mμ transmission filter. The blank was made by diluting three ml of the diazotizing materials to 50 cc with ten per cent acetic acid. Readings had to be made within one hour.

Reagents.

1. Potassium standard:
   (a) 0.2228 gm of potassium sulfate was made up to one liter with water.
   (b) To 10.0 ml of this standard, 1.0 ml each of 2/3 N sulfuric acid and ten per cent sodium tungstate was added, and this mixture made up to 50 ml with distilled water. One ml of this solution is equivalent to 0.02 mgm of the potassium ion.
2. Precipitating reagent:

(a) 120 gms of sodium nitrite was dissolved in 180 ml of distilled water.

(b) 25 gms of cobalt nitrate was dissolved in a mixture of 50 ml of distilled water and 12.5 ml of glacial acetic acid.

(c) 210 ml of the sodium nitrite solution was added to the cobalt nitrate solution; and this mixture was aereated for one to two hours.

(d) One-tenth volume of the mixture in (c), usually 28 ml, of forty per cent silver nitrate was added, a few ml at a time. The mixture was shaken vigorously after each addition. The reagent was stored in a glass-stoppered bottle in the refrigerator and warmed to room temperature and filtered before use. It was found best to reareate it weekly and discard at the end of one month.

3. Protein precipitants:

(a) 2/3 N H_2SO_4 -- 18.5 ml of concentrated sulfuric acid was diluted to one liter with distilled water.

(b) 10% Na_2WO_4 -- 10 gms of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) was dissolved and made up to 100 ml with distilled water.

4. 1.80% $AgNO_3$ -- 1.80 gms of silver nitrate was dissolved and made up to 100 ml with distilled water.

5. Wash reagent -- a mixture of one part of "O"-free diethyl ether, two parts of 95 per cent ethanol, and two parts of distilled water was used. It was made fresh daily.

6. 0.2 N NaOH -- 8.0 gms of sodium hydroxide was dissolved and made up to one liter with distilled water.

7. 10% acetic acid -- 100 ml of glacial acetic acid was diluted to one

liter with distilled water.

8. 0.5% α-naptholamine -- 0.5 gm of α-naptholamine was dissolved in 30 ml of glacial acetic acid and diluted to 100 ml with distilled water. This reagent had to be prepared fresh every few days.
9. 0.5% sulfanilic acid -- 0.5 gm of sulfanilic acid was dissolved and made up to 100 ml with 30 per cent acetic acid. It was prepared fresh every week.

Sodium Determination

The analytical procedure used, with a few slight modifications, was that of Salit (51).

In the analysis of serum, 1.0 ml of the material was added to 9.0 ml of distilled water in a 50 cc Erlenmeyer flask. Then, with constant shaking, 10.0 ml of 20 per cent trichloroacetic acid was added. The flask was permitted to stand for five minutes; and, then, the mixture was filtered through Whatman #40 filter paper. A 2.0 ml aliquot, containing 0.3 to 0.4 mgm of the sodium ion was used in the analytical procedure.

In the analysis of whole blood, 1.0 ml of the material was added to 15 ml of distilled water in a 50 cc Erlenmeyer flask immediately following withdrawal of the sample from the animal. Then, with constant shaking, 4.0 ml of 20 per cent trichloroacetic acid was added. The flask was permitted to stand for five minutes; and, then, the mixture was filtered through Whatman #40 filter paper. A 2.0 ml aliquot, containing 0.2 to 0.3 mgm of the sodium ion was used in the analytical procedure.

Analytical Procedure. A 2.0 ml aliquot of the filtrate prepared as directed above was placed in a conical-tipped 15 cc centrifuge tube. A like aliquot of the standard

was treated in the same manner. To each tube, then, was added six cc of freshly filtered precipitating reagent.

With a 1 cc Mohr pipette graduated to 0.1 cc, exactly 0.3 ml of absolute ethanol was added to each tube. The alcohol was stirred into the solution with a platinum wire stirring rod. The bulk of the precipitate was allowed to settle to the bottom of the tube and another 0.3 ml of ethanol added. The stirring was repeated without disturbing the precipitate on the bottom. After a few minutes another 0.3 ml was added, and the stirring repeated. This procedure was repeated four more times until 2.1 ml of ethanol had been added over a period of at least 30 minutes.

The tubes were centrifuged for 10 minutes at 3000 r.p.m. and the supernatant liquid decanted. The tubes were returned to an upright position and were <u>not</u> allowed to drain. Five ml of freshly filtered wash reagent was added to the tubes; and the precipitate was thoroughly stirred and distributed throughout the solution.

After centrifuging again, the tubes were decanted and drained on coarse, dry filter paper. The mouth of each tube was wiped with a moist cloth to remove all traces of precipitating reagent and a drop of glacial acetic acid added to each tube.

The tube was then filled with distilled water to a point one inch from the top. The precipitate was dissolved by vigorous stirring, and the contents of the tube transferred, by means of a small funnel, to a 50 cc volumetric flask. This procedure was repeated twice more; and, finally, the funnel rinsed into the flask, and the volume made up to mark with distilled water. The contents of the flask were well mixed.

A 10.0 ml aliquot of the contents of the flask was pipetted into a 50 cc graduated cylinder. This was made up to 50 ml with distilled water and

mixed. To the cylinder, then, was added, 0.5 ml of 20 per cent potassium ferrocyanide; and the contents were well mixed by inversion. The samples were allowed to stand for one hour and then compared with the standard in an Evelyn photoelectric colorimeter using a 620 mμ transmission filter. The blank was made by adding 0.5 ml of the color reagent to 50 ml of water.

Reagents.

1. Precipitating reagent:
   (a) 46 ml of 30 per cent acetic acid (by volume) was added to 80 gms of uranyl acetate. Distilled water was then added to make the mixture up to 520 gms; and the salt was dissolved on a steam bath.
   (b) 23 ml of 30 per cent acetic acid (by volume) was added to 220 gms of zinc acetate. Distilled water was then added to make the mixture up to 520 gms; and the salt was dissolved on a steam bath.
   (c) Solutions (a) and (b) were mixed while hot, allowed to stand 24 hours, and filtered. This reagent kept indefinitely and was re-filtered before use.
2. Wash solution -- 15 cc of the uranyl zinc acetate precipitating reagent was added to one cc of five per cent sodium chloride with subsequent additions of about five cc of 95 per cent ethanol in small portions. This mixture was filtered with suction and washed, alternately, with ether and alcohol, the last washing being made with ether. The filter residue was added to one liter of glacial acetic acid which was shaken vigorously for several hours to dissolve the salt. The reagent was filtered before use.
3. Sodium standard -- exactly 5.000 gm of c. p. sodium chloride was

dissolved in distilled water and made up to one liter. 1.0 ml of this solution contains 1.967 mgm of the sodium ion. This stock solution was diluted 1 to 10 for comparison in the serum determination, and 1 to 20 in the whole blood determination.

4. Color reagent -- 0.5 gm of potassium ferrocyanide was dissolved and made up to 100 ml with distilled water. The reagent was discarded after one week.

5. 20% CCl_3COOH -- 20 gms of trichloroacetic acid was dissolved and made up to 100 ml with distilled water. It was stored in the refrigerator.

Calcium Determination

The procedure used was a slight modification of the method proposed by Wang (52).

To 5.0 ml of whole blood, serum, or plasma in a 50 cc Erlenmeyer flask, 10.0 ml of distilled water, and 10.0 ml of 20 per cent trichloroacetic acid were added. The flask was stoppered, shaken vigorously, and allowed to stand for five minutes. The solution was then filtered through a Whatman #40 filter paper into a clean, dry test tube.

Analytical Procedure. A 5.0 ml aliquot of this filtrate was pipetted into a conical-tipped 15 cc centrifuge tube. Then the following materials were added: 1.0 ml of 20 per cent sodium acetate; 0.25 ml of 0.016 per cent bromcresol green indicator; 1.0 ml of 0.1 M ammonium oxalate; and 0.45 ml of 1 + 3 ammonium hydroxide (in the case of serum or plasma; with whole blood, 0.35 ml of the base was added). This mixture was thoroughly stirred with a platinum wire stirring rod; and the resulting solution showed a color indicative of the required pH of 5. The tube was allowed to stand overnight.

A precaution in the addition of the oxalate solution was observed; in that, considerable difficulty arose in washing out the excess precipitating reagent if this reagent was not added directly to the solution in the tube.

At the end of the precipitation period, the tube was centrifuged for eight minutes at 2000 r.p.m. The supernatant liquid was carefully decanted into a conical-tipped 15 cc centrifuge tube and analyzed for magnesium as outlined in the analytical procedure for that element.

Approximately five ml of wash solution was then added to the tube and thoroughly mixed with the precipitate. The solution was poured into the tube such that the sides of the tube were rinsed of any excess oxalate reagent. This mixture was centrifuged, the supernatant liquid decanted, and the washing repeated.

After the final centrifugation and decantation, the inverted tube was placed in a 35° C oven for at least six hours to remove the last traces of any organic solvent.

Then 2.0 ml of approximately normal sulfuric acid was delivered from a pipette into the tube with care to wash the sides of the tube with the acid. The mixture was stirred in order to get the calcium oxalate into solution, and then placed in a boiling water bath for one minute. The oxalic acid was immediately titrated with 0.01 N potassium permanganate from a microburette graduated in 0.01 cc and with a tip delivering not more than 0.010 cc at a drop.

Reagents.

1. 20% CCl_3COOH -- 20 gms of trichloroacetic acid was dissolved and made up to 100 ml with distilled water. It was stored in a refrigerator.
2. 20% $NaCH_3COO$ -- 20 gms of sodium acetate was dissolved and made

up to 100 ml with distilled water. This reagent was discarded after a week.

3. 0.1 M $(NH_4)_2C_2O_4$ -- 1.42 gm of ammonium oxalate was dissolved and made up to 100 ml with distilled water. This reagent was prepared fresh daily.

4. 1 + 3 NH_4OH -- one part of concentrated ammonium hydroxide was mixed with three parts of distilled water.

5. 1 N H_2SO_4 -- 27 ml of concentrated sulfuric acid was diluted to one liter with distilled water.

6. 0.01 N $KMnO_4$ -- 15.8 gms of c.p. potassium permanganate was dissolved and made up to one liter with distilled water. This stock solution was diluted one to ten before each day's work in the laboratory and standardized with 0.003 N sodium oxalate. The reference solution of sodium oxalate was stored in the refrigerator and remained in good condition for an indefinite length of time.

Magnesium Determination

This procedure is basically the original method of Briggs (53) with a number of modifications as set forth by Duncan (27), Kuttner and Cohen (54), Kuttner and Lichtenstein (55), and Bodansky (56).

The preparation of the protein and calcium free filtrate of serum, plasma, or whole blood has already been discussed in the method for the determination of calcium.

This filtrate was decanted into a conical-tipped 15 cc centrifuge tube. 1.0 ml of the potassium phosphate solution and 1.0 ml of concentrated ammonium hydroxide were added. The inside of the tube was then rubbed with a glass stirring rod to initiate precipitation of the magnesium ammonium

phosphate. The stirring rod was rinsed into the tube with 1.0 ml of 1 + 2 ammonium hydroxide. The tube, then, was stoppered and placed in the refrigerator for at least 16 hours.

Following precipitation, the phosphate was centrifuged down at 3000 r.p.m. for 15 minutes. The supernatant liquid was drawn off to 0.2 ml with suction by means of a capillary tube and 8.0 ml of wash solution was poured into the tube in such a manner that the sides of the tube were washed down. The precipitate was distributed thoroughly throughout the wash solution by stirring and the tube was again centrifuged at the same speed for ten minutes. The supernatant liquid was removed to 0.2 ml as before and the washing repeated. After the final centrifugation and removal of the supernatant liquid, the tube was placed in an oven at 100° C until the precipitate was completely dry and free from ammonia (about two hours).

The precipitate was then dissolved by the addition of 5.0 ml of 1 N sulfuric acid. 4.0 ml of molybdate reagent was added and the contents of the tube mixed by rolling the tube between the palms of the experimenter's hands. Then 1.0 ml of the dilute stannous chloride reagent was added and the tube immediately inverted. A standard was prepared by substituting 5.0 ml of the diluted stock standard solution for the 1 N sulfuric acid above. The samples were compared in the Dubosq colorimeter with the standard set at 20.0 mm.

Due to the gradual deterioration of the molybdate and stannous chloride solutions, a blank containing the color developing materials was prepared daily.

Reagents.

1. 2% KH_2PO_4 -- 2.0 gms of monopotassium hydrogen phosphate was dissolved and made up to 100 ml with distilled water. This reagent

was prepared fresh daily.

2. Rinse solution -- one part of concentrated ammonium hydroxide was diluted with two parts of distilled water.

3. Wash solution -- a mixture of 20 parts of 95 per cent ethanol, 20 parts of concentrated ammonium hydroxide, and 60 parts of distilled water was prepared fresh daily.

4. 10 N H_2SO_4 -- 279 ml of concentrated sulfuric acid (sp. gr. = 1.84) was poured into 600 ml of distilled water. After cooling, the solution was made up to one liter.

5. 1 N H_2SO_4 -- the reagent listed above was diluted one part to nine parts of distilled water.

6. Molybdate reagent -- cold 10 N sulfuric acid, 0.5 volume; and 7.5 per cent molybdate stock solution, one volume, were mixed. While mixing, 2.5 volumes of distilled water were quickly added. This reagent was discarded after one week.

7. 7.5% molybdate stock solution -- 90 gms of molybdic acid was dissolved in 250 ml of 5 N sodium hydroxide in a two liter flask. This was made to volume with distilled water, permitted to stand, and decanted before use.

8. Stannous chloride stock solution -- 15 gms of stannous chloride was dissolved and made up to 25 ml with concentrated hydrochloric acid. This reagent was stored in the refrigerator and prepared fresh at the end of a month.

9. Dilute stannous chloride reagent -- the stock solution was diluted 200 times. This reagent is good for only a few hours.

10. Standard phosphate stock solution -- 0.110 gm of monopotassium hydrogen phosphate was dissolved in water. 2.0 ml of concentrated

sulfuric acid was added and the solution diluted to 250 ml with distilled water.

11. Standard phosphate solution -- 10 ml of the stock solution was diluted to 250 ml with normal sulfuric acid. This standard contained an equivalent of 0.02 mg phosphorous per 5.0 ml of solution.

Calculations

Potassium in Serum or Plasma.

$$\frac{2\text{-log } G_x}{2\text{-log } G_s} \times \text{mgm } K^+ \text{ in stan.} \times \frac{100}{3 \times 0.1} = \text{mgm \% } K^+$$

Potassium in Whole Blood.

$$\frac{2\text{-log } G_x}{2\text{-log } G_s} \times \text{mgm } K^+ \text{ in stan.} \times \frac{100}{3 \times 0.05} = \text{mgm \% } K^+$$

Potassium in Plant Material.

$$\frac{2\text{-log } G_x}{2\text{-log } G_s} \times \text{mgm } K^+ \text{ in stan.} \times 100 = \text{per cent } K^+$$

Sodium in Serum and Whole Blood.

$$\frac{2\text{-log } G_x}{2\text{-log } G_s} \times \text{mgm } Na^+ \text{ in stan.} \times \frac{100}{.1} = \text{mgm \% } Na^+$$

Calcium in Plasma, Serum, and Whole Blood.

$$\frac{\text{Titration} \times 0.2 \times \text{correction} \times 100}{1} = \text{mgm \% } Ca^{++}$$

Magnesium in Plasma, Serum, and Whole Blood. Magnesium was determined as phosphorous. A considerable deviation from Beer's law is present; and, consequently, the formula for calculation contained a correction for this. All samples were compared against a 0.02 mgm standard set at 20 mm.

$$\frac{0.48}{\text{Reading of unknown}} - 0.0040 = \text{mgm P in aliquot}$$

Table 1 lists the corrected amounts of phosphorous at various colorimetric readings. The values in this table were multiplied by a factor to convert them to mgm of magnesium.

Table value x .785 x 100 = mgm % Mg^{++}

Table 1. Inorganic phosphorous in aliquot, at stated colorimetric readings, corrected for deviation from Beer's law, 0.02 mgm standard set at 20 mm.

mm	mgm 0.0	mgm 0.1	mgm 0.2	mgm 0.3	mgm 0.4	mgm 0.5	mgm 0.6	mgm 0.7	mgm 0.8	mgm 0.9
8	0.0560	0.0552	0.0545	0.0538	0.0531	0.0525	0.0518	0.0512	0.0505	0.0499
9	493	487	482	476	471	465	460	455	450	445
10	440	435	431	426	422	417	413	408	404	400
11	396	392	389	385	381	377	373	370	367	363
12	360	357	353	350	347	344	341	338	335	332
13	329	326	324	321	318	316	313	310	308	305
14	303	301	298	296	293	291	289	287	284	282
15	280	278	276	274	272	270	268	266	264	262
16	260	258	256	254	253	251	249	248	246	244
17	242	241	239	237	236	234	233	231	230	228
18	227	225	224	222	221	220	218	217	215	214
19	213	211	210	209	207	206	205	204	203	201
20	200	199	198	197	196	194	193	192	191	190
21	189	188	186	185	184	183	182	181	180	179
22	178	177	176	175	174	173	172	171	170	170
23	169	168	167	166	165	164	163	163	162	161

Table 1 (concl.)

mm	mgm 0.0	mgm 0.1	mgm 0.2	mgm 0.3	mgm 0.4	mgm 0.5	mgm 0.6	mgm 0.7	mgm 0.8	mgm 0.9
24	160	159	158	158	157	156	155	154	153	152
25	152	151	151	150	149	148	147	147	146	145
26	145	144	143	143	142	141	140	140	139	138
27	138	137	137	136	135	135	134	134	133	132
28	132	131	130	130	129	128	128	127	127	126
29	126	125	124	124	123	123	122	122	121	121
30	120	119	119	118	118	117	117	116	116	115
31	115	114	114	113	113	112	112	111	111	110
32	110	110	109	109	108	108	107	107	106	106
33	105	105	105	104	104	103	103	102	102	102
34	101	101	100	100	100	099	099	098	098	098
35	097	097	096	096	096	095	095	095	094	094
36	093	093	093	092	092	092	091	091	090	090

EXPERIMENTAL PROCEDURE

The potassium content of the pasture grass on which cattle were fed in the early spring appeared to be a possible factor in the etiology of grass tetany. As a result, the first part of this study involved the determination of a number of wheat grass samples. The samples analyzed were divided into several series. The first set was obtained from Texas pastures where a number of cases of grass tetany had been reported in the past and where, in some instances, animals had succumbed the same day of the sampling. The second series included a number of varieties and crosses of varieties of Kansas wheat grasses and other pasture grasses. The third were obtained from different parts of the state of Kansas to demonstrate a geographical variation, if any existed. The final part of the study on the potassium content of pasture grasses involved the analyses of grass samples at various stages of their physiological development.

It had been proposed in the literature concerning grass tetany that the ratio of monovalent mineral elements to divalent mineral elements in the blood was a causal factor in this disorder. The second part of this study, then, was concerned with the analysis of blood samples obtained from diseased animals for their potassium content, as well as for the amounts of calcium and magnesium.

The third portion of the experimental work involved an attempt to produce the symptoms of grass tetany in a calf by means of intravenous injections of potassium chloride. The salt solution containing one gram of potassium per ten cubic centimeters of solution was injected by means of a syringe into the jugular vein. All blood samples were drawn from the same vein, alternating the injections and the withdrawals between the left and right sides of the animal. A study of the blood mineral picture was made

throughout the experiment to permit observation of any significant changes.

Since it had been pointed out that the disorder occurred most frequently in lactating cows shortly after parturition, and that the mineral imbalance attendant to this normal physiological process might be a factor responsible for the disease, the final portion of the work involved the determination of potassium, sodium, magnesium, and calcium in the blood of several cows through the post-partum period.

RESULTS

The Potassium Content of Pasture Grasses

Eighteen samples of wheat grass were obtained from the state of Texas from pastures which had a reported record of a number of cases of grass tetany. Later clinical reports on the animals, however, indicated that many of them had died of "bloat". The results are given in Table 2. They are, in general, lower than those obtained in the analysis of samples from the state of Kansas.

Table 2. Potassium content of Texas wheat grass

Sample No.	Potassium (% of dry weight)
1	2.1
2	1.5
3	3.4
4	2.6
5	2.5
6	2.7
7	2.9
8	2.6
9	2.6
10	2.7
11	3.2
12	3.3
13	3.1
14	2.6
15	3.4

Table 2 (concl.)

Sample No.	Potassium (% of dry weight)
16	3.3
17	3.5
18	3.8

Samples 16, 17, and 18 were obtained from pastures where several animals had displayed the symptoms of the disorder a day or two previously.

A number of Kansas pasture grasses of various varieties and cross-breeds of varieties were examined for their potassium content with the results tabulated in Table 3.

Table 3. Potassium content of various varieties of Kansas grasses

Sample No.	Variety	Potassium (per cent of dry weight)
1	Kawvale Wheat	2.9
2	Kawvale Wheat	2.8
3	Kawvale Wheat	3.4
4	Turkey Wheat	3.4
5	Pawnee Wheat	3.6
6	Chieftan Wheat	3.3
7	Red Chief Wheat	2.8
8	Tenmarq Wheat (1)	4.1
9	Tenmarq Wheat (2)	4.2
10	Tenmarq Wheat (3)	4.2
11	Pawnee Wheat (1)	4.3
12	Pawnee Wheat (2)	4.2
13	Pawnee Wheat (3)	4.0

Table 3 (concl.)

Sample No.	Variety	Potassium (per cent of dry weight)
14	Marquillo x Tenmarq Wheat (1)	4.7
15	Marquillo x Tenmarq Wheat (2)	3.9
16	Marquillo x Tenmarq Wheat (3)	4.4
17	Marquillo-Tenmarq x Kawvale-Tenmarq Wheat (1)	4.3
18	Marquillo-Tenmarq x Kawvale-Tenmarq Wheat (2)	4.4
19	Marquillo-Tenmarq x Kawvale-Tenmarq Wheat (3)	3.8
20	Oro x Illinois Wheat (1)	3.7
21	Oro x Illinois Wheat (2)	3.9
22	Oro x Illinois Wheat (3)	4.1
23	Gladden Wheat (1)	4.2
24	Gladden Wheat (2)	4.1
25	Gladden Wheat (3)	3.5
26	Reno Barley	3.4
27	Reno Barley	2.5
28	Reno Barley	3.0
29	Beardless Barley	2.9
30	Common Rye	2.8
31	Common Rye	2.9
32	Common Rye	3.6
33	Lawrence Rye	2.4
34	Balbo Rye	3.3
35	Balbo Rye	3.5
36	Balbo Rye	2.6
37	Cereophyll Rye	3.4
38	Cereophyll Rye	3.3

Samples 8 to 25 include three replicates of each variety.

A series of samples of Tenmarq wheat was taken from various places in the state of Kansas, to permit study of the variations in potassium content as related to geographical distribution. The samples were picked at a time such that the plants were all approximately equal in physiological age. Table 4 and Figures 1 and 2 show the results of the investigation.

Table 4. Geographical variations in the potassium content of Kansas grown Tenmarq wheat grass (1944)

Sample No.	Location of Field	Potassium (% of dry weight)
1	Garden City	3.0
2	Garden City	3.3
3	Meade	3.8
4	Meade	3.9
5	Dodge City	4.5
6	Dodge City	4.3
7	Hays	3.6
8	Hays	3.5
9	Smith Center	4.5
10	Smith Center	4.7
11	Kingman	3.4
12	Kingman	3.5
13	Belleville	4.8
14	Belleville	4.7
15	Wichita	3.3
16	Wichita	3.3
17	Manhattan	3.7

Table 4 (concl.)

Sample No.	Location of Field	Potassium (% of dry weight)
18	Manhattan	4.3
19	Thayer	3.5
20	Thayer	3.4
21	Columbus	2.5
22	Columbus	2.4
23	Hutchinson	3.4
24	Hutchinson	3.8

The results of the analyses on three samples of wheat grass which were picked at three different times during the years, 1944-45, in Manhattan, Kansas are shown in Table 5.

Table 5. Seasonal fluctuation in the potassium content of wheat grasses

Sample No.	Season	Month	Potassium (% of dry weight)
1	Late Fall	November	5.3
2	Late Fall	November	5.0
3	Late Fall	November	3.4
4	Winter	January	3.4
5	Winter	January	2.7
6	Winter	January	2.1
7	Spring	March	3.8
8	Spring	March	4.1
9	Spring	March	3.8

In 1945, three varieties of Kansas wheat grasses were picked at different stages of their physiological development in a number of fields

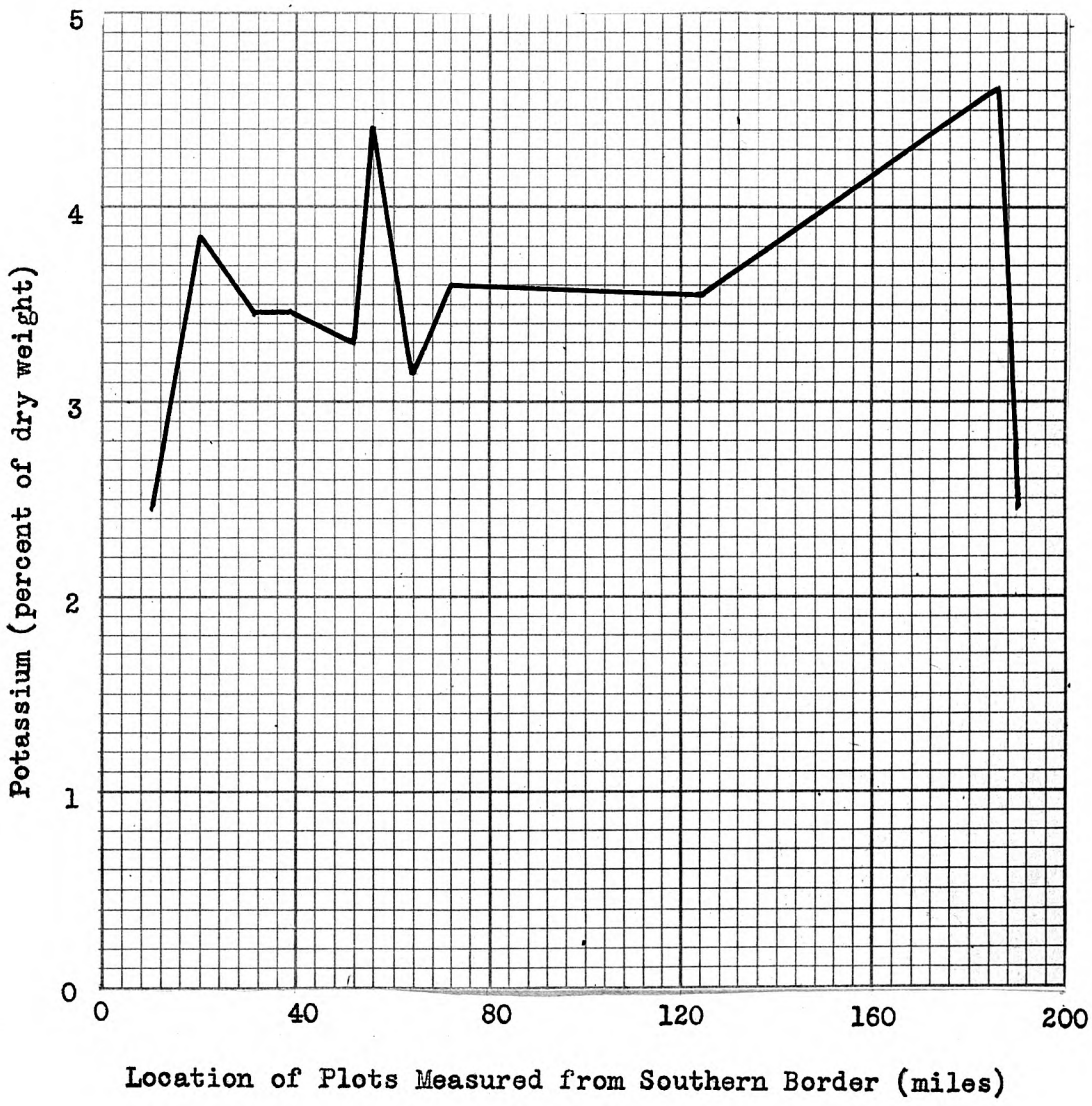

Figure 1. Geographical variations in the potassium content of Kansas wheat grasses with regard to the north-south distribution of plots.

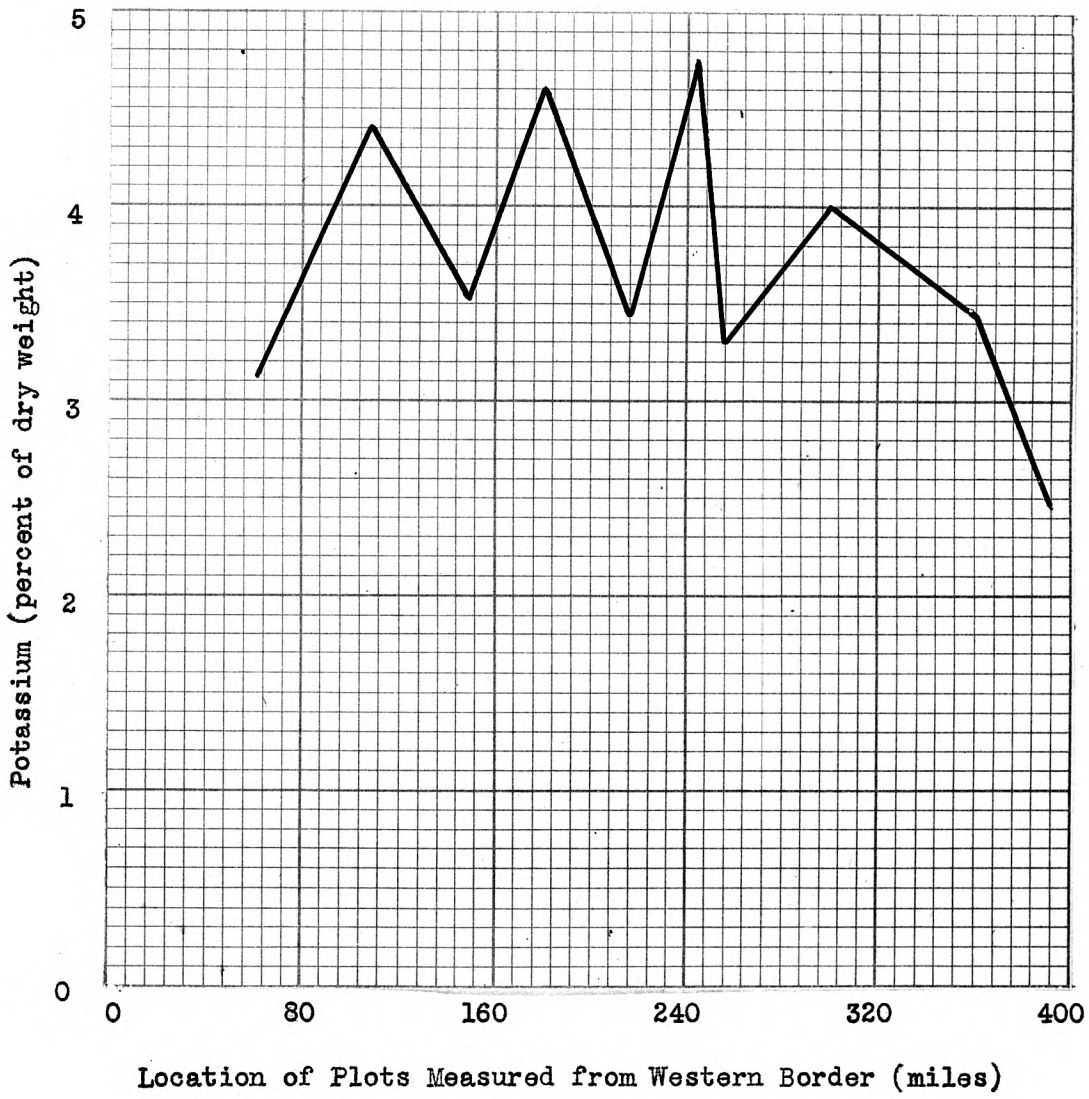

Figure 2. Geographical variations in the potassium content of Kansas wheat grasses with regard to the east-west distribution of plots.

over the state. The results are given in Table 6.

Table 6. The variation of the potassium content of three varieties of wheat grass at different stages of growth

Location	Variety	Date Picked	% K
Columbus	Turkey	3-26-45	2.2
Columbus	Turkey	4-5-45	2.0
Columbus	Turkey	4-15-45	1.6
Columbus	Blackhull	3-26-45	2.1
Columbus	Blackhull	4-5-45	1.9
Columbus	Blackhull	4-15-45	1.8
Columbus	Tenmarq	3-26-45	2.7
Columbus	Tenmarq	4-5-45	2.2
Columbus	Tenmarq	4-15-45	1.9
Thayer	Turkey	3-26-45	3.2
Thayer	Turkey	4-5-45	2.6
Thayer	Turkey	4-15-45	3.0
Thayer	Blackhull	3-26-45	3.2
Thayer	Blackhull	4-5-45	2.8
Thayer	Blackhull	4-15-45	2.8
Thayer	Tenmarq	3-26-45	3.1
Thayer	Tenmarq	4-5-45	3.0
Thayer	Tenmarq	4-15-45	2.8
Wichita	Turkey	3-27-45	3.6
Wichita	Turkey	4-5-45	3.0
Wichita	Turkey	4-15-45	2.9
Wichita	Blackhull	3-27-45	3.8
Wichita	Blackhull	4-5-45	3.3
Wichita	Blackhull	4-15-45	3.1
Wichita	Tenmarq	3-27-45	3.4
Wichita	Tenmarq	4-5-45	3.0
Wichita	Tenmarq	4-15-45	3.2
Kingman	Turkey	3-27-45	3.5
Kingman	Turkey	4-5-45	3.3
Kingman	Turkey	4-15-45	2.9
Kingman	Blackhull	3-27-45	3.7
Kingman	Blackhull	4-5-45	3.0
Kingman	Blackhull	4-15-45	2.9

Table 6 (cont.)

Location	Variety	Date Picked	% K
Kingman	Tenmarq	3-27-45	3.5
Kingman	Tenmarq	4-5-45	2.9
Kingman	Tenmarq	4-15-45	2.9
Hutchinson	Turkey	3-27-45	3.5
Hutchinson	Turkey	4-5-45	3.2
Hutchinson	Turkey	4-15-45	2.8
Hutchinson	Blackhull	3-27-45	3.6
Hutchinson	Blackhull	4-5-45	3.1
Hutchinson	Blackhull	4-15-45	3.4
Hutchinson	Tenmarq	3-27-45	3.9
Hutchinson	Tenmarq	4-5-45	3.0
Hutchinson	Tenmarq	4-15-45	2.8
Dodge City	Turkey	3-28-45	4.1
Dodge City	Turkey	4-5-45	3.6
Dodge City	Turkey	4-15-45	3.4
Dodge City	Blackhull	3-28-45	3.9
Dodge City	Blackhull	4-5-45	3.9
Dodge City	Blackhull	4-15-45	3.2
Dodge City	Tenmarq	3-28-45	3.5
Dodge City	Tenmarq	4-5-45	3.4
Dodge City	Tenmarq	4-15-45	3.1
Meade	Turkey	3-28-45	4.1
Meade	Turkey	4-5-45	3.7
Meade	Turkey	4-15-45	3.4
Meade	Blackhull	3-28-45	3.6
Meade	Blackhull	4-5-45	3.7
Meade	Blackhull	4-15-45	3.4
Meade	Tenmarq	3-28-45	3.7
Meade	Tenmarq	4-5-45	4.2
Meade	Tenmarq	4-15-45	3.3
Garden City	Turkey	3-28-45	3.8
Garden City	Turkey	4-5-45	3.3
Garden City	Turkey	4-15-45	3.3
Garden City	Blackhull	3-28-45	3.6
Garden City	Blackhull	4-5-45	3.6
Garden City	Blackhull	4-15-45	2.9

Table 6 (cont.)

Location	Variety	Date Picked	% K
Garden City	Tenmarq	3-28-45	3.7
Garden City	Tenmarq	4-5-45	3.8
Garden City	Tenmarq	4-15-45	3.0
Tribune	Turkey	3-28-45	3.4
Tribune	Turkey	4-10-45	3.5
Tribune	Turkey	4-20-45	3.1
Tribune	Blackhull	3-28-45	3.3
Tribune	Blackhull	4-10-45	3.3
Tribune	Blackhull	4-20-45	2.6
Tribune	Tenmarq	3-28-45	3.6
Tribune	Tenmarq	4-10-45	3.6
Tribune	Tenmarq	4-20-45	2.8
Colby	Turkey	3-29-45	3.7
Colby	Turkey	4-10-45	3.7
Colby	Turkey	4-20-45	3.0
Colby	Blackhull	3-29-45	3.6
Colby	Blackhull	4-10-45	3.4
Colby	Blackhull	4-20-45	2.8
Colby	Tenmarq	3-29-45	3.7
Colby	Tenmarq	4-10-45	3.7
Colby	Tenmarq	4-20-45	3.2
Hays	Turkey	3-29-45	3.5
Hays	Turkey	4-5-45	3.5
Hays	Turkey	4-15-45	3.1
Hays	Blackhull	3-29-45	3.5
Hays	Blackhull	4-5-45	3.3
Hays	Blackhull	4-15-45	3.2
Hays	Tenmarq	3-29-45	3.2
Hays	Tenmarq	4-5-45	3.3
Hays	Tenmarq	4-15-45	2.4
Smith Center	Turkey	3-29-45	3.6
Smith Center	Turkey	4-10-45	3.5
Smith Center	Turkey	4-20-45	3.0
Smith Center	Blackhull	3-29-45	3.7
Smith Center	Blackhull	4-10-45	3.6
Smith Center	Blackhull	4-20-45	3.2
Smith Center	Tenmarq	3-29-45	4.1

Table 6 (concl.)

Location	Variety	Date Picked	% K
Smith Center	Tenmarq	4-10-45	3.5
Smith Center	Tenmarq	4-20-45	2.9
Belleville	Turkey	3-29-45	3.5
Belleville	Turkey	4-10-45	3.8
Belleville	Turkey	4-20-45	2.8
Belleville	Blackhull	3-29-45	3.3
Belleville	Blackhull	4-10-45	3.6
Belleville	Blackhull	4-20-45	2.6
Belleville	Tenmarq	3-29-45	3.4
Belleville	Tenmarq	4-10-45	3.6
Belleville	Tenmarq	4-20-45	3.0
Manhattan	Turkey	3-30-45	2.9
Manhattan	Turkey	4-5-45	3.2
Manhattan	Turkey	4-15-45	3.0
Manhattan	Turkey	4-25-45	3.0
Manhattan	Turkey	5-7-45	2.9
Manhattan	Turkey	5-14-45	2.7
Manhattan	Turkey	5-21-45	2.4
Manhattan	Turkey	5-28-45	2.2
Manhattan	Blackhull	3-30-45	3.3
Manhattan	Blackhull	4-5-45	3.2
Manhattan	Blackhull	4-15-45	3.0
Manhattan	Blackhull	4-25-45	3.1
Manhattan	Blackhull	5-7-45	2.8
Manhattan	Blackhull	5-14-45	2.5
Manhattan	Blackhull	5-21-45	2.6
Manhattan	Blackhull	5-28-45	2.3
Manhattan	Tenmarq	3-30-45	3.3
Manhattan	Tenmarq	4-5-45	3.2
Manhattan	Tenmarq	4-15-45	3.0
Manhattan	Tenmarq	4-25-45	3.1
Manhattan	Tenmarq	5-7-45	3.0
Manhattan	Tenmarq	5-14-45	2.8
Manhattan	Tenmarq	5-21-45	2.9
Manhattan	Tenmarq	5-28-45	2.5

Blood Minerals of Grass Tetany Affected Animals

A few blood samples from cattle which had succumbed to the disorder were obtained. A summary of the incomplete mineral analyses are shown in Table 7.

Table 7. Mineral content of bovine blood

Sample No.	Remarks	Type of Sample	Potassium (mgm %)	Calcium (mgm %)	Mg (mgm %)
1	4 yr. old lactating Jersey, 1½ months post-partum, milk fever	Serum	22.8		
2	8-10 yr. old lactating Hereford, 2 weeks post-partum, grass tetany	Serum	30.0		
3	Same sample as 2	Whole Blood	52.4	3.7	1.77
4	Animal was unable to rise	Serum	25.5		
5	Same sample as 4	Whole Blood	97.2	4.2	1.89
6	Animal had recovered spontaneously	Serum	22.5		
7	--	Serum	54.0	10.1	2.26
8	--	Serum	30.0	9.8	1.76
9	Non-lactating cow showing some signs of convulsions	Serum		7.8	
10	Same sample as 9	Whole Blood	175.1	5.0	
11	Animal showed typical symptoms of grass tetany	Serum	74.3	11.6	3.36
12	Same sample as 11	Whole Blood	67.4	5.4	

Table 7 (concl.)

Sample No.	Remarks	Type of Sample	Potassium (mgm %)	Calcium (mgm %)	Mg (mgm %)
13	Animal showed typical symptoms of grass tetany	Serum		6.2	
14	Same sample as 13	Whole Blood	107.8	4.8	
15	Animal recumbent, unable to rise	Serum	48.0	7.7	
16	Animals from same herd as 9-15, but showed no symptoms	Serum	36.0	10.1	
17	of grass tetany	Serum		10.8	
18	Same sample as 16	Whole Blood		7.0	
19	Same sample as 17	Whole Blood	46.0	6.8	
20	Normal, healthy animals with no apparent pre-disposition to	Plasma	20.0	11.4	2.3
21	grass tetany	Whole Blood	40.8	7.2	2.4
22	Normal, healthy animal	Plasma	29.8	11.1	2.61
23	Same sample as 22	Whole Blood	50.0	8.6	2.62
24	Normal, healthy animal, one month before calving period	Plasma	31.8	10.4	2.96
25	Same sample as 24	Whole Blood	55.4	7.9	3.09

Artificially Induced "Grass Tetany"

Two animals were subjected to potassium chloride injections in an attempt to produce the symptoms typical to grass tetany.

The first animal selected was an Ayrshire bull weighing 250 pounds and aged six months.

On the first day of the experiment, the injection of one gram of potassium produced no visible effect.

The animal showed slight signs of muscular tension and some twitching of the tail muscles with the second dosage which contained three grams of potassium and was given two days later.

On the fourth day of the experiment, an injection of four grams was introduced into the vein. The calf fell to the stable floor immediately following the injection and was unable to rise. A blood sample was drawn quickly, as the animal became subject to definite convulsive movements. A calcium gluconate injection was started, but the heart was weak and respiration quite labored, and the animal succumbed five minutes after the original potassium chloride injection. The symptoms observed, since they were of such short duration, could not be readily classed as typical of grass tetany.

Table 8 contains the results of the mineral analyses on the blood samples taken.

One of the major difficulties in this experiment was the failure to control the rate of injection. To overcome this and to obtain further data, a second attempt was made. This time the injection rate was standardized at one gram of potassium per minute. This animal was a Jersey bull calf, aged six months and weighing 200 pounds.

Three injections were given to the animal at two day intervals; the first one containing one gram of potassium and the next two, three grams. All of these produced no visible effects.

The fourth injection of four grams of the mineral resulted in some signs of hyperirritability as the animal staggered for a short time; but these signs were short-lived; and the animal appeared to be normal within a few minutes after the end of the injection.

During the next dosage, the calf went to its knees at the end of four

minutes (an injection of four grams of potassium), but recovered and rose again at the end of the complete five gram injection. He staggered several times and finally fell to the ground unable to rise. His muscles, particularly those of the neck and legs, displayed the typical tetanic convulsions of grass tetany. There was also a violent contraction of the smooth muscles as was indicated by excessive urination and defecation. Meanwhile the heart weakened and finally stopped after a 30 cc injection of twenty per cent calcium chloride. The complete picture was one of grass tetany in which the symptoms of the attack had been telescoped into a very short period of time.

The results of the determination of the blood minerals are given in Table 9. Post mortem examination of the carcass produced no evidence of a conclusive nature.

Table 8. Fluctuations in blood minerals resulting from potassium injections.

Date of Injection	Amount & Rate of Injection	Time of Blood Sample	Sample No.	Cell Volume	K^+ (mgm/100 cc) Whole Blood	K^+ Plasma	K^+ Cells	Na^+ (mgm/100 cc) Whole Blood	Na^+ Serum	Na^+ Cells	Ca^{++} (mgm/100 cc) Whole Blood	Ca^{++} Plasma	Ca^{++} Cells	Mg (mgm/100 cc) Whole Blood	Mg Plasma	Mg Cells
May 21 1945	1 gm K^+ 0.5 gm per min.	Before Injection	G-49	36%	40.8	20.0	77.8	242	304	131	7.2	11.4	0	2.4	2.3	2.6
		3 Min. After	G-50	36%	41.6	25.0	71.1	244	319	111	6.6	11.5	0	2.2	2.5	1.7
		4 Hr. After	G-51	36%	----	21.4	----	245	348	64	---	11.8	---	---	2.1	---
May 23 1945	3 gm K^+ 1.5 gm per min.	Before Injection	G-52	36%	40.6	18.6	79.8	266	308	192	6.7	11.1	0	2.6	2.0	3.7
		3 Min. After	G-53	38%	40.6	23.0	69.3	272	323	189	6.6	11.0	0	2.5	2.1	3.2
		4 Hrs. After	G-54	35%	41.8	21.4	79.8	294	339	212	8.9	11.7	3.7	2.4	2.1	2.9
May 25 1945	4 gm K^+ 4.0 gm per min.	Before Injection	G-55	31%	31.9	20.1	58.1	277	329	161	8.8	11.9	1.9	2.1	2.0	2.3
		½ Min. After	G-56	30%	53.7	40.9	83.7	---	---	---	8.4	11.5	1.2	2.0	2.1	1.8
		8 Min. After	G-57		----	36.5	----	---	349	---	---	13.4	---	---	2.2	---

Table 9.

Date of Injection	Amount of Injection	Time of Blood Sampling	Sample No.	Cell Volume	Potassium (mgm/100 cc) Whole Blood	Potassium (mgm/100 cc) Plasma	Potassium (mgm/100 cc) Cells	Sodium (mgm/100 cc) Whole Blood	Sodium (mgm/100 cc) Serum	Sodium (mgm/100 cc) Cells	Calcium (mgm/100 cc) Whole Blood	Calcium (mgm/100 cc) Plasma	Calcium (mgm/100 cc) Cells	Magnesium (mgm/100 cc) Whole Blood	Magnesium (mgm/100 cc) Plasma	Magnesium (mgm/100 cc) Cells
July 16 1945	1 gm K^+	Before Injection	G-86	33%	50.0	29.8	90.9	284	329	194	8.6	11.1	3.5	2.62	2.61	2.64
		3 Min. After	G-87	31.5%	50.0	30.0	93.4	277	323	178	8.3	10.7	3.0	2.62	2.50	2.89
		3 Hour After	G-88	31.5%	47.7	28.3	89.9	291	323	222	7.7	10.3	2.1	2.60	2.54	2.73
July 18 1945	3 gm K^+	Before Injection	G-93	34.5%	44.9	24.1	84.4	302	319	269	8.5	11.1	3.6	2.64	2.57	2.78
		3 Min. After	G-94	33.5%	46.0	25.3	87.3	302	---	---	8.5	10.0	5.5	2.63	2.39	3.10
		3 Hour After	G-95	33.5%	44.8	24.9	84.2	285	344	167	8.7	11.3	3.6	2.71	2.55	3.01
July 20 1945	3 gm K^+	Before Injection	G-97	31.5%	41.7	22.8	82.9	311	317	298	8.6	11.0	3.4	2.68	2.36	3.36
		3 Min. After	G-98	30.5%	46.9	29.5	86.5	299	323	246	8.6	10.8	3.6	2.65	2.37	3.28
		3 Hour After	G-99	31.0%	43.2	22.3	89.7	280	---	---	8.6	10.7	3.9	2.66	2.32	3.42
July 25 1945	4 gm K^+	Before Injection	G-106	30.5%	43.5	36.0	60.6	291	321	223	8.1	11.0	1.5	2.90	3.04	2.56
		3 Min.45 Sec. After	G-107	31.0%	51.8	43.0	71.7	277	317	190	8.1	10.6	2.6	2.91	2.86	3.03
		3 Hour After	G-108	30.5%	46.4	35.6	70.9	300	327	239	8.6	11.1	2.9	3.05	2.76	3.70
July 27 1945	5 gm K^+	Before Injection	G-109	29.5%	45.6	25.7	93.3	291	325	210	8.8	11.4	2.5	2.89	2.65	3.46
		$2\frac{1}{2}$ Min. After	G-110	--	--	50.3	--	273	322	---	---	13.0	---	----	3.16	----
		14 Min. After	G-111	--	--	34.6	--	---	440	---	---	50.7	---	----	3.07	----

Mineral Composition of the Dairy Cow Immediately Post-Partum

The potassium, sodium, calcium, and magnesium content of several samples of blood drawn from six dairy cows in the first few days after parturition was determined. Four samples were drawn from each animal; the first, immediately following parturition; the second, 24 hours later; the third, 72 hours post-partum; and the fourth, 168 hours after the birth of the calf.

Six animals were used in the experiment:

(1) 371A, a Jersey heifer that had been on standard winter rations with a pasture supplement, calved at 6:45 A.M., July 2, 1945.

(2) 366A, a Jersey heifer that had been on standard winter rations only, calved at 12:45 P.M., July 2, 1945.

(3) 365A, a Jersey heifer that had been on standard winter rations with a supplement of vitamin A, calved at 4:00 P.M., July 5, 1945.

(4) 347A, a Jersey cow that had been on standard winter rations with a pasture supplement, calved at 6:45 A.M., July 6, 1945.

(5) 191A, a Holstein cow that had had the same feed as 347A, calved at 4:00 P.M., July 16, 1945.

(6) 124A, a Holstein heifer that had had the same feed as 347A, calved at 2:30 P.M., July 20, 1945.

Table 10 gives the potassium content of the whole blood, plasma, and cells of the various samples drawn from the six animals. Tables 11, 12, and 13 show similar results for the sodium, calcium, and magnesium determinations. Figures 3, 4, 5, and 6 give a graphical interpretation of the post-partum changes in the blood minerals, as based on the averages of the values obtained from all six animals.

Table 10. Changes in blood potassium of cows in the post-partum period

Animal	Sample No.	Sample Withdrawn (hours post-partum)	Potassium (mgm %) Whole Blood	Plasma	Cells
371A	G-61	9	64.4	20.6	137.4
	G-63	34	76.3	30.2	156.5
	G-67	82	49.9	19.0	117.0
	G-79	178	47.5	29.9	85.8
366A	G-62	3	62.6	32.1	108.3
	G-64	28	66.3	24.6	143.8
	G-66	76	53.8	22.0	115.8
	G-78	172	44.3	23.9	86.7
365A	G-65	1	54.5	16.7	116.2
	G-73	25	54.2	18.7	133.4
	G-76	73	52.8	18.2	129.8
	G-84	168	54.6	36.1	89.3
347A	G-70	4	63.5	23.6	118.5
	G-74	29	53.8	18.8	112.4
	G-77	82	60.6	27.6	107.0
	G-85	178	51.0	35.6	78.4
191A	G-89	1	59.1	29.8	116.0
	G-91	24	51.5	30.5	95.0
	G-96	73	43.6	29.4	75.2
	G-105	168	43.9	25.3	87.4
124A	G-100	2	49.4	26.8	88.0
	G-103	26	56.3	27.4	107.8
	G-104	74	46.3	25.0	87.8
	G-112	171	41.1	24.9	74.8

Table 11. Changes in blood sodium of cows in the post-partum period

Animal	Sample No.	Sample Withdrawn (hours post-partum)	Sodium (mgm %) Whole Blood	Serum	Cells
371A	G-61	9	280	344	173
	G-63	34	292	370	156
	G-67	82	293	370	124
	G-79	178	287	350	149
366A	G-62	3	285	347	193
	G-64	28	295	365	166
	G-66	76	303	358	197
	G-78	172	301	340	218

Table 11 (concl.)

Animal	Sample No.	Sample Withdrawn (hours post-partum)	Sodium (mgm %) Whole Blood	Serum	Cells
365A	G-65	1	288	371	153
	G-73	25	301	393	97
	G-76	73	303	---	---
	G-84	168	286	325	214
347A	G-70	4	285	345	202
	G-74	29	281	362	147
	G-77	82	286	336	214
	G-85	178	282	324	206
191A	G-89	1	275	331	168
	G-91	24	307	331	255
	G-96	73	289	320	219
	G-105	168	272	302	200
124A	G-100	2	277	329	189
	G-103	26	285	308	244
	G-104	74	291	336	203
	G-112	171	283	317	212

Table 12. Changes in blood calcium of cows in the post-partum period

Animal	Sample No.	Sample Withdrawn (hours post-partum)	Calcium (mgm %) Whole Blood	Plasma	Cells
371A	G-61	9	7.2	10.3	2.0
	G-63	34	7.1	10.5	1.2
	G-67	82	8.2	11.1	1.9
	G-79	178	8.5	11.5	1.9
366A	G-62	3	7.1	10.5	2.0
	G-64	28	7.0	9.9	1.5
	G-66	76	7.8	10.5	2.6
	G-78	172	7.9	9.8	4.0
365A	G-65	1	7.7	11.0	2.3
	G-73	25	8.5	10.5	4.0
	G-76	73	8.8	10.6	4.8
	G-84	168	8.3	11.3	2.7
347A	G-70	4	5.7	8.5	1.8
	G-74	29	5.7	7.4	2.9
	G-77	82	7.8	10.4	4.1
	G-85	178	7.3	10.0	2.5

Table 12 (concl.)

Animal	Sample No.	Sample Withdrawn (hours post-partum)	Calcium (mgm %) Whole Blood	Plasma	Cells
191A	G-89	1	7.6	9.9	3.1
	G-91	24	8.2	10.2	4.0
	G-96	73	8.0	10.5	2.4
	G-105	168	8.4	10.5	3.5
124A	G-100	2	7.6	10.6	2.5
	G-103	26	7.6	10.5	2.5
	G-104	74	7.3	10.1	1.9
	G-112	171	7.7	10.4	2.1

Table 13. Changes in blood magnesium of cows in the post-partum period

Animal	Sample No.	Sample Withdrawn (Hours post-partum)	Magnesium (mgm %) Whole Blood	Plasma	Cells
371A	G-61	9	3.08	2.48	4.09
	G-63	34	2.93	3.89	1.26
	G-67	82	2.69	3.73	0.42
	G-79	178	2.46	2.56	1.94
366A	G-62	3	3.11	4.95	0.35
	G-64	28	3.14	4.30	0.97
	G-66	76	2.87	4.42	----
	G-78	172	2.77	3.10	2.06
365A	G-65	1	2.58	3.80	0.58
	G-73	25	2.75	3.14	1.90
	G-76	73	2.45	2.57	2.19
	G-84	168	2.72	2.68	2.80
347A	G-70	4	3.12	3.86	1.86
	G-74	29	3.49	4.58	1.68
	G-77	82	2.32	2.67	1.81
	G-85	178	3.01	3.08	2.89
191A	G-89	1	2.78	3.05	2.24
	G-91	24	2.50	2.72	1.91
	G-96	73	2.63	2.64	2.61
	G-105	168	2.40	2.43	2.33
124A	G-100	2	2.80	3.05	2.38
	G-103	26	2.91	2.85	3.03
	G-104	74	2.76	2.62	3.04
	G-112	171	3.03	2.85	3.39

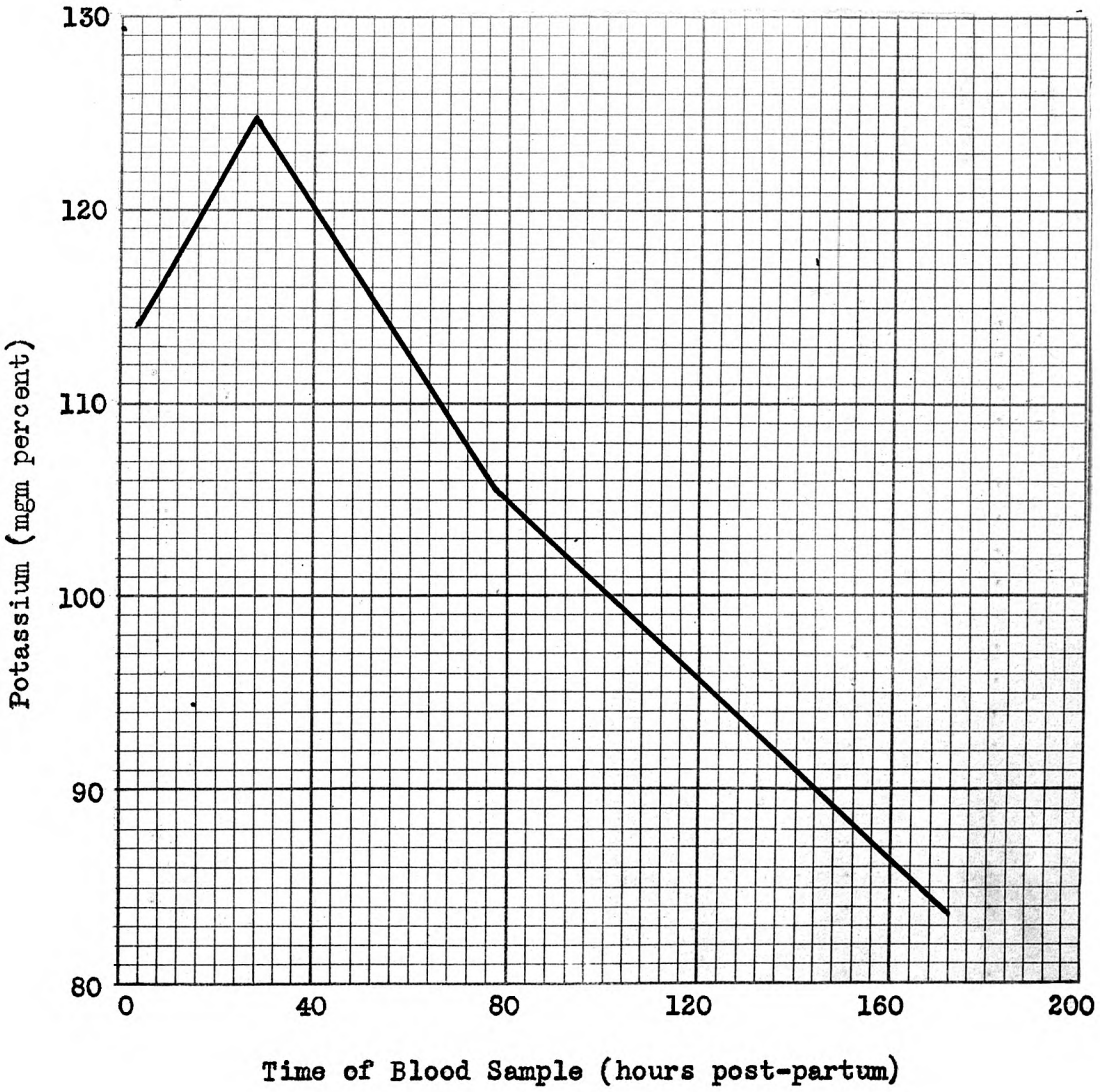

Figure 3. The fluctuations in the potassium content of the red cells of six cows immediately post-partum.

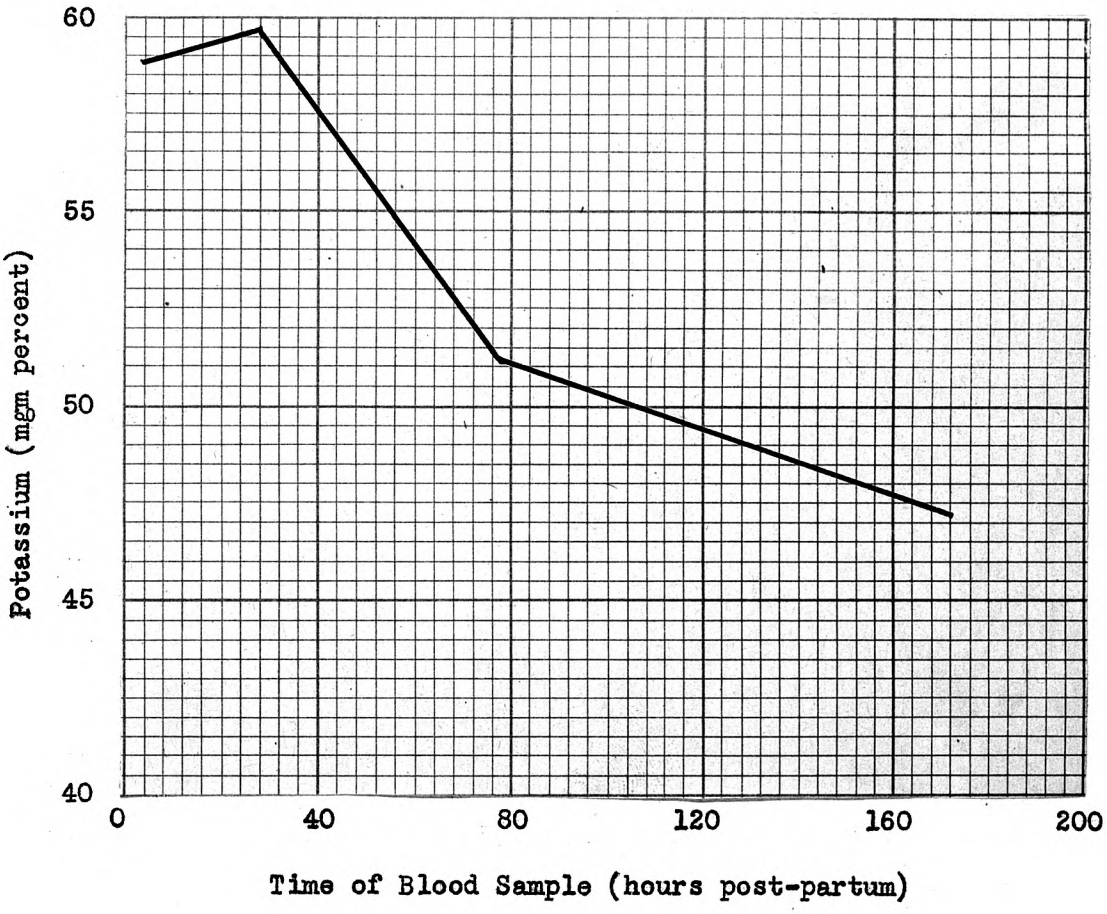

Figure 4. The fluctuations in the potassium content of the whole blood of six cows immediately post-partum.

Figure 5. The fluctuations in the calcium content of the plasma of six cows immediately post-partum.

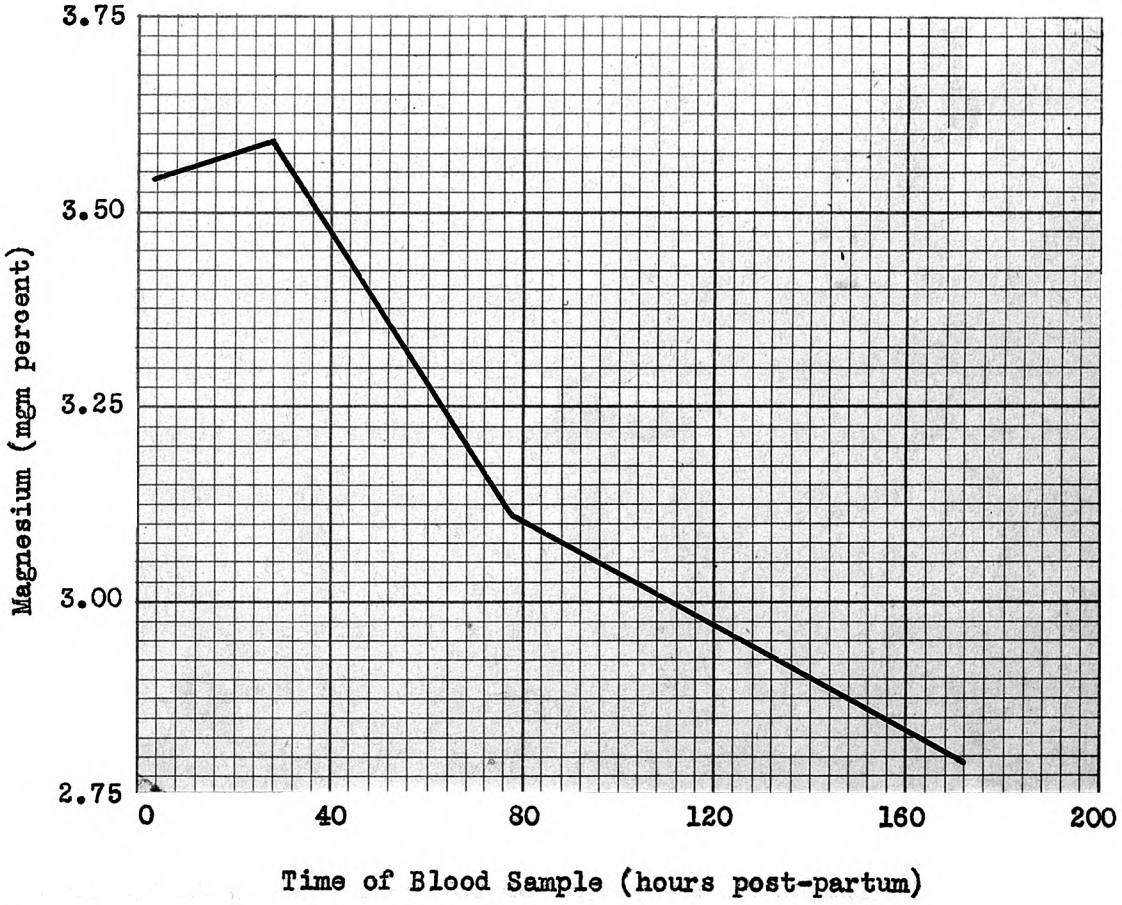

Figure 6. The fluctuations in the magnesium content of the plasma of six cows immediately post-partum.

DISCUSSION

The determination of a number of samples of wheat grasses substantiates the reports of other investigators in that values up to 5.3 per cent potassium on a dry weight basis were found.

In studying the data presented in Table 6, the maximum potassium content of the grass is exhibited in the early spring, regardless of the pasture location, at a time coincident with the highest incidence of cases of grass tetany. A decline following this maximum occurs. Table 5 indicates the same increase in early spring, with a high in the late fall as well. These facts also agree with the findings of other workers. In general, these results strengthen the theory that the potassium content of wheat grass is a fundamental factor in the etiology of the disorder under discussion.

Little significance apparently can be attached to the fluctuations of potassium in wheat grass as a function of the geographical distribution of the pastures.

It seems that the change from a diet low in potassium to one containing relatively large amounts of that element has more influence in the causation of the disease than the fact that some grasses have an exceptionally high potassium content. Many animals become subject to the disease on pastures where analysis shows the potassium content to be at normal levels. However, as is shown in Table 3, where a number of samples composed of three replicates taken from several small plots were analyzed, considerable variation may exist in the potassium content of samples taken from various parts of the same field. Hence, it is quite possible that a sample may not be representative of the plot from which it came; and, in particular, may not be the same as that which a "poisoned" animal ingested.

The average values for blood minerals in normal cows based on deter-

minations made on whole blood and plasma samples in this study and on work completed by other investigators are assumed to be:

Table 14. Mineral content of normal bovine blood

Mineral Element	Whole Blood (mgm per cent)	Plasma (mgm per cent)
Potassium	48.0	25.0
Calcium	8.0	10.5
Magnesium	2.5	2.4

These values when compared with those in Table 7 substantiate the hypothesis of an increase in the ratio of monovalent to divalent elements in the blood during the symptoms of grass tetany. Abnormal increases in serum or whole blood potassium as a result of the disease are shown in a number of samples. The average value for whole blood in the afflicted animals was approximately 100 mgm per cent and in plasma, 44 mgm per cent. It is noted that several of these cases failed to show any hypomagnesemia or hypocalcemia, but show only an increase in the monovalent mineral element. This gives further support to the thought that grass tetany is involved with the ratio of the amounts of the mineral elements and not their absolute values. The element most consistent in its deviation from normal is potassium.

The provocation of tetanic convulsions by injections of potassium chloride gives more support to the theory which proposes potassium as a causal factor in grass tetany.

In both experiments the plasma potassium increased following each injection. When this increase approached a one-hundred per cent rise, the animal succumbed in convulsions. There also appears to be increases in the potassium content of the cells in proportion to the dosage. At the same

time, a decrease in the sodium of the cells occurs. This decrease is reflected by a rise in serum sodium or by an overall decline in the sodium of the whole blood. In general, no consistent changes can be observed in the calcium and magnesium contents. The plasma of the second experimental animal exhibited slight decreases in both these elements following the first four injections; but they were not of sufficient magnitude as to be significant.

The production of definite symptoms of grass tetany, particularly in the second animal, attendant to which a rise of blood potassium was the only specific change observed, indicates the importance of this element in the disorder.

In the studies involving the blood mineral picture of a cow during the first week of the post-partum period, an upset in the mineral balance is seen which may aid in an explanation of the almost exclusive occurrence of grass tetany in lactating cows shortly after parturition.

The potassium level in the plasma was slightly above normal; however, the amount of this element in the cells was much higher than usual; in some cases, it was almost double the average normal value of 80 - 90 mgm per cent. At the end of the first week in the post-partum period, the plasma potassium remained at a level on the high side of the normal range, which, according to the theory in discussion, would predispose the animal to grass tetany.

The blood calcium values, both plasma and whole blood, are in agreement with the findings of other investigators, in that, they appear to relatively lower at parturition than a week later. The reverse is true for magnesium, and again this is in agreement with the report of other workers.

This hyperpotassemia and hypocalcemia, both of which have been shown

to be involved as factors in grass tetany, may offer an explanation to the high incidence of the disease in dairy cattle.

As indicated in the review of the literature on this subject, potassium has been recognized as having the effect of increasing the irritability of nerves. Since grass tetany is an involvement of the nervous system in which hyperirritability is an apparent symptom, it follows that potassium may be a factor. This study has been undertaken to find substantiation, if any, of this idea. Although many more studies must be made before any definite conclusions can be made, it would appear from the data collected here, that potassium, if not a direct causal factor, at least plays a fundamental role in the etiology of the disease.

It is suggested that future studies on the etiology of this disease should include pH determinations on the blood; since it has been an accepted fact for sometime that slight increases in the pH of the blood will result in tetany. It is possible that the ratio $\frac{K\text{-}Na}{Ca\text{-}Mg}$ may be expanded to include the hydrogen ion concentration in an explanation of grass tetany, such that the ratio $\frac{K\text{-}Na\text{-}OH}{Ca\text{-}Mg\text{-}H}$ will be considered to be involved in this disorder.

SUMMARY

A theory for the etiology of grass tetany involving the increase of the potassium content of the blood as a causal factor has been studied.

It has been found that wheat grass reached a maximum peak in potassium content of approximately five per cent in some samples on a dry weight basis; and that this peak occurred in the spring at a time coincident with the highest incidence of grass tetany.

Several cases of grass tetany have been examined; and the blood potassium was found to be consistently high.

Intravenous injections of potassium chloride into two calves have been made. The production of typical grass tetany symptoms in one and of terminal tetanic convulsions in the other has been taken as further substantiation of the original theory.

The upset in the mineral blood picture of cows immediately following parturition has been studied for a possible explanation of frequent occurrence of grass tetany in lactating dairy cows in this period. The indication of hyperpotassemia and hypocalcemia are considered as predisposing factors.

It is concluded that potassium plays an important role in the etiology of grass tetany; and that further studies with regard to this element in the health and disease of animals should be made.

ACKNOWLEDGEMENTS

To Dr. J. S. Hughes, my advisor, for many hours spent advising the author, and for the many valuable suggestions during the course of this experiment.

To Dr. G. H. Wise, for the time spent in obtaining the blood samples required for this work.

To Mr. M. J. Caldwell, for the advice given the author in the development of the necessary techniques and procedures used in this study.

BIBLIOGRAPHY

(1) Dayus, N. Z. Jour. Agric., Oct. 20, (1928).

(2) Sjollema, B., On the Nature and Therapy of Grass Staggers, Vet. Rec., 10, 425-430, (1930).

(3) Sjollema, B. and Seekles, L., Über Störungen des mineralen Regulationmechanismus bei Krankheiten des Rindes, Biochem. Zeit., 229, 358-380, (1930).

(4) Seekles, L., Sjollema, B. and van der Kaay, F., Die Wirkungweise des Calciums, Biochem. Zeit., 243, 316-329, (1931).

(5) Warringholz, Die Wiedetetanie der Rinder und die Tetanie bei Maul-und Klauenseuche, Berl. Tierarzt., Wochenschr., 47, 745-750, (1931).

(6) Sjollema, B., Nutritional and Metabolic Disorders in Cattle, Nutr. Abstr. and Rev., 1, 621-31, (1932).

(7) Hare, T., A Disease of Lactating Cows, Vet. Rec., 12, 837-839, (1932).

(8) Dryerre, H., Lactation Tetany, Vet. Rec., 12, 1163-1168, (1932).

(9) Dehecq, Le Syndrome d' Hypocalcémie Aigue en Pathologie Bovine. Son Traitement par les Injections Intraveneuse de Gluconate de Calcium, Rec. Méd. Vét, École d'Alfort, 108, 81-90, (1932).

(10) Sjollema, B., Onderzoekingen over de Oorzaken van Grastetanie, Tijdschr. v. Diergeneesk., 59, 57-80 and 329-351, (1932).

(11) Sjollema, B. and Seekles, L., Untersuchungen über die Atiologie der Grastetanie, Arch. f. wiss. u. prakt. Tierheilk., 65, 331-343, (1932).

(12) Sjollema, B. and Seekles, L., ibid, 66, 60-69, (1933).

(13) Seekles, L. and Sjollema, B., ibid, 66, 117-123, (1933).

(14) Broersma, S., Grastetanie en het gebrink van kunstmest in den landbouw, Tijdshr. v. Diergeneesk, 60, 68-71, (1933).

(15) Kuipers, K., Grastetanie, Tijdshr. v. Diergeneesk, 60, 1-6, (1933).

(16) Johnstone, R., Grass Tetany, Jour. Department. Agric. Victoria, 31, 396-400, (1933).

(17) Sjollema, B., Is A. T. 10 een geschickt middel om recidive van tetanie, Tijdschr. v. Diergeneesk., 61, 927-929, (1934).

(18) Blakemore, F. and Stewart, J., Some Observations on Several Outbreaks of so-called Lactation Tetany in Cattle, Univ. Cambridge, Inst. Animal Pathol., Rep. Director, 159-168, (1932-33).

(19) Allcroft, W. and Greene, H., Blood Magnesium and Calcium in the Cow in Health and Disease, Biochem. Jour., 28, 2220, (1934).

(20) Hopkirk, C., Marshall, D. and Blake, T., Grass Tetany of Dairy Cows, Vet. Rec., 13, 355-361, (1933).

(21) Carlstrom, B., Hypocalcemic Morbid Conditions in Domestic Animals, Skand. vet. Tidskr., 23, 229-268, (1933).

(22) Hopkirk, C., Staggers in Livestock, N. Z. Jour. Agric., 51, 18-21, (1935).

(23) New Zealand Dept. Agric., Grass Staggers in Dairy Cows, Ann. Rep., 31-32, (1935-36).

(24) Wright, J., A Disease of Metabolism in Sheep, Vet. Rec., 15, 1253-1255, (1935).

(25) De Gier, C., Reisziekte Bij pony's, Tijdschr. v. Diergeneesk., 62, 1186-1196, (1935).

(26) Blumer, C., Madden, F. and Walker, D., Hypocalcemia, Grass Tetany or Grass Staggers in Sheep, Austral. Vet. Jour., 15, 24-27, (1939).

(27) Duncan, C., Huffman, C. and Robinson, C., Magnesium Studies in Calves, Jour. Biol. Chem., 108, 35-44, (1934).

(28) Allcroft, W. and Green, H., Seasonal Hypomagnesemia of the Bovine Without Clinical Symptoms, Jour. Comp. Pathol., 51, 176-191, (1938).

(29) Allcroft, W. and Godden, W., Changes in the Calcium and Magnesium of the Serum and the Inorganic Phosphorous of the Blood of Cows at Calving and of the Calf During Early Life, Biochem. Jour., 28, 1004-1007, (1934).

(30) Robinson, C. and Huffman, C., Composition of Bovine Blood, Jour. Biol. Chem., 67, 257, (1926).

(31) Hibbs, J., et al, Blood Changes in Normal and Milk Fever Cows at Parturition, Jour. Animal Sci., 3, 437-438, (1944).

(32) Sjollema, B., Mineral Studies in Calves, Vet. Rec., 15, 1253-1255, (1935).

(33) Duncan, C. and Huffman, C., Jour. Dairy Sci., 21, 111, (1938).

(34) Nicholson, J. and Shearer, G., The Occurrence of Lactation Tetany in Ireland, Vet. Jour., 94, 388-398, (1938).

(35) Nolan, A. and Hull, F., Grass Tetany in Cattle, Amer. Jour. Vet. Res., 2, 41-45, (1941).

(36) McMillen, W. and Langham, W., Grazing Winter Wheat with Special Reference to the Mineral Blood Picture, Jour. Animal Sci., 1, 14-21, (1942).

(37) Caldwell, M. and Hughes, J. S., A Suggested Explanation for the Action of Mineral Elements on Nerve Irritability, Jour. Amer. Vet. Med. Assoc., 106, 298-300, (1945).

(38) Clowes, G., Protoplasmic Equilibrium, Jour. Phys. Chem., 20, 407-450, (1916).

(39) Lillie, R., Protoplasmic Action and Nervous Action, Chicago, University of Chicago Press, 417 p., (1932).

(40) Starling, E., Principles of Human Physiology, Philadelphia, Lea & Febiger, 1247 p., (1941).

(41) Best, C. and Taylor, N., The Physiological Basis of Medical Practice, Baltimore, Williams and Wilkins, 1872 p., (1939).

(42) Schoenthal, L., Family Periodic Paralysis, A Review, Amer. Jour. Dis. Child., 48, 799, (1934).

(43) Herrington, M., Successful Treatment of Two Cases of Familial Periodic Paralysis with Potassium Citrate, Jour. Amer. Med. Assoc., 108, 1339, (1937).

(44) Fenn, W., The Role of Potassium in Physiological Processes, Physiol. Rev., 20, 377-415, (1940).

(45) Zwemer, R. and Truszkowski, R., Potassium in Cortico-Adrenal Insufficiency, Sci., 83, 558, (1936).

(46) Spiegal, A., Spiegal, E., Ashkenoz, E., and Lee, A., Physico-chemical Effects of Electrically Induced Convulsions, Jour. Neuropath. and Exptl. Neur., 4, 277-290, (1945).

(47) Miller, E., A Physiological Study of the Winter Wheat Plant at Different Stages of Its Development, Kansas Agric. Exper. Sta. Tech. Bull. 47, (1939).

(48) Adie, R. and Wood, T., Jour. Chem. Soc., 77, 1076-80, (1900).

(49) Kramer, B., Jour. Biol. Chem., 41, 263, (1920).

(50) Kramer, B. and Tisdall, F., Ibid., 46, 339, (1921)

(51) Salit, Ibid., 96, 659, (1932).

(52) Wang, Chi Che, Ibid., 111, 443, (1935).

(53) Briggs, A., Colorimetric Method for the Determination of Small Amounts of Magnesium, Ibid., 52, 349-355, (1922).

(54) Kuttner, and Cohen, Micro-Colorimetric Studies, Ibid., 75, 517, (1927).

(55) Kuttner, and Lichtenstein, Micro-Colorimetric Studies, Ibid., 86, 671, (1930).

(56) Bodansky, A., Phosphatase Studies, Ibid., 99, 197, (1933).

(57) Harris, A Modified Silver Cobaltinitrite Method for Determination of Potassium in Blood, Ibid., 136, 619, (1940).