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TIMBER MILK VETCH AS A POISONOUS PLANT

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TIMBER MILK VETCH AS A POISONOUS PLANT*

By I. E. NEWSOM, FLOYD CROSS, B. R. McCRORY, and A. H. GROTH, Pathology and Bacteriology; J. W. TOBISKA and EARL BALIS, Chemistry; L. W. DURRELL and E. C. SMITH, Botany; and E. N. STOUT, Extension Veterinarian

During the last few years stockmen in the western part of Moffat County have reported a disease of sheep and cattle running on summer range on Blue and Douglas Mountains. At first it was thought that the symptoms might be due to a deficiency of phosphorus or calcium or the presence of selenium, but feeding experiments at Fort Collins and extensive chemical tests proved that this was not the case.

The similarity of the symptoms to a disease reported by E. A. Bruce[1] in "Stock Poisoning Plants of British Columbia" suggested that one of the milk vetches might be responsible. A survey of the Blue Mountain area revealed the presence on the range of two milk vetches, *Astragalus junciformis* and *Astragalus hylophilus,* the last-named being very abundant and closely resembling the description and figure of *Astragalus campestris* given by Bruce. Controlled feeding experiments on the range proved conclusively that the symptoms and death could be produced in both sheep and cows by feeding large quantities of *Astragalus hylophilus,* the determination of which species had been confirmed by the Gray Herbarium at Cambridge, Mass. This plant may well be identical with the *A. campestris* of Bruce, since his description was taken from Henry's Flora of Southern British Columbia and does not agree with the descriptions of *Astragalus campestris* in the manuals this side of the border.

Description of Plant

The "timber milk vetch," *Astragalus hylophilus* (fig. 1), responsible for the poisoning of stock in western Colorado, occurs in tufts of slender stems which branch freely from the base, forming clumps 8 to 16 inches high and as wide or wider. The leaves are compound, with 7 to 19 narrow leaflets rather distant from each other and about half an inch long. The flowering stems are about the length of the leafy ones, with long stems and 6 to 12 flowers. These flowers are white with purple tips, small and pea-like, and may be seen from June to late in July. The fruit is a straight, narrow, smooth pod, less than an inch in length, and when mature usually bent down.

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*A cooperative project between the Sections of Pathology and Bacteriology, Chemistry, and Botany, with the assistance of the extension veterinarian.

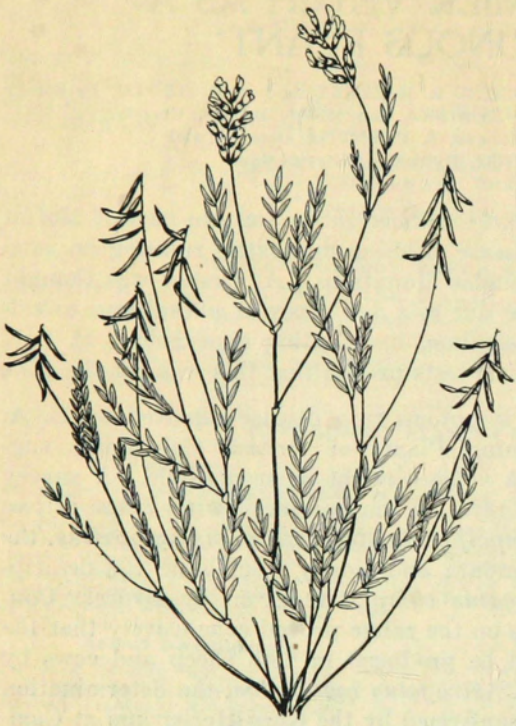

Figure 1.—Sketch of several stems of timber milk vetch, showing clusters of pea-like flowers and small pods.

The plant is common in the mountain districts of Idaho, Montana, Wyoming, Colorado, and Utah. On the eastern side of the mountains in Colorado it is more commonly found in aspen groves; near the Utah boundary it is more abundant under and between the sagebrush growth.

Technical Description

Astragalus hylophilus (Rydb.) Nelson. Stems clustered, slender, freely branched from base, sparingly strigose; leaves pinnately compound; leaflets 13 to 25 (decidedly fewer in our specimens), elliptic to lance-oblong (rather narrower in our specimens); the terminal leaflet well-developed, 1 to 2 cm long, glabrous above, sparingly strigose below; racemes 6- to 12-flowered, about as long as the leafy branches; calyx strigose with dark hairs, tube 3 mm long, teeth subulate, 1 to 1.5 mm long; corolla about 1 cm long, white, tinged with purple at the tip, keel purple-tipped; pod linear, glabrous, 2 cm long, usually more or less drooping.

Since there is some uncertainty as to whether this is the same as the plant described by Bruce as *Astragalus campestris*, a description of the latter as understood by American botanists is appended.

Astragalus campestris Gray. Stem often solitary, branched, slender, sparingly strigose; leaves pinnately compound, the upper reduced to phyllodes or the rachis greatly produced without a terminal leaflet; leaflets 7 to 19, linear-subulate to narrowly linear on lower leaves; raceme few-flowered; calyx strigose with some dark hairs, lobes less than half as long as the tube; flowers ochroleucous with faint purple tips; pods linear, strigose, 3 cm long.

Description of Range

The area surveyed lies mostly between elevations of 7,500 feet and 8,500 feet on Blue Mountain in Moffat County, Colo. It includes rounded summits, high valleys, narrow ravines, and draws. Springs and waterholes are few. The range has been over-grazed and has suffered several successive seasons of drought. The typical sagebrush cover (fig. 2) is diversified by strips of aspen in the ravines, aspen groves on the flatter summits, and scattered masses of shadbush (*Amelanchier alnifolia*) and snowberry (*Symphoricarpus pauciflorus*) which sometimes cover whole hillsides. The usual grasses

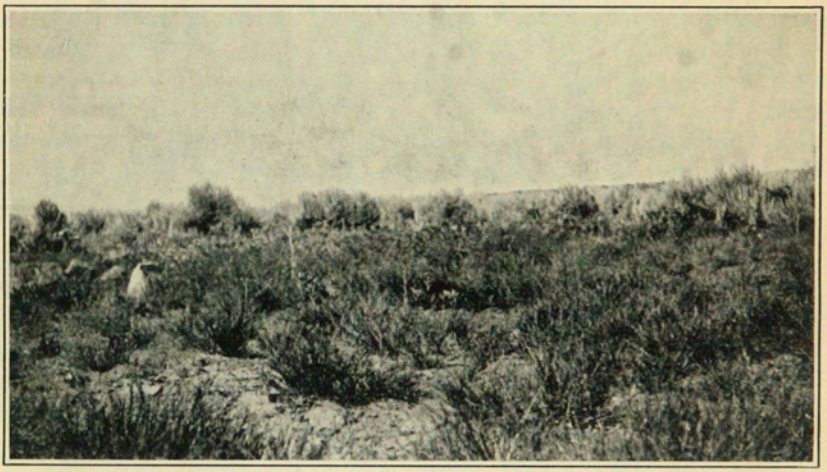

Figure 2.—Clumps of timber milk vetch growing in a clear area in sagebrush.

and herbs of the western Colorado range are present but show severe effects of over-grazing and drought. *Balsamorrhiza* is present in considerable quantities and is eaten freely early in the season, but by the middle of July is dried up.

The milk vetch is very abundant over most of the range, occurring in the aspen groves, the sagebrush cover, and the openings in that cover, being most abundant in the last situation and taking almost exclusive possession of these areas (fig. 3). In July, when almost all other vegetation but the shrubs is dried up, the vetch is still fresh and green. Not until then do the animals begin to eat this plant and then not exclusively, but adding leaves and twigs of the shadbush and snowberry and the remnants of green grass in the closely-grazed draws near the waterholes.

Name of Disease

Bruce says that in Canada the trouble is variously designated

as "timber trouble, timber grass poisoning, jack pine fever, husky, Kamloops cattle disease, knocking disease, timber paralysis, and roaring disease" as applied to cattle and sheep, and "mountain fever, roaring disease, Clinton horse disease, and timber paralysis" as applied to horses. Beath [2] adds to this list the name "cracker heels," thus calling attention to a peculiar symptom observed in cattle. Other names given have been "wheezers" and "lungers." The Colorado cattlemen seem not to have given a name, but they speak of the affected animals as having been "poisoned."

Seasonal Occurrence

All authorities agree that the disease develops from the latter part of June to the last of August, which seems to conform to the Colorado experience. This corresponds to the time cattle are grazed on the higher ranges and to the seasonal growth of the plant. Animals once affected may continue to show symptoms throughout the fall and winter and may even come down again the following summer.

Animals Affected

Bruce describes the disease in horses, cattle, and sheep. Beath says it affects sheep and cattle mainly. In this state it has been reported only in cattle and sheep and has been reproduced only in those animals. Cattlemen in the district where the disease exists insist that only cows giving milk are affected. No dry cows, bulls, or steers show evidence of the disease. This observation first led to the supposition that the disease was a phosphorous deficiency. Bruce,

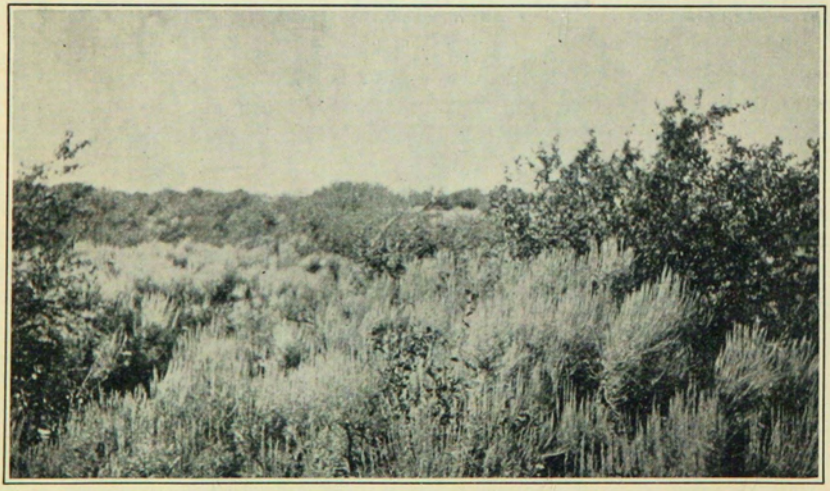

Figure 3.—Typical range where timber milk vetch is found growing under sagebrush and shrubs. It also grows in aspen groves.

however, calls attention to the same reports in British Columbia. Under experimental feeding the disease can be readily produced in both dry cows and sheep. The explanation of the natural disease being limited to lactating animals is not at hand. It offers an intriguing field for further investigation. Calves nursing diseased mothers show no symptoms of vetch poisoning unless they themselves eat the weed. Since cows acutely affected may cease giving milk, the calves may show evidence of malnutrition. Apparently the poisonous principle is not secreted through the milk.

Bruce says that native animals are less affected than outsiders, probably because they eat less of the plant.

Symptoms

Cattle

Probably the acute symptoms are seldom observed on the range. In experimental animals the symptoms may appear as early as the second day after feeding. They consist of a generally anxious appearance of the face; dilation of the pupils and impairment of vision; drooling from the mouth, with the tip of the tongue protruding between the lips and the muzzle dry, followed by a cracking and peeling of the epithelial layer. In eating, the cud may fall from the mouth. The animal is greatly depressed but on being disturbed shows signs of irritation, resenting interference. There is considerable anxiety about the calf; the cow bawls frequently, and the voice soon becomes husky and later fails entirely. General signs of malnutrition develop early—the hair stands out, giving the animal a rough appearance; the flanks are tucked up; rumination ceases; and the cow is indifferent about eating the vetch but continues a desire for other food. Constipation is followed by diarrhoea, and the feces are covered with mucus.

There is little desire to move, but when urged the cow shows incoordination affecting all four limbs; the hind quarters weave from side to side; knuckling of the posterior limbs may be noticed, thus producing a very irregular gait.

The legs may not support the weight, and the cow goes down, showing typical spasms of the musculature. On moving, the respiration is greatly increased, and wheezing sounds are emitted. The heart becomes extremely rapid, weak, and irregular. Urinary irritation is frequently noticed. The urine is expelled in spurts as the cow walks. This was especially stressed by Bruce and was noticeable in our own cases. Muscular tremors are sometimes noticed, especially in the region of the flanks. Exhaustion and paralysis come quickly on being driven, and death may occur suddenly.

The chronic symptoms are more commonly observed on the range and are easily recognized by the cattlemen. The coat stares; the flanks are tucked up; the cow loses flesh; the nose peels off; the voice is described as whispering or husky; incoordination of motion is evident; there is weaving of the hind quarters; the animal tires easily and on being pushed may go down. On walking, the claw strikes the fetlock of the opposite hind leg, thus producing a clicking sound and giving the Wyoming name of "cracker heels." On driving, the urine is passed in spurts. Coughing is a frequent symptom, especially when being driven. Vomiting was not mentioned by the cattlemen but frequently occurred in one of the natural cases brought to Fort Collins. These animals may become completely paralyzed and die on the range, or they may show varying degrees of severity of the disease throughout the winter, with recovery toward spring. Some may come down again the following summer.

Sheep

While the authors have seen no natural cases in sheep, it is our impression from the description of the symptoms by herders, borne out by the experimental cases, that the disease is almost always acute in these animals. Bruce stresses the acuteness of the disease is sheep, describing especially the urinary irritation, the incoordination of motion, the change in character of the voice, and the paralysis of the heart. Three of our animals died on the fifth day after the beginning of the feeding experiment. The symptoms were not particularly apparent until the animals were driven. Incoordination was then very noticeable; the respiration increased; the heart fairly raced; and the animals collapsed after a short distance. One of them died without spasms; one showed violent spasms prior to death; and another had a violent fit of coughing. Apparently, moving sheep that have eaten vetch is a dangerous undertaking.

Horses

Since neither Beath nor we have seen the disease in horses, it is apparent that we must take the description from Bruce, and even his description is made up from reports of others and not from personal observation. His account is quoted as follows:

> "As usually seen, the animal appears to be normal when standing undisturbed, but just as soon as it is excited or made to move it shows signs of distress: the breathing becomes laboured; the nostrils fully extended; roaring well marked and of an expiratory type (which is very rare in ordinary roarers); the animal breaks into a profuse perspiration, staggers, falls, may roll, usually defaecates, the mouth wide open emitting foam, and the tongue cyanotic; in fact, death from asphyxia appears imminent and may occur. Such a paroxysm lasts from

5 to 15 minutes, but has been known to last half an hour. During the attack the temperature rises 2 or 3 degrees but drops back to normal when the animal has recovered. After an attack there is evidence of exhaustion, but otherwise the horse appears normal. Very slight exertion will bring on such attacks; we know of one animal that had three when being driven a short distance from pasture, the third proving fatal. One horse is reported as running about 300 yards and then dropping dead with a gush of blood from the nostrils. These animals are usually in fair condition, but repeated attacks bring on emaciation, and pneumonia is a common sequel. We are unable to say whether aphonia (loss of voice) occurs as in cattle and sheep, but upon one occasion we heard an affected animal neigh, and the voice was certainly affected, being unnaturally hoarse."

Course of the Disease

More is known of the course of the disease in cattle than in other species. It is believed that these rarely die in the first week of the disease—at least not in Colorado. Possibly driving in this early stage would cause the loss of more of them. Generally, however, the disease comes on gradually, and the animals develop the chronic type which may last for months or even a year or more. Some do badly during the following winter and then die in the second summer. Even under good feeding, many make no increase in weight. Although some undoubtedly do recover, the general belief is that for the most part the fattening of such animals is not very profitable. Of the 6 animals brought to Fort Collins, only 1 died; 4 were slaughtered at the conclusion of our observations because they were considered to be of little value; and only 1 was kept over for use the next year. Bruce reports an extreme case where 100 percent of the milking animals showed evidence of the disease, with a death loss of 44 percent. He thinks, however, that 10 percent loss is not uncommon, but that 2 percent to 3 percent is more nearly the average in vetch-infested districts. On Douglas and Blue Mountains in this state, an annual morbidity of 10 percent, with a mortality of 3 percent, seems well within the range of probability.

Sheep die more readily and have fewer chronic cases. Bruce estimates the average loss in these animals in the affected districts of British Columbia at not more than 10 percent. From such reports as can be gathered, ours is about the same.

In horses we have only the figures given by Bruce. He says losses have been excessive in a few outbreaks, but generally the horse suffers from a chronic type of the disease without high mortality but with little tendency toward recovery, thus rendering the animal useless for work.

Post-mortem Findings

There are no distinctive lesions. Some animals develop a bronchitis and die of pneumonia. Others are greatly emaciated and anemic, and those dying in the acute stage usually show a dilated heart.

Treatment and Prevention

It is very doubtful if any medicinal treatment is of value. Even if the sick animals should be seen, which is often not feasible on the range, no drug seems likely to be effective. Since driving may bring on an acute attack, it is better to keep the animals as quiet as possible. Where the vetch is prolific, and especially in dry years when grass is short, if losses are to be prevented the animals must be removed from the range. Since the vetch usually dries up in Colorado about the last of August, it seems feasible to pasture the worst-infested areas after that time. Good feed and good care will bring some of them through and allow them to be slaughtered for meat. The worst should be killed early to save feed.

EXPERIMENTAL

On August 25, 1933 the following letter was received from R. T. Buffham of Greystone, a postoffice in the extreme northwestern part of Colorado:

> "Several cattle growers in the western section of Moffat County have been troubled for the last few years with a poison of some sort. We thought it was death camas that caused the poison but have decided it was something else. Here are the facts:
>
> "1. Cows with young calves are the only animals affected. Never any 'dry animals' are affected. Never bulls, steers, etc.
>
> "2. The symptoms are: One day the cows will be perfectly all right, and the next day they break down in the back or lose control of their hind legs, making them weave from side to side as they walk. They immediately begin to lose flesh until they become quite poor. They urinate continuously when being driven. Most of them cough when being moved.
>
> "3. The first signs of poison are when the feed dries up. The cattle then eat sarvus brush, which we believe to be the cause of the poisoning.

> "I would like to have the opinion of the college on this matter, as to the cause, if it is caused from eating sarvus brush or other vegetation. And if there is any remedy we would like to know it."

A short time thereafter a visit was made to Mr. Buffham at his ranch, and a further description of the disease was obtained from him. His cattle ran on Douglas Mountain during the summer and in the Valley of Vermillion Creek during the winter. Some of the cattle were already down from the mountain, and Mr. Buffham tried to show some that were affected. Having no horse, it was impossible to get sufficiently near the animals to make an adequate examination. It was, however, possible to hear the noisy breathing on being driven, which he said was characteristic of the disease.

Because only female cattle with calves were affected, a phosphorus deficiency was strongly suspected, and inquiry was made as to the craving of the animals for bones. At that time neither Mr. Buffham nor his son had known of any of the sick animals chewing bones. Later, however, information came that bone-chewing was noticed by the men who handled the cattle on the range. In view of this history, and in the light of the recent work on phosphorus deficiency by du Toit, Malan et al.[3] in South Africa, and that of Eckles[4] et al. in Minnesota, a cooperative project was drawn up by the Sections of Pathology and Bacteriology and Chemistry for a study of the disease. This project contemplated a chemical study of the blood of affected animals taken at regular intervals; a comparison of this with normals taken near Fort Collins; and a study of the soil and forage in the affected district, with special reference to phosphorus and lime. This work was carried on during the summer of 1934, and while it is now apparent that phosphorus deficiency is not the sole cause of the disease, it seemed wise to put the findings on record in case anyone in the future might wish to use them for comparison in similar lines of investigation.

Blood Study

Arrangements were made with the owners to make monthly bleedings of cattle during the summer of 1934, and accordingly 10 cattle were selected on Douglas Mountain and 20 on Blue Mountain. Four bleedings were made on the Douglas Mountain cattle, and three on the first 10 and two on the remainder of the Blue Mountain animals. Approximately 40 cc of blood were drawn from the jugular vein of each animal into a vial containing 1 cc of a 20 percent sodium citrate solution in distilled water. There is some inconvenience in carrying on this kind of chemical investigation at long range, as was experienced by workers in South Africa and elsewhere. The town of

Craig, in Moffat County, which was headquarters in making the sampling trips, is 85 miles distant from the Douglas Mountain area and 120 miles from the Blue Mountain range. Samples were taken one day at Douglas Mountain and the second day at Blue Mountain. The samples were stored under refrigeration at Craig during our stay. They were then transported to the laboratory, a distance of some 300 miles, and were worked up approximately 48 hours later. They were always in good condition upon arrival at the laboratory.

The procedure in testing these blood samples for Ca and P was mainly that of Malan and Van der Lingen[5] of South Africa. It was found advisable, however, to make some changes in their methods; viz., in the Denige method for determining "P" colorimetrically. The use of Zinzadze's Mo Blue reagent, instead of $SnCl_2$, to accomplish the reduction of phosphates is a decided advantage. Stannous chloride solutions are temperamental, whereas the Zinzadze reagent remains constant over a period of 6 months or more. Upon addition of Mo Blue reagent to a test, the color first disappears, and then on warming it returns and remains quite stable over a period of several hours. The methods in detail are reported in the appendix.

The entire matter of the value of inorganic Ca and P determinations in the blood serum of dairy cattle as a means of determining systemic deficiencies is ably discussed in a series of papers from the Divisions of Agricultural Biochemistry and Dairy Husbandry of the University of Minnesota.[6-7-8] From these discussions it appears that a host of variable factors enters into the question. Since our animals were necessarily at such a distance from the laboratory, it was not feasible to make as many samplings of blood as was desired. However, the cattle were corralled several hours before bleeding, and all unnecessary exercise of the animals was avoided.

Palmer and Eckles placed the normal range of Ca in their dairy animals as between 9.0-12.0 mgm percent, with extreme values of 4 to 24.4 mgm percent. The "P" or phosphorus content of blood from six dairy cows (Palmer, Cunningham, and Eckles), showed a variation from 4.06 to 8.7 mgm percent, with extreme low values for other animals of 2 to 3 mgm percent.

An interesting tabulation of average results obtained for inorganic blood Ca and P at various localities is copied below from the 1931 Report of the Ontario Veterinary College (pp. 23-24):

Blood normals of dairy cattle for calcium (Ca) and inorganic phosphorus (P) in milligrams per 100 cc of blood:

British Columbia results—21 animals (dairy cows and heifers), 64 determinations:

Ca—Average 10.59. Extremes 8.7 to 12.8.
P —Average 4.18. Extremes 2.67 to 6.7.

Australian results—29 animals (cows), 29 determinations:

Ca—Average 10.54. Extremes 9.38 to 12.92.
P —Average 4.44. Extremes 2.5 to 6.67.

New York State results—14 dry cows, 124 determinations, monthly tests from April to December:

Ca—Average 10.72.
P —Average 6.25.

Twenty-three milk cows, 210 determinations, monthly tests from April to December:

Ca—Average 10.77.
P —Average 4.65.

Ontario—5 cows, 5 determinations:

Ca—Average 11.62. Extremes 10.00 to 12.4 .
P —Average 3.54. Extremes 2.53 to 4.22.

From all the literature obtainable, it is evident that the values for blood calcium are much more uniform than those of phosphorus, and they average between 10.5 and 11.0 mgm percent. The values for phosphorus vary rather widely, largely due to age and diet. The averages for normal animals appear to be 4.2 to 6.5 mgm percent, with extremes extending in either direction from these values to as low as 1.5 mgm and as high as 7.8 mgm percent.

It is only stating the matter in the words of many investigators in this field to say that the outward evidences of phosphorus deficiency—such as depraved appetites for bone, wood, etc.—are not always corroborated by the findings of low inorganic P in the blood. In this matter, one must take into consideration that nature tends to maintain the circulating medium (the blood) as nearly constant as is possible, and that major deviations from this constant might be expected only after a rather prolonged unbalance of the diet.

Douglas Mountain Cattle

The data on the bloods of cattle from the Douglas Mountain range are presented in table I.

TABLE I.—BLOOD OF DOUGLAS MOUNTAIN CATTLE—1934
Values in mg/100 cc

Date of sampling:	5-14	6-25	8-1	9-3	5-14	6-25	8-1	9-3	
Number of cow	Calcium				Phosphorus				Remarks
407	13.4	8.7	9.1	9.6	3.9	4.8	3.7	4.8	In very bad condition September 3.
408	11.7	9.4	9.5	8.5	4.4	5.0	4.8	4.8	No illness.
409	11.9	9.8	9.2	9.4	3.6	5.7	4.5	5.1	No illness this year.
410	8.2	8.4	.. ..	.. ..	4.4	5.3	.. ..	.. ..	In extremely bad condition August 1.

Continued on page 14

TABLE I.—Continued from page 13
Values in mg/100 cc

Date of sampling:	5-14	6-25	8-1	9-3	5-14	6-25	8-1	9-3	
Number of cow	Calcium				Phosphorus				Remarks
411	10.4	9.0	9.9	9.6	4.3	5.2	4.4	4.0	Mild symptoms September 3.
412	9.2	9.5	9.6	9.4	4.0	5.4	4.7	3.8	In bad condition August 1 and September 3.
413	10.6	8.3	.. ..	8.9	4.5	5.3	.. ..	4.5	In bad condition August 1. Improved September 3.
414	11.6	9.4	9.1	9.5	5.1	5.8	4.8	4.8	No illness this year.
415	12.0	9.1	8.9	10.0	4.7	4.8	4.6	4.5	Very bad August 1. Better September 3.
416	11.0	9.4	9.0	10.4	5.7	5.8	4.5	4.8	Steer calf.

PROTOCOLS—DOUGLAS MOUNTAIN CATTLE

No. 407.—May 14. Five-year-old cow with second calf by her side; had not been sick previously.
June 25. No change.
August 1. Showing definite symptoms of the disease.
September 3. Could walk only a few yards without going down. Very weak.

No. 408.—May 14. Aged cow with her sixth calf. Never had been sick. Showed no signs of illness up to September 3, when last bled.

No. 409.—May 14. Cow 9 or 10 years old, with calf by her side. Was sick 6 years ago but has had 3 calves since. Showed no signs of illness up to September 3, when last bled.

No. 410.—May 14. Three-year-old heifer. Probably with first calf. Normal.
June 25. No change.
August 1. Was so bad with the disease that owner refused further bleedings.

No. 411.—May 14. Six-year-old cow; has had 3 calves but is now dry. Was sick last summer. Showed some evidence of the disease at time of last bleeding on September 3.

No. 412.—May 14. Four-year-old cow with her second calf by her side. Was sick last summer and was given bonemeal and cottoncake during winter.
June 25. No change.
August 1. In very bad condition.
September 3. Still gaunt and in bad shape.

No. 413.—May 14. Four-year-old cow with her first calf last year. Had a very bad attack. Is now with calf.
August 1. Cow is in such bad shape that bleeding was refused by owner.
September 3. Cow is better, so bleeding was allowed.

No. 414.—May 14. Four-year-old cow—first calf last year—had a bad attack of the disease. Now bred. Showed no evidence of the disease up to September 3, 1934.

No. 415.—May 14. Three-year-old cow; first calf by her side.
July 27. Normal.
August 1. Has very bad attack.
September 3. Somewhat improved.

No. 416.—May 14. Eight-month-old steer. Normal. So remained throughout the summer.

Blue Mountain Cattle

The data on the bloods of cattle from the Blue Mountain range are presented in table II.

TABLE II.—BLOOD OF BLUE MOUNTAIN CATTLE—1934
Values in mgm/100 cc

Date of sampling:	6-27	8-1	9-4	6-27	8-1	9-4	
Number of cow	Calcium			Phosphorus			Remarks
428	9.2	.. ..	.. ..	4.6	.. ..	.. ..	Unable to walk August 1; died few days later.
429	9.3	9.0	.. ..	3.8	4.9	.. ..	Affected last year, not this.
430	9.4	9.7	8.7	5.7	4.5	4.5	Dry cow, not affected.
431	9.0	8.8	9.9	4.9	4.8	3.8	No disease.
432	8.9	8.7	9.1	5.1	4.8	4.1	No disease.
434	9.2	9.2	9.1	5.2	4.8	4.4	Yearling heifer, no disease.
435	9.6	9.2	8.4	5.2	5.2	4.5	Two-year-old heifer, not diseased.
436	7.8	9.2	.. ..	3.9	4.3	.. ..	Was sick last year.
438	7.8	9.2	8.2	4.2	4.7	3.4	In bad condition on September 4.
439	7.8	9.4	.. ..	4.2	4.4	.. ..	Two-year-old heifer, not diseased.
440	7.5	9.4	.. ..	4.7	4.9	.. ..	Young cow, lightly affected.
441	8.9	9.4	.. ..	4.5	4.7	.. ..	Died before September 4.
442	8.2	8.6	.. ..	4.2	4.7	.. ..	Young cow, lightly affected.
443	8.8	8.5	.. ..	4.6	4.1	.. ..	Young cow, lightly affected.
444	8.6	9.2	.. ..	4.3	4.6	.. ..	Young cow, lightly affected.
445	8.1	9.0	.. ..	4.9	4.9	.. ..	Yearling heifer, not sick.
446	7.8	9.1	.. ..	4.2	5.2	.. ..	Died before September 4.
447	8.6	8.9	.. ..	4.0	5.2	.. ..	Dry, no disease.
450	7.9	9.4	.. ..	4.6	4.2	.. ..	Died before September 4.
500	8.7	8.6	.. ..	6.1	5.0	.. ..	Died before September 4.
424	.. ..	.. ..	8.1	.. ..	.. ..	2.1	Advanced case.
425	.. ..	.. ..	8.2	.. ..	.. ..	2.8	Advanced case.

PROTOCOLS—BLUE MOUNTAIN CATTLE

No. 428.—June 27. Three-year-old; had one calf; thought to be affected.
August 1. Said to be so bad she could not walk. Was not brought in with others.
September 4. Cow had died since August 1.

No. 429.—June 27. Dry cow, 9 years old. Has had 5 or 6 calves. Affected last year.

No. 430.—June 27. Four-year-old cow; has had one calf; never affected.
September 4. Still dry and fat.

No. 431.—June 27. Three-year-old cow, with calf by side. Has never been affected. Developed no trouble.

No. 432.—June 27. Four-year-old cow; has had two calves; never affected.
September 4. Dry and in good condition.

No. 434.—June 27. Yearling heifer; never affected.
September 4. Not diseased.

No. 435.—June 27. Two-year-old heifer; never affected.

No. 436.—June 27. Eight-year-old cow; has had 5 calves; in poor flesh; was sick last fall.

No. 438.—June 27. Three-year-old cow, calf by side; never affected.
September 4. Calf weaned; cow in bad condition. Cannot bawl.

No. 439.—June 27. Two-year-old heifer; never affected.

No. 440.—June 27. Young cow, calf by side; lightly affected.

No. 441.—June 27. Young cow, calf by side; limp in gait of hind quarters.
September 4. Had died since last bleeding.

*No. 442.—June 27. Young cow, calf by side; light case; heavy breath.
August 2. Still shows the disease.

No. 443.—June 27. Young cow, calf by side; light case.

No. 444.—June 27. Young cow, calf by side; light case.

No. 445.—June 27. Yearling heifer; not sick.

No. 446.—June 27. Young cow, carrying calf; shows symptoms: heavy breathing, weaving gait.
September 4. Had died since last bleeding.

No. 447.—June 27. Dry cow; no disease.

No. 450.—June 27. Young cow, calf at side; has advanced case of disease.
August 2. Shows symptoms.
September 4. Had died since last bleeding.

No. 500.—June 27. Young cow, calf at side; heavy breathing. Visible symptoms.
August 2. Still shows evidence of disease.
September 4. Had died since last bleeding.

*No. 424.—September 4. Old roan cow, dry for 3 years; has had 5 or 6 calves previously; shows disease.

*No. 425.—September 4. Seven-year-old cow with calf; shows disease.

Control Animals

In order to have comparisons, 4 normal steers from the college feeding experiments were bled 10 times over a period of 9 months. The first bleeding was in June and the last in March. Until October 1 these animals were running on a foothill pasture, and after that time they were fed full rations in pens on the college farm.

Thirteen steers that were on merely a maintenance ration were also bled 3 times from December to May. The record of these check animals will be found in tables III and IV.

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*These cows were trucked to Fort Collins on October 8. Their further history appears in connection with that lot.

TABLE III.—BLOOD OF THE FOUR CHECK STEERS AT THE COLLEGE
Values in mgm/100 cc

	Calcium				Phosphorus			
Date	No. 43	No. 55	No. 48	No. 51	No. 43	No. 55	No. 48	No. 51
6-13-34	10.3	10.0	10.8	9.6	3.4	3.5	3.4	3.3
8-2-34	9.4	9.1	9.6	9.2	4.9	4.4	5.3	4.2
9-1-34	9.8	10.0	9.7	9.4	4.3	4.1	4.5	4.6
10-1-34	8.5	8.9	8.9	8.7	3.3	3.2	3.4	3.2
10-18-34	8.6	9.0	9.4	8.4	4.6	4.9	4.1	4.1
10-30-34	7.8	7.9	7.8	7.7	4.4	4.1	4.2	4.5
11-9-34	9.3	8.7	9.0	7.8	4.3	3.9	3.5	4.1
11-20-34	9.2	8.5	8.9	9.0	3.9	4.1	3.7	4.1
12-18-34	8.0	8.1	8.1	6.9	4.2	4.4	4.0	4.2
3-18-35	7.7	7.9	8.4	7.2	3.9	3.9	3.6	3.9

TABLE IV.—BLOOD OF THE THIRTEEN CHECK STEERS AT THE COLLEGE
Values in mgm/100 cc

Steer number	Calcium			Phosphorus		
	12-14-34	3-4-35	5-22-35	12-14-34	3-4-35	5-22-35
3	.. ..	8.7	9.0	.. ..	4.3	3.8
13	8.0	7.6	8.7	3.7	3.4	3.3
25	8.3	8.0	.. ..	3.4	4.2	.. ..
27	7.7	8.2	9.2	3.6	4.1	4.4
46	7.5	8.6	.. ..	3.5	3.5	.. ..
50	7.7	8.5	.. ..	3.9	3.5	.. ..
57	7.6	8.2	8.5	4.0	4.2	5.0
63	8.2	8.6	.. ..	4.3	3.9	.. ..
66	8.7	8.0	9.4	4.1	3.9	4.7
69	8.5	8.0	8.4	3.9	4.1	5.1
70	8.5	8.4	9.1	3.9	3.7	4.8
80	8.7	7.8	9.3	4.5	4.3	5.9
82	8.1	.. ..	.. ..	3.8	.. ..	.. ..

Cows Brought to Fort Collins

On October 8, 1934 six affected cows, 3 from the herd of Frank Bourdette and 3 from that of Pierre Morgan, both lots from the

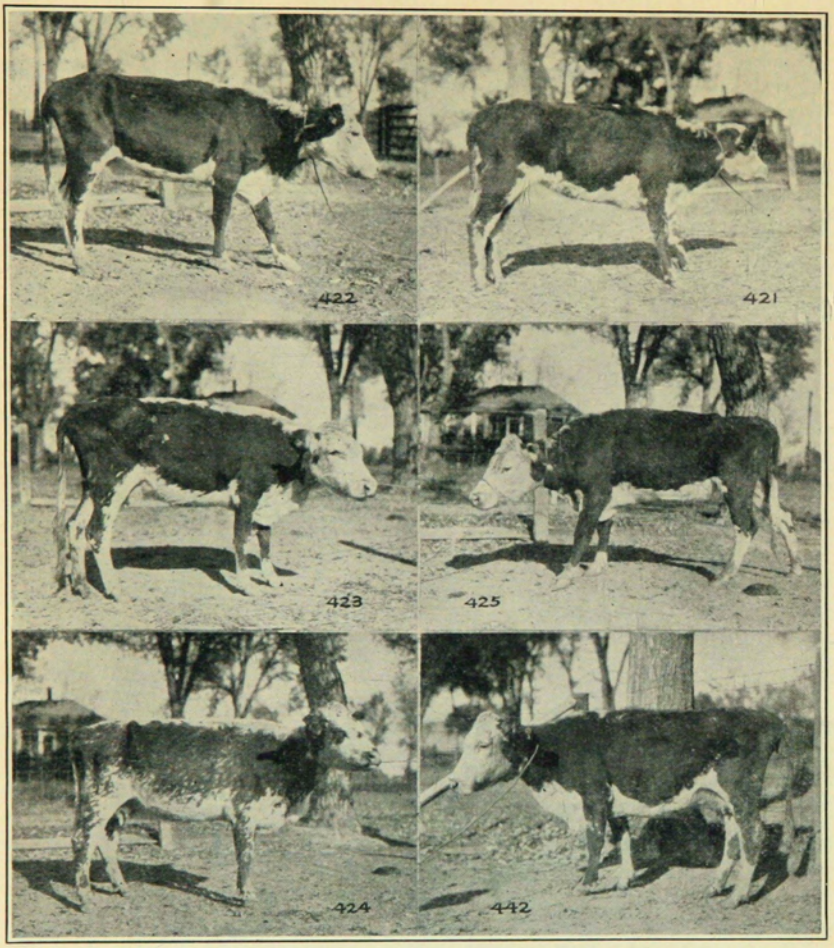

Figure 4.—Cows brought to Fort Collins; photographs taken three days after arrival.

Blue Mountain range, were trucked to Fort Collins. They were divided into two lots, one given only alfalfa hay, and the other alfalfa with the addition of bonemeal.

TABLE V.—RATIONS FED COWS AT FORT COLLINS

Lot number	Cow number	Ration
1	422 423 424	Alfalfa only
2	421 425 442	Alfalfa and bonemeal

All showed the typical symptoms of the disease; that is, emaciation, loss of voice, weaving, and the clicking sound of the hind feet when walking. Numbers 422 and 423 had not been previously bled. Numbers 424, 421, and 425 had been bled on September 4, and number 442 had been bled along with the other Bourdette cattle on June 27 and on August 2. She had shown symptoms at the time of the June bleeding. The others had developed the disease later in the summer.

The analysis of a sample of the alfalfa taken on November 8, 1934 was as follows:

FODDER ANALYSIS

Moisture	7.35 percent
Ash	7.52 percent
Protein	13.74 percent
Ether extract	1.11 percent
Fiber	39.38 percent
Nitrogen-free	30.90 percent
On Air-Dry Hay	
CaO	1.57 percent
P_2O_5	0.45 percent

The bonemeal was placed in a box in the pen and the cows allowed to take it at liberty. Five pounds were placed before them on October 12, 1934. As they did not consume it readily, further feedings were mixed with equal parts of common salt, and 5 pounds of that mixture were given on November 20, 1934, December 7, 1934, and January 2, 1935. On the same dates the other pen was given 2½ pounds of salt without the bonemeal.

At the time the cows arrived, 2 or 3 bones were thrown into the pen, but at no time were the cattle seen to pay any attention to them.

Bleedings were made of all six cattle at intervals as shown in the table, and phosphorus and calcium determinations made.

At the conclusion of this experiment on January 12, 1935, all cows except number 442, which had died in the meantime, were showing considerable improvement. Number 424 was evidently carrying a calf, had gained 184 pounds, and showed very little evidence of weaving or clicking when walking. She was sold as a normal animal. The other four were not with calf but were still showing some symptoms of the disease and were used for dissection. The symptoms were in inverse proportion to the gain in weight, number 425 being the least affected, and numbers 422 and 423 the worst.

Vomitus was frequently found in pen number 2 and was presumed to have come from number 442. However, it continued after the death of that animal and probably came from number 421, although no one ever saw that animal expelling the stomach content.

TABLE VI.—CALCIUM AND PHOSPHORUS IN BLOOD OF CATTLE BROUGHT TO FORT COLLINS

	Ca in mgm/100 cc blood						P in mgm/100 cc blood					
	Lot 1 Alfalfa			Lot 2 Alfalfa and bonemeal			Lot 1 Alfalfa			Lot 2 Alfalfa and bonemeal		
Date bled	Number of Animal											
	422	423	424	421	425	442	422	423	424	421	425	442
6-27-34	..	..	..	..	..	7.9	..	..	..	..	..	4.2
8-2-34	..	..	..	..	..	8.6	..	..	..	..	..	4.7
9-4-34	..	..	8.0	9.9	8.3	..	..	..	2.1	3.8	2.8	..
10-9-34	8.7	9.2	7.9	8.7	8.5	8.4	3.9	3.7	1.8	2.0	2.5	3.6
10-16-34	8.8	8.3	8.3	9.3	8.9	7.5	3.9	3.4	4.5	3.4	4.9	5.7
10-23-34	7.9	8.2	7.5	8.9	7.3	7.1	3.8	3.7	4.5	4.8	4.5	4.8
10-30-34	8.0	8.6	7.4	7.6	7.6	6.9	5.5	4.4	4.5	4.2	3.8	4.0
11-9-34	9.1	8.1	8.1	8.0	9.3	8.6	4.2	3.9	3.9	3.7	3.8	3.2
11-20-34	7.9	8.2	7.6	8.1	7.6	7.6	4.8	3.8	4.4	3.6	3.8	4.1
12-4-34	7.1	7.5	7.1	7.9	7.8	..	4.0	3.6	4.0	3.9	4.0	..
12-12-34	8.3	8.4	7.8	8.5	8.2	..	4.7	4.1	4.5	3.8	4.8	..
12-18-34	7.6	7.6	7.1	8.9	8.3	..	4.1	3.6	3.5	5.0	4.5	..
12-27-34	7.1	7.5	7.3	7.7	7.6	..	4.1	3.6	4.3	4.1	4.0	..
1-10-35	8.2	9.0	7.8	8.7	8.4	..	4.4	3.8	4.5	4.6	4.4	..

The cows were weighed at monthly intervals, according to the following table:

TABLE VII.—WEIGHTS OF COWS

Lot number	Cow number	10-11-34	11-13-34	12-12-34	1-12-35	Gain
1	422	685	710	670	760	75
Alfalfa	423	590	630	625	665	75
only	424	786	902	930	970	184
2	421	480	535	540	585	105
Alfalfa and	425	830	890	875	960	130
bonemeal	442	672	610	Died 11-29-34		

Number 442 was known to have had the disease longer than the others and did not respond to the changed food. She lost 62 pounds the first month and died on November 29. The postmortem examination revealed little that was distinctive. Emaciation was extreme. There was an excessive amount of synovia in several of the articulations, particularly noticeable in the coxo-femoral joint. The liver was quite small, weighing only 2,940 grams without the gall bladder.

The spleen weighed 363 grams and one kidney 475 grams. There was a 5-months fetus in the uterus. No parasite eggs were found in the feces.

While three of these cows were low in phosphorus on arrival, they did not chew bones nor did they crave bonemeal. Apparently they ate the bonemeal only to get the salt. The phosphorus content of the blood was practically normal at the end of the first week and was almost as high in the group not getting the bonemeal. After the first week there was no significant difference between the two lots with respect to the phosphorus content. Number 442 died of the disease without the phosphorus content ever running below 3.2 mgms percent.

Conclusions from Blood Study

With the exception of two animals (nos. 424 and 425) there were no significant differences either in calcium or phosphorus content between the healthy and the diseased cattle. With the same exceptions, there were no significant differences between the Moffat County cattle and the controls at Fort Collins. The two exceptions showed a normal phosphorus content within 2 weeks after being placed on alfalfa hay, the one getting the bonemeal showing no more rapid improvement than the one on alfalfa alone. Several animals died without showing any marked decrease in either phosphorus or calcium.

It is obvious, therefore, that the disease could not be attributed to either a phosphorus or a calcium deficiency.

Soil Analyses

The matter of determining the availability of minerals to plants is a question which has been widely discussed over an extended period of years. There is no unanimity of opinion among soil chemists today regarding the correct manner of estimating such quantities.

Weak acids and alkalies have been used, as well as neutral salt solutions. The various methods of soil extraction have produced columns of data, varying widely in actual quantities as expressed by the methods used. Doubtless many compounds both organic and inorganic within the soil, and even products elaborated by plant roots and bacteria, play a part in determining the quantities and qualities of foods available for plant nutrition. It is probable that no single acid constituent within the soil outranks CO_2 in its importance as solvent.

We accordingly decided to use the CO_2 extraction method in the study of availability of CaO and P_2O_5 in our range pasture soils. As controls we used (1) natural soils from the college foothills pasture, (2) tame pasture soils, and (3) cultivated soils in the environs of Fort Collins.

Our method is described in the appendix, and the results obtained are expressed in parts per million of oxides in the tables which follow.

In so far as the soils are concerned, the available lime content appears to be more than sufficient. The P_2O_5, however, fluctuates widely. On the basis of the use of the CO_2 extraction method over a period of 3 years in our laboratory, we have adopted certain criteria which, while arbitrary, seem to speak the truth for many Colorado soils; viz.,

For native range pastures 3.0 parts per million P_2O_5 appears sufficient.

For tame pastures 3.0 to 5.0 parts per million P_2O_5 appears sufficient.

For cultivated soils all values below 5.0 parts per million P_2O_5 have proved deficient, and values above 5.0 parts per million up to 10 parts per million have been found in cultivated soils of the Fort Collins area to correspond fairly well with productive soils under field crop conditions.

Discussion—The data presented, although not exhaustive, appear to show that the available P_2O_5 varies quite widely during the growing season. On the basis of these observations, the soil moisture at any given time appears to exercise a major influence upon the availability of phosphorus. Our native pasture soils in the foothills near Fort Collins do not differ widely from the range soils of the sections of Moffat County studied with respect to available phosphorus. The tame pastures of the college farm reflect the effects of cultivation in their higher content of available phosphorus. The range pasture soils on either side of the divide are represented by the altered products of disintegration of gray and red sandstones with admixtures from the various shales. It is observable that the available P_2O_5 decreases with the increase in red color (Fe^{+++} content) of the soils. The differences in availability observed as between the Blue Mountain and Douglas Mountain area and the eastern foothills region may be traced in large part to the slightly favorable moisture conditions prevailing during the growing season in the eastern foothills.

Soil Analyses

TABLE VIII.—ANALYSES OF SOILS
Available CaO and P_2O_5 extraction. Values in parts/million air-dry soil.
DOUGLAS MOUNTAIN

May 14, 1934			June 26, 1934			August 1, 1934			September 3, 1934		
Sample number	CaO	P_2O_5	Sample number	CaO	P_2O_5	Sample number	CaO	P_2O_5	Sample number	CaO	P_2O_5
3327	754	nil	3342	588	trace	3371	636	trace	3413	789	2.8
3328	4308	3.1	3341	950	1.3	3372	629	trace	3414	674	nil
3329	642	nil	3343	762	2.2	3374	487	nil	3415	543	nil
3330	3450	2.1	3344	2407	2.5	3373	1070	3.0	3416	1284	3.4

BLUE MOUNTAIN

June 28			August 2			September 4		
3345	656	2.9	3375	583	nil	3417	701	nil
3346	535	4.1	3376	821	trace			
3347	468	trace	3377	757	nil			
3348	730	5.3	3378	388	nil			

COLLEGE FOOTHILL PASTURE

May 25			July 3			August 17			September 29		
Sample number	CaO	P_2O_5	Sample number	CaO	P_2O_5	Sample number	CaO	P_2O_5	Sample number	CaO	P_2O_5
3337	4559	1.8	3350	4198	2.0	3389	4354	3.5	3424	4367	4.0
3338	4407	1.2	3349	4124	2.3	3390	4525	3.9	3425	4589	3.9

COLLEGE FARM—CULTIVATED TAME PASTURE

May 29			July 9			August 17			September 29		
Sample number	CaO	P_2O_5	Sample number	CaO	P_2O_5	Sample number	CaO	P_2O_5	Sample number	CaO	P_2O_5
3339	3201	2.0	3351	1565	5.4	3387	851	trace	3422	1920	5.6
3340	4319	1.6	3352	4011	4.1	3388	4434	4.1	3423	4570	4.7

Grass Analyses

Our method of sampling and handling the grass samples, like those of the soils, had to be adapted to the circumstance of carrying them out of a rather inaccessible region on horseback. While the soils were carried in closely-woven canvas bags, the grasses were carried in 1-pound tin cans packed in large bags which could be carried from the saddle. The methods of analysis described in the appendix were the usual approved A. O. A. C. methods.

TABLE IX.—ANALYSES OF SHRUBS AND WEEDS

DOUGLAS MOUNTAIN

	H_2O	Ash	CaO	P_2O_5
Balsam root weed	12.12	14.09	1.89	0.208
Sarvis berries and leaves	3.39	5.99	1.53	0.302
Short rabbit brush		8.36	2.25	0.235

BLUE MOUNTAIN

Cow parsnip	41.91	11.36	2.39	0.452
Helianthus		10.13	2.29	0.818

TABLE X.—ANALYSES OF GRASS

H_2O percentage on natural grass. All other values percentage on air-dry grass.

DOUGLAS MOUNTAIN

May 14, 1934					June 26, 1934				
Sample number	H_2O	Ash	CaO	P_2O_5	Sample number	H_2O	Ash	CaO	P_2O_5
3331	70.08	9.38	1.82	0.595	3354	44.31	11.02	0.642	0.336
3332	59.33	9.76	1.49	0.421	3353	44.49	9.06	1.27	0.200
3333	58.97	11.02	0.950	0.437	3355	41.62	12.28	1.00	0.472
3334	60.91	8.14	1.28	0.341	3356	46.13	8.04	0.819	0.200

August 1, 1934					September 3, 1934				
Sample number	H_2O	Ash	CaO	P_2O_5	Sample number	H_2O	Ash	CaO	P_2O_5
3380	30.51	9.89	1.31	0.332	3407	26.09	9.35	0.674	0.326
3379	30.55	12.57	0.729	0.348	3408	28.73	10.96	0.599	0.351
3382	19.75	9.75	0.696	0.359	3409	38.02	9.08	0.588	0.582
3381	14.61	8.59	0.770	0.098	3410	20.45	7.24	0.760	0.132

BLUE MOUNTAIN

June 28, 1934					August 2, 1934				
Sample number	H_2O	Ash	CaO	P_2O_5	Sample number	H_2O	Ash	CaO	P_2O_5
3357	46.13	7.91	1.06	0.317	3383	32.70	7.14	0.663	0.243
					3384	34.06	15.95	1.03	0.491
3358	36.18	8.60	0.704	0.417	3385	21.14	14.49	0.775	0.367
					3386	39.41	12.97	0.841	0.505

September 4, 1934				
Sample number	H_2O	Ash	CaO	P_2O_5
3411	48.11	8.34	0.753	0.365

FORT COLLINS VICINITY, FOOTHILL PASTURE

May 7					June 13				
Sample number	H_2O	Ash	CaO	P_2O_5	Sample number	H_2O	Ash	CaO	P_2O_5
3336		10.68	1.07	0.567	3359		6.50	0.698	0.304

July 16					August 6				
Sample number	H_2O	Ash	CaO	P_2O_5	Sample number	H_2O	Ash	CaO	P_2O_5
3368	19.67	8.93	1.01	0.209	3393		8.18	1.64	0.276

September 29				
Sample number	H_2O	Ash	CaO	P_2O_5
3428	17.91	5.75	0.610	0.144

COLLEGE FARM

H_2O percentage on natural grass. All other values percentage on air-dry grass.

May 29, 1935					July 9, 1935				
Sample number	H_2O	Ash	CaO	P_2O_5	Sample number	H_2O	Ash	CaO	P_2O_5
3335-A	74.30	10.33	0.783	0.800	3365	69.26	10.78	0.967	0.723
B					3366	77.91	9.81	2.54	0.538

August 17					September 29				
Sample number	H_2O	Ash	CaO	P_2O_5	Sample number	H_2O	Ash	CaO	P_2O_5
3391	64.58	9.23	1.11	0.682	3426	60.17	10.10	1.09	0.719
3392	72.87	10.12	2.84	0.477	3427	67.10	9.97	3.20	0.413

A—Timothy, brome grass—tame pasture, irrigated.
B—Sweet clover pasture—in plowed ground.

Discussion

The question of an adequate supply of lime and phosphorus in roughage depends on several factors, and it is rather difficult to determine deficiencies in specific cases. As our range pastures go, we know that in early spring, when nothing but dry grass is present from the previous growing season, this residue is very low in nutriment. By the first of June, when the young grasses are lush and at their best, the mineral content is highest. In irrigated pastures the growing conditions are favorable throughout the growing season, but on the range in a semi-arid region the herbage begins to die down

as early as July for lack of moisture, and by September there is little green material left.

Our analyses of grasses from the Douglas Mountain and Blue Mountain areas cannot be interpreted as inordinately low in lime and phosphorus content, even in late summer. This is accounted for by the fact that grass was scarce and grew only in protected places in the shelter of shrubbery. Here it may be reiterated that in all probability the deficiencies of the range consisted in the scarcity of grasses of any kind, rather than in the poor quality of those grasses which were present.

The gradual deterioration of the quality of range pasture is illustrated in our analyses of grasses from our foothills pasture. These grasses are largely of the grama, buffalo, and wheat grass varieties. The gradual diminution of the percentage of P_2O_5 as the season progresses is evident. In this respect, in our foothills pasture near the college the grasses are more abundant, but their mineral content is even less than that in the Moffat County ranges under observation.

Also, the effect of irrigation is quite apparent in the Ca and P content of herbage from tame pastures of the college farm.

In the table, A represents a timothy-brome grass pasture, and due to irrigation and limited grazing it maintained not only a fair stand of grass, but this remained of good quality throughout the growing season.

The set of results marked B in the table represents the lime and phosphorus content of a sweet clover pasture adjacent to the grass pasture A.

From the data presented, as well as from numerous analyses made in our laboratory over a period of years, it would appear that a content of .30 percent or more of P_2O_5 on the basis of air-dry grass cannot be considered as representing a deficiency in the plant. Likewise a lime content of .6 to .8 percent (CaO) for grasses would not appear to indicate deficiency.

From the rather limited quantity of data presented, it would appear that any deficiency of lime and phosphorus in the grasses of the Douglas and Blue Mountain ranges might more appropriately be ascribed to the quantity, and not to the quality, of these grasses.

Analysis of Plants for Selenium

During the past few years considerable attention has been given to the study of the content of rare elements in plants as a possible cause of their toxicity. The so-called "alkali disease" of cattle in

TABLE XI.—SELENIUM CONTENT OF PLANTS

COLORADO

Name of plant	Date taken	Location	Selenium content
Purple aster	July to September, 1934	Douglas Mountain	None
Chrysothamnus sp.	" " " "	" "	"
Lupinus sp. (blue)	" " " "	" "	Faint trace
Lupinus argenteus (short)	" " " "	Blue Mountain	None
Lupinus sp.	" " " "	" "	"
Atriplex (annual)	" " " "	Foot Blue Mountain	"
Atriplex campestris	" " " "	" " " "	"
Lupinus argenteus	July 20, 1935	West Moffat County	Faint trace
Astragalus lancelarius	" " "	" " "	" "
Ximenesia exauriculata	" " "	" " "	" "
Astragalus hylophilus (campestris)	" " "	Moffat County	Trace
Ximenesia exauriculata	" " "	" "	Faint trace
Lupinus argenteus	" " "	" "	None
Ximenesia exauriculata	" " "	South of Craig	Faint trace
Balsam orrhiza sagittata	" " "	" " "	None
Stanleya (mancos shale)	" " "	" " "	Faint trace
Astragalus hylophilus	" " "	" " "	None
Lithospermum sp.	" " "	West Moffat County	Faint trace
Crepis acuminata	" " "	" " "	None
Ligusticum officinale	" " "	" " "	"
Astragalus lancelarius	" " "	K Ranch	Faint trace
Astragalus hylophilus	" " "	" "	" "
Erigeron macranthus	" " "	" "	" "
Chrysopsis pedunculata	" " "	" "	" "
Chrysothamnus (rabbit brush)	" " "	" "	" "
Comandra pallida	" " "	" "	None
Chrysothamnus	" " "	" "	Faint trace
Eriogonum heracleoides	" " "	" "	" "
Cordylanthus ramosus	" " "	" "	" "
Chrysopsis hirsutissima	" " "	Blue Mountain	None
Gutierrezia sarothrae	" " "	" "	"
Pentstemon	" " "	" "	"
Stenotus acaulis	" " "	" "	"
Stenotus acaulis	" " "	" "	"

WYOMING

(From Dr. O. A. Beath)

Name of plant	Date taken	Location	Selenium content
Astragalus bisulcatus			0.5 ppm
Atriplex nuttallii			Faint trace
Astragalus bisulcatus		Laramie formation	Very heavy
Astragalus bisulcatus		Laramie formation	1,950 ppm
Astragalus bisulcatus		Niobrara formation	260 ppm
Oonopsis condensata		Niobrara formation	11 ppm

certain localities of the plains section has been investigated by O. A. Beath et al.[9] Wyoming, and K. W. Franke et al.[10] South Dakota; and related studies dealing with selenium have been made by A. M. Hurd-Karrer[11], Horace G. Byers[12], and the United States Department of Agriculture, and others. Since parts of Colorado fall within the area where plants may be affected by selenium content, it was thought advisable to study some of the plants, and particularly the weeds of the Douglas Mountain and Blue Mountain areas, for selenium content. This was the more advisable since, on an impoverished range, stock is often driven by hunger to eat plants which it would refuse under normal conditions. Accordingly, numerous samples of weeds from the previously-mentioned areas were collected, as well as a few samples of weeds from Wyoming which were known to carry selenium.

The weeds were brought to the laboratory, dried and tested according to the methods of Horn, M. J.[13] and Robinson.[14] In table XI are recorded the results obtained. All weed samples were first subjected to the qualitative test[9], and only those showing rather pronounced positive tests were subsequently tested in a quantitative manner.

The samples from Wyoming, by courtesy of O. A. Beath, were selected from the Niobrara soils because of their known positive reaction for selenium, and all these were determined quantitatively. In no case did the weeds from our Moffat County area (Colorado) show more than a trace of selenium (less than 0.5 part per million). For this reason it did not appear probable that the cattle on this range were suffering from selenium poisoning.

Timber Milk Vetch

It having become apparent that the cattle disease in Moffat County was not a phosphorus deficiency, the cooperation of the Botany Section of the station was secured, and a plant survey of the area was made in July 1935. It was then found that not only was the timber vetch present in the area, but that owing to repeated drouth and over-grazing it constituted a rather large percentage of the forage. Since the plant was then rapidly drying up, quick action had to be taken if any feeding experiments were to be conducted in that season.

Feeding Experiments

These were carried out on the Blue Mountain range, beginning on July 27, 1935. Four cows and five sheep were assembled by Pierre Morgan, and the vetch was gathered by Mr. and Mrs. Frank

E. Bourdette. The animals were kept in corrals, the cattle separately from the sheep; but the individuals were not separated, so that the amount of vetch consumed by each animal could not be ascertained. The cattle had to be driven 600 yards to water once daily but were not allowed to graze on the way. The sheep were watered in the corral. No food other than vetch was given during the time of the experiment.

Experimental Animals

Cattle

1. Light red cow, 3 years old, with calf 4 to 5 weeks old; good condition.
2. Roan cow, 3 years old, with calf 3 weeks old; good condition.
3. Small, dark red cow, 3 years old, with calf 2½ months old; good condition.
4. Large, dark red cow, 3 years old, dry; in better condition than others.

Sheep

1. Both ears cropped, 2 years old, with lamb 35 pounds weight.
2. Crop-eared ewe, 2 years old, with lamb 35 pounds weight.
3. Paint line ewe, 2 years old, with lamb 35 pounds weight.
4. Paint brand H ewe, 4 years old, dry.
5. Left-ear crop ewe, 3 years old, with lamb 35 pounds weight.

TABLE XII.—AMOUNTS OF VETCH FED AND EATEN

Date	Pounds vetch fed	Pounds vetch eaten
CATTLE		
7-28	27	All
7-29	96	All
7-30	87	All
7-31	95	75
8-1	60	20
8-2	74	44
8-3	41	37
SHEEP		
7-28	28	All
7-29	56	All
7-30	40	All
7-31	47	42
8-1	34	34
8-2	33	28
8-3	39	36

PROTOCOLS—CATTLE

COW NUMBER 1.

July 30. Bleary eyed; slight drooling from mouth, tip of tongue protruding; skin over nose dry and cracked. Continues to eat and drink and bawl for calf.

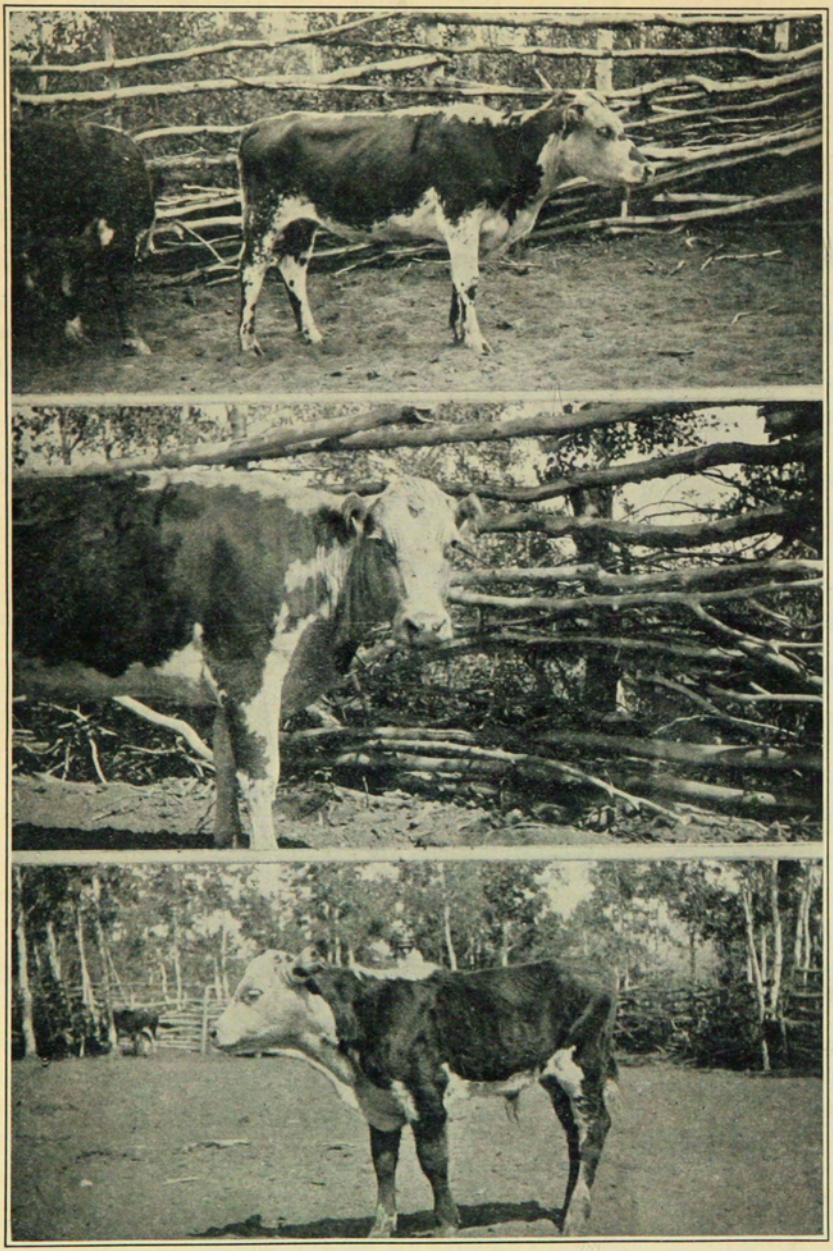

Figure 5.—Some of the animals observed in the vetch-feeding experiments. Top, cow number 2; center, cow number 1, with nose peeling; bottom, calf of cow number 3.

July 31. Depressed but irritable when disturbed. Drooling slightly increases, not a profuse type. Tongue tip protruding to lip edge, not enough for picture. Abnormal control of tongue, frequent licking of nose; skin over nose starting to peel off; nasal discharge serous and slight. Lower lip slightly drooping. Frequent swallowing of saliva; cheeks distend upon expiration movements. Slight incoordination of rear quarters after being driven to water. Very little milk secreted; calf hungry. Eating, drinking; bawls for calf; voice husky.

August 1. More depressed; drinks only small quantity of water. Calf scouring and hungry.

August 2. Respiration 40. Very depressed; lazy; slight coughing; loses cud; drooling, green-tinged saliva; very little milk secretion; not eating while observed; muscular tremors; would not drink; urinary irritation; calf gaunt, hungry, scouring.

August 3. Very depressed; small quantities of urine frequently voided in normal manner; slight coughing; respiration 37; no wheezing heard; gait remains slightly unsteady; muscular tremors; fails to show interest in fresh vetch. Would graze if allowed. Bawls for calf, voice husky; better control of tongue; drinks considerable water. Calf very gaunt; no other symptoms.

August 4. Will not move very far, even when hit with rope. Increased incoordination of posterior legs and knuckling of posterior fetlock when walking. Better control of tongue; most of skin has peeled from nose; coughing some. Eating dirt, vetch. Calf gaunt, hungry. Released.

September 3. This cow was found on the range and corraled September 3. Her general condition and appearance had changed considerably. The vertebral processes, scapular spine, hip joints and the tuber coxae were very prominent. The abdomen was a typical picture of "pot belly," being distended out of proportion to the balance of the body contour. A very marked loss of weight had occurred, without symptoms of gauntness in the flank region. The hair was dry, dull, and lusterless. The respiration was 58 before the animal was forced to move. A slight mucous discharge from both nostrils; an audible wheezy respiratory expiration sound that could easily be heard at a distance of 30 feet. Occasional coughing, which was dry and raspy or harsh.

Upon forcing the animal to walk around in the corral, the respiratory rate increased to 69, being short, shallow, rapid type. The wheezing became noticeably louder and the coughing more frequent. However, the character of neither changed sufficiently to be recorded. After walking approximately 60 yards she stopped and urinated, the first half of urine being normal, then the spasmodic voiding which was very irregular as to time and quantity. Another 50-yard drive resulted in an uncontrolled spasmodic voiding of small quantities of urine, followed by frequent attempts to urinate when forced to walk. Incoordination of the hind legs, especially manifested by knuckling of the fetlock and turning the toes inward at the time in the forward stroke to cause a solid impact with the posterior part of the ankle of the opposite leg. Sometimes only one claw would contact the opposite ankle. However, this striking the opposite ankle or claw resulted consistently in a ratio of 3 out of 4 steps taken. The knuckling of the ankle resulted in a very pronounced drop in the loin region, exhibiting symptoms comparable to those when an animal trips and is temporarily thrown off balance. This cow's calf has grown and fleshened normally and does not exhibit any evidence of vetch poisoning.

COW NUMBER 2.

July 28. Observed eating and drinking normally during the day.

August 1. Slight incoordination of hind legs; nose dry and peeling. Eating vetch; will not drink. Bawls for calf; voice normal. Decreased milk flow; calf hungry, beginning to scour, becoming gaunt.

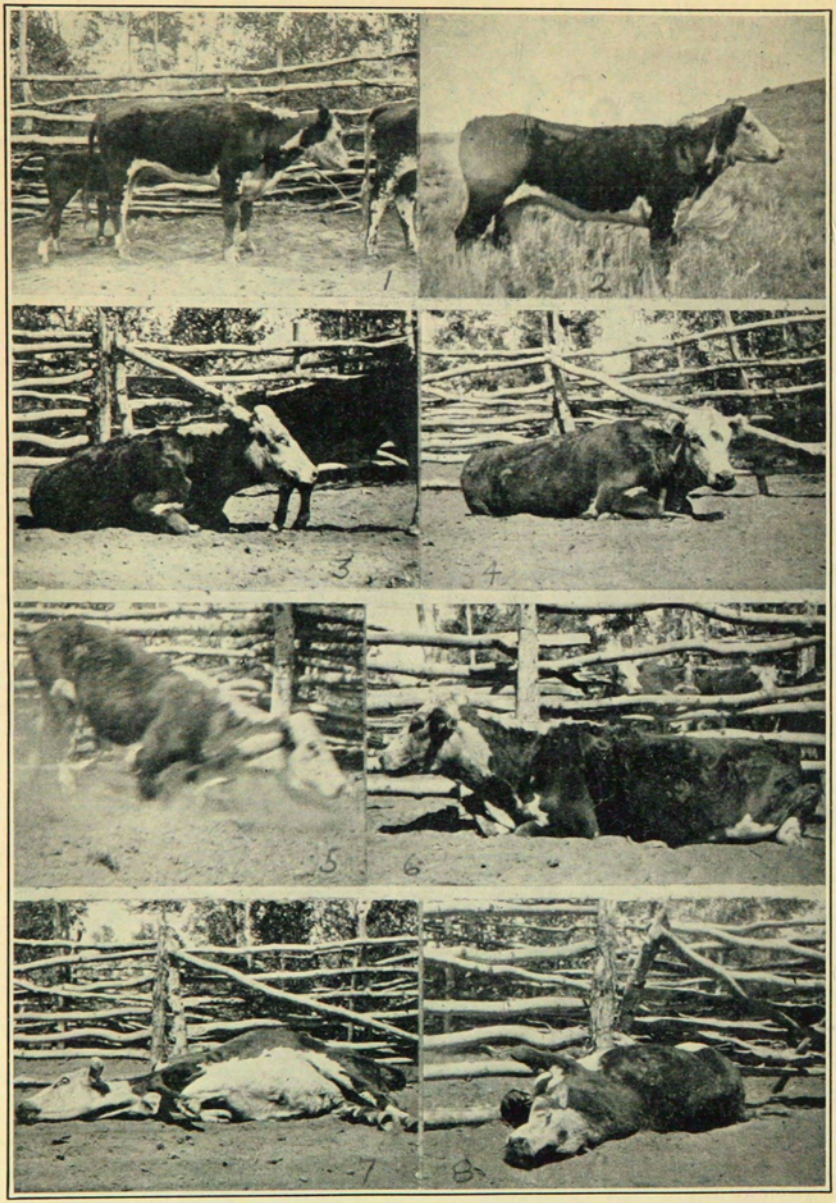

Figure 6.—Progress of the disease resulting in death of cow number 3 in vetch-feeding experiments. 1, before being driven to water; 2, period of excitement before falling; 3, after she went down and bawled for her calf; 4, she remains down; 5, she attempts to rise; 6, in attempting to rise she crawls along the ground; 7, she falls over on her side; 8, she rolls over.

August 2. Incoordination increased. Tremors of flank and thigh muscles. Difficulty in chewing and retaining cud. Slight salivation; becoming depressed. Irritable when exerted by driving. Eating vetch. Frequent swallowing; drinks water; milk flow decreased; notices calf; voice husky; calf scouring, very gaunt.

August 3. Depressed, lazy; eyes bleary; staggers, showing incoordination of front and hind legs after being driven to water. Drank water and wanted to leave others. Respiration 48; half of nose peeled off; eating vetch during observation period. Not much interest shown when calf was moved. Calf frequently attempts to nurse.

August 4. Muscular tremors very pronounced, especially of posterior quarters. Eating vetch; drinks water; marked incoordination of posterior quarters after being driven to water. Voice husky; frequent swallowing; very little drooling; calf growing weaker, not eating vetch. Released.

September 3. This cow and her calf were moved to the home ranch, placed in a corral, and fed alfalfa hay. Neither showed any evidence of vetch poisoning. The cow had completely recovered and was fatter than at the time she was placed on the experiment. The owner stated her milk flow had returned; from the appearance of the calf it was getting all the milk it needed, for a good growth had been made since it was released.

COW NUMBER 3.

July 28. Eating and drinking normally.

July 29. Eating and drinking normally.

July 30. Very nervous, irritable; wants to fight man on foot. Butts other cows and calves if they get near. Very anxious about her 3-months-old calf. Extremely alert to every moving object; eats and drinks very little. Calf eating vetch.

July 31. Previous symptoms; marked urinary irritation, especially noticeable while being driven to and from water. Had to rest 4 times on the way; went down twice on return trip after resting 5 to 10 minutes. Arose and followed after other cattle and her calf; voice husky and faint. Little interest shown in calf. Very marked incoordination of all legs. Posterior fetlocks knuckled over when walking, slight when standing. Generally stands with legs well under body. Muscular tremors of shoulder, flank, and thigh regions for an hour after returning from water. Respiration 62 upon return from water; one hour later, 47. Calf eating vetch. Seldom attempts to nurse.

August 1. Found down; forced to arise but went down after a few steps. Symptoms more pronounced; slight interest in calf; voice very faint, husky; slight drooling; frequent swallowing, nose peeling; very irritable. Calf observed eating vetch more frequently than other cattle. Calf drinks much water.

August 2. Muscular tremors pronounced. Staggering gait when forced to move. Went down after few steps. Refused to get up, even when teased. Respiration 110, short and difficult; cheeks distend upon expiration; slight drooling; had typical convulsion, got tangled in pole fence, pictures taken. Removed from fence; would not get up; no voice sound, while several apparent attempts were made to bawl; urinary irritation very pronounced. Jerking of leg whenever touched with the hand or stick; refused to drink water carried to her; very irritable if man approached; did not notice other cattle or horses; could not be made to arise. Calf showed incoordination of posterior quarter and knuckling over of posterior fetlocks before arriving back at the corral; following watering, laid down immediately after reaching corral; would get up if forced, walk or stagger a few steps, and stop with feet about normally set under body.

August 3. Cow unable to arise; tried 3 times and then refused to move even when man approached. Respiration 46, short, shallow type. Pulse full and very rapid, weak. Nose nearly peeled; very slight drooling; wheezy respiration; cheeks distend at irregular intervals upon expiration. No voice; no interest in calf; not eating; will not drink.

Calf eating little. Very marked incoordination of all legs when driven to and from water. Had to rest 5 times; nearly went down twice, but got up on feet and stood for a few minutes before going on. Did not notice dam. Voice faint and husky; nose peeling; no drooling; eyes bleary; marked muscular tremors of all body muscles, especially when forced to trot. Went down; would not get up.

August 4. No change in cow.

Calf, after being driven 100 yards, staggered and went down; not observed eating. Other symptoms as on August 3.

August 5. Cow still down; dying; destroyed.

September 3. Calf made rapid recovery.

COW NUMBER 4.

July 28 to August 1. Normal; eating vetch and drinking water during daily observations. Nose peeling July 31.

August 1. Irritable; steps very high with hind feet; fetlock knuckles over frequently, causing cow temporarily to lose her balance and drop down in the loin region. Incoordination of posterior quarters after walk of 200 yards; cows allowed to rest until returned to corral, then driven around inside corral a few times. Respiration increased rapidly, rate 72, short and shallow in character. Very marked staggering gait; generally stopped with feet in about normal position or would immediately reset them; muscular tremors of flank and thigh regions; voice normal. Becoming gaunt.

August 2. Staggered to and from water. Had to rest 4 times; if forced on would have gone down. Eating some during the day. Drinks considerable quantity of water, yet noticeably gaunt. Frequent voiding of small quantities of urine. Muscular tremors more pronounced. Less irritable; wants to be alone; would try to leave other cattle.

August 3. Not observed eating vetch during the day; wants to eat grass growing around water hole; went down once but after resting got up and staggered on back to the corral; very gaunt. Apparently lost weight faster than any of the other cattle. Rapid respiration; voice normal.

August 4. No marked change. Released.

September 3. Corralled on range and allowed to rest. Before forcing animal to move, respiration rate was 25. After walking approximately 200 yards, interrupted by three short rests while observations of cow number 1 were made, respiration rate was 33. This cow was extremely nervous. Muscular tremors of flank musculature pronounced after short exercise. Urination practically normal. Incoordination of hind legs and knocking of the opposite ankle on the medial surface was in a ratio of about 1 out of 4 steps. Cow in good state of flesh. Hair normal.

PROTOCOLS—SHEEP

EWE AND LAMB NUMBER 1.

July 28 and 29. Eating vetch and drinking; no symptoms. Lamb nursing and eating vetch.

July 30. Ears drooping; head lowered; slight raspy cough upon exertion. Eating vetch.

July 31. Above symptoms more pronounced; somewhat depressed. Respiration normal; increases slightly if animal is exercising.

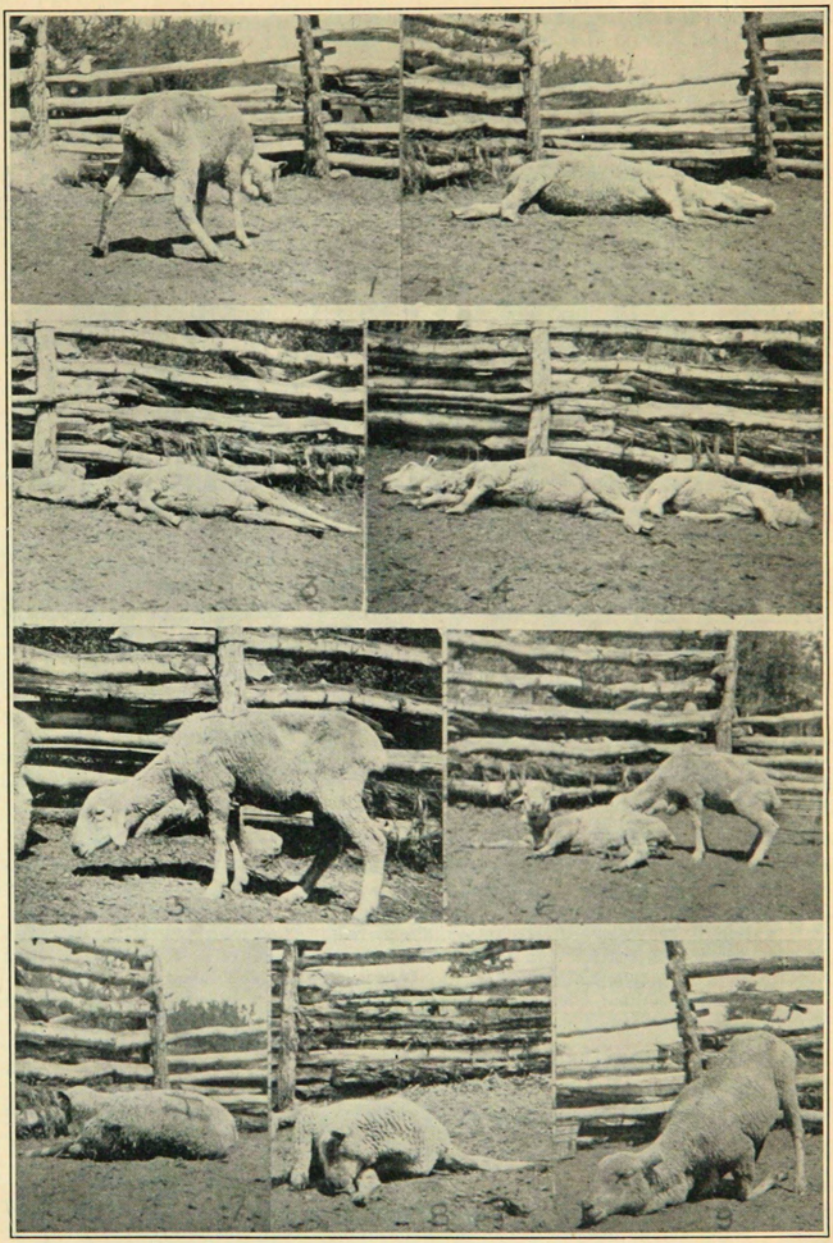

Figure 7.—Studies of sheep used in the vetch-feeding experiments. 1, Ewe number 1 staggering; 2, ewe number 1 after falling; 3, ewe number 2 just before death; 4, ewe number 2 after death; the lamb dropped dead immediately afterward; 5, ewe number 3; 6, ewe number 3 standing, and ewe number 5 down; 7, ewe number 4 goes down; 8, ewe number 4 just before death; 9, ewe number 5 in a frequent attitude.

August 1. Incoordination of all legs; down after being driven twice around a 25 by 25 foot corral. Respiration 38; coughing; after 5-minute rest got up; started eating vetch.

August 2. Head lowered; standing apart from other sheep. More depressed; staggered and fell on second drive around corral; voice normal; observed eating vetch; frequent swallowing of saliva.

August 3. Very depressed. Would move only a couple of steps when forced or pushed. Respiration 41; slight drooling; frequent swallowing; incoordination of all legs. Other sheep being driven around corral stimulated this ewe to attempt to follow; took a few steps and fell. Died almost instantly; no definite lesions found upon post mortem. Lamb eating vetch; no symptoms.

EWE AND LAMB NUMBER 2.

July 28 to August 1. Observed eating vetch; apparently normal .

August 1. Incoordination posterior legs. On second trip around corral went down; short rest; arose and walked to vetch pile and started eating; respiration 51, after getting up; voice normal; lamb eating vetch, stays close to ewe.

August 2. Somewhat depressed; lazy when attempts made to drive sheep. Incoordination of all legs on second trip around corral; laid down. Respiration 64 at that time; observed eating vetch. Lamb continued normally along with other sheep to vetch pile; started eating.

August 3. On first drive around corral staggered few steps, fell, and died instantly; no sound or convulsion. Lamb fell 3 feet in the rear and died instantly. No lesions on post mortem.

EWE AND LAMB NUMBER 3.

July 28 to August 2, incl. Eating vetch; drinking water.

August 3. Incoordination of all legs; frequent swallowing; active. Lamb eating vetch; no symptoms. Attempts to nurse; very little milk.

August 4. No change. Lamb shows no symptoms. Released.

EWE NUMBER 4.

July 28 to August 2. Appeared normal; observed eating vetch and drinking.

August 3. Forced to move with other sheep, dropped behind after 50 feet; left the others, walked unsteadily across 25-foot corral, and stopped with head lowered, feet normally placed. Other sheep driven over to that side of corral immediately and allowed to stand and rest. After approximately 2 minutes, this ewe raised her head and galloped back across corral, turned and headed back to other sheep but fell at about center of corral and died in convulsions with loud coughing. Post mortem: About ½ pint fluid in pericardial sac. No other lesions.

EWE AND LAMB NUMBER 5.

July 28 to August 2 incl. Eating vetch; lamb eating vetch.

August 3. Incoordination, especially of posterior quarters. Respiration increased to 41 after being driven twice around corral. Very alert, nervous; voice raspy.

August 4. Incoordination more pronounced; lamb no symptoms. Released.

September 3. The sheep released August 4 and placed in the band on this range could not be identified. At the time animals were released, several sheep in the main flock were showing the typical symptoms of acute poisoning. When driven short distances some would lag behind, go down, and die. Many ewes had the wheezy-type respiration and dry, harsh cough. The percentage loss during the period between August 1 and September 3 could not be accurately ascertained, yet the history obtained indicated a loss of from 1-3 a day out of a band of 1,200 ewes.

EXPERIMENTS WITH GUINEA PIGS

Beath[2] says that full strength alcohol is necessary for the preparation of a timber vetch extract, and that the minimum lethal dose of such extract when given intraperitoneally represents 1 gram of the moisture free plant. Both watery and alcoholic extracts were made by us and evaporated under vacuum to a gummy consistency.

Guinea pigs were given on successive days 1, 5, and 10 grams of the watery extract by the mouth, with no deleterious result.

Five guinea pigs were given each 1 gram of the alcoholic extract by the mouth, followed the next day with 10 grams of the same material, without any of them showing symptoms of poisoning.

Five grams of the air-dried ground plant were mixed with an equal quantity of ground barley, and each of 2 guinea pigs consumed this amount without evidence of illness. Each of the 2 pigs then consumed 10 grams of the plant, prepared in the same manner, daily for 11 days, with no untoward result.

It should be pointed out that these results are not directly contradictory to Beath's, since he administered his extracts intraperitoneally.

General Summary and Conclusions

A chronic disease of cattle occurring on Douglas and Blue Mountains, in the extreme northwestern part of Colorado, developing in the late summer and manifesting itself mostly in lactating animals, is described. The more prominent symptoms are incoordination of gait, weaving, clicking the heels together, emaciation, weakness, husky voice, the urine passed in spurts on driving, and finally paralysis and death. Because the disease was confined to cows with calves, and for the further reason that the animals were said to chew bones, it was assumed that there was a phosphorus or a lime deficiency. Exhaustive chemical tests of blood, soil, and forage proved that to be erroneous.

Suspicion then fell upon the timber milk vetch (*Astragalus hylophilus*). Feeding experiments showed that the disease could be produced in both cattle and sheep by feeding this plant. It then developed that severe losses in sheep in the same territory, which formerly had not been associated with the cattle loss because they were more acute, could also be attributed to the same weed. Numerous examinations of the forage to determine whether selenium played any part were negative. No toxic substance was demonstrated by feeding either the plant itself or the watery or alcoholic extracts to guinea pigs.

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APPENDIX

COLORIMETRIC METHODS FOR DETERMINATION OF INORGANIC CALCIUM AND PHOSPHORUS IN BLOOD SERUM

BLOOD

SAMPLE

About 25 cc of blood is drawn by means of a canula from the jugular vein of an animal into a small sample bottle containing 1.0 cc of 20 percent sodium citrate solution (lithium citrate is used when sodium is to be determined in the blood). The sample freshly taken is well mixed with the citrate by slow shaking, to avoid all possibility of clotting, and then transferred directly to the laboratory or stored at low temperature (30°-40°C.) until ready for use. Storage for more than 24 hours is to be avoided if blood is to be analyzed fractionally.

In the laboratory the sample is shaken and 20 cc pipetted into a 100 cc centrifuge tube, containing 80 cc of 5 percent trichloracetic acid solution. Shake well and centrifuge mixture for 10 minutes at a speed of 2000 r.p.m., then filter and centrifuge again, if necessary, to obtain a clear solution. This clear solution of blood serum may then be stored in a refrigerator for relatively long periods of time without affecting the subsequent analysis for phosphorus and calcium.

INORGANIC PHOSPHORUS DETERMINATION

REAGENTS

(1) Molybdenum Blue Reagent—Zinzadze*.

(2) Standard Phosphate Solution—0.4394 g dry KH_2PO_4 dissolved in 1 liter of H_2O—i. e., 1 cc=0.1 mgm P

A dilute standard is made by diluting 5 cc of above standard to 100 cc —i. e.,—1 cc= .005 mgm P.

PROCEDURE

Eight cc of blood serum solution is pipetted into a 50 cc centrifuge tube. 1.5 cc of Molybdenum Blue reagent is added and the contents diluted to 50 cc and stirred. Six cc of the dilute standard phosphate solution is treated similarly. (Or, if the P content is small, 3 cc of standard is made up in the same way.)

The tubes in a suitable wire basket are placed in a simmering water bath for 20 minutes. (Occasional stirring during this period is advantageous.) The tubes are then cooled in running water to laboratory temperature, centrifuged for 5 minutes at about 1800 r.p.m. to precipitate any colloidal suspension, and after standing for an hour are ready for color comparison.

With the 6 cc standard set at 30 mm, (standard colorimeter), the calculation is

$$\frac{30}{R} \times 0.03 \times \frac{100}{1.6} = P \text{ (in milligrams per 100 cc bloods)}$$

$$\frac{56.25}{R} = P$$

*Zinzadze's reagent as prepared for our work. (Also Mo Blue reagent.)

PREPARATION

Bring 50 cc of H_2SO_4(sp.gr. 1.84) to a boil (in hood) and add 3 grams of MoO_3. Continue heating until complete solution of MoO_3.

Cool to laboratory temperature and pour cautiously into 50 cc of distilled water. While this solution is still hot, transfer to a short-necked flask, add 0.15 gram of powdered Molybdenum metal and boil for 10 minutes. If convenient, allow to stand overnight and then decant carefully, leaving any undissolved residual metal (Mo).

Titrate the strength of this solution by adding it dropwise from burette into 5 to 7.5 cc n/10 $KMnO_4$. Then dilute this reagent with a 50 percent H_2SO_4 solution of MoO_3 prepared according to procedure described above (50 cc (1.84) H_2SO_4+3 grams MoO_3+50 cc H_2O), until 2.5 cc Mo Blue reagent = 0.2 cc n/10 $KMnO_4$. Store this reagent in clean glass-stoppered bottle in cool dark place. It will remain constant for several months.

CALCIUM DETERMINATION

REAGENTS

(1) Mo Blue Reagent (Zinzadze).

(2) 5 percent Na_3PO_4 solution (freshly prepared) 23.1 gm $Na_3PO_4 \cdot 12H_2O$/200 cc H_2O.

(3) NaOH solution 18 percent.

(4) A calcium standard made from C. P. $CaCO_3$ dissolved in HCl and diluted to such a volume that 1 cc solution 0.1 mgm Ca.

(5) Washing mixture: 580 cc of 96 percent alcohol.
320 cc distilled water.
100 cc amyl alcohol.

This mixture is made alkaline to 1 drop phenolphthalein by addition of NaOH.

PROCEDURE

Ten cc of serum solution is pipetted into a 50 cc centrifuge tube. With phenol-phthalein as indicator, the 18 percent NaOH is added dropwise until the solution is alkaline and then 1 cc in excess is added. After standing 5 minutes, 1 cc of 5 percent Na_3PO_4 is added to each tube and the contents thoroughly mixed by twirling.

One and .7 cc of the calcium standard solution is treated in a similar manner.

After one hour is allowed for precipitation (with occasional twirling), the tubes are then centrifuged for a 10-minute period at a speed of about 2,000 r.p.m. (Care is observed not to stir up the precipitates after centrifuging.) The tubes are then carefully decanted and finally inverted on clean absorbent paper to drain. The mouths of the tubes should be carefully wiped, and then the precipitate is broken up by playing a fine stream of washing mixture upon it from the wash bottle.

The precipitates are thus washed and centrifuged 3 times. After the final washing the tubes are carefully drained and then dried for 3 or 4 minutes in a 100°C. oven.

One and .8 cc of Mo Blue reagent is now added, which amount should readily dissolve and precipitate. The contents are made up to 50 cc and thenceforth treated in a similar manner as described under phosphorus determination.

With the 1.7 cc standard placed at 30 mm on the colorimeter, the calculation becomes $\frac{30}{R} \times \frac{0.17}{2} \times \frac{100}{1}$ mgm ca. per 100 cc blood $\frac{255}{R} = Ca$

REFERENCES

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DETERMINATION OF AVAILABLE CALCIUM AND PHOSPHORUS IN SOILS (CO_2 EXTRACTION METHOD)

SAMPLING

Representative samples of soil to 12 inches depth are taken in pasture or plowed field. A 2-quart fruit-jarful is usually adequate for all tests which are to be made.

The soil, when brought into the laboratory, is put through a 6-mesh screen, and the coarse gravel is discarded. The soil is then laid out to air dry and is stored.

PROCEDURE

Fifty gram portions of air dry soil, after being ground and put through a 20-mesh screen, are weighed into duplicate (2 liter) balloon flasks and 500 cc distilled water added. After the soil has become thoroughly disintegrated and saturated with water (about 1 hour), the mixture is saturated for 15 minutes with frequent shaking with a stream of CO_2 (freshly generated), and then the flasks are stoppered and allowed to stand over night.

The samples are again saturated with CO_2 for 15 minutes with frequent shaking, then stoppered and shaken for 60 minutes on a mechanical shaker. The mass is centrifuged (at 2,000 r.p.m.) in 200 cc bottles and subsequently filtered through a Buechner funnel.

The amount of extract usually recovered is in excess of 450 cc, and 10 cc of this extract is taken to represent 1 gm air dry soil. (The above total procedure should be calculated to consume 24 hours).

This extract is divided into two 200 cc portions and one 50 cc portion. One aliquot of 200 cc is used for P_2O_5 determination, while the other is held in reserve, and the 50 cc aliquot is used for CAO determination.

PHOSPHORUS

A 200 cc aliquot of CO_2 extract (representing 20 gm soil) is evaporated to dryness in a smooth silica dish and then ignited free of organic matter. The heating may be at incipient dull redness in electric muffle, or over an electric hot-plate, but must not be carried to fusion.

When cool, the mass is moistened with 2 cc (1:6) H_2SO_4 and about 25 cc of distilled water. By means of a rubber policeman, all the solid material is transferred from the sides of the dish to the bottom. The solution is then heated on a water bath for 30 to 60 minutes, cooled and filtered into 100 cc beakers. Evaporate to about 30 cc, using 2-4 dinitrophenol (saturated aqueous) solution as an indicator, neutralize with NaOH (10 percent), and transfer to a 50 cc graduate.

One and .5 cc of (Zinzadze) Mo Blue reagent is now added, the solution made up to 50 cc, stirred, and transferred to 50 cc centrifuge tubes. These are heated by immersion in simmering water bath for 20 minutes, then removed and cooled to laboratory temperature by running water.

After 1 to 1.5 hours of elapsed time, the color readings are made against a 6 cc (KH_2PO_4) color standard and calculated to parts P_2O_5 per million.

CALCIUM

In the third aliquot (50 cc) the CaO is determined in accordance with the titrimetric method described in Methods (A. O. A. C.), Chapter XXVII, paragraph 36, where the Ca is precipitated as oxalate and titrated with n/10 $KMnO_4$ solution.

METHOD FOR CA AND P DETERMINATION IN GRASSES AND HERBAGE

When the grasses or herbs sampled are at relatively great distances from the laboratory, 1-pound tin cans with close fitting lids are suitable to use as containers. These, when reasonably packed with the mixed herbage of pastures which the animals are grazing, will hold about 200 grams of material.

The air-dried grasses are weighed, then cut up fine by running through a Wiley mill and stored in suitable glass jars.

Five gram samples of dried grasses are charred in large porcelain crucibles in a muffle furnace, taking care not to raise the temperature above the point of the first incipient dull red heat of the furnace.

The ashes are taken up with (1+4) HCl and evaporated to dryness on water bath as described in "Methods of Analysis" A. O. A. C., chapter XII, Plants. The solutions free from silica are made up to 200 cc (solution A) in volumetric flasks, and 25 cc aliquots of this solution are taken for analysis.

CALCIUM

This is determined by the titrimetric method described in Methods, A. O. A. C., by precipitating the Ca as oxalate and titration with standard $KMnO_4$ reagent (25 cc aliquots are used).

Sample of results obtained:

	CaO	
	On dry grass	
Buffalo grass	1.018 percent	1.033 percent
Sleepy grass	2.615 percent	2.675 percent
Number 3288, Rush	0.494 percent	0.477 percent
Number 3290, Sedge	0.477 percent	0.475 percent
Number 3293, Sedge	0.618 percent	0.599 percent

PHOSPHORUS

In a 25 cc aliquot of the above solution A, determine the P_2O_5 as outlined in A. O. A. C. Methods, chapter II, No. 10, by precipitating the phosphorus as the phospho-molybdate and titrating the molybdate with standard NaOH. Below are some of the sample results:

	P_2O_5	
Buffalo grass	0.234 percent	0.226 percent
Sleepy grass	0.261 percent	0.264 percent
Number 3288, Rush	0.645 percent	0.691 percent
Number 3290, Sedge	0.550 percent	0.571 percent
Number 3293, Sedge	0.366 percent	0.382 percent