AN INVESTIGATION OF THE CAUSE PREVENTION AND TREATMENT OF CATTLE DISORDERS IN THE SWAN RIVER VALLEY OF MANITOBA

A Thesis

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by

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HISTORICAL

A disorder of cattle has been known to exist for over forty years in the South-Western portion of the Swan River Valley of Manitoba. This condition usually occurs during pasture seasons of wet years with the affected animals exhibiting a persistent "pining", or debility. It was found that horses, sheep, and pigs remain unaffected but that young calves and cows in milk are most susceptible. At timesthe malady has been so severe as to cause as high as thirty per cent death loss in calves and several deaths among mature stock. Very characteristic of the disease is the fact that scouring ceases abruptly if the stock are removed to unaffected areas. Hay from affected fields is usually regarded as "safe" though in some cases such hay has been known to cause looseness and even scouring.

Some farmers outside the area attributed the condition to poor feeding practices, but despite good feeding regimes on many farms the disease persisted. Agricultural experts and veterinarians were unable to diagnose the condition or to suggest remedial or preventive measures. Farmers in the circumstances found it economically expedient to reduce their herds or to obtain use of pastures outside the affected area. Farmers were therefore involved in loss of revenue by reason of reduced cattle production, and at the same time, the other chief source of revenue - grain growing - was not feasible, the greater part of the affected area being of hilly topography and subject to severe erosion under cultivation.

Members of the University of Manitoba Animal Science Department and officials of the Manitoba Department of Agriculture first became

cognizant of these disorders about twenty-five years ago. The findings of Theiler (1924) in South Africa, and Eckles (1930) in Minnesota, had brought to light the existence of a wide-spread-area deficiency of phosphorus in soils and forage, which in turn give rise to a pathological condition in cattle. The clinical symptoms of such a condition in some respects (notably debility) simulate those manifested by cattle in the Swan River area, and hence, the earliest recommendations to allow cattle access to a phosphorus supplement. Following the failure of such therapy either to prevent or ameliorate the condition, various theories as to cause - parasitism, bacteria, water supply, poisonous plants, selenium - were advanced over the years but postulation of causes and application of remedial measures did not result in recognizable abatement of the disease. Thus it became increasingly evident that the problem would not be resolved by any procedure other than an intense study conducted in situ.

Funds for the conduct of such became available in the early summer of 1948, and accordingly, a study was inaugurated by the Animal Science Department of the University of Manitoba and concurrently and separately by the Soils Department of the University.

REVIEW OF LITERATURE

Cobalt

The existence of a disease clinical entity variously designated "Bush Sickness" (Kidson, 1937), Morton Main's Disease (Breirem, 1944), Moor Disease (Kivimae, 1945), and Coast Disease (Marston, 1948), has been reported from many countries. Deficiency of cobalt alone in some cases, and in others a combination of cobalt and copper deficiencies, have been established as the causal agents. Several cobalt deficiency areas have been reported in Canada; by Hancock (1945) in Nova Scotia, McIntosh (1945) in Ontario, and Hamilton (1946) in British Columbia. Cobalt deficiency symptoms, similar to those described by Cunningham (1946) for copper deficiency, or to those of molybdenum excess as described by Ferguson (1940) differ but little from those exhibited by cattle in the Swan River Valley. A typical syndrome for cobalt deficiency is described by Hancock (1945) and Keener (1944). It usually includes a depraced or depressed appetite, constipation or diarrhoea, progressive debility, disinclination to drink water, staring coat, dry scaly skin, muscular incoordination, anemia, anorexia, and sometimes death. The Swan River Valley disorder differs in that sheep are not noticeably affected and there is a definite craving for salt that increases as the disease progresses.

Askew (1944) states that cobalt deficiency occurs when stock are pastured for long periods on cobalt deficient pastures, and that the deficiency is overcome if the animals spend two or three weeks of their summer on "safe" pastures. He found also that the disorder can be prevented or treated in several ways. The most practical method for large

ranges is to supply the stock with about 1 to 2 mg. of cobalt per day. Top dressing the soil with 16 ounces of cobalt sulphate every three years is also a very effective method. Oral dosage (Geyer, 1945) is more cumbersome but desirable for precise experimental work or for badly affected animals. Feeds high in cobalt such as brewers or bakers yeast will also provide some additional cobalt.

Selenium

The symptoms exhibited by cattle in the affected area were found to be similar in many respects to those of selenium poisoning of cattle in South Dakota, Montana, Nebraska, and Kansas (Franke, 1934). In horses, cattle, and swine, selenium poisoning manifests itself clinically by an alteration in the growth of the horn, of the hoofs, and a loss of hair from the mane and tail of horses, switch of cattle, and the hair of swine. In more severe cases, the animals exhibit marked impairment of the locomotor function and unless given careful attention may die of thirst or starvation. Many of the most severely affected animals die or are destroyed. Some plants, including wood aster, certain vetches, and Nuttall's saltbush, accumulate in their tissues the selenium and perhaps other toxic minerals, and are poisonous only when growing on seleniferous soils. Selenium containing grain and hay is toxic and should not be used for livestock.

The disease in the Swan River Valley was then similar to selenium poisoning only in respect of the coat and hoof changes. The Swan River disorder differed from selenium poisoning in that a persistent diarrhoea was involved, horses and pigs were not affected, hay was not usually toxic, the area did not lie on seleniferous soils, and the plants

associated with selenium poisoning were absent. It was then evident after a preliminary survey of the affected area in the early summer of 1948, that selenium poisoning was not the cause of the disorder.

Molybdenum

The first case of molybdenum poisoning of cattle was reported in Somerset in England. Three workers, Ferguson, Lewis, and Watson, (1940) found that large quantities of molybdenum in the herbage of affected pastures was correlated with the severity of the disorder. The disease was marked by a persistent diarrhoea, fading of coat color, decreased milk flow, stiffness of limbs, and extreme emaciation. An intensive study was started in 1947 and in the succeeding years the disease was proved to be caused by an excess of molybdenum in the pasturage.

A similar disorder in the San Joaquin Valley of California was later reported by Barshad (1948) to be caused by molybdenum poisoning. The symptoms shown by the affected cattle were identical with those observed in Somerset.

In England, "teart" herbage was found to contain from 20 to 100 parts per million of molybdenum while nearby "non-teart" herbage usually contained less than 5 p.p.m. Consequently, the English investigators set the lowest toxic level at 20 p.p.m. In the San Joaquin Valley the disorder was found to be caused by much lower concentrations of molybdenum in the herbage. Barshad (1948) reported that disturbances appeared in cattle on Ladindo clover containing 15 p.p.m. Britton (1946) reported that there was a persistent scouring with fading of coat around Buena Lake in California on alfalfa containing 6

to 36 p.p.m. and Britton (1946) reported chronic molybdenum poisoning in cattle in the Kearn County of California on alfalfa pasturage containing only 10.3 p.p.m. Britton thus formulated the theory that the 20 p.p.m. limit set by the English workers was too high and believed that 10 p.p.m. would be a more accurate limit.

The effects of still smaller quantities of molybdenum were noticed in New Zealand where copper deficiencies were believed to exist. Here, Alcroft (1946) suggests that an excess of molybdenum may be partly responsible. A few analysis of pastures showed 2.5 to 305 p.p.m. in copper deficient areas where no scouring occurs. While unthriftiness, anemia and difficulty in producing and rearing young may be due mainly to copper deficiency, it is believed that the severe and persistent diarrhoea of "peat scours" may be associated with excess of molybdenum superimposed on copper deficiency.

Experiments in artificially producing the disease in cattle with the administration of a molybdenum salt have been undertaken in at least three countries. In Somerset, Ferguson (1940) reports that cattle on winter rations which received a daily molybdenum dose equivalent to 30 pounds of dry matter containing 150 p.p.m. of molybdenum scoured in 4 to 10 days. Cattle pastured on unaffected areas only required one third of this amount to produce the same effects. On either ration the symptoms were identical to those of cattle on "teart" pastures but about one third of the cattle appeared to be resistant to the toxicity and did not scour on these levels. In Australia, (Dick, 1945) a group of stall fed cattle and a group of pastured cattle received ammonium molybdate daily for three years and seven months at a rate equi-

valent to 28 and 20 p.p.m. respectively. Though no information was given about any digestive disturbances, it is reported that the livers of these cattle showed very small copper contents. In California, scouring, emaciation, and greying of coat were produced in a 6-month-old Holstein Friesian calf by feeding sodium molybdate daily for 7 months at a rate equivalent to that used in the winter trials in England. A month after the cessation of molybdenum feeding the animal had almost recovered.

These trials would indicate that the disorder can be induced artificially in cattle but that the resistance among individuals to the toxicity varies greatly.

Analysis of toxic vegetation in Britain and America show that legumes tend to absorb more molybdenum than the grasses and grains. Newly sown pastures on "teart" land are said to be "non-teart" for one or two years indicating that the older the plant the greater the absorption. Like the degree of "teartness", the molybdenum content of the herbage is highest in autumn. The molybdenum content of plants was also found to be highest in the parts that have a high metabolic activity such as leaves, growing points, runners, nodules, and lowest in old stems and roots.

In both England and U.S.A. the molybdenum content of the plants was found to vary with the molybdenum content and p.H. of the soil. Alkaline soils were found to be far more toxic than acid soils with the same molybdenum content. The molybdenum was found to leach down in the acid soils and to remain near the surface in the alkaline soils. This would explain the fact that in the acid soils in England the molybdenum content of the soil was found to increase with depth, while on alkaline

soils in California the molybdenum content of the soil was found to decrease with depth.

Prevention and Treatment of "Teartness"

English and American investigators have experimented with, and have recommended, several methods of reducing the effects of "teartness." English workers recommend 2 grams of copper sulphate per animal per day for mature stock and 1 gram per day for calves. They also suggest avoidance of sowing pastures with plants that absorb large quantities of molybdenum and recommend ploughing and reseeding of the older pastures which are more toxic. California workers found that by supplementing the pastures with liberal amounts of dry roughage, approximately normal condition of cattle can be maintained even when pasture plants contain as much as 100 p.p.m.

THE DISEASE

The symptoms in affected cattle in the Swan River Valley are similar to those of a disease in cattle reported by Ferguson (1940) in England and by Barshad (1948) in California. In both countries the cause was demonstrated to be excessive molybdenum in the pasture plants.

The main symptoms of the disorder common to the Swan River Valley disorder are severe diarrhoea, anaemia, and excessive salt consumption. The feces become exceedingly thin, bubbly, dark in color, and often contain large amounts of undigested matter. The hemoglobin content of the blood was also observed to drop significantly as the disease progressed.

The diarrhoea and anaemia are accompanied by many bodily changes. There is a rough, staring coat of hair, characteristic stiffness of legs, severe loss of weight, and noticeable decrease in milk flow. The animal becomes emaciated and in the advanced stage the lower joints may become swollen and the animal may become so stiff that it has great difficulty in rising. The hair color fades and may shed in spots or over the entire body. The hooves have been reported to grow rapidly and at times become abnormally long and turned up in front. Also there appears to be a permanent change in size, and especially so if the animal has been severely affected for several months. Calves appear to remain somewhat stunted and it is not uncommon to hear good breeders complain that their cattle do not attain the size expected by virtue of their breeding.

The disorder is more severe during wet seasons than during dry seasons. The summers of 1948 and 1949 were exceptionally dry and as a result the severe diarrhoea did not appear until late in July and August.

However, other symptoms such as unthriftiness and high salt consumption appeared in a milder form earlier in the season. If the season is dry, onset of the disorder is delayed and does not become severe until several days after a heavy rain. If cattle are changed from a short pasture to a lush pasture in an adjacent field, scouring commences in most cases within one to five days. The lushness of the pasture would therefore appear to be related to the development of the symptoms.

The degree of toxicity varies greatly. Some pastures are so toxic as to cause scouring during the entire grazing season while less affected pastures, even near the centre of the area, cause scouring only during a long wet season. It was also found in the survey, that the legumes, which are deep rooted plants, were more toxic in previous years than were the grasses. Alfalfa and clover pastures on some farms induced severe scouring throughout the grazing season of a dry summer.

A most peculiar symptom that has not been reported in other areas of molybdenum poisoning, is the increased craving for salt shown by affected animals. Severely affected cattle in the Swan River Valley have been known to consume for several days up to sixteen to twenty times their normal salt consumption. This extreme salt consumption tends to aggravate the disorder and even in the absence of the disorder would ordinarily cause scouring and irritability (Frens, 1946, and Steyn, 1946). Nevertheless, salt toxicity is a secondary factor, as when salt is denied or in circumstances of controlled feeding of salt, the disorder still manifests itself.

Different animals show different degrees of susceptibility to the disorder under identical conditions. Some individuals scour on dry

pasture, others do not become affected until the approach of rainy seasons, and still others withstand the most toxic herbage in the area. This resistance does not appear to be inherited, though there is some evidence from later experiments that some type of resistance may be acquired.

Some animals appear to be unaffected though their thriftiness and capacity for production seems to be lowered. This is also true in the winter season when animals are fed on hay from the affected area. They become exceptionally "hard keepers", and some farmers find that livestock fed copper sulphate during the entire winter, come through in better condition and on less feed than those that receive no treatment. When copper sulphate theropy is discontinued for a few days, there is an immediate decline in the physical condition and production of animals.

THE 1948 INVESTIGATIONAL PROCEDURE

From June 1st to July 15th, 1948, the disorder was not in evidence in the affected area. During this period a survey was conducted to test the validity of certain theories as to cause of the disorder. The selenium poisoning theory was first investigated. This investigation included a search for plants associated with selenium poisoning and a survey of the manifestations of the disorder. As demonstrated in "the Review of Literature", the selenium poisoning theory was found invalid and attention was then focused on cobalt deficiency.

Cobalt

The most direct method of determining the existence of a cobalt deficiency seemed to be the oral administration of cobalt to affected cattle. Nineteen cattle pastured on four of the most severely affected pastures in the area were used in the experiment. 63 mg. of cobalt was administered weekly as a drench to alternate animals. This daily intake of nine milligrams, according to Geyer (1945) is adequate for normal health. As anemia is one of the main symptoms of cobalt deficiency (Neal, 1937) hemoglobin determinations were made on each animal using the Fisher Electro Hemometer. This experiment was run from June 21st to August 2nd.

As the spring and early summer of 1948 were exceptionally dry, the disorder was manifested in a mild form with only a few animals pastured near the center of the area developing the typical syndrome. At no time during the seven weeks of the trial did the treated animals show any improvement over the checks. The scouring and other typical symptoms of the disorder were equally in evidence in both groups and increased in

severity with the advent of the rainy season.

As two heifers in one herd became so severely affected at the end of four weeks it was necessary to move the herd to unaffected pastures outside the area. For this reason, the hemoglobin determinations recorded in Table No. 1 are complete only for 13 cattle. The data in Table No. 1 are not statistically significant and hence, on the basis of this experiment, cobalt treatments had no significant effect on hemoglobin levels. Though the July rains appeared to aggravate the condition, (cattle on Farm 1, some of the worst affected in the district, showed a gradual decrease in hemoglobin levels from July to August) the hemoglobin levels did not significantly change.

Molybdenum

By August 2nd it became evident that cobalt therapy had failed and hence that the disorder must be caused by some other factor. Attention was next directed towards a study of the possibility of excess molybdenum in pasture plants being the causal agent. Ferguson (1940) demonstrated the high therapeutic value of a daily intake of 2 grams copper sulphate in the instance of cattle affected with symptoms almost identical with those manifested by cattle in the Swan River area. It was therefore considered advisable to experiment with the same therapy.

Accordingly, copper sulphate therapy was given to half the controls and to half the animals that had received cobalt in the previous experiment. As cobalt therapy was also continued with the same animals that had received it previously, the group of thirteen cattle was divided into four treatment groups; controls, cobalt, copper sulphate, and cobalt

TABLE 1

HEMOGLOBIN DETERMINATIONS

(Grams per 100 c.c. blood serum)

Animal No.	Herd No.	June 21	June 28	July 5	July 12	July 19	July 26	August 2
1		9.00	9.50	10.25	10.25	8.75	9.50	8.25
2		11.50	10.25	9.00	10.00	11.25	10.00	9.25
3 x	1	8.50	7.75	7.50	8.25	7.50	8.50	8.25
4		12.00	10.75	10.75	10.25	11.25	11.50	8.50
5 x		12.00	11.50	10.50	10.75	10.75	9.25	9.00
6 x		6.25	6.25	7.00	10.00	8.00	8.75	9.25
7	2	10.00	9.75	9.25	9.50	9.25	9.50	10.00
8 x		8.00	8.50	8.75	8.50	8.75	9.00	10.25
9		8.25	9.00	9.25	10.25	9.50	9.25	11.00
10		10.00	9.00	10.25	11.00	9.00	9.50	10.00
11 x	3	9.00	9.00	9.00	10.00	9.00	9.50	10.00
12 x		7.00	9.50	8.75	9.50	7.75	7.75	9.25
13		9.75	10.00	8.75	9.50	9.75	10.75	11.00

x - Received Cobalt Dosage

See Appendix III for analysis of variance and "t" test.

and copper sulphate. The copper-sulphate group and the copper-sulphate-cobalt group showed an immediate and marked improvement. Within two to three days the scouring subsided, the milk flow increased and the animals assumed a more thrifty appearance. The cattle that received both cobalt and copper sulphate showed no improvement over those that received copper sulphate only. There was no difference in the intensity of the disorder in the cobalt and check groups though there was a gradual improvement by fall when the pastures became sparse and the toxicity was not so great.

It was evident that cobalt theropy was without value either as a curative or preventive agent. It was likewise evident that copper sulphate was an effective therapy.

Experimental Causation of the Disease

During the fall of 1948 experiments were conducted with cattle at the University of Manitoba to determine if molybdenum would produce the same syndrome as observed in the affected cattle in the Swan River Valley. Five 2-year-old Holstein heifers constituted the experimental group. These were weighed before and after the experiment and hemoglobin determinations were made at weekly intervals. The molybdenum dosage followed that used by Ferguson (1940) in Somerset in England. 5.145 grams of solium molybdate equivalent to molybdenum content of 150 p.p.m. of 30 pounds of dry matter, was administered in a daily drench to each animal.

Within four days, the molybdenum had produced a stiffness of legs, a slight looseness of feces, and a general listlessness. These symptoms became more intensified each day until the fourteenth day, at which time, two animals were scouring so severely and the others were in

such poor condition that it was necessary to discontinue the drenches. Failure to cause severe diarrhoea in all heifers was attributed to the exceptionally binding nature of the winter feed. Though weight loss in Table 2 was not found to be significant, all animals but one lost weight during the 25 day period with the average weight loss for the five being 44 pounds. The hemoglobin determinations recorded in the following table also indicate no significant change at the 1 percent level though a continuous drop was recorded for animal No. 5.

TABLE 2

Hemoglobin Determinations and Weight Loss

of Animals on Molybdenum Dosage

Weights (in lbs)

	Animal No.				
Date	1	2	3	4	5
Nov. 8, 1948	660	845	720	645	575
Dec. 3, 1948	695	725	690	625	490
Gain or loss	35	-120	-30	-20	-85

Hemoglobin Determinations (grams per 100 c.c. blood)

	Animal No.				
Date, 1948	1	2	3	4	5
Nov. 8	10.00	8.75	9.00	8.75	10.00
Nov. 14	10.75	9.00	9.75	12.00	9.50
Nov. 24	9.00	9.25	8.75	8.75	9.00
Dec. 3	10.00	8.25	10.00	8.25	8.75

See Appendix III for analysis of variance and "t" test.

FEASIBILITY OF SALT AS A CARRIER IN COPPER SULPHATE THERAPY

In copper sulphate therapy it is important that each animal receive a definite quantity of this compound each day. The margin between optimum copper sulphate therapy and copper sulphate poisoning is not wide, and for this reason copper sulphate must be administered with care. Copper sulphate has a bitter metallic taste and can not be fed in the pure form. Administration by drenching, though effective and accurate in experimental procedure, was found in previous investigations (Edie, 1948) to be impracticable in commercial cattle production.

As affected cattle exhibit an abnormal craving for salt it was hoped that a salt lick containing copper sulphate would not only supply the corrective agent, but would provide an effective regulator in the therapy whereby stock would tend to consume more of the mix at a time when they required it most. It was then evident that if salt consumption varied with the degree of herbage toxicity, it could be used as a reliable criterion of the degree of herbage toxicity. With this objective in mind an accurate record was kept throughout the summer of the salt consumption of all herds under observation.

Two trials were set up in the area in June, 1949 with the intention of discovering if cattle on pasture would consume sufficient copper sulphate in the form of a salt lick to constitute an effective therapeutic. In these and in all future experiments, copper sulphate levels in the salt were based on a normal salt consumption as stated by Morrison (1947) of approximately 1 ounce per adult animal per day. In later trials this was found to be very close to the normal consumption

of cattle in the affected area. Investigations (Ferguson, 1940; Edie, 1948) indicate that two grams of copper sulphate per adult animal per day was sufficient to prevent the disorder. To furnish this amount in a salt medium it was necessary to incorporate two grams of powdered copper sulphate in 14 parts of salt. A 1:14 mix was then supplied ad libitum to Herd 1 and a 1:28 mix was similarily made available to Herd 2. Graph 1 indicates that Herd 1, which had received copper sulphate in the feed throughout the winter, and was receiving salt ad libitum up to the time of the experiment, consumed more salt than was needed to supply two grams of copper sulphate per animal per day. Graph 2 shows that Herd 2, which had received no copper sulphate therapy during the previous months and had received limited amounts of salt prior to the experiment, consumed excessive quantities of salt during the first few days of the trial. The first week of both trials was adequate to demonstrate that sufficient copper sulphate to prevent development of the disorder could be administered to cattle in the form of a salt lick.

Another trial on the palatibility of copper sulphate in salt was conducted during the later part of the summer. This experiment was designed to find if cattle would descriminate against any of the ratios of copper sulphate to salt used in the trials in the area. As the most severely affected farms were already in use for experiments it was necessary to conduct this experiment on Herd 4.

A trough with two compartments 16"x8"x4" was used. The treated and untreated salt was put in separate compartments and made available ad libitum to five mature cattle. Each week the unconsumed salt was weighed back and replaced by a more concentrated mix. The results appear

Graph 1

Herd 1

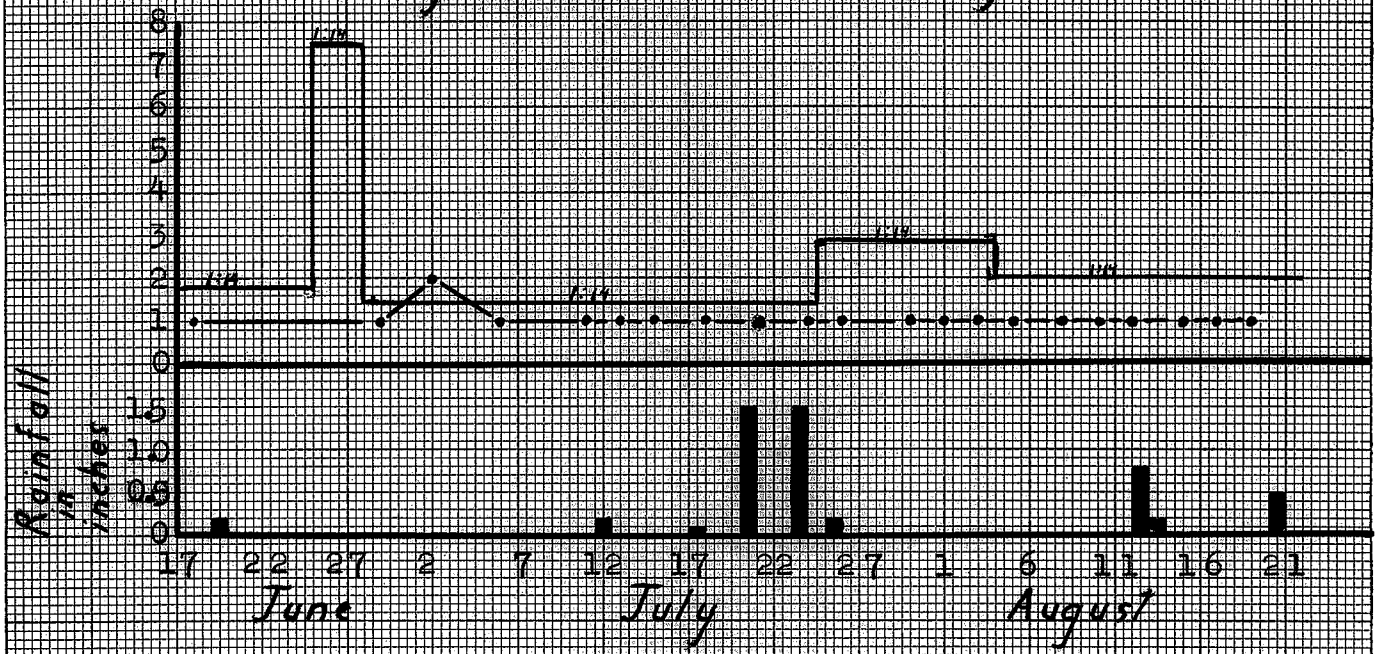
4 cows

Pasture-grade 2

Legend

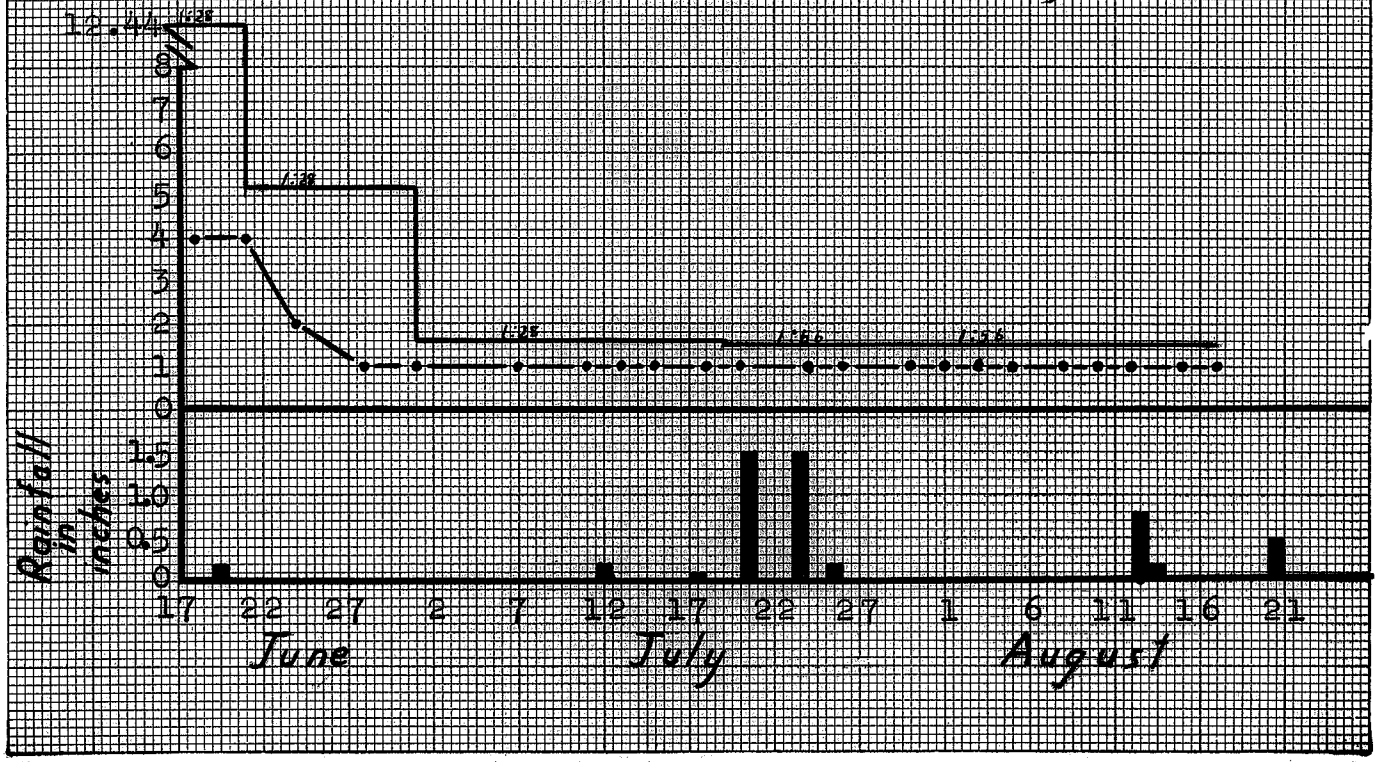
Salt-oz:animal:day

Feces-grade



Graph 2

Herd 2 4 cows Pasture grade 2



in Table 3.

It is evident from Table 3 that cattle showed no discrimination between the treated and untreated salt until the offering of the 1:14 mix was reached. The total salt consumption then showed very little change from the previous week but there was an immediate decrease in consumption of treated salt and a subsequent increase in consumption of untreated salt. The increase in salt consumption by the second week was probably due to more lush pasturage produced by rains. However, the lower level of copper sulphate during the first week could have depleted the animals copper reserves and hence provoked an increased salt consumption during the second week.

As this farm was only moderately affected it was not possible to determine whether severely affected cattle had an instinctive craving for copper-sulphate-fortified salt. This experiment then indicated that copper sulphate incorporated in salt at low levels is sufficiently palatable by stock grazing on moderately affected pastures to insure freedom from disease symptoms.

TABLE 3

Palatibility of Copper-Fortified Salt

Week	Ratio of $CuSO_4$ to salt	Salt consumption in ozs per animal per day		
		Treated	Untreated	Total
1	1:56	.80	.80	1.60
2	1:28	1.10	1.10	2.20
3	1:14	.67	1.60	2.28

ESTABLISHING CRITERIA FOR APPRAISING TRIALS

In determining the corrective value of any level of copper sulphate therapy it was necessary to establish a number of reliable criteria by which the degree of toxicity of the pasture could be evaluated and by which the degree that an animal was affected could be estimated.

It was first necessary to take into consideration factors such as rain, condition of pasture, and species of pasture plants, all of which have an effect on the toxicity of the pasture. If two herds of cattle are pastured on equally toxic herbage, the herd that is on the more closely grazed pasture will not require as much copper sulphate to overcome the toxicity of the pasture. If this difference in yield of pastures is not taken into consideration, the soil of the undergrazed pasture will be considered to be more toxic than the soil of the closely grazed pasture. Similarily, a soil associated with a legume pasture might be assumed erroneously to be more toxic than an equally affected soil growing a non leguminous pasture. Rains also tend to increase the toxicity of pastures and unless taken into consideration, copper sulphate requirements for cattle established during dry seasons will be expected to be sufficient to prevent the disorder during wet seasons.

As the forage yield of all pastures steadily decreased from spring to fall with only slight increases manifested with the advent of rains, the grazing condition of each pasture was classified as Poor, Good, Very good, or Excellent. These grades correspond to Grades 1, 2, 3, and 4. The predominant species of each pasture was also noted and recorded along with the condition of the pasture. All cases where stock

was moved to fresh pastures or had their pastures accidentally or purposely supplemented with green fodder were noted and included in the record of the trial. In order to demonstrate the effect of rains on the severity of the disorder, all precipitation was measured and plotted against the consistency of feces and salt consumption of each trial.

Though there are many symptoms common to the disorder, there are few that can be measured quantitively to represent the degree that an animal is affected by the disorder. The symptoms of greatest value in this respect are the consistency of feces and consumption of salt.

As scouring was observed to become more severe as the disease progressed, the consistency of the feces was graded in order to indicate varying degrees of toxicity. The grades established were numbered from 1 to 4 and correspond to firm, loose, scouring, and severe scouring. Though these grades are relative, even as is degree of thriftiness, they were of great value in estimating the effects of copper sulphate therapy.

As cattle tend to crave more salt as the disease progresses it was evident that salt consumption should also prove to be a valuable criterion in respect to the extent to which a herd was affected. In all trials with salt, the salt was allowed ad libitum in small weighed quantities, (usually $3\frac{1}{2}$ pounds) and combined with copper sulphate in a definite ratio. In allowing only small lots and replenishing as soon as finished, an indication of the trend of the experiment was obtained and losses through waste from rains and other factors were reduced to a minimum. Allowing the salt ad libitum was necessary to obtain a record of the total salt that a herd would consume under given conditions. This method of administering copper sulphate was also of practical value

to the farmer in that it was readily adaptable to local conditions.

In analyzing the results from trials on copper sulphate levels in salt it was found that neither of the two criteria was highly accurate when used by itself to determine the extent that an animal was affected, but when used together, they made possible a very accurate estimate of the extent to which a herd was affected.

In most cases, toxic effects were indicated by an increase in both scouring and salt consumption. However, in cases where either one remained normal and the other changed they were still of value in determining the benefits of a given level of copper sulphate. For example, Graph 1 shows that Herd 1 pastured on one of the worst affected pastures in the area and on a copper sulphate to salt ratio of 1:14 exhibited no signs of scouring all summer but had an above-normal salt consumption which increased after the heavy August rains. The above-normal salt consumption indicated that the herd was affected during most of the summer, being most highly affected after the rains, while the absence of scouring indicated that there was a sufficient intake of copper sulphate to prevent manifestation of the disorder.

When it was found that salt consumption could be used as a measure of the severity of the disorder, it was obviously advantageous to obtain some idea of the normal consumption of treated salt by unaffected cattle in this area. This figure could then be compared with the normal 1 ounce per animal per day level upon which all trials were based. To establish this, two herds representing approximately average conditions for the area were selected. Herd 3, located near the centre of the area, was supplied with a 1:28 mix of copper sulphate to salt plus

an additional 2 grams of copper sulphate per animal per day in the grain. Herd 2, located near the outskirts of the area, was supplied with the same level of copper sulphate in the salt, but as the pasture in this part of the area was considered to be less toxic than in the centre, only 1 gram of copper sulphate per animal was given daily in the grain. The additional copper sulphate administered in the grain insured that the stock would remain in an unaffected condition, while if it was added to the salt the stock might refuse the salt due to the objectionable taste arising from the high concentration of copper sulphate. The moderate amount of copper sulphate in the 1:28 salt mix represented the average ratio of copper sulphate to salt used in further trials. It was later found in the trial on palatability of copper sulphate in salt that cattle showed no discrimination between salt fortified at this rate and untreated salt. Herd 3 was kept on this trial for 5 weeks and Herd 2 for 4 weeks, a time period in each case which was considered adequate to obtain an accurate estimate of the average normal salt consumption per animal.

At no time during these trials did the animals exhibit any symptoms of the disorder. Graphs 2 and 3 show that the average salt consumption during the trials remained at 1 ounce per animal per day and was thus in conformity with the figure for normal salt consumption on which all trials were based.

Salt consumption of cattle varies with season, sex, and lactation period (Morrison, 1947). It was then often necessary to make allowance for some of these factors in analyzing the results from salt trials with herds in the Swan River Valley where such conditions were not normal.

Graph 3

Herd 3

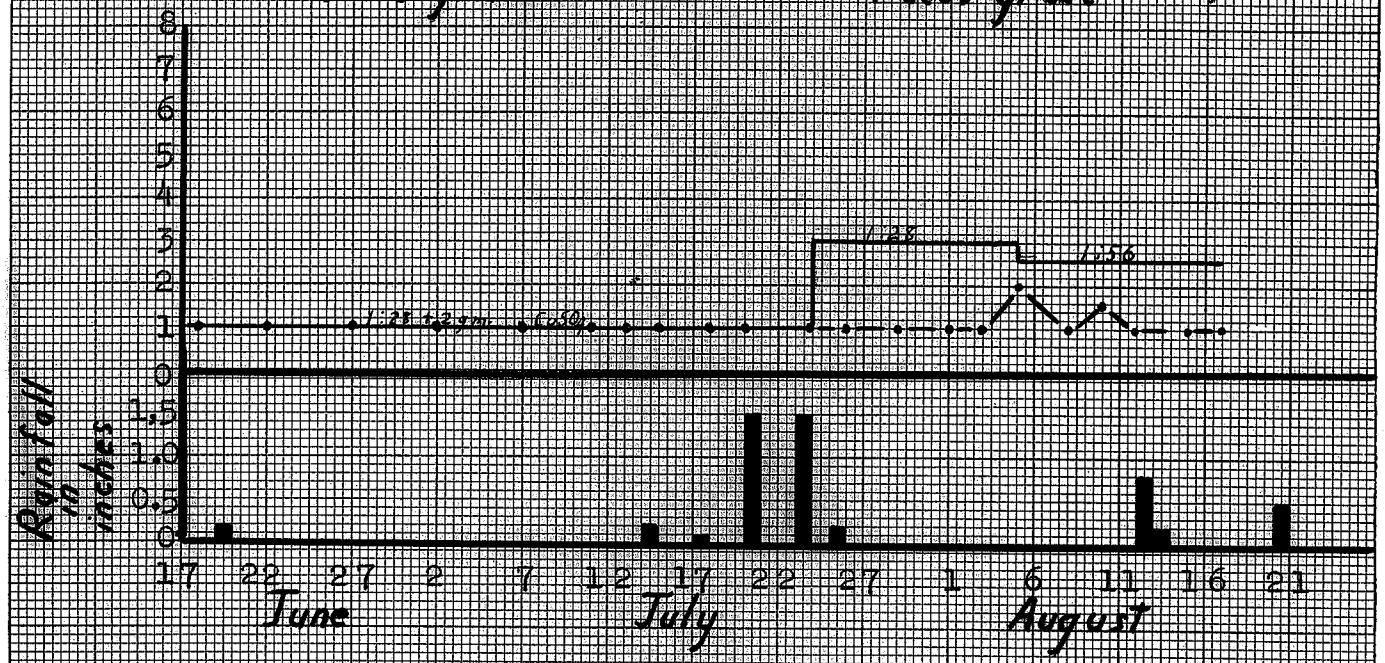
3 cows

Pasture grade 2

Legend

Salt-oz:animal:day

Feces grade

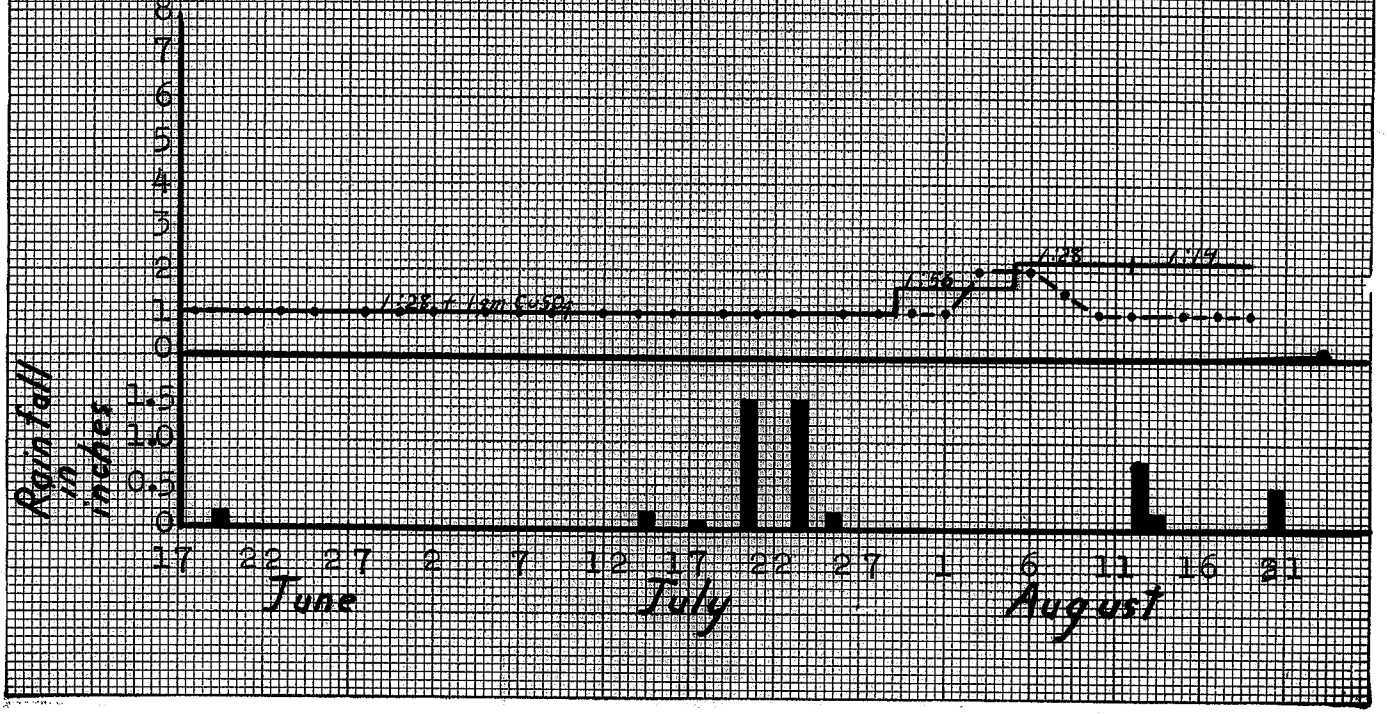


Graph 4

Herd 4

4 cows, 1 bull

Pasture grade 3



DETERMINATION OF APPROPRIATE LEVELS OF COPPER SULPHATE INCORPORATION IN SALT TRIALS

An investigation was conducted during the pasture season to determine the level of copper sulphate necessary in a salt lick to maintain normal health of pastured cattle in each part of the affected area. Nine herds consisting of 38 cattle distributed over the area were subjected to experiments constantly under supervision of the experimentalist, with copper sulphate administered in salt. Several other herds in the affected area, which, for various reasons could not be used for accurate experimental work, were treated by this method and observed at weekly intervals.

In all of these experiments, four ratios of copper sulphate to salt were used, 1:14, 1:28, 1:56, and 1:112. The concentration of 1:14 was selected as the upper limit in order that an animal ingesting a normal daily amount of 1 ounce of salt would obtain 2 grams of copper sulphate. This quantity was shown in previous investigations by Edie, 1948, and Ferguson, 1940, to be sufficient to cure or alleviate the scouring associated with the disorder.

Due to the small number of cattle in the area, very few trials could be conducted with each of the different levels of copper sulphate. Assuming that lighter rates would be sufficient near the outside of the area, the heavier concentrations of copper sulphate in salt were confined to the centre of the area. In this way, it was possible to distribute the lighter rates over the entire area. Each herd was kept on the same rate throughout the summer except in cases where the level of copper sulphate in salt proved insufficient to maintain health. In such

cases, animals were restored to normal condition with a higher level of copper sulphate in the salt and again put on the assigned level to determine if such was sufficient to prevent recurrence, the toxicity having once been overcome. It was then possible to determine the minimum level of copper sulphate necessary in the salt to provide affected animals with adequate protection against toxicity.

Three times a week observations were made and recorded on precipitation, consistency of feces, yield of pastures, salt consumption, and general condition. These data were recorded and graphed for each herd so that the effects of each factor could be estimated. The number of each graph is the same as that of the corresponding herd, pasture, and farm.

Trial 1

Response of Herd 1, pastured on what surveys had established as the worst affected pasture in the area, and having access to a 1:14 mix, is shown in Graph 1. Scouring was not in evidence at any time during the summer. However, the salt consumption was above normal at all times and showed substantial increases after heavy rains. These data indicate that the level was sufficient to control the scouring but was insufficient to maintain a normal salt consumption during wet seasons. All animals remained in a healthy condition throughout the summer and no toxic symptoms were observed. As the salt consumption was greatly in excess of normal for only a short period, the rate of 1:14 was considered adequate for cattle on this pasture.

Trial 2

Graph 2 shows the results of experiments with Herd 2 which

was pastured near the centre of the affected area. Though part of the pasture extended down on to the unaffected river flats, it was learned that in previous years when the stock was pastured on the high land only, the scouring was most severe. The extremely high consumption of 1:28 salt mix during the first few days of this experiment may be accounted for in that previous to the experiment the herd was affected by the disorder and animals were not receiving salt ad libitum. As abatement of scouring was rapid with the continued treatment, and as the effects of the toxicity were offset in all respects, it was evident that the 1:28 copper sulphate-salt mix was satisfactorily therapeutic. This concentration of copper sulphate was later reduced by fifty per cent, and when succeeding rains failed to produce any indication of the disorder, it was concluded that this pasture was not as toxic as hitherto assumed.

Thus, the rate of 1:28 was considered to be adequate for this herd provided that this mix was constantly available and that animals were given the run of the entire pasture.

Trial 3

Graph 3 shows the results secured from Herd 3 which was used as a check on salt consumption for the first seven weeks of the experiments. This graph not only illustrates the amount of copper sulphate required on this farm but indicates how the herd's salt consumption increased immediately when the needs for a corrective agent were not met. When the stock received the greater part of the copper sulphate in their grain, their salt consumption was normal, but when the only source was the salt mix, their salt consumption rose until they were

ingesting daily, an amount of copper sulphate equivalent to the amount formerly obtained from the copper sulphate-supplemented-grain. It was realized, however, that the amount of rainfall at this time had an additional effect on salt consumption. Later, when the level of copper sulphate in the salt was reduced by one half, instead of increasing the salt consumption until they obtained sufficient copper sulphate to prevent toxicity, the cattle showed the typical signs of the disorder.

It is concluded from these data, that an intake of three grams of copper sulphate per animal per day, which intake can be achieved by allowing animals free access to a 1:14 mix, is essential to maintenance of health on this pasture.

Trial 4

Herd 4, situated in the northern extremity of the area exhibited very slight manifestations of the disorder during two successive years. Nevertheless, in previous years, this herd was more severely affected than any of the herds in the southern portion of the area. From June 17 until July 28, this herd was used for a check on salt consumption and from July 29 to August 18, on copper sulphate palatability trials, both of which have been discussed under the heading of "Adaptability of Copper Sulphate in Salt." Graph 4 shows that a rate of 1:28 was sufficient to prevent slight scours of stock on high yielding alfalfa pasture.

It is then evident that during dry years a level of 1:28 would be adequate for this herd but during wet seasons a narrower ratio would be called for.

Trials 5 and 6

Trials 5 and 6 were conducted to determine the copper sulphate intake necessary for prevention of the disorder in moderate and slightly affected parts of the area. Herd 5, pastured near the edge of the area and Herd 6 located between Herd 5 and the centre of the area were tested with a mix of 1:56. The only noticeable difference in the two trials besides the difference in location was that the pasture on which Herd 5 was grazed was a grade lower than that of Herd 6. A duplicate of these experiments was begun in June with Herd 10 in the centre of the area but as the herd was moved to pasture outside the area before the trial had been in progress a week, no data were obtained.

Graphs 5 and 6 indicate that this level of copper sulphate in salt (1:56) was not sufficient to prevent some manifestation of the disorder. The salt consumption of Herd 6 remained almost constant throughout the summer and though the level was sufficient to prevent scouring during dry weather, it was not adequate after rainy weather. Also, the herd exhibited a listlessness and unthriftiness during the entire summer and at no time appeared to be completely free of the disorder. Graphs 5 and 6 show that the 1:56 level more closely met the requirements of Herd 5 than Herd 6. There was no evidence of scouring until after the rains and the herd remained in a more thrifty state during the trial period. However, the above normal salt consumption at all times indicated that the copper sulphate level was entirely satisfactory. The data yielded by Trials 5 and 6 therefore indicate that the level of 1:56 was insufficient to meet the requirements of either herd and that Pasture 6 was more toxic than Pasture 5. As

Graph 6

Herd 6

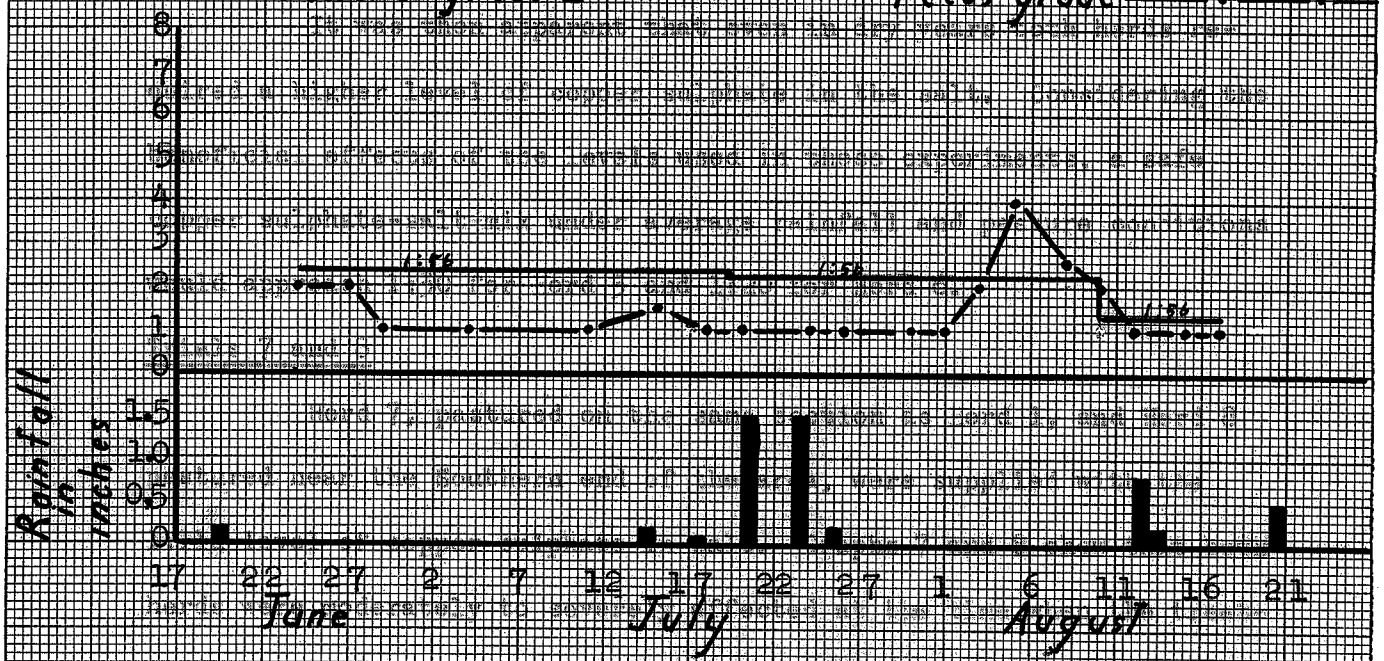
4 cows

Pasture-grade 2

Legend

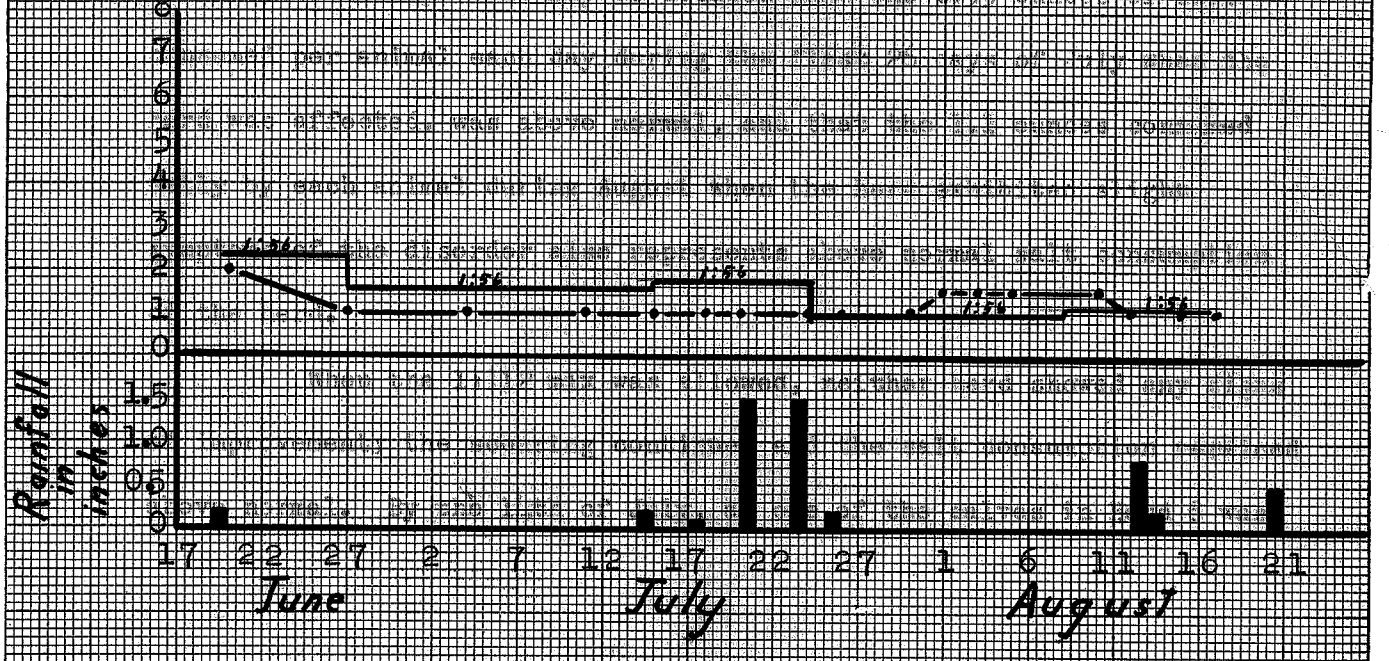
Salt-oz: animal: day

Feces grade



Graph 5

Herd 5 4 cows Pasture grade 1



Pasture 6 was closer to the centre of the area, it is apparent that the difference in the yield of the pastures might not be the only cause of this difference but that more molybdenum might be available in the soil of Pasture 6 than in Pasture 5.

It was then apparent that even in dry years both herds required a higher level of copper sulphate in the salt. Considering the beneficial effects of the levels used in these experiments, a safe copper sulphate-salt-mix under average rainfall and pasture conditions would approach 1:30 for Herd 5 and 1:20 for Herd 6.

Trials 7 and 8

Herd 7, pastured on the same section as Herd 1, and Herd 8 pastured near the Southern end of the area, were supplied with the 1:112 level of copper sulphate in salt. Graphs 7 and 8 show that both herds were moderately to severely affected at the time when the treatments were begun. As five of the cattle in Herd 8 were under 12 months of age, it is expected a normal salt consumption for this herd would be less than 1 ounce per day established by the checks. In the light of this, from Graph 8 it may be deduced that the 0.93 ounces of salt consumed per animal each day during the first 26 days of July when the herd was affected, was above normal, and that the 1.1 ounces consumed daily by each animal during August when the herd exhibited slight symptoms of the disorder also represents above normal salt consumption of the herd.

When the 1:112 mix was allowed, neither herd showed any signs of improvement; the scouring continued and the salt consumption remained above normal. By the 14th of July, as one of the calves in Herd 8 was

Graph 7

Herd 7

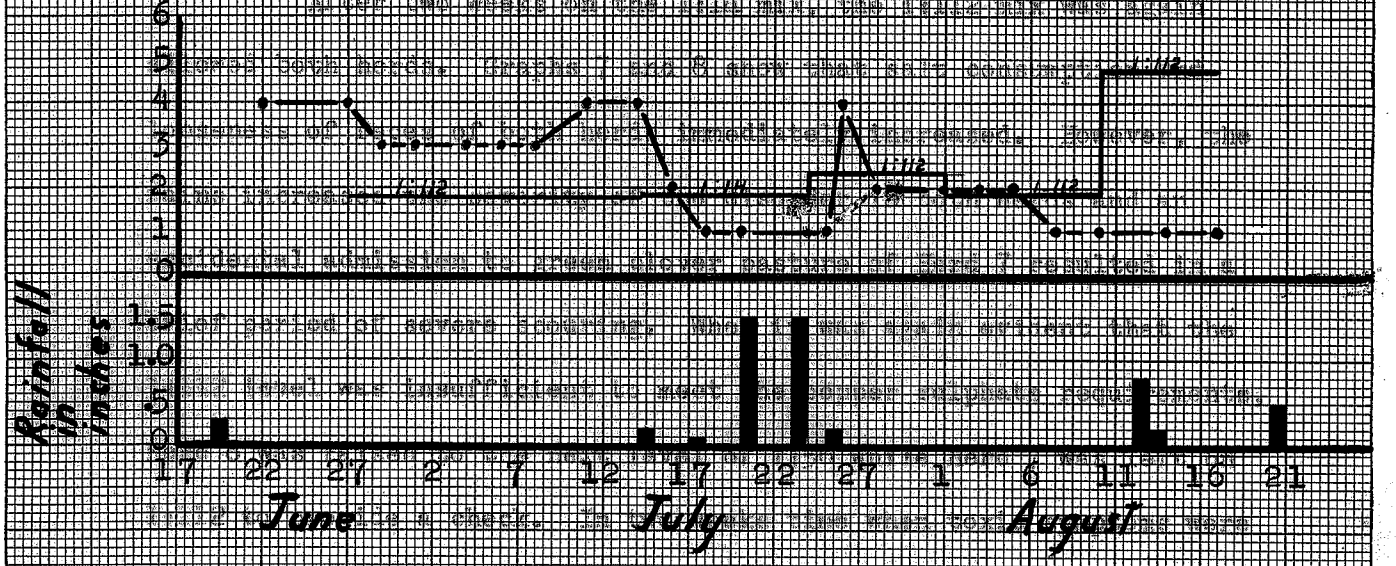
3 cows

Pasture - grade 2

Legend

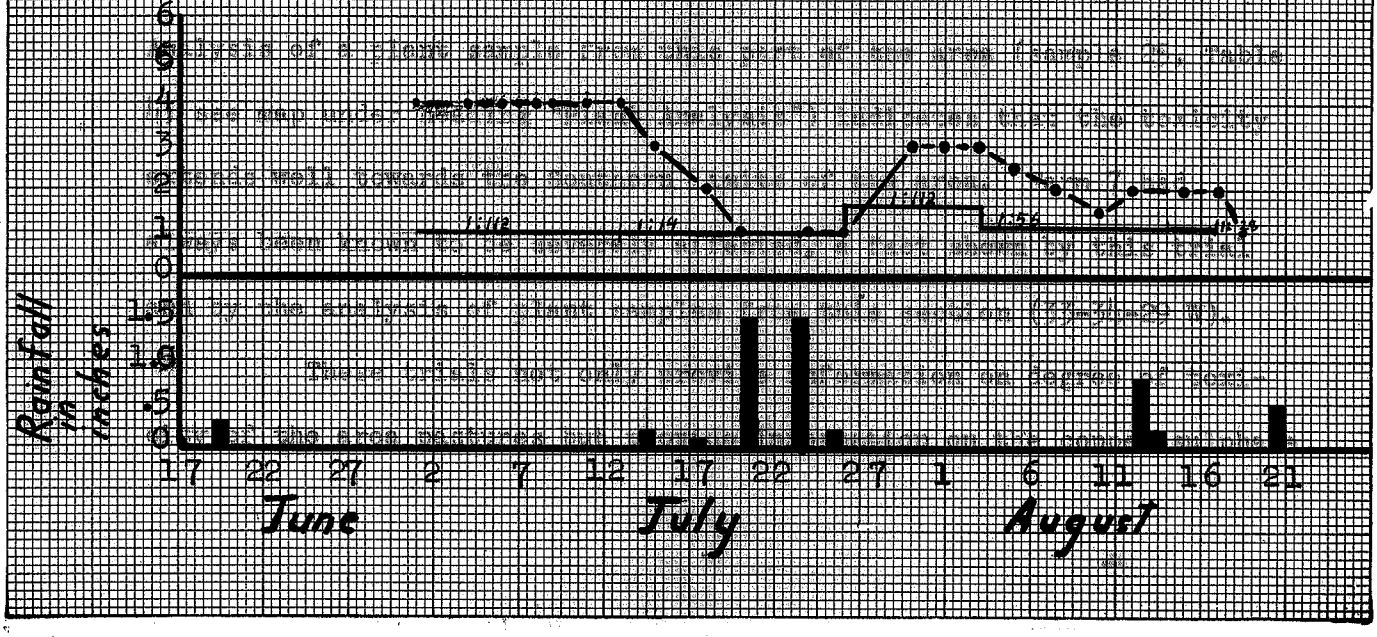
Salt - oz / animal / day

Feces - grade



Graph 8

Herd 8 2 cows 5 yearlings Pasture grade 2



in critical condition and as all other animals in both herds were scouring severely, it was considered advisable to supply both herds with a 1:14 mix in order to restore health. Following the ingestion of the 1:14 mix both herds showed a marked improvement attaining a normal consistency of the feces in four to six days.

After two weeks on the 1:14 mix, the 1:112 mix was again allowed both herds. Graphs 7 and 8 show that salt consumption and looseness of feces of both herds immediately increased. However, the rains increased the severity of the disorder in both herds and an accidental admission to green clover pasture of Herd 7 resulted in a brief period of severe scouring. When it was again evident that the 1:112 level was insufficient to meet the copper sulphate requirements, Herd 8 was raised to the next level of 1:56 while Herd 7 was left on 1:112 to provide a check. In two weeks time when toxic symptoms were still present in Herd 8, the copper sulphate level in the salt was raised to 1:28. The scouring disappeared at this level and the herd took on a thriftier appearance. It was then apparent that the pasture land in the southern end of the area on which Herd 8 was grazing was much more toxic than was supposed at the time the trials began. Analysis of a plant sample from this part of the area (sample 25, Table 4; see map under heading "Plant Analysis") indicates that the toxicity extends well towards the Southern limits of the area. Farm 7 had always been known to be severely affected, a fact shown by this trial and by the analysis of plant samples from this section (33-34-29 W).

These trials not only provide information on degree of toxicity of the area pastures but provide information on the copper sulphate

resulting from a 1:112 ratio of copper sulphate to salt was found to be insufficient to prevent the disorder or effect recovery of affected animals.

Trial 9

Herd 9 was the worst affected herd during the summer of 1949. The severity of the disorder was probably due to the lush alfalfa pasture on which this herd grazed. Such was found in other investigations (Ferguson, 1940; Barshad, 1940) to be most toxic. At the same time water supply contained excessive concentrations of salts which, along with a high salt consumption, (Frens, 1946) might have resulted in ingestion of salt in toxic amount and hence aggravated the condition. The ratio of copper sulphate to salt assigned to the herd at the commencement of the trial was 1:28. With the continued dry weather in the first half of the trial, the disorder practically disappeared but after the heavy rains there was an enormous increase in salt consumption. As this excessive salt consumption was accompanied by only moderate rather than severe scouring, it was evident that this level of copper sulphate had some influence in reducing the toxicity. The ratio of copper sulphate to salt was doubled during the last few days of the experiment. This resulted in marked abatement of symptoms of the disorder but not in complete cessation thereof.

It is therefore evident that for preventive and curative purposes on this farm, rather than increase the ratio of copper sulphate to salt, a practice that might lead to copper sulphate poisoning, it would be advisable in future to replace the alfalfa pasture by a non leguminous pasture. The disorder could then be corrected by a 1:14

Graph 9

Herd 9

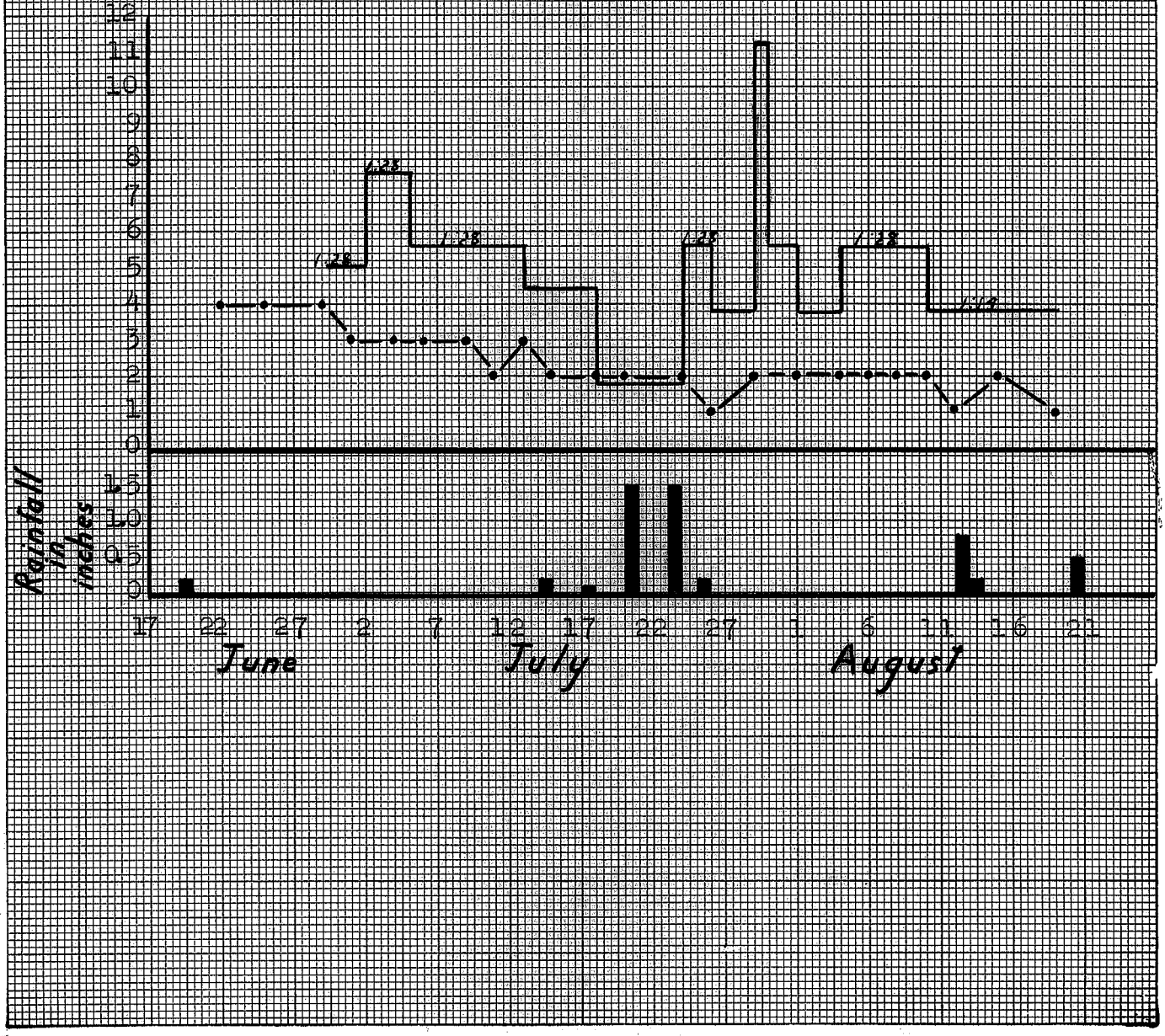
5 cows

Pasture grade 4

Legend

Salt-oz:animal:day

Feces grade



level as shown to be sufficient for Herds 1, 3, and 8, pastured on the remainder of the same section of land (see map).

Other Experiments with Copper Sulphate in Salt

Besides the closely conducted trials described above, copper sulphate in salt was allowed to a total of 38 cattle in seven additional herds. Herd 11, one mile south of Herd 1, was in very poor condition at the time the trials were inaugurated in June. When a 1:14 ratio was allowed, improvement was rapid, milk production increased within three days, and the condition of the feces was back to normal in less than 4 days. Similar results were obtained with Herd 12 pastured 1 mile North of Herd 1. A 1:14 mix promoted rapid improvement of the affected animals, and restored normal condition within five to seven days.

A 1:56 rate was supplied to Herds 13, 14, and 15, which were subjected only to slight toxic pasture during the summer. In all cases this level was found to be sufficient to prevent symptoms of the disorder.

The 1:112 rate was supplied to Herd 16. This herd had been pastured on very toxic pasture during the previous summer but was moved to an adjacent pasture on the same farm which was found to be only slightly affected. On this mildly toxic pasture the 1:112 rate was found to be an adequate preventive.

These trials, though not closely conducted by the experimenter, were of added value in recommending appropriate copper sulphate-in-salt levels for each part of the area, and, as the salt lick was usually prepared by the farmer, the method was practical.

OTHER METHODS OF ADMINISTERING COPPER SULPHATE

As young calves do not consume sufficient salt to insure adequate intake of copper sulphate by this medium, and as it was preferable to have another method of administering copper sulphate to stock in the barn during winter, it was necessary to do some experimenting with other therapeutic methods.

For Calves

During the summer, twelve calves distributed over the area were given regular doses of copper sulphate in milk, water, or grain for periods varying from 1 week to 2 months. As the experimenter could not always be present at feeding time, the farmers were supplied with the necessary weighed amounts of copper sulphate and were instructed in how to mix and feed the required amounts with the milk. For exact experimental work on some farms, the copper sulphate was dissolved in water and an accurately measured volume of this solution was added to the milk, water, or feed. For less exact but more practical purposes, a tubular measure of approximately 1 gram of powdered-copper-sulphate capacity was used to measure out the dose. In these trials, rates of $\frac{1}{2}$, 1, and 2 grams per calf per day were used. It found that in most cases $\frac{1}{2}$ gram showed some indication of improvement but was not conducive to rapid or complete recovery. 1 gram was usually sufficient to produce recovery in from 3 to 4 days and in only a few cases was more than this amount required. One 5 month old Jersey calf receiving its copper sulphate quota via the grain, showed little or no improvement on 1 gram per day, moderate improvement with 2 grams, and complete recovery with 4 grams. When the proven 4 gram intake in this case was

reduced to 2 grams, symptoms of toxicity were again in evidence. Another Jersey calf on Farm 9 required about the same amount of copper sulphate before recovery was effected. As all calves of the beef breeds required only from 1 to 2 grams to bring about recovery, there appeared to be a difference between type in resistance towards the toxicity. It was also found that the taste of copper sulphate was less disguised by water than by milk or grain. Milk was found to be the most acceptable, grain next, and water the least acceptable medium to calves.

Adult Stock

The two cows in Herd 17 were fed copper sulphate in grain all summer for purposes of determining the efficacy of this therapeutic method as well as the quantity of copper sulphate necessary for this part of the district. It was also advisable to see if stock took more copper sulphate in the salt than they required. These cows had been severely affected the previous year and the scouring had been brought under control with 2 grams of copper sulphate a day in the form of a drench. It was found in the summer of 1949 that 1 gram was sufficient during the dry part of the season but that 2 grams were required each day after the heavy rains. As this rate was below that consumed by similarly affected animals on salt trials, it was evident that the cattle on salt trials often ingested more copper sulphate than they required. However, it was found that if the appropriate level of copper sulphate in salt is used, the copper sulphate consumption does not reach toxic limits.

Several cows on Farm 4 were given copper sulphate in the grain. A great difference in the palatability of copper sulphate by

different animals was observed. The maximum voluntary intake was found to vary from 2 grams to 7 grams in one feed (4 pounds) of grain. As the cattle had an abundance of good alfalfa pasture, these intakes of $CuSO_4$ are lower than they would be in the winter when cattle have a better appetite for grain.

COPPER SULPHATE AND SALT TOLERANCE

Ingestion of toxic quantities of either copper sulphate or salt produces symptoms quite similar to those of the disorder. It is then difficult to distinguish between a deficiency or excess of the corrective agent. Copper poisoning described by Dun (1907) is marked by an impaired appetite, constipation, alternated with diarrhoea, colic, muscular weakness, and haemoglobinuria. Salt poisoning symptoms described by Steyn (1946) and Frens (1946) include retching, twitching, diarrhoea, muscular irritability, and even death.

The toxic limits of copper sulphate ingestion by cattle have been reported by several workers. Becker in Florida (1938) showed that copper sulphate fed daily for seven months at the rate of 5.8 grams per 1,000 pounds live weight did not hinder growth of an animal, whereas 7.8 grams per 1,000 pounds live weight fed to another animal proved toxic. Cunningham in New Zealand (1946) fed two 7 month-old calves 0.78 and 1.17 grams of copper sulphate daily for 12 and 16 months respectively. Their health remained normal and they yielded satisfactory carcasses of good flavor. He also drenched 2 cows daily with 1.95 and 4.88 grams of copper sulphate; "all calved normally and gave excellent carcasses." Single doses of 80 grams administered by stomach tube were not fatal but 400 grams proved lethal. The daily copper sulphate intake per animal in the Swan River Valley exceeded 5.8 grams on one farm only, and for very short periods.

It is also important to note that a severely affected animal requires and consumes more copper sulphate in the form of a salt lick fed ad libitum than a slightly affected animal. This suggests that

most of the copper sulphate is used to counteract the effects of the toxic agent and that the copper sulphate toxic limit for an affected animal is higher than for a non affected animal. This suggests that most of the copper sulphate is used to counteract the effects of the toxic agent and that the copper sulphate toxic limit for an affected animal is higher than for a non affected animal. This coupled with the fact that animals consume large quantities of copper sulphate-salt mix only during a few weeks following wet weather, indicates that there is no danger of copper poisoning in cattle if the proper ratios of copper sulphate to salt are used.

Salt, however, if consumed in sufficiently large quantities may also be harmful to an animal, and, as none is used to counteract the toxicity, the toxic limits for an affected animal are no higher than for a non affected animal. Frohner (1936), sets the lethal doses of salt for mature animals at 3.3 to 6.5 pounds and Dun (1907) sets the lethal dose for yearlings at 1 pound. Frens (1946), states that continued ingestion of 1.5 per cent of salt in drinking water produced typical salt poisoning in mature stock while 1 per cent had no effect. In Herds 2 and 9 in which from 11 to 12 ounces of salt were consumed daily per animal for short periods, it is quite possible that the intake was approaching toxic amounts and may have tended to aggravate the condition. If the ratio of copper sulphate to salt be too wide such as the mix allowed on Farm 9, then in order to secure sufficient copper sulphate to correct the disorder, stock are likely to ingest toxic quantities of salt. It is important also in allowing salt ad libitum to affected cattle to make certain that the mix is available

at all times throughout the grazing season. A precaution taken in June when the trials began was to supply only treated salt ad libitum previous to the trials. When affected cattle are not given free access to salt for several weeks they develop such a craving for it that when salt is supplied they may ingest toxic quantities, and if the salt contains copper sulphate the added toxicity may cause death. In cases where herds had received very little salt previous to the trials, small daily allotments were given for the first few days until the herd failed to completely consume a day's supply. Nevertheless, herds 1 and 2 which had been receiving salt previous to the trials, showed excessively high salt consumption during the first few days of the trials.

Precautions in Administration of Copper Sulphate

Copper sulphate has a very corrosive effect on many metals and should not be mixed in iron, tin, or steel containers. All mixes and solutions containing copper sulphate should be held in granite, enamel, crockery, wooden, or plastic containers. As rains tend to leach the copper sulphate out of the salt it is advisable to keep all mixtures sheltered from the weather.

PLANT ANALYSIS

To determine the nature and concentration of the toxic agent in the pasturage it was necessary to analyze plant samples from the affected areas. Nine such samples gathered during the summer of 1948 were analyzed in the spring of 1949. This analysis revealed an excessive molybdenum content of several samples with the concentration varying greatly between samples. Thirty additional plant samples collected from within the affected area and from different non-affected areas of the province during the following summer were selected in such a manner as to furnish information on analysis of the severity and area-incidence of the condition and the effects of season, rain, species and age of pasture plants, topography, and fertilizer pasture treatment. The plant samples were analyzed for molybdenum by the stannous chloride thiocyanide method of Marmoy (1939). Duplicate determinations on dry weight basis were made on all samples. The molybdenum content of these samples is shown in Table 4 and on the Map.

Severity and Area-Incidence of Molybdenum Poisoning

The chief purpose of plant analysis was to determine if sufficient molybdenum was contained in affected pasturage to establish excess molybdenum as the causal agent. As the molybdenum content of plants has been shown in investigations by Ferguson (1940), and Barshad (1948), to vary greatly from sample to sample, from month to month, and from year to year, in order to establish definitely the extent and severity of the disorder, analysis of hundreds of plant samples is called for. It is then evident that plant samples gathered during one or two seasons only were inadequate for the purpose. For this reason,

the analysis of plant samples was intended only to provide some indication of the most severely affected parts of the area.

Table 4 indicates that toxic quantities of molybdenum were found in samples from the affected area. Nine out of 28 plant samples collected from affected parts of the area showed molybdenum contents above the 10 p.p.m. lower limit of toxicity set by the American workers (Barshad, 1948) and 2 samples showed molybdenum contents above the 20 p.p.m. lower limit of toxicity set by the English workers (Ferguson, 1940). The average molybdenum content of affected herbage was found to be lower than that found in England or the United States, but as the disorder was comparatively mild in both years in which the samples were gathered, it was not surprising to find a low molybdenum content of the herbage.

The Map (Page 50) indicates that molybdenum content of the samples varied with the degree of severity of the disorder manifested by cattle in the area. Samples containing the highest molybdenum content were taken from the pasture of Herd 1 locally known to be one of the worst affected pastures in the district. No sample secured from an unaffected pasture either around the affected area or from other parts of the province showed molybdenum content above normal. Of greatest significance, was the low molybdenum content of unaffected pastures on the river flats running through the centre of the area. As previously pointed out, a sample from one of these pastures showed a molybdenum content equivalent to that found in samples from the Brandon and Winnipeg areas.

It is apparent that the degree of toxicity of area pastures

TABLE 4

PLANT ANALYSIS

(all determinations done in duplicate and based on dry weight)

Sample No.	Location	Predominant Species	Molybdenum P.P.M.	Date Collected
1	NW 33-34-29 W	alfalfa, timothy	8.2	23/6/48
2	NE 32-34-29 W	brome	6.3	25/6/48
3	NE 33-34-29 W	brome	11.2	30/6/48
4	NE 28-34-29 W	brome, crested wheat, alfalfa	4.4	2/7/48
5	SE 33-34-29 W	alfalfa	3.9	3/7/48
6	NE 33-34-29 W	brome	17.8	7/7/48
7	NE 33-34-29 W	brome	25.6	18/8/48
8	SE 34-34-29 W	timothy	8.3	27/7/48
9	SE 32-34-39 W	brome	14.5	1/9/48
10	NE 33-34-29 W	timothy	7.2	24/7/49
11	NE 33-34-29 W	clover	7.0	24/7/49
12	NE 33-34-29 W	alfalfa	4.6	24/7/49
13	NE 33-34-29 W	brome	20.7	24/7/49
14	NE 33-34-29 W	couch	7.6	24/7/49
15	NE 33-34-29 W	green oats	5.7	24/7/49
16	SW 17-35-29 W	8 yr. alfalfa (valley)	4.6	16/7/49
17	SW 17-35-29 W	8 yr. alfalfa (hill)	7.9	16/7/49
18	SW 17-35-29 W	2 yr. alfalfa (hill)	5.2	16/7/49
19	SW 17-35-29 W	8 yr. alfalfa (hill after rain)	6.9	25/7/49
20	SW 33-34-29 W	brome	17.0	24/7/49
21	NW 27-34-29 W	brome, alfalfa	4.8	3/8/49
22	NE 32-34-29 W	brome (fertilized)	6.7	3/8/49

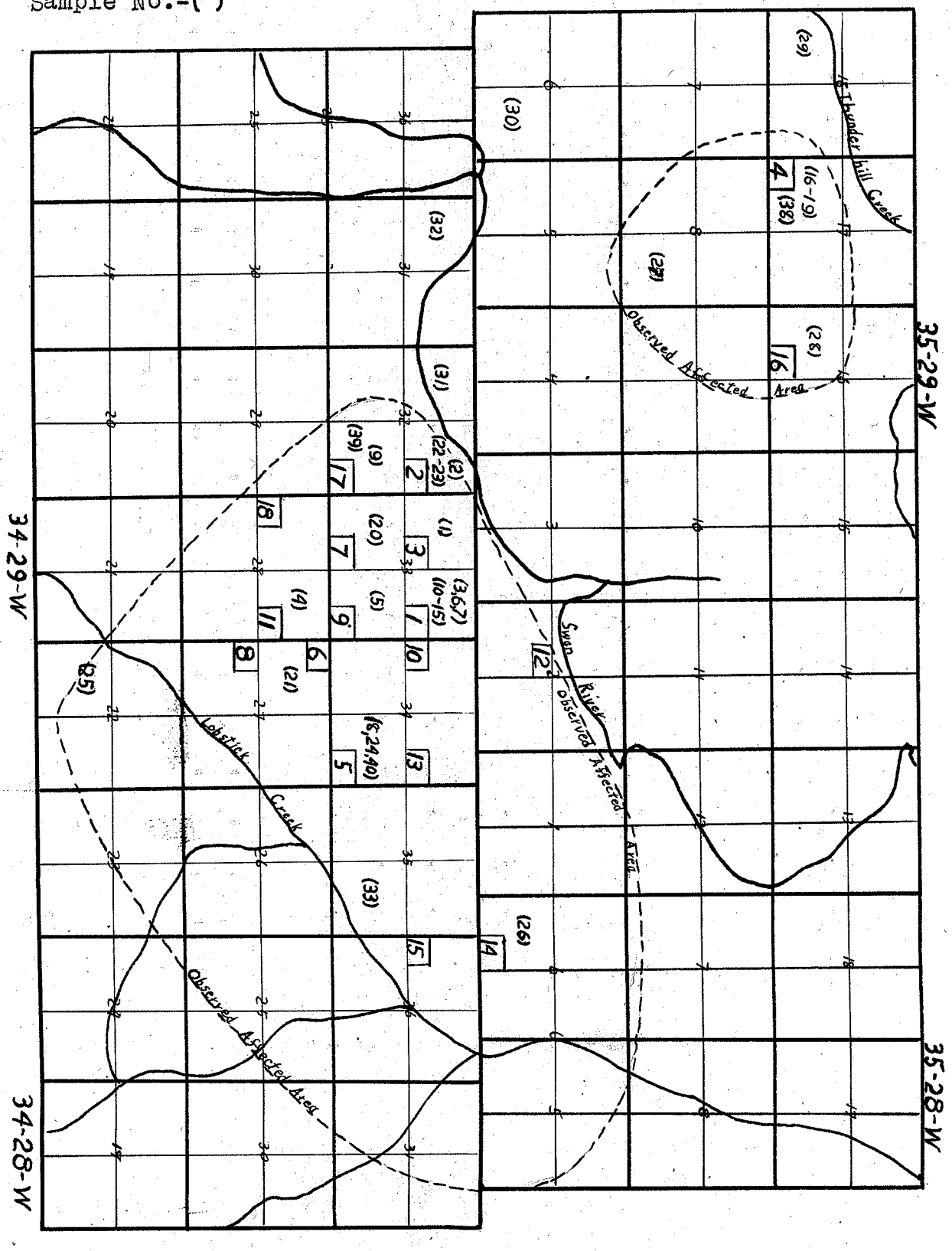
TABLE 4 (con't.)

Sample No.	Location	Predominant Species	Molybdenum P.P.M.	Date Collected	
23	NE 32-34-29 W	brome (not fertilized)	6.9	3/8/49	
24	SE 34-34-29 W	timothy	7.4	27/8/49	
25	SE 21-34-29 W	brome	15.1	24/7/49	
26	SW 6-35-28 W	brome, couch	6.2	26/7/49	
27	SE 8-35-29 W	alfalfa	11.7	15/8/49	
28	SW 16-35-29 W	couch	15.6	12/8/49	
29	SW 18-35-29 W	couch	6.4	5/8/49	x
30	SW 5-35-29 W	couch	2.5	10/8/49	x
31	NW 32-34-29 W	swamp grass	1.0	15/8/49	x
32	NW 31-34-29 W	couch	5.8	26/7/49	x
33	NE 22-34-29 W	couch	5.2	24/7/49	x
34	SW 30-35-28 W	brome	5.5	24/6/49	x
35	Fort Garry	Alfalfa	1.9	6/7/49	x
36	SW 27-8-19 W	brome	.5	7/8/49	x
37	NW 24-8-19 W	swamp grass	1.1	7/8/49	x
38	SW 17-35-29 W	oats (grain)	4.0	7/48	
39	SE 32-34-29 W	barley (grain)	5.6	8/48	
40	SE 34-34-29 W	barley (grain)	2.9	8/48	
41	SW 27-8-19 W	barley (grain)	.7	8/49	x
42	Fort Garry	oats (grain)	1.2	8/48	x

x - no observed symptoms

Map of Affected Area

Herd No. -□
Sample No.-( )



depends upon the rainfall. Twenty-four herds or more may be affected during seasons of high precipitation while only one or two herds may be affected during extremely dry seasons. The disorder usually occurs during the grazing season becoming most severe in July and August, with the greatest toxicity appearing two weeks after a heavy rain. In the majority of herds the heavy rains caused an increase in salt consumption and a greater incidence of scouring. The analysis of plant samples collected from Pasture 1 at different times in the summer of 1948 showed an increase in the molybdenum content of the pasture plants as the season advanced. Table 4 shows that the 3 samples taken from Pasture 1 contained 11.2 p.p.m. on June 30, 17.8 p.p.m. on July 5, and 25.6 p.p.m. on August 18. The pasture was observed to be unaffected at the time that the first sample was taken and highly toxic after the heavy rains when the third sample was taken. It was then evident that the molybdenum content of the pasturage was related to the severity of the disorder and was dependent upon the rainfall.

Topography

Topography was also found to have a direct relation to the extent and severity of the affected area. The area lies in a "rolling" region of numerous gullies and ravines. As this "hilly" land levels out to the flat plains of the Swan River Valley, the toxicity of the affected land also appears to fade out. It was observed that though many of these gullies drained into the Swan River, the flats along the river remained unaffected. This observation was supported by the low molybdenum content of the plant sample from the river flats.

Lower lying portions of small pastures were also noticed to

be less toxic than adjacent hills. As few pastures were found to lie entirely within valleys it was difficult to find herds on such pastures where observations of this nature could be made. However, two samples of alfalfa were taken from the same field, one from the valley and the other from a hill. The sample on the hill contained 7.9 p.p.m. of molybdenum and the sample from the valley contained 4.6 p.p.m.

It was concluded from these observations that the topography had a direct effect on the extent and severity of the disorder. Higher land was found to be more toxic than the lower areas, and the "rolling" land was found to be more toxic than the level land.

Species of Plants

It was observed in the affected area, likewise in California and England that certain species of pasture plants were more toxic and contained greater quantities of molybdenum than others. Legumes were among the most toxic pastures in English and American investigations and were found to absorb the largest quantities of molybdenum. In the Swan River area, legumes, during the summer of 1949, were not found to be more toxic than non legumes. Herd 9 on Grade 4 alfalfa pasture was the only herd on legume pasture that showed more toxic effects than neighboring herds on grass pastures. Of the two legumes, three grasses, and one grain, (Table 4, samples 10-15) collected from within an area of four square rods on Farm 1, brome grass was found to have a molybdenum content of 20.7 p.p.m., and alfalfa 4.6 p.p.m. Of all the samples collected from the area during two years the molybdenum content averaged 6.4 p.p.m. for alfalfa, and 13.5 p.p.m. for brome. It is then evident that during the summers of 1948 and 1949, alfalfa contained less molyb-

denum than brome grass. However, as legumes were reported to have been more toxic than non legumes in previous years, it may be concluded that the greater toxicity of legumes is caused by high precipitation and that during dry years they are no more dangerous than non legumes.

Age of Plants and Toxicity

The theory advanced by Ferguson (1940) that older plants are more toxic than young plants was investigated by collecting and analyzing plant samples from an eight-year-old-alfalfa hay field and a two-year-old-alfalfa hay field. The samples were collected within one rod of each other on adjacent fields on the same day. Sample 17 from the older crop contained 7.9 p.p.m. molybdenum and Sample 18 from the younger crop contained only 5.2 p.p.m. Though these results confirm the findings of Ferguson (1940) as these were the only samples collected in which differences in molybdenum content in respect to the age of plants were found, the experiment can not be regarded as furnishing conclusive proof that older plants contain more molybdenum than younger plants.

Toxicity of Grain

Table 4 shows that Samples 38, 39, and 40 collected from various parts of the affected area contained 4.0, 5.6, and 2.9 p.p.m. of molybdenum respectively. Samples 41 and 42 collected from unaffected parts of the province contained only .7 and 1.2 p.p.m. respectively. Though the grain samples from the affected area did not contain toxic quantities of molybdenum, they contained sufficiently more molybdenum than the check samples to supply additional evidence to the theory that the soil of the affected contains more molybdenum than unaffected areas.

Fertilizers

Fertilizing with ammonium phosphate at 25 lbs. per acre was found by some farmers in the affected area to reduce the toxicity of the pasture. English experiments with ammonium sulphate and nitrogen phosphate fertilizers showed that a rate of 1 cwt. per acre reduced the percentage of molybdenum in the pasturage by half to two-thirds throughout the entire grazing season. Plant samples were taken from a fertilized (2 years previously) pasture in the area and from an adjacent non fertilized pasture. The fertilized sample contained 6.7 p.p.m. molybdenum, and the non fertilized sample contained 6.7 p.p.m. molybdenum. This indicates that the fertilizer was without influence on the molybdenum content of the pasture during the third summer after fertilizing. The possibility remains of fertilizer having had the influence in question in the season of its application. This could not be determined since pasture fertilized in the summer season was not available for study.

WINTER EXPERIMENTS

During the winter of 1949-50 several experiments were conducted for the purpose of inducing the disease in cattle by oral administration of molybdenum and concurrently determining if the toxicity could be transmitted through milk. Farmers in the affected area reported several cases of stall fed calves receiving milk from pastured and affected cows having manifested scours, and furthermore, that recovery of calves was coincident with recovery of cows. The experimenter observed one such case during the summer of 1949 in Herd 7 and found that when the administration of copper sulphate corrected the scouring of a cow, a calf in the barn receiving the milk of this cow and manifesting scours, also ceased scouring. This observation suggested the possibility of the toxicity of molybdenum being transmitted through the milk of affected cows.

The procedure followed to test the validity thereof was administration of molybdenum to a cow, analyzing her milk for molybdenum, and observing any toxic effects shown by a calf receiving ten pounds of this milk daily. Only one cow was used in the first or pilot experiment. Molybdenum was first administered in the form of a daily ammonium molybdate drench containing an amount of molybdenum equivalent to the content of 30 pounds of dry feed containing 25 p.p.m. molybdenum. This dosage was equivalent to the highest concentration of molybdenum found in plant samples from the affected area, (Sample 7, Table 3). The milk was analyzed for molybdenum by the stannous chloride thiocyanide method (Maramoy, 1939, see Appendix) which was modified by the experimenter to render it applicable to the analysis

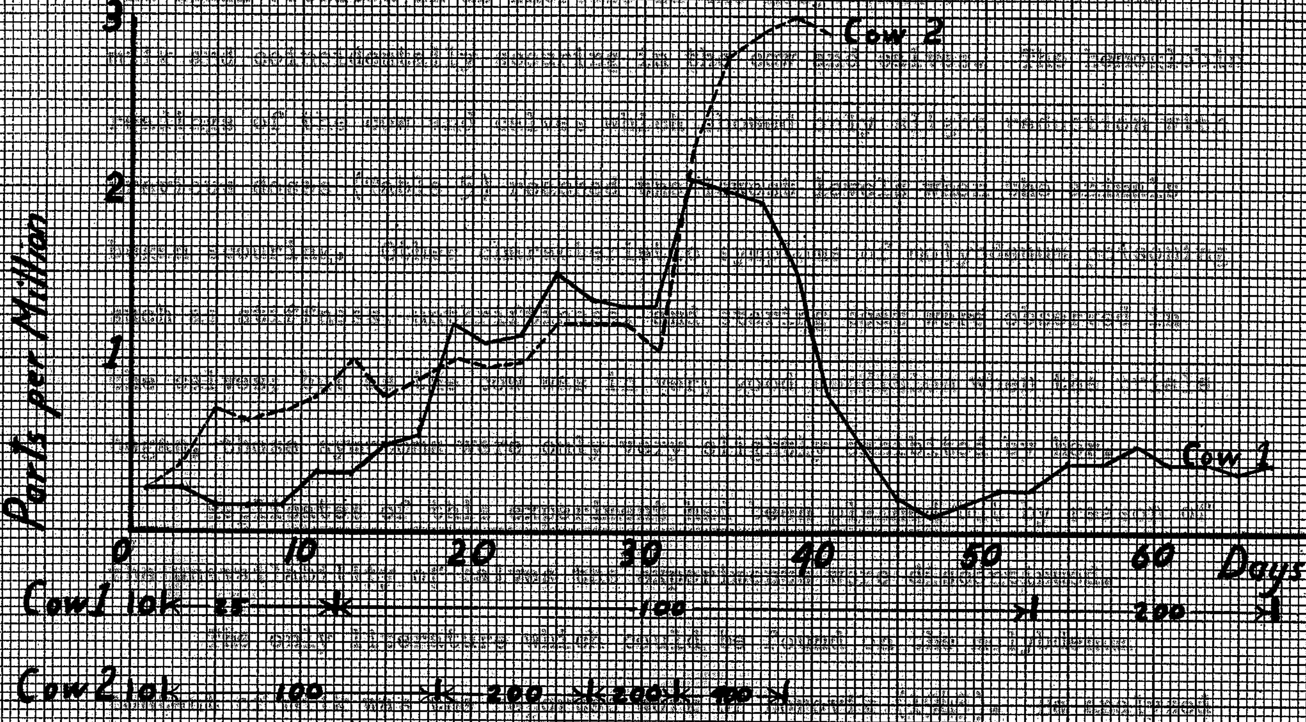
of milk. All determinations were done in duplicate and adequate checks were made on the method to assure accuracy. Determinations were also made on the milk of ten other cows managed under the same conditions to obtain an accurate check on the normal molybdenum content of cows milk. The roughage fed to all animals consisted of corn silage and medium quality alfalfa-brome hay.

Graph 10 shows that ten days of a daily molybdenum dosage equivalent to 25 p.p.m. in 30 pounds of dry matter failed to cause any change in the condition of the cow and produced an increase on only 15 p.p.m. in the molybdenum content of the milk. The dosage was then raised to an equivalent of 100 p.p.m. in 30 pounds of dry matter. This caused a gradual increase for the following 20 days in the molybdenum content of the milk. The molybdenum content then decreased until normality was reached within eight days. Neither the cow nor the calf showed any symptoms of molybdenum poisoning. As older animals are known to be more resistant than younger animals, it was considered advisable to experiment with a younger cow.

Accordingly, a five year old cow in her second laction was put on the 100 p.p.m. molybdenum-intake level and ten pounds of her milk was supplied daily to each of two four-month-old calves. Hemoglobin readings were made twice weekly on the cow and calves to determine whether the molybdenum produced anemia.

Again, there was a gradual increase and subsequent decrease in the molybdenum content of the milk and neither cow nor the calf showed any toxic symptoms. The molybdenum dosage was then increased to an equivalent of 200 p.p.m. in 30 pounds of feed. Again, there was

Graph 10
Molybdenum Content of the Milk of Cows 1 and 2



Daily Molybdenum Dosage ≡ to p.p.m. in 30 lbs. hay

an increase in the molybdenum content of the milk. When the molybdenum content of the milk began to decrease the second time, the dosage was changed to 200 p.p.m. of molybdenum in the form of solium molybdate to find whether this salt is more toxic than ammonium molybdate. (Sodium molybdate was used in investigations by Ferguson in 1947, and ammonium molybdate was used by Dick (1945). However, there was no decrease in the molybdenum content of the milk, indicating that there was no difference in the effect of the two salts in this particular trial.

The dosage was raised to 400 p.p.m. in thirty pounds of feed and again resulted in an increase in the molybdenum content of the milk and coincidentally scouring in the cow and calves. The hemoglobin readings of the cow and calves which showed only slight reduction with previous doses (Table 5) reached the lowest levels when the animals began scouring. Other characteristic symptoms of molybdenum poisoning such as stiffness, unthriftiness, and staring coat were observed in the calves; but as the cow was in very good condition when the trials began, these symptoms were only very slightly exhibited by her.

Replicates of this experiment had been planned but by reason of the unavailability of calves the experiments were discontinued.

The only literature which could be found on the molybdenum content of milk was the reported work of Borovik (1945). He analyzed human milk and found a molybdenum content of .005 to .001 per cent of the ash. Since ash constitutes .7 per cent of milk (Roadhouse, 1941; Eckles, 1943) this would be equivalent to .35 to .07 p.p.m. of molybdenum of the whole milk. The writer found the normal molybdenum content of the milk from ten cows to be from .35 to .03 p.p.m. with an

TABLE 5

Hemoglobin Determinations

(Grams per 100 c.c.)

Date	Mo. p.p.m.	Hb. Cow	Hb. Calf 1	Hb. Calf 2
Jan. 1	0.23			
3	0.36			
5	0.70	11.00	11.00	10.50
7	0.63			
9	0.68	11.00	10.75	10.50
11	0.82			
12		10.50	8.75	8.50
13	1.00			
15	0.78			
16		11.25	10.00	9.75
17	0.63			
19	0.98	9.00	9.50	10.25
21	0.95			
22		10.00	9.50	9.00
23	1.00			
25	1.18			
26		9.50	9.00	8.50
27	1.18			
29	1.18			
30		10.50	9.00	8.50
31	1.04			
Feb. 2	2.33	10.50	9.00	8.00
4	2.73			
6	2.90	9.00	9.50	7.00

Jan. 1 - Daily ammonium molybdate dosage begun, equivalent to 100 p.p.m. of molybdenum in 30 pounds of dry feed.

Jan. 20 - Daily ammonium molybdate dosage begun, equivalent to 200 p.p.m. of molybdenum in 30 pounds of dry feed.

Jan. 27 - Daily sodium molybdate dosage begun, equivalent to 200 p.p.m. of molybdenum in 30 pounds of dry feed.

Feb. 1 - Daily ammonium molybdate dosage begun, equivalent to 400 p.p.m. of molybdenum in 30 pounds of dry feed.

average of .12 p.p.m. and standard deviation of .10.

The reason for a higher intake of molybdenum being required to produce toxic symptoms when animals are confined to dry feed is not known. Ferguson (1940) attributed it to the binding nature of winter feed, but the writer found that animals on ensilage and alfalfa hay were almost as loose as on pasture, and hence should not have required such large quantities of molybdenum to induce comparable symptoms of disorder. Ferguson (1940) reported that a 150 p.p.m. level (based on 30 pounds of dry feed) produced scouring in most cattle on winter rations and a 50 p.p.m. level produced scouring on unaffected pasture. The University of California in 1948 reported that a six month old calf received 5 grams of sodium molybdate daily (145 p.p.m. in 30 pounds of dry feed) for seven months before it became severely affected. Investigation by Edie (1948) showed that two out of five 2-year old heifers, scoured after 14 days on a 150 p.p.m. level and all animals exhibited symptoms of the disorder after the fourth day.

It is therefore apparent that there is a great variation in the resistance of individuals to molybdenum poisoning.

If molybdenum in the milk is capable of affecting calves, it would require only 3 p.p.m. in 10 pounds of milk (the concentration found in milk when the calves began scouring) to produce the disorder. This is equivalent to .67 p.p.m. of molybdenum in 30 pounds of feed and is very low in comparison to the 146 p.p.m. level required to produce scours in a California calf (Bardshad, 1948). It is therefore probable that if the milk of a cow scouring as a consequence of molybdenum poisoning is toxic to calves, that the toxicity is due to

some factor produced by the molybdenum poisoning of the cow rather than by the molybdenum in the milk.

CONCLUSIONS

Molybdenum Theory Substantiated

The investigation has established that molybdenum poisoning is the cause of cattle disorders in the Swan River Valley. The experimental work also demonstrated that other factors such as cobalt or copper deficiencies could have little or no contributing effect on the disorder. Our reasoning here is as follows:

(1) The symptoms of the disorder are similar to those exhibited by cattle affected by molybdenum poisoning in England and California, and the treatment for such, copper sulphate therapy was found to be a preventative, a copper deficiency is not considered likely because sheep have never been known to be affected in the area. New Zealand investigations (Cunningham, 1946) showed that sheep are just as susceptible to copper or cobalt deficiency as cattle; also English investigations (Ferguson, 1940) showed that sheep were more resistant to molybdenum poisoning than cattle.

(2) Plant analysis not only demonstrated toxic quantities of molbydenum (above the lower limits of toxicity set by Ferguson and Barshad) in the affected pastures, but the concentrations of molybdenum in the herbage were found to follow closely the varying degrees of severity of the disorder exhibited by herds in the district.

The first observation indicative of the disorder being caused by an excess rather than a deficiency of the causative agent, was that amimals were more severely affected when they consumed large amounts

of herbage from a high yielding pasture than when they obtained only small amounts of herbage from an adjacent closely grazed pasture. Were the disease caused by a deficiency, cattle would have received more of the deficient element from the high yielding pasture and consequently, would not have been as severely affected as when pastured on the low-yielding pasture. Again, in the instance of unaffected animals newly introduced into the district, the disease was manifested in less than two to three weeks on the most severely affected farms, whereas in copper or cobalt deficiency diseases, animals brought into the affected area are not observed to be affected for several months (Marston, 1948).

The disease was also artificially produced in cattle on winter rations in a non-affected area by the administration of toxic quantities of molybdenum. These experiments indicate that the toxicity was transmitted through the milk, but insufficient replicates of the experiment were conducted to establish the findings as significant.

Recommendations

Copper sulphate was found to be an effective agent in treatment and prevention of the disorder. Copper sulphate requirements of cattle were demonstrated to be dependent on the position of the herd in the affected area, the species, yield, and age of pasture, rainfall, and resistance of individual animals. Because of these variations it was difficult to recommend a definite copper sulphate treatment for individual herds. However, recommendations were made as to the quantities of copper sulphate necessary under average conditions, for the guidance of farmers in adjustment of the treatment in accordance with the degree of severity of the disorder manifested by their cattle. A copper-sulphate-salt mix of 1:14 was recommended for the centre or

most severely affected parts of the area; a 1:28 mix for moderately affected parts; a 1:56 mix for the remaining portions. In winter, as hay is not as toxic as grass, the $CuSO_4$ intakes represented by the above ratios, might be reduced by 50 percent. If copper sulphate is administered in the grain, two to three grams per animal per day is recommended for the centre of the area, 1 gram for moderately affected parts, and $\frac{1}{2}$ gram on the remaining portions. Severely affected calves should receive from 2 to 4 grams of copper sulphate a day in the milk, water or grain, until recovery is complete and then should be maintained on 1 to 2 grams a day as long as they are exposed to the toxicity. For slightly affected calves these allowances may be decreased by 50 percent.

Legumes were not found to be more toxic than non legumes during dry weather, but as the survey showed that they were much more toxic during wet years and as other investigators (Ferguson, 1940, and Barshad, 1948) showed that they absorbed greater amounts of molybdenum, it is recommended that they be reduced or eliminated from pastures in the affected area. It might also be advisable as shown by Barshad (1948) to supplement the pasture during wet seasons with dry feed which is known to be less toxic than green plants.

Very few methods of reducing the toxicity by treating the soil can be recommended without further experimentation. Fertilizers, though observed to have some beneficial effect might not be sufficiently beneficial to render their use economical. Application of copper sulphate to the soil is being experimented with in England (Ferguson, 1940) but results have not been reported. Ferguson points out that

the copper content of the herbage can be increased, but only a small proportion of the copper applied is absorbed by the herbage, the greater part being bound in the soil.

It was found in plant analysis by the writer, and also by Ferguson (1940) that older pastures contain more molybdenum than younger pastures, hence ploughing and reseeding of old pastures is indicated.

It is then evident that until other means of reducing the molybdenum toxicity of plants in the affected area are found, copper sulphate therapy will remain the most effective preventive of the disorder.

APPENDIX 1

Molybdenum Analysis of Milk and Plants

REAGENTS

(1) Acid HCl (conc), S.G. 1.16

(2) Potassium thiocyanate solution; dissolve 10 grams KCNS in water and dilute to 100 ml.

(3) Stannous chloride solution: dissolve 10 grams of $SnCl_2.2H_2O$ in 100 ml. dilute HCl (1:9). Prepare this solution each day as it is required.

(4) Ethyl ether: shake pure ether with one tenth of its volume of a mixture of KCNS and $SnCl_2.2H_2O$ reagents immediately before use.

(5) Standard molybdenum solution: dissolve 0.368 grams of ammonium molybdate $(NH_4)6.Mo_7O_{24}.4H_2O$ in water, dilute to 1 litre. 20 mls. of this solution must be diluted to 200 ml. to obtain a solution containing 20 micrograms of molybdenum per ml.

Method of Obtaining Standard Curve

Make up a set of standards from the standard molybdenum solution to contain concentrations varying from 0 to 20 parts per million. Appropriate aliquots of these are taken and the Mo extracted by the following method.

Transfer the respective aliquots to separatory funnels and dilute to a volume of 50 ml. Add sufficient HCl (conc) to make the concentration of HCl seven ml. in 50 ml. of solution. Mix thoroughly and add 3.0 ml. of KCNS and 3.0 ml. of stannous chloride solution in the order named, shaking between each addition. After one minute add 10 ml. of pure ether and shake the mixture vigorously for about 30 seconds, taking care to release internal pressure without loss of solution. When the layers have separated, drain the aqueous phase into a beaker and collect the ether in a 25 ml. volumetric flask. Return

the aqueous phase to the separatory funnel and extract once more using 10 ml. of ether, adding the ether layer to volumetric flask used in the first extraction. Make up to volume, using ether that has previously been shaken with stannous chloride and potassium thiocyanate solutions.

The wave length at which minimum transmission of light takes place is obtained. Using the Universal Spectrophotometer (Coleman Co.) with the violet filter 11-214 (PC4) a value of 460 mμ was obtained. The transmittance curve is obtained by plotting on semi-log paper transmittance values obtained in comparing the prepared standard concentrations of molybdenum against ether shaken with one tenth of its volume of stannous chloride and potassium thiocyanate.

Method for Plant Samples (Marmoy)

Transfer 2 grams of plant material to a silica basin and ignite in a muffle furnace at 450°C. When cool moisten ash with a few drops of water, add 10 ml. of aqua regia and evaporate to dryness on a hot plate taking care to avoid sputtering. Ignite a second time at 450°C and when cool add 10 ml. of HCl to the silica basin. Warm and transfer to a 100 ml. beaker diluting to approximately 40 ml. Boil for a few minutes and filter through a Whatman No. 44 filter into a 100 ml. graduated flask. Wash with hot water until the volume is approximately 80 ml., cool, dilute to the mark.

Method for Milk Samples (writer)

Pipette 20 ml. of well shaken milk sample into a silica basin and evaporate to dryness at approximately 130°C. Ignite in a muffle furnace at 450°C to a white ash. When cool, moisten ash with 1 ml. of

aqua regia, evaporate to dryness on a hot plate and ignite again in a muffle furnace at 450°C. When cool, add 10 ml. of HCl (conc) and then 20 ml. of hot water. Transfer to a 100 ml. graduated flask, washing the silica basin with hot water, cool, and bring up to volume.

Determination of Mo content

Transfer an aliquot containing not more than 20 mgms. of molybdenum (usually 50 ml.) to a separatory funnel, diluting to 50 ml. if necessary. Add sufficient HCl (conc) to make the concentration 7.0 ml. of acid in 50 ml. of solution. Proceed as in making up the standards by adding potassium thiocyanate and stannous chloride in the order named, shaking between each addition and extracting with successive portions of ether until all color is removed from the aqueous solution. (Two extractions are usually sufficient). As before, make up to the 25 ml. volume by addition of ether shaken with stannous chloride and potassium thiocyanate solutions.

The color concentration is then measured by the use of the spectrophotometer to give transmittance values at the concentrations of molybdenum present in the plant tissue. Reference is made to the transmittance versus concentration chart obtained from the standard concentrations and the parts per million of molybdenum determined by multiplying by the appropriate conversion factor as determined by aliquot size.

APPENDIX II

Salt Trial Tables

Table 6 - Herd 1

Date	Total salt in lbs.	$CuSO_4$ to salt	Salt, oz: animal: day	$CuSO_4$, gm: animal day	Grade of feces
June 18	7.0	1:14	1.86	3.72	1
29	5.0	1:14	7.50	15.00	1
July 2	7.0	1:14	1.40	2.80	2
6		1:14	1.40	2.80	1
11		1:14	1.40	2.80	1
13		1:14	1.40	2.80	1
15		1:14	1.40	2.80	1
18		1:14	1.40	2.80	1
20		1:14	1.40	2.80	1
24	3.5	1:14	2.72	5.54	1
26		1:14	2.72	5.54	1
30	3.5	1:14	2.72	5.54	1
Aug. 1		1:14	2.72	5.54	1
3		1:14	2.72	5.54	1
5	3.5	1:14	2.00	4.00	1
8		1:14	2.00	4.00	1
10		1:14	2.00	4.00	1
12	3.5	1:14	2.00	4.00	1
15		1:14	2.00	4.00	1
17		1:14	2.00	4.00	1
19		1:14	2.00	4.00	1

Table 7 - Herd 2

June 18	7.0	1:28	12.44	12.44	4
21	7.0	1:28	5.33	5.33	4
24		1:28	1.62	1.62	2
28		1:28	1.62	1.62	1
July 1		1:28	1.62	1.62	1
7		1:28	1.62	1.62	1
11		1:28	1.62	1.62	1
13		1:28	1.62	1.62	1
15		1:28	1.62	1.62	1
18		1:28	1.62	1.62	1
20		1:28	1.62	1.62	1
24	3.5	1:56	1.55	.78	1
26		1:56	1.55	.78	1
30		1:56	1.55	.78	1
Aug. 1		1:56	1.55	.78	1
3		1:56	1.55	.78	1
5	3.5	1:56	1.55	.78	1
8		1:56	1.55	.78	1
10		1:56	1.55	.78	1
12		1:56	1.55	.78	1
15		1:56	1.55	.78	1
17		1:56	1.55	.78	1

Table 8 - Herd 3

Date	Total salt in lbs.	$CuSO_4$ to salt	Salt, oz: animal: day	$CuSO_4$, gm: animal day	Grade of feces
June 18	7.0	1:28	1.04	3.04	1
22		1:28	1.04	3.04	1
27		1:28	1.04	3.04	1
July 2		1:28	1.04	3.04	1
7		1:28	1.04	3.04	1
11		1:28	1.04	3.04	1
13		1:28	1.04	3.04	1
15		1:28	1.04	3.04	1
18		1:28	1.04	3.04	1
20		1:28	1.04	3.04	1
24	3.5	1:28	3.11	3.11	1
26		1:28	3.11	3.11	1
30	3.5	1:56	3.11	1.56	1
Aug. 1		1:56	3.11	1.56	1
3		1:56	3.11	1.56	1
5	3.5	1:56	2.66	1.33	2
8		1:56	2.66	1.33	1
10		1:56	2.66	1.33	2
12	3.5	1:56	2.66	1.33	1
15		1:56	2.66	1.33	1
17		1:56	2.66	1.33	1
19		1:56	2.66	1.33	1

Table 9 - Herd 4

June 17	7.0	1:28	1.06	2.06	1
21		1:28	1.06	2.06	1
23		1:28	1.06	2.06	1
27		1:28	1.06	2.06	1
30		1:28	1.06	2.06	1
2		1:28	1.06	2.06	1
5		1:28	1.06	2.06	1
8	3.5	0	1.12	2.00	1
11		0	1.12	2.00	1
14		0	1.12	2.00	1
18	3.5	0	1.02	2.00	1
20		0	1.02	2.00	1
24		0	1.02	2.00	1
26		0	1.02	2.00	1
29	3.5 x	1:56	1.60	.40	1
Aug. 1		1:56	1.60	.40	1
3		1:56	1.60	.40	2
5	3.5 x	1:28	2.20	1.10	2
8		1:28	2.20	1.10	1
10		1:28	2.20	1.10	1
12	5.0 x	1:14	2.28	.67	1
15		1:14	2.28	.67	1
19		1:14	2.28	.67	1

x - includes untreated salt.

Table 10 - Herd 5

Date	Total salt in lbs.	$CuSO_4$ to salt	Salt, oz: animal: day	$CuSO_4$, gm: animal day	Grade of feces
June 21	3.5	1:56	2.32	1.16	2
27	7.0	1:56	1.55	.78	1
July 4		1:56	1.55	.78	1
11		1:56	1.55	.78	1
15	3.5	1:56	1.66	.83	1
18		1:56	1.66	.83	1
20		1:56	1.66	.83	1
24	3.5	1:56	.93	.47	1
26		1:56	.93	.47	1
30		1:56	.93	.47	1
Aug. 1		1:56	.93	.47	2
3		1:56	.93	.47	2
5		1:56	.93	.47	2
8	2.5	1:56	1.11	.56	1
10		1:56	1.11	.56	2
12		1:56	1.11	.56	1
15		1:56	1.11	.56	1
17		1:56	1.11	.56	1

Table 11 - Herd 6

June 25	14.0	1:56	2.43	1.21	2
27		1:56	2.43	1.21	2
29		1:56	2.43	1.21	1
July 4		1:56	2.43	1.21	1
11		1:56	2.43	1.21	1
15		1:56	2.43	1.21	2
18	3.5	1:56	2.33	1.16	1
20		1:56	2.33	1.16	1
24	3.5	1:56	2.33	1.16	1
26		1:56	2.33	1.16	1
30	3.5	1:56	2.33	1.16	1
Aug. 1		1:56	2.33	1.16	1
3		1:56	2.33	1.16	2
5	3.5	1:56	2.33	1.16	4
8		1:56	2.33	1.16	3
10		1:56	2.33	1.16	2
12	3.5	1:56	1.28	.64	1
15		1:56	1.28	.64	1
17		1:56	1.28	.64	1

Table 12 - Herd 7

Date	Total salt in lbs.	$CuSO_4$ to salt	Salt, oz: animal: day	$CuSO_4$, gm: animal day	Grade of feces
June 22					4
27	7.0	1:112	1.77	.44	4
29		1:112	1.77	.44	3
July 1		1:112	1.77	.44	3
4		1:112	1.77	.44	3
6		1:112	1.77	.44	3
8		1:112	1.77	.44	3
11		1:112	1.77	.44	4
14	3.5	1:14	1.86	3.72	4
16		1:14	1.86	3.72	2
18		1:14	1.86	3.72	1
20		1:14	1.86	3.72	1
24	3.5	1:112	2.33	.58	1
26		1:112	2.33	.58	4 x
28		1:112	2.33	.58	2
Aug. 1	3.5	1:112	1.86	.46	2
3		1:112	1.86	.46	2
5		1:112	1.86	.46	2
8		1:112	1.86	.46	1
10	3.5	1:112	4.75	1.19	1 x
14	3.5	1:112	4.75	1.19	1 x
18		1:112	4.75	1.19	1 x

x - pasture supplemented with green clover

Table 13 - Herd 8

July 1	5.0	1:112	.95	.24	4
4		1:112	.95	.24	4
6		1:112	.95	.24	4
8		1:112	.95	.24	4
11		1:112	.95	.24	4
13	3.5	1:14	.96	1.92	4
15		1:14	.96	1.92	3
18		1:14	.96	1.92	2
20		1:14	.96	1.92	1
24		1:14	.96	1.92	1
26	3.5	1:112	1.60	.40	2
30		1:112	1.60	.40	3
Aug. 1		1:112	1.60	.40	3
3	3.5	1:56	1.14	.57	3
5		1:56	1.14	.57	3
8		1:56	1.14	.57	2
10	3.5	1:56	1.14	.57	2
12		1:56	1.14	.57	2
15		1:56	1.14	.57	2

Table 14 - Herd 9

Date		Total salt in lbs.	$CuSO_4$ to salt	Salt, oz: animal: day	$CuSo_4$, gm: animal day	Grade of feces
June	22					4
	25					4
	29	3.5	1:28	5.06	5.06	4
July	1	7.0	1:28	7.46	7.46	3
	4	7.0	1:28	5.60	5.60	3
	6		1:28	5.60	5.60	3
	9	7.0	1:28	5.60	5.60	3
	11		1:28	5.60	5.60	2
	13	7.0	1:28	4.48	4.48	3
	15		1:28	4.48	4.48	2
	18	3.5	1:28	1.86	1.86	2
	20		1:28	1.86	1.86	2
	24	3.5	1:28	5.60	5.60	2
	26	3.5	1:28	3.73	3.73	1
	29	3.5	1:28	11.20	11.20	2
	30	3.5	1:28	5.60	5.60	2
Aug.	1	3.5	1:28	3.73	3.73	2
	4	3.5	1:28	5.60	5.60	2
	6	3.5	1:28	5.60	5.60	2
	8	3.5	1:28	5.60	5.60	2
	10	3.5	1:14	5.60	11.20	2
	12	3.5	1:14	3.73	7.46	1
	15	3.5	1:14	3.73	7.46	2
	18		1:14	3.73	7.46	1

Statistical Analysis of Table 1

The weekly variations of hemoglobin readings were recorded and subjected to an analysis of variance and "t" test as follows.

Cobalt Treated Animals

Weekly Variations	3	5	6	8	11	12
1	-0.75	-0.50	0.00	0.50	0.00	2.50
2	-0.25	-1.00	0.75	0.25	0.00	-0.75
3	0.75	0.25	3.00	-0.25	1.00	0.75
4	- .75	0.00	-2.00	0.25	-1.00	-1.75
5	1.00	-1.50	0.75	0.25	0.50	0.00
6	-0.25	-0.25	0.50	1.25	0.50	1.50

Untreated Animals

Weekly Variations	1	2	4	7	9	10	13
1	0.50	-1.25	-1.25	-0.25	0.75	-1.00	0.25
2	0.75	-1.25	0.00	-0.50	0.25	1.25	-1.25
3	0.00	1.00	-0.50	0.25	1.00	0.75	0.75
4	-1.50	1.25	1.00	-0.25	-0.75	-2.00	0.25
5	0.75	-1.25	0.25	0.25	-0.25	0.50	1.00
6	-1.25	-0.75	-3.00	0.50	1.75	0.50	0.25

Analysis of Variance

	Degrees of Freedom	Mean Square	Calculated F value	F value at:- 5% point	1% point
Treatments	1	.82	.95	3.99	7.04
Weeks	5	2.12	2.47	2.36	3.31
Treatments X Weeks (interaction)	5	.98	1.15	2.36	3.31
Error	66	.86			
Total	77				

t value of differences between first and seventh week in treated and untreated animals - 1.07

t value to be significant at 5% - 2.20

t value to be significant at 1% - 3.11

Statistical Analysis of Table 2

Weights

t of weights was calculated to be .71

t of weights to be significant at 5% is 2.31.

Hemoglobin Determinations

As molybdenum dosage was continued for only two weeks the variations in hemoglobin readings for the first two weeks only were subjected to an analysis of variance while the variations between the first and third readings and the first and fourth readings were tested with the t test.

Animals

Weekly Variations	1	2	3	4	5
1	0.75	0.25	0.75	3.25	-0.50
2	-0.75	0.25	-1.00	-3.25	-0.50

Analysis of Variance

	Degrees of Freedom	Mean Square	Calculated F value	F value at:- 5% point	1% point
Between Weeks	1	11.56	3.25	6.61	16.16
Between Animals	4	.21	.06	6.26	15.52
Error	4	3.56			
Total	9				

Calculated t value - .21

t value to be significant at 5% point - 2.31

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