I. MINERAL CONTENT OF SOME OKLAHOMA FORAGE IN RELATION TO THE NUTRITION OF BEEF CATTLE

II. EFFECT OF MANGANESE UPON CERTAIN BLOOD CONSTITUENTS OF BEEF CATTLE

By

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TABLE OF CONTENTS

I. MINERAL CONTENT OF SOME OKLAHOMA FORAGE IN RELATION TO THE NUTRITION OF BEEF CATTLE

INTRODUCTION 1

REVIEW OF LITERATURE 3

EXPERIMENTAL 13
Development of Methods 13
Methods adopted 25

RESULTS AND DISCUSSION 29

SUMMARY 35

BIBLIOGRAPHY 37

II. EFFECT OF MANGANESE UPON CERTAIN BLOOD CONSTITUENTS OF BEEF CATTLE

INTRODUCTION 44

LITERATURE REVIEW 45

EXPERIMENTAL 47

RESULTS AND DISCUSSION 49

SUMMARY 64

BIBLIOGRAPHY 65

APPENDIX 67

VITA 78

I. MINERAL CONTENT OF SOME OKLAHOMA FORAGE IN RELATION TO THE NUTRITION OF BEEF CATTLE

INTRODUCTION

Lack of thriftiness is commonly observed in cattle produced in certain areas of southeastern Oklahoma. This unthriftiness is characterized by a thin condition during winter months, by poor reproductive performance, and by the inferior growth rate of calves. Mild anemia has also been observed. A marked deficiency of available phosphorus exists in the soil in one area, near Wilburton; and phosphorus in the forage supported by this soil is low and not readily available to ruminants. That cattle in this area suffer from phosphorus deficiency is indicated by the occasional display of pica.

An experiment was begun in 1947 to study the phosphorus requirements of range cattle at the Wilburton location. The performance of cattle at that location was compared with that of a similarly managed herd on the Lake Carl Blackwell range near Stillwater. Improvement in reproductive performance was noted in the Wilburton cattle when a mineral phosphorus supplement was provided; yet, none of the cattle at Wilburton performed as satisfactorily as those at Stillwater.

As phosphorus supplementation was only partially effective in improving performance, it was suggested that there might be other contributing dietary factors. Examination of the Wilburton forage at that time revealed amounts of the major nutrients similar to those found in Stillwater forage. Manganese, however, was found to be

present in relatively high concentration. Since manganese is a trace mineral nutrient affecting phosphorus metabolism, its high concentration was considered to be a possible causative factor abetting the phosphorus deficiency.

Trace minerals other than manganese affect the metabolism of phosphorus; and some others, when ingested in improper amounts, cause the development of symptoms similar to those observed in the Wilburton cattle. For that reason it appeared desirable to determine the distribution of certain trace minerals in the forage of the Wilburton and Stillwater areas.

LITERATURE REVIEW

At this time five metals, cobalt, copper, molybdenum, manganese and zinc, are known to be essential micronutrients for animals. Each has been identified with one or more specific enzyme systems. However, the role of a metal in a specific enzyme system and the nutritional disturbances caused by an improper dietary amount of that metal have not been clearly related.

Cobalt

Cobalt has not been shown to have any relationship to phosphorus metabolism. The known specific physiological function of cobalt is related to its presence in vitamin B_{12} (67). The ruminant's demand for vitamin B_{12} can be supplied by synthesis from inorganic cobalt within the rumen organisms (28)(39)(44). Other animals appear to be unable to utilize inorganic cobalt for this purpose.

Cobalt deficiency in ruminants has been known for a very long time. The outstanding feature of the deficiency in sheep is the profound anemia and appetite failure. Blood volume, the concentration of plasma protein, and the oxygen capacity of the blood decrease as a macrocytic anemia appears and becomes progressively more severe (51). There is little doubt that the syndrome of cobalt deficiency in sheep is the result of a severe vitamin B_{12} deficiency (6)(54).

Cattle, deficient in cobalt, show similar symptoms, namely, appetite failure, loss of condition, and anemia. Nutritional diseases of cattle responding to cobalt therapy have been reported from several

areas of this country: New Hampshire (8)(49), Massachusetts (8), North Carolina (8), Wisconsin (34), Michigan (43)(46), and Florida (60). Killham (46) points out the similarity between symptoms of these nutritional diseases and the symptoms of phosphorus deficiency; in fact, early Wisconsin and Michigan workers considered these diseases to be due to phosphorus deficiency.

The minimum amount of cobalt that must be present in forage to prevent a deficiency is believed to be about 0.07 ppm. (9)(64).

Excess cobalt is detrimental to animal life. Becker and Smith (5) found that daily dosages of 250 to 500 mg. per 100 lbs. of body weight produced depressed appetite and loss of body weight in sheep. Oral and parenteral cobalt administration causes polycythemia in many animals. The polycythemia is thought to be the result of a passive response to the partial anoxia induced by an inhibition of respiration in the blood forming tissues (51). However, the concept that erythropoiesis is largely regulated by the oxygen tension in the bone marrow has been challenged. Grant and Root (3) observed the oxygen saturation of blood in red bone marrow during periods of constant and severe erythropoietic stimulus. They found that vigorous erythropoiesis was not necessarily associated with lowered oxygen tension of the blood circulating through the marrow.

In the rat, hemoglobin formation remains essentially unchanged in cobalt-induced polycythemia (81). The toxic effects of cobalt have been suggested to be due to the binding of sulfhydryl groups essential in respiratory systems (63). Because the toxic limit is 3000 to 5000 times greater than the usual amount of cobalt in forage spontaneous cases of cobalt toxicity are unlikely to occur.

Copper

Copper, like cobalt, plays an important role in blood formation. The function of copper in erythropoiesis was first reported by Hart's group in 1928 (40). Subsequent studies have shown that copper promotes iron absorption in the gastrointestinal tract, iron mobilization from the tissues, and is involved in the direct utilization of iron in hemoglobin synthesis (91).

During the progressive depletion of the sheep's copper reserves, the first sign of impaired function is lack of crimp in wool. This condition results in the newly-formed wool being stringy and hair-like. Such wool has a large content of free sulfhydryl groups; normally these groups are oxidized, crimping the wool fiber by the closure of disulfide linkages (51). This is particularly striking in the case of the merino breed; other breeds do not necessarily show the stringy wool (17). With black sheep the wool becomes depigmentized in copper deficiency; in fact, achromotrichia is associated with copper deficiency in rats (45), in cattle and sheep (51), and in cats and rabbits (36). These observations point to a role for copper in some oxidative systems of animals.

Lambs from copper-deficient ewes develop a nervous disorder characterized by incoordination. The disorder occurs in widely separated areas of the world but chiefly in Australia (52) and New Zealand (17). The lesion is a diffuse demyelination of the central nervous system, the degree of which determines the range of symptoms from spastic paralysis of the legs to general paralysis. Mature animals usually succumb before these symptoms appear. The lamb from a copper-deficient ewe may also develop an acute osteoporosis leading

to fractures; this malady apparently results from a less pronounced copper depletion than is necessary to cause paralytic symptoms (17).

In cattle, a state of copper deficiency is characterized by a harsh, depigmentized hair coat and such relatively non-specific symptoms as anemia, appetite failure, weight loss, poor reproductive capacity, and slow growth of calves. The bones fracture easily (17) (18). The liver and blood contain less than normal amounts of copper -- a useful measure in diagnosis of copper deficiency.

Unlike cobalt, copper does have some slight relationship to phosphorus metabolism. In extreme cases of copper deficiency cattle have brittle bones and high levels of plasma phosphatase and plasma inorganic phosphorus in the blood (19). Copper administration lowers the enzyme and phosphorus levels to normal.

The state of copper deficiency in ruminants and its relationship to phosphorus metabolism is complicated by the relative amount of molybdenum ingested. If molybdenum levels are normal, pastures containing 6 to 8 ppm. of copper are generally considered to be adequate for satisfactory growth, health, and reproduction of ruminants. Three to 5 ppm. are marginal levels. For wool production, slightly higher levels have been suggested as necessary (55) (64). In the United States copper deficiencies have been discovered only in certain areas of Florida (20).

Molybdenum

Molybdenum has a fairly definite relationship to phosphorus metabolism. Osteoporosis tends to develop when cattle and sheep ingest excessive amounts of molybdenum. This would seem to be an

inhibition of calcification although blood calcium and phosphorus are normal (17)(18). An apparent increase in phosphorus excretion has been observed following molybdenum feeding to cattle (75), but not to rats (74).

A reciprocal antagonism exists between copper and molybdenum such that dosing with molybdate causes depletion of copper reserves and restricts copper assimilation (23). This antagonism is considered to be a significant factor in the "complicated" copper deficiency disease called peat scours in New Zealand (17) and in the disease called teartness in England (26)(56). In these diseases an excessive molybdenum content of the forage (20 ppm. or more on the dry basis) produces fo predisposes the severe diarrhea (scouring) which is the outstanding symptom. Dosing with copper salts relieves the scouring of teart and cures peat scours which is fundementally a copper deficiency disease that has been modified by the molybdenum-induced scouring. Copper is also active in reducing the effects of molybdenum toxicity in the rat (34)(61)(75).

The converse of the above effect, that abnormally low levels of molybdenum in forages with normal copper levels might lead to chronic copper poisoning, has been suggested as a factor in the development of a syndrome exhibited by sheep in certain areas of Australia. Although the pasture copper content is essentially normal, sheep in those areas accumulate abnormally high concentrations of liver copper and die with a crisis resembling that of a copper toxicity (23). However, there is not any direct evidence that deficiency of molybdenum induces abnormal copper accumulation in the liver (51).

In general, the effects of excess molybdenum resemble those of

copper deficiency, however, these effects are not in equivalence. In some cases a relatively small amount of molybdenum will exert a profound effect on the copper metabolism of sheep and in others a proportionally small amount of copper will overcome the effect of a massive amount of molybdenum (51).

Singer et al. (75) found evidence of an interrelationship of copper, molybdenum, and phosphorus in the rat. Simultaneous administration of molybdenum with phosphorus tended to increase phosphorus assimilation; copper administration with phosphorus had little effect on phosphorus assimilation, but administration of molybdenum and copper together with phosphorus reduced phosphorus uptake. Simultaneous administration of molybdenum and/or phosphorus with copper reduced the accumulation of copper in the liver. Phosphorus and copper administration had no effect on molybdenum accumulation. Shirley et al. (73) studied the deposition and alimentary excretion of radio-phosphorus in steers on high molybdenum and copper diets. Molybdenum alone, or in the presence of copper, changed the pathway of phosphorus excretion toward the feces, the magnitude of the effect being somewhat less in the latter case.

The Florida group suggested, as possible mechanisms for the toxic action of molybdenum, either (a) indirect interference in essential enzyme systems involved in skeletal metabolism due to an induced copper deficiency, or (b) direct inhibition of these enzyme systems by molybdenum itself (75). Neither of these postulated mechanisms has received support.

The minimum toxic level of molybdenum in pasture forage is considered to be about 5 ppm.; above this concentration signs of copper deficiency appear (64). In the United States, cases of molybdenum toxicity

have been reported in parts of Florida (18) and California (13). A minimum requirement by animals for molybdenum has never been established (83); in fact, the presence of molybdenum in the list of essential trace elements is subject to question. The first direct evidence for the essentiality of molybdenum in animal metabolism came with the report of Richert and Westerfeld (65) that molybdenum is the co-factor for xanthine oxidase in rat intestine. A later paper extended this report, stating that in molybdenum deficiency xanthine oxidase was completely absent in the intestine (66). DeRenzo and co-workers (22) suggested that molybdenum played a role in the activation of both intestinal and liver xanthine oxidase; but, its action on the liver enzyme has not been confirmed (48)(66).

Manganese

Manganese has a definite relationship to phosphorus metabolism. Its deficiency leads to poor development of the skeletal system in rabbits and poultry (1)(30)(80)(85). The bones become weakened and porous and are shorter than those in animals with adequate manganese.

In poultry, manganese deficiency causes the appearance of the "perosis" syndrome, a leg disorder characterized by enlargement and malformation of the tibiometatarsal joint with slipping of the gastrocnemius tendon from its normal position (30)(89). Subnormal hatchability of eggs and inferior growth of chicks have also been noted (88).

Impaired reproduction is observed in manganese-deficient rats; the specific lesions are testicular degeneration and irregular or absent estrus cycles (12)(72).

Growth is less than normal for mice, rats, and rabbits fed manganese-deficient rations (24)(72)(85). The minimum requirement for growth is small and subject to modification by the relative amounts of calcium and phosphorus in the ration (89).

Combs et al. (16) found that the abnormally high bone phosphatase activity, usually seen in rachitic chicks, could be decreased to the normal level if manganese were omitted from the ration. On the other hand, Wachtel and his associates (85) were unable to show any influence of manganese deficiency on bone phosphatase in the rat, although bone formation was not normal. Serum phosphatase was definitely increased, however, while blood calcium and phosphorus remained normal. Serum phosphatase decreases, as does the bone enzyme, in manganese-deficient chicks (86).

Evidence has pointed to an effect of manganese on blood formation, but little information bearing directly on this point is available. Robscheit-Robbins and Whipple (68) reported that manganese, although toxic to anemic dogs, brought an irregular hemoglobin response, sometimes favorable and sometimes not. Manganese was less effective than copper salts. Wachtel et al. (85) noted a slight progressive anemia and retarded hemoglobin regeneration in deficient rats. Skinner and McHargue (77) obtained evidence supporting a role for manganese in hemoglobin formation by showing that manganese, in combination with iron and copper, produced more marked hemoglobin regeneration than iron and copper alone.

Manganese deficiencies in grazing ruminants have never been reported, probably because the requirements are so very low in relation to the usual manganese content of pastures. Phillips (64), however,

suggested that rations should contain 25 to 50 ppm. which approximates the usual content in pasture grasses.

Effects produced by consumption of large amounts of manganese have not been extensively studied. Those that have been noted to date are similar to effects of manganese deficiency. For example, inhibition of calcification in rats caused by high manganese supplementation was reported by Blumberg et al. (11) and Chornock et al. (15). This inhibition was most pronounced in incipient rickets produced by a ration with a wide calcium-to-phosphorus ratio.

Nance (58) observed depressed birth and weaning weights for rabbits born of does that received large amounts of manganese.

Excessive manganese in pasture plants is held responsible for the forage anemia of horses encountered in parts of Sweden. Hay from an anemia-producing locality assayed 1270 ppm. manganese as compared to the average 440 ppm. for anemia-free localities (82). High forage copper sometimes accompanies the anemia.

Manganese is detrimental to swine when fed at high levels. Grummer and his associates found that a ration containing 500 ppm. manganese caused appetite failure and retarded growth when forced on growing pigs.

Toxic effects of manganese may not be manifested if the ration supplies enough phosphorus. Becker and McCollum (7) could observe no toxic effects in rats from feeding manganese at high levels. They believed that the high concentration of phosphorus in the ration (0.72%) depressed manganese absorption. A slight growth retardation was observed at the high level of intake (100 mg. manganese per day). Skinner (76) could not demonstrate toxicity in rats fed either 1000 or 2000 ppm. manganese in a high phosphorus ration.

Zinc

The effects of zinc deficiency do not appear to be correlated with the fact that zinc is a constituent of the enzyme carbonic anhydrase and the hormone insulin. Hove et al. (41) have provided the bulk of the information concerning the nature of a zinc deficiency. Poor mineralization of bones, poor intestinal absorption, and reproductive disturbances characterize the syndrome.

The effects of excess zinc are similar to those of zinc deficiency. There is a poor intestinal absorption and poor bone development; growth is retarded and a microcytic, hypochromic anemia appears (69). Copper administration corrects the zinc-induced anemia suggesting a zinc-copper interaction (79).

Ruminant deficiencies of zinc are unlikely since most plants contain ample amounts.

From the foregoing discussion it is apparent that improper dietary amounts of one or more of the above mentioned trace metals could well be related to the rather non-specific symptoms encountered in the affected cattle at Wilburton. Thus, without regard to interspecies differences, poor reproductive performance is noted in manganese and zinc deficiencies; modified phosphorus metabolism in copper, manganese, and zinc deficiencies and in zinc, manganese and molybdenum excesses; anemia in cobalt, copper and possibly manganese deficiencies; and depressed growth rates when any of the trace metals are not present in the proper amounts.

EXPERIMENTAL

Development of Analytical Methods

Cobalt

Some cobalt assays of Oklahoma forage plants have been reported (84), however the procedure used was not considered to give accurate results with the low cobalt concentrations encountered. For this reason, further exploration was conducted in search of a method that would provide accurate cobalt estimates at extremely low concentrations.

Sandell (70) has reviewed the colorimetric methods and reagents used for cobalt determinations at the microgram level. Methods based on the colored chelate formed with nitroso-R-salt (1-nitroso-2-naphthol-3,6-disulfonate) have been most widely used for biological materials. Both the chelate and reagent absorb light strongly at 420 mμ consequently excess reagent must be destroyed. In previous work conducted in the Department of Agricultural Chemistry Research the method described by Beeson and Gregory (10) was used. This procedure provides for destruction of the excess reagent by boiling with nitric acid which oxidizes the reagent to the lighter colored nitro acid and decomposes the nitroso-R chelates of most other metals. The nitric acid method, however, gives erratic results. The length of boiling time and the amount of acid used are somewhat critical (84). Oxidation of the excess reagent with bromine in the presence of nitric acid is an accepted alternative (57), but, according to Gallego et al. (29), bromine oxidation is better in hydrochloric acid than in nitric acid.

The initial approach in this study was made with the nitroso-R method. Tidwell (84) experienced considerable difficulty due to the interference of iron and especially manganese. The literature shows conflicting opinions concerning the interference of ferric iron. Hiscox (41) and MacPherson (50), who used acetate buffers, stated that ferric iron didn't interfere; Marston and Dewey (53) used a citrate buffer and reported that ferric iron, in a concentration ten times that of cobalt, was permissible although an excessive amount interfered because of its color in citrate medium. Tidwell, with an acetate buffer, obtained slightly augmented cobalt values in the presence of added iron (11.6 to 92.9 mg. Fe^{+++} per 100 ml. of solution). Although the ferric iron can be removed by specific treatment, the preferable method is to isolate the cobalt thereby eliminating iron and any unrecognized sources of interference. Isolation of cobalt by extraction as the dithizonate (the chelate formed with diphenylthiocarbazone) preceding formation of the nitroso-R chelate eliminates ferric iron interference. The procedure was tested with solutions containing 3 µg. cobalt and varying amounts of ferric iron. The curves in Figure 1 show the influence of ferric iron on the amount of cobalt measured by the nitroso-R method with and without cobalt isolation as the dithizonate. The procedure of Gallego _et al._ (29) was used which employs hydrochloric acid for stabilization of the cobalt chelate and bromine for oxidation of excess reagent. Dithizone extraction also eliminates the severe negative interference of manganese. It can be seen in Table 1 that the optical densities of solutions containing 3 µg. cobalt were not appreciably changed in the presence of manganese when using the isolation procedure. Without isolation of cobalt,

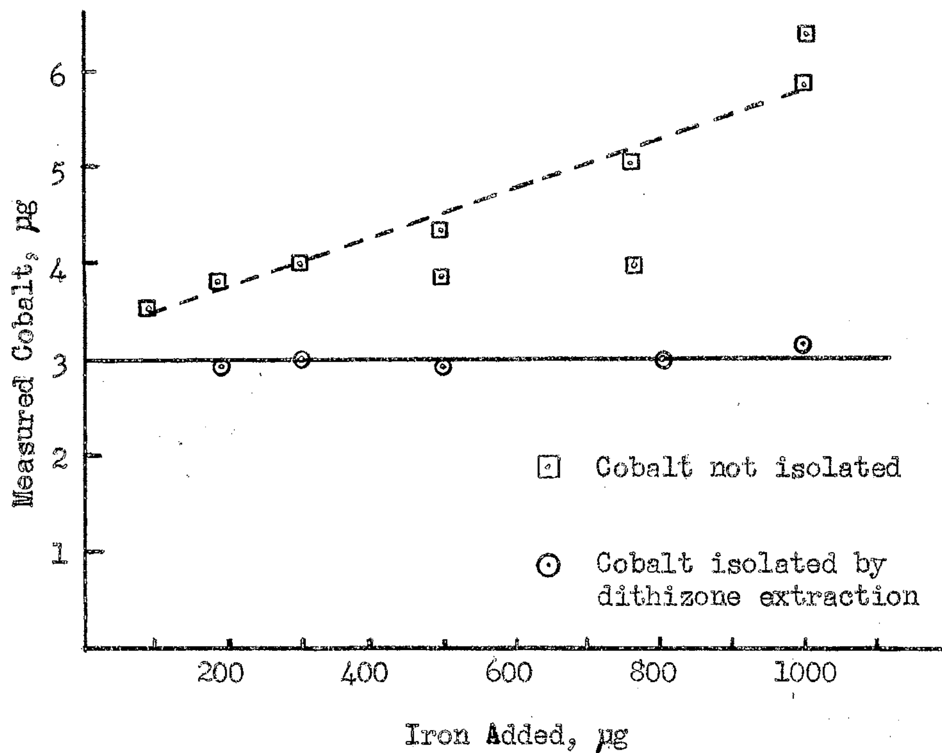

Figure 1. Effect of iron in the nitroso-R method for cobalt, with and without dithizone extraction.

Tidwell (84) found that 5 mg. manganese reduced the optical density by approximately one-third.

TABLE 1

EFFECT OF MANGANESE IN THE NITROSO-R SALT METHOD FOR COBALT (COBALT ISOLATED BY DITHIZONE EXTRACTION)

Cobalt Present µg	Manganese Added mg	Optical Density
3	0.0	.119
3	0.5	.124
3	0.75	.124
3	1.25	.119
3	2.5	.116
3	5.0	.114

It appeared that the Gallego method (29) with dithizone isolation of the cobalt would be satisfactory, but nitroso-R methods are cumbersome. The complex is unstable in the light so all of the boilings had to be done in the dark. It was also necessary to use bromine with its attendant hazards. A principal drawback, however, was the dilution that became necessary. Each sample was boiled for five minutes and, with the quantity of salts present, a final volume of 20 to 25 ml. was mandatory in order to keep the salts in solution. With this dilution the spectrophotometer transmission range from 100 to 50 per cent is spanned by cobalt concentrations up to 15 µg. Since the amount of cobalt in these samples is so small this dilution requires that one work near 95 per cent transmission with little precision attainable.

One means of surmounting the dilution and salt concentration problems is a chromatography approach such as worked out by Dean (21) or King *et al.* (47). However, many possible errors are consequent to such an approach so it seemed more satisfactory to achieve concentration of the cobalt chelate by extraction. The nitrosocresol method introduced by Ellis and Thompson (25) is an extremely sensitive method employing solvent extraction. The cobalt-nitrosocresol chelate is formed in buffered aqueous solution and extracted into 5 ml. of petroleum ether. The chelate in petroleum ether absorbs strongly at 360 mμ and the transmission range of the spectrophotometer from 100 to 40 per cent is spanned by a concentration range up to 3 μg cobalt. This method provides about a five-fold increase in sensitivity over the nitroso-R method.

Nitrosocresol is synthesized from m-cresol by means of the Baudisch reaction (4) and is extracted from the reaction mixture into petroleum ether. This solution in petroleum ether is stable for several months under refrigeration and serves as stock reagent. Before use, the stock solution is extracted with an equal volume of sodium borate buffer. The aqueous solution of sodium o-nitrosocresol so obtained is the reagent used in the cobalt procedure. It has been observed that this aqueous solution is not stable but changes progressively from day to day. Such instability would cause marked errors, if not recognized, in cases where sample absorbancies are compared with a fixed reference. However, calibration curves prepared daily following the extraction of sodium o-nitrosocresol are parallel and perfectly reliable (Figure 2). The curves shown in Figure 2 are regression lines taken from several sets of data. The similarity of their slopes is

interpreted to mean that the properties of the chelate are not changing, rather, the absorption of the reagent itself increases with time.

With plant samples it is necessary to remove ferric iron, whose o-nitrosocresol chelate is appreciably soluble in petroleum ether. Isolation of cobalt by dithizone extraction removes the possibility of ferric ion interference.

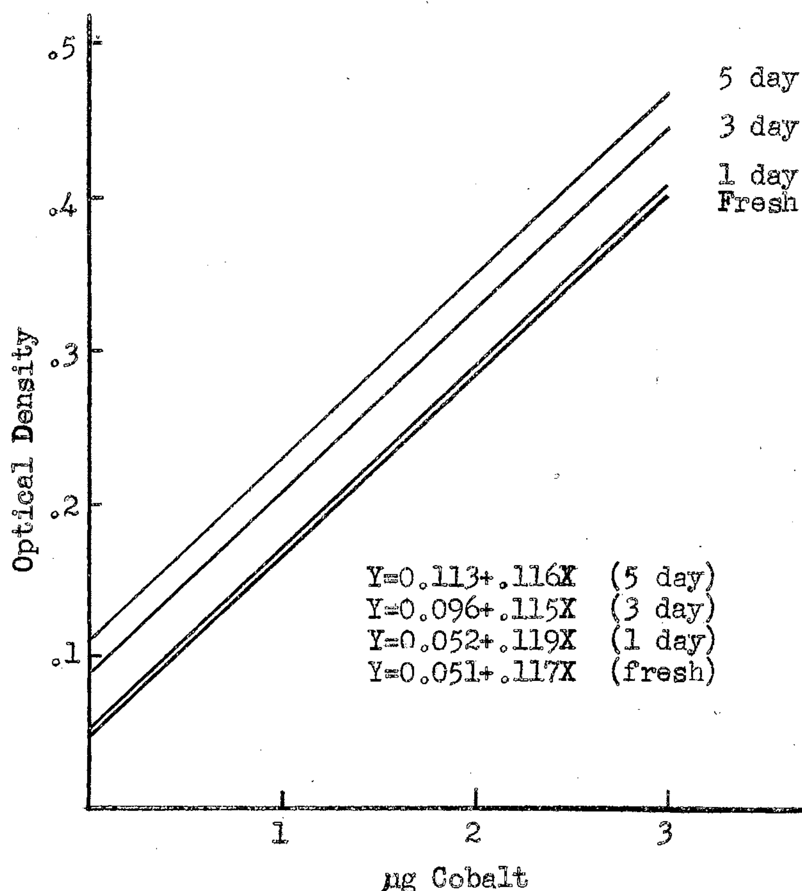

Figure 2. Effect of age of reagent on cobalt calibration curves. (nitrosocresol method)

Copper

Copper analyses have not previously been reported for plants from the Wilburton and Stillwater areas. It was necessary to select a copper procedure capable of sufficient accuracy for our purpose and yet not prohibitive in terms of labor and equipment.

Two organic reagents, diphenylthiocarbazone (dithizone) and diethyldithiocarbamate (carbamate) have been widely used for the estimation of copper (70). Neither one has been particularly satisfactory. Dithizone is a colored compound itself and this necessitates elaborate procedures for the removal of excess reagent before comparing absorbancies, or else a mixed-color method must be used. Furthermore, dithizone is not selective (78). Other ions chelate with it producing colors of similar hues. An additional disadvantage is the instability of dithizone solutions, particularly in the presence of light.

Carbamate is also a very sensitive reagent for copper. Its chelate is insoluble in water but soluble in many organic solvents. Like dithizone, this reagent is not selective, but complexes with many divalent metal ions. Iron, manganese, and nickel are the principal ions interfering either by reaction with the reagent or by imparting an interfering coloration to the organic solvent. These principal interferences may be removed by specific treatments for each (70).

A general treatment, that provides protection from many metal ions, employs ethylenediamine tetracetic acid (EDTA) which forms water-soluble complexes with interfering ions. Sedivec and Vasek (71) first reported that the specificity of the copper reaction with the carbamate reagent could be increased in the presence of EDTA. Cheng and Bray (14) recently reported that only bismuth gives an interfering color with carbamate in the organic phase when extracted in the presence of EDTA. EDTA serves to keep interfering ions other than bismuth in the aqueous phase while the copper-carbamate is extracted into carbon tetrachloride. This is demonstrated for iron by the data

in Table 2 which show that the augmentation of the copper assay caused by the presence of 2 mg. ferric iron does not exist when EDTA is present.

TABLE 2

EFFECT OF EDTA AND ADDED CALCIUM ON THE INTERFERENCE OF IRON IN THE CARBAMATE METHOD FOR COPPER

Copper Present µg	Fe^{+++} Present mg	EDTA Added mM	Calcium Added mM	Copper Found µg
20	2.08			(300)
20	2.08	1.5		20
30	2.08			45
30	2.08	1.5		30
20	2.08	1.5	4	28.5
30	2.08	1.5	2.5	33

The usual plant sample, however, contains from 1 to 2 millimoles of ions which complex with EDTA -- chiefly calcium and magnesium. Excessive amounts of these ions relative to the quantity of EDTA present, tend to negate any advantage being gained by the use of EDTA (Table 2).

A serious drawback to the use of EDTA is that it interferes with the dithizone extraction of cobalt from the same sample in which copper is being determined. Dithizone extraction of cobalt from an alkaline solution containing EDTA is incomplete as shown in Figure 3. Attempts to release cobalt from its apparent EDTA-chelate by the addition of excess calcium were partially successful; however, any attempt to develop this into a procedure for plant samples seemed pointless. Because of the foregoing disadvantages in the use of EDTA it seemed

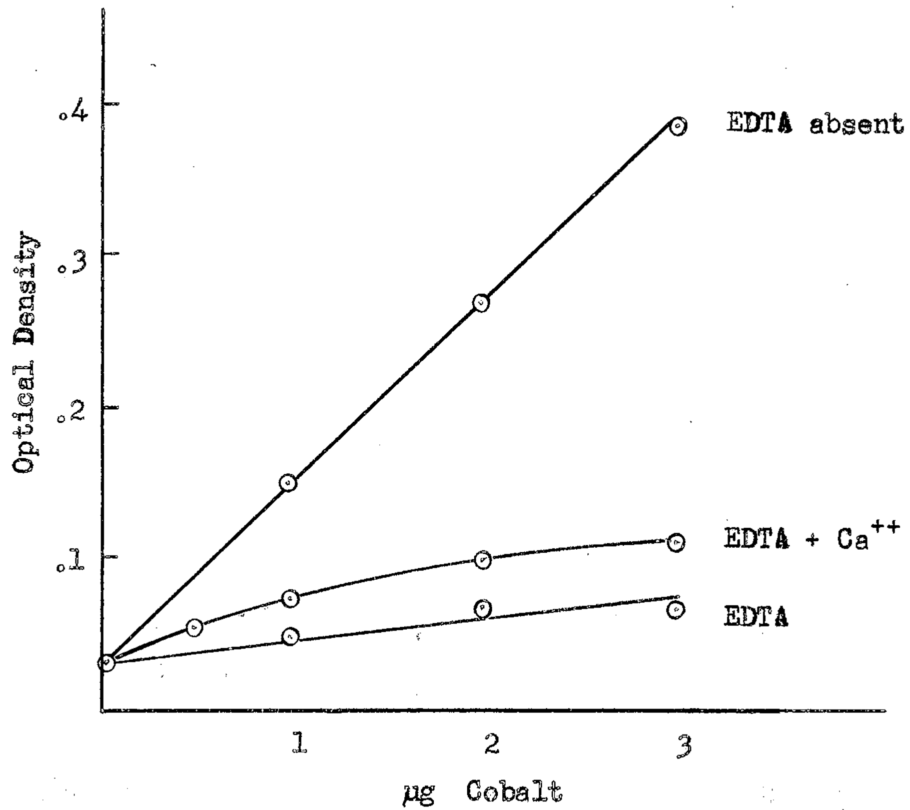

Figure 3. Effect of EDTA on the dithizone extraction of cobalt.

preferable, first, to isolate the copper by dithizone extraction from acidic solution and then extract cobalt after making the solution alkaline. Copper may be separated from cobalt by extracting with dithizone in a solution of pH 1 to 2.

Briefly, the procedure that has been found satisfactory for the determination of cobalt and copper in a single sample of plant material, both from the standpoint of sensitivity and reliability in the presence of interfering ions, may be outlined as follows (see also Figure 4):[1]

1. Copper is extracted from the solution of plant ash with dithizone solution. The acidity of plant ash solution is between pH 1 and 2 for this extraction.

2. The extract is evaporated to dryness and the copper-dithizonate oxidized with perchloric acid.

3. Copper is determined in the residue by the carbamate procedure with EDTA present for added protection from extraneous ions. The absorbancy of the copper chelate in carbon tetrachloride solution is measured at 515 mμ in the Evelyn colorimeter.

4. For cobalt isolation, the original plant ash solution is make alkaline and cobalt, zinc, and lead are extracted with dithizone solution.

5. The extract is washed with 0.01 N hydrochloric acid to decompose zinc and lead dithizonates; these elements may be determined in the acid washings.

6. The washed dithizone extract is evaporated and the cobalt-dithizonate oxidized with perchloric acid.

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[1] The procedure is given in detail in the Appendix.

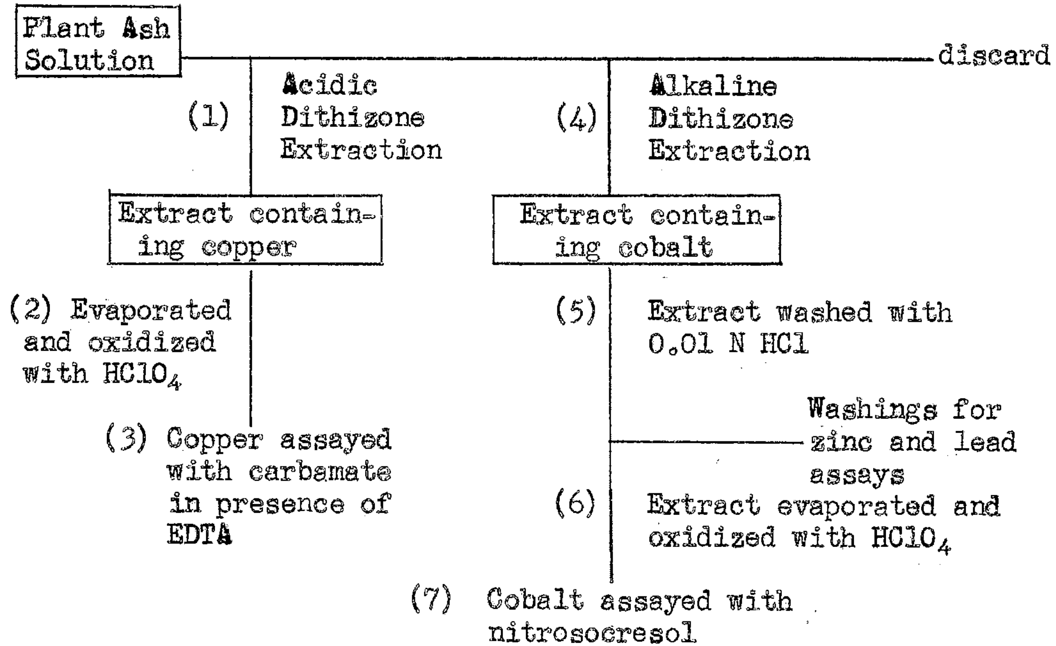

Figure 4. Scheme of Analysis for Cobalt and Copper.

(The numbers refer to the steps in the outline on page 22)

7. Cobalt is determined in the residue by the nitrosocresol procedure. The optical density of the cobalt chelate in petroleum ether is measured at 360 mμ with the Beckman spectrophotometer.

Recovery experiments, in which cobalt standards were added to plant samples before destroying organic matter with nitric and perchloric acids, gave good results (see Table 3). Copper recoveries were also satisfactory; one sample gave a spuriously high value possibly due to a variation in composition of the backing sample.

TABLE 3
RECOVERY OF ADDED COBALT AND COPPER FROM PLANT SAMPLES

Sample Assay, %	Standard Added, %	Found %	Recovery %	Recovery %
		Cobalt		
0.40	1.0	1.40	1.0	100
0.40	1.0	1.35	.95	95
0.40	1.0	1.38	.98	98
0.40	0.5	0.93	.93	106
0.40	0.5	0.88	.48	96
		Copper		
40	30	68	28	93
40	30	67	27	90
40	30	80	40	133
40	40	77	37	92
40	40	79.5	39.5	98

Dry ashing in a muffle furnace generally resulted in low cobalt recoveries (70 to 92 per cent) and very erratic copper recoveries. Unless silica was decomposed, following dry ashing, only about two-thirds of the added cobalt and copper could be recovered.

ZINC

A satisfactory procedure was not obtained. Some analyses were made using a mixed-color dithizone method. The semi-quantitative values obtained with this method indicated ample zinc in the samples examined.

Methods Adopted

Sampling

Forage samples were cut by hand from pastures located near Wilburton and on the experimental range at Lake Carl Blackwell. Some samples were of the individual grass species and others were representative of the general grass growth at a particular time, e.g., early spring grass.

All samples were oven-dried, if necessary, and cut into short lengths with shears to avoid contamination with metal from the grinding mill. Sheared samples were stored in sealed glass jars. The samples were not cleaned except to remove any foreign material. In certain cases the major mineral constituents were determined on sample portions that had been ground in a Wiley mill. All samples were analyzed for proximate composition.

Major Minerals

A complete analysis of the bulk mineral nutrients was made on most samples using standard analytical procedures. For calcium, magnesium, and phosphorus determinations, samples were ashed overnight in a muffle furnace at 425° C. followed by extraction of the ash with hot, dilute hydrochloric acid. Calcium was precipitated from an aliquot of the ash extract as the oxalate. The calcium oxalate was filtered by suction, dissolved in sulfuric acid and the oxalic acid so obtained titrated with 0.05 N potassium permanganate. Magnesium was precipitated as $MgNH_4PO_4$ from the combined filtrate and washings from the calcium determination; this precipitate was ignited and weighed as $Mg_2P_2O_7$. Phosphorus was determined in a diluted aliquot of the ash extract by the colorimetric method of Fiske and Subbarow (27).

For the determination of sodium and potassium, samples were ashed overnight in platinum dishes in the muffle furnace at 425° C. The ash was extracted with hot, dilute hydrochloric acid and the remaining silica decomposed with hydrofluoric acid. The acid-soluble material following this treatment was added to the ash extracts. After appropriate dilutions the sodium and potassium concentrations were determined with the aid of a Perkin-Elmer flame photometer.

When iron was determined, the ferric thiocyanate complex was formed in an aliquot of the ash extract used for sodium determination. The color intensities of sample solutions were compared to those of standard iron solutions with the aid of a visual comparator. Destruction of silica was found to be essential in order to free the bulk of the iron.

Trace Minerals

Of the various methods available for the determination of trace amounts of metals, we were essentially limited to those based on colorimetry.

Manganese was determined by the periodate method of Willard and Greathouse (90) in an aliquot of ash extract. Previous workers in this laboratory did not destroy silica in preparation of their samples (32)(84). When silica is destroyed about 10 per cent higher manganese values are obtained.

Molybdenum was determined according to the directions of Tidwell (84). This method is essentially that of Barshad in which the thiocyanate complex of quinquevalent molybdenum is formed in the presence of stannous chloride (3). The stannous ion serves to reduce iron to the ferrous state. The molybdenum complex is extracted into ethyl

ether and the color intensity of the ether solution determined photometrically. Samples were prepared for molybdenum analysis by ashing overnight in a muffle furnace and extracting the ash as described above. Although molybdenum retention by the silica residue is said to be negligible (3), silica was routinely destroyed with hydrofluoric acid as a precautionary measure. Recovery of standard amounts of molybdenum (2 to 4 µg), added before ashing, was near 100 per cent.

Cobalt and copper were assayed by the method outlined in the preceding section. Ashing was done by the following wet method: A five gram, oven-dry sample was placed in a 600 ml. tall-form beaker with 25 ml. of concentrated nitric acid and digested overnight on the steam plate. Digestion was completed on a hot plate after the addition of 10 ml. of concentrated nitric acid, 5 ml. of perchloric acid, and 0.5 ml. of concentrated sulfuric acid. Occasionally it was necessary to add more perchloric acid to completely eliminate charred material from the residue. When the digestion mixture had become clear and approached dryness the beaker was removed, let cool, and the residue taken up by boiling with distilled water. After filtration the solution was evaporated down to a volume suitable for analysis.

In all of the trace mineral analyses, precuations were taken to avoid contamination. Different sets of separatory funnels were used for each operation in the cobalt and copper procedure; one set was used for dithizone extractions, one for cobalt-nitrosocresol extraction and one for copper-carbamate extractions. All glassware was rinsed with nitric acid after each period of use to remove any adsorbed ions. Laboratory distilled water, redistilled in glass, was used in all procedures. Acids, excepting the hydrochloric used for neutralizations,

were not purified nor was ammonium hydroxide. With these precautions, the reagent blanks averaged 0.08 µg cobalt and 5 µg copper; such a blank was carried with each set of samples.

RESULTS AND DISCUSSION

Results of analyses for the minerals contained in comparable grass samples from the Wilburton and Lake Carl Blackwell areas are presented in Table 4. The predominant grass species were Big Bluestem (Andropogon furcatus), Little Bluestem (Andropogon scoparius), and Indian grass (Sarghastrum nutans). Some of the samples were laboratory composites of the three species and others were representative of the general pasture growth at the sampling date. In some cases separate analyses were made of the three individual species.

Differences between comparable samples in major mineral content were small and inconsistent. Calcium concentrations in spring and summer samples from the two areas ranged from 0.27 to 0.43 per cent. Likewise, magnesium values ranged from 0.15 to 0.25 per cent.

Neither area supplied sufficient phosphorus in most of these samples to meet the recommended level for cattle (59)[1]. Phosphorus values for Wilburton forage ranged from 0.15 to 0.06 per cent and decreased with the age of the grass. The Wilburton values were often lower than those for comparable Lake Blackwell samples in which phosphorus ranged from 0.16 to 0.07 per cent. The calcium-to-phosphorus ratio ranged from 2:1 to 7:1; wide ratios tend to decrease the availability of phosphorus.

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[1]Supplementary data are presented in the Appendix.

TABLE 4 MINERAL CONTENT OF COMPARABLE RANGE GRASS FROM WILBURTON AND LAKE CARL BLACKWELL AREAS

Sample	Date	Location	Ca %	Mg %	P %	Na %	K %	Mn ppm.	Mo ppm.	Co ppm.	Cu ppm.
51-526-B	6/51	B[1]	0.35	0.17	0.11	0.026	1.17	174	0.16	0.04	7.3
51-526-A	6/51	W[1]	0.38	0.15	0.13	0.076	1.77	328	0.12	0.05	8.1
51-526-E	7/51	B[1]	0.43	0.17	0.10	0.053	1.65	99	–[2]	0.05	5.7[3]
51-526-S	7/51	W[1]	0.31	0.20	0.08	0.035	1.08	144	0.09	0.05	5.7
52-526-17	5/52	B	0.41	0.15	0.16	0.005	1.72	173	0.25	0.07	11.0
52-526-10	4/52	W	0.43	0.22	0.08	0.023	2.00	340	0.31	0.10	12.0
52-526-20	6/52	B	0.33	–[2]	0.10	0.010	1.08	41	0.15	0.10	5.8
52-526-18	6/52	W	0.32	0.20	0.10	0.021	1.16	204	0.11	0.09	10.1
52-526-L	7/52	B[1]	0.33	–[2]	0.07	0.014	1.65	63	0.05	0.07	5.4
52-526-K	7/52	W[1]	0.37	–[2]	0.06	0.007	0.78	177	0.06	0.03	6.4
52-526-N	9/52	B[1]	0.32	–[2]	0.08	0.008	0.83	36	–[2]	0.07	6.2
52-526-M	9/52	W[1]	0.42	–[2]	0.06	0.012	1.00	225	0.06	0.04	11.0
52-526-58	6/53	B	0.32	0.18	0.11	–[2]	–[2]	33	0.11	0.03	5.3
52-526-57	6/53	W	0.27	0.18	0.15	–[2]	–[2]	270	0.09	0.15	7.4
52-526-71	7/53	B	0.36	0.17	0.11	–[2]	–[2]	43	–[2]	–[2]	–[2]
52-526-72	7/53	W	0.38	0.25	0.07	–[2]	–[2]	208	0.05	0.03	7.3

[1] Major minerals determined in laboratory composite; trace minerals determined in the three individual species and averaged.

[2] Insufficient sample for analysis or otherwise omitted.

[3] Little Bluestem contained 42 ppm. copper at this sampling; it was not included in the average.

Potassium concentrations ranged from 0.78 to 2.00 per cent with no consistent difference between grass samples from the two areas. Generally, the sodium concentration in Wilburton forage was slightly greater than in samples from Lake Blackwell. The range in Wilburton samples was 0.007 to 0.076 per cent while in Lake Blackwell samples it was 0.005 to 0.053 per cent.

Iron was found in generous amounts whenever assayed. Values ranged from 0.045 to 0.293 per cent.[1] These values are considerably higher than those reported by Gibson (32) probably because silica destruction was routine in the present series.

In all samples molybdenum was present in amounts far below the minimum toxic concentration of 5 ppm. The values ranged from 0.31 ppm. in the spring to 0.05 ppm. in late summer. Tidwell (84) reported similar values for samples from these areas.

In both areas the cobalt and copper content of forage approached the minimal levels for adequate nutrition of ruminants. Many of the cobalt values were less than 0.07 ppm. which is considered to be the minimum level. Copper was present in amounts near the minimum level of 5 ppm. However, the copper supplied by these forage is probably sufficient in view of the extremely low molybdenum concentrations. Published values for similar grass in Kansas are much higher in cobalt, copper, and molybdenum; the average values are about five times those found in the Oklahoma forage (33). That report serves to emphasize the comparatively low content of these elements in Wilburton and Lake Blackwell forage. However, Nelson et al. (62) found that supplementing

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[1] Supplementary data are presented in the Appendix.

the feed of Wilburton cattle with cobalt and copper had no apparent beneficial effect. Therefore, a deficiency of copper and/or cobalt is probably not the primary cause of the poor performance of cattle at Wilburton.

Manganese was invariably found to be present in concentrations some two to nine times higher in Wilburton forage than at Lake Blackwell.[1] Manganese values in Wilburton samples ranged from 144 ppm. to 340 ppm. but the range in Lake Blackwell samples was only 33 to 174 ppm. Manganese concentrations decreased with the age of the grass. Certain weedy grasses and weeds were also found to accumulate large amounts of manganese.[1] To date, the accumulation of manganese has been the only marked, consistent difference noted between the Wilburton and Lake Blackwell forage.

In Table 5 are listed the cobalt and copper content of grass from the two areas by species. Indian grass contained slightly more cobalt than Big Bluestem, but with so few samples the difference may be insignificant. The copper content was more nearly alike for the three species than the cobalt content. There was probably no significant species difference in the accumulation of manganese although at times Little Bluestem had somewhat greater concentrations than the other species. Species differences in manganese content are shown in Table 6.

The possibility that the greater concentration of manganese in the Wilburton forage is a contributing factor to the poor performance is discussed in Part II of this thesis. Much of the manganese

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[1]Supplementary data are presented in the Appendix.

TABLE 5

COBALT AND COPPER CONTENT OF GRASSES BY SPECIES
(values in ppm.)

Date	Big Bluestem		Little Bluestem		Indian	
	Cobalt	Copper	Cobalt	Copper	Cobalt	Copper
			Lake Carl Blackwell			
6/51	.03	6.8	.06	5.8	.03	9.3
7/51	.05	5.0	.04	(42)	.06	6.5
7/52	-	-	.06	5.0	.08	5.8
9/52	.05	6.9	-	-	.09	5.5
			Wilburton			
5/51	.04	6.9	.07	9.6	.04	7.8
7/51	.05	5.0	.05	4.8	.06	7.4
7/52	.00	4.6	.03	4.4	.05	8.2
Range	0-.05	4.6-6.9	.03-.07	4.4-9.6	.03-.09	5.5-9.3
Ave.	.04	5.0	.05	6.0	.06	7.2

TABLE 6

MANGANESE CONTENT OF GRASSES BY SPECIES
(values in ppm.)

Date	Big Bluestem	Little Bluestem	Indian
	Lake Blackwell		
10/50	47	47	47
6/51	174	217	131
7/51	107	112	77
	Wilburton		
6/50	168	219	-
10/50	205	224	210
5/51	-	342	314

contained in these plant samples appears to be readily available. The quantities of manganese obtained from typical samples by various extraction procedures are shown in Table 7.

TABLE 7

SOLUBILITY OF MANGANESE IN TYPICAL PLANT SAMPLES

Sample	Total Manganese ppm	Mn Extracted %	Procedure[1]
53-526-58	33	48	W
"	33	45	A
53-526-57	270	57	W
"	270	98	A
"	270	65	Ac
53-526-73	253	65	W
"	253	83	W-C
"	253	79	A
"	253	79	Ac

[1] The procedures were: W, sample refluxed with water overnight; W-C, continuous extraction with water overnight; A, refluxed with 0.022 N HCl overnight; Ac, extracted by shaking with 2% acetic acid for 15 minutes.

From these data it seems likely that a large portion of the manganese is present in soluble form and could be dissolved by digestive fluids.

SUMMARY

Determinations were made of the minerals contained in forage samples from the Range Cattle Mineral Station near Wilburton, Oklahoma and, for comparison, from the experimental range at Lake Carl Blackwell. Analytical results were obtained for the calcium, phosphorus, magnesium, sodium, potassium, iron, manganese, molybdenum, cobalt, and copper concentrations in representative samples.

Preliminary work was conducted in search of methods for cobalt and copper that would give accurate results at low concentrations of these minerals. The procedure that was developed permitted analysis of a single sample for both cobalt and copper. A double extraction with diphenylthiocarbazone was used to isolate cobalt and copper. Cobalt, after its isolation, was determined photometrically as the chelate formed with o-nitrosocresol; copper was determined photometrically as its diethyldithiocarbamate chelate formed in the presence of ethylenediamine tetraacetic acid. Recovery experiments, in which cobalt and copper were added to plant samples prior to oxidation of organic matter, served to establish the reliability of the procedure.

Analytical results indicated that cobalt and copper were present in amounts near the minimal level for adequate nutrition of ruminants; low amounts were found in samples from both of the areas surveyed. Molybdenum was found in amounts far below the toxic level in all samples. The other minerals, excepting manganese, were found to be

present in similar amounts in comparable samples from the two areas or else the differences observed were inconsistent. Phosphorus concentrations in most samples were inadequate to meet the recommended intake for cattle.

The only consistent difference between samples from the two areas was the accumulation of manganese by Wilburton forage. Wilburton samples contained two to nine times as much manganese as Lake Blackwell samples. The greater concentration of manganese in the Wilburton forage is a possible factor affecting the performance of cattle. In view of the low cobalt concentration in the forage it is possible that a marginal cobalt deficiency abets the effects of excess manganese and low phosphorus.

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II. EFFECT OF MANGANESE UPON CERTAIN BLOOD CONSTITUENTS OF BEEF CATTLE

INTRODUCTION

Many cattle produced near Wilburton, Oklahoma, are unthrifty. A premise was made that the unthriftiness manifests a state of phosphorus deficiency resulting from the low phosphorus content of the forage. However, attempts made in 1947 to 1950 to improve the condition of cattle at Wilburton by feeding a phosphorus supplement were only partially successful. In particular, reproduction was considered to be subnormal.

Manganese was found to be present in high concentration in Wilburton forage. In view of reported effects of manganese on phosphorus metabolism, this concentration was considered to be a possible causative factor abetting the phosphorus deficiency.

The experiment described herein was conducted to explore the effect of high levels of manganese on certain blood constituents related to phosphorus metabolism and anemia in beef cattle. Two supplementary experiments were conducted, one with cattle to show the effect of exercise on plasma phosphorus, and the other to show the effect of manganese in preventing the recovery of rachitic rats when dosed with vitamin D. The results of these two experiments are presented and discussed in this section.

LITERATURE REVIEW

It was pointed out in Part I of this thesis that effects of excess manganese are similar to those of manganese deficiency. Reports of inhibited calcification, retarded growth, appetite failure and anemia caused by high levels of manganese were cited therein.

In additional reports it has been stated that excess manganese disturbs both calcium and phosphorus metabolism. For example, De and Basu (4) obtained elevated fecal calcium and phosphorus excretion in human subjects by supplementing diets with 100 to 200 mg. manganese. Calcium balances were very negative in some instances. Similarly, Chornock et al. (2) observed increased excretion of both calcium and phosphorus in rats receiving high levels of manganese. In that experiment negative phosphorus balances were common.

Reid and his associates (12) found that manganese sulfate supplementation markedly decreased calcium absorption in lactating dairy cows. Phosphorus metabolism was unaffected. It further appeared that calcium absorption was not disturbed when iodine, magnesium, copper, cobalt, zinc, and iron were included with the manganese supplement. In the latter case, however, the manganese was provided as a commercial mineral mixture; whether or not the apparent beneficial effect of the additional minerals was real or merely indicative of lower manganese availability in the mineral mixture was not precisely ascertained. Reid and Ward (13) did report balance data which indicated that manganese

availabilities were similar for the sulfate and the mixed mineral supplement. However, the supposition of Reid and Ward that differences in amount absorbed reflect differences in manganese availability in the intestine is not necessarily valid. Manganese retention was low and practically constant with daily intakes which ranged from 622.4 mg. to 1325.6 mg. The amount retained was 154.4 $\pm$ 9.8 mg. per day.

In a balance study conducted by Gallup _et al._ (6) six young steers were fed four levels of supplemental manganese. Manganese, calcium, and phosphorus balances were determined for each animal at each manganese level. Manganese sulfate was added to the basal ration in order to establish the 250, 500, 1,000, and 2,000 ppm. supplemental levels. Results of this study indicated that supplemental manganese decreased the absorption of both calcium and phosphorus. The decrease in absorption was more pronounced in the case of calcium.

EXPERIMENTAL

The following experiment, conducted on the experimental range at Lake Carl Blackwell, was initiated in the fall of 1950. Sixty-four grade Hereford cows were divided into four comparable groups. During wintering periods each group was confined in a small trap. The basal ration fed during the winter consisted of prairie hay, salt, and 1.4 lbs. of corn gluten meal per head daily. The cows of Group 1 served as controls; Group 2 received the basal ration supplemented with enough manganese sulfate to make the manganese content of the ration 250 ppm.; Group 3 received the basal ration supplemented with manganous sulfate to a concentration of 500 ppm. manganese; and Group 4 received the same ration as Group 3 plus 400 mg. iron and 40 mg. copper per head daily.

During the summer periods, from April to mid-November, each group grazed in individual native grass pastures; the groups were periodically rotated among the pastures throughout the summer. The manganese and mineral supplements were continued during the summer by mixing the mineral salts with corn which was fed on alternate days at the rate of 1 lb. per head daily.

The cows in all groups were bred in early summer and the winter crop of calves was weaned in October. Production data for this experiment were collected and analyzed by the Animal Husbandry Department; birth and weaning weights, cow weights, and the number of calves born

and weaned are on record in that Department. In general, production was satisfactory in all groups.

Venous blood was collected in citrated tubes from one-half of the cows in each group at regular intervals throughout the year. Plasma inorganic phosphorus was determined in each sample as a means of ascertaining nutritional status with respect to phosphorus in the various groups. Because excess manganese had been suggested to induce anemia, erythrocyte counts, hemoglobin, and hematocrit determinations were made on each sample. Plasma protein concentrations were also determined.

Erythrocyte counts were determined indirectly by measuring the turbidity of a diluted sample at a wavelength of 660 mμ with the Evelyn colorimeter. The dilution was 0.02 ml. of blood in 10 ml. of isotonic saline solution. A standard curve relating turbidity and red cell count had previously been established by measuring samples that had also been counted directly. Hemoglobin was determined by the acid hematin procedure (3) in the same diluted sample that had been used for the erythrocyte count. The color density was read at 540 mμ in the Evelyn colorimeter. This procedure had been previously standardized by measuring hemoglobin in a series of samples using the iron method of Wong (16). Hematocrits were determined by centrifugation at 2000 r.p.m. for thirty minutes in Wintrobe tubes. So that valid comparisons of hematocrits could be made, all blood samples for each collection were centrifuged at the same time. Plasma protein concentration was determined indirectly by its correlation with the specific gravity of the plasma (11). Plasma inorganic phosphorus (trichloroacetic acid-soluble phosphorus) was determined by the method of Fiske and Subbarow (5).

RESULTS AND DISCUSSION

Summarized blood data are graphically presented in Figures 5 to 9. These graphs were prepared by averaging the blood values obtained for each group of cows during the summer and winter periods. Each point on the graphs represents a seasonal mean for one group of cows. A complete tabulation of the blood data is in the Appendix.

Hemoglobin data are summarized in Figure 5. The cows comprising Group 3, whose ration contained 500 ppm. manganese, were found to maintain lower hemoglobin values than those of other groups. Seasonal means for Group 3 ranged from 9.9 to 11.3 per cent hemoglobin as compared with 10.5 to 12.1 for the other groups. This tendency toward low hemoglobin values appears to be a characteristic of that group and not an effect due to high manganese consumption. Group 3 entered the experiment with a lower mean hemoglobin level than the other groups. During the winter periods in 1953 and 1954 certain individual hemoglobin values dropped as low as 5.3 to 8.4 per cent; at those times such cows were considered to be anemic. In all groups the seasonal means for hemoglobin fluctuated mildly.

Erythrocyte counts, expressed as seasonal means, did not closely follow the hemoglobin pattern (see Figure 6). The counts for Group 3 cows dropped noticeably during 1952 and the winter of 1953, a period in which the other groups maintained steady or slightly increased counts. In 1953 the winter mean for Group 3 was 6.03 million per cmm.

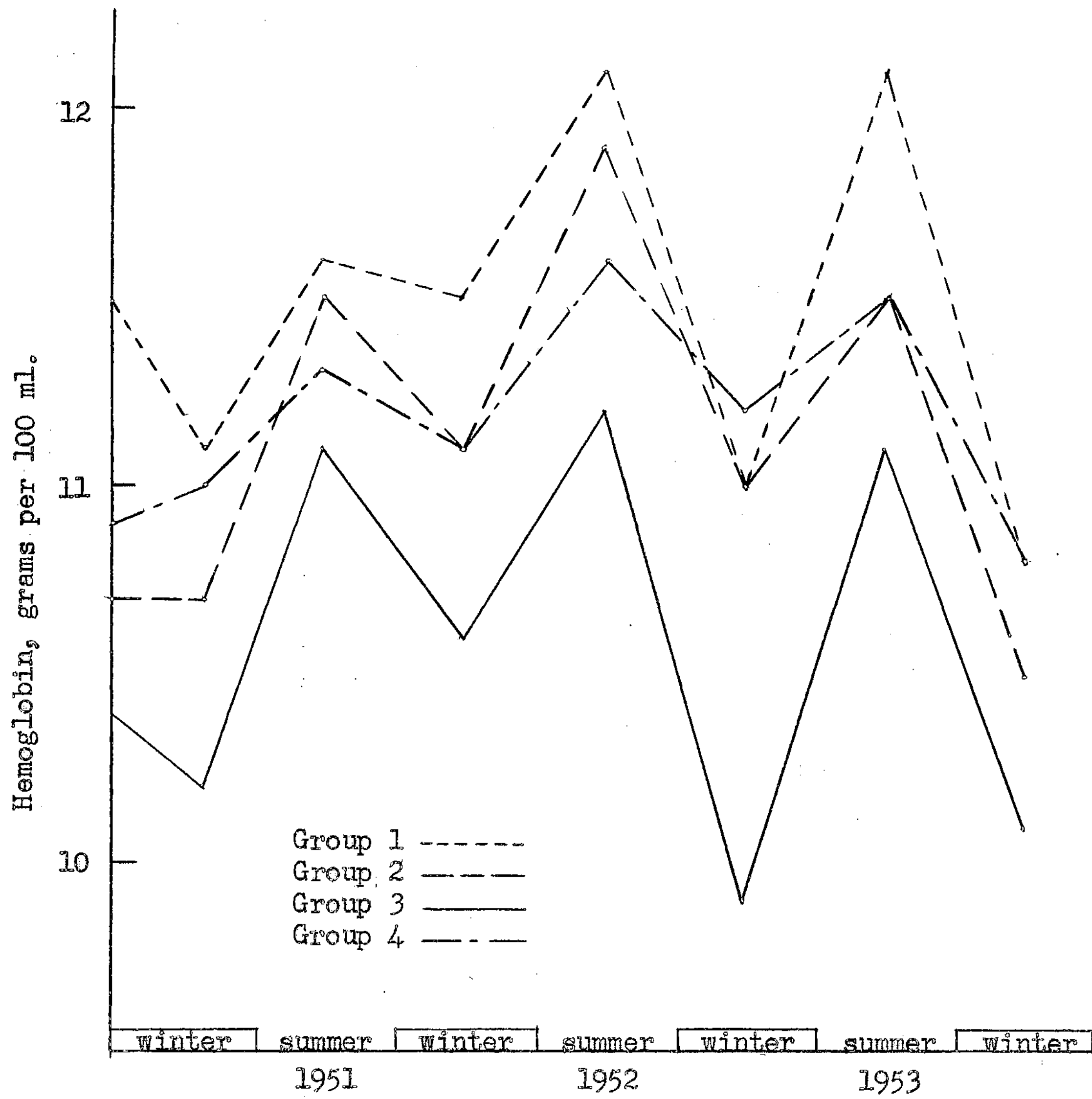

Figure 5. Seasonal hemoglobin levels by groups.

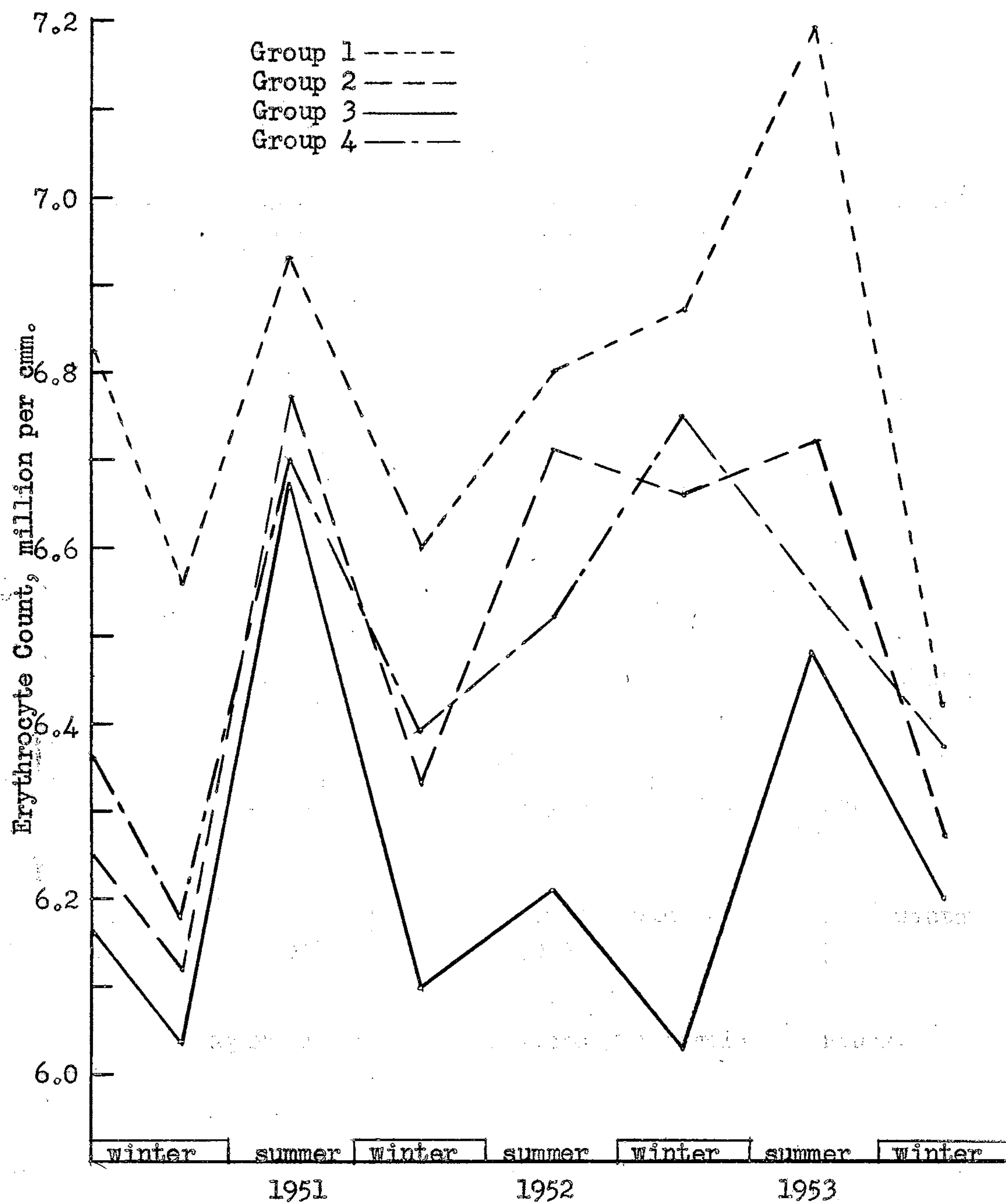

Figure 6. Seasonal erythrocyte levels by groups.

which was about 700,000 below the other groups. This period of low values for Group 3 was transitory, however, and in the winter of 1954 all of the groups maintained similar erythrocyte counts.

The hematocrit curves (see Figure 7) resemble those for hemoglobin. This resemblence emphasizes the individuality of the four groups of cattle as indicated by the preceding graphs; in each graph the relative standings of the four groups remain essentially unchanged from the experiment's inception. Persistent experimental effects are not apparent in these measures of blood constituents. Group 3 hematocrit values ranged from 30 to 34 per cent and were from 1 to 2 per cent lower than those of the other groups. The anemia that occurred in some individuals was also indicated by hematocrit values which were as low as 18 to 25 per cent.

Plasma protein concentrations among the four groups were very similar; however, the seasonal fluctuations were marked (see Figure 8). The values for the groups usually varied less than 0.3 percentage unit.

If high manganese supplementation intensifies phosphorus deficiency, it would be expected that the plasma phosphorus level would be decreased by the experimental treatments. The data suggest that such an effect did occur in Group 3 during 1952; the mean plasma phosphorus for this group was about 40 per cent lower than that for any of the other groups during that summer (Figure 9). However, since this trend was not observed in the following year, it is doubtful that the decrease in plasma phosphorus was an experimental effect. In fact, rather than showing a definite trend, the plasma phosphorus levels were extremely erratic during the following summer (1953). At each collection that summer some one group showed a markedly lower concentration of plasma phosphorus

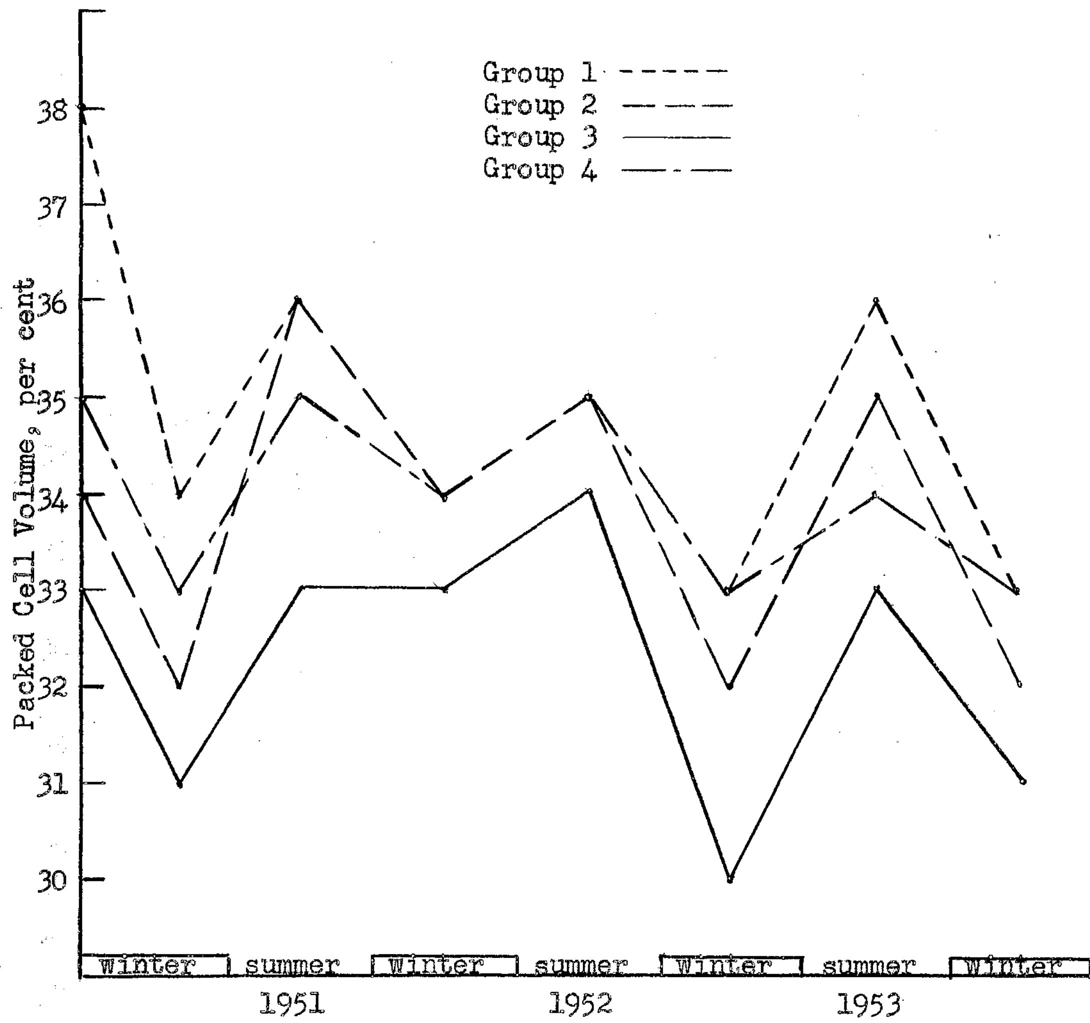

Figure 7. Seasonal hematocrits by groups.

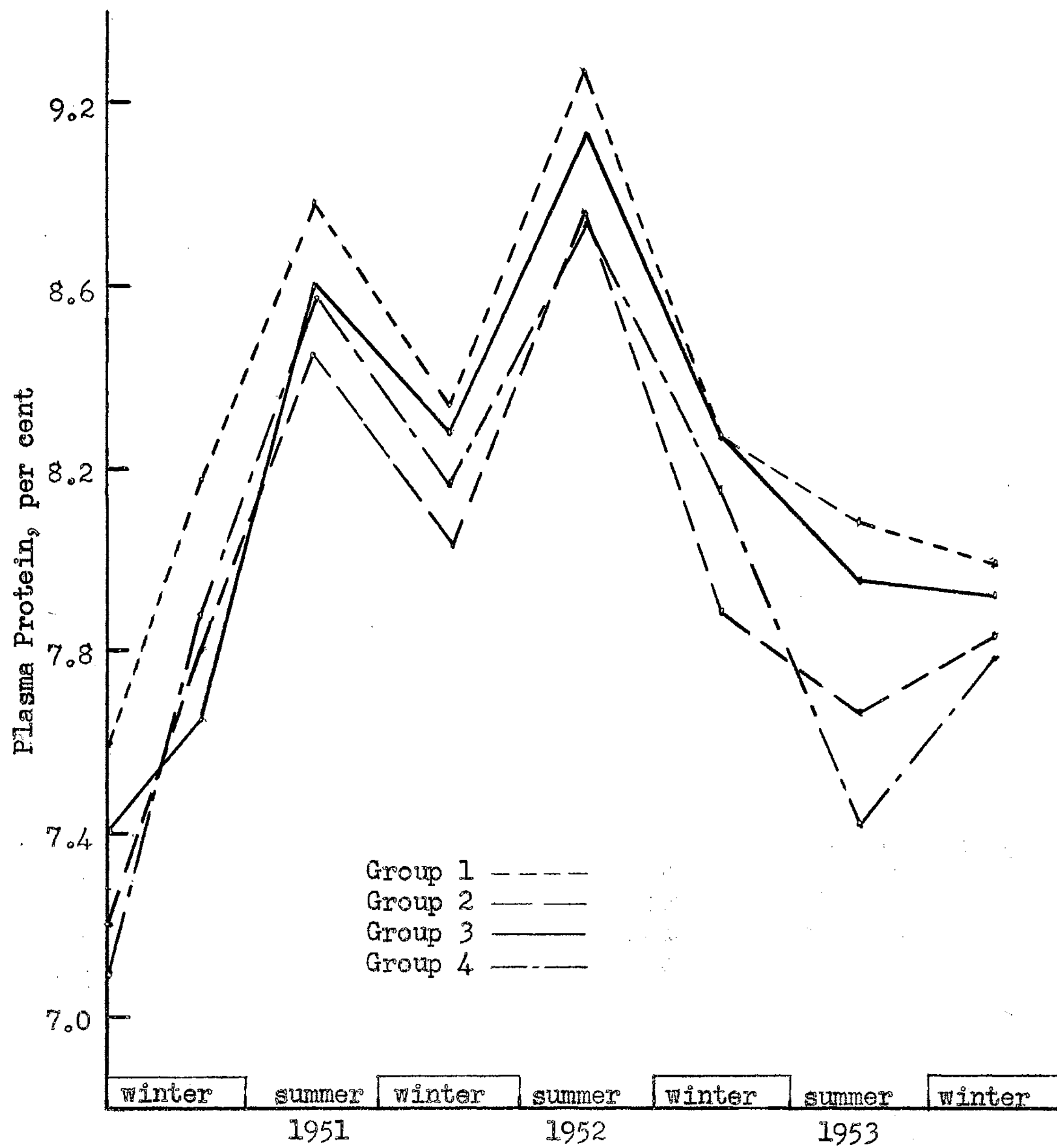

Figure 8. Seasonal plasma protein levels by groups.

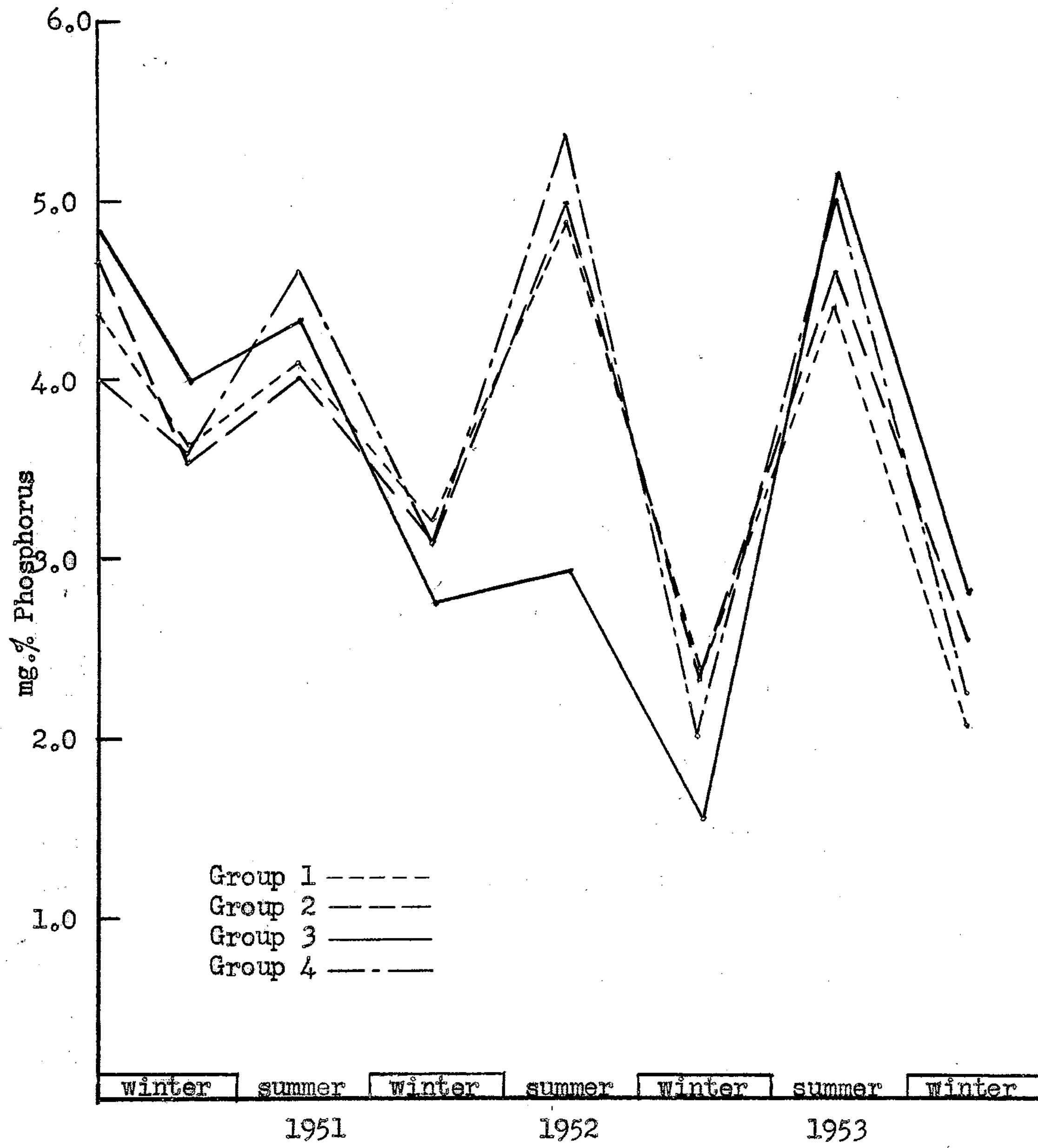

Figure 9. Seasonal plasma inorganic phosphorus levels by groups.

than the others. On July 2, Group 2 had the lowest phosphorus value; but on July 14 and August 4, Group 1 was the lowest. On September 8, Group 3 was the lowest; and on November 10, Group 4. Preceding each of these dates, the group indicated had suffered a large drop in plasma phosphorus. Each one of these groups had occupied the same pasture during the period in which their plasma phosphorus decreased with one exception, that being Group 1 on the July 14 bleeding date. One year earlier Group 3 grazed in that same pasture for the entire summer. During that period the cows in Group 3 had constantly low plasma phosphorus values.

The pasture in question is somewhat isolated and on each bleeding date it was necessary to drive the cattle approximately one and one-half miles to a corral. None of the other groups had to make such a trip. It is suggested that moving the cattle from the isolated pasture was partially responsible for the low plasma phosphorus concentrations that were observed. Palmer et al. (10) showed that exercising dairy cattle caused decreases in plasma phosphorus averaging 22 per cent of the normal values when measured 30 minutes following the exercise period.

The following experiment was conducted in order to determine the effect of exercise on the plasma phosphorus of beef cattle. Blood samples were obtained for plasma phosphorus determination from cows that had been at rest overnight. Some of the cattle were then exercised by driving them approximately two and one-half miles. Blood samples were again obtained following the exercise and the plasma phosphorus concentrations compared to those obtained from the cows at rest. This experiment was conducted on February 3, 1954 and again on June 16, 1954.

The data obtained are summarized in Table 8[1]. In the June trial, exercise caused a marked decrease in plasma phosphorus; the average decrease was 27 per cent of that present before exercise. During the winter, when the phosphorus level had fallen relatively low, such marked decreases did not immediately follow exercise (February trial). However, during the two hours after exercise the level decreased by an average of 0.56 mg. per cent in the exercised cows, but only 0.15 mg. per cent in those not exercised.[1] It was observed that the variation in plasma phosphorus without exercise was considerable. In the June trial this variation amounted to about 15 per cent in approximately one hour. Because of day-to-day fluctuations, Palmer et al. (10) used the mean plasma phosphorus for three consecutive days as the true phosphorus value. Henderson and Landingham (7) pooled alternate-day samplings over a period of 12 days for their representative blood sample.

TABLE 8

EFFECT OF EXERCISE ON PLASMA INORGANIC PHOSPHORUS

Date	June 16, 1954		February 3, 1954	
Treatment	Exercised	Not Exercised	Exercised	Not Exercised
No. of animals	16	16	8	8
Initial plasma phosphorus, mg.%	4.54	4.57	2.69	2.34
Mean change, mg.%	-1.26*	-0.69*	-0.42	-0.33
Range, mg.%	+0.40 to -2.88	+1.40 to -1.84	+0.20 to -0.96	+0.36 to -0.68

* The difference in means is significant at the 2.5% level.

It is possible that other factors influenced the plasma phorphorus of cattle grazing in the isolated pasture. Although Group 3 was

[1] A complete tabulation of the data is in the Appendix.

pastured there, those animals were not driven to the corral for bleeding during 1952. Exercise would not have been as important a factor effecting the low plasma phosphorus that summer. That pasture may contain less phosphorus than the others; in July, 1953, grass in that pasture contained only 0.08 per cent phosphorus and 34 ppm. manganese while the other pastures contained an average of 0.11 per cent phosphorus and 44 ppm. manganese.

The experiment at Lake Carl Blackwell has not served to demonstrate any persistent effect of manganese on phosphorus or certain other blood constituents. The low hemoglobin and hematocrit values of Group 3 seemed to be characteristic of those animals and unrelated to high manganese supplementation. In this experiment, manganese has not caused persistent aphosphorosis or anemia.

Although this experiment has furnished little evidence of an effect by manganese on phosphorus metabolism, such an effect has been well established by other investigators. The mechanism by which excess manganese exerts its influence on phosphorus is not clear. Some workers have suggested that insoluble manganous phosphate is precipitated in the intestine thereby decreasing phosphorus availability (1). If such were the case, the quantity of manganese fed in this experiment, like that contained in Wilburton forage, could remove 20 per cent of more of the total phosphorus consumed. Such precipitation would certainly promote phosphorus deficiencies at Wilburton where phosphorus is already limited in amount.

However, it is not certain whether such a mechanism adequately explains the effects of manganese. Slight solubility of manganous phosphate may be relatively unimportant since calcium phosphate, a

poorly soluble salt, readily satisfies the physiological demands for calcium and phosphorus. Whether manganous phosphate would satisfy the demand for phosphorus is not known. As a source of manganese, it is no less effective for prevention of perosis (a manganese deficiency disease) than other more soluble manganese salts (15). However, manganese requirements are small in comparison to phosphorus requirements; the relative availability of insoluble salts for a trace mineral and a bulk mineral may be quite different.

Any mechanism explaining the effect of manganese on phosphorus metabolism should also account for the disturbed metabolism of calcium. De and Basu (4), Chornock _et al._ (2), Reid _et al._ (12), and Gallup _et al._ (6) all observed increased fecal calcium excretions in the presence of excess manganese. It is not clear why intestinal precipitation of manganous phosphate should increase calcium excretion. In the experiment of Gallup _et al._ (6) the ration used had an unfavorable calcium to phosphorus ratio of 0.54. Precipitation of phosphate from this ration in an unavailable form should, in fact, tend to improve the ratio and thereby promote calcium absorption rather than increase calcium excretion.

The effect of manganese on calcium absorption could be either indirect, by means of disturbed phosphorus absorption not involving intestinal precipitation of phosphorus, or the direct antagonism of calcium absorption by excess manganese. An indirect effect on calcium absorption may be considered as follows: When excess phosphorus is present in the intestine calcium absorption is reduced. This is seen in high phosphorus rickets and in uremia when phosphorus excretion is necessarily by way of the intestine. Consequently, if phosphorus were

caused to accumulate in the intestine as the result of interference with the mechanism of phosphorus absorption, it would indirectly cause increased calcium excretion. Sadisavan (14) found that excess zinc interfered with phosphorus absorption and that increased fecal excretion of phosphorus accompanied high zinc supplementation, but the increase in phosphorus excretion was not as insoluble zinc phosphate. He observed that the activity of alkaline phosphatase in the intestine was decreased by zinc which implicates this enzyme in the process for phosphorus absorption. Other workers have suggested that intestinal alkaline phosphatase participates in the active absorption of phosphorus, but evidence is indirect and based mostly on histological data (8). Excess manganese might interfere with active phosphorus absorption by inhibiting phosphatase but this has not yet been established.

A direct antagonistic influence on calcium metabolism is the other possible effect of manganese involving calcium. Blumberg *et al.* (1) and Chornock *et al.* (2) noticed improvement in manganese-induced rickets when vitamin D was provided. Chornock's group also found that citrate feeding was of some value in treating manganese rickets. Both of these treatments tend to reduce free calcium in the intestine and consequently stimulate phosphorus absorption. Since calcium absorption is largely controlled by vitamin D, the above observations are suggestive of a manganese-vitamin D interaction.

A preliminary experiment was conducted in order to examine this last point. Fifty weanling rats were rendered rachitic by feeding the Steenbock rachitogenic diet for four weeks. This ration was constructed as follows: corn, 75 per cent; corn gluten, 20 per cent; calcium

carbonate, 3 per cent; sodium chloride, 1 per cent; and dried yeast, 1 per cent. Lysine was added as the racemate at a level of 0.25 per cent. The ration assayed 1.20 per cent calcium, 0.30 per cent phosphorus, and contained 12.7 ppm. manganese. At the end of four weeks on this ration, the colony had developed severe rickets; tibiae line tests on four individuals confirmed their rachitic condition. At that time, the remaining rats were placed at random into nine groups of five rats each. The experiment had three treatments consisting of (a) the basal rachitogenic ration, (b) the basal ration plus 0.15 per cent manganese, and (c) the basal ration plus 0.50 per cent manganese. Manganese was supplied as the carbonate. Vitamin D (irradiated ergosterol in corn oil solution) was given at levels of 0, 5, and 50 units per rat on the second and fourth days. On the seventh day of the experiment, the surviving rats were killed and the tibiae collected. During the week of Vitamin D supplementation the colony became infected and several rats died with a pneumonic disease. The presence of this disease relegated the experiment to a preliminary status. The severity of rickets after seven days was determined by measurements of the extent of metaphyseal recalcification in the tibiae. These measurements were taken from a radiogram of the tibiae. This data is presented in Figure 10. Each point represents the average of the measurements on the tibiae from all of the surviving rats in a group; most groups had four survivors. Vitamin D administration prompted immediate recalcification but the effectiveness of the vitamin was reduced by the presence of manganese. The 0.5 per cent level of manganese reduced the efficacy of the vitamin to about one-third. All the rats that received no vitamin D showed severe

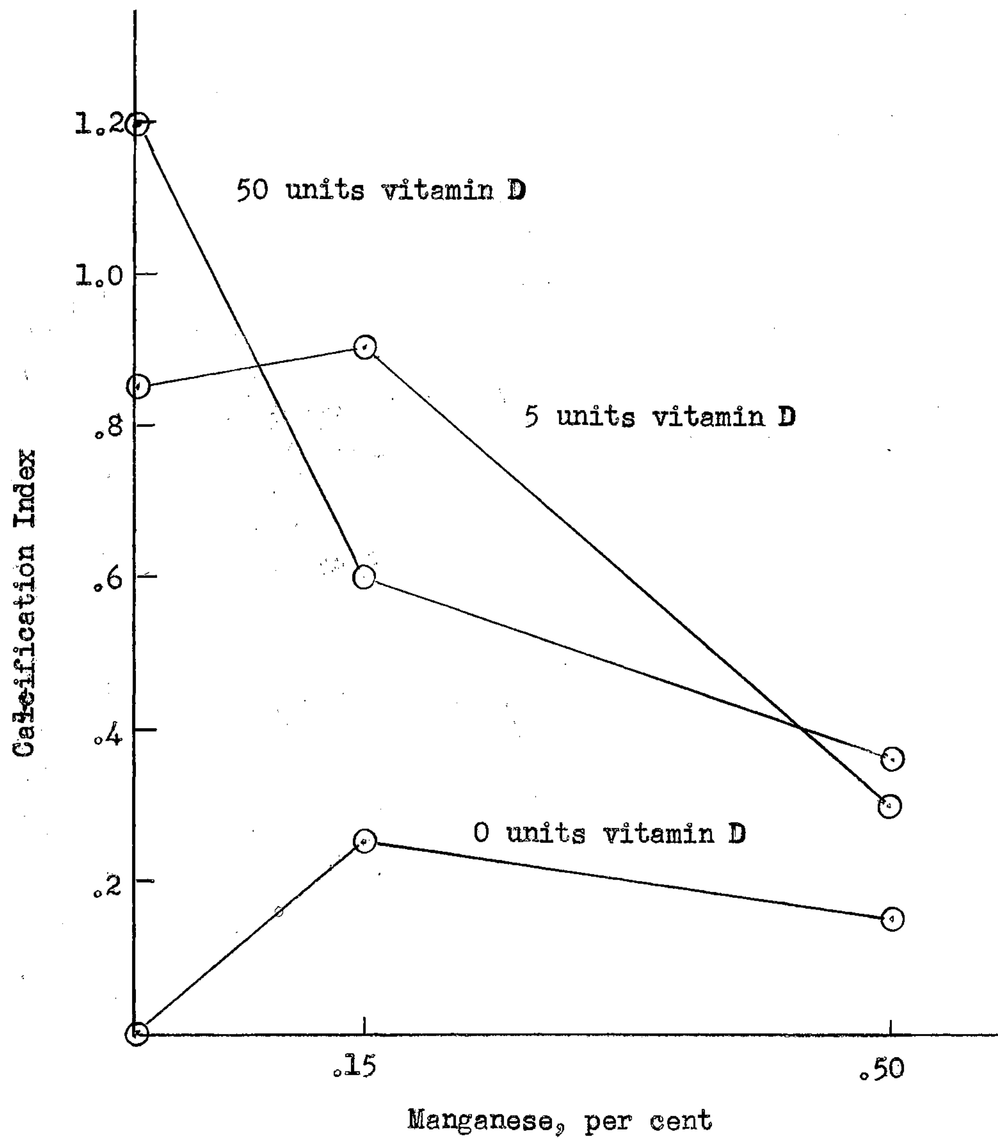

Figure 10. Effect of added manganese on the response of rachitic rats to vitamin D.

rickets at the end of the experiment with the exception of one at each level of supplementary manganese.

Since the action of vitamin D is believed to be directly on calcium absorption (9) this experiment indicates that excessive manganese interferes directly with calcium absorption by decreasing the efficacy of vitamin D. Decreased calcium absorption would help to account for the decreased absorption of phosphorus. This is suggested as a partial explanation for the increased calcium and phosphorus excretions observed when feeding excess manganese. It is recognized that the amount of manganese provided is sufficient to precipitate the phosphorus in the ration provided such a process is of importance. In that way, the action of manganese might be considered to amplify the unfavorable calcium to phosphorus ratio already existent in the ration. However, the data of Chornock et al. (2) indicate that interference with calcium and phosphorus metabolism was marked only when the calcium to phosphorus ratio was unfavorable. Narrowing this ratio permitted positive phosphorus balances although the calcium plus manganese to phosphorus ratio remained wide.

SUMMARY

The effect of supplementary manganese on hemoglobin, hematocrit, erthrocyte count, plasma protein, and plasma inorganic phosphorus in beef cattle blood has been examined. Supplemental manganous sulfate at levels of 0, 250, 500 ppm. manganese, and 500 ppm. manganese plus iron and copper was fed to similar groups of Hereford cows. Blood was sampled periodically over a three year period for determination of the different constituents. No persistent effects due to the consumption of manganese were noted on these blood constituents. The low hemoglobin and hematocrit values observed in blood samples from the group receiving 500 ppm. manganese seemed to be characteristic of the animals comprising that group and not due to manganese.

Low plasma phosphorus values were observed at times for all of the groups, but these appeared to be unrelated to manganese supplementation. It was found that exercise caused lowered plasma phosphorus values in beef cattle as had previously been reported for dairy cows. Exercise may have been responsible for some of the low phosphorus values in this experiment.

Data obtained from a preliminary experiment with rachitic rats indicated that manganese may interfere with the action of vitamin D. Such an interaction may help explain the decreased absorption of calcium and phosphorus in the presence of excess manganese that had been noted by other investigators.

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APPENDIX

A. DETAILED PROCEDURE FOR THE DETERMINATION OF COBALT AND COPPER.

Oxidize a five gram, oven-dry sample of plant material by the wet method described in the text. After dissolution of the soluble residue and removal of the silica by filtration, evaporate the solution to a small volume and transfer to a 125 ml. separatory funnel. The total volume should be 35 to 45 ml. at this point. Neutralize the contents of the funnel with ammonium hydroxide to the methyl red endpoint and add enough standardized hydrochloric acid to give a pH between 1 and 2. Extract with repeated 10 to 15 ml. portions of diphenylthiocarbazone solution (saturated in carbon tetrachloride) until the extract attains the green color of the dithizone solution. Two extractions are usually sufficient. Evaporate the combined extracts to dryness, then add 1 ml. of perchloric acid, cover with a watch glass, and heat on a hot plate until the solution is colorless. Then remove the cover and fume off the remaining acid. Small, 100 ml. beakers are convenient for the preceding steps; toward the end of the fuming-off process they may be laid on their sides in order to keep the temperature low on the bottom of the beaker where the residue of copper perchlorate is deposited. Wash the residue into a 60 ml. separatory funnel reserved for this step. The volume should be held to about 10 ml.

The procedure for determination of copper follows that of Cheng and Bray (14). Add 10 ml. of a mixture of ethylenediamine tetracetic

acid and ammonium citrate (20 grams of ammonium citrate and 5 grams of the disodium salt of ethylenediamine tetracetic acid in 100 ml. of purified water), and add ammonium hydroxide until the solution is basic. Then add 2 ml. of 1 per cent, filtered, aqueous diethyldithiocarbamate solution and extract with 15 ml. of carbon tetrachloride. Run the extract into a test tube and keep the tube stoppered. It is necessary to filter the extracts before measuring their absorbancy; this may be done conveniently using a small funnel and filtering directly into a colorimeter tube. Measure the absorbancy of the filtered extracts at 515 mμ and compare with standards treated in the same manner.

For cobalt determination, add 300 ml. of purified 20 per cent sodium citrate solution to the solution in the large separatory funnel remaining after isolation of the copper with dithizone. Neutralize the solution with ammonium hydroxide to the phenolphthalein endpoint and extract repeatedly with dithizone solution. Repeat the extractions until the extract has the original green color of the dithizone solution.

If zinc or lead are desired they may be isolated from the dithizone extracts by washing with 0.01 N hydrochloric acid; this may be omitted. Evaporate the combined extracts to dryness and oxidize with perchloric acid as before. Take up the residue with hot water and transfer to a 60 ml. separatory funnel reserved for this step. The volume should be held to about 15 ml. at this stage.

The determination of cobalt in this solution is carried out as directed by Gregory <u>et al.</u> (35). Add 5 ml. of pH 7.8 sodium borate buffer, 2 ml. of o-nitrosocresol (in buffered solution prepared by extraction of the stock petroleum ether solution), and 5 ml. of

petroleum ether. Shake vigorously for 10 minutes and discard the aqueous layer. Wash out excess reagent by shaking the petroleum ether extract with 1 ml. of 1 per cent cupric acetate solution; and wash the extract once with 5 ml. of water and then with 5 ml. of hydroxylamine solution which will reduce any ferric iron that was carried over into the petroleum ether. Determine the absorbancy of the petroleum ether extract at 360 mμ using solvent as the blank. Take care to correct for the age of the reagent by using a calibration curve prepared with reagent of the same age as that used in the determination.

B. Composition of Various Pasture Samples from Wilburton and Lake Carl Blackwell Areas

Sample		Description	Ash	Protein	Fat	Crude Fiber	N-free extract
			Proximate Composition of Dry Matter				
			%	%	%	%	%
			Wilburton				
1.	50-526-102	Big Bluestem	6.70	5.67	1.74	32.93	52.96
2.	103	Little Bluestem	5.65	4.83	1.51	31.51	56.50
3.	112	Big Bluestem	7.52	3.48	1.59	37.37	50.31
4.	113	Little Bluestem	9.40	3.38	1.82	34.58	50.82
5.	114	Indian grass	9.26	3.48	1.82	34.15	51.30
6.	51-526-139	Spring grass	8.90	11.58	2.94	26.25	50.33
7.	141	Big Bluestem					
8.	142	Little Bluestem[1]	8.03	9.59	2.03	36.41	43.94
9.	143	Indian grass					
10.	175	Big Bluestem	6.12	5.59	1.85	35.45	50.99
11.	176	Little Bluestem	6.81	4.99	1.51	35.91	50.78
12.	178	Indian grass	8.70	5.61	1.75	35.23	48.71
13.	52-526- 10	Spring grass	10.32	17.91	2.44	22.33	47.00
14.	18	Summer grass	7.65	8.62	1.83	31.91	49.99
15.	21	Big Bluestem					
16.	22	Little Bluestem[1]	6.84	4.86	2.02	31.23	55.05
17.	23	Indian grass					
18.	30	Big Bluestem					
19.	31	Little Bluestem[1]	6.87	4.52	2.17	33.59	52.85
20.	32	Indian					
21.	53-526- 57	Early summer grass	7.03	9.18	1.75	32.49	49.55
22.	72	Summer grass	6.00	4.94	3.36	34.85	50.85
23.	73	Late summer grass	7.14	6.21	2.98	34.03	48.64
Weeds and Miscellaneous Grasses							
24.	50-526-115	"Dog hair" grass	6.38	9.21	1.82	28.31	54.28
25.	116	Broom weed	6.79	8.38	6.17	30.47	48.19
26.	117	False dandelion	7.90	4.25	6.50	32.21	49.14
27.	51-526-144	Tickle grass[2]	-	-	-	-	-
28.	145	"Dog hair" grass[2]	-	-	-	-	-
29.	146	Vetch grass	8.07	25.94	3.12	28.55	34.32
30.	184	Wire grass	8.21	5.75	3.17	35.76	47.11
31.	188	"Dog hair" grass	8.01	6.22	1.55	34.93	49.29
32.	52-526- 11	Yarrow[2]	-	-	-	-	-
33.	12	Mullen[2]	-	-	-	-	-
34.	13	Cone flower[2]	-	-	-	-	-
35.	14	Indian paint brush[2]	-	-	-	-	-
36.	19	Wire grass[2]	-	-	-	-	-
37.	24	Brome sage [2]	-	-	-	-	-
38.	25	A Panicum species[2]	-	-	-	-	-
39.	26	Ragweed[2]	-	-	-	-	-

Mineral Composition of Dry Matter

	Ca %	P %	Mg %	Na %	K %	Fe %	Mn ppm.	Mo ppm.	Co ppm.	Cu ppm.
					Wilburton					
1.	.33	.07	.22	.005[3]	.54[3]	.009[3]	168	.06[4]	.09[4]	–[2]
2.	.32	.07	.18	.003[3]	.46[3]	.010[3]	219	.13[4]	.08[4]	–[2]
3.	.32	.04	.11	.006[3]	.25[3]	.028[3]	205	.15[4]	.17[4]	–[2]
4.	.36	.04	.08	.003[3]	.20[3]	.020[3]	224	.14[4]	.12[4]	–[2]
5.	.29	.03	.10	.007[3]	.31[3]	.015[3]	210	.14[4]	.12[4]	–[2]
6.	.50	.11	.14	.037	2.70	.024[3]	269	.11[4]	–[2]	–[2]
7.			.18	.140	2.11	–[2]	–	.13	.04	6.9
8.	.38[1]	.13[1]	.14	.063	1.60	–[2]	342	.13	.07	9.6
9.			.12	.025	1.60	.055	334	.09	.04	7.8
10.			.23	.068	1.30	–[2]		.12	.05	5.0
11.	.31[1]	.08[1]	.19	.034	1.16	–[2]	144[1]	.09	.05	4.8
12.			.20	.003	0.77	–[2]		.07	.06	7.4
13.	.43	.23	.23[5]	.023	2.00	.100	340	.30	.16	12.0
14.	.32	.10	.20[5]	.021	1.16	.100	204	.11	.06	10.1
15.				.012	.55	–[2]		.07	.00	4.6
16.	.37[1]	.06[1]	–[2]	.012	.76	–[2]	177[1]	.06	.03	4.4
17.				.022	.50	–[2]		.04	.05	8.2
18.				.012	1.20	–[2]		.07	.06	15.4
19.	.42[1]	.06[1]	–[2]	.015	.82	–[2]	225[1]	.02	.05	8.4
20.				.037	1.08	–[2]		.10	.02	9.2
21.	.27	.15	.18	–[2]	–[2]	–[2]	270	.09	.15	7.5
22.	.34	.07	.25	–[2]	–[2]	–[2]	208	.05	.03	7.3
23.	.34	.08	–[2]	–[2]	–[2]	–[2]	253	–[2]	–[2]	–[2]
24.	.37	.09	.15	.007[3]	.61	.053[3]	730	2.05	.90[4]	–[2]
25.	.75	.12	.21	.046[3]	1.07	.009[3]	881	.03[4]	.00[4]	–[2]
26.	1.11	.04	.34	.029[3]	.72	.000[3]	231	.00[4]	.03[4]	–[2]
27.	.19	.17	.18	.153	1.95	.089	334	.85	.05	9.0
28.	.35	.15	.27	.152	2.33	.151	556	.53	.53	10.3
29.	1.27	.52	.38	.184	3.88	.077	76	.32	.24	22.0
30.	.26	.07	–[2]	–[2]	–[2]	–[2]	385	.23	.09	7.2
31.	.26	.06	.23	.010	.67	–[2]	305	.12	.15	9.5
32.	1.00	.41	.39	.105	3.35	.107	545	–[2]	–[2]	–[2]
33.	1.49	.34	.45	.017	3.95	.114	209	–[2]	–[2]	–[2]
34.	.68	.29	.32	.061	3.60	.074	297	–[2]	–[2]	–[2]
35.	1.37	.31	.43	.045	3.92	.109	195	–[2]	–[2]	–[2]
36.	.36	.08	.19	.076	.61	.293	308	.26	–[2]	–[2]
37.	.16	.11	.18[5]	.020	.87	–[2]	218	.03	.06	9.8
38.	–[2]	.08	–[2]	.048	.93	–[2]	298	–[2]	.11	8.2
39.	2.30	.17	1.04	.066	2.16	–[2]	173	.04	.07	25.2

Sample		Description	Proximate Composition of Dry Matter				
			Ash	Protein	Fat	Crude Fiber	N-free extract
			%	%	%	%	%
		Lake Carl Blackwell					
39.	50-526-120	Big Bluestem	5.97	2.28	1.32	40.71	49.72
40.	121	Little Bluestem	4.72	1.78	1.39	39.36	52.75
41.	122	Indian grass	6.46	1.81	1.13	40.77	49.83
42.	51-526-147	Big Bluestem					
43.	148	Little Bluestem[1]	8.11	8.07	2.04	33.35	48.43
44.	149	Indian grass					
45.	180	Big Bluestem[2]	-	-	-	-	-
46.	181	Little Bluestem	9.76	5.73	2.13	33.83	48.55
47.	182	Indian grass[2]	-	-	-	-	-
48.	52-526- 17	Spring grass	10.49	13.30	1.20	33.59	41.52
49.	20	Early summer grass	8.46	7.94	1.94	32.85	48.81
50.	27	Big Bluestem					
51.	28	Little Bluestem[1]	6.61	4.82	2.11	33.10	53.36
52.	29	Indian grass					
53.	37	Big Bluestem					
54.	38	Little Bluestem[1]	6.78	5.03	2.01	33.01	53.17
55.	39	Indian grass					
56.	53-526- 58	Early summer grass	6.82	7.73	2.76	30.80	51.89
57.	71	Summer grass	7.27	6.89	3.40	32.51	49.93
58.	52-526- 16	Broomsedge[2]	-[2]	-	-	-	-

Mineral Composition of Dry Matter

	Ca %	P %	Mg %	Na %	K %	Fe %	Mn ppm	Mo ppm	Co ppm	Cu ppm
					Lake Carl Blackwell					
40.	.22	.03	.14	.004[3]	.60[3]	.016[3]	47	.11[4]	.03[4]	–[2]
41.	.25	.02	.07	.004[3]	.33[3]	.021[3]	47	.06[4]	.07[4]	–[2]
42.	.25	.02	.15	.003[3]	.35[3]	.040[3]	47	.11[4]	.12[4]	–[2]
43.	.34		.15	.029	1.17	.045	174	.20	.03	6.8
44.	.35	.11[1]	.18	.035	1.18	.061	217	.17	.06	5.8
45.	.33		.17	.012	1.16	.042	131	.11	.03	9.3
46.	.44	.12	.18	.037	1.84	093	107	–[2]	.05	5.0
47.	.42	.09	–[2]	.011	1.77	–[2]	112	–[2]	.04	42.
48.	.43	.10	–[2]	.053	1.65	–[2]	77	–[2]	.06	9.5
49.	.41	.16	.15[3]	.004	1.72	.070	173	.25	.07	11.0
50.	.33	.10	.07[3]	.010	1.08	.116	41	.15	.10	5.8
51.			–[2]	–[2]	–[2]	–[2]			–[2]	–[2]
52.	.33[1]	.07[1]	–[2]	.008	.72	–[2]	63	.05	.06	5.0
53.			–[2]	.007	.56	–[2]			.05	5.8
54.			–[2]	.005	1.21	–[2]		–[2]	.05	6.9
55.	.32[1]	.08[1]	–[2]	.011	.32	–[2]	36[1]	–[2]	–[2]	–[2]
56.			–[2]	.007	.96	–[2]		–[2]	.09	5.5
57.	.32	.10	.18	–[2]	–[2]	–[2]	33	.11	.03	5.4
58.	.36	.10	.17	–[2]	–[2]	–[2]	43	–[2]	–[2]	–[2]
59.	.21	.18	.18	.011	.94	.153	223	.14	.04	14.0

[1] Big Bluestem, Little Bluestem, and Indian grass were combined for analysis.

[2] Insufficient sample for proximate analysis, or analysis otherwise omitted.

[3] Value is inaccurate because of a faulty method.

[4] Value obtained by Tidwell (84).

[5] Semi-quantitative result obtained.

C. Blood Constituents of Cows at Lake Carl Blackwell

Date	Phosphorus mg. % of plasma				Hemoglobin %			
	1[1]	2	3	4	1	2	3	4
11-1-50	4.38	4.68	4.84	4.00	11.5	10.7	10.4	10.9
1-4-51	3.62	3.76	4.76	4.71	10.8	10.6	10.1	11.3
2-6-51	3.64	3.21	3.35	3.58	10.9	10.6	10.2	11.0
3-29-51	4.13	3.69	4.04	3.38	11.6	11.0	10.9	11.1
5-1-51	3.28	3.50	3.83	2.67	11.1	10.5	9.6	10.5
6-20-51	4.32	4.64	5.21	4.96	10.8	10.7	10.1	10.5
8-2-51	4.49	4.91	5.02	5.00	11.8	12.0	12.0	11.7
9-11-51	3.86	3.17	3.52	3.81	11.8	11.6	11.4	11.7
10-25-51	3.69	3.42	3.60	4.63	12.1	11.6	11.1	11.4
12-20-51	3.64	3.29	3.41	4.17	11.5	10.8	10.0	10.4
1-30-52	3.02	3.12	2.71	2.78	11.4	11.0	10.8	10.9
3-16-52	3.45	2.96	2.42	2.93	11.3	11.3	10.9	11.8
4-17-52	2.78	3.00	2.48	2.50	11.7	11.3	10.8	11.4
6-5-52	4.96	4.98	3.06	5.23	11.3	10.9	9.9	11.3
7-25-52	5.17	5.00	3.29	6.48	11.9	12.1	11.7	11.6
9-9-52	5.17	4.63	2.94	5.17	12.2	12.3	11.5	11.7
11-4-52	4.28	5.39	2.40	4.54	12.9	12.2	11.7	12.0
1-8-53	2.54	3.13	1.66	2.58	11.2	11.9	10.5	10.9
1-31-53	2.35	2.43	1.59	2.04	11.3	11.5	9.9	11.6
3-20-53	2.49	1.98	1.03	1.59	11.0	10.5	9.3	11.6
4-23-53	2.09	1.78	1.92	1.82	10.7	10.2	9.8	10.9
7-2-53	5.35	3.00	5.74	5.92	12.1	11.3	11.0	11.0
7-14-53	3.82	5.17	4.98	4.48	12.8	11.9	11.0	12.2
8-4-53	2.97	4.90	5.92	5.20	11.8	11.0	11.0	11.1
9-8-53	4.51	4.20	3.36	5.40	12.2	12.5	11.5	11.7
11-10-53	5.33	5.68	5.78	3.98	11.8	10.9	10.8	11.6
2-3-54	2.50	2.50	2.92	2.45	11.1	11.1	10.3	11.2
4-21-54	1.63	2.59	2.70	2.05	10.4	10.0	10.0	10.3

[1] Column headings are the group numbers (see text).

Erythrocyte Count millions per cmm				Hematocrit % packed cells				Plasma Protein %			
1	2	3	4	1	2	3	4	1	2	3	4
6.82	6.25	6.16	6.36	38	34	33	35	7.6	7.2	7.4	7.1
6.25	5.85	5.95	6.55	33	31	31	34	7.4	6.9	7.2	7.4
6.40	6.22	6.03	5.60	34	32	31	33	8.5	7.5	7.7	7.9
6.96	6.38	6.41	6.44	36	33	33	33	8.4	8.4	7.8	8.0
6.64	6.03	5.75	6.12	33	31	29	32	8.4	8.4	7.9	8.2
6.57	6.27	6.01	6.02	35	32	31	32	9.0	8.7	8.5	8.8
7.07	7.04	7.12	6.95	34	35	34	35	8.8	8.5	8.6	8.6
6.99	6.96	6.79	6.87	38	39	33	38	8.3	8.0	8.4	8.4
7.07	6.81	6.76	6.94	38	37	36	36	9.0	8.6	8.9	8.5
6.28	5.94	5.56	6.00								
7.10	6.57	6.56	6.63								
6.58	6.64	6.20	6.67	35	35	34	36	8.1	8.0	8.2	8.1
6.42	6.16	6.06	6.26	32	32	31	32	8.6	8.1	8.4	8.2
6.67	6.38	5.67	6.61	33	33	32	34	9.0	9.1	9.1	8.9
6.41	6.54	6.13	6.16	34	35	34	33	9.1	8.9	9.2	8.7
6.95	6.93	6.61	6.50	37	37	35	36	9.2	8.9	8.7	8.8
7.16	6.98	6.43	6.79	37	37	34	35	8.9	8.2	8.8	8.6
7.06	7.21	6.60	6.68	34	35	32	32	8.6	7.9	8.1	8.1
7.19	7.08	6.26	7.06	33	33	30	33	8.3	8.1	8.4	8.4
6.73	6.31	5.47	6.89	34	33	30	36	7.8	7.9	8.3	8.1
6.49	6.02	5.77	6.38	31	29	28	32	8.4	7.7	8.2	8.1
7.37	6.64	6.52	6.56	35	35	32	32	8.5	7.8	8.0	7.8
7.12	6.41	6.10	6.87								
6.76	6.34	6.33	6.37								
7.19	7.41	6.80	6.87	38	37	34	34	8.1	8.0	8.2	8.2
7.53	6.80	6.63	7.13	36	34	33	36	7.6	7.3	7.6	7.9
6.49	6.55	6.30	6.71	34	33	31	35	8.1	7.5	7.9	8.3
6.35	5.99	6.11	6.03	31	31	31	31	7.8	8.1	7.9	7.2

D. EFFECT OF EXERCISE ON PLASMA INORGANIC PHOSPHORUS

February 3, 1954

Initial Phosphorus mg.%	Treatment	Second Bleeding mg.%	Phosphorus change mg.%	Phosphorus after 2 hour rest mg.%	Phosphorus change mg.%
1.84	Driven for 2.5 miles	2.04	+0.20	1.12	-0.92
2.52		2.36	-0.16	1.32	-1.04
2.76		2.28	-0.48	1.92	-0.36
3.64		2.68	-0.96	2.28	-0.40
2.92		2.56	-.036	2.40	-0.16
2.56		1.96	-0.60	1.52	-0.44
1.72		1.44	-0.28	1.20	-0.24
3.56		2.84	-0.72	1.96	-0.88
Ave. 2.69		2.27	-0.42	1.71	-0.56
2.12	Rested for 1.5 hours	2.04	-0.08	2.12	+0.08
2.00		1.44	-0.56	1.32	-0.12
1.16		1.52	+0.36	1.52	0.00
2.00		1.68	-0.32	1.44	-0.24
2.76		2.40	-0.36	2.24	-0.16
2.28		1.96	-0.32	1.56	-0.40
3.36		2.68	-0.68	2.28	-0.40
3.04		2.36	-0.68	2.40	+0.04
Ave. 2.34		2.01	-0.33	1.86	-0.15

Mean total change for exercised animals = -0.98 mg.%

Mean total change for non-exercised animals = -0.48 mg.%

EFFECT OF EXERCISE ON PLASMA INORGANIC PHOSPHORUS (continued)

Initial Phosphorus mg.%	Treatment	Second Phosphorus mg.%	Phosphorus change mg.%	Average change mg.%
		June 16, 1954		
3.24	Driven about 2.5 miles	2.04	-1.20	
2.40		2.12	-0.28	
3.80		2.12	-1.68	
2.48		1.56	-0.92	
4.20		3.52	-0.68	
6.36		4.72	-1.64	
5.76		3.56	-2.20	
6.32		5.12	-1.20	
5.20		3.56	-1.64	
5.20		2.56	-1.64	
4.08		3.12	-0.96	
4.40		3.68	-0.72	
4.60		3.00	-1.60	
4.88		3.52	-1.36	
4.12		4.52	+0.40	
5.64		2.76	-2.88	-1.26
3.56	Rested	3.20	-0.36	
4.20		4.72	+0.52	
1.92		2.24	+0.32	
2.41		2.40	-0.01	
5.04		3.96	-1.08	
6.80		6.32	-0.48	
5.60		6.44	+0.84	
4.40		3.44	-0.96	
4.24		4.88	+0.64	
4.36		4.12	-0.24	
5.32		6.04	+0.72	
4.64		5.64	+1.00	
4.72		6.12	+1.40	
6.04		5.20	-0.84	
6.00		5.20	-0.80	
5.40		3.56	-1.84	-0.69

VITA

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candidate for the degree of
Doctor of Philosophy

Thesis: I. MINERAL CONTENT OF SOME OKLAHOMA FORAGE IN RELATION TO THE NUTRITION OF BEEF CATTLE
II. EFFECT OF MANGANESE UPON CERTAIN BLOOD CONSTITUENTS OF BEEF CATTLE

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