SOME FACTORS AFFECTING PLASMA INORGANIC PHOSPHORUS AND ITS DETERMINATION IN THE BLOOD OF BEEF CATTLE

By

DARLE L. NIENEKER

Bachelor of Arts

Hope College

Holland, Michigan

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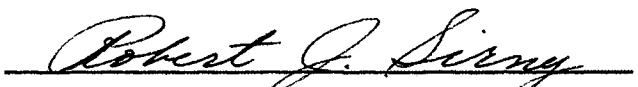
Thesis Adviser



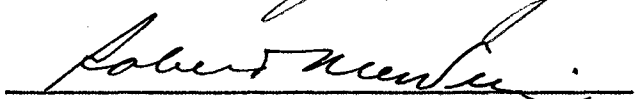


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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Certain inorganic elements rank with the vitamins in their importance in human and animal nutrition, and among these elements phosphorus is one of the most important.

Phosphorus is of importance in the body framework; it enters into such a great variety of reactions in the body as to give rise to the saying, "If the biographies of the elements could be written, that of phosphorus would be the most interesting of all", Sherman (1947).

Among the ways in which phosphorus functions are the following: As a constituent of the mineral structures of the bones and teeth, as phosphate ions in body fluids and as a constituent of soft tissues, as phospholipids, creatine phosphate, phosphoric acid esters of carbohydrates and their intermediates, nicotinic acid amide phosphate, riboflavin enzymes and coenzymes, diphosphothiamine, and pyridoxal phosphate.

The well-known types of phosphorus compounds in blood are grouped as phospholipids and acid soluble phosphorus compounds, the latter group being subdivided into inorganic, ester, and nucleotide phosphorus compounds.

Since phosphorus functions in so many vital processes of the animal, its accurate determination in biological material and factors affecting its determination are of particular importance.

REVIEW OF LITERATURE

Apparently normal levels of inorganic phosphorus in the blood of cattle cover a wide range and depend upon such factors as phosphorus intake, age, sex of the animal, environmental temperature, and exercise.

Age

Green and Macaskill (1928), Allcroft and Godden (1934), Johnson (1939), Van Landingham, Henderson, and Bowling (1942), MacVicar, Long, and Ross (1949), Rusoff, Frye, and Scott (1951), McSherry and Gringer (1954) observed that the plasma inorganic phosphorus varies with the age of the animal. There is an increase in plasma inorganic phosphorus from the time of birth until the age of 5 or 6 months. It then decreases until the normal range for mature cattle is reached (about 5.8 mg. percent).

Payne et al. (1946) reported that the normal levels for blood serum inorganic phosphorus, in mg. percent, were 7.30, 4.76, 5.07, and 4.89 for Hereford yearling bulls, herd bulls, 2-year old heifers, and aged cows, respectively.

Haag and Jones (1935) found that the inorganic phosphorus content of the blood plasma of normal dairy cattle was 7.74, 7.68, 7.05, 6.48, and 5.20 mg. percent for dairy cattle at ages of six months, twelve months, eighteen months, twenty-four months, and older, respectively.

Palmer et al. (1930) observed that the inorganic phosphate of the blood of dairy calves increases from 5.97 mg. percent at birth to 7.68 mg. percent at 150 days, after which it decreases. This decrease continues

until the normal range for mature cattle is reached (about 5.8 mg. percent).

Temperature

The exact effect of exterior temperature on the inorganic phosphorus content of the blood of cattle is undetermined.

Rusoff et al. (1954) reported that the inorganic phosphorus of the blood of cattle was not affected by enviromental conditions. McCay (1931) found that the summer sunshine and pasture had no effect on the plasma inorganic phosphorus. Riek et al. (1948) observed that high temperature and humidity lowered the plasma inorganic phosphorus.

Phosphorus Intake

Palmer et al. (1930) observed a significant rise in plasma inorganic phosphorus three-fourths of an hour after feeding in six of ten trials, a significant fall in one trial, with no significant change in the other three trials. In one of the latter trials, a significant rise had occurred by the end of one and three-fourths hours, and in another there was a significant fall which was not manifested until the end of two and three-fourths hours. There were not enough significant changes at the end of one and three-fourths hours to indicate any single physiological effect. At the end of two and three-fourths hours, however, a significant fall had occurred in eight of the ten trials in comparison with the plasma inorganic phosphorus the previous hour. Therefore the mean magnitude of significant change in blood phosphate at the end of the three-fourths hour interval over and above the maximum analytical difference amounted to 0.363 mg. or 5.7 percent of the mean plasma inorganic phosphorus in these cases. This is not a great effect but is sufficient to warrant consideration in determining a procedure

for taking blood samples from cattle.

Van Landingham et al. (1935) found that an average daily intake of 3.8 gm. of phosphorus per 100 lb. of body weight was sufficient to maintain normal inorganic phosphorus in the plasma of growing dairy animals 6 to 25 months of age, and that 1.3 gm. was not sufficient.

Henderson and Weakly (1930) fed dairy heifers rations varying in phosphorus content from 0.131 to 0.298 gm. per 100 gm. of ration. They found that rations containing less than 0.20 percent phosphorus would decrease the amount of inorganic phosphorus of the plasma. Changes in the dietary levels of phosphorus could be detected by changes in plasma inorganic phosphorus within one week.

Beeson et al. (1938) observed that a definite phosphorus deficiency was produced in fattening beef steers by feeding a ration containing 0.12 percent phosphorus. The plasma inorganic phosphorus level dropped from 6.71 to 4.40 mg. percent. Outward symptoms of aphosphorosis were manifested by poor conditions, chewing boards, and eating dirt. The addition of 0.66 percent steamed bone meal to the ration increased daily gains from 1.37 to 1.90 lb., caused a 25.3 percent reduction in feed required per 100 lb. of gain, and resulted in maintaining a normal (about 6.50 mg. percent) plasma phosphorus. The authors concluded that the beef steer requires about 2.0 gm. of phosphorus daily per 100 lb. of live weight for normal growth and fattening.

Beeson et al. (1941) observed that steers receiving in excess of 2.0 gm. of phosphorus per 100 lb. of live weight daily, did not make any more rapid or efficient gains than those receiving a 2.0 gm. daily intake. However, steers that received 1.63 gm. daily or less of phosphorus per 100 lb. liveweight failed to make good gains or to make

efficient utilization of their feed.

Black et al. (1943) found that range cattle weighing 600 lb. and given 6.5 gm. of phosphorus by hand dosing six times a week as bonemeal or disodium phosphate did not develope phosphorus deficiency.

Haig (1935), Wallis, Palmer, and Gullickson (1935), Van Landingham, Henderson, and Bowling (1935), Riddell, Atkeson, Peterson, and Thompson (1939), Knox, Benner, and Watkins (1941), Rusoff and Piercy (1946), Watkins and Knox (1948), Reid, Ward, and Salsbury (1948) also found that the plasma inorganic phosphorus varies directly with the phosphorus intake.

Exercise

Sherman (1947) stated that a very important function of the phosphate ions in the blood is to enter into combination with the organic substances which are undergoing metabolism. Some of these phosphorylations represent a brief functioning of the phosphorus as a constituent of an intermediate compound in the metabolism of the organic substance, while in other cases organic phosphorus compounds formed in the body form organic nutrients and blood phosphate may be built into structural material. The formation of phosphoric acid esters of glucose and some of its metabolic intermediates are typical of phosphorylations from which the phosphorus soon returns to the condition of blood phosphate. On the other hand, the compounds derived in part from the soluble phosphate of the blood serum which become characteristic structural constituents may be illustrated by the nucleic acids of cell nuclei. When, however, a fat or fatty acid is phosphorylated by soluble phosphate of blood, lymph, or cell fluid, the phospholipid formed may function in both ways; part of it as an intermediate in fat metabolism,

and part of it as a very important constituent of cell structure and particularly of cell membranes. Thus the soluble phosphates of the blood, lymph, and tissue fluids, in addition to their function in the buffering and removal of fixed acids, are in dynamic interrelationship, both with the mineral matter of the skeletal system and with the organic compounds of the soft tissues and the phosphorylized intermediates and catalysts of the active processes of the energy metabolism.

Harvard and Reay (1925) noted marked periodic fluctuations of plasma inorganic phosphorus of humans. They reported that the concentration of plasma inorganic phosphorus is very unsteady in human blood studied at hourly intervals when the subject is not kept completely at rest. Ordinary movements of the subject about the laboratory cause large variations. They believe that these changes are the result of exercise which they later showed causes first a small rise and then a rapid fall in blood phosphorus. In these studies the lowest level was reached in about three-fourths of an hour after the period of exercise.

Palmer (1930) showed that there was a marked increase of the inorganic phosphorus content of the blood of cattle just after exercise, followed by a still more marked decrease a half-hour later, and in most cases a still further, but less marked decline after another half-hour. In most of the animals tested a minimum value was reached at the end of two hours.

Viewed from a percentage standpoint the mean significant changes, found by Palmer (1930), are as follows. Immediately after exercise there was a rise of 6.34 percent compared with the value before exercise. One-half hour later there was a drop of 22 percent compared with the value immediately after exercise. After another half-hour there was a

further drop of 4.9 percent, using the first half-hour value as a basis.

Anticoagulant

According to Hawk (1954) the anticoagulants in common use and the amount usually recommended per ml. of blood are potassium, lithium and sodium oxalate (1 to 2 mg.), sodium citrate (5 mg.), sodium fluoride (10 mg.), and heparin (0.2 mg.). Only an amount of anticoagulant sufficient for the quantity of blood to be received should be employed; excessive amounts of anticoagulant may interfere with some analyses, cause hemolysis, or produce an abnormal distribution of water and electrolytes between cells and plasma. However, this is not true of heparin. Of the various anticoagulants, heparin is by far the most satisfactory and should be more widely used.

Heller and Paul (1934) found that the ammonium salts produced an increase in cell volume with an increase in the percentage of anticoagulant used, while sodium, potassium, and lithium salts produced a cell-volume decrease with percentage increase of salts used. Various determinations indicated that about 0.2 percent of the oxalates and 0.4 percent of the citrates produced the most favorable results from the standpoints of anticoagulation and hemolysis. Since ammonium salts produced an increase in cell volume and the other salts had an opposite effect, various mixtures were tested, and it was found that with the use of a 0.2 percent concentration of a mixture of 40 percent potassium oxalate and 60 percent ammonium oxalate there was a minimum change in cell volume. When heparin was used as an anticoagulant in the analysis for inorganic constituents of blood, results were less favorable than when inorganic anticoagulants were used.

Time Interval Between Collection and Analysis

Palmer et al. (1930) reported in their experiments that the blood used in the analyses was drawn from the jugular vein, and allowed to flow into 100 ml. glass tubes, containing 1 ml. of a saturated solution of sodium citrate. The size of sample drawn was 50 ml. The blood was mixed thoroughly with the sodium citrate solution immediately to prevent coagulation. It was then centrifuged to separate the plasma from the corpuscles. Upon completion of the centrifugation, sufficient plasma for the test was pipetted off, and preserved in an ice box until tested.

Rusoff et al. (1951) reported that citrated samples were drawn from the animals in the mornings at Jeanerette, La., refrigerated and shipped in ice to Louisiana State University in Baton Rouge, where the samples were analyzed for plasma inorganic phosphorus and other components.

Watkins and Knox (1948) reported in their experiment that blood samples were centrifuged on the range, placed on ice, and without delay taken to the laboratory for analysis of the plasma inorganic phosphorus.

It was not reported, in any of the literature reviewed for this thesis, that an experiment had been conducted on the affect of the time interval between collection and analysis on the plasma inorganic phosphorus content of blood.

EXPERIMENTAL

The following experiments were conducted in an investigation of some of the factors that might affect the results of the analysis of cattle blood for inorganic phosphorus content of plasma.

The phosphorus determinations in all of the experiments were conducted as follows: Blood samples were taken from the jugular vein of each animal used in the experiments. The samples were collected in heavy-wall pyrex test tubes containing either heparin or lithium citrate as an anticoagulant. For every 30 ml. of blood 1.5 ml. of lithium citrate solution containing 0.1 gm. per ml. was used. The 1.5 ml. of lithium citrate solution was evaporated to dryness on a hot plate, leaving a fluffy residue that dissolved immediately when the blood came in contact with it. The concentration of heparin solution, used as an anticoagulant, was 1 mg. per ml. Two drops of the heparin solution were placed in each tube used for the collection of blood.

The blood samples were chilled and brought to the laboratory for analysis. After centrifugation at 2000 r.p.m. for 30 minutes in a centrifuge with a 16 inch head, the clear plasma was analysed for inorganic phosphorus by a modification of the Fiske and Subbarow method (1925). The plasma proteins were precipitated by pipetting 0.5 ml. of clear plasma into 9.5 ml. of a 10 percent solution of trichloroacetic acid. The precipitated proteins were spun down (1500 r.p.m. for 15 minutes), and a 5 ml. aliqout of the clear supernatant liquid was placed in a colorimeter tube. One ml. of molybdate II reagent, made by dissolving 25 gm. of ammonium molybdate in 300 ml. of 10 N sulfuric acid and diluting to a liter with water, was added to form phosphomolybdic acid. This compound

was then reduced with 0.4 ml. of aminonaphtholsulfonic acid solution. This latter reagent contains 0.25 gm. of 1-amino-2-naphthol-4-sulfonic acid, 0.5 gm. of sodium sulfite and 14.25 gm. of sodium bisulfite in 100 ml. of water. Reduction of the phosphomolybdic acid with the amino-naphthol-sulfonic acid reagent produces a blue color. The volume in the colorimeter tube was made to 10 ml. with water, and after standing 20 minutes the color intensity was determined with an Evelyn colorimeter at a wave length of 660 mμ. The concentration of phosphorus in the tube was determined from a standard curve. In this method a dilution factor of 400 was necessary to convert the micrograms of phosphorus in the tube to milligrams phosphorus per 100 ml. of plasma.

All of the results of the following experiments were statistically analyzed according to methods described by Snedecor (1955).

Hand-feeding versus Self-feeding of Animals

Experiment I. Procedure. Fifteen grade Hereford steers were used to determine the effect of hand-feeding as compared to self-feeding on hourly variations of the inorganic phosphorus content of the blood plasma of cattle. In hand-feeding the animals receive a constant amount of feed twice daily. In self-feeding the animals have feed before them at all times during the day.

The steers were divided into two groups, and two experiments conducted on each group. Group I, the hand-fed group, was composed of seven grade Hereford steers which weighed from 402 to 486 pounds. Of these, three were hand-fed 3 pounds of a ration containing 0.12 percent phosphorus twice a day. The other four were hand-fed 4.1 pounds of a ration containing 0.16 percent phosphorus twice a day. The animals were fed their ration at 7:00 a.m. and 7:00 p.m. Group II, the self-fed group, was composed of

eight grade Hereford steers which weighed from 565 to 680 pounds. This group was self-fed a ration containing 0.15 percent phosphorus. The feed was placed before the animals at 7:00 a.m. and removed at 7:00 p.m.

The blood samples were taken from the juglar vein of each of the animals of both groups at 6:30, 8:30, 10:30, 2:30, and 6:30 as indicated in Table I. All of the trials of this experiment were conducted on different days. Trials 1 and 2 were conducted with the hand-fed group on two different days. Trials 3 and 4 were conducted with the self-fed group on two different days after completing Trials 1 and 2.

Experiment I. Results. The average results obtained in Trials 1 through 4 of this experiment are shown in Table I.

TABLE I

AVERAGE INORGANIC PHOSPHORUS CONTENT OF THE PLASMA OF HAND-FED AND SELF-FED STEERS

(mg. per 100 ml.)

Group	No. of steers	Trial	Time of Day 6:30am	8:30am	10:30am	2:30pm	6:30pm
I (hand-fed)	7	1	4.27	4.13	3.96	3.90	3.78
		2	4.58	4.23	4.05	3.87	3.78
		Average	4.42	4.18	4.01	3.89	3.78
II (self-fed)	8	3	3.61	3.26	3.11	3.17	2.92
		4	3.96	3.51	3.38	3.63	3.67
		Average	3.79	3.39	3.25	3.40	3.30

In both Trials 1 and 2, there was a general decrease in plasma inorganic phosphorus between the hours of 6:30 a.m. and 6:30 p.m. Because of individual variations, the difference between the intitial and final values was not statistically significant. Individual values

are shown in the Appendix, Table X.

In both Trials 3 and 4, the general decrease in plasma inorganic phosphorus that was observed in Trials 1 and 2 didn't occur. Individual values are shown in the Appendix, Table X.

There was a tendency for the plasma inorganic phosphorus of the self-fed group to fluctuate more than that of the hand-fed group. This fluctuation was probably the result of variable phosphorus intake during the day. Palmer (1930) reported that at 45 minutes after feeding, plasma inorganic phosphorus was increased to a maximum, and at 2 hours and 45 minutes after feeding, it was decreased to a minimum.

Experiment II. Procedure. The same fifteen grade Hereford steers that were used in Experiment I were used in Experiment II to determine the effect of hand-feeding as compared to self-feeding on the day to day variations in plasma inorganic phosphorus.

The animals were fed in the same manner as described in Experiment I, Group I being hand-fed and Group II self-fed. They were bled every morning at 6:30 a.m. immediately before feeding for five days.

Experiment II. Results. Average results obtained in this experiment are shown in Table II.

TABLE II

AVERAGE INORGANIC PHOSPHORUS CONTENT OF THE PLASMA OF HAND-FED AND SELF-FED STEERS

(mg. per 100 ml.)

Group	No. of steers	Days 1	2	3	4	5
I (hand-fed)	7	4.54	4.57	4.33[1]	4.50	4.58
II (self-fed)	8	3.78	4.01	4.07	4.07	4.96

[1]The heating system was out-of-order for several hours on the night before the blood was taken for this analysis. There was about a 20° drop in temperature in the pens which might account for this low plasma inorganic phosphorus value. Since this experiment was conducted in two different weeks Group II was not affected by this temperature drop.

A statistical analysis of these results showed that there was no significant change in the plasma inorganic phosphorus of either group from day to day. However, as in Experiment I, there was more fluctuation of the plasma inorganic phosphorus in the self-fed group than in the hand-fed group. Individual values are given in the Appendix Table XI.

Frequency of Bleeding the Animals

Procedure. Two trials were conducted with six grade Hereford steers, which weighed from 380 to 557 pounds, to determine the effect of frequent bleeding on the inorganic phosphorus content of the blood plasma. In Trial 1 the steers were hand-fed 10 pounds of a ration containing 0.15 percent phosphorus per day. They were fed this ration in two equal portions at 6:30 a.m. and 6:30 p.m.

The experiment was started at 6:00 a.m. Three of the steers were used as an experimental group and the other three as a control group. The animals of the two groups were matched as evenly as possible according to weight. The experimental group was bled seven times in a period of twelve hours. The control group was bled three times in the same twelve hour period. This experiment was conducted twice.

In Trial 2 the same procedure was followed as in Trial 1. The two trials were conducted one week apart.

Results. Average results obtained in each of the two trials are shown in Table III.

TABLE III

AVERAGE PLASMA INORGANIC PHOSPHORUS AS AFFECTED BY FREQUENCY OF BLEEDING

(mg. per 100 ml.)

Group	Trial	Animal No.	Time 6:00am	8:00am	10:00am	12:00am	2:00pm	4:00 pm	6:00pm
Experimental	1	00	7.20	7.14	6.46	7.04	6.36	6.28	5.68
"	"	55	6.40	6.76	6.88	6.48	5.92	5.56	5.54
"	"	88	5.66	6.78	6.00	5.58	5.58	5.82	4.86
"	"	Ave.	6.42	6.89[2]	6.45	6.37	5.95	5.89	5.36
Control	"	0	6.58			5.88			6.06
"	"	5	5.82			5.44			5.16
"	"	8	7.22			6.70			5.80
"	"	Ave.	6.54			6.01			5.67
Experimental	2	00	7.74	7.90	7.04	6.20	6.78	6.98	5.80
"	"	55	6.22	6.64	6.38	5.28	5.52	5.78	6.06
"	"	88	5.34	5.64	4.54	3.94	4.14	4.70	4.40
"	"	Ave.	6.43	6.73[2]	5.99	5.14	5.48	5.82	5.09
Control	"	0	5.88			5.80			5.84
"	"	5	5.68			5.08			4.64
"	"	8	7.52			6.90			6.58
"	"	Ave.	6.36			5.92			5.69

[2]At eight o'clock in both trials of this experiment there was an increase in the average plasma inorganic phosphorus of the experimental group. This increase two hours after feeding was also observed by Palmer (1930).

A statistical analysis of these results showed that there was no signficant difference in the plasma inorganic phosphorus of the experimental group as compared to the control group over the twelve hour period. However, the results in Table III show that there were decreases over a twelve hour period of 1.08 and 1.34 mg. percent for the experimental group and 0.87 and 0.67 mg. percent for the control group.

It was the large variation of the plasma inorganic phosphorus of the individual animal that caused the results of this experiment to be statistically insignificant.

Exercise of Animals

Procedure. Six grade Hereford steers, ranging in weight from 380 to

557 pounds, were used to determine the effect of exercise on plasma inorganic phosphorus. The steers were hand-fed 10 pounds of a ration containing 0.16 percent phosphorus per day. The feed was given in two equal portions twice daily, at 6:30 a.m. and 6:30 p.m.

Three of the steers picked at random served as an experimental group and three as a control group. Because of earlier results the steers in both groups were bled initially at 2:20 p.m., eight hours after feeding. The experimental group was then vigorously exercised in the arena for 5 minutes. During this period the steers were driven around the arena by men on horseback. They were then brought back to their pen, adjacent to the arena, and bled at 2:30 p.m. This exercise treatment was then repeated and the animals bled at 2:40 p.m. During these periods the control group was kept in their pen and blood samples were taken at the same time intervals. Both groups were kept in their pens and fed at 6:30 p.m. Blood samples were taken at 6:00 p.m. and again at 6:00 a.m. the next morning.

Results. The results of this experiment are shown in Table IV.

TABLE IV

PLASMA INORGANIC PHOSPHORUS CONTENT OF EXERCISED AND CONTROL STEERS

(mg. per 100 ml.)

Group	Animal No.	Time 2:20pm	2:30pm	2:40pm	6:00pm	6:00am
Exercised[3]	00	9.00	9.58	8.96	7.40	8.28
"	5	8.44	9.18	8.36	6.72	6.94
"	88	7.94	7.40	7.36	5.32	5.12
"	Ave.	8.46	8.72	8.23	6.48	6.78
Control	0	9.06	9.68	9.78	8.96	9.24
"	55	8.60	8.34	7.96	6.68	8.32
"	8	7.20	7.56	7.40	7.70	8.06
"	Ave.	8.29	8.53	8.38	7.78	8.54

[3]Exercised for 5 minutes between 2:20 and 2:30 p.m. and between 2:30 and 2:40 p.m.

A statistical analysis of the results showed that there was a significant change in plasma inorganic phosphorus of the exercised steers between 2:40 and 6:00 p.m. (8.23 to 6.48 mg. percent). The control steers showed a drop at this same time, but it was not a significant drop (8.38 to 7.78 mg. percent). The results also showed that the plasma inorganic phosphorus of the control steers was at a normal level the next morning. The plasma inorganic phosphorus of the exercised steers was not. It may be concluded from this that the exercise caused a drop in plasma inorganic phosphorus from 8.23 mg. percent at 2:40 p.m. to 6.48 mg. percent at 6:00 p.m. The experiment also showed that the animals had not returned to their normal plasma inorganic phosphorus level (about 8.40 mg. percent) 15 hours after exercise.

Time Interval Between Collection and Analysis of Blood

Procedure. Blood from seven steers was used to determine the effect on plasma inorganic phosphorus of allowing blood to stand for several hours after collection.

The samples were collected in the usual manner, chilled by placing in a refrigerator at 2-5°C for about 20 minutes, centrifuged and replaced in the refrigerator. Three hours after collection the samples were taken out of the refrigerator, allowed to come to room temperature, analyzed in duplicate, and then replaced in the refrigerator. This procedure was repeated at 6, 30, 50, and 100 hours after the samples had been collected.

Results. The results obtained in this experiment are shown in Table V.

TABLE V

PLASMA INORGANIC PHOSPHORUS AS AFFECTED BY THE TIME INTERVAL BETWEEN COLLECTION AND ANALYSIS

(mg. per 100 ml.)

Animal No.	Time after collection of samples 3 hr.	6 hr.	30 hr.	50 hr.	100 hr.
156	5.12	5.00	5.12	4.88	5.04
"	4.92	4.88	4.92	4.88	5.00
122	4.12	4.08	4.32	4.12	4.36
"	4.08	3.92	4.24	4.24	4.28
136	3.04	3.04	3.24	3.12	3.12
"	2.92	2.98	3.16	3.04	3.28
120	4.92	4.96	4.84	4.76	5.00
"	4.76	4.84	4.80	4.72	4.88
68	4.92	5.12	5.20	5.20	5.20
"	5.00	4.92	5.20	5.28	5.28
199	5.64	5.56	5.72	5.64	5.64
"	5.44	5.32	5.48	5.44	5.40
140	4.44	4.44	4.24	4.08	4.24
"	4.24	4.20	4.24	4.04	4.04
Total	63.56	63.26	64.72	63.44	64.76
Ave.	4.54	4.52	4.62	4.53	4.62

A statistical analysis of the results showed that allowing the centrifuged blood to stand for as long as 100 hours had no significant effect on the inorganic phosphorus content of the plasma. The initial analysis gave an average plasma inorganic phosphorus value of 4.54 mg. percent and final value of 4.62 mg. percent. The statistical analysis also showed that the standard deviation of the duplicates was 0.06 mg. percent. Therefore it may be concluded that if the samples are collected, chilled, centrifuged, and placed in a refrigerator the time interval before analysis does not have any effect on the results.

Anticoagulant

The purpose of these experiments was to determine if the concentration of the lithium citrate used as an anticoagulant has any effect on the

plasma inorganic phosphorus of the blood.

Experiment I. Procedure. Ten grade Hereford steers picked at random were used as a source of blood in Experiment I. Two blood samples (10 ml. ea.) were collected from each steer in graduated centrifuge tubes containing lithium citrate and heparin. The samples were centrifuged at 2000 r.p.m. for 30 minutes, and then placed in a refrigerator until analyzed 16 hours later.

The concentrations of the lithium citrate and heparin solutions used were 0.1 gm. per ml. and 1 mg. per ml., respectively. One ml. of lithium citrate was used as anticoagulant in one tube and two drops (approx. 0.1 ml.) of heparin were added to the other tube. The lithium citrate in the sample tubes was evaporated to dryness on a hot plate before the blood samples were collected.

Experiment I. Results. The results obtained in Experiment I are given in Table VI.

TABLE VI

PLASMA INORGANIC PHOSPHORUS AS AFFECTED BY ANTICOAGULANT

(mg. per 100 ml.)

Animal No.	Anticoagulant	
	Lithum Citrate	Heparin
1	6.04	6.72
2	7.02	7.88
3	6.84	7.54
4	5.76	6.82
5	5.30	6.38
6	9.24	10.40
7	8.28	8.32
8	6.94	7.92
9	8.32	9.28
10	8.06	8.82
Total	71.80	80.08
Ave.	7.18	8.01

As shown in Table VI higher values for inorganic phosphorus content of blood plasma were obtained when heparin was used as an anticoagulant. For example the plasma inorganic phosphorus for animal no. 4 was 6.82 mg. percent when heparin was the anticoagulant and 5.76 when lithium citrate was the anticoagulant. A statistical analysis of the results showed this difference to be significant.

Experiment II. Procedure. Eight sheep, picked at random, served as a source of blood for this experiment. Four graduated centrifuge tubes were used to collect the blood from each animal. Two drops of heparin solution (1 mg. per ml.) were added to one tube, 1.0, 0.4, and 0.2 ml. of lithium citrate solution (0.1 gm. per ml.) were added to the other three tubes. The lithium citrate in the sample tubes was evaporated to dryness on a hot plate before the blood samples were collected. Ten ml. of blood was collected in each tube. The samples were centrifuged and placed in a refrigerator until analyzed 16 hours later.

Experiment II. Results. The results of Experiment II are given in Table VII.

TABLE VII

PLASMA INORGANIC PHOSPHORUS AS AFFECTED BY ANTICOAGULANT

(mg. per 100 ml.)

Animal No.	Heparin	Lithium Citrate 1.0 ml.	0.4 ml.	0.2 ml.
1	5.40	——[4]	4.88	5.24
2	6.28	5.04	5.96	6.12
3	5.96	5.20	5.64[5]	5.68
4	5.40	5.04	——[5]	5.40
5	5.12	4.56	5.00	5.12
6	6.68	6.12	6.60	——[5]
7	5.52	4.68	——[5]	——[5]
8	5.48	5.24	5.40	5.44

[4]Tube broke in centrifuge and the sample was lost.

[5]The plasma clotted in these samples.

In the samples with the lower concentration of citrate there was a large amount of plasma clotting. A low concentration of citrate allows calcium ions to be present which activates the formation of thrombin from prothrombin. Fibrinogen in the presence of thrombin forms fibrin.

As shown in Table VII, the highest values for inorganic phosphorus content of blood plasma were obtained when heparin was used as an anticoagulant. The lowest values were obtained when 1 ml. of lithium citrate equivalent to 0.1 gm. per 10 ml. of blood was used as the anticoagulant. As the amount of lithium citrate was decreased, the values for plasma inorganic phosphorus were increased. For example, the plasma inorganic phosphorus for animal no. 2 was 6.28 mg. percent when heparin was the anticoagulant and 5.04, 5.96, and 6.12 when lithium citrate in amounts equivalent to 0.1, 0.04, and 0.02 gm. per 10 ml. of blood, respectively, was the anticoagulant.

A statistical analysis of the results showed that only the differences between the values obtained with heparin and 1 ml. of lithium citrate were significant. These results confirm those in Experiment I.

Experiment III. Procedure. The purpose of this experiment was to observe if the anticoagulant caused a difference in the volume of packed red cells. Ten sheep, picked at random, served as a source of blood for this experiment. Two tubes were used to collect the blood from each animal. In one tube two drops of heparin solution (1 mg. per ml.) were added and in the other 1 ml. of lithium citrate solution (0.1 gm. per ml.). Graduated centrifuge tubes were used for collecting the samples. The

lithium citrate in the sample tubes was evaporated to dryness before the blood samples were taken. Ten ml. of blood were collected in each tube. A small amount of this sample was used for determining the volume of packed red cells. The volume of packed red cells was determined by centrifuging the sample at 2000 r.p.m. for 30 minutes in Wintrobe tubes. The sample remaining in the centrifuge tubes was then centrifuged and placed in a refrigerator until analyzed 16 hours later.

Experiment III. Results. Values obtained for plasma inorganic phosphorus and volume of packed red cells are shown in Table VIII.

TABLE VIII

PACKED RED CELLS (percent) AND PLASMA INORGANIC PHOSPHORUS (mg. per 100 ml.) AS AFFECTED BY ANTICOAGULANT

Animal No.	Phosphorus		Packed red cells	
	Heparin	Lithium Citrate	Heparin	Lithium Citrate
1	6.08	4.96	42	33
2	5.76	5.16	43	39
3	5.20	4.92	40	33
4	6.28	5.48	45	38
5	5.48	4.68	48	42
6	5.00	4.28	46	39
7	5.08	4.72	47	41
8	4.32	3.96	46	41
9	4.80	4.36	42	39
10	4.40	3.64	47	41
Total	52.40	46.16		
Ave.	5.24	4.62		

As in the first two experiments significantly lower results were obtained for plasma inorganic phosphorus when lithium citrate was used as anticoagulant than when heparin was used. It was also found that there was a significant lowering of the volume of packed red cells by lithium

citrate.

Hawk (1947) stated that it is important to remember that unless special precautions are taken, the anticoagulant may significantly alter the distribution of diffusible substances between cells and plasma. Heparin is an excellent anticoagulant in this respect, since it does not have this drawback. Heparin inhibits coagulation in three ways: (1) In conjunction with a co-factor found in serum albumin, it is a powerful antithrombin. (2) It prevents the conversion of prothrombin to thrombin (a co-factor of the plasma again is required). (3) It inhibits the agglutination of platelets and prevents this disintegration.

Therefore from this experiment it may be concluded that lithium citrate at a concentration of 0.1 gm. per 10 ml. of blood will cause a significant lowering of the plasma inorganic phosphorus as well as the volume of packed red cells. The decrease of the volume of packed red cells in turn causes an increase in plasma which might account for the decrease in plasma inorganic phosphorus.

SUMMARY

In these experiments an attempt was made to determine, (1) the effect of hand feeding versus self-feeding, (2) frequent bleeding, (3) exercise, (4) time-interval after bleeding, and (5) concentration of lithium citrate on the final results of the determination of the plasma inorganic phosphorus content of the blood of cattle.

It was found that there was no significant difference in plasma inorganic phosphorus between a hand-fed group and a self-fed group. However, there was a tendency for the self-fed group to show considerable more fluctuation of plasma inorganic phosphorus from hour to hour and day to day.

Frequent bleeding caused a trend toward a decreasing plasma inorganic phosphorus content over a twelve-hour period. This effect of frequent bleeding may be the result, in part, of excitement and activity of the animals.

There was a significant drop of plasma inorganic phosphorus due to exercise.

The amount of time the blood was stored in a refrigerator after being collected and centrifuged had no significant effect on the plasma inorganic phosphorus.

Lithium citrate was found to have several effects on blood depending on the amount used. It was found that 0.1 gm. of lithium citrate per 10 ml. of blood caused some hemolysis, a significant decrease in plasma

inorganic phosphorus, and a decrease in the volume of packed red cells. Lithium citrate concentrations of 0.04 and 0.02 gm. per 10 ml. blood allowed the plasma of the samples to clot upon standing in the refrigerator.

Although some of these results are not statistically significant, the factors brought forth in this thesis should be considered when making a determination of plasma inorganic phosphorus in the blood of cattle.

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APPENDIX

TABLE IX

PERCENTAGE COMPOSITION OF EXPERIMENTAL RATIONS

Ingredients	Percent Phosphorus of Rations 0.12%	0.15%	0.16%
Cerelose	22.10	22.10	22.10
Cottonseed hulls	37.05	36.67	37.05
Dried beet pulp	27.10	27.10	27.10
Dehydrated alfalfa meal	9.10	9.10	9.10
Corn gluten meal	3.70	3.70	3.70
Urea	.35	.35	.35
A and D supplement[1]	.10	.10	.10
Salt	.50	---[4]	.50
Calcium carbonate[2]	.47	.56	.95
Reagent dicalcium phosphate[3]	.18	.32	.36
P from supplement	0.14	0.07	0.08
P from basal	0.08	0.08	0.08

[1]Contributed vitamin A, 2724 I.U. and vitamin D, 340 I.U., per 1 lb. of total ration.

[2]Calcium carbonate 38.84 percent calcium (1955 analysis).

[3]Reagent dicalcium phosphate 22.25 percent phosphorus (1957 analysis).

[4]Salt was fed free choice.

TABLE X

HOURLY PLASMA INORGANIC PHOSPHORUS VALUES OF THE HAND-FED AND SELF-FED ANIMALS

Group	No. of steers	Animal no.	Trial	6:30am	8:30am	10:30am	2:30pm	6:30pm
I (hand-fed)	7	156	1	5.14	4.92	4.66	4.60	4.52
		122		4.48	3.78	3.64	3.80	3.64
		136		3.34	3.32	2.86	2.92	2.26
		120		4.22	4.42	4.32	4.00	3.72
		68		4.38	4.32	3.96	3.54	3.76
		199		4.52	4.28	4.40	4.60	4.50
		140		3.80	3.84	3.88	3.84	3.70
		Average		4.27	4.13	3.96	3.90	3.78
I (hand-fed)	7	156	2	5.20	4.70	4.32	4.28	4.00
		122		4.08	3.74	3.44	3.38	3.44
		136		3.16	3.02	2.54	2.52	2.52
		120		4.68	4.24	4.62	4.18	4.14
		68		5.12	4.84	4.60	4.30	4.32
		199		5.14	4.58	4.72	4.24	4.20
		140		4.66	4.48	4.14	4.16	3.84
		Average		4.58	4.23	4.05	3.87	3.78
II (self-fed)	8	97	3	3.66	3.64	2.92	3.68	3.22
		118		3.32	2.74	2.78	2.84	2.68
		186		3.08	2.64	2.62	2.36	2.22
		160		3.72	3.52	3.28	3.48	3.36
		114		3.72	3.50	3.04	3.16	2.92
		0		4.14	3.54	3.88	3.94	3.54
		185		3.06	2.38	2.26	2.22	2.04
		158		4.16	4.10	4.10	3.66	3.38
		Average		3.61	3.26	3.11	3.17	2.92

TABLE X (continued)

Group	No. of steers	Animal no.	Trial	6:30am	8:30am	10:30am	2:30pm	6:30pm
II (self-fed)	8	97	4	4.06	3.64	2.86	3.76	3.68
		118		4.10	3.48	3.26	3.54	3.58
		186		3.24	2.66	2.40	2.78	3.02
		160		4.24	3.90	4.04	4.08	4.14
		114		4.02	4.16	4.00	4.28	3.90
		0		4.46	3.76	3.72	3.70	3.82
		185		3.26	2.64	2.40	3.18	3.04
		158		4.28	4.22	4.32	4.20	4.20
		Average		3.96	3.51	3.38	3.63	3.67

TABLE XI

DAILY PLASMA INORGANIC PHOSPHORUS VALUES OF THE HAND-FED AND SELF-FED ANIMALS

Group	No. of steers	Animal no.	Days 1	2	3	4	5
I (hand-fed)	7	156	5.02	5.14	4.94	5.20	5.20
		122	4.10	4.18	3.68	4.02	4.08
		136	2.98	3.08	2.92	3.52	3.16
		120	4.84	4.64	4.42	4.42	4.68
		68	4.96	5.49	4.86	4.94	5.12
		199	5.54	5.24	4.78	4.96	5.14
		140	4.34	4.24	4.54	4.64	4.66
		Average	4.54	4.57	4.33	4.50	4.58
II (self-fed)	8	97	3.96	4.16	4.72	4.00	4.06
		118	3.32	3.56	4.16	3.64	4.10
		186	3.14	3.04	2.54	2.80	3.24
		160	4.22	4.54	3.92	4.06	4.24
		114	4.28	4.54	4.16	4.96	4.02
		0	3.82	4.50	4.82	4.60	4.46
		185	3.20	3.16	3.34	3.18	3.26
		158	4.30	4.60	4.90	4.54	4.28
		Average	3.78	4.01	4.07	3.97	3.96

VITA

Darle L. Nieneker

Candidate for the Degree of

Master of Science

Thesis: SOME FACTORS AFFECTING PLASMA INORGANIC PHOSPHORUS AND ITS DETERMINATION IN THE BLOOD OF BEEF CATTLE

Major Field: Chemistry

Biographical:

Personal data: Born in Allegan, Michigan, January 26, 1934, the son of Marinus and Lena Nieneker.

Education: Attended grade school in Burnips, Michigan; graduated from Hudsonville High School, Hudsonville, Michigan, in May 1952; received the Bachelor of Arts degree from Hope College, Holland, Michigan, with a major in Chemistry in June, 1956; graduate study at Oklahoma State University 1956-58.

Member of Phi Sigma and Phi Lambda Upsilon.

Date of Final Examination: May, 1958.