This is to certify that the

thesis entitled

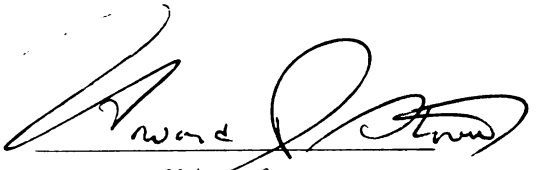
Effects of Sampling and Specimen Treatment on Element Concentrations in Bovine Liver and Serum

presented by

Michael Ross Slanker

has been accepted towards fulfillment
of the requirements for

M.S. degree in Large Animal Clinical Sciences



Major professor

Date 6/7/85

Howard Stowe

EFFECTS OF SAMPLING AND SPECIMEN TREATMENT ON ELEMENT CONCENTRATIONS IN BOVINE LIVER AND SERUM

By

Michael Ross Slanker

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Large Animal Clinical Sciences

1985

ABSTRACT

EFFECTS OF SAMPLING AND SPECIMEN TREATMENT ON ELEMENT CONCENTRATIONS IN BOVINE LIVER AND SERUM

By

Michael R. Slanker

Effects of sample site, perfusion, lipid content and formalin fixation on element concentrations in the bovine liver and the effects of hemolysis and the serum-to-clot contact time on bovine serum element concentrations were determined by inductively coupled plasma-atomic emission spectrometry. Differences in hepatic concentrations of calcium, manganese, molybdenum, phosphorus, potassium and zinc were associated with sample site. Liver hemoglobin iron (perfusion) was negatively correlated with hepatic phosphorus and potassium. Liver lipid was negatively correlated with hepatic phosphorus. Formalin fixation altered hepatic concentrations of cadmium, calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium, sodium and zinc. Hemolysis decreased serum calcium, copper, magnesium and sodium and increased serum iron, phosphorus, potassium and zinc concentrations. Serum-to-clot contact time increased serum phosphorus and potassium concentrations. Some of the changes in element concentrations induced by sample handling procedures were large enough to alter the clinical interpretation of the element concentration data.

ACKNOWLEDGEMENTS

I am grateful to Dr. Stowe for accepting the responsibility of major professor in the middle of the program. His guidance and encouragement in the preparation of this thesis has been invaluable. A special thanks to Dr. Braselton for his help with the experimental design, use of the laboratory, advice with the analytical procedures and especially for his enthusiastic support, understanding and personal friendship. I would like to thank Dr. Trapp for his advice with the experimental design and his assistance with the tissue collection procedures.

The author's gratitude is extended to Dr. Keahey for the arrangements and support of this research. I would like to take this opportunity to express my appreciation to Dr. Kaneene for his help with the experimental design and statistical analyses.

I would like to thank Beth Martin for her technical assistance with the analytical procedures.

Thanks Eileen for your personal friendship and patience.

This research was supported in part by the Michigan State University Agricultural Experiment Station.

TABLE OF CONTENTS

Table of Contents (Continued)

LIST OF TABLES

TABLE Page

LIST OF FIGURES

FIGURE Page

INTRODUCTION

The utilization of elemental analysis has become increasingly important since the discovery that even elements which occur in the body in small amounts can have biological effects which are directly related to their concentration. Marginal to severe trace element imbalances can be considered risk factors for several diseases, but proof of cause and effect relationships depends on understanding the basic mechanisms of actions and an ability to determine and interpret a marginal element concentration.[1]

With the advent of neutron activation, atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry and other methods of analysis, detection capabilities for tissue element concentrations in parts per billion and parts per trillion are now common. These developments in instrumentation for single and multielement quantitation have provided an effective means of assessing tissue element concentrations in biopsies or necropsy samples.

Several factors must be considered in the process of interpreting tissue element concentration analysis results. The common factors of species, age, and sex of the animal are usually known and the effects of these factors on some element concentrations in tissues have been determined.[2] Tissue samples for analysis in our laboratory, usually comprise a small fraction of the organ or part of one of the lobed or paired organs. When these samples are received at the laboratory, only subjective evaluations from the gross appearance of the tissue are

possible. Sample site, sampling, and handling procedures cannot be determined and such information is rarely available in the accompanying clinical history. Therefore it is necessary to determine the effects of these variables in order to know if the data will be of value in determining the clinical diagnosis.

The use of inductively coupled argon plasma atomic emission spectrometry (ICP) at Michigan State University to determine the multi-element profile on serum and tissue for veterinary diagnostic purposes in the Animal Health Diagnostic Laboratory requires responsible interpretation. To this end, this research was conducted to establish the effects that sample site, residual perfusion, lipid content and formalin fixation have on element concentrations in the bovine liver. The effects of hemolysis and serum-to-clot contact time on the element concentrations in bovine serum were also determined.

LITERATURE REVIEW

Influences on Tissue Element Concentrations

Sample Site

Several researchers have determined the effects of sample site on tissue element concentrations. Kaldor[3] determined there was no statistically significant difference in the iron concentration in samples of rat liver taken from different locations within the liver. Frey et al.[4] reported that multiple samples from different locations of the same human liver contained 17-27 mg of total iron per 100 mg of tissue. Barry et al.[5] analyzed duplicate liver biopsy samples from 8 people in one study and 12 people in another study and detected a coefficient of variation for total iron of 8.6% and 7.1%, respectively. Cassidy et al.[6] reported significant differences in the concentrations of iron and copper between different sample sites in pig liver. Webb et al.[7,8,9] determined the variations in element concentrations for different anatomical sites of the pig, cow and dog heart. The concentration of aluminum, barium, cesium, copper, lead, manganese, molybdenum, strontium, and tin were determined. The anatomical sites of the heart were the aorta, main pulmonary artery, tricuspid valve, mitral valve, right and left coronary arteries, os cordis, right atrum, left atrial appendage, the free wall of both the right and left ventricles, left ventricle-papillary muscle, interventricular septum, crista supraventricularis and left bundle branch. While the array of trace metals in the individual tissues was found

consistently in all 3 species, the trace metals were unevenly distributed throughout the heart and blood vessels.

Perfusion

The amount of residual perfusion in a tissue is usually described by the terms blanched or pale, hyperemic, vascular engorgement and congestion. The great variation in the size of the adult bovine liver, often seen at necropsy, is largely due to the degree of residual perfusion.[10] Hepatic congestion is marked by severe organ enlargement, a greatly increased content of blood, and marked accentuation of the lobular pattern.[11] Variations in elemental concentrations may be two- to threefold, or more, if the blood content is not taken into account.[12] Kaldor[3] determined that at least 24% of the liver iron came from hemoglobin (perfusion) whereas hematin enzymes contributed a negligible quantity of liver iron. Frey et al.[4] dismissed perfusion as a problem in determining the liver iron content.

The intracellular concentration of sodium for bovine erythrocytes is 88 mM/liter of red blood cells whereas the plasma concentration is approximately 145 mM/liter. Comparing these values to the general interstitial fluid sodium concentration of 135 mM/liter and the tissue intracellular sodium concentration of 10 mM/liter,[13] it can be seen that increased perfusion may cause an increase in the tissue sodium concentration. Little information was found regarding the effects of perfusion on other element concentrations.

Lipid Content

Fatty infiltration of liver may be as significant as the amount of residual perfusion because displacement of the element storage tissue by

fat would be expected to cause a decrease in concentrations of stored tissue elements. Herdt et al.[14] determined that bovine liver fat content can range from less than 4% to at least 34%. When there is fatty infiltration in the liver, the fat is usually equally distributed throughout the organ and causes organ enlargement, light color, light weight and friability.[15]

Formalin Fixation

Element concentrations in solid tissues stored as frozen sections are not as easily affected by the loss of elements or by contamination as are the liquid samples. Formalin fixation, however, may have a significant effect on the concentrations of several elements. Frey et al.[4] reported that when 50 g of iron-rich tissue were fixed for 24 hours in 500 ml of 15% formalin solution, the iron content of the formalin increased from 54 to 784 µg per 100 ml of formalin. This represented a loss of 80 µg of iron/gram of tissue. In tissue samples analyzed for zinc, cadmium, copper, lead, calcium, and magnesium after fresh freezing and formalin fixation, Gunson et al.[16] found no effects of formalin fixation on the element measurements except for magnesium. The amount and direction of change for the change in magnesium concentration was not reported. Zook et al.[17] found no significant difference between the lead concentrations in fresh frozen and formalin fixed dog liver samples. One-half of each sample was frozen at the time of necropsy and the other half was stored in 10% neutral buffered formalin for 2 to 4 months.

Causes of Alterations in Serum Element Concentrations

Hemolysis

The concentrations of many elements are greater in the erythrocyte than in serum.[2,18] Thus, a slight amount of hemolysis can cause a large increase in the serum iron concentration and some increase or decrease in the concentration of other serum elements. Colvin et al.[19] determined that individual human erythrocytes from the same sample may vary in their content of mercury, iron, and copper by a factor of 5, 6 and 10, respectively. Variations observed for one-day-old chick definitive erythrocytes and for four-day-old chick embryo primitive erythrocytes were similar to the variations in the human erythrocytes.[19] Hove et al.[20] determined that zinc concentration of rat erythrocytes varied only slightly during zinc deficiency whereas the plasma zinc concentration ranged from 2.5 ppm in the deficient rat to 4.0 ppm in the rat with adequate zinc intake. Release of the contents of the erythrocyte, through lysis, should have a dilution effect upon some serum elements and increase the concentration of other serum elements depending upon the erythrocyte to serum element concentration ratios. The amount of change will also depend upon the degree of hemolysis.

Contamination

Sample collection, handling and interim storage are the major procedures during which alterations in element concentrations in serum samples occur. The elements cobalt, chromium, and manganese are contaminants introduced by stainless steel utensils.[21] There was a significant ($P<0.01$) increase in bovine serum mean zinc concentrations

from 1.33±0.07 ppm to 2.29±0.15 ppm within a two-hour period of exposure to the Vacutainer®[a] tubes.[22] There was no significant further increase in the serum zinc concentration from 2 to 32 hours of exposure time. It was also determined that there was a significant ($P<0.05$) decrease in the serum copper and iron concentrations during the first two hours of exposure. The mean serum copper and iron concentrations decreased from 0.587±0.02 and 2.79±0.39 ppm, respectively, to 0.529±0.02 and 1.70±0.24 ppm, respectively.[22]

Nackowski et al.[23] evaluated twenty different types of whole-blood/plasma collection tubes from three major manufacturers for their effects on human blood lead, copper, zinc and cadmium concentrations. These tubes utilized disodium ethylenediaminetetraacetic acid (EDTA) and potassium oxalate as anticoagulants. All twenty tube types caused increased zinc concentration in the test solution ranging from 7 ppm to 100 ppm. Six of the twenty tube types caused increases in lead concentrations ranging from 7 ppm to 33 ppm. A significant cadmium contamination (0.25 ppm) occurred in "lead free" brown-stoppered tubes. These tubes also were associated with a significant loss of lead from the blood sample after 4 days of exposure. This lead was quantatively recovered when the tube was washed with a 1% nitric acid solution. Unger et al.[24] determined that 52% of the lead was lost from aqueous standards after 5 days of storage time. None of the tubes contributed significant copper contamination.

---

[a]Becton Dickinson Co., Oxnard, CA 93030.

Williams[25] evaluated several different vacutainer tube types including the plain (red-stoppered), lead-free (amber-stoppered), heparinized (green-stoppered) and the special "metal-free" (blue-stoppered) tubes for their ability to contaminate serum, plasma, distilled deionized water and 0.1 M/L hydrochloric acid with iron, copper, and zinc. There was no significant contamination of the test solutions with iron or copper from any of the tube types. All tubes, however, significantly ($P<.01$) contaminated the test solutions with zinc. The serum zinc concentration in the acid-washed control tubes ranged from .77 to 1.33 ppm. The serum zinc concentrations in the "metal-free" (blue-stoppered) tubes ranged from .88 to 1.44 ppm, an increase of approximately 10% over the serum zinc concentrations in the acid-washed tubes. It was concluded that the metal-free (blue-stoppered) tubes may be adequate for large scale general screening procedures but only acid-washed glass tubes will be adequate for studies which require great accuracy. The butyl rubber stoppers of the vacutainer tubes are manufactured by a process which uses zinc salts and therefore serves as the source of zinc contamination.

Meranger et al.[26] examined the effects of storage times, temperatures and container types on the concentrations of cadmium, copper, mercury, lead and zinc in whole heparinized blood. They demonstrated considerable variations in element concentrations with all six container types and at all four temperatures of storage. In general, the cadmium, copper, and zinc concentrations in whole blood increased while the lead and mercury concentrations decreased as a function of time of storage, regardless of container type or temperature of storage. The

concentrations of cadmium, copper, and zinc increased up to 35%, 93% and 93%, respectively, while the concentrations of mercury and lead decreased by 60% and 86%, respectively.

Helman et al.[27] tested three types of Vacutainer®[a] tubes, silicone-coated #4787, uncoated #4799 and minimal lead #4808 for their ability to contaminate the collected blood sample with magnesium, calcium, zinc, iron, copper, cobalt, manganese, lithium and lead. The results of their work showed that only zinc and lead were affected significantly. The serum concentrations of zinc and lead were increased in all 3 tubes by up to 100% and 20%, respectively. This contamination was traced to the glycerin coating on the closure tops of the tubes. Helman et al.[27] also found that Perry needles[b] were associated with up to 100% contamination in zinc determinations, while Monoject®[c] needles added no significant amounts of contamination.

Narayanan[28] evaluated the Vacutainer®[a] brand evacuated, 3200 series, red-stoppered blood collection tube for its possible role in contaminating serum samples with calcium, magnesium, iron, nickel, copper, cadmium, and lead. It was determined that the tube itself added 0.075 ppm calcium, 0.035 ppm magnesium, and 0.015 ppm iron. The stopper contributed 0.125 ppm calcium, 0.044 ppm magnesium, and 0.028 ppm iron. The contributions of the elements nickel, copper, cadmium and lead by the tube and stopper were below the detection limits of the atomic absorption spectrophotometer utilized for analysis.

---

[b]Affiliated Hospital Products, Inc., Massillon, OH 44648.

[c]Sherwood Med. Industries, Inc., Delano, FL 32720.

Reimold et al.[29] examined in detail all the procedures involved in the analysis of serum for zinc and found some degree of zinc contamination in almost all the steps of the procedure. They also determined the Becton-Dickinson and Monoject[d] plastic disposable syringes and polypropylene tubes to be zinc free. They concluded that the best way to collect blood for zinc determination is with a plastic syringe and that the sample should be stored in polypropylene tubes with polythene stoppers.[e]

Serum-to-Clot Contact Time

Little information seems available concerning the shift in elements between the erythrocytes and the plasma or serum when these fluids remain in contact with the erythrocytes during shipment or storage. Rich[30] reported a twofold increase in phosphorus concentrations in serum remaining in contact with the erythrocytes over a three-day period. Prasad et al.[31] and Foley et al.[32] reported an increase in the sample zinc concentration as a function of platelet destruction during the clotting process.

From the information available in the literature, it became apparent that considerable variations in tissue element concentrations were attributed to contamination or loss of elements during the sample collection, handling and interim storage procedures. Less information was available concerning the effects of physiological factors on tissue

---

[d]Sherwood Medical Industries, St. Louis, MO 63103.

[e]Walter Sarstedt, Inc.

element concentrations. Therefore, this research was conducted to determine the effects that sample site, residual perfusion, lipid content, and formalin fixation have on element concentrations in the bovine liver. The effects of hemolysis and the effects of serum-to-clot contact time on the element concentrations in the bovine serum were also determined.

EXPERIMENT 1

Effects of sample site, lipid content, residual perfusion and formalin fixation on element concentrations in bovine liver samples.

Materials and Methods

Ten lactating Holstein cows were randomly selected from the animals presented for necropsy at the Michigan State University Animal Health Diagnostic Laboratory. Each animal had been dead for 24-72 hours. The entire liver was removed from each animal. The gall bladder and fat were trimmed from the surface of all livers. Each liver was placed on a stainless steel table with the diaphragmatic surface of the liver facing upward and the caudate process placed to the left. The diaphragmatic surface was divided into 3 sampling areas, A, B and C (Figure 1). A stainless steel coring device[f] was used to collect 200 gm (25-35 cores) of tissue from each of the 3 areas. Tissue cores were placed in eight ounce plastic cups[g] with the corresponding A-B-C labels. A composite sample for each cow was prepared by blending together, in a stainless steel blender,[h] 50 gm of tissue from each of the 3 areas.

---

[f]Stainless steel thin wall tubing, 15 cm in length, 1.25 cm outside diameter, sharpened at one end with an inside bevel.

[g]#4020, Falcon, Oxnard, CA 93030.

[h]Waring Products Division, New Hartford, CT.

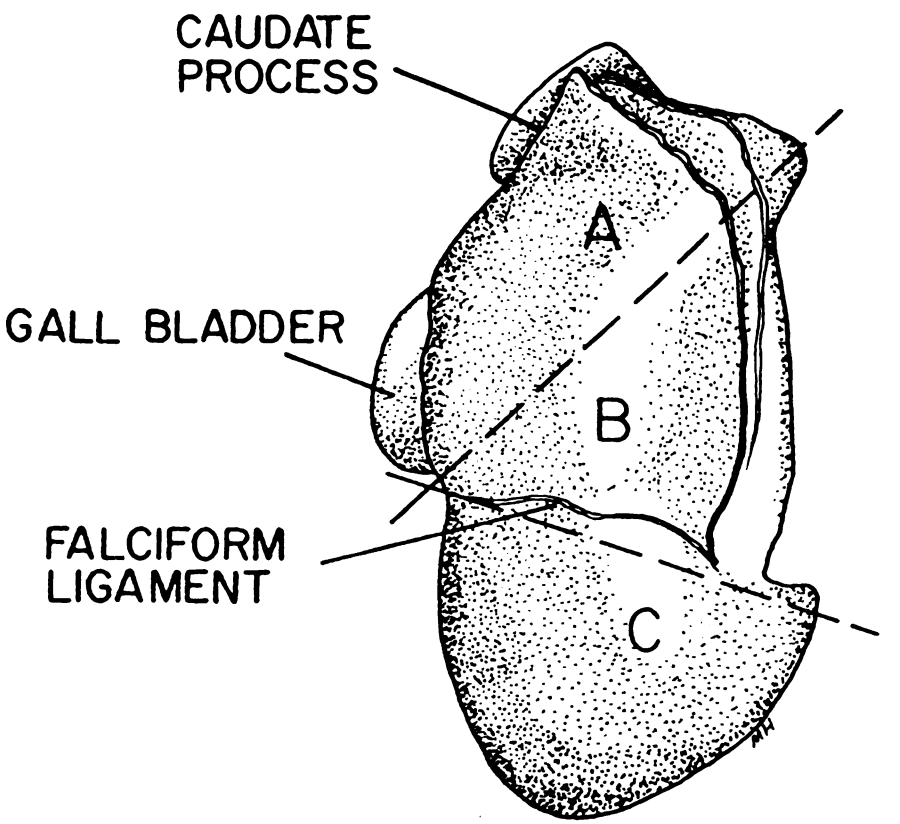


Figure 1. Drawing of a cow's liver (diaphragmatic surface) with the area designations A, B and C.

The blending process was considered complete when a pasty, homogenous consistency was achieved.

Five core samples from area A for each of the 10 cows were divided lengthwise. Half of each core was weighed and then immersed for 24 hours in ten times its weight of buffered 10% formalin[i] to create 50 samples of fixed tissue. The other half of each of the 50 cores represented the fresh tissue samples. One gram of tissue from each of the 50 fresh and the 50 fixed tissue samples was selected from a portion of the cores devoid of large blood vessels and analyzed by ICP[33] for aluminum (Al), arsenic (As), cadmium (Cd), calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg), molybdenum (Mo), phosphorus (P), potassium (K), selenium (Se), sodium (Na), thallium (Tl) and zinc (Zn). Five one-gram samples from areas B and C and the composite from each cow were also analyzed by ICP for the previously listed elements.

---

[i]Formalin formulation: 1600 ml, 37% formaldehyde; Fisher Scientific Company, Chemical Manufacturing Division, Fairlawn, NJ 07410.

104 gram, sodium phosphate dibasic, anhydrous #7917, Mallinkrodt Inc., Paris, KY 40361.

64 gram, sodium phosphate monobasic #7892, Mallinkrodt Inc., Parks, KY 40361.

Q.S., 4 gallons with distilled water.

The lipid content of three, 10-gram aliquots from each cow's composite sample was determined using the hexane-isopropanol fat extraction procedure.[34]

The hemoglobin iron content of three 5-gram aliquots from each cow's composite sample was determined using the methyl-ethyl ketone hemoglobin extraction procedure.[35] The hemoglobin extract was analyzed for iron concentration using the ICP.[33]

The data for the element concentrations in sample areas A, B, and C were analyzed by the two-way analysis of variance (ANOVA) to determine the significance of differences in element concentrations between areas and between cows. Data for each element from each cow's sample areas were transformed into percentages by the following procedure. For each cow, the fifteen respective element concentrations for each element were summed together and the grand mean concentration was determined ($\frac{\Sigma A + \Sigma B + \Sigma C}{15} = \bar{X}_{15}$).* The grand mean for each element was designated to represent 100% ($\bar{X}_{15} = 100\%$). For each element, the mean concentration from each sample areas was determined ($\frac{\Sigma A}{5} = \bar{X}A$, $\frac{\Sigma B}{5} = \bar{X}B$, $\frac{\Sigma C}{5} = \bar{X}C$) and then divided by their respective grand mean concentrations ($\bar{X}A/\bar{X}_{15}$, $\bar{X}B/\bar{X}_{15}$, $\bar{X}C/\bar{X}_{15}$). The 3 quotients for each element were multiplied by 100 and transformed into percentages. The transformed percentage values were then used in place of their respective mean concentrations in the one-way ANOVA and Duncan's multiple range test used to determine the significance of the differences

---

* $\Sigma_{i=1}^{5} A_i = \Sigma A$; $\Sigma_{i=1}^{5} B_i = \Sigma B$; $\Sigma_{i=1}^{5} C_i = \Sigma C$

in element concentrations between sample areas. The raw data for the element concentrations in fresh and fixed tissue were subjected to the paired-t test to determine the significance of differences in mean element concentrations between fresh and fixed tissue. The tissue element concentrations were correlated with hemoglobin iron (residual perfusion) and with hepatic lipid content. The element concentrations, lipid concentrations and hemoglobin iron concentrations for these correlations were all derived from the composite sample.

RESULTS

Effects of Sample Site

Table 1 shows the mean element concentrations and standard deviations for each sample area in the bovine liver (A, B, C and composite) and the mean of means concentration for each element. The mean element concentrations for each sample area were all within 5% of the mean of means concentration.

The summary data for the two-way ANOVA for the significance of element concentration differences between cows and between areas are presented in Table 2. The significant interactions between the cow effect and sample area effect are also presented in Table 2. There was a significant difference ($P<0.001$) in element concentrations between cows for every element and a significant difference ($P<0.05$) in element concentration between sample areas for calcium, magnesium, molybdenum, phosphorus, potassium, and zinc. There was a significant interaction between the cow effect and the sample area effect for calcium, iron, magnesium, molybdenum, phosphorus, potassium, sodium, and zinc.

The mean and standard deviations of the transformed data are presented in Table 3. The variations within the sample areas (standard deviations) were greater than the differences between sample areas for all elements except molybdenum and phosphorus. The maximum difference between areas was less than 6.5% (cadmium, areas B-C).

The summary data for the one-way ANOVA for the significance of the difference in element concentrations between sample areas and

Table 1. Mean element concentrations and standard deviations for liver samples from different sample sites in the bovine liver.

Element	(Cows)	Area A	Area B	Area C	Means A, B & C	Composite[a]
			(ppm wet weight basis)			
Cadmium[b]	6	.213±.08	.216±.09	.205±.08	0.21	.215±.09
Calcium	10	41.8±7.5	42.5±7.7	43.8±8.9	42.73	43.5±7.8
Copper	10	53.9±66.6	54.9±59.8	56.4±56	54.96	57.7±59
Iron	10	134.5±45	136±39	133±39	135.10	137±40
Magnesium	10	143±18	139±19	138.5±20	140.50	139±20
Manganese	10	1.75±.47	1.67±.48	1.68±.50	1.70	1.67±.47
Molybdenum	10	.721±.25	.699±.25	.687±.26	0.70	.707±.25
Phosphorus	10	3163±277	3132±270	3038±309	3101.00	3101±288
Potassium	10	2782±487	2700±325	2701±309	2728.00	2700±340
Sodium	10	1163±287	1156±266	1160±267	1159.00	1166±258
Zinc	10	88.1±65	87.7±56	85.3±56	88.10	87.6±57

[a]The composite sample consists of 50 g aliquots from each area. (A, B and C) blended together.

[b]Only 6 of the 10 bovine livers contained detectable amounts of cadmium.

Table 2. Summary data from the two-way analysis of variance for the significance of element concentration differences in bovine livers.

Element	Cow Effect P Values	Lobe Effect P Values	Interaction P Values
Cadmium[a]	.001	NS[b]	NS
Calcium	.001	.022	.001
Copper	.001	NS	NS
Iron	.001	NS	.001
Magnesium	.001	.001	.001
Manganese	.001	NS	NS
Molybdenum	.001	.013	.002
Phosphorus	.001	.003	.027
Potassium	.001	.001	.001
Sodium	.001	NS	.001
Zinc	.001	.001	.001

[a]Only 6 of the 10 bovine livers contained detectable amounts of cadmium.

[b]NS=P>0.05.

Table 3. Mean and standard deviations of the transformed bovine liver element concentration data from 3 sample areas

Element	Area A %	Area B %	Area C %
Cadmium[a]	101.0±5.4	103.0±5.7	96.8±7.4
Calcium	98.1±4.9	99.7±2.0	102.3±5.9
Copper	98.0±7.6	100.0±6.4	102.0±11.3
Iron	99.6±6.0	101.0±4.6	99.2±7.9
Magnesium	102.0±3.1	99.0±1.4	98.6±3.2
Manganese	103.0±3.1	98.4±4.2	98.7±4.0
Molybdenum	103.0±3.4	99.8±1.9	98.2±3.8
Potassium	102.0±3.7	99.0±1.4	99.0±4.6
Phosphorus	102.0±3.1	101.0±1.0	98.0±3.4
Sodium	100.3±4.3	99.8±3.1	100.1±4.8
Zinc	100.0±6.6	99.7±2.9	96.9±6.5

[a]Only 6 of the 10 bovine livers contained detectable amounts of cadmium.

the results of Duncan's multiple range test are presented in Table 4. There was a significant difference ($P<0.05$) between sample areas for the element concentrations of magnesium, manganese, molybdenum, phosphorus and zinc. The element concentrations in area A were consistently significantly different from the concentrations in area C and, in most cases, were also significantly different than area B concentrations.

Effects of Formalin Fixation

The mean and standard deviations of the concentrations for each element in both the fresh and fixed tissue samples are presented in Table 5. There was a significant decrease ($P<0.05$) in the tissue element concentrations caused by formalin fixation for each element except cadmium, phosphorus and sodium. The mean percent decreases in concentrations caused by formalin fixation were -18%, -22% and -23% for copper, iron and zinc, respectively, while the elements calcium, magnesium, manganese, molybdenum, and potassium had larger mean percent decreases at -33%, -60%, -35%, -49% and -91%, respectively. The sodium concentration was significantly increased ($P<0.001$) in the fixed tissue and there was no significant change in the cadmium and phosphorus concentrations.

There was not a significant correlation ($P>0.05$) between the amount of weight change for the formalin fixed tissue and the weight of the sample placed in the formalin solution. There also was not a significant correlation ($P>0.05$) between the amount of weight change for the formalin fixed tissue and the tissue lipid content. The formalin fixed tissues averaged 3.3% lighter than their corresponding fresh weights.

Table 4. The summary data for the one-way ANOVA and Duncan's multiple range test for the significant differences in elemental concentrations between bovine liver sample areas.

Element	Lobe effect P Value	Duncan test
Cadmium	NS[a]	-----
Calcium	NS	-----
Copper	NS	-----
Iron	NS	-----
Magnesium	0.01	A-B,C[b]
Manganese	0.01	A-B,C
Molybdenum	0.01	A-B,C
Phosphorus	0.01	A,B-C
Potassium	NS	-----
Sodium	NS	-----
Zinc	0.03	A-B,C

[a]NS=P>0.05.

[b]The elemental concentration in area A is significantly (P<0.05) different from the elemental concentration in both areas B and C but the elemental concentrations in areas B and C are not significantly different from each other.

Table 5. Mean and standard deviations of element concentrations in samples of fresh and formalin fixed bovine liver.

Element	Cows[a]	Fresh (ppm)	Fixed (ppm)	Paired-T P Values	% Change (Mean & SD)	Range of % Change
Cadmium[b]	6	.213±.08	.193±.06	NS[c]	-10.6±6.6	2-19
Calcium	10	41.8±7.5	27.9±5.3	.0001	-33.4±4.9	25-42
Copper	10	55.6±60.6	48.5±52.9	.035	-17.6±6.4	6-27
Iron	10	135.8±44.6	106.6±36.6	.0001	-22.0±8.2	8-32
Magnesium	10	143.2±17.7	56.9±9.5	.0001	-60.1±5.9	54-73
Manganese	10	1.75±.47	1.13±.29	.0001	-35.0±7.0	20-44
Molybdenum	10	.715±.25	.365±.15	.0001	-49.5±6.1	38-59
Phosphorus[d]	10	3154±277	3484±66	NS	+14.9±13.6	-12-(+43)
Potassium	10	2841±487	256±89.4	.0001	-91±2.4	87-96
Sodium[d]	10	1166±287	3064±702	.0001	+180±105	59-402
Zinc	10	92.5±65.2	70.9±48.7	.003	-22.8±5.4	14-34

[a]The mean fresh tissue concentration and the mean fixed tissue concentration for an element in a single cow represent one pair, i.e. the mean fresh tissue calcium concentration and the mean fixed tissue calcium concentration in cow #1 represent 1 pair.

[b]Only 6 of the 10 bovine livers contained detectable amounts of cadmium.

[c]NS=P>0.05.

[d]Sodium and phosphorus are elements present in the buffer for the 10% formalin solution.

Effects of Residual Perfusion and Lipid Content

The summary data of the element concentrations correlated with hemoglobin iron (perfusion) and lipid concentrations are presented in Table 6. There was a significant negative correlation ($P<0.02$) between the magnesium, phosphorus and potassium concentrations and hemoglobin iron concentrations and a significant negative correlation ($P<0.02$) between phosphorus concentrations and lipid concentration.

Table 6. Summary of element concentrations in bovine livers correlated with the hemoglobin iron and lipid concentrations.

Elements and Lipid	n	Element vs Hemoglobin Iron[a]		Element vs Lipid Content[b]	
		r	P Value	r	P Value
Cadmium[c]	6	-.550	NS[d]	-.226	NS
Calcium	10	-.336	NS	-.318	NS
Copper	10	-.075	NS	-.467	NS
Lipid	10	.501	NS	-----	-----
Total Iron	10	.176	NS	.233	NS
Magnesium	10	-.826	.01	-.494	NS
Manganese	10	.055	NS	-.518	NS
Molybdenum	10	.024	NS	-.610	NS
Phosphorus	10	-.866	.01	-.750	.02
Potassium	10	-.736	.02	-.407	NS
Sodium	10	-.139	NS	-.472	NS
Zinc	10	-.469	NS	-.244	NS

[a]Hemoglobin iron ranged from 26.8 ppm to 84 ppm on a wet weight basis.

[b]Lipid content ranged from 3% to 25.4% on a wet weight basis.

[c]Only 6 of the 10 bovine livers contained detectable amounts of cadmium.

[d]NS=P>0.05.

DISCUSSION

Effects of Sample Site

The significant interactions between cow effect and sample area effect (Table 2) prevented the use of the one-way ANOVA and Duncan's multiple range test. The data were therefore transformed into percentages to eliminate the cow effect and the interaction. From the small percentage differences between areas and the larger percentage differences within areas (Table 3), it was concluded that the statistically significant differences (Table 4) would not have a significant effect on the clinical interpretation of the data. The toxic, adequate, marginal and deficient concentrations for all the elements could be detected, regardless of sample site.

Effects of Formalin Fixation

By splitting the core samples lengthwise, the maximum ratio of surface area to weight of sample, within practical limits, was achieved. This process also produced paired samples which were as close to identical as possible with respect to their element content and allowed for the use of the paired-t statistical test.

By determining the percent of change in concentration for each element (Table 5) caused by formalin fixation, the effects of the large, between-cow variations in concentrations were minimized. The large, between-cow variations in concentrations of copper, iron, and zinc did not appear to affect the amount (%) of change in their concentrations.

The mean percent change in concentrations for copper, iron and zinc were all decreases of less than 25% (Table 5). These decreases would affect the clinical interpretation of the data if the tissue concentrations were in the low normal range. The mean percent change in concentration for calcium, magnesium, manganese, molybdenum and potassium were all decreases of greater than 30% (Table 5). These decreases would affect the clinical interpretation of the data even if the tissue element concentrations were well within the expected ranges. The increase in tissue sodium concentration caused by formalin fixation is the result of sodium being used in the formalin solution as one of the buffering agents. The mean percent increase of 180% (Table 5) would have an effect on the clinical interpretation of the tissue sodium concentration.

Effects of Residual Perfusion

The significant negative correlations ($P<0.02$) (Table 6) between phosphorus, potassium and magnesium and the hemoglobin-iron concentrations were expected when the tissue element concentrations[36] were compared with the whole blood element concentrations.[13,18] The liver concentrations of magnesium and phosphorus were approximately 10 times the whole blood magnesium and phosphorus concentrations while the liver potassium concentration was approximately 2 times the whole blood potassium concentration. However, the expected ranges for the concentrations of phosphorus, potassium and magnesium in the bovine liver were so wide that, from a diagnostic point of view, only the interpretation of marginal deficiencies of these elements will be affected by severe congestion.

Effects of Lipid Content

There was a significant negative correlation ($P<0.02$) between phosphorus concentrations and lipid content (Table 6). The clinical interpretation of the phosphorus concentrations will only be affected by the lipid content when marginal phosphorus concentrations are accompanied by a lipid content greater than 15%.

SUMMARY

There were significant differences in the concentrations of magnesium, manganese, molybdenum, phosphorus and zinc between the 3 designated areas of bovine liver. None of these differences appeared to affect the clinical interpretation of the data. There were no significant differences in concentrations between areas for cadmium, calcium, copper, iron and potassium.

Formalin fixation produced significant decreases in the concentrations of calcium, copper, iron, magnesium, manganese, molybdenum, potassium, and zinc when bovine liver tissue was immersed in a sodium phosphate buffered 10% formalin solution. These decreases were large enough to affect the clinical interpretation of the element concentration data. The increase in sodium concentration caused by formalin fixation was also great enough to affect the clinical interpretation of the sodium concentration data.

There was a significant negative correlation between the concentrations of phosphorus, potassium and manganese and the hemoglobin-iron concentration (perfusion). The clinical interpretation of the concentration data for these 3 elements may be affected when marginal concentrations coincide with severe congestion of the liver tissue. There was a significant negative correlation between the concentrations of phosphorus and the lipid content of the liver which may also affect the clinical interpretation of the phosphorus concentration data if a marginal concentration of phosphorus were present in a "fatty" liver. The

correlations of the concentrations of calcium, copper, total iron, sodium, zinc, cadmium, molybdenum and manganese and the hemoglobin-iron concentrations were not significant. The correlations of the concentrations of cadmium, calcium, copper, iron, magnesium, manganese, molybdenum, potassium, sodium and zinc with tissue lipid content were not significant.

EXPERIMENT 2

Effects of hemolysis on element concentrations in serum samples from lactating cows.

Materials and Methods

Six lactating Holstein cows were selected as blood donors from the Michigan State University teaching animals. Five hundred milliliters of blood were obtained from the jugular vein of each animal using a 3-inch, 10 gauge stainless steel needle.[j] Half of the blood was collected in plastic, acid-washed culture tubes[k] which were then vigorously shaken and frozen to induce hemolysis. After freezing for 20 hours, these tubes were allowed to set at room temperature 4 hours, centrifuged and the hemolyzed serum contents separated from the cells 24 hours post-collection. The other 250 ml of blood from each cow were collected into glass, acid-washed culture tubes[l] and stored at room temperature. These tubes were centrifuged and the serum was separated from the clot 4 hours postcollection.

A range in degree of hemolysis in serum was established for each cow by combining varying amounts of hemolyzed serum with its

---

[j]B-D®, Becton Dickinson Co., Oxnard, CA 93030.

[k]Fifty milliliter plastic, tissue culture tubes with screw caps, #2070 Falcon®, Division Becton Dickinson, Oxnard, CA 93030.

[l]Twenty-five by 150 mm (40 ml) glass, tissue culture tubes with teflon-lined screw caps, ART #4066-A, Kimble Products Kimax®, Vineland 08360.

corresponding nonhemolyzed serum in plastic culture tubes.[m] The hemoglobin content of each of the 99 samples was determined by the cyanmethemoglobin method utilizing a hemophotometer.[n] A 1 ml aliquot from each of the 99 samples was analyzed utilizing the ICP.[33]

Correlation analyses between serum element concentrations and hemolysis (hemoglobin and iron) were conducted.

---

[m]Sixteen by 125 mm (15 ml) plastic, tissue culture tubes with screw caps, #3033 Falcon, Division Becton Dickinson, Oxnard, CA 93030.

[n]Fisher Hemophotometer, Flo-thru Model 54, Fisher Scientific Co., Pittsburg, PA 15219.

RESULTS

Table 7 contains the correlation coefficients and their significance values for the element concentrations in bovine serum correlated with serum hemoglobin and iron concentrations. There were significant correlations ($P<0.05$) between the hemoglobin content and concentrations of every element. For the elements calcium, copper, magnesium, and sodium, the correlations were negative whereas the correlations for phosphorus, potassium and zinc were positive. There were no significant correlations ($P>0.05$) between the copper and iron concentrations in the serum samples. Calcium, magnesium and sodium concentrations were significantly ($P<.001$) negatively correlated with the iron concentrations. Phosphorus, zinc, and potassium were significantly ($P<.001$) correlated with the iron concentrations.

Figure 2 represents the bovine serum iron concentrations correlated with the hemoglobin concentrations in the samples. Figure 3 and Figures 5-10 represent the serum iron concentrations correlated with the serum concentrations of calcium, copper, magnesium, phosphorus, potassium, sodium and zinc. Figure 4 represents the serum copper concentrations correlated with the serum hemoglobin concentrations.

Table 7. Summary of bovine serum element concentrations correlated with serum hemoglobin and serum iron concentrations (hemolysis).

Element	Samples n	Element vs Hemoglobin r	Element vs Hemoglobin P Value	Element vs Iron r	Element vs Iron P Value
Calcium	99	-.576	0.001	-.606	0.001
Copper	98	-.215	0.050	-.144	NS[a]
Iron	99	.973	0.001	-----	-----
Magnesium	99	-.392	0.001	-.402	0.001
Phosphorus[b]	99	.327	0.001	.327	0.001
Potassium	53	.666	0.001	.696	0.001
Sodium	99	-.576	0.001	-.569	0.001
Zinc	99	.712	0.001	.688	0.001

[a]NS=P>0.05.

[b]Total phosphorus.

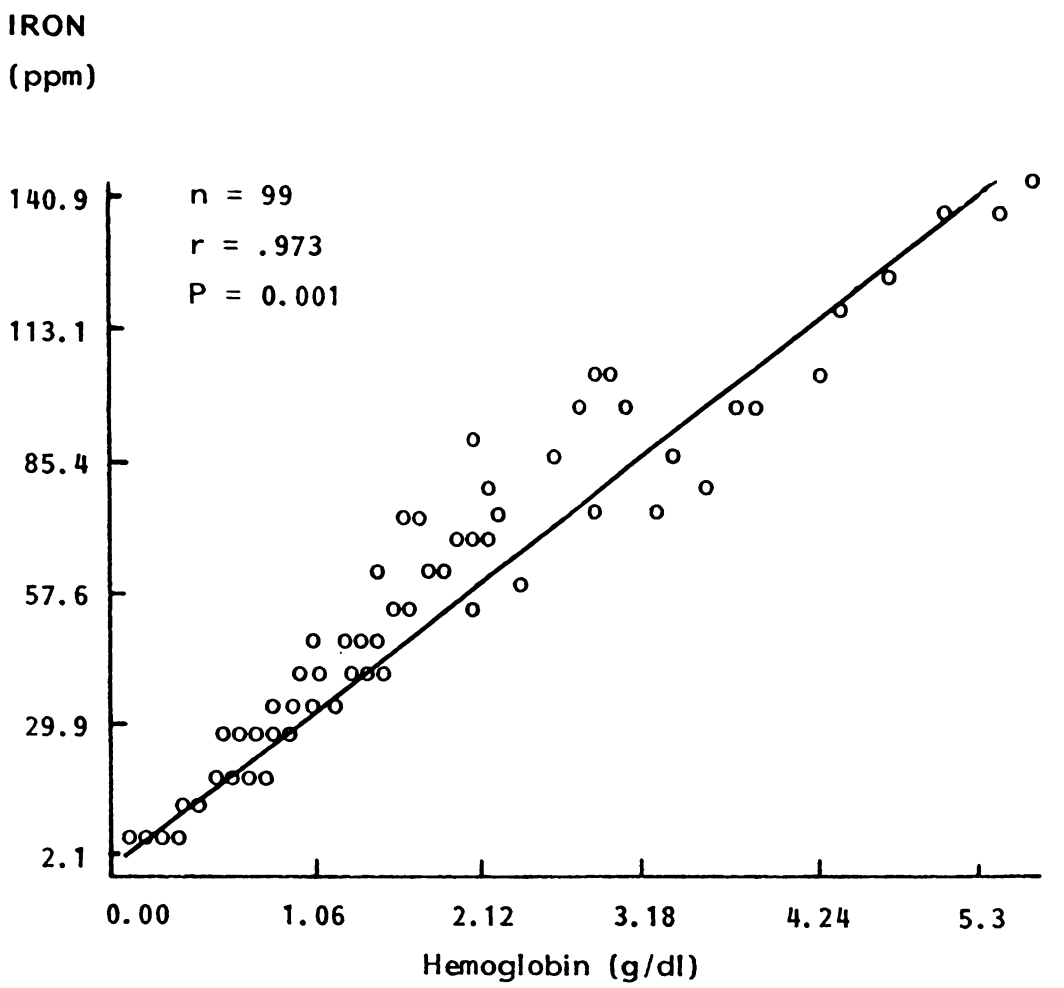


Figure 2. Bovine serum iron concentrations correlated with the serum hemoglobin concentrations.

Hemoglobin $\bar{X}$ = 1.53 g/dl, SD=1.23 g/dl; iron $\bar{X}$ = 48.2 ppm, SD=34.6 ppm; slope=27.4.

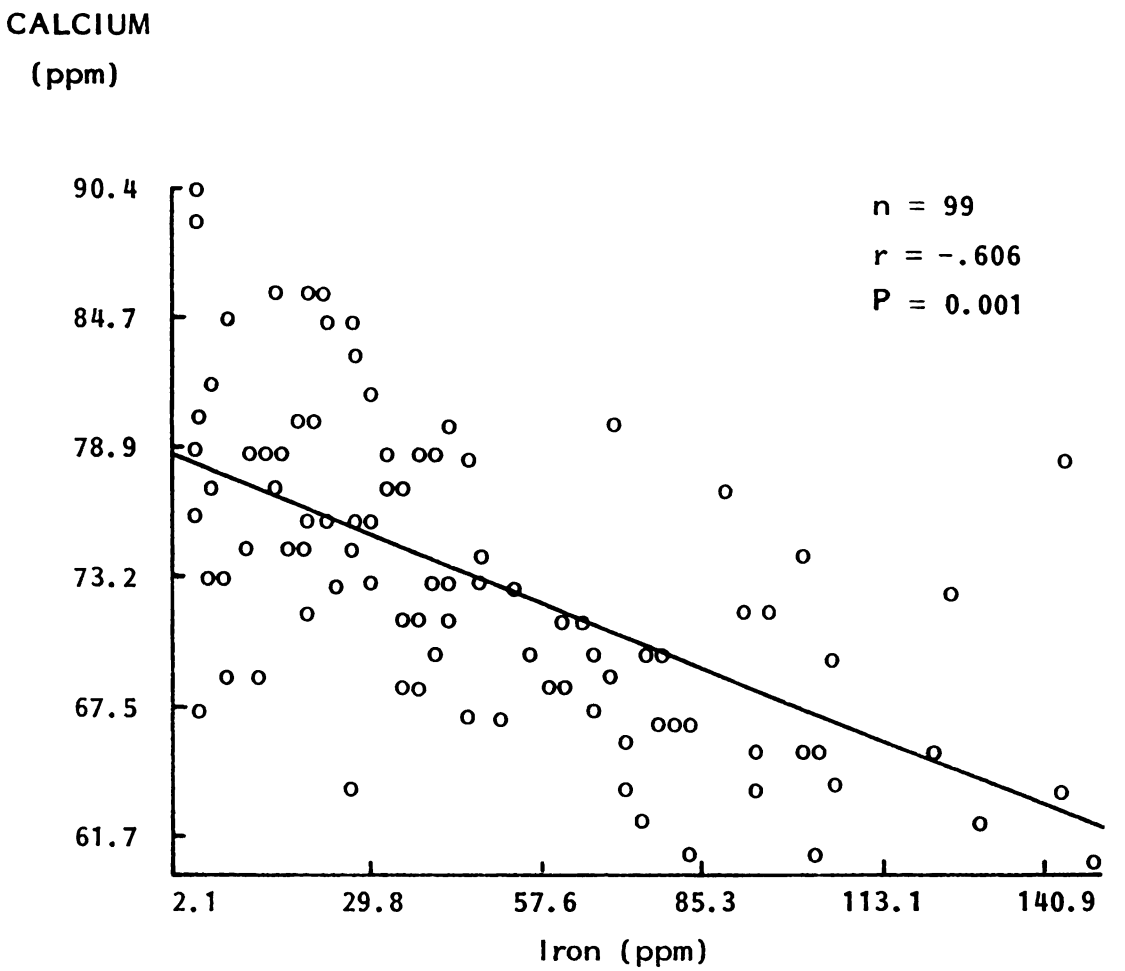


Figure 3. Bovine serum calcium concentrations correlated with the serum iron concentrations (hemolysis).

Calcium $\bar{X} = 74.3$, SD=6.5 ppm; iron $\bar{X} = 48.2$ ppm, SD=34.6 ppm; slope=-0.113.

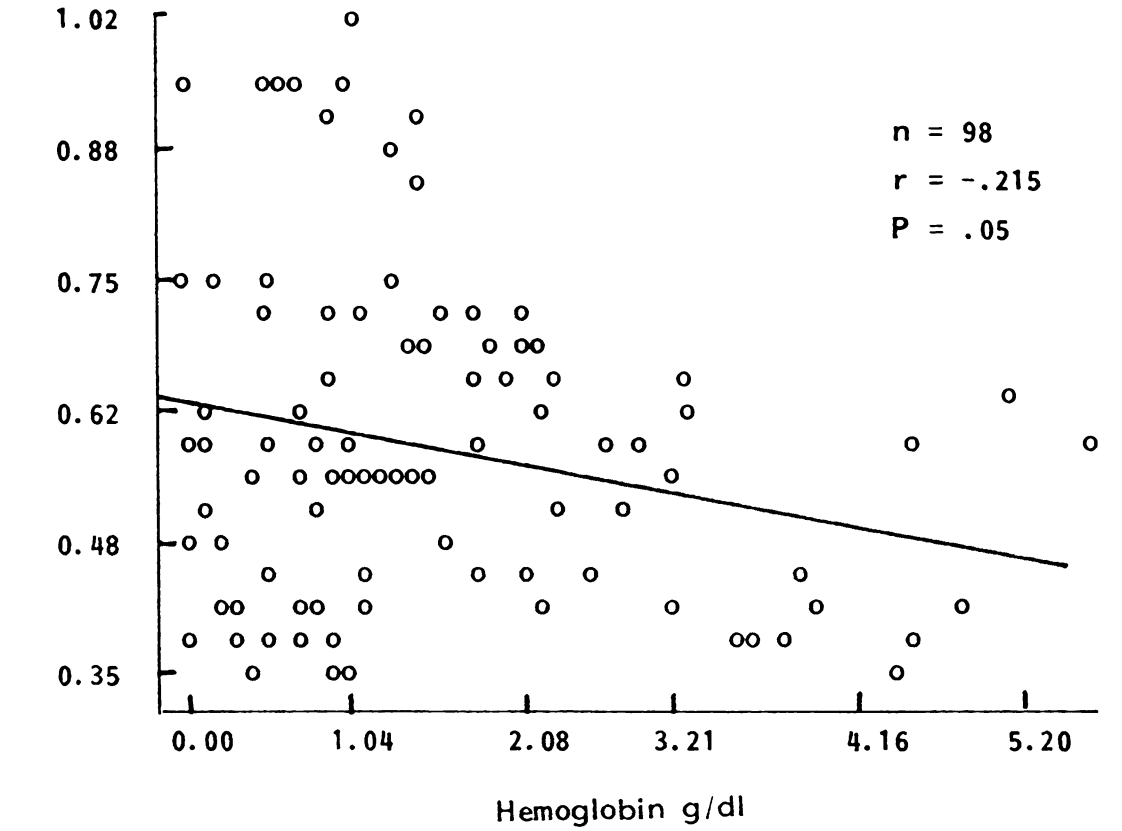


Figure 4. Bovine serum copper concentrations correlated with the serum hemoglobin concentration (hemolysis).

Copper $\bar{X}$ = 0.61 ppm, SD=0.17 ppm; hemoglobin $\bar{X}$ = 1.53 g/dl, SD=1.23 g/dl; slope=-.031

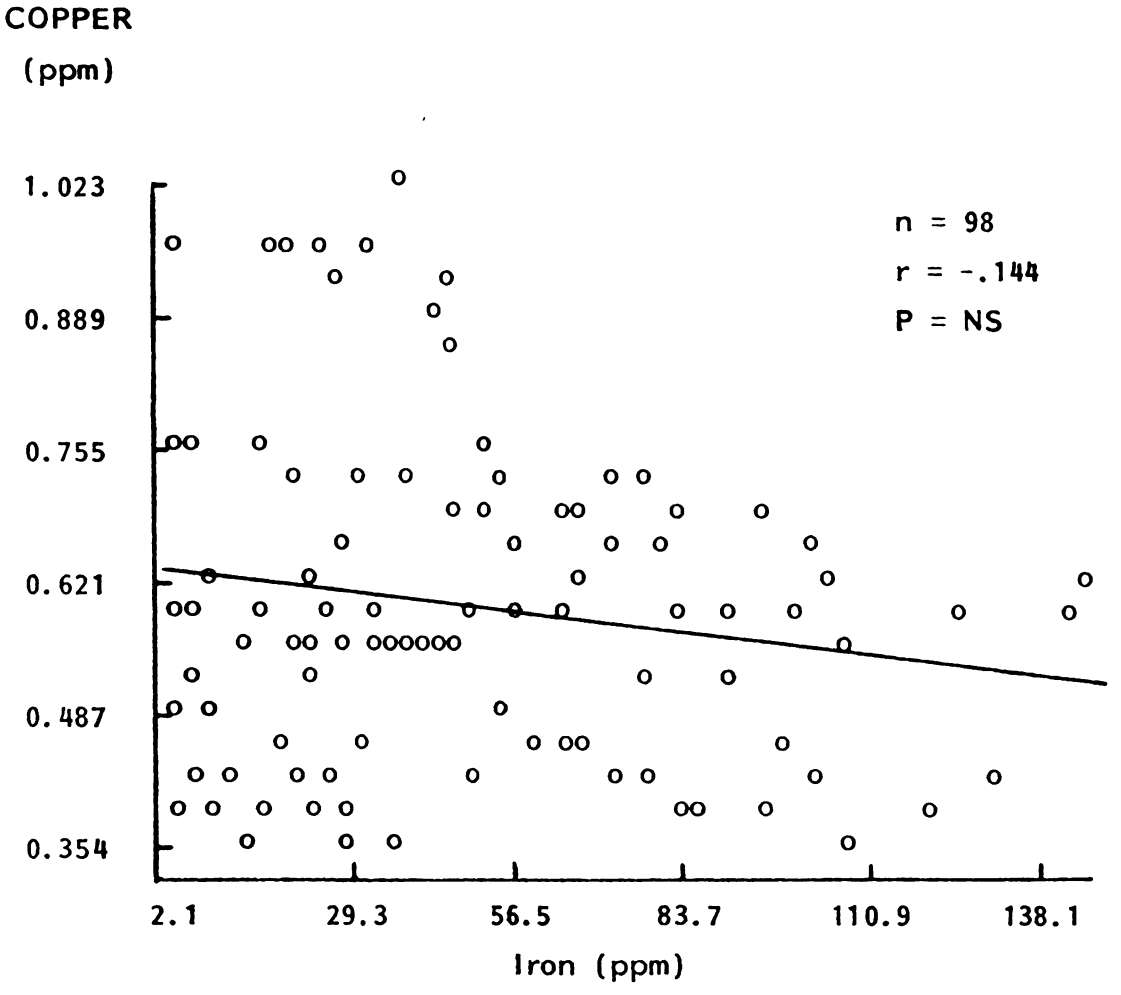


Figure 5. Bovine serum copper concentrations correlated with serum iron concentrations (hemolysis).

Copper $\bar{X}$ = 0.61 ppm, SD=0.17 ppm; iron $\bar{X}$ = 48.2 ppm, SD=34.6 ppm; slope=-7.16; NS=P>0.05.

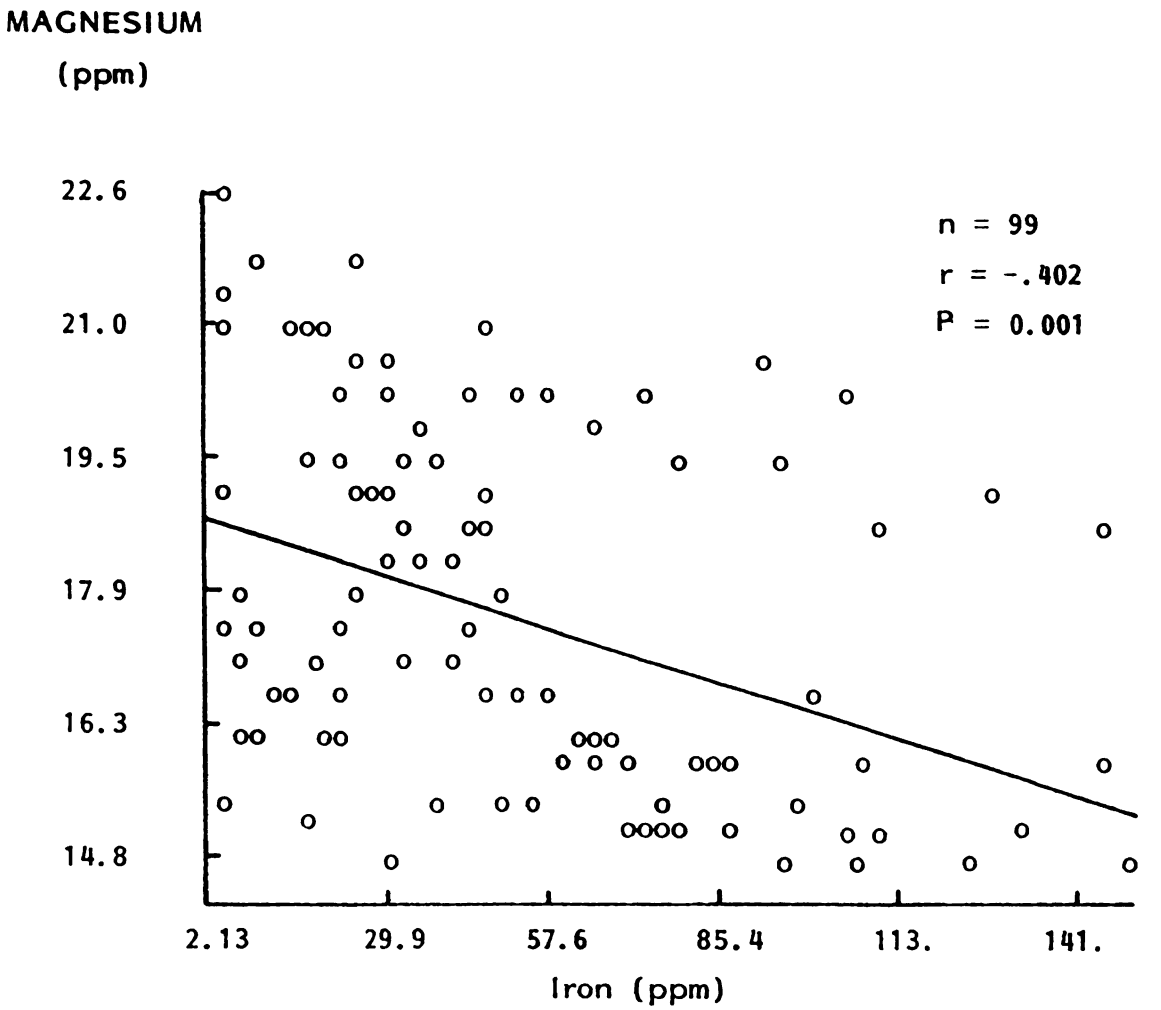


Figure 6. Bovine serum magnesium concentrations correlated with serum iron concentrations (hemolysis).

Magnesium $\bar{X}$ = 17.9 ppm, SD=2.1 ppm; iron $\bar{X}$ = 48.2 ppm, SD=34.6; slope=-0.024.

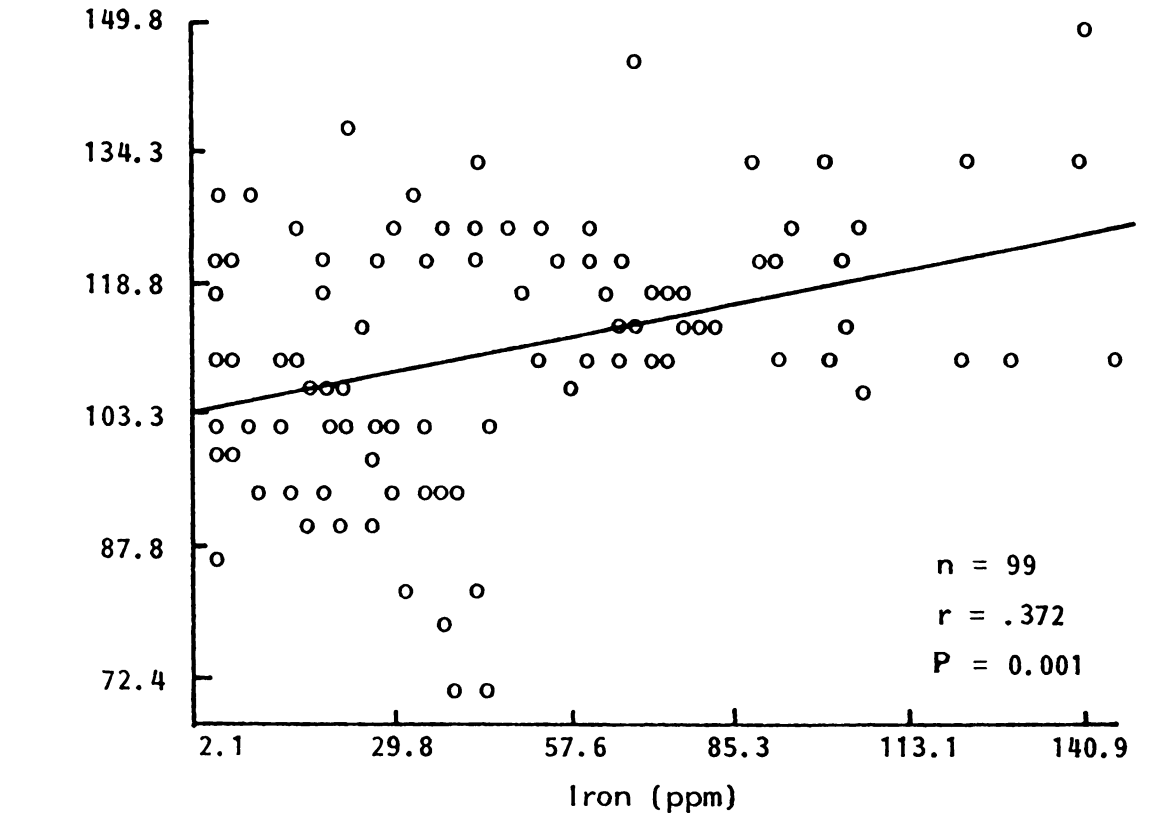


Figure 7. Bovine serum phosphorus concentrations correlated with serum iron concentrations (hemolysis).

Phosphorus $\bar{X}$ = 114.4, SD=14.9 ppm; iron $\bar{X}$ = 48.2 ppm, SD=34.6; slope=0.160.

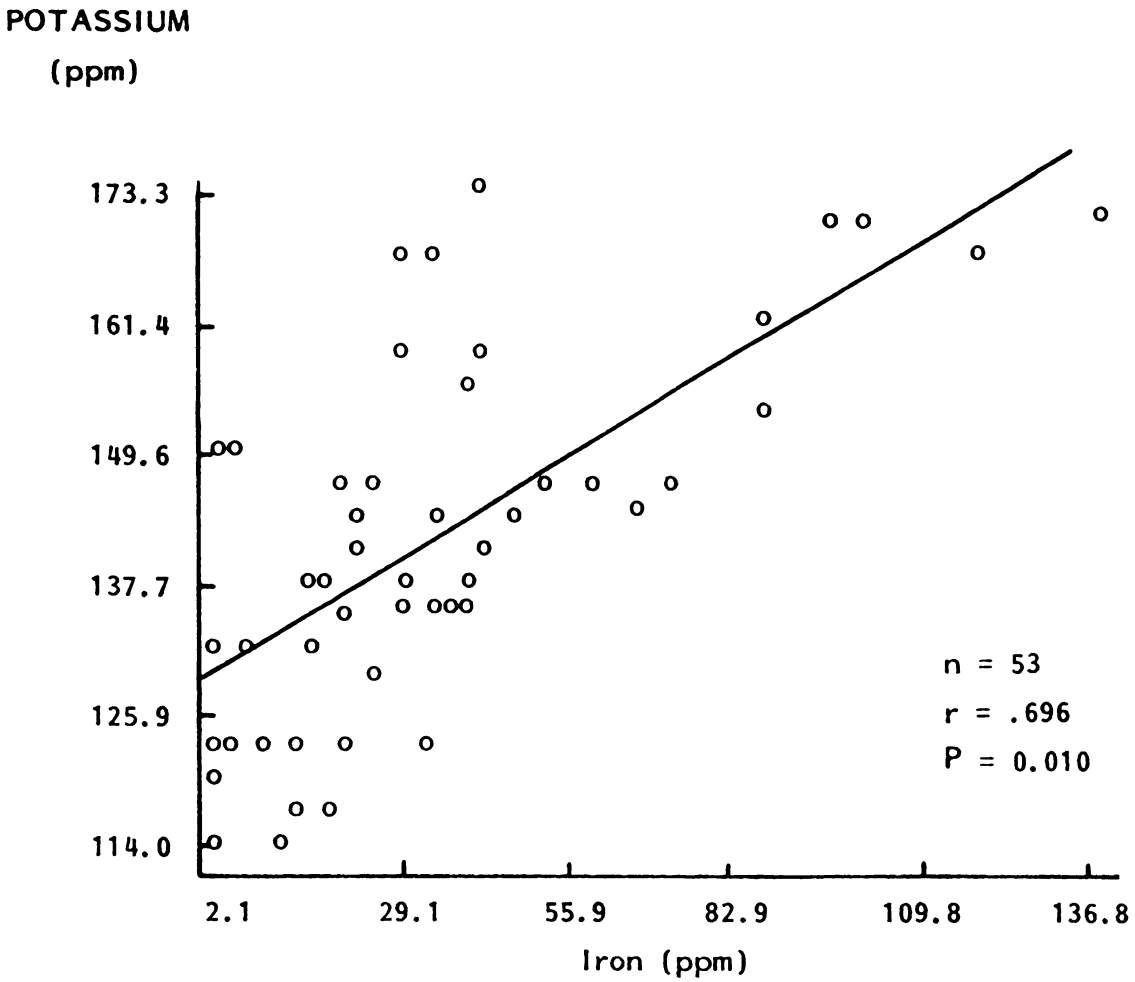


Figure 8. Bovine serum potassium concentrations correlated with serum iron concentrations (hemolysis).

Potassium $\bar{X}$ = 142.9 ppm, SD=15.7 ppm; iron $\bar{X}$ = 48.2 ppm, SD=34.6 ppm; slope=0.369.

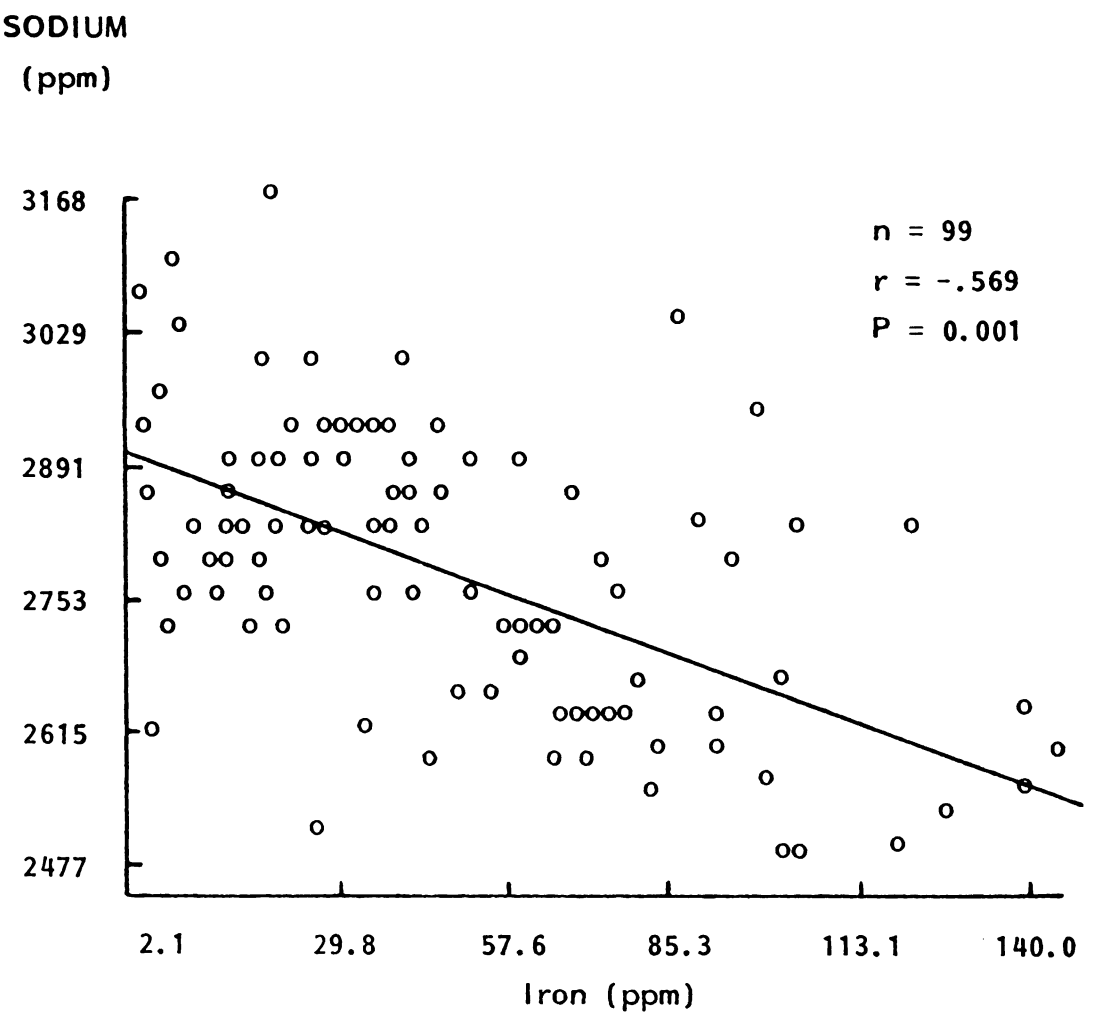


Figure 9. Bovine serum sodium concentrations correlated with the serum iron concentrations (hemolysis).

Sodium $\bar{X}$ = 2796 ppm, SD=149 ppm; iron $\bar{X}$ = 48.2 ppm, SD=34.6 ppm; slope=-2.45.

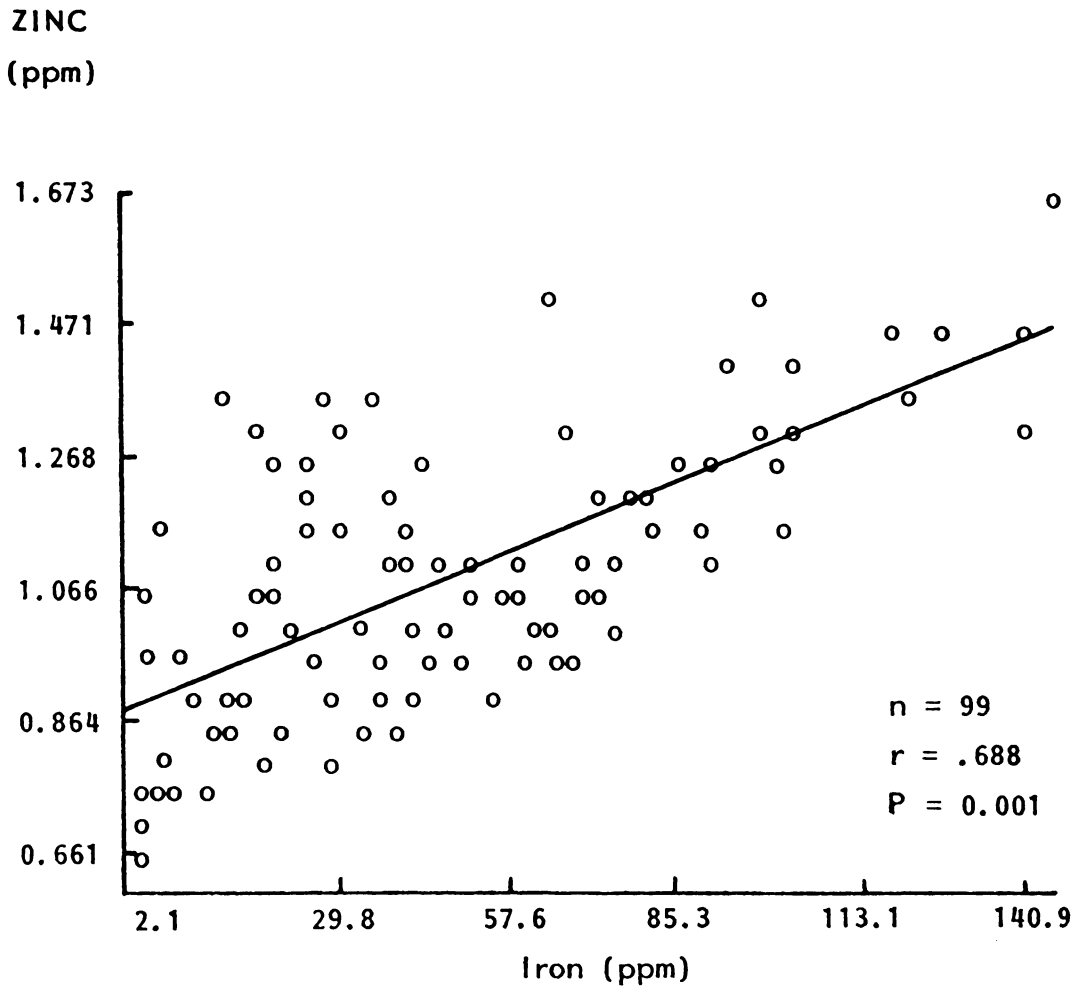


Figure 10. Bovine serum zinc concentrations correlated with the serum iron concentrations (hemolysis).

Zinc $\bar{X}$ = 1.11 ppm, SD=0.21 ppm; iron $\bar{X}$ = 48.2 ppm, SD=34.6 ppm; slope=4.10.

DISCUSSION

The elements calcium, copper, iron, magnesium, potassium, phosphorus, sodium and zinc are routinely detected in bovine serum by the ICP method.[33] The phosphorus concentration, when determined by the ICP, represents total serum phosphorus, not just inorganic phosphorus as commonly measured in serum. The negative correlations and the r-values (Table 7) indicate the effect of hemolysis was one of simple dilution of the serum elements calcium, copper, magnesium and sodium with the contents of the erythrocytes. Almost all of the blood calcium is contained in the plasma and very little is contained in the erythrocytes.[37] The erythrocyte concentrations of copper and magnesium are nearly identical to the serum concentrations.[18] The bovine serum concentration of sodium is approximately 2 times the erythrocyte sodium concentration. The positive correlations and r-values (Table 7) were the effects of adding the erythrocyte contents of iron, phosphorus, potassium and zinc to the serum. The ratio of hemoglobin-iron in the erythrocyte to transferrin iron in the plasma is $1000:1^{2}$ accounting for the near perfect correlation (Table 7) between the serum hemoglobin concentration and the serum iron concentration. The total phosphorus concentration in the human erythrocyte is approximately 5 times the total phosphorus concentration in the plasma.[18] The potassium concentration in the bovine erythrocyte is approximately 6 times the serum potassium concentration.[13,18] All the zinc in the erythrocyte is found in the carbonic anhydrase enzyme[20] and the ratio between the erythrocyte zinc concentration and the serum zinc concentration is 5:1.[18]

The effects of hemolysis on the clinical interpretation of bovine serum element concentrations were evaluated by correlating serum iron concentrations with other serum element concentrations. Because there was a high correlation (0.973) between serum hemoglobin and serum iron concentrations, the effects of hemolysis on serum element concentrations could also have been assessed in relation to serum hemoglobin changes.

When 11 g/dl is considered as the normal hemoglobin concentration for bovine whole blood,[37] a serum value of 5.2 g/dl hemoglobin (Figure 2) represented the lysis of approximately 50% of the erythrocytes and correlated with a serum iron concentration of 140 ppm. A serum hemoglobin concentration of 1.0 g/dl represented the lysis of approximately 9% of the erythrocytes and correlated with a serum iron concentration of 29 ppm. In a survey of more than 600 bovine serum analyses performed in our laboratory, 99% of the serum iron concentrations were less than 30 ppm and 95% of the samples contained less than 15 ppm iron.

The amount of decrease in serum calcium concentrations caused by hemolysis can be predicted from the correlation line (Figure 3). When the serum iron concentration remains below 30 ppm, the amount of decrease in serum calcium concentrations would be less than 5 ppm. This small decrease would not cause a change in the clinical interpretation of the serum calcium data. If hemolysis produced a serum iron concentration of greater than 100 ppm (35% hemolysis), the expected 10 ppm or greater decrease in the serum calcium concentration could cause the clinical interpretation of serum calcium concentrations to change from low normal to deficient.

The amount of decrease in serum copper concentrations caused by hemolysis can be predicted from the correlation line (Figure 4)

produced when serum copper concentrations were correlated with serum hemoglobin concentrations. When the serum hemoglobin concentration remains below 1 g/dl (30 ppm iron), the amount of decrease in serum copper will be less than 0.01 ppm. If the serum hemoglobin concentration reached 5.2 g/dl (140 ppm iron) the amount of decrease in serum copper concentration would be less than 0.2 ppm. This small decrease in serum copper concentration caused by 50% of the erythrocytes being lysed could cause the clinical interpretation of serum copper concentrations to change from low normal to deficient. There was no significant correlation ($P>0.05$) between serum copper and serum iron concentrations (Figure 5).

The amount of decrease in serum magnesium concentrations caused by hemolysis can be predicted from the correlation line (Figure 6) produced when serum magnesium concentrations were correlated with serum iron concentrations. The decrease of less than 1 ppm caused by lysis of 9% of the erythrocytes (30 ppm iron) would not effect the clinical interpretation of the magnesium concentration data. The clinical interpretation would not be changed until more than 50% of the erythrocytes (140 ppm iron) were lysed. At that point what would originally have been interpreted as a marginally deficient serum magnesium concentration would be decreased, by 3 ppm, into the deficient range.

From the correlation line (Figure 7) produced when serum phosphorus concentrations were correlated with serum iron concentrations, the serum phosphorus concentration was predicted to increase by 23 ppm when 50% of the erythrocytes were lysed (140 ppm iron). This amount of increase could cause a marginal concentration of phosphorus to increase into the

normal range. The large range for expected serum total phosphorus concentrations (139±44 ppm)[36] and the apparent lack of information concerning the cut-off values for marginal-to-deficient concentrations, prevent a precise interpretation of the effects of severe hemolysis on phosphorus. When the serum iron concentration remained below 30 ppm the predicted increase in serum phosphorus concentration was less than 5 ppm. This small increase would have no effect on the clinical interpretation of the serum total phosphorus concentration data.

The amount of increase in serum potassium concentrations caused by hemolysis can be predicted from the correlation line (Figure 8) produced when serum potassium concentrations were correlated with serum iron concentrations. An increase in serum iron concentration to 30 ppm correlated with an increase in serum potassium concentration of 10 ppm. This small change in potassium concentration would not cause a change in the clinical interpretation of the potassium concentration data. The large range of expected serum potassium concentration (180-270 ppm)[18] and the apparent lack of information concerning the cut-off values for marginal-to-deficient serum potassium concentrations, prevent a precise interpretation of the effect of hemolysis on the clinical interpretation of potassium when the serum iron concentration is between 30 ppm and 80 ppm. When the serum iron concentration is greater than 80 ppm, the predicted potassium concentration increase of 30 ppm or greater would be expected to affect the clinical interpretation of the potassium concentration data by causing marginal concentrations to increase into the normal range.

The amount of decrease in serum sodium concentrations can be predicted from the correlation line (Figure 9) produced when serum sodium

concentrations were correlated with serum iron concentrations. When the serum iron concentration is below 30 ppm, the decrease in the serum sodium concentration would be less than 70 ppm. With the large range of expected serum sodium concentrations (3250-3650 ppm)[18] a decrease of 70 ppm correlated with a serum iron concentration of 30 ppm would not be expected to cause a change in the clinical interpretation of the sodium concentration data. When the serum iron concentration is between 30 ppm and 60 ppm, the effect of the concurrent sodium concentration decrease of 185 ppm would be difficult to determine but when the serum iron concentration is greater than 60 ppm, the concurrent decrease in serum sodium concentration would be expected to cause a marginal sodium concentration to be in the deficient range.

The zinc versus iron correlation line (Figure 10) lies between the zinc concentrations of 0.86 ppm and 1.47 ppm. There was an increase in serum zinc concentration of 0.59 ppm correlated with the lysis of 50% of the erythrocytes (140 ppm iron). If a serum sample contained 0.2 ppm zinc without hemolysis, it would be possible to raise the zinc concentration into the normal range with 50% hemolysis, and thus change the clinical interpretation of the zinc concentration data. The segment of the correlation line (Figure 10), which lies between 2 ppm and 29 ppm iron, corresponds to a change in zinc concentration of .14 ppm (0.86 ppm-1.0 ppm). With the small change of .14 ppm, it would only be possible to raise a borderline concentration of zinc into the low normal range. The amount of hemolysis would have to be greater than 18% (60 ppm iron) to have an effect on the clinical interpretation of the serum zinc concentration data.

SUMMARY

Hemolysis produced significant ($P<0.05$) decreases in serum calcium, copper, magnesium and sodium and significant increases in serum phosphorus, potassium and zinc. A serum iron concentration of 29 ppm correlated to 1 g/dl of hemoglobin released by lysis of approximately 9% of the erythrocytes. The data indicate that when serum iron concentrations remain below 30 ppm, there is no effect on the clinical interpretation of the element concentration data for calcium, copper, magnesium, phosphorus, potassium, sodium and zinc.

EXPERIMENT 3

Effects of serum-to-clot contact time on element concentration in adult bovine serum samples.

Materials and Methods

Six adult Holstein animals consisting of one bull, one freemartin and 4 lactating cows were selected as blood donors from the Michigan State University teaching animals. Five hundred milliliters of blood were collected from the jugular vein of each animal using a 3-inch, 10 gauge, stainless steel needle.[j] The blood was collected in glass, acid-washed, culture tubes[l] and stored at room temperature. Some of serum from each animal was separated from the clot and prepared for analysis at 4, 8, 24, 48, 72, 96, 120 and 144 hours postcollection. The rest of the serum from each animal was separated from the clot at 4 hours postcollection and maintained in the initial glass collection tubes for 144 hours postcollection before being prepared for analysis. All samples were centrifuged and any hemolyzed samples were discarded.

Five 1-ml aliquots of serum from each cow for each time period were analyzed utilizing the ICP[33] and the mean concentration for each element was calculated for each group of 5 aliquots. A correlation analysis was conducted to determine the influence of serum-to-clot contact time upon the concentrations of serum elements. The paired-t test was conducted to determine the significance of the difference in element concentrations between the serum that was in contact with the clot and the glass collection tubes for 4 hours and the element concentrations in the

serum that was in contact with the glass collection tubes, without the clot, for 144 hours.

RESULTS

The data are presented in Table 8 and Figures 11-18. Serum phosphorus and potassium concentrations correlated significantly ($P<0.05$) with serum-to-clot contact time. Serum calcium, copper, iron, magnesium, sodium and zinc were unaffected ($P>0.05$) by serum-to-clot contact time.

There was a significant decrease ($P<0.01$) in the serum zinc concentration for the samples maintained in contact with the initial glass collection tubes for 144 hours when compared to the zinc concentrations in the serum samples with only 4 hours of sample container contact. There were no significant differences ($P>0.05$) between the paired concentrations (144 hour contact versus 4 hour contact) for the elements calcium, copper, iron, magnesium, phosphorus, potassium and sodium.

Table 8. Summary of correlation analyses of bovine serum-to-clot contact time (range 4-144 hours) with serum element concentrations.

Element	Time Periods n	Element/Time r	Element/Time P-Value
Calcium	8	.263	NS[a]
Copper	8	-.537	NS
Iron	8	-.341	NS
Magnesium	8	.588	NS
Phosphorus[b]	8	.771	0.05
Potassium	8	.982	0.001
Sodium	8	-.653	NS
Zinc	8	.130	NS

[a]NS=P>0.05.

[b]Total phosphorus.

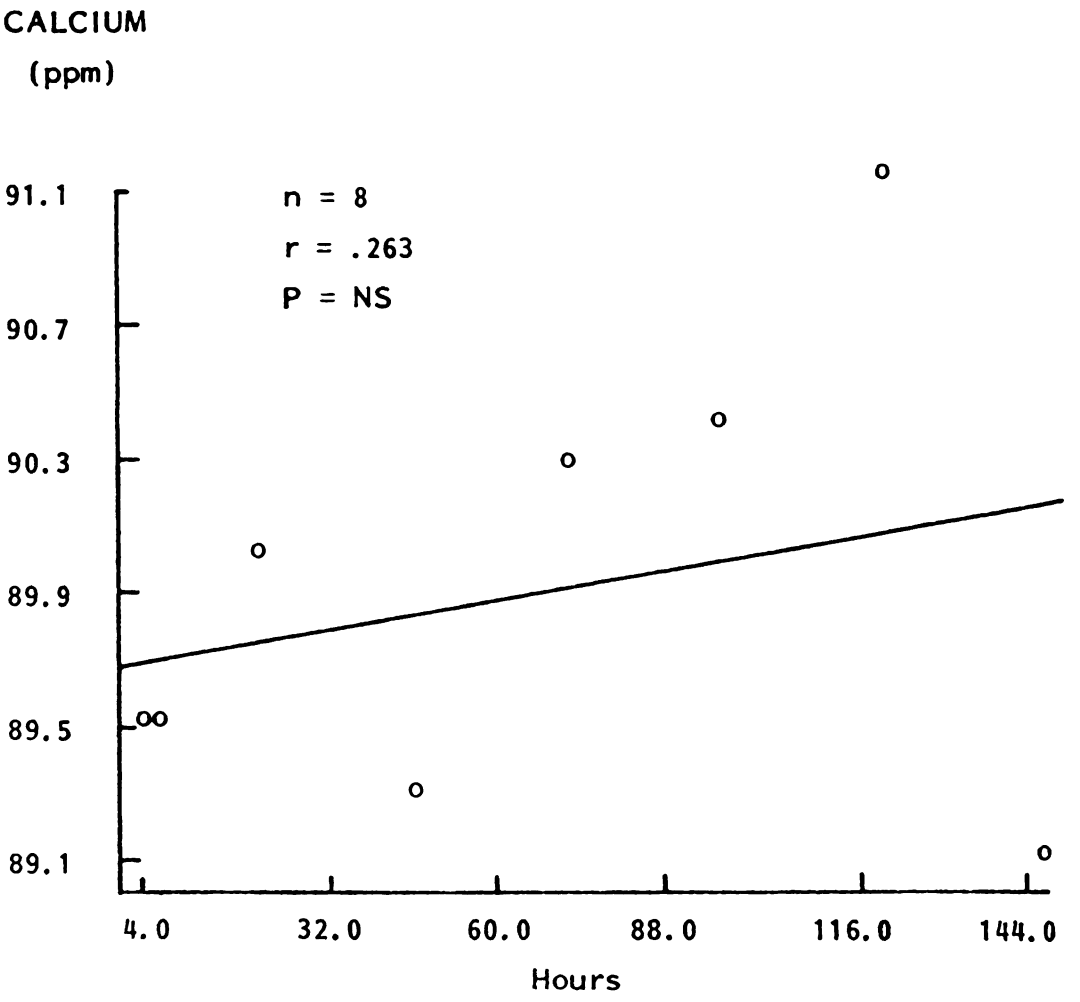


Figure 11. Bovine serum calcium concentrations correlated with the amount of time the serum remained in contact with the clot.

Calcium $\bar{X}$ = 89.9 ppm, SD=0.63 ppm; time $\bar{X}$ = 64.5 hours, SD=49.0 hours; slope=3.39.

NS=P>0.05.

0=mean calcium concentration of 6 animals at each time period.

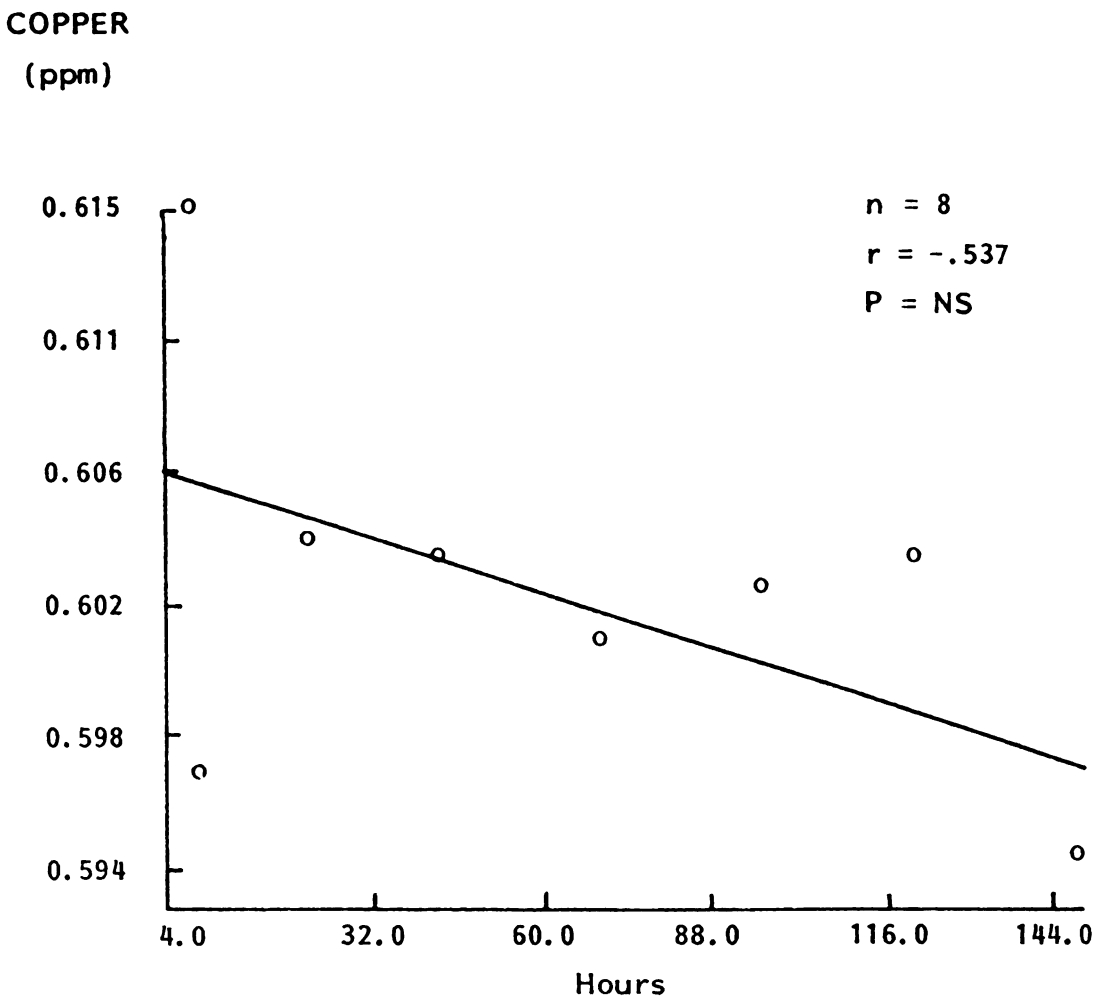


Figure 12. Bovine serum copper concentrations correlated with the amount of time the serum remained in contact with the clot.

Copper $\bar{X}$ = 0.603 ppm, SD=0.006 ppm; time $\bar{X}$ = 64.5 hours, SD=49.0 hours; slope=-6.31.

NS=P>0.05.

o=mean copper concentration of 6 animals at each time period.

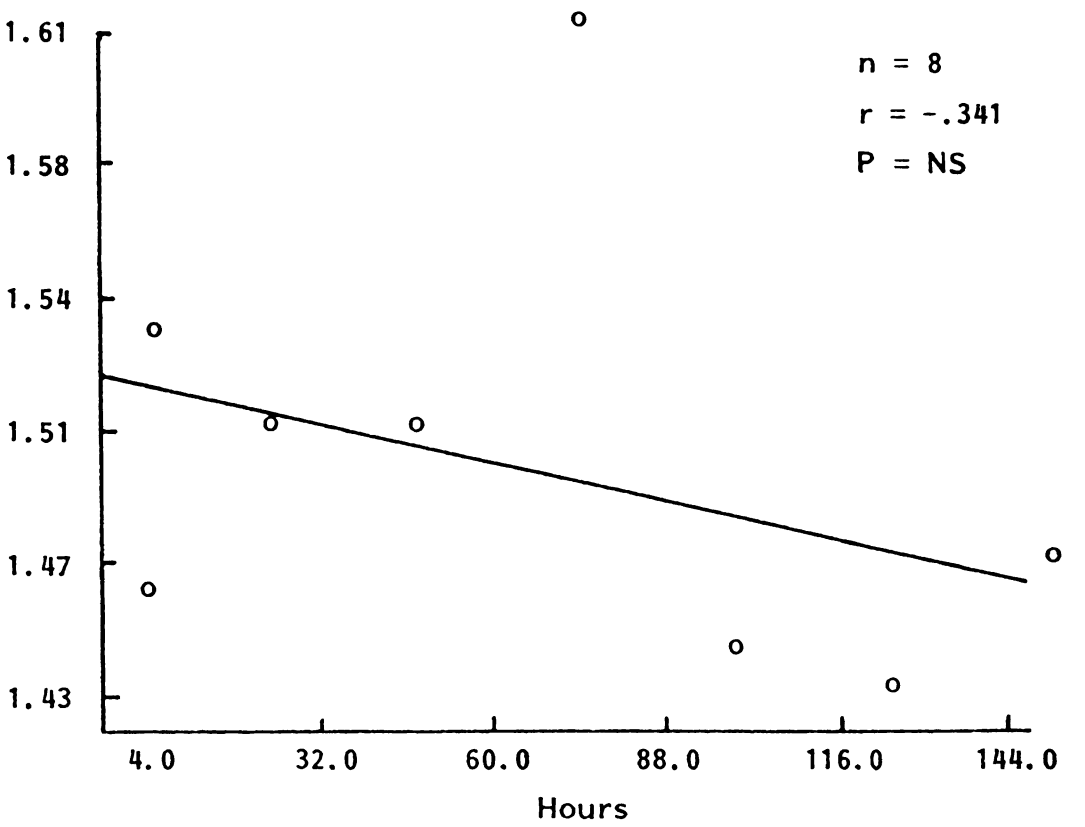


Figure 13. Bovine serum iron concentrations correlated with the amount of time the serum remained in contact with the clot.

Iron $\bar{X}$ = 1.499 ppm, SD=0.054 ppm; time $\bar{X}$ = 64.5 hours, SD=49.0 hours; slope=-3.75.

NS=P>0.05.

o=mean iron concentration of 6 cows at each time period.

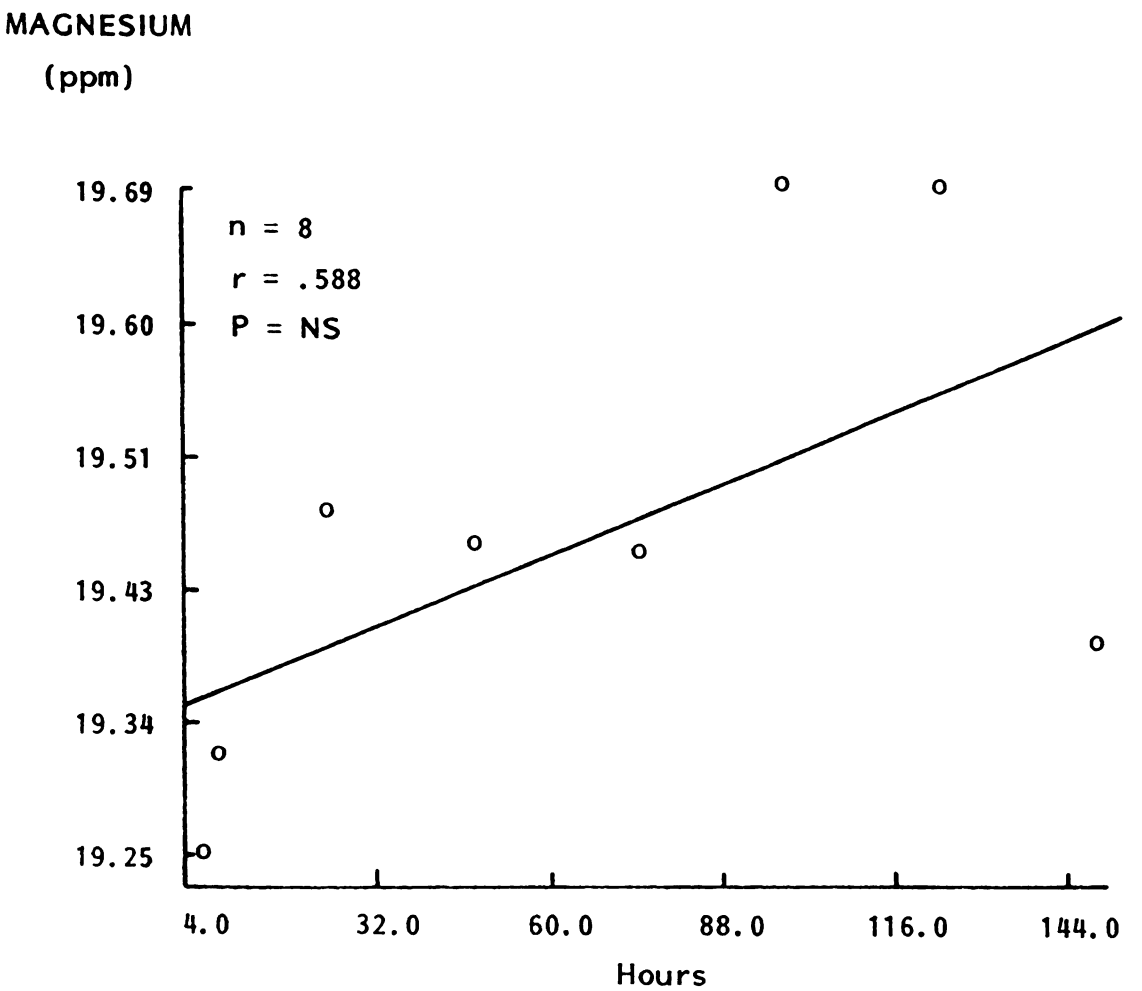


Figure 14. Bovine serum magnesium concentrations correlated with the amount of time the serum remained in contact with the clot.

Magnesium $\bar{X}$= 19.47, SD=0.15 ppm; time $\bar{X}$= 64.5 hours, SD=49.0 hours; slope=1.78.

NS=P>0.05.

o=mean magnesium concentration of 6 animals at each time period.

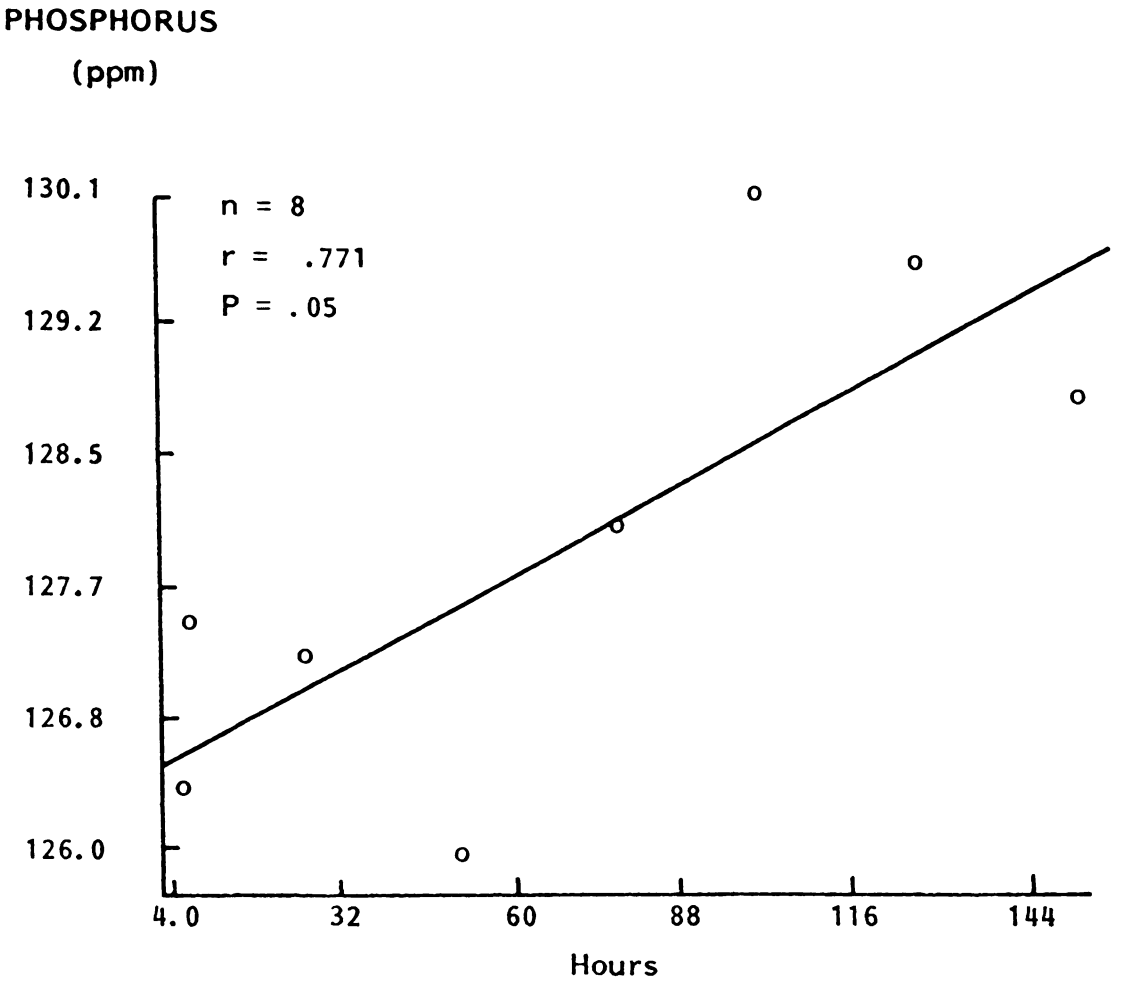


Figure 15. Bovine serum phosphorus concentrations correlated with the amount of time the serum remained in contact with the clot.

Phosphorus $\bar{X}$= 128.1 ppm, SD=1.4 ppm; time $\bar{X}$= 64.5 hours, SD=49.0 hours; slope=0.022.

o=mean phosphorus concentration of 6 animals at each time period.

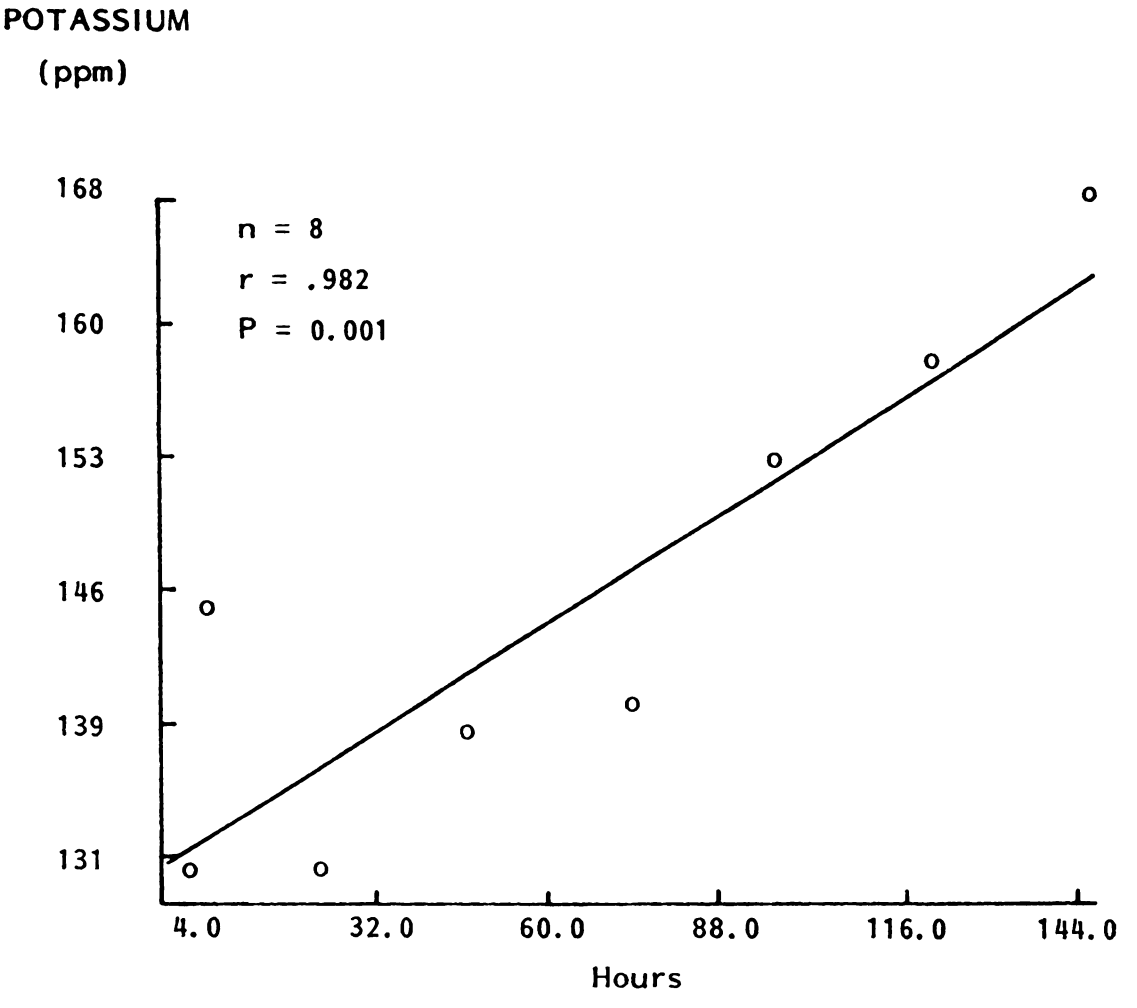


Figure 16. Bovine serum potassium concentrations correlated with the amount of time the serum remained in contact with the clot.

Potassium $\bar{X}$= 144.9 ppm, SD=13.0 ppm; time $\bar{X}$= 64.5 hours, SD=49.0 hours; slope=0.22.

o=mean potassium concentration of 6 animals at each time period.

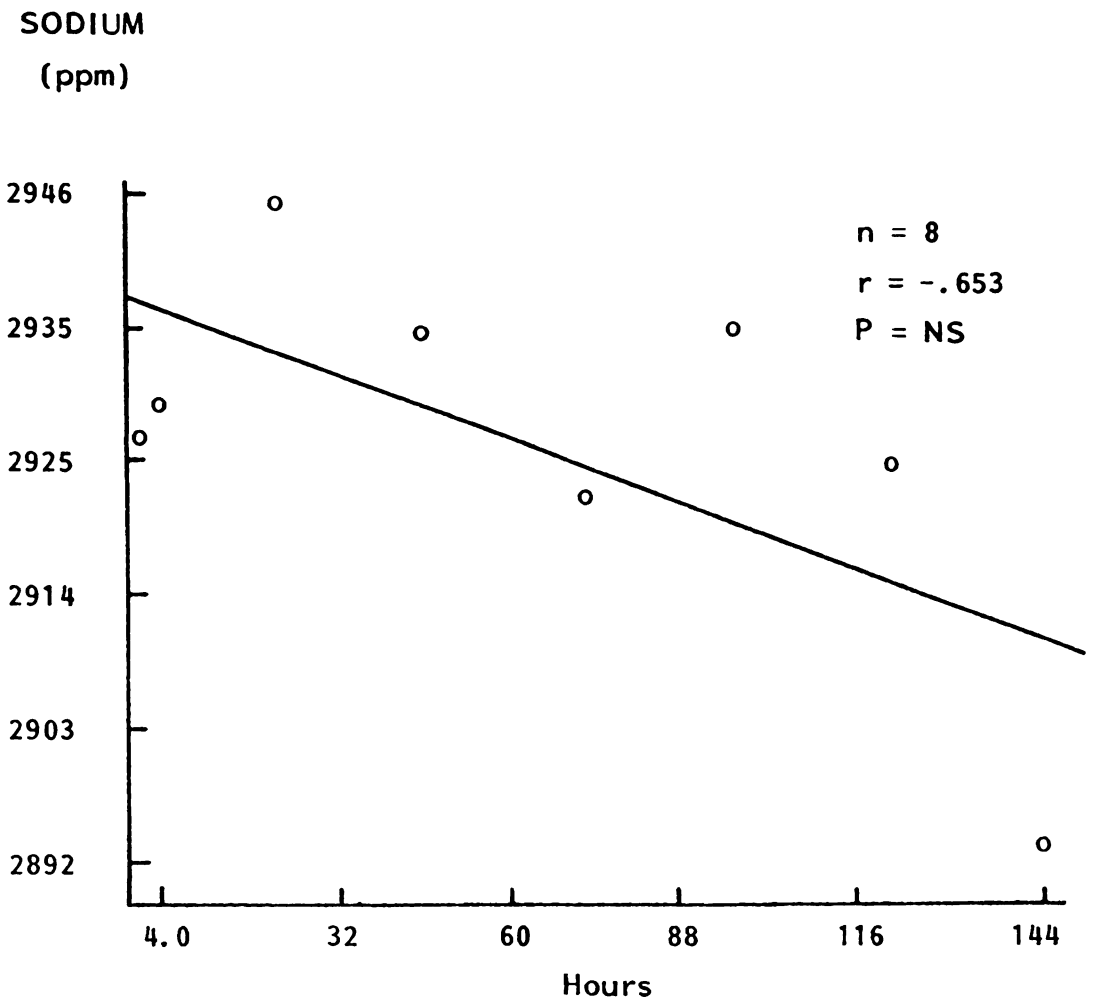


Figure 17. Bovine serum sodium concentrations correlated with the amount of time the serum remained in contact with the clot.

Sodium $\bar{X}$ = 2928 ppm, SD=14.9 ppm; time $\bar{X}$ = 64.5 hours, SD=49.0 hours; slope=-0.198.

NS=P>0.05.

o=mean sodium concentration of 6 animals at each time period.

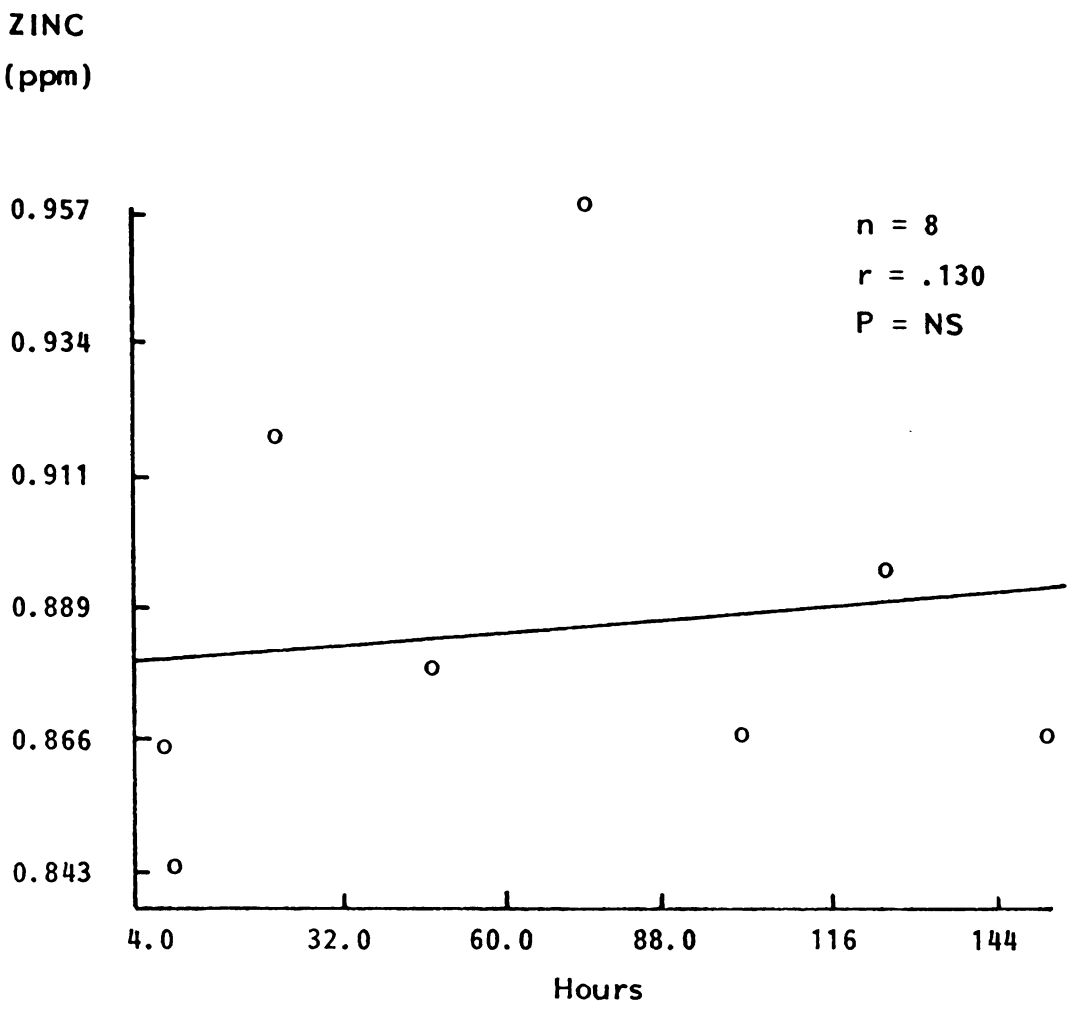


Figure 18. Bovine serum zinc concentrations correlated with the amount of time the serum remained in contact with the clot.

Zinc $\bar{X}$= 0.888 ppm, SD=0.033 ppm; time $\bar{X}$= 64.5 hours, SD=49.0 hours; slope=8.85.

NS=P>0.05.

o=mean zinc concentration of 6 animals at each time period.

DISCUSSION

Changes in the concentrations of elements in serum associated with serum-to-clot contact time may be attributed to elements adhering to the container wall, the cellular membranes or the fibrin portion of the clot. The elements may also diffuse across the cell membranes either into, or out of, the cellular components of the clot. The ratio of red blood cells to white blood cells is usually such that only the element concentrations in the erythrocytes and serum need to be considered in this study. The significant decrease ($P<0.01$) in only serum zinc concentrations for the (4 hour-144 hour) container-contact samples indicates that there was an apparent net loss of serum zinc to the container wall and all other element concentration changes were the result of the interaction between the serum and the clot.

Table 9 demonstrates the element concentrations in both the erythrocyte and the serum or plasma and the ratios of these concentrations. From these data, it should be possible to predict the direction of change for the serum element concentration as a result of diffusion across a concentration gradient. On this basis, the influence of serum-to-clot contact time on phosphorus and potassium was anticipated (Table 8) (Figures 15 and 16). It was not determined in this study whether the increase in serum phosphorus concentration with increasing clot contact time was due to inorganic phosphorus or both organic and inorganic phosphorus diffusion out of the erythrocyte. The small increase (3 ppm) in serum total phosphorus concentration, as predicted from the correlation line (Figure 15), would not cause a change in the clinical interpretation of

Table 9. Element concentrations in the erythrocyte and in plasma or serum with the calculated concentration ratios.

Element	RBC	Plasma or Serum	RBC/ Serum Ratio
Calcium[a,1]	2 mg/dl	9.8 mg/dl	1:4.9
Copper[a,1]	115 µg/dl	119 µg/dl	1:1.03
Iron[a,1]	2.5 µg/dl (non-hemoglobin)	105 µg/dl	1:42
Magnesium[a,1]	4.5 mg/dl	2.1 mg/dl	2.14:1
Magnesium[b,1]	1.5 mg/dl	1.96 mg/dl	1:1.3
Phosphorus[a,c,1]	52 mg/dl	11.4 mg/dl	4.6:1
Potassium[a,1]	437 mg/dl	16 mg/dl	27:1
Potassium[b,1]	150 mg/dl	22 mg/dl	6.8:1
Potassium[b,2]	22 mM/liter (85 mg/dl)	5 mM/liter (19.5 mg/dl)	4.4:1
Sodium[b,1]	180 mg/dl	330 mg/dl	1:1.8
Sodium[b,2]	88 mM/liter (200 mg/dl)	145 mM/liter (333 mg/dl)	1:1.6
Zinc[a,1]	1500 µg/dl	300 µg/dl	5:1

[a]Human.

[b]Bovine.

[c]Total phosphorus

[1](Altman et al.)

[2](Hays et al.)

serum total phosphorus concentration data because of the large range for expected concentrations (139±44 ppm)[36] and the lack of a cut-off value for marginal-to-deficient concentrations. Comparing the predicted increase (30 ppm) (Figure 16, correlation line) in serum potassium concentration with the reported normal range (180 ppm to 270 ppm),[18] it can be seen that marginally low serum potassium concentrations could be increased by prolonged serum-to-clot contact time to values well within the expected range. The large difference between the 4-hour mean potassium concentration (131 ppm) (Figure 16) and the reported normal range (180 ppm to 270 ppm) is probably due to the small number of animals (6 cows) used in this study as compared to the 100 animals used in the referenced study.

SUMMARY

Serum concentrations of calcium, magnesium and zinc tended to be increased while serum phosphorus and potassium were significantly increased by prolonged serum-to-clot contact time. The serum-to-clot contact time effect on serum potassium could be sufficient to alter the clinical interpretation of serum potassium concentrations. Serum concentrations of copper, iron and sodium tended to be decreased by extended serum-to-clot contact time. However, neither ion was affected sufficiently by clot contact to alter clinical interpretations of their serum concentrations.

APPENDIX

APPENDIX

Liver element concentrations in individual core samples.

Table A1. Liver cadmium concentrations in individual core samples (ppm wet weight).

	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	Cow 7	Cow 8	Cow 9	Cow 10
	.2551	NDA	.1609	.1985	.1761	NDA	NDA	.2598	.1344	NDA
Area	.3502	NDA	.1659	.2521	.1855	NDA	NDA	.2315	.1158	NDA
	.4043	NDA	.1933	.2589	.1850	NDA	NDA	.2504	.1140	NDA
A	.3586	NDA	.1564	.2233	.1565	NDA	NDA	.2514	.1117	NDA
	.3803	NDA	.1636	.2167	.1511	NDA	NDA	.2254	.1046	NDA
	.3252	NDA	.1926	.2373	.1412	NDA	NDA	.2866	.1081	NDA
Area	.3727	NDA	.1951	.2505	.1474	NDA	NDA	.2423	.0865	NDA
	.3311	NDA	.1823	.2015	.1842	NDA	NDA	.2398	.1135	NDA
B	.3717	NDA	.2006	.2559	.1412	NDA	NDA	.2607	.1045	NDA
	.3893	NDA	.1745	.2242	.1718	NDA	NDA	.2927	.1269	NDA
	.3296	NDA	.1549	.2631	.1428	NDA	NDA	.2278	.1165	NDA
Area	.3310	NDA	.1664	.2174	.1262	NDA	NDA	.2087	.1315	NDA
	.3943	NDA	.1773	.2313	.1217	NDA	NDA	.2221	.1333	NDA
C	.3793	NDA	.1719	.2379	.1314	NDA	NDA	.2317	.1133	NDA
	.2824	NDA	.1600	.2277	.1315	NDA	NDA	.2446	.1299	NDA

NDA--Non-detectable amounts.

Table A2. Liver calcium concentrations in individual core samples (ppm wet weight).

	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	Cow 7	Cow 8	Cow 9	Cow 10
	50.45	31.61	36.73	54.82	40.06	46.54	41.19	34.69	36.99	39.86
Area	49.07	32.85	34.97	57.18	39.85	45.15	39.14	34.03	39.61	42.61
	54.47	30.55	33.40	53.21	41.45	44.66	39.69	34.20	41.09	42.83
A	53.29	30.81	37.41	55.01	47.70	45.79	38.96	35.26	38.14	44.59
	54.66	28.58	37.93	51.04	44.34	48.07	39.52	35.45	35.05	47.00
	48.77	30.87	38.30	60.40	45.34	41.02	41.42	41.56	40.20	41.58
Area	48.86	35.11	40.52	55.64	46.73	64.69	36.69	34.20	38.37	44.47
	51.15	30.85	36.46	59.47	45.37	46.12	37.31	36.68	37.31	43.87
B	49.47	32.34	35.27	55.62	48.84	45.79	38.67	34.26	35.61	39.86
	48.44	30.60	37.61	55.77	46.43	42.62	37.95	35.17	36.41	40.96
	49.80	32.20	41.81	57.55	51.33	42.50	38.51	36.97	43.97	39.02
Area	50.13	33.87	40.24	62.87	57.01	41.86	38.47	35.21	41.14	43.10
	48.94	31.65	39.05	60.24	66.40	51.83	38.16	36.63	36.92	44.06
C	45.19	30.22	39.74	55.58	57.66	45.29	36.71	34.25	36.95	38.61
	43.62	30.25	37.85	54.21	50.89	66.84	34.48	34.65	40.74	43.13

Table A3. Liver copper concentrations in individual core samples (ppm wet weight).

	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	Cow 7	Cow 8	Cow 9	Cow 10
	41.68	86.36	34.68	5.375	32.38	117.9	16.46	3.211	144.7	2.071
Area	66.49	81.32	33.53	6.292	30.84	175.3	17.11	3.010	125.0	2.046
	64.27	84.06	34.59	5.984	29.34	183.7	18.12	3.248	119.8	1.802
A	67.44	90.05	36.27	5.426	46.15	192.2	17.52	2.761	131.1	1.970
	60.49	85.00	32.12	5.799	37.88	176.7	15.32	2.701	141.1	1.894
	41.86	77.73	36.88	6.557	40.49	160.3	18.25	2.644	119.4	1.808
Area	67.93	111.40	37.32	6.555	38.21	154.1	18.80	2.967	132.1	1.739
	31.63	85.19	35.44	5.362	34.86	190.2	17.46	2.990	140.6	1.818
B	68.74	92.33	35.05	5.281	46.32	188.9	17.37	2.761	126.6	1.641
	82.33	73.84	39.29	6.585	47.19	197.4	17.89	2.740	144.5	1.661
	42.52	92.32	32.97	7.773	24.79	165.1	25.52	2.884	162.1	1.715
Area	46.62	74.40	34.67	9.617	44.62	153.8	21.30	3.031	145.6	1.712
	56.54	74.27	41.31	8.568	33.48	141.7	19.69	3.042	147.0	1.657
C	60.65	85.04	43.75	9.011	28.62	164.9	22.37	2.868	139.8	1.525
	52.12	81.99	37.58	8.439	42.76	141.3	19.79	2.748	135.6	1.622

Table A4. Liver iron concentrations in individual core samples (ppm wet weight).

	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	Cow 7	Cow 8	Cow 9	Cow 10
	133.5	86.57	149.2	120.8	115.0	132.2	78.03	219.9	105.70	188.0
Area	186.7	75.50	150.4	109.2	117.8	130.0	67.32	159.0	90.26	199.4
	201.2	86.81	137.2	148.6	125.1	127.8	65.73	183.0	104.60	213.4
A	189.3	86.75	155.1	147.1	161.1	127.1	68.43	188.1	87.80	203.2
	188.7	84.64	151.1	120.8	124.0	121.3	72.92	190.6	102.5	211.0
	131.9	88.54	141.6	133.4	109.3	118.1	87.05	225.5	111.2	197.0
Area	178.0	78.91	160.3	126.5	121.5	116.1	73.12	163.3	122.2	190.2
	135.8	120.10	145.9	134.3	118.3	144.1	78.22	178.9	98.96	208.3
B	176.8	97.29	148.1	147.1	132.3	125.1	72.09	186.5	111.6	194.6
	189.6	79.97	145.0	129.7	120.1	153.2	75.84	189.3	105.9	193.0
	157.3	81.57	145.9	102.2	130.3	140.4	100.5	193.5	137.6	224.5
Area	141.5	78.10	144.7	125.6	125.8	132.6	80.01	141.2	109.4	235.0
	164.5	86.50	152.2	105.9	137.8	128.8	83.34	170.5	123.6	203.0
C	163.4	82.18	135.1	94.99	137.7	124.0	83.16	158.2	123.3	188.9
	142.0	79.01	138.1	104.1	131.6	118.5	80.06	171.2	108.6	214.3

Table A5. Liver magnesium concentrations in individual core samples (ppm wet weight).

	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	Cow 7	Cow 8	Cow 9	Cow 10
Area A	162.6	150.4	105.8	131.4	166.4	131.1	162.3	157.6	135.6	149.9
	158.6	146.3	106.0	138.3	165.1	139.6	163.2	151.3	131.7	152.5
	171.5	134.0	98.79	138.3	167.5	140.2	159.4	144.5	130.4	142.2
	167.9	142.4	106.1	136.3	141.6	141.5	160.7	148.8	130.7	145.9
	163.0	145.0	107.5	134.5	156.8	132.7	166.1	143.7	128.6	153.8
Area B	161.2	139.4	102.0	139.1	153.9	120.1	165.3	160.5	128.7	147.7
	156.3	126.0	90.45	136.2	159.6	134.4	158.1	145.2	125.2	145.1
	174.9	124.5	103.1	135.7	156.5	131.7	155.9	148.1	131.0	146.0
	161.5	136.4	84.39	140.3	156.5	134.9	160.1	145.0	126.8	133.3
	150.4	130.9	106.9	146.8	151.2	133.3	158.1	147.4	126.2	131.9
Area C	168.4	131.2	95.67	142.8	155.8	136.7	153.8	157.5	130.1	122.9
	169.9	139.8	91.12	137.6	156.7	130.8	154.2	147.9	133.3	130.5
	169.8	127.5	100.2	145.3	153.2	133.6	152.2	149.5	128.4	138.0
	170.9	130.8	105.1	148.6	146.9	131.7	151.7	147.8	131.0	119.4
	167.8	127.4	87.86	144.9	153.5	137.8	152.6	143.9	127.6	135.5

Table A6. Liver manganese concentrations in individual core samples (ppm wet weight).

	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	Cow 7	Cow 8	Cow 9	Cow 10
	2.633	2.651	1.379	1.139	1.415	1.611	1.503	1.969	2.184	1.184
Area	2.141	2.663	1.366	2.690	1.380	1.730	1.490	1.832	2.242	1.239
	2.098	2.424	1.328	1.189	1.309	1.737	1.483	1.836	2.204	1.143
A	2.388	2.503	1.416	1.127	1.184	1.663	1.250	1.882	2.260	1.235
	2.093	2.665	1.730	1.121	1.300	1.650	1.460	1.867	2.213	1.307
	2.419	2.610	1.527	1.263	1.366	1.369	1.511	2.022	2.188	1.161
Area	2.356	2.239	1.377	1.217	1.306	1.551	1.430	1.677	2.178	1.193
	2.843	2.193	1.491	1.149	1.271	1.497	1.357	1.697	2.319	1.273
B	2.214	2.513	1.362	1.123	1.110	1.621	1.379	1.642	2.101	1.129
	2.131	2.441	1.535	1.329	1.129	1.598	1.375	1.790	2.140	1.125
	2.329	2.438	1.220	1.382	1.304	1.701	1.233	1.977	2.197	1.069
Area	2.537	2.475	1.161	1.282	1.413	1.579	1.108	1.747	2.244	1.123
	2.268	2.344	1.490	1.428	1.301	1.472	1.270	1.804	2.236	1.230
C	2.161	2.500	1.478	1.454	1.396	1.510	1.315	1.745	2,346	1.060
	2.532	2.310	1.237	1.366	1.327	1.649	1.301	1.798	2.289	1.141

Table A7. Liver molybdenum concentrations in individual core samples (ppm wet weight).

	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	Cow 7	Cow 8	Cow 9	Cow 10
	.432	1.163	.492	.772	.705	1.045	.490	.641	.928	.414
Area	.467	1.138	.480	.791	.721	1.016	.595	.723	.934	.428
	.517	1.067	.475	.828	.699	1.057	.580	.645	.907	.335
A	.482	1.115	.551	.786	.624	1.085	.578	.646	.923	.449
	.465	1.137	.471	.778	.663	1.001	.607	.601	.903	.434
	.388	1.145	.476	.898	.661	.954	.505	.571	.876	.394
Area	.437	1.198	.469	.821	.683	1.001	.599	.679	.885	.389
	.441	1.091	.507	.798	.670	1.039	.568	.674	.922	.401
B	.453	1.169	.479	.746	.642	1.018	.567	.596	.857	.345
	.462	1.069	.517	.901	.661	1.015	.562	.587	.911	.362
	.421	1.173	.419	.882	.608	.978	.472	.542	.959	.369
Area	.411	1.095	.414	.802	.641	.990	.586	.673	.947	.320
	.445	1.071	.471	.920	.641	.906	.545	.649	.956	.351
C	.478	1.105	.454	.886	.688	.984	.527	.600	.935	.370
	.480	1.048	.478	.906	.641	1.008	.528	.587	.947	.374

Table A9. Liver potassium concentrations in individual core samples (ppm wet weight).

	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	Cow 7	Cow 8	Cow 9	Cow 10
Area A	3247	3231	2168	2227	3135	2333	2803	2629	2605	3266
	2932	3077	2276	2267	3240	2515	2952	2571	2410	3384
	3135	2901	2153	2317	3118	2618	2897	2601	2486	3266
	3135	3189	2284	2238	2975	2734	3045	2650	2520	3342
	3115	3259	2223	2292	3129	2477	3125	2628	2591	3392
Area B	3137	2919	2108	2381	2862	2218	2825	2690	2286	3032
	2925	2702	2106	2359	3058	2700	2872	2516	2370	3170
	3343	2803	2249	2357	3045	2445	2923	2582	2529	3292
	3063	2993	2071	2350	2911	2542	2975	2637	2515	3000
	2883	2979	2392	2386	2964	2492	3012	2665	2532	3024
Area C	3172	2874	2072	2526	2850	2331	2735	2703	2474	2653
	3316	2761	1965	2392	2679	2591	2845	2581	2521	2889
	3389	2876	2096	2476	2815	2493	2878	2664	2646	2929
	3241	2982	2233	2567	2716	2556	2965	2731	2632	2740
	3244	2940	2021	2499	2819	2646	3007	2685	2506	2972

Table A8. Liver phosphorus concentrations in individual core samples (ppm wet weight).

	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	Cow 7	Cow 8	Cow 9	Cow 10
	3266	3487	2503	3035	3354	3351	3411	3180	2845	3109
Area	3311	3437	2566	3005	3324	3526	3342	3093	3079	3234
	3532	3234	2313	3110	3204	3469	3348	2906	3088	3090
A	3512	3313	2637	3055	3029	3267	3360	2966	3085	3142
	3420	3453	2504	2985	3335	3229	3395	2935	3016	3373
	3247	3461	2391	3129	3142	2875	3519	3131	3109	3001
Area	3259	2991	2306	2938	3201	2569	3361	2960	2981	3137
	3594	3097	2470	3064	3313	3429	3279	3016	3164	3204
B	3299	3367	2251	3395	3211	3534	3306	2835	3011	2945
	3137	3306	2611	3403	3122	3523	3325	2920	3028	2954
	3320	3241	2223	3309	2862	3626	3369	3212	3013	2851
Area	3372	3200	2170	3129	3137	3100	3315	3040	2940	2946
	3384	3028	2374	3326	2875	2486	3217	3029	3081	2856
C	3475	3315	2469	3285	3062	3062	3311	2964	3136	2545
	3441	2985	2225	3222	3211	2537	3224	2925	3248	2922

Table A10. Liver sodium concentrations in individual core samples (ppm wet weight).

	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	Cow 7	Cow 8	Cow 9	Cow 10
	1262	888	1018	1663	1203	1260	717	872	1273	1346
Area	1294	895	979	1721	1216	1271	749	868	1296	1401
A	1297	937	897	1776	1271	1261	727	884	1299	1292
	1302	905	986	1762	1316	1277	762	909	1186	1346
	1333	933	987	1695	1251	1292	791	886	1169	1380
	1232	979	909	1566	1264	1204	757	904	1201	1321
Area	1292	1016	999	1590	1318	1421	778	931	1209	1273
B	1207	984	935	1634	1356	1305	771	943	1184	1250
	1200	926	905	1686	1325	1286	772	946	1201	1150
	1419	945	956	1545	1316	1245	784	952	1174	1134
	1256	890	1089	1529	1450	1190	733	865	1270	1222
Area	1274	928	1037	1539	1493	1313	745	842	1234	1298
C	1232	922	1038	1641	1537	1433	745	873	1128	1263
	1174	940	1052	1510	1547	1363	747	883	1075	1183
	1168	927	1017	1573	1446	1386	743	881	1113	1241

Table A11. Liver zinc concentrations in individual core samples (ppm wet weight).

	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6	Cow 7	Cow 8	Cow 9	Cow 10
	166.6	27.44	33.69	118.9	74.98	83.22	50.33	93.24	33.69	206.8
Area	208.1	27.94	33.76	142.7	75.31	80.18	51.56	92.22	33.11	212.1
	206.4	25.54	34.51	142.6	72.92	82.83	50.34	92.44	32.74	163.0
A	189.8	27.14	32.63	140.2	65.60	95.91	50.36	92.44	32.53	223.5
	185.7	26.24	36.20	122.4	67.19	80.43	52.12	88.22	31.86	234.0
	161.7	27.51	38.23	126.8	72.15	84.43	47.48	96.90	31.15	187.1
Area	190.9	25.70	34.11	122.4	76.20	107.8	49.61	90.36	33.77	152.0
	161.6	24.30	32.96	114.7	73.59	85.98	48.41	91.92	32.62	168.8
B	202.1	26.60	30.87	129.9	69.63	80.75	48.07	90.63	30.90	161.0
	195.2	24.82	38.52	125.0	71.13	87.94	48.37	95.63	31.29	172.6
	186.6	25.86	29.10	142.3	72.84	84.52	45.21	88.90	32.91	130.9
Area	201.9	24.67	27.98	117.3	72.18	74.77	47.97	84.69	34.70	136.3
	198.4	23.89	35.80	133.0	65.67	87.75	47.10	87.39	34.14	142.8
C	198.0	25.26	34.73	140.6	66.93	89.83	47.15	88.73	35.07	131.8
	189.1	24.30	27.96	134.6	67.50	90.50	47.39	90.00	34.27	153.3

LIST OF REFERENCES

LIST OF REFERENCES

1. Mertz W: The essential trace element. Science 213:1332-1338, 1981.

2. Underwood EJ: Trace elements in human and animal nutrition, 4th Edition, Academic Press, NY, 1977.

3. Kaldor I: Studies on intermediary iron metabolism. V. The measurement of non-haemoglobin tissue iron. Austral J exp Bio 32:795-800, 1954.

4. Frey WG, Gardner MH, Pillsburg JA: Quantitative measurement of liver iron by needle biopsy. J Lab Clin Med 72:52-57, 1968.

5. Barry M, Sherlock S: Measurement of liver iron concentrations in needle-biopsy specimens. Lancet 16:100-103, 1971.

6. Cassidy J, Eva JK: The variations in concentrations of copper and iron within and between lobes of pig's liver. Proc Nutr Soc 17:30, 1958.

7. Webb J, Kirk KA, Niedermeier W, Griggs JH, Turner ME, James TN: Distribution of 13 trace metals in pig heart tissue. Bioinorg Chem 3:61-75, 1973.

8. Webb J, Kirk KA, Niedermeier W, Griggs JH, Turner ME, James TN: Distribution of 8 trace metals in beef heart tissue. Bioinorg Chem 5:253-259, 1976.

9. Webb J, Kirk KA, Niedermeier W, Griggs JH, Turner ME, James TN: Distribution of 9 trace metals in 17 anatomical sites of dog heart tissue and their classification using pattern recognition. Bioinorg Chem 7:303-314, 1977.

10. Blood DC, Henderson JA: Veterinary Medicine, 4th Edition, Williams and Wilkins Co., Baltimore, 1974, p 118.

11. Blood DC, Henderson JA: Veterinary Medicine, 4th Edition, Williams and Wilkins Co., Baltimore, 1974, p 122.

12. Koirtyohann SR, Hopps HC: Sample selection, collection, preservation and storage for a data bank on trace elements in human tissue. Fed Proc 40:2143-2148, 1981.

13. Hayes VE: Duke's Physiology of Domestic Animals, 8th Edition, Cornell University Press, Ithaca, 1970.

14. Herdt TH, Goeders L, Liesman JS, Emery RS: Test for estimation of bovine hepatic lipid content. J Am Vet Med Assoc 182: 953-955, 1983.

15. Smith HA, Jones TC, Hunt RO: Veterinary Pathology 4th Edition, Lea and Febiger, Philadelphia, 1972, p 36.

16. Gunson DE, Kowalczyk DF, Shoop CR, Ramberg CF: Environmental zinc and cadmium pollution associated with generalized osteochondrosis, osteoporosis and nephrocalcinosis in horses. J Am Vet Med Assoc 180:295-299, 1982.

17. Zook BC, Kopito L, Carpenter JL, Cramer DV, Shwachman H: Lead poisoning in dogs: Analysis of blood, urine, hair and liver for lead. Am J Vet Res 33:903-909, 1972.

18. Altman PL, Ditterman DS: Blood and other body fluids. First Edition, Federation of American Societies for Experimental Biology, 1961.

19. Colvin JR, Sowden LC, Male RS: Variability of the iron and mercury content of individual red blood cells. National Research Council of Canada Publication 14561:329-341, 1974.

20. Hove E, Elvehjem CA, Hart EB: The relation of zinc to carbonic anhydrase. J Biol Chem 136:425-430, 1940.

21. Behne D: Source of error in sampling and sample preparation for trace element analysis in medicine. J Clin Chem Clin Biochem 19: 115-120, 1981.

22. Minnick PD, Braselton WE, Slanker MR: Altered serum element concentrations due to laboratory use of vacutainer tubes. Vet Hum Tox 24:413-414, 1982.

23. Nackowski SB, Putnam RD, Robbins DA, Varner MO, White, LD, Nelson KW: Trace metal contamination of evacuated blood collection tubes. Am Ind Hyg Assoc J 39:503-508, 1977.

24. Unger BC, Green VA: Blood lead analysis—lead loss to storage containers. Clin Toxicol 11:237-243, 1977.

25. Williams DM: Trace metal determination in blood obtained in evacuated collection tubes. Clin Chim Acta 99:23-29, 1979.

26. Meranger BC, Holleborn BR, Blanchette GA: The effects of storage times, temperatures and container types on the accuracy of atomic absorption determinations of Cd, Cu, Hg, Pb, and Zn in whole heparinized blood. J Anal Toxicol 5:33-41, 1981.

27. Helman EZ, Wallick KD, Reingold IM: Vacutainer contamination in trace element studies. Clin Chem 17:61-62, 1981.

28. Narayanan S: The influence of blood collection medium on trace metal analysis. Becton Dickinson and Company, Rutherford, NJ.

29. Reimold EW, Besch DJ: Detection and elimination of contaminations interfering with the determination of zinc in plasma. Clin Chem 24:675-680, 1978.

30. Rich LJ: Update on veterinary clinical pathology. Southeastern Michigan Veterinary Medical Association. Committee for Continuing Education, Mercy College Conference Center, 1980.

31. Prasad AS, Oberlesas D, Halsted JA: Determination of zinc in biological fluids by atomic absorption spectrophotometry in normal and cirrhotic subjects. J Lab Clin Med 66:508, 1965.

32. Foley B, Johnson S, Hackley B, et al: Zinc content of human platelets. Proc Soc Exp Biol Med 128:265, 1968.

33. Braselton WE, Meerdink GL, Stowe HD, Tonsager SR: Experience with multielement analysis in diagnostic clinical toxicology and nutrition. Amer Assn Veterinary Laboratory Diagnosticians, 24th Annual Proceedings, 1981, pp. 111-125.

34. Hara A, Radin NS: Lipid extraction of tissue with low-toxicity solvent. Analyt Biochem 90:420-426, 1978.

35. Teale FWJ: Cleavage of the haem-protein link by acid methyl-ethyl ketone. Biochem Biophys Acta 35:543, 1959.

36. Stowe HD, Braselton WE, Kaneene JB, Slanker MR: Multi-element assays of bovine tissue specimens by inductively coupled argon plasma emission spectrometry. Am J Vet Res 46:561-565, 1985.

37. Coles EH: Veterinary Clinical Pathology, 2nd Edition, W. B. Saunders Company, Philadelphia, 1974.