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Brisket Disease: Influence of Hypoxia and an Induced Calcium-Potassium Imbalance on the Mineral Composition of Blood, Heart, Liver, Kidney, and Bone

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BRISKET DISEASE: INFLUENCE OF HYPOXIA AND AN INDUCED CALCIUM-POTASSIUM IMBALANCE ON THE MINERAL COMPOSITION OF BLOOD, HEART, LIVER, KIDNEY, AND BONE

by

David Eugene Bailey

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David E. Bailey

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ABSTRACT

Brisket Disease: Influence of Hypoxia and an Induced Calcium-Potassium Imbalance on the Mineral Composition of Blood, Heart, Liver, Kidney, and Bone

by

David E. Bailey, Doctor of Philosophy

Utah State University, 1969

Major Professor: Dr. Joseph T. Blake
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Brisket disease, an affliction of cattle, is important because of: (1) economic losses, (2) similarities to chronic mountain sickness in humans, and (3) the provision of experimental animals for cardiac research. In afflicted cattle, right cardiac ventricular hypertrophy and dilatation occur and are manifestations of attempted compensation for reduced alveolar oxygen by increasing pulmonary circulation.

Geographic variations in occurrence of brisket disease in Utah indicate that hypoxia is not the sole causative factor. From the findings that afflicted cattle exhibit hypocalcemia and hyperkalemia, and the disease occurs most commonly in wet meadowland environments where potassium is high and calcium low in browse, a dual stress theory of cause was hypothesized; i.e., altitude-induced hypoxia plus ionic calcium-potassium imbalance.

To test the hypothesis, 40 Hereford calves were randomized into four equal groups, two at 1,372 meters (normal) and two at 2,745 meters (hypoxic) elevation. At each elevation there were control

(balanced) and treated (calcium-potassium) groups. For 16 weeks, treated calves received, by diet, one-fourth the calcium and 10 times the potassium requirements; also, repeated injections of dipotassium ethylenediaminetetraacetate, potassium chloride, and an aldosterone inhibitor to further induce hypocalcemia and hyperkalemia. Control groups at each elevation received a balanced diet and no injections. Since optimal myocardial function is dependent upon proper ion balance, and concentrations of calcium, potassium, sodium, phosphorus, magnesium, chloride, iron, zinc, and copper in blood, heart, liver, kidney, and bone are indices, these elements were quantitated.

Calcium concentration in serum was reduced by 1.6 milligrams per 100 milliliters from an initial value of 9.4 milligrams per 100 milliliters, and an average increase of 1.8 milliequivalents per liter in potassium concentration in whole blood, from the initial concentration of 12.4 milliequivalents per liter, occurred in treated calves. Elevation caused an increase of 1.7 milliequivalents per liter in potassium concentration in serum from the initial concentration of 6.2 milliequivalents per liter. Iron concentration in whole blood increased in response to hypoxia and decreased due to treatment. In the serum, sodium and copper decreased and chloride increased due to treatment.

Compared to low elevation, significant tissue compositional changes in calves at high elevation were as follows: (1) calcium: kidney 12 percent higher, heart 9 percent lower; (2) sodium: liver 5 percent lower, kidney 3 percent higher; (2) phosphorus: kidney 2 percent higher.

More profound changes occurred in cattle subjected to treatment: compared to controls, the tissue compositions in imbalanced cattle were as follows: (1) calcium: heart 10 percent and liver 13 percent lower, kidney 92 percent higher; (2) potassium: heart 13 percent higher, liver and kidney 6 percent lower; (3) sodium: heart 18 percent, liver 8 percent, and kidney 14 percent lower; (4) magnesium: heart 20 percent and liver 5 percent higher, kidney 11 percent lower; (5) phosphorus: heart 6 percent and kidney 21 percent higher, liver 2 percent lower; (6) absolute dry matter: liver 5 percent and kidney 13 percent lower; (7) total ash: kidney 4 percent lower. In addition, iron, zinc, and copper were decreased in both cardiac and hepatic tissues of treated calves.

Treatment influenced bone ash composition as follows: compared to controls, calcium decreased to 25.3 from 32.5 percent; phosphorus decreased to 16.5 from 19.0 percent; potassium increased to 0.16 from 0.08 percent; and zinc increased to 319 parts per million from 227 parts per million. High altitude was also influential. Compared to controls, phosphorus increased to 18.1 percent from 17.5 percent, potassium decreased to 0.112 from 0.129 percent, sodium to 1.09 from 1.17 percent, and magnesium to 0.64 from 0.70 percent.

(207 pages)

INTRODUCTION

Numerous studies concerning the effects of high altitude on man and domestic animals and studies of simulated high altitude on laboratory animals have contributed to an abundant literature. Most of the research has been done on man or animals located in the Andean Mountains of South America or in the Rocky Mountains in Utah and Colorado, at elevations above 2,400 meters.

During the summer months (June through October) cattle in the Rocky Mountain States are pastured on national forest, state, or privately owned lands at elevations from 2,400 to 3,200 meters. While in habitation at high altitude, an illness occurs in some of these cattle, the cause of which is not well understood. The disease has been called brisket disease, pulmonary hypertensive heart disease, and high altitude disease. The former name is most commonly used. It is a descriptive term coined by ranchers, and identifies the part of the body most conspicuously abnormal, i.e., a swollen brisket (area between and anterior to the forelegs).

In Utah, the disease reaches epizootic proportions in some geographic areas. It is subacute in onset and most afflicted animals succumb unless they are taken to a lower altitude. Even removal to a lower elevation has little value in alleviating symptoms of the disease in cattle with advanced cases. Brisket disease is important because of the economic loss to cattlemen. The disease has also become important because of its similarities to chronic mountain sickness in humans

and the opportunity of using afflicted cattle as experimental animals to learn more concerning heart disease.

The right cardiac ventricular hypertrophy and dilatation, which occur in afflicted cattle, are manifestations of attempts by them to compensate for reduced alveolar oxygen by increasing pulmonary circulation. Pulmonary hypertension, right heart failure, and generalized body fluid imbalance are cardinal pathologic characteristics of the disease and indicate an inability to adequately compensate for altitude-induced hypoxia.

It has been hypothesized that hypoxia is the cause of brisket disease (Alexander et al., 1960). However, the disease does not occur with equal frequency on all cattle grazing ranges at high altitude, nor is it uniformly related to elevation (Blake, 1968). From the pattern of geographic variation of incidence of the disease, it is evident that hypoxia is not the sole causative factor.

A proper balance of ions in the animal body is vital for normal physiological functions and when an imbalance exists, the animal is in a diseased state. Of particular importance in maintaining physiological homeostasis are calcium (Ca), potassium (K), sodium (Na), and magnesium (Mg). It has been shown that in brisket disease of cattle there is an imbalance of serum electrolytes (Blake, 1965a). Afflicted cattle exhibit a decreased level of serum Ca and increased levels of serum K and phosphorus (P). Such a calcium-potassium (Ca-K) ionic imbalance in the environment of the myocardial fibers would produce effects similar to those produced by hypoxia, i.e., enlargement of the right side of the heart and generalized body fluid imbalance. Thus, one can hypothesize that a disturbance of Ca-K homeostasis may be contributory to brisket disease.

From the above described circumstances, one is justified in determining whether brisket disease is inducible in cattle experimentally subjected to the combined stresses of hypoxia and ionic imbalance. Experimentally producing the combination of hypocalcemia and hyperkalemia enables the *in vivo* measurement of such an ionic imbalance on right cardiac ventricle performance. In the literature, a good method is not described for experimentally inducing a prolonged combination of hypocalcemia and hyperkalemia in cattle. A technique for doing this has been developed as a part of a research plan, of which the present dissertation is a part (Blake et al., 1969). By placing cattle with the Ca-K imbalance at high altitude, the stresses of ion imbalance and hypoxia are imposed coincidentally.

REVIEW OF LITERATURE

Healthy Animals: Cardiac Function and Body Fluid Balance

The heart has the fundamental task of providing positive pressure which propels blood throughout the circulatory system. In the pulmonary circulation, the right ventricle provides the pumping action to force deoxygenated blood through the lungs, where its oxygen (O_2) supply is renewed. In the systemic circulation, the left ventricle provides the pumping action to propel oxygenated blood to all body tissues.

Any pathologic situation which interferes with the normal pattern of circulation of blood results in an upset of body fluid balance. Situations causing this might be heart, lung, or vascular structural abnormalities, rarefied atmosphere, or any factors that deleteriously influence contraction of the myocardium.

Role of oxygen (O_2)

The importance of O_2, water, and food to the animal organism is fundamental. Of these three basic essentials for the maintenance of life, the deprivation of O_2 leads to death most rapidly. Oxygen is normally carried by the circulating blood from the lungs to the tissues, mainly as oxyhemoglobin with a small amount dissolved in physical solution. Tissue cells must be continually supplied with O_2 by the circulation, for they have little reserve (Ganong, 1963).

The major function of O_2 in the animal body is to supply energy through the oxidation of foodstuffs. Energy is required for the

maintenance of body temperature, for the performance of external work, and for the muscular movements of the heart and of respiration (Bell, Davidson, and Scarborough, 1961).

Role of minerals

Mineral matter of the body is comprised of a large number of elements, present in varying amounts in different parts of the body, according to the functions they perform. These elements are absolutely essential for life and must be present in correct proportions. Interrelationships among certain minerals, as well as actual amounts, govern both their usefulness and their harmful effect.

Calcium (Ca) and potassium (K)

Of all minerals, Ca is most abundant in the body (Thomas and Howard, 1964). Of the Ca in the body, 99 percent is found in bones and teeth, where it gives rigidity and strength. The 1 percent which occurs outside the bones or teeth is widely distributed throughout the body. Much of it is in blood plasma. The Ca in plasma exists in three fractions: (1) protein bound; (2) nonionized and complexed, but soluble; and (3) unbound and ionized (Goodman and Gilman, 1965). The unbound ionized fraction is the most physiologically active. Since the blood vessel walls are relatively impermeable to the first two fractions, the interstitial fluid Ca is composed principally of the ionized component.

Calcium plays several important roles in the animal body. The level of ionized Ca governs the excitability of nervous and muscle tissues (Shanes, 1958). An excess decreases and a deficit increases excitability.

Calcium plays an additional role in coupling excitation with muscle contraction (Shanes, 1963). This phenomena is observed with both cardiac and skeletal muscle. Actomyosin, a muscle protein complex involved in contraction, causes greater muscle contraction when in the presence of Ca than when in the presence of Mg and adenosine triphosphate. As muscle alternately contracts and relaxes, Ca moves in and out of the myoplasm. During depolarization (contraction), Ca is displaced from a bound site and enters the myoplasm. The greater the quantity of Ca that enters the myoplasm, the greater the strength of the contraction (Reiter, 1964; Berne and Levy, 1964). Subsequent relaxation is associated with a re-binding of Ca by a "relaxing factor" (Berry, 1961). It is known that Ca causes increased phosphorylase activity, and in turn, increased glucose-1-phosphate which results in increased availability of energy. Since muscle contraction requires energy, it has been hypothesized (Freisen, Allen, and Valadores, 1966) that the increased muscle contraction that occurs when Ca concentration is increased is due to the effect of the increased Ca on phosphorylase activity.

In development of the action potential, Ca plays an important role in regulating the permeability of the cell membrane to Na and K (Hoffman and Suckling, 1956). An excess of Ca diminishes and a decrease augments the permeabilities.

Potassium is the major cation in the intracellular fluid, and it has about the same importance within the cell as does Na in the extracellular fluid. It combines reversibly with many buffers of the cell and can be ionized or deionized. It is essential for many of the chemical reactions occurring within cells (Guyton, 1966). Potassium

is necessary for the conduction of nerve impulses in voluntary and involuntary nervous systems (Bland, 1956).

Other minerals: sodium (Na), phosphorus (P), magnesium (Mg), and chloride (Cl)

Sodium comprises 90 percent of the total cations of the extracellular fluid (Bland, 1956). Several authors have studied the effects of the Na concentration on myocardial contractility. Clark (1913) studied the effects of ions on frog heart and reported that reduction of the Na concentration leads to a strengthening of contraction of the myocardium. Later, Niedergerke and Luttgav (1957) showed that every known feature of the action of Ca ions on the contraction of heart muscle could be simulated by a decrease of the Na concentration. More recently, Reiter (1964), and Jewell and Blinks (1968) have shown that Na antagonizes the action of Ca and that the force of contraction of the myocardium depends on the ratio of the Ca ion concentration to the Na ion concentration squared, i.e., $[Ca^{++}]/[Na^{+}]^{2}$.

Phosphorus has many important functions in the biochemistry and physiology of the body. It is ubiquitous in anatomical terms and is of great significance in reactions throughout all organs and tissues (Guyton, 1966). Most of the P in the body is present in bone, where it plays a key role in osteoblastic and osteoclastic activities (McLean and Urist, 1961). A proper concentration of phosphate is important in assuring an orderly biochemical sequence, as phosphate is transferred from molecule to molecule in the metabolism of carbohydrates, lipids, and proteins (Fruton and Simmonds, 1961). Throughout all the tissues, the storage of energy in biochemical terms is largely as "high-energy phosphate" compounds. Since the heart requires much energy for

contraction, these "high-energy phosphate" compounds are vital for normal cardiac function. The function of the kidney in the renal excretion of phosphate has been studied, but the precise details of the mechanism have not been defined. It is believed that the phosphate in plasma is filterable, and the net tubular reabsorption ranges from virtually all of the phosphate that is filtered to as low as 80 percent (Pitts, 1963). There are many modifying influences that tend to alter the excretion of phosphate to maintain the normal level of P in plasma.

Magnesium is the second most plentiful cation within cellular fluids (Goodman and Gilman, 1965). Glaser and Brandt (1959) studied the localization of Mg in the myocardium of dogs, rabbits, and calves using radioactive Mg. They report that the concentration of Mg is 10 times greater in the myocardium than in skeletal muscle, and the interventricular septum had a higher concentration than either of the ventricular walls.

Chlorine differs from Na and K in that it is found in large concentrations both in intracellular and extracellular fluids. However, the Cl ion is the major anion in the extracellular fluid (Guyton, 1966). Chlorides of the blood, principally NaCl, make up two-thirds of its acidic ions. Chloride ions, in concert with Na and K, function in maintaining osmotic pressure and acid-base equilibrium, in controlling the passage of nutrients into cells, and in water metabolism in general (Maynard and Loosli, 1962). Very little has been done concerning the effects of Cl ions on the myocardium. However, a recent study of the effect of anion substitution on the membrane resistance of the Purkinje fibers indicates that for this excitable tissue, Cl ion conductance is

small at rest but becomes significant during excitation (Cotlove and Hogben, 1962).

Hematinic elements: iron (Fe), zinc (Zn), and copper (Cu)

A minimum quantity of Fe is needed to complete the structure of hemoglobin. Atomic Fe in the ferrous state is incorporated into an Fe porphyrin compound called heme, which combines with globin to form the hemoglobin molecule (Ingram, 1965). The hemoglobin molecule is vitally involved in the transport of O_2 from the lungs to the tissues and organs of the body.

Copper acts as a catalyst in the formation of hemoglobin. It is not concerned with assimilation or storage of Fe, but with the transformation of Fe into hemoglobin (Elvehjem, 1935; Marston, 1950). There is an interrelationship in the action of Cu and Zn and a balance of the two must exist for normal Fe prophyrin formation.

Chemical composition of tissues

Dry matter

The normal dry matter content of the hearts of various mammals has been reported. The dry matter content of the heart of the rat was reported to be 22.4 percent (Ledingham, 1953). The hearts of the dog and cat contain 19.2 percent dry matter (Darrow, Harrison, and Taffel, 1939; Yannet and Darrow, 1940). There is disagreement concerning the normal dry matter content of human heart. This may be attributable to the fact that studies of normal human hearts are infrequent. Widdowson and Dickerson (1964) report the dry matter content of adult human heart to be 17.3 percent; whereas, Tipton (1960) reported the mean dry matter

composition to be 25 percent with a range of 20 to 29 percent dry matter. Pig heart reportedly contains 17.5 percent dry matter (Widdowson and Dickerson, 1960). Bovine heart was found to contain 20.6 percent dry matter (Elvehjem and Peterson, 1927) and 22.4 percent dry matter (Wooster, 1956).

The dry matter content of liver from several species has been reported. The normal dry matter content of adult liver is reported as follows: rat, 25.5 percent (Harrison, 1953); rabbit, 26.2 percent (Gaudino, 1956); cat, 25.0 percent (Yannet and Darrow, 1938); dog, 22.6 percent (Eichelberger and McLean, 1942); and pig, 24.5 percent (Widdowson and Dickerson, 1960). The dry matter content of normal adult human liver is reported by Widdowson and Dickerson (1960) to be 23.0 percent; whereas, Tipton (1960) reports a somewhat higher value, 35.0 percent. Concerning ruminants, sheep liver contains 25.1 percent dry matter (Mounib and Evans, 1960). Elvehjem and Peterson (1927) reported the dry matter content of bovine liver to be 28.4 percent; whereas, Wooster (1956) reported a slightly higher value of 30.3 percent for bovine liver.

The reported dry matter content of the kidneys of the adults of several different mammalian species ranges from 19 to 25 percent. The kidney of the rat contains 24.3 percent dry matter (Manery and Hastings, 1939; Widdowson and Dickerson, 1964); cat, 18.8 percent (Darrow, Harrison, and Taffel, 1939); dog, 19.8 percent (Eichelberger and Bibler, 1940); and pig, 18.8 percent (Widdowson and Dickerson, 1960). There is disagreement concerning the dry matter content of normal human kidney: Widdowson and Dickerson (1960) reported 19.0 percent dry matter, while Tipton (1960) reported 23.0 percent dry matter. Concerning

ruminant species, the sheep kidney reportedly contains 22.6 percent dry matter (McCance and Widdowson, 1946). The dry matter content of bovine kidney was reported as being 25.1 percent (Wooster, 1956) and 24.5 percent (McCance and Widdowson, 1946). Elvehjem and Peterson (1927) reported a somewhat lower level of 18.9 percent dry matter.

The cleaning of bone samples for analysis is quite tedious, and careful precautions are necessary if the percentage of water and dry matter are to be accurately determined (Robinson and Elliott, 1957). It has, therefore, become customary to express the composition of bone tissue on a dry, fat-free basis (Widdowson and Dickerson, 1964). The dry matter content of bone lies within a range of 85 to 90 percent for the human (Neuman and Neuman, 1958) and for the bovine (Maynard and Loosli, 1962).

Ash content

The ash content of normal hearts has not been extensively reported. The hearts of normal adult humans contained 1.1 percent ash (Tipton, 1960). Bovine heart also reportedly contained 1.1 percent ash (Wooster, 1956).

The ash content of liver from various species lies within a very close range. The ash content of livers from normal adult human beings was found to be 1.3 percent (Tipton, 1960). Swine liver contained 1.5 percent ash and bovine liver contained 1.4 percent ash (Wooster, 1956).

The ash content of kidneys of few animal species has been reported. The ash content of normal adult human kidney (Tipton, 1960) and of normal adult bovine kidney (Wooster, 1956) are reportedly the same, 1.1 percent.

On a dry, fat-free basis the rat femur was found to contain 56.6 percent ash (Widdowson and Dickerson, 1964) and the human femur contained 56.7 percent ash. The human tibia was higher, containing 66.3 percent ash. McLean and Urist (1961) have reported the analysis of bovine cortical bone and found that on a dry, fat-free basis, it contains 56.7 percent ash.

Mineral elements

Whole blood and serum: Ca, K, Na, P, Mg, Cl, Fe, Zn, and Cu. The normal Ca level in blood serum lies within the range of 9.0 to 11.4 mg/100 ml for the adult human being (Widdowson and Dickerson, 1964), pig (Widdowson and McCance, 1956), dog (Bernstein, 1954; Carr and Schloerb, 1959), rabbit (Bernstein, 1954; Economou-Mavrou and McCance, 1958), and fowl (Barlow and Manery, 1954). Serum from cattle blood reportedly contains an average of 10.0 mg/100 ml Ca with a range of 9.4 to 12.2 mg/100 ml (Altman, 1961; Spector, 1956).

The K levels in the whole blood and in the blood serum of several species have been reported. The K concentration of whole blood for the monkey is 54 mEq/L, for the dog 25 mEq/L, and for man 46 mEq/L (Altman, 1961). For cattle the K content of whole blood ranges from 15-18 mEq/L (Altman, 1961). The K concentration of blood serum of several species has been reported as follows: rat, 5.5 mEq/L (Bernstein, 1954); dog, 4.0 mEq/L (Darrow and Yannet, 1935); pig, 6.0 mEq/L (Widdowson and McCance, 1956); horse, 4.8 mEq/L (Bernstein, 1954); and sheep, 4.5 mEq/L (Eveleth, 1937). Several authors have reported the K concentration of bovine blood serum as follows: Spector (1956) reported 4.7 mEq/L, Altman (1961) reported 5.7 mEq/L, and Blake (1965a) reported 5.0 mEq/L.

The Na content of whole blood for several species is reported as follows: monkey, 91 mEq/L; dog, 121 mEq/L; man, 94 mEq/L; and cattle, 109 mEq/L (Altman, 1961). The Na concentration in blood serum of several species has been reported as follows: for the rat and horse, 142 mEq/L (Bernstein, 1954); for the dog, 144 mEq/L (Darrow and Yannet, 1935); for the pig, 143 mEq/L (Widdowson and McCance, 1956); for the sheep, 148 mEq/L (Eveleth, 1937); and for man, 135-145 mEq/L (Widdowson and Dickerson, 1964). For healthy cattle, the Na concentration in serum was reported to be 144 mEq/L (Altman, 1961), and 139 mEq/L (Spector, 1956; Blake, 1965a).

The P concentration of blood serum of the rabbit (Economou-Mavrou and McCance, 1958), dog (Bernstein, 1954), and pig (Widdowson and McCance, 1956) has been reported to be 7.1 mg/100 ml. For man, the P level in blood serum is reportedly in the range of 2.5 to 4.5 mg/100 ml (Widdowson and Dickerson, 1964). The P concentration in serum of cattle is reported to be 6.0 mg/100 ml (Spector, 1956), 6.5 mg/100 ml (Altman, 1961), and 6.9 mg/100 ml (Blake, 1965a).

The Mg content of blood serum for several mammalian species is as follows: rat, 7.3 mg/100 ml (Bernstein, 1954); rabbit, 5.3 mg/100 ml (Economou-Mavrou and McCance, 1958); dog, 3.6 mg/100 ml (Darrow and Yannet, 1935); horse, 2.9 mg/100 ml (Eveleth, 1937); and man, 3.9 mg/100 ml (Widdowson and Dickerson, 1964). Bernstein (1954) and Eveleth (1937) reported the serum Mg concentration for both cattle and sheep to be 5.8 mg/100 ml. Spector (1956) reported a level of 5.6 mg/100 ml, whereas Altman (1961) reported a level of 5.3 mg/100 ml of Mg for blood serum of cattle.

The Cl content of normal blood serum is 390 mg/100 ml for the rat (Bernstein, 1954) and dog (Darrow and Yannet, 1935), 362 mg/100 ml for the rabbit (Economou-Mavrou and McCance, 1958), 385 mg/100 ml for the pig (Widdowson and McCance, 1956), 380 mg/100 ml for the sheep (Eveleth, 1937), and 355 mg/100 ml for both the horse (Bernstein, 1954) and man (Widdowson and Dickerson, 1964). For blood serum of cattle, Bernstein (1954) and Eveleth (1937) reported a level of 359 mg/100 ml of Cl; similarly, Altman (1961) reported 391 mg/100 ml and Spector (1956) reported 369 mg/100 ml.

The Fe content of whole blood has not been too extensively reported. In man it is reported to be between 46 and 52 mg/100 ml (Altman, 1961), and in cattle between 45 and 55 mg/100 ml (Forbes and Swift, 1926; Gessert et al., 1952). In cattle the value decreases with age (Underwood, 1962).

There is much disagreement concerning the Zn content of blood serum. For man, Albritton (1952) reported a level of 800 µg/100 ml and Daum (1954) reported 239 µg/100 ml; however, these values are apparently higher than normal. Several investigators have reported the level to lie within the range of 109 to 130 µg/100 ml (Vikbladh, 1950; Vallee and Gibson, 1948; Koch et al., 1956; Widdowson and Dickerson, 1964; Berfenstam, 1952; Vallee et al., 1956). The Zn concentration in serum of cattle blood is reported to lie within the range of 160 to 220 µg/100 ml (Underwood, 1962). Others report normal values to be 300 µg/100 ml for cattle (Gessert et al., 1952; Altman, 1961).

The normal Cu concentration in human blood serum is reported to be 110 µg/100 ml (Widdowson and Dickerson, 1964; Herring et al., 1960). The Cu concentration in blood serum from normal cattle blood is 95 to

110 µg/100 ml (Elvehjem, 1935; Haag and Adams, 1958; Underwood, 1962; Widdowson and Dickerson, 1964).

Heart, liver, and kidney: Ca, K, Na, P, Mg, Fe, Zn, and Cu. Data are available for the Ca, K, Na, and P concentrations in normal or non-diseased heart (cardiac) tissue for man, rat, and cattle. All reported values are on a dry matter basis. For cardiac tissue from man, the reported levels of these elements are as follows: Ca, 448 ppm (parts per million); K, 7.6 ppt (parts per thousand); Na, 14 ppt; and P, 9.1 ppt (Widdowson and Dickerson, 1964). For cardiac tissue from the rat, the reported values are as follows: Ca, 410 ppm (McAleese and Forbes, 1961); K, 9.4 ppt; Na, 6.2 ppt; and P, 11.7 ppt (Ledingham, 1953). For cardiac tissue of the bovine, the reported values are as follows: Ca, 312 ppm; K, 6.5 ppt; Na, 3.6 ppt; and P, 9.1 ppt (Wooster, 1956).

The normal Mg concentration in cardiac tissue of the human is 810 ppm on a dry matter basis (Widdowson and Dickerson, 1964) and from the pig heart is 902 ppm (Widdowson, Dickerson, and McCance, 1960). The Mg content of bovine heart has not been reported.

The normal content, on a dry matter basis, of hematinic elements in cardiac tissue from man has been reported to be: Fe, 710 ppm; Zn, 145 ppm; and Cu, 23.8 ppm (Butt et al., 1960; Tipton, 1960). The Fe concentration in bovine heart dry matter is reported to be 205 ppm (Wooster, 1956). Values for Zn and Cu in bovine heart are unavailable from the literature.

Data are available for the mineral composition of normal or non-diseased liver (hepatic) tissue of mammalian, non-ruminant species as follows: Ca, K, Na, P, and Mg contents of human and rat livers; Ca, P,

and Mg contents of swine liver; and P content of cat liver. All of the reported values are on a dry matter basis. The reported values for hepatic tissue from man are: Ca, 296 ppm (Butt et al., 1960); K, 13.7 ppt; Na, 4.6 ppt; P, 12.5 ppt; and Mg, 865 ppm (Widdowson and Dickerson, 1960). The values for the rat are as follows: Ca, 321 ppm; K, 132 ppt; Na, 3.2 ppt; P, 12.1 ppt; and Mg, 856 ppm (Harrison, 1953). The reported values for liver from the dog are the following: Ca, 150 ppm (Widdowson and Dickerson, 1960); K, 12.7 ppt; Na, 4.0 ppt; and Mg, 788 ppm (Eichelberger and McLean, 1942). The following values are reported for swine liver: Ca, 220 ppm; P, 15.7 ppt; and Mg, 1,050 ppm (Widdowson and Dickerson, 1960). The reported P concentration in liver from the cat is 11.9 ppt (Yannet and Darrow, 1938).

Data concerning the mineral composition of normal hepatic tissue from ruminants, particularly cattle, are most pertinent to this thesis. The data are limited. For sheep, the K and Na contents of liver were reported to be 12.6 ppt and 3.3 ppt, respectively (Mounib and Evans, 1960). For cattle, the reported values are as follows: Ca, 264 ppm; K, 14.6 ppt; Na, 4.9 ppt; and P, 15.1 ppt (Wooster, 1956). The Mg content of bovine liver is unreported.

Butt et al. (1960) has reported the concentration of hematinic elements in human liver: Fe, 188 ppm; Zn, 28 ppm; and Cu, 4.5 ppm on a dry tissue basis. For cattle, Underwood (1962) found these hepatic concentrations: Fe, 64 ppm; Zn, 125 ppm; and Cu, 77 ppm on a dry matter basis. However, values reported by other investigators differ considerably for Fe and Cu concentrations in cattle liver. Gessert et al. (1952) reported a mean of 208 ppm Fe (dry matter basis) with a range of 138 to 332 ppm; and Kohler, Elvehjem, and Hart (1936) reported a range

of 181 to 260 ppm. Haag and Adams (1958) reported that the Cu concentration in bovine liver varied from 2 to 300 ppm depending upon the diet of the animal.

Data are available concerning the mineral composition of normal or non-diseased kidney (renal) tissue of mammalian, non-ruminant species as follows: Ca, K, Na, P, and Mg contents of human and rat kidneys; K, Na, and Mg contents of dog kidney; and Ca content of swine kidney. All reported values are on a dry matter basis. The reported values for renal tissue of man are as follows: Ca, 735 ppm; K, 7.8 ppt; Na, 18.7 ppt; P, 9.7 ppt; and Mg, 372 ppm (Widdowson and Dickerson, 1960). The values for the rat are the following: Ca, 346 ppm; K, 7.5 ppt; Na, 12.4 ppt; P, 12.7 ppt; and Mg, 502 ppm (Manery and Hastings, 1939). The reported values for renal tissues from the dog are as follows: K, 7.6 ppt; Na, 18.1 ppt; and Mg, 235 ppm (Eichelberger and Bibler, 1940). The reported Ca content of swine renal tissue was 468 ppm (Widdowson and Dickerson, 1960).

For cattle and sheep, the Ca, K, Na, P, and Mg contents of renal tissue have been reported. For cattle: Ca, 280 to 312 ppm (Wooster, 1956; McCance and Widdowson, 1946); K, 9.6 ppt (Wooster, 1956) and 9.2 ppt (McCance and Widdowson, 1946); Na, 8.4 ppt (Wooster, 1956); P, 7.1 ppt (Wooster, 1956) and 8.1 ppt (McCance and Widdowson, 1946); and Mg, 510 ppm (McCance and Widdowson, 1946). For sheep: Ca, 594 ppm; K, 8.1 ppt; Na, 13.2 ppt; P, 11.2 ppt; and Mg, 478 ppm (McCance and Widdowson, 1946).

Bone: Ca, P, K, Na, Mg, and Zn. The concentration of Ca and P in dry, fat-free bone (femur) has been reported for several non-ruminant species: man, 26.0 percent Ca and 11.5 percent P (Baker, Butterworth,

and Langley, 1946); rat, 28.4 percent Ca and 13.0 percent P (Weidman and Rogers, 1950; Dickerson, 1962); rabbit, 28.0 percent Ca and 12.6 percent P (Weidman and Rogers, 1958); and swine, 27.1 percent Ca and 12.5 percent P (Dickerson, 1962). The reported values for cancellous bone from cattle are 27 percent Ca and 12 percent P on a dry, fat-free basis (McLean and Urist, 1961).

The K, Na, and Mg contents of bone have been reported on a dry fat-free basis. The K content was reported to be 0.14 percent for the femur of man (Agna, Knowles, and Alverson, 1958), 0.08 percent for the rat (Bergstrom and Wallace, 1954), and 0.06 percent for cattle (McLean and Urist, 1961). The Na content was reported to be 0.63 percent for the femur of man (Agna, Knowles, and Alverson, 1958), 0.71 percent for the femur of the rabbit (Davies, Kornberg, and Wilson, 1952), and 0.73 percent for cattle (McLean and Urist, 1961). The Mg content was reported to be 0.36 percent for the rat femur (Widdowson and Dickerson, 1964), and 0.44 percent for the bovine femur (McLean and Urist, 1961). Blincoe and Bohman (1966) studied the concentration of Zn in bone of range cattle. They found that the Zn concentration in the femur, humerus, and rib ranged from 172 to 196 ppm of bone ash.

Chemicals Influencing Body Ca-K Balance

Ethylenediamine (or ethylenedinitrilo) tetraacetic acid (EDTA)

The use of chelating agents to bind certain metals and metalloids and to eliminate them from the mammalian body for therapeutic purposes has become quite common in human and veterinary medicine. Of the numerous chelating agents in existence, EDTA has received the greatest

attention from biologists (Chenoweth, 1956). EDTA displays a variable affinity for a whole series of divalent ions, including particularly Ca since it is the most abundant cation in the animal body (Chaberek and Martell, 1959).

When EDTA is administered parenterally, a Ca-EDTA complex is formed with ionic Ca in the blood plasma. The Ca-EDTA complex is then excreted in the urine by the human (Spencer et al., 1952) and the rat (Larsen et al., 1960). However, the presence of large amounts of bound Ca in the blood plasma indicates that the elimination of the Ca-EDTA is slower than the binding (Foreman and Trujillo, 1954). The ionic Ca in the plasma is thus decreased, but is replenished by body stores, most likely from the skeleton (Spencer et al., 1956). The excess calciurea reflects the magnitude of this Ca influx from the body stores.

EDTA has been used with success in the treatment of hypercalcemia in human patients (Holland, Danielson, and Schagian-Edwards, 1953; Dudley et al., 1955). In normocalcemic adult human patients, intravenous infusion of the disodium salt of EDTA at the rate of 1 gram per hour causes a decrease of ionic Ca in plasma (Spencer, 1960). In cases of both hypercalcemia and normocalcemia, the bound Ca was eliminated from the body. Popovici et al. (1950) have shown that Ca levels in plasma can be controlled in rabbits and humans by successive intravenous or intraperitoneal doses of Na_2-EDTA. Continuous intravenous infusion of EDTA into cows was shown by Ramberg et al. (1967) to elicit a rapid decrease in ionic Ca concentrations in plasma.

Aldosterone, aldosterone inhibitors, and KCl

Aldosterone is by far the most potent of the naturally occurring

corticosteroids in regard to controlling electrolyte balance (Turner, 1966). It is secreted from the zona glomerulosa of the adrenal cortex and plays an important role in the renal regulation of Na and K balance in the body. Aldosterone acts directly on the kidney where it causes a decrease in the Na excretion and an increase in K and hydrogen ion excretion (Barger, Berlin, and Tulenko, 1958).

The mechanism of action of aldosterone antagonists is considered to be competitive inhibition (Goodman and Gilman, 1965). Aldosterone antagonists cause an increase in Na excretion and a decrease in K excretion (Liddle, 1958). It was reported that aldosterone antagonists induced hyperkalemia when administered to human patients (Manning and Behrle, 1961; Ogden et al., 1961).

The intravenous infusion of KCl at a dosage of 500 mg per 45 kg body weight in a 4-hour period results in dramatic recovery from hypokalemia and may result in hyperkalemia if administered to normokalemic patients (Darrow et al., 1949). Laragh and Capeci (1955) found that intravenous KCl administration at the rate of 2.8 mEq/kg over a 24-hour period elevated the concentration of Na in the plasma of K-deficient patients after surgery. The Na evidently came from soft tissues and bone in exchange for K. The Na-rich, K-poor cells of hypokalemic patients would theoretically lessen any elevation in plasma K which one would expect from a given dose of KCl based on a normal diffusion rate.

Brisket Disease; Cardiopathology; Body Fluid Imbalance

In the study of cardiopathology and brisket disease, the tissues and organs governing homeostasis and body fluid balance are important.

The blood, heart, and kidneys are vitally involved in the regulation of homeostasis and body fluid balance (Guyton, 1966). Blood has a number of functions including: (1) transport of minerals from the gastro-intestinal tract to body tissues, (2) transport of O_2 from the lungs to body tissues by the use of hemoglobin, of which Fe is an integral part, and (3) distribution and equalization of water throughout the body (Dukes, 1955). The levels of mineral elements in the blood are indicative of the "mineral status" of an animal (Maynard and Loosli, 1962). The heart acts as a pump to force blood; first to the lungs where it becomes oxygen-laden, and second to all tissues of the body where the O_2 and food nutrients are utilized. Specific concentrations of certain minerals in the myocardium are essential for normal cardiac function (Reiter, 1964). The kidneys are involved in the excretion of minerals and are fundamental in controlling the level of electrolytes and water present in the body (Bell, Davidson, and Scarborough, 1961).

Liver and bone are intimately involved in general body homeostasis (Guyton, 1966). The liver stores many of the minerals, including the hematinic minerals, and releases them into the general body circulation when the need arises (Ganong, 1963). Bone is the major storage area for minerals in the body and these minerals are in a constant dynamic state (McLean and Urist, 1961). When the level of a mineral in the blood becomes lower than normal, it is mobilized from bone, and when the level becomes higher than normal, some is deposited in bone, thus maintaining a hemic balance.

Role of hypoxia

General effects of hypoxia

The barometric pressure is inversely related to altitude. The

decreased barometric pressure at high altitude is accompanied by a decrease of O_2 pressure, since O_2 remains constant at a level of 21 percent of the total barometric pressure (Guyton, 1966). Not only is O_2 pressure less, but the quantity of O_2 per unit of atmosphere is less at high elevation. Breathing air at high altitude where O_2 tension is low results in a decrease in the O_2 supply to body tissues unless the heart and lungs can compensate. Prolonged exposure to an inadequate supply of O_2 results in tissue hypoxia. Early compensatory responses to hypoxia are increases in heart rate and in the depth and rate of respiration (Van Liere, 1942).

Small differences in barometric pressure have a definite effect on water distribution and fluid balance in the body. Water retention in dogs and rats increased in the periphery after being exposed to a decrease in barometric pressure from 760 to 662 mm Hg (Smith, 1928).

In a hypoxic animal, the partial pressure of O_2 in the blood and the saturation of hemoglobin are both reduced, depending upon the severity of the hypoxia. Consequently, the blood less effectively supplies O_2 to the tissues. The reduced partial pressure of O_2 in arterial blood is more serious than is the lowered O_2 saturation because the velocity of oxidative processes in the tissues is proportional to partial pressure of O_2 delivered to the tissues (Van Liere, 1942). The increased respiratory rate in response to hypoxia washes the carbon dioxide (CO_2) out of the lungs, and as a consequence, the arterial pressure of CO_2 decreases. It is known that CO_2 tension in the arterial circulation is one of the important factors in the dissociation of O_2 from oxyhemoglobin at the tissue level (Barcroft, 1914). Due to the decreased CO_2 tension following increased respiration, the hemoglobin

gives up its O_2 less readily, and as a consequence, tissues become hypoxic even though there may be adequate O_2 in the blood. The tissues in anoxic anoxia (hypoxia) are hampered in three ways: (1) the rate of oxidation is diminished because of the low O_2 tension in the blood, (2) there is a deficiency of O_2 in the blood, and (3) the dissociation of oxyhemoglobin is lessened due to the low CO_2 tension (Wright, 1937).

Berne and Levy (1964) have reported that in contrast to skeletal muscle, the myocardium normally functions exclusively aerobically and cannot incur an O_2 debt. It is evident then, that a level of hemoglobin in the blood sufficient to supply adequate O_2 for normal cardiac function is necessary.

At high altitude there is an increase in the minute volume of blood flow because of increased systolic discharge, increased heart rate, and reduced peripheral resistance (Van Liere, 1942). This condition remains until the O_2 content of arterial blood reaches 9 percent or lower; then a circulatory crisis occurs. The heart rate becomes slower, the ejection phase becomes abbreviated, and circulatory collapse becomes imminent.

Among afflictions partially attributed to the effects of hypoxia are mountain sickness in lambs (Cuba-Caparo, 1949), brisket disease in cattle (Alexander and Jensen, 1959; Hecht et al., 1959), and chronic mountain sickness in humans (Monge, 1943). The occurrence of altitude-dependent diseases is widespread. Arango (1949) reported the occurrence of a high altitude disease (brisket disease) in cattle which resided in the Andes at elevations of 2,600 to 4,000 meters. Their only chance for recovery was relocation at a lower elevation.

Brisket disease in Peru was reported by Cuba-Caparo, Copaira, and Vega (1955). It occurred in cows and calves that were kept at 3,500 to 4,000 meters elevation. Also in Peru, Hultgren and Spickard (1961) noted circulatory disorders in horses, cattle, pigs, and lambs. The disorders were attributed to high altitude, and hydropericardium was a common finding at necropsy. In 1945, 500 cattle were imported into Colombia from the United States and placed at 2,600 meters elevation (Velasquez, 1947). After 2 to 3 months at high altitude, they developed symptoms similar to brisket disease. The symptoms were attributed to cardiac insufficiency at the high altitude. Imported goats, sheep, donkeys, and horses were similarly affected (Velasquez, 1948).

The weights of hearts of animals from different elevations were compared by Glover and Newsom (1918). They found that the hearts of cattle from high altitudes (2,582 to 3,048 meters) were 11 percent heavier than were the hearts of cattle from lower elevations (12 to 90 meters).

Hypoxia as related to brisket disease

The first reported scientific investigation of brisket disease in cattle was reported by Glover (1913). It had been recognized in the South Park and North Park areas of Colorado as early as 1889 (Glover and Newsom, 1914). These authors concluded that brisket disease was a high altitude disease of cattle, occurring above 2,438 meters elevation, and that the principal symptoms were swelling under the jaw and in the brisket area. At necropsy, the most pronounced features were general edema and a dilated heart (Glover, 1914; Glover and Newsom, 1914). The only recognized treatment was to remove the animals to lower elevation

and then, in advanced cases the animals did not survive (Glover and Newsom, 1917).

In Utah, studies began in 1948 with an investigation of deaths of range cattle located at high elevation on the UM and 7-Mile ranges of the Fishlake National Forest (Madsen, 1948). The deaths occurred in the late summer months after the cattle had been at high elevation for several months. Upon investigation, it was learned from ranchers that deaths had occurred as early as 1913.

A mass of literature has been published dealing with brisket disease since these early investigations. Alexander and Jensen (1959) described brisket disease as a syndrome in which all signs and lesions are attributed to congestive right heart failure. These authors observed enlargement of the right heart under conditions of hypoxia in experimental animals as well as in field cases of brisket disease.

It is generally accepted that the cause of death in brisket disease is congestive failure of the right ventricle of the heart (Blake, 1961; Puntriano, 1954; Pierson, 1957; Hecht et al., 1959; Alexander and Jensen, 1959). The total heart weight is greatly increased in brisket disease cases, primarily due to an increase in weight and size of the right ventricle and to a lesser extent the interventricular septum (Blake, 1965b). The same author found that the lumen of the right ventricle was also greatly enlarged in cattle afflicted with brisket disease. By electron microscopy, the increase in size of the heart in brisket disease has been shown to be hypertrophy, due to intracellular edema, granulation, and hyalinization (Epling, 1968).

Hecht et al. (1959) demonstrated that cattle afflicted with brisket disease have near normal systemic blood pressure but an increased

pulmonary blood pressure. Alexander et al. (1960) postulated that the pulmonary hypertensive response of cattle at high altitudes was due to chronic hypoxia. They demonstrated a strong positive correlation between mean pulmonary arterial blood pressure and right ventricular cardiac mass. The increase in viscosity of blood which occurs at high altitude (Hurtado, 1932) and the increase in blood volume in the pulmonary circulation are also considered to be causative factors in brisket disease (Alexander and Jensen, 1959). Increases in pulmonary vascular pressure, viscosity, and volume of blood in the pulmonary circulation would place an increased work load on the heart, especially when the heart rate is already increased.

Although several authors have investigated the cause of the increased pulmonary blood pressure, and have observed the extra-thick muscularis of bovine pulmonary blood vessels (Hecht et al., 1959; Alexander and Jensen, 1963), it has been difficult to demonstrate gross anatomical peculiarities in the pulmonary tissues of afflicted cattle as compared to normal cattle (Blake, 1968).

It must be stressed that neither pulmonary hypertension nor right congestive heart failure is a pathognomonic sign for brisket disease, since cattle as well as other animals may develop these symptoms from many chronic conditions of the lung. Diseases such as chronic pneumonia and multiple abscessation (Pierson, 1957); and traumatic pericarditis pulmonary neoplasm, pulmonary infestation, diaphragm rupture, and pulmonary emphysema can be differentiated on bases other than enlarged right heart ventricle and edema (Blake, 1968). Hecht et al. (1962) have differentiated the high altitude disease of cattle (brisket disease)

from certain other causes of right ventricular enlargement and edema (brisket syndrome).

Exposure of rats and cattle to hypoxia at high elevation has been observed to increase the activity and thus produce hypertrophy of the adrenal glands (Sundstrom and Michaels, 1942; Langley, Scokel, and Whiteside, 1952; Puntriano, 1954; Van Liere and Fedor, 1955). Puntriano (1954) attributed brisket disease in cattle to adrenal cortical insufficiency following hypertrophy of the gland. A reduction in thyroid activity (Gordon and Tornetta, 1943; Van Middlesworth, 1949) was found to aid in adjustment of rats to simulated high altitude. High altitude appears to exert a deleterious effect on the kidney and liver. Increased activity and weight of the kidney were observed by Silvette (1943) and Kindred (1943) when rats were subjected to low barometric pressure. Several workers have noted degenerative changes in the liver of cattle suffering from brisket disease (Bourne, 1950; Pierson, 1957; Washburn et al., 1960). Liver, spleen, lungs, kidneys, and adrenal glands were also reported to be enlarged in cattle with brisket disease (Blake, 1965b).

Role of mineral imbalance

Calcium ions in the tissues govern the excitability of muscle and nervous tissue (Shanes, 1958). When the hypercalcemic state exists, the excitability of the tissues is decreased (Maxwell, Elliott, and Robertson, 1964). However, a modest diminution in the level of ionized Ca increases the excitability of nervous and muscle tissue, leading to tetanic seizures.

All the effects of excess K are believed to be caused by decreased membrane potentials resulting from high K levels in the extracellular

fluids (Szent-Gyorgyi, 1952). The most significant consequence of hyperkalemia is related to its effects on cardiac function (Goodman and Gilman, 1965). It may contribute to cardiac failure and ultimately be responsible for fatal arythmia or asystole. Bland (1956) reported that a high concentration of the K ion decreases nerve excitability and depresses the myocardium. In the myocardium, excess K influences both impulse conduction and muscle contractility resulting in heart block and poor muscle tone. Reiter (1964) has studied the effects of varying concentrations of several ions on myocardial contractility. He reported that an increased concentration of the K ion diminishes the force of contraction of mammalian heart muscle, whereas a decreased K concentration causes a marked positive effect. D'Silva (1937) reported that the release of epinephrine during the crisis of an illness caused liberation of glucose and K from the liver into the blood plasma. Furthermore, if blood circulation became stagnated, the muscles would discharge K into the circulation (Fenn et al., 1939). Severe or terminal illness is often accompanied by a raised rather than a depressed plasma K level (Wilde, 1962).

Sodium is a key factor in producing the clinical features of congestive heart failure (Bland, 1956). Intracellular Na as well as K are concerned with the fundamental chemical disturbances which are primary bases for inefficient cardiac contraction and cardiac failure (Friedberg, 1956). Magnesium deficiency induces alterations in plasma including hypomagnesemia, hypercalcemia, and hypophosphatemia (MacIntyre, 1963). Low Mg concentration in the plasma causes greatly increased irritability of the peripheral nerves and can cause tetany in skeletal muscle (Guyton, 1966). Hypomagnesemia also occurs

in congestive heart failure (Bland, 1956). Hypermagnesemia induces a fall in blood pressure, and if the level gets high enought, the heart stops in diastole (Wacker and Vallee, 1964). Bland (1956) also reports that hypermagnesemia is associated with progressive depression of neuromuscular conduction in the heart.

Since Cu deficiency diseases occur where the forage is normally low in Cu, several investigators have studied the serum level in blood of cattle (Beck, 1941; Beeson and Matrone, 1950; Cunningham, 1950). It has been shown that a level of 60 µg/100 ml indicates a Cu deficiency in cattle (Marston, 1952).

Either deficiencies or excesses of Cu result in anemia (Runnells, Monlux, and Monlux, 1965). Underwood (1959) reports that Cu deficiency causes cardiac myopathy which results in hypertrophy and cardiac failure. There was a marked depletion of cytochrome oxidase activity in the myocardium, and the hypertrophy was believed to be a compensation of the reduction in tissue respiration. There is an interrelationship in the action of Cu and Zn. Excess Zn inhibits the normal function of Cu in catalyzing Fe porphyrin formation (Jones, 1965; Adelstein and Vallee, 1962).

Blake (1965a) has studied the hematopathological conditions associated with brisket disease. It was found that cattle with brisket disease had abnormal levels of serum electrolytes. Calcium content was lower and K content was higher than normal. The author hypothesized that a Ca-K imbalance combined with the stress of hypoxia at high altitude could cause right ventricular hypertrophy and dilatation under conditions too mild for either stress alone to cause the cardiopathology. This hypothesis would reconcile the lack of a high, positive

correlation between incidence of brisket disease in Utah and the elevation at which the cattle reside (Blake, 1968).

To further support the hypothesis that mineral imbalance is related to brisket disease, other authors have reported related findings. Puntriano (1954) believed the pathogenesis of brisket disease to stem from deranged metabolism of blood electrolytes causing loss of plasma from the blood. The plasma loss was thought to result in increased blood viscosity, decreased volume, and hemoconcentration. Raleigh et al. (1955) suspected brisket disease to be due to nutritional deficiency and/or toxicity. The Colorado Experiment Station (Colorado Contributing Project No. 238, 1957), reported that brisket disease was associated with mineral deficiencies, especially of Fe and Cu, coupled with the stress of high altitude.

A recent study was conducted to determine the concentration of various minerals in the plant species found on ranges with wet meadowland type browse where brisket disease consistently occurs in Utah (Abaza, Blake, and Fisher, 1967). It was found that these plant species fail to provide a dietary balance of minerals for cattle. Their Ca content was approximately one-third of that needed to furnish the nutritional requirement of Ca for cattle and their oxalate contents were high enough to cause further deprivation of Ca. Potassium content of the plant species was approximately five times the level needed to supply the K requirement of cattle.

Chemical compositional imbalances in blood

Blake (1965a), in his report of hematopathologic conditions associated with brisket disease, found that the contents of blood serum

electrolytes were altered, depending on the severity of the disease. Calcium in the serum of healthy range cattle was 8.5 mg/100 ml, whereas those cattle with moderately severe cases of brisket disease showed decreased serum Ca concentrations to 7.7 mg/100 ml and those with severe cases of the disease had Ca concentrations of 5.5 mg/100 ml of serum. Potassium level of the blood serum was greatly increased with increase in severity of the disease, as follows: healthy cattle, 5.2 mEq/L; moderately affected cattle, 5.6 mEq/L; and severely affected cattle, 9.3 mEq/L. Sodium level of the serum was slightly reduced with increase in severity of the disease, as follows: healthy cattle, 142 mEq/L; and severely affected cattle, 137 mEq/L. Serum P content increased significantly with increase in severity of the disease, as follows: healthy cattle, 7.0 mg/100 ml; moderately affected cattle, 7.4 mg/100 ml; and severely affected cattle, 13.8 mg/100 ml.

EXPERIMENTAL METHODS

Experimental Animals

To determine the effects of hypoxia and a Ca-K ionic imbalance on the mineral content of bovine tissues, 40 Hereford calves approximately 3 months of age, of similar breeding, and from the same herd were obtained. They were transported to the Veterinary Science Research Laboratory at Utah State University, where they were given usual prophylaxis against infectious diseases, weighed, and individually identified. The prophylaxis consisted of vaccination with Clostridium Chauvei-Septicum and Clostridium Hemolyticum bacterins. The calves were then randomized into four groups, 10 calves per group, with five steers and five heifers per group. A two by two factorial design was employed and the two factors involved were altitude (low; 1,372 meters vs. high; 2,745 meters) and ion balance (balanced vs. imbalanced; low Ca--high K). The high altitude was considered adequate to cause hypoxia; whereas, the calves were native to the lower altitude. The four groups were arbitrarily assigned to treatments as follows: (A) lower altitude, ion balanced; (B) lower altitude, ion imbalanced; (C) high altitude, ion balanced; and (D) high altitude, ion imbalanced. The experimental design with groups and treatments is shown in Table 1, page 96.

The calves were confined in small enclosures at the two elevations for the duration of the 16-week study. Water and feed pellets were available *ad libitum* and oat hay was given at a fixed rate. The basal

feed pellets contained 32.5 percent oat hay, 35.5 percent barley, 4.0 percent molasses, 8.0 percent wheat bran, 16.0 percent cottonseed meal, 1.0 percent bakers flour, 1.0 percent oxytetracycline, 1.0 percent sodium chloride, and 1.0 percent dicalcium phosphate. The composition of the rations and comparison of amounts of electrolytes consumed and required by the calves are listed in Table 2, page 96.

Altitude-induced hypoxia

The two groups of calves, A and B, that were arbitrarily assigned at lower altitude of 1,372 meters were kept at Logan, Utah. These two groups were housed in separate corrals at the Veterinary Science Research Facility where they were supplied Logan city water in automatic waterers.

The other two groups, C and D, assigned to the high altitude of 2,745 meters were transported to the UM cattle allotment on the Fishlake National Forest. There they were housed in two wire corrals and allowed access to water from a spring.

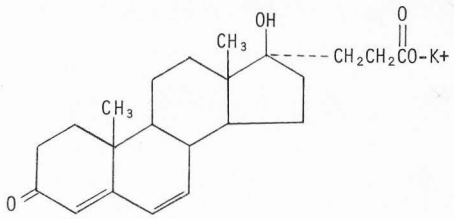
Experimental imbalancing of body Ca-K

To induce a Ca-K imbalance, an imbalanced diet and chemotherapeutic agents were given to the calves in group B at the lower elevation and group D at the high elevation. Calves in groups A and C received the basal feed pellets and oat hay; whereas, the calves in groups B and D received oat hay and an imbalanced feed pellet. These latter feed pellets were composed the same as the basal pellets, except KCl replaced NaCl and monobasic potassium phosphate replaced dicalcium phosphate. These alterations resulted in a diet that contained less than one-fourth the requirement of Ca and more than 10 times the requirement for K (National Academy of Science--National Research Council, 1963). In

manipulation, the Na content was also decreased to provide less than one-half the requirement of Na.

In addition to the Ca-K imbalance imposed by the diet, the calves in the ion imbalanced groups were subjected to chemotherapeusis as follows: parenteral administration of (1) K_2-EDTA (dipotassium salt of EDTA) to lower blood serum ionic Ca, (2) an aldosterone antagonist (SC-14266)[1] to impinge upon the normal rate of renal K elimination, and (3) KCl (potassium chloride) to further flood the circulatory system with K. SC-14266 was used rather than spironolactone, as it was more suitable to circumstances of the experiment, more versatile in use, yet equipotent. Its effects are more rapid and sustained and it is more stable and soluble than is spironolactone, as described by the manufacturer.

As described by the manufacturer, SC-14266 is a water-soluble compound with the ability to inhibit the renal electrolyte effects of aldosterone and other mineralocorticoids in laboratory animals. SC-14266 is the K salt of a steroid acid, and the structure and chemical name of the compound are as follows:



Potassium 3-(3-Oxo-17β-hydroxy-4,6-androstadien-17α-yl)propanoate

[1]G. D. Searle and Company, Chicago, Illinois

All dosages were calculated on a basis of 45 kg body weight per day, but administered at double this dosage and one-half the frequency for convenience. The K_2-EDTA was administered Monday, Wednesday, and Friday and the SC-14266 and KCl on Tuesday, Thursday, and Saturday each week. Initially, the drugs were given intramuscularly, but as soon as it was learned that much larger dosages would be necessary for the desired response, the route was changed to intraperitoneal via the right paralumbar fossa.

The dosages of K_2-EDTA used are shown in Table 3, page 97. The lower rate of 400 mg per 45 kg body weight was used the first 10 weeks and the higher rate of 1,700 mg per 45 kg body weight was used the last six weeks of the trial. The total quantity of K_2-EDTA administered was approximately 127 grams per calf and there were 44 injections over the period of 113 days. The K_2-EDTA was dissolved in distilled water and administered intramuscularly as a 30 percent solution and intraperitoneally as a 10 percent solution.

The lower intramuscular dosage rates of SC-14266 were 455 mg, then 910 mg and the ultimate intraperitoneal rate was 1,365 mg per 45 kg body weight, each injection. The 455 mg dosage was used the first three weeks, the 910 mg dosage the next seven weeks, and the 1,365 mg dosage the last six weeks of the experimental period. The proper dosage rate was not known and when the dosage suggested by the manufacturer did not induce hyperkalemia, the dosage was doubled, and then tripled. The posology and total quantity of SC-14266 administered are shown in Table 3, page 97. The SC-14266 had to be stored in an atmosphere of nitrogen to prevent its oxidation and had to be injected without delay once placed in solution. To reconcile the special problems

of the drug, to the special problems posed by the primitiveness of the geographic area where the calves at high altitude were kept, the powder was repackaged into quantities sufficient for each treatment, then resealed in nitrogen. On the treatment day, the proper amount of distilled water was added to each vial just prior to use. For uniformity, the same procedure was used for the treatment of calves more conveniently located at the lower altitude.

The last four weeks of the experimental period, a 10 percent solution of KCl in distilled water was administered intraperitoneally on the same days as the SC-14266 was given. The dosage was 400 mg per 45 kg body weight, each injection. The posology and total quantity of KCl administered are shown in Table 3, page 97.

Criteria for Evaluating Influences of Hypoxia and Induced Ca-K Imbalance

The criteria used to evaluate the influences of the combined stresses of hypoxia and induced ionic imbalance were the levels of minerals in body tissues vital for normal cardiac function and body fluid balance. The tissues involved were blood, heart, liver, kidney, and bone. The minerals quantitatively determined were Ca, K, Na, P, Mg, Cl, Fe, Zn, and Cu. (See Table 4, page 98.)

Sampling Procedures

Collection

Whole blood and serum samples from each calf were collected nine times during the 16-week experimental period, at approximately 2-week intervals. At each collection the blood samples were taken 4 hours

after treatment with EDTA, which was 28 hours after treatment with SC-14266 and KCl. Three times, blood samples were also collected 28 hours after treatment with EDTA, which was 4 hours after treatment with SC-14266 and KCl. The purpose was to determine the sustained effects of the treatments during the 48-hour treatment interval. Venous blood (5 ml) was drawn via jugular-puncture into 11 x 75 mm plastic test tubes that contained 5 mg of heparin. After collection, the tubes were capped tightly and retained for future analysis.

Blood for serum separation was also collected via vena-puncture. Approximately 15 ml of whole blood were collected in Whirl Pak Bags,[2] allowed to clot and retract, and then 5 ml of blood serum were poured into 11 x 75 mm plastic test tubes and the tubes tightly sealed.

At the end of the experimental period, all calves were killed and necropsied. Tissue samples of approximately 100 grams each were taken from heart, liver, and kidney and placed in tightly sealed plastic bags. The cardiac tissue samples comprised complete cross-sections of the interventricular septum at the point of attachment of the moderator band in the right ventricle. Liver samples comprised complete cross-sections at a point just distal to the attachment of the gall bladder. A central cross section was taken from the right kidney of each calf.

Three cross section discs, one midpoint and one at each end of the diaphysis, were taken from the right metatarsal of each calf for mineral analysis. The bone marrow, endosteum, and periosteum were removed, and the discs were then sealed in plastic bags.

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[2]Scientific Products, Evanston, Illinois.

Storage

The whole blood and serum samples in sealed plastic tubes were refrigerated at 4 C until chemical analyses could be conducted. Heart, liver, kidney, and bone samples in sealed plastic bags were stored in a freezer at approximately -20 C until tissue preparation for chemical analysis could be completed.

Tissue Preparation for Chemical Analyses

Whole blood and serum

The whole blood and serum were prepared for analysis by removing the samples from the refrigerator and allowing them to come to room temperature and then pipetting the required amount into chemically cleaned glass test tubes containing double distilled water to give the proper dilution.

Heart, liver, and kidney

The frozen samples of heart, liver, and kidney were removed from the freezer and allowed to come to room temperature. Each sample was then carefully cut into several smaller pieces and placed in a Waring Blendor[3] with semi-micro stainless steel container and blades. The samples were completely homogenized, transferred to Whirl Pak Bags, and refrigerated at 4 C until subsamples were taken for chemical analysis.

Bone

The bone discs were allowed to come to room temperature, then extracted with ether to remove the lipid material according to the

[3]Waring Products Company, Winsted, Connecticut.

method of Saxon (1914). The defatted bone discs were then ground through a Wiley Mill[4] with stainless steel blades and the ground composite of the three discs from each calf was transferred to a Whirl Pak Bag and stored at 4 C until subsamples were taken for chemical analysis.

Chemical Analysis

Glassware for all of the chemical procedures in this research was chemically cleaned. The cleaning procedure was as follows: wash with Haemo-sol,[5] rinse with tap water, rinse with distilled water, wash in hydrochloric acid bath, and finally rinse twice with double distilled water.

Standards

Stock standards

Stock solutions of 100 mg/100 ml concentration were prepared for the elements studied except P and Cl. Calcium carbonate was dissolved in 5 percent nitric acid to prepare the Ca stock standard. Potassium and sodium chlorides separately dissolved in double distilled water were used for the K and Na stock standards. Phosphorus stock standard was prepared by dissolving sufficient potassium phosphate in double distilled water to make a solution containing 40 mg/100 ml. Magnesium sulfate was dissolved in a mixture of nine parts ethanol and one part of 5 percent nitric acid to prepare the Mg stock standard. Iron and Zn stock standards were prepared by dissolving Fe wire and Zn powder in

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[4]Model 4276M, Arthur H. Thomas Company, Philadelphia, Pennsylvania.

[5]Scientific Products, Evanston, Illinois.

5 percent nitric acid. Copper wire was dissolved in 50 percent nitric acid to prepare a Cu stock standard. The Cl stock standard was prepared by dissolving sufficient reagent grade NaCl in double distilled water to make a solution containing 100 mEq/L (355 mg/100 ml).

Working standards

Each time determinations were made, working standards were prepared from the stock standards by diluting with double distilled water. The working standards were in the ranges of 0.1 to 2.0 mg/100 ml for Ca, 0.1 to 1.0 mg/100 ml for K, 0.01 to 0.10 mg/100 ml for Na, 0.01 to 0.10 mg/100 ml for Mg, 0.4 to 2.0 mg/100 ml for Fe, 0.05 to 0.50 mg/100 ml for Zn, and 0.01 to 0.10 mg/100 ml for Cu. The P working standard contained 8 mg/100 ml P and the stock standard for Cl served undiluted as the working standard. The accuracy of the analytical method is as follows: P and Cu, 0.2 ppm; Ca, K, Cl, and Fe, 0.1 ppm; Na and Zn, 0.05 ppm; and Mg, 0.01 ppm.

Dry matter

Dry matter percents (dried sample weight x 100/wet sample weight) of cardiac, hepatic, renal, and osseous (right metatarsal bone) tissues were determined. Duplicate samples of approximately two grams for each tissue were carefully weighed into clean, dry pyrex glass weighing bottles, and the bottles were placed in a drying oven.[6] The oven was maintained at 90 C and a pressure of 150 mm of mercury was maintained inside the oven by use of a vacuum pump.[7] After the samples had been in the drying oven for six hours, they were removed, placed in a

[6]Model 31468, Precision Scientific Company, Chicago, Illinois.

[7]Model A557, Arthur H. Thomas Company, Philadelphia, Pennsylvania.

dessicator, and cooled to room temperature. The samples were again weighed and the percent dry matter calculated.

Ash content

The percent ash (ashed sample weight x 100/wet sample weight) of cardiac, hepatic, renal, and osseous tissues was determined. Duplicate two-gram samples of each tissue were carefully weighed into clean, dry pyrex glass ashing crucibles. The crucibles were then placed in a muffle furnace[8] and maintained at approximately 500 C overnight. The ashed samples in the crucibles were then cooled to room temperature in a dessicator and weighed again. The percent ash was calculated.

Mineral elements

Whole blood and serum: Ca, K, Na, P, Mg, Cl, Fe, Zn, and Cu

The concentrations of K and Na in both whole blood and blood serum, Fe in whole blood, and Ca in blood serum were determined for samples obtained each of the nine times. The concentrations of P, Mg, Cl, Zn, and Cu in blood serum were determined only for samples obtained the first, fifth, and eighth sampling times. Potassium, Na, and Fe were the only mineral elements determined in whole blood. The concentrations of Ca, K, Na, P, Mg, Cl, Zn, and Cu were determined in blood serum. The methods and dilutions used for the chemical analyses of whole blood and blood serum are shown in Table 5, page 98. An atomic absorption spectrophotometer[9] was used to determine the concentrations of K, Na,

[8]Electric Multiple Unit Furnace, Type 62, Denver Fire Clay Company, Denver, Colorado.

[9]Perkin-Elmer Model 303.

and Fe in whole blood; and the concentrations of K, Na, Mg, Zn, and Cu in blood serum, according to the methods of Allan (1961), David (1962), and Willis (1963). Calcium concentrations in blood serum were determined using an ultramicro modification[10] of the method of Diehl and Ellingboe (1956). The inorganic P concentration in blood serum was determined according to the method of Hycel, Inc. (1965) by the use of a colorimeter.[11] Chloride concentration in blood serum was determined by the method of Schales and Schales (1941), using ultramicro equipment.[12]

Heart, liver, and kidney: Ca, K, Na, P, Mg, Fe, Zn, and Cu

Calcium, K, Na, P, and Mg concentrations were determined in cardiac, hepatic, and renal tissues. Iron, Zn, and Cu concentrations were also determined in cardiac and hepatic tissues. Concentrations of all of these elements, except P, were determined by atomic absorption spectrophotometry.[13] The P concentrations in cardiac, hepatic, and renal tissues were determined colorimetrically, according to the method of Fiske and Subbarow (1925).

The same tissue samples that were used to determine the dry matter content were wet ashed and used for the mineral analyses. The dried sample in weighing bottle was treated with 4 ml of concentrated nitric acid and 2 ml of 30 percent hydrogen peroxide and to prevent violent

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[10]Beckman/Spinco Model 150 Ultramicro Analytical System, Beckman Instruments, Inc., Fullerton, California.

[11]Bausch and Lomb Spectronic 20, Type 33-29-40, Bausch and Lomb, Inc., Rochester, New York.

[12]Beckman/Spinco Model 150 Ultramicro Analytical System, Beckman Instruments, Inc., Fullerton, California.

[13]Perkin-Elmer Model 303.

boiling, allowed to set overnight without heating. The samples were then cautiously heated to boiling, cooled, and 4 ml of concentrated nitric acid and 2 ml of 30 percent hydrogen peroxide were again added. The samples were again heated, kept at boiling temperature until the volume was reduced to approximately 2 ml, then cooled. The procedure of adding nitric acid and hydrogen peroxide, boiling, and cooling was repeated three times to permit complete digestion of samples. The resultant clear solution of approximately 2 ml was then quantitatively transferred to a 25 ml volumetric flask and diluted to volume with double distilled water. This 25 ml solution was then stored in 30 ml capped pyrex test tubes. Chemical analyses were then conducted on dilutions of this 25 ml solution. (See Table 6, page 99.)

Bone: Ca, K, Na, P, Mg, and Zn

Phosphorus was determined by the method of Fiske and Subbarow (1925). The other mineral elements were quantitatively determined using atomic absorption spectrophotometric methods (David, 1962; Willis, 1963). The bone samples that were previously dry-ashed were wet-ashed, diluted, and used for chemical analyses. To each dry-ashed bone sample were added 6 ml of concentrated nitric acid and 3 ml of 30 percent hydrogen peroxide. The samples were allowed to set for two hours without heating to prevent rapid boiling. The bone samples were completely dissolved and the resultant clear solutions were then heated to boiling, cooled, and quantitatively transferred to 25 ml volumetric flasks and diluted to volume with double distilled water. The 25 ml solution was then transferred to, and stored in, 30 ml capped pyrex test tubes. Further dilutions of this 25 ml solution were used for chemical analyses. (See Table 6, page 99.)

Statistical Procedure

The statistical procedure followed was the analysis of variance for factorial experiments as described by Cochran and Cox (1957). An analysis of variance for a two-by-two factorial experiment was conducted for the concentration of each element and dry matter and ash content in cardiac, hepatic, renal, and osseous tissues. The two factors were elevation and Ca-K balance.

The concentrations of mineral elements in whole blood and blood serum were statistically analyzed utilizing the analysis of variance for the split-plot in time design for factorial experiments (Steel and Torrie, 1960). The factors involved were elevation, Ca-K balance, and periods (times). The 16 weeks of the experiment were divided into three periods. Period I was the pretreatment period. Period II consisted of the first 10 weeks of the trial in which the chemotherapeutic agents were given at the low dosage level. Period III was the last 6 weeks of the experimental trial in which the chemicals were given at the high dosage level.

EXPERIMENTAL RESULTS

In this study, nearly 6,000 analyses were made to determine the mineral content of blood, heart, liver, kidney, and bone samples from the calves used (Table 4). The results are tabulated in the appendix. The analytical errors involved in the various determinations was 5 percent or less.

The data shown in Tables 7 through 59 are on an individual calf basis and concern the mineral composition of blood at periodic intervals during the study and the mineral composition and other properties of cardiac, hepatic, renal, and osseous tissues at the termination of the study. In Tables 7-18 are shown the individual Ca, K, Na, P, Mg, Cl, Fe, Zn, and Cu content of blood obtained from each calf. In Tables 19-30, the concentrations, determined in duplicate analyses, of Ca, K, Na, P, Mg, Fe, Zn, and Cu; and the Ca/P ratio, Ca/Mg ratio, dry matter, and ash content of cardiac tissue from each calf are shown. Similarly, duplicate analyses of the Ca, K, Na, P, Mg, Fe, Zn, Cu, dry matter, and ash content of hepatic tissue from each calf are shown in Tables 31-40. Duplicate analyses of renal tissue from each calf were made and the Ca, K, Na, P, Mg, Na/K ratio, dry matter, and ash content are shown in Tables 41-48. In Tables 49-59 are listed results of duplicate analyses made to determine the Ca, K, Na, P, Mg, Zn, Ca/P ratio, dry matter, and ash content of osseous tissue (right metatarsal bone) from each calf.

In Tables 60 through 70 are shown data similar to those shown in the series of Tables 7 through 59, except the values in the latter group of tables are averages per group of 10 calves, for the four groups used

in this study. Group means for the Ca, K, Na, P, Mg, Cl, Fe, Zn, and Cu content of blood are shown in Tables 60-66. Shown in Tables 67-70 are group mean concentrations of certain minerals in cardiac, hepatic, renal, and osseous tissue.

An analysis of variance was conducted on the data for the mineral content of tissues, and the mean squares and variance ratios (F values) are listed for blood (Tables 71-74), cardiac (Tables 75-77), hepatic (Tables 78-80), renal (Tables 81-82), and osseous (Tables 83-84) tissues.

Summaries of the mineral content in the various tissues are shown in Tables 85-92. In Figures 2-6, the summary of influences of hypoxia and ion imbalance on concentration of minerals in blood and other body tissues are depicted. Direction of change and statistical significance are shown.

Degree of Ca-K Imbalance Attained in Blood

Blood serum Ca concentration was unaffected at the low K_2-EDTA dosage level used during period II as compared to the pretreatment values and the values for untreated calves (Tables 60 and 71). However, at the high K_2-EDTA dosage level of period III, the Ca concentration in serum decreased 1.7 mg/100 ml from the initial value of 9.5 mg/100 ml in treated calves at high elevation and 1.4 mg/100 ml from the initial value of 9.2 mg/100 ml in treated calves at low elevation. The lowering of Ca in serum of calves in groups B and D during period III was statistically significant at the $P = 0.01$ level (Table 72). There was a sustained decrease of Ca concentration in serum during the high level intraperitoneal administration of K_2-EDTA during the six weeks of

period III (Figure 1). There were no statistically significant changes resulting from elevation differences in serum Ca concentration.

Increases in K concentration of 1.2 mEq/L and 2.0 mEq/L in treated calves at low elevation (Group B) and of 1.6 mEq/L and 1.5 mEq/L in treated calves at high elevation (Group D) occurred in blood serum (Table 61) and whole blood (Table 62), respectively. These increases were not statistically significant (Table 72) for $P < 0.05$, but the difference in whole blood was significant for $P < 0.10$. Large variations of K concentration from blood sampling time to blood sampling time reduced the sensitivity of the statistical measurement of drug effect.

There was a trend for K concentrations in both whole blood and serum to increase during the 16-week trial period, irrespective of drug treatment level. High elevation had an effect on K concentrations in both serum and whole blood. The K concentration in serum of calves at high elevation increased significantly ($P < 0.05$, Table 72) compared with that of calves at low elevation during period III (Table 61).

Influences of Hypoxia and Induced Ca-K Imbalance on Composition of Body Tissues

Hypoxia

The effects described in this section are those attributed to the single factor of hypoxia (low elevation compared to high elevation), and the reported data are averages of the two groups of calves, C and D, at high elevation, compared to averages of the two groups of Calves,

A and B, at the lower elevation as shown in Table 1. In some instances, the effects were quite pronounced; whereas, in others, there was little distinguishable effect.

Dry matter

Hypoxia had a very slight effect on the dry matter content of cardiac, hepatic, renal, and osseous tissues. Although the dry matter content of cardiac tissue was less in calves at high elevation than in calves at low elevation (Table 67), the difference was not statistically significant (Table 77). There was essentially no difference in the dry matter content of either hepatic (Tables 68 and 80) or renal (Tables 69 and 82) tissues due to high elevation. The dry matter content of osseous tissue (right metatarsal bone) was higher in calves at high elevation (Table 70).

Ash content

Hypoxia did not have a marked effect on the ash content of cardiac, hepatic, renal, or osseous (metatarsal bone) tissues in calves at the two elevations. The ash content of cardiac (Table 67) and renal tissue (Table 69) was lower, and of hepatic (Table 68) and osseous tissue (Table 70) was higher in calves at high elevation than in calves at low elevation. However, none of these changes were statistically significant (Tables 77, 82, 80, and 84, respectively).

Mineral elements

Whole blood and serum: Ca, K, Na, P, Mg, Cl, Fe, Zn, and Cu. Hypoxia had varied effects on the concentration of minerals in whole blood and blood serum. Although the Ca concentration in serum was slightly

less in the calves at high elevation than in calves at the lower elevation during period II (Table 60), there was no statistically significant difference (Table 71).

The K concentration in blood serum of calves at high elevation was 7.85 mEq/L as compared to 7.50 mEq/L for calves at low elevation (Table 61) during period III. This difference was statistically significant at the P = 0.05 level (Table 72). Although the trend was for concentrations of K in blood serum and whole blood of calves at high elevation to increase with the passing of time (see Tables 61 and 62), the only statistically significant change was the one indicated above.

The concentration of Na in whole blood and blood serum was variable from blood sampling time to blood sampling time and did not show a trend as did K. The only statistically significant difference ($P < 0.05$) between the Na concentrations in blood of calves at the two elevations occurred in Na concentrations in blood serum during period II (Table 71). During this period, the mean Na concentration in blood serum of calves at low elevation was 157.1 mEq/L, compared to a level of 148.3 mEq/L in blood serum of calves at high elevation (Table 63).

Although there were small differences in P, Mg, Cl, Zn, and Cu concentrations in blood serum, both between and within calf groups at the two elevations (Table 66), the elements were, ostensibly, uninfluenced by elevation (Table 74).

The effect of hypoxia, due to elevation, on the Fe concentration of whole blood was highly significant statistically ($P < 0.01$, Table 72). The mean Fe concentration of whole blood of calves at low elevation during period III was 48.1 mg/100 ml as compared to a concentration of 49.4 mg/100 ml in the blood of calves at high elevation (Table 65).

Heart, liver, and kidney: Ca, K, Na, P, Mg, Fe, Zn, and Cu. The effect of hypoxia on the Ca concentrations in both cardiac (Table 75) and renal (Table 81) tissue was highly significant ($P < 0.01$). The Ca concentration in cardiac tissue was 249.6 ppm for calves at low elevation and was 227.5 ppm for calves at high elevation (Table 67). The renal tissue of calves at high elevation contained 433.3 ppm of Ca, compared to 357.2 ppm for calves at low elevation (Table 69). The Ca concentration in hepatic tissues was essentially the same for the calves at low and high elevation (Table 68).

There were only small differences, nonsignificant statistically, in the K concentration in cardiac (Tables 67 and 75), hepatic (Tables 68 and 78), and renal (Tables 69 and 81) tissues of calves at the two elevations.

The Na concentration in cardiac tissue was less in calves at high elevation than in calves at lower elevation (Table 67), but not significantly (Table 75). The decreased concentration of Na in hepatic tissue (Table 68) of calves at high elevation (4.7 ppt) compared to those at low elevation (4.4 ppt) was highly significant (Table 78). The Na concentration in renal tissues of calves at high elevation was significantly greater ($P < 0.05$, Table 81) than for those at low elevation (Table 69).

Phosphorus values for cardiac, hepatic, and renal tissues were similar for all calves. The only statistically significant difference ($P < 0.05$) was increased P in renal tissue of calves at high altitude (Tables 69 and 82).

The effect of hypoxia was to increase the Mg concentration in cardiac and hepatic tissues and decrease Mg concentration in renal tissue

(compare groups A and B to C and D, Table 89). None of the effects were statistically significant.

The concentrations of Fe, Zn, and Cu in cardiac and hepatic tissues were comparable for calves at the two elevations (Tables 67 and 68).

Bone: Ca, K, Na, P, Mg, and Zn. There was little effect of hypoxia on the Ca concentration in osseous tissue. The mean for the calves at both high and low elevation was 28.9 percent Ca for bone ash (Table 70).

The decrease in K concentration and increase in P concentration in osseous tissues of calves at high elevation (Table 70) were significant ($P < 0.01$, Table 83).

Sodium (Tables 70 and 83), Mg, and Zn (Tables 70 and 84) concentrations were significantly ($P < 0.05$) decreased in calves at high elevation compared to calves at lower elevation.

Induced Ca-K imbalance

The results described in this section are those effects attributable to the induced Ca-K imbalance due to an imbalanced diet and the administration of the chemotherapeutic agents employed. As a rule, these effects were more profound than those due to hypoxia. The data reported are based on averages of two groups of untreated calves, A and C, compared to averages of the B and D groups of calves which were "treated" with the unbalanced diet and chemotherapeutic agents.

Dry matter

The Ca-K imbalance caused a slight increase in the dry matter content of cardiac tissue (Table 67), from 22.28 percent (mean of groups A and C) to 22.54 percent (mean of groups B and D), which was

not statistically significant. There was a highly significant ($P < 0.01$) decrease in the dry matter content of both hepatic (Table 80) and renal (Table 82) tissues, attributable to the Ca-K imbalance. Hepatic tissue (Table 68) dry matter averaged 25.30 percent in the treated calves compared to 26.75 percent in the untreated calves; similarly, dry matter of renal tissue (Table 69) averaged 17.79 percent and 20.38 percent, respectively. The dry matter content of osseous tissues (metatarsal bone) was not significantly altered due to the Ca-K imbalance (Table 70).

Ash content

The ash content of cardiac (Table 67), hepatic (Table 68), and osseous (Table 70, metatarsal bone) tissues was less for the calves stressed with the Ca-K imbalance than for the other calves, but these decreases were not statistically significant. The renal tissues of calves subjected to the Ca-K imbalance had a significant ($P < 0.01$, Table 82) decrease in ash content. The ash content of renal tissue averaged 1.15 percent in Ca-K imbalanced calves as compared to 1.24 percent for untreated calves (Table 69).

Mineral elements

Whole blood and serum: Na, P, Mg, Cl, Fe, Zn, and Cu. Although there was a generalized trend for the Na concentration in both whole blood and blood serum to increase during the experimental period in calves that did not receive the chemotherapeutic agents (Table 87), the values were inconsistent and varied considerably, both within and between groups of calves. The only statistically significant ($P < 0.01$, Table 72) difference in Na concentration between treated and untreated calves

was for serum Na during period III. The Na concentration in serum was less in the treated calves.

The P (Table 88) and Mg (Table 89) concentrations in serum of Ca-K imbalanced calves were less during period III than for control calves, but the differences were not significant statistically.

A highly significant ($P < 0.01$) increase in the Cl concentration during period III was observed in the Ca-K imbalanced calves (Table 74). The mean concentration of Cl in serum of untreated calves was 397 mEq/L; whereas, the mean Cl concentration was 435 mEq/L in the serum of Ca-K imbalanced calves (Table 66).

There was a general tendency for the Fe concentration in whole blood to decrease in all calves during the 16-week experimental period (Table 65). However, the Ca-K imbalanced calves exhibited a greater decrease than did untreated calves. The Fe concentrations in whole blood were 55.5 mg/100 ml and 55.4 mg/100 ml for period I; 52.9 mg/100 ml and 49.5 mg/100 ml for period II; and 51.5 mg/100 ml and 46.0 mg/100 ml during period III for untreated and Ca-K imbalanced calves, respectively (Table 65). The greater decrease of the Fe concentration in whole blood of Ca-K imbalanced calves was highly significant ($P < 0.01$, Table 73).

The Zn concentration in blood serum was approximately the same initially (period I) and decreased for all calves during period II; however, Zn in the serum of Ca-K imbalanced calves continued to decrease during period III, whereas it did not in the untreated calves (Table 66). As a result, for period III, the Zn concentration in Ca-K imbalanced calves was down to 137 μg/100 ml, compared to a mean concentration of 183 μg/100 ml for the untreated calves. Due to the great

variability among animals, this difference was not statistically significant.

A highly significant ($P < 0.01$, Table 74) decrease occurred in the serum concentration of Cu during both periods II and III for the Ca-K imbalanced calves as compared to the untreated calves. The Cu concentrations in serum changed as follows: period I, untreated calves 106 µg/100 ml and Ca-K imbalanced calves 107 µg/100 ml; period II, untreated calves 102 µg/100 ml and Ca-K imbalanced calves 91 µg/100 ml; period III, untreated calves 109 µg/100 ml and Ca-K imbalanced calves 91 µg/100 ml (Table 92).

Heart, liver, and kidney: Ca, K, Na, P, Mg, Fe, Zn, and Cu. The changes in Ca concentration in cardiac, hepatic, and renal tissues for Ca-K imbalanced calves were highly significant ($P < 0.01$, Tables 75, 78, and 81, respectively). The group mean Ca concentration in cardiac tissues for Ca-K imbalanced calves was decreased to 225 ppm as compared to 251 ppm for untreated calves (Table 85), and for hepatic tissue Ca concentration was decreased to 247 ppm for Ca-K imbalanced calves compared to 286 ppm for untreated calves.

In renal tissue there was a large increase in Ca concentration due to the Ca-K imbalance. Untreated calves had a mean Ca concentration of 280 ppm and Ca-K imbalanced had a mean of 538 ppm Ca.

An increase of K concentration in cardiac tissue and decreases of K concentration in hepatic and renal tissues were statistically highly significant ($P < 0.01$) in the tissues from Ca-K imbalanced calves as compared to untreated calves (Tables 75, 78, and 81, respectively). The increase in the K concentration of cardiac tissue was from 5.1 ppt (untreated calves) to 5.8 ppt (Ca-K imbalanced calves), the K

concentration in hepatic and renal tissues for untreated and Ca-K imbalanced calves decreased from 14.5 ppt to 13.7 ppt; and from 10.3 ppt to 9.7 ppt, respectively (Table 86).

The Ca/K ratio and the Ca/Mg ratio of cardiac tissue (Table 77), and the Na/K ratio of renal tissue (Table 81) were all smaller ($P < 0.01$) in Ca-K imbalanced calves than in control calves. In cardiac tissue, the Ca/K ratio decreased from 0.049 to 0.039 and the Ca/Mg ratio decreased from 0.27 to 0.20 for Ca-K imbalanced as compared to untreated calves (Table 67). The Na/K ratio for renal tissue decreased from 1.02 to 0.94 for Ca-K imbalanced calves (Table 69).

The concentration of Na in cardiac (Table 75), hepatic (Table 78), and renal (Table 81) tissues was less in Ca-K imbalanced calves than control calves by a highly significant amount ($P < 0.01$). The differences were 3.5 ppt and 2.9 ppt for cardiac tissue; 4.8 ppt and 4.4 ppt for hepatic; and 10.6 ppt and 9.1 ppt for untreated calves and Ca-K imbalanced calves, respectively (Table 87).

The P concentrations in cardiac (Table 76) and renal (Table 82) tissues were greater ($P < 0.01$) and in hepatic tissue (Table 78) less ($P < 0.05$) in Ca-K imbalanced calves than in control calves. The mean concentrations of P in cardiac, hepatic, and renal tissues were 8.35 ppt and 8.85 ppt; 16.2 ppt and 15.9 ppt; and 7.52 ppt and 9.05 ppt for untreated and Ca-K imbalanced calves, respectively (Table 88).

The increase in Mg (Table 89) concentration in cardiac tissue from 923 ppm for untreated calves to 1,104 ppm for Ca-K imbalanced calves was highly significant ($P < 0.01$, Table 75). The concentration of Mg in hepatic tissues increased significantly ($P < 0.05$, Table 79) from 730 ppm for untreated calves to 765 ppm for Ca-K imbalanced calves

(Table 89). The concentration of Mg in renal tissue was decreased in Ca-K imbalanced calves, also by a highly significant amount ($P < 0.01$, Table 82). Means were 532 ppm and 473 ppm for untreated and Ca-K imbalanced calves, respectively (Table 89).

The concentration of Fe was little influenced in cardiac tissue but greatly decreased ($P < 0.01$, Table 79) in hepatic tissue from the Ca-K imbalanced calves. In hepatic tissue, the concentration of Fe was 250 ppm for untreated calves and 199 ppm for Ca-K imbalanced calves (Table 90).

The decrease of Zn and Cu in both cardiac (Table 76) and hepatic (Tables 79 and 80, respectively) tissues was highly significant statistically ($P < 0.01$) for Ca-K imbalanced calves as compared to untreated calves. The Zn concentration (Table 91) was reduced from 116 ppm to 92 ppm in cardiac tissue and from 230 ppm to 163 ppm in hepatic tissue for untreated and Ca-K imbalanced calves, respectively. The concentration of Cu (Table 92) was reduced from 21 ppm to 16 ppm in cardiac tissue, and from 82 ppm to 44 ppm in hepatic tissue for untreated and Ca-K imbalanced calves, respectively.

Bone: Ca, K, Na, P, Mg, and Zn. The induced Ca-K imbalance had a marked influence on the mineral composition of bone ash (Table 70). The Ca, P, and Mg concentrations were reduced ($P < 0.01$, Tables 83 and 84) and the K concentration increased ($P < 0.01$, Table 83). The Na concentration was little influenced. Inasmuch as Ca reduction was greater than P reduction, the Ca/P ratio (Table 70) was also decreased ($P < 0.05$, Table 83). The Zn concentration was significantly greater ($P < 0.01$, Table 84) in bone of Ca-K imbalanced calves than in bone of control calves (Table 70).

Changes in mineral composition of bone ash due to the induced Ca-K imbalance were as follows (Table 70): Ca decreased to 27.3 percent from 32.5 percent; K increased to 0.161 percent from 0.080 percent; P decreased to 16.5 percent from 19.0 percent; Mg decreased to 0.508 percent from 0.828 percent; and Zn increased to 319 ppm from 227 ppm.

DISCUSSION

In Utah, brisket disease in cattle has been under investigation for several years. Pulmonary hypertension, right heart failure, and generalized body fluid imbalance are pathologic characteristics of the disease and indicate inadequate compensation for hypoxia. The right cardiac ventricular hypertrophy and dilatation which occur in afflicted cattle are results of attempts to compensate for reduced alveolar oxygen by increasing pulmonary circulation.

The imbalance of hypocalcemia and hyperkalemia, which has been demonstrated in afflicted cattle (Blake, 1965a) would also subject cardiac tissue to the same pathologic imbalance. A deficiency of Ca ions and an excess of K ions in the environment of the myocardial fibers would deleteriously influence contractility and thus quicken and exaggerate enlargement of the right side of the heart of an animal that was under the stress of hypoxia.

To study the magnitude of the effect of Ca deficiency and K excess in cardiac tissue on right cardiac ventricle performance, it was imperative to find a method of experimentally producing such an imbalance. By inducing a hemic Ca-K imbalance it was assumed that the same imbalance would also occur in vital organs, including the heart, providing the hemic imbalance could be sustained for a prolonged period of time.

In the present experiment the imposition of the two stresses, hypoxia and ion imbalance, did cause signs and lesions which simulated brisket disease. There occurred structural changes in the right side

of the heart; i.e., right ventricular hypertrophy and dilatation. There were also fluid imbalances but to a lesser extent than occur in natural cases of the disease. There was hypertrophy of the lungs, adrenal glands, liver, kidney, and thyroid-parathyroid glands. These findings will be published later. Physiological measurements were also made on the calves; i.e., blood gaseous composition, blood pH, and electrocardiograms. These findings were reported in a thesis (Lu, 1968). It is not pertinent to this dissertation to enlarge further upon symptomatic similarities to the natural disease, or physiopathological changes in the experimental calves; the intent of the present work being to report on tissue chemical changes.

Induction of Hemic Mineral Imbalance

The chemotherapeutic agents employed in this study were given to imbalance blood electrolytes, particularly Ca and K. It was presumed that K_2-EDTA would chelate primarily Ca in the plasma since this is the most abundant hemic divalent ion. It was known that the EDTA would also chelate Mg, Fe, Zn, and Cu, thus increasing the total ion imbalance.

Although it is difficult to produce a hyperkalemia (Drescher et al., 1958), an effective way might be to inhibit aldosterone. It was known that an aldosterone inhibitor would exert an influence on Na concentration. The purpose of repeatedly injecting KCl into the body was to "flood" the body with K, and concommitantly alter the Cl content of the body.

It was presumed that further imbalance would occur as an indirect consequence, because of interrelationships between various minerals in

the animal body. An increase in Ca is usually accompanied by a decrease in Mg and vice versa (Smith and Jones, 1966). An excess or deficit of Zn causes Cu deficiency and a Cu deficiency induces Fe deficiency anemia (Jones, 1965).

Compared to nutritional requirements (National Academy of Science--National Research Council, 1963), the ration of the Ca-K imbalanced calves was low in Ca, marginally low in Na, and excessively high in K. The imbalanced ration contained only one-fourth of the amount of Ca, one-half of the Na, and 10 times the K required. The ration of untreated calves was well balanced in these elements except for an excess of K. It was not possible to keep the K level within recommended nutritional bounds in the basal ration, but the basal ration contained approximately only one-half the amount of K as in the imbalanced ration. Both rations were relished by the calves, and transition was made from milk of the dam without difficulty. Calves in all four groups were soon consuming more than 1.4 kg of dry matter per 45 kg of body weight.

Hypocalcemia

The high dosage level of K_2-EDTA given during period III proved to be the maximum dose that could be safely given. Even at this dose, some of the calves became prostrate and remained so for several hours after an inoculation was given. They appeared clinically similar to cows with milk fever. A larger dosage rate may have been fatal.

The dosage of 1,700 mg of K_2-EDTA per 45 kg of body weight administered intraperitoneally three times per week on alternate days proved adequate to sustain a decreased Ca concentration in serum. Although there was some undulation of Ca level with the repeated injections and intervals between injections, there was a constant marginal hypocalcemia

in the treated calves for the 6-week duration of period III. Reduction of Ca concentration in serum attained by this method was approximately the same as was attained by other investigators (Brown and Smith, 1964; Pischke and Stott, 1964) with an intravenous drip method. Their dose levels were slightly higher at 2 to 3 grams per 45 kg of body weight, as compared with the dose level of 1.7 grams per 45 kg used in the present experiment. Their higher levels were permissable because of the slow infusion technique. The easier procedure of rapid intraperitoneal administration at two-day intervals and at a lower dosage level of EDTA to achieve the same degree of hypocalcemia has advantages over the dangerous, laborious, and limited intravenous drip method. Results of the present experiment indicate that cattle can tolerate repeated intraperitoneal injections of EDTA over a period of at least several months.

In the EDTA-chelated Ca molecule, the Ca is tightly bound. The complexing of other ions in solution by EDTA also occurs and is dependent upon the pH (Blaedel and Meloche, 1963). At the pH of blood, chelation of Ca, Fe, Zn, Cu, and certain other ions occur. The effects of EDTA on blood Fe have been reported in rats (Larsen et al., 1960) and cattle (Bailey, Blake, and Fisher, 1967). The affinity of EDTA for Fe is greater than for Ca, and the concentration of Fe in blood decreased in calves given EDTA, which indicates a disadvantage of its use to produce prolonged hypocalcemia. Some of the Mg in serum was also complexed and resulted in hypomagnesemia. The chelation of Mg was less than Ca, since the affinity of EDTA is greater for Ca.

Although the Ca is tightly bound, the Ca-EDTA complex is soluble. A small amount of the Ca-EDTA complex is metabolized; however, the major

portion is eliminated in the urine as the complex. Foreman, Vier, and Magee (1953) found that after intraperitoneal injections of carbon-14-labeled EDTA into rats, 98 percent was excreted as a Ca-EDTA complex in the urine.

The oral route of administration of EDTA is unacceptable for cattle and other species. The molecule is poorly absorbed from the rumen, depending upon the pH; the lower the pH the better the absorption. Furthermore, either the Na or K salt of EDTA in the presence of Ca in the rumen would form a Ca-EDTA complex; and even though absorbed, little, if any, would exist as ionic Ca in the serum. Results of experiments by Larsen et al. (1960) corroborate this reasoning. They were unable to influence Ca concentration in serum or bone by oral administration of EDTA, but did influence Fe concentrations in the blood and tissues of rats.

It may not be correct to extrapolate from species to species, since the pH of the stomach of the rat is lower than the pH of the rumen. Thus it would be expected that absorption of an oral dose of EDTA would be greater for the rat than for cattle.

The role of oxalate in complexing Ca can be an important factor in Ca metabolism, since many plants consumed by cattle contain large amounts of oxalates (Abaza, Blake, and Fisher, 1967). Oxalate salts, when ingested, have a deleterious influence on metabolism of Ca already in the body (Fincke and Sherman, 1935), and ingested oxalates interfere in the absorption of Ca by forming insoluble Ca-oxalate (MacKenzie and McCollum, 1937). Calcium assimilation in cattle on an oxalate-rich diet was investigated and it was found that the cattle were in a negative Ca balance due to the poor Ca absorption (Talapatra, Ray, and Sen, 1948).

The dietary requirement of Ca for cattle is approximately 0.30 percent of the dry matter intake (Cunha et al., 1964; National Academy of Sciences--National Research Council, 1963). Although the Ca-deficient pellets consumed by the "imbalanced" calves contained only one-fourth of the nutritional requirement for Ca, the principle factor in reducing and maintaining a reduced Ca concentration in serum was undoubtedly the repeated administration of EDTA.

Hyperkalemia

The attainment of significant increased K concentration in either whole blood or serum did not clearly occur as a direct consequence of flooding the system with exogenous K or blocking the action of aldosterone in the elimination of K. There were these factors which reduced the sensitivity of statistical measurements of drug effect: (1) variation from blood sampling time to blood sampling time, (2) elevation effect on K concentration, and (3) trend for the K concentrations in both whole blood and serum to increase with time during the experimental period. The intersampling variation was expected, since reported values of K concentration in serum of cattle were from 5 to 10 mEq/L and values of K concentration in whole blood were from 10 to 50 mEq/L (Altman, 1961). By the use of the statistical technique whereby each calf was its own control, and by partitioning out the influences mentioned, the variance ratio for whole blood, although not for $P < 0.05$, was significant for $P < 0.10$, which indicates that the KCl and SC-14266 at the large-dose levels did promote hyperkalemia (Table 73).

The statistically significant ($P < 0.05$) higher K concentration in serum is in agreement with the results of Ayres et al. (1961). They

reported that high elevation caused an increase in total body K and aldosterone. The presumed reason for the increased aldosterone was to compensate for increased K.

The progressive increase of K in both whole blood and serum of all calves during the experiment, irrespective of treatment or altitude, detracted from the statistical significance. This increase of K is unexplainable, unless it was a consequence of continuous ingestion of the copious quantities of K consumed by calves given basal and treatment rations (Table 2). Even though the quantity of K given parenterally to treated calves was large, and their ration contained 10 times the K requirement, the quantity of K consumed by the untreated calves was also substantial (six times the requirement). From reports in the literature, one would assume that a dietary level of six times the normal K requirement would not be sufficient to cause hyperkalemia. Van Koetsveld (1964) fed a ration to cattle that provided 11 times the normal K requirement, yet the K concentrations in plasma were unaltered. In the present experiment, the calves were approximately 3 months old at the start and 7 months old at the end. During this period, important changes occur in the digestive and reproductive systems of the ruminant. Whether these changes were related to the progressive increases of K in whole blood and serum is not known.

The repeated 4 gram injection of KCl should have been sufficient to cause hyperkalemia if calves reacted as did rats (Rigo et al., 1963). Yet, this result was not obtained. Possibly, the increase in tissue stores of K, especially in the skeleton, was responsible for the absence of severe hyperkalemia. The concentration of K in the skeleton was more than doubled in Ca-K imbalanced calves (Table 86). This

highly significant increase could account for a general excess of K in the body without it being manifested as a hyperkalemia.

The administration of aldosterone promotes the elimination of K and retention of Na. With adrenalectomy in rats, the K in serum increased and Na in serum decreased (French and Manery, 1964), and injection of aldosterone prevented the effect. Similar results were obtained by Bert et al. (1963) in man. Calves given the SC-14266 should have had an altered renal threshold with retention of K and elimination of Na. In the present experiment, the Na levels in serum and whole blood were highly variable, and a definite hyponatremia caused by the treatment was not demonstrated. However, according to the literature, there are wide variations in Na and K concentrations in whole blood and serum of normal cattle. The reported Na concentrations in whole blood range between 80 and 135 mEq/L, and in serum range between 133 and 170 mEq/L (Altman, 1961). Seemingly, the treatment differences which were imposed were not radical enough to alter Na concentrations in blood beyond the wide normal range.

The role of mineralocorticoids in regulating Na-K balance is not completely understood. Arons et al. (1958) found comparable decreases of total Na and K in the body after administration of deoxycorticosterone and they obtained contradictory results concerning balance and total exchangeable Na and K in man during administration of ACTH or deoxycorticosterone for 4 weeks. They concluded that some Na may have become non-exchangeable, and that exchangeable K may have been released from normally non-exchangeable stores.

The ability of the body of the animal to maintain blood homeostasis of K and Na, even during periods of imposed radical imbalances of

exogenous sources of these electrolytes, is phenomenal. Experimental production of a high level of hyperkalemia is, ostensibly, difficult to accomplish.

Other elements in blood

It is interesting to note that the effects of hypoxia and Ca-K imbalance were a decrease in Fe, Zn, and Cu concentrations in blood, except that the concentration of Fe increased in calves at high elevation. Since EDTA has a strong affinity for Fe, Zn, and Cu, the decrease in concentration of these minerals in blood was expected.

As expected, hypoxia did cause an increase in Fe content of the blood of animals in the groups at high altitude. In both Ca-K imbalanced and untreated groups, calves at the 2,745 meters elevation had higher blood Fe levels than did those at the 1,372 meters elevation. Iron concentrations in the blood were also altered as a consequence of EDTA administration. Initially, the concentration of Fe in the blood of calves in all four groups averaged essentially the same. As periodic treatment with EDTA continued, the treated calves at both elevations experienced marked decreases in blood Fe concentrations. It was a progressive decrease during the entire experimental period (Figure 7).

Some decrease of blood Fe occurred, not attributable to EDTA, as evidenced by a decrease of approximately 6 mg/100 ml in untreated calves at low elevation (group A, Table 65) from the beginning to the end of the study. This coincides with the findings of Greatorex (1954) that the concentration of Fe in blood decreases as calves get older.

The altitude effect of increasing blood Fe concentration opposed: (1) the EDTA effect, and (2) the age-time effect of reducing it (Figure 7). The EDTA-treated calves that were located at high elevation did

not show as much effect from the drug treatment as did calves that received EDTA and were at low elevation. However, the combined EDTA effect plus age-time effect of lowering blood Fe concentrations were more profound than the high altitude effect of increasing it.

During period III, the groups (groups B and D, Table 66) of calves that received EDTA had lower serum Zn levels than did the untreated calves by 46 µg/100 ml. Although this was a decrease of 25 percent for treated calves, it was not statistically significant. There was great individual variability within groups which decreased the sensitivity of the statistical test. This is a probable case of statistical non-significance, but yet practical importance, since the values for treated calves during period III are well below the normal values reported for Zn concentration in serum (Underwood, 1962).

Other investigators have also found that EDTA exerts an effect on blood Zn levels. Millar et al. (1954) administered EDTA intraperitoneally to rats to hasten the onset of Zn deficiency. Ilin, Arkhangelskaya, and Norets (1965) also used EDTA to accelerate the elimination of Zn from rats. Urinary excretion of Zn was increased as much as 100 times and the concentration of Zn in blood and other soft tissues was greatly decreased.

Imbalances in Heart, Liver, Kidney, and Bone

A summary of the comparative effects of hypoxia and Ca-K imbalance on the composition of cardiac tissue is illustrated in Figure 3. To mimic the Ca-K imbalance in cattle afflicted with brisket disease, it was important that a deficiency of Ca and an excess of K existed in cardiac tissue. This Ca-K imbalance was established as expected.

Apparently, there was a substitution of K for Na in cardiac tissue, since as the concentration of K increased the concentration of Na decreased (Figure 3). The same opposing change occurred with Ca and Mg, the Ca decreasing and the Mg increasing.

The liver is a storage area for the hematinic elements, i.e., Fe, Zn, and Cu. In the calves that were treated with EDTA, the hepatic concentrations of these three elements were greatly decreased (Figure 4). This was expected, since EDTA has an affinity for these elements. EDTA binds these divalent ions in the plasma and forms a metal-EDTA complex. The complex is then excreted in the urine, thus removing the metal from the body (Larsen et al., 1960). The minerals that are removed from the plasma in this way must then be replaced from body stores. Apparently, this was the case in this experiment, thus causing a decrease in the concentration of Fe, Zn, and Cu in the liver.

The effects of hypoxia and Ca-K imbalance on the composition of renal tissue are summarized in Figure 5. The changes in renal composition due to the Ca-K imbalance were in the same direction (except Na), but much more extensive ($P < 0.01$) than any caused by hypoxia. An important change due to drug treatment is the massive increase in the Ca concentration in renal tissue. As compared to untreated calves, the calves that received EDTA exhibited nearly a two-fold increase in the Ca concentration in renal tissue from 281 ppm to 528 ppm (Table 69). This is a finding that is consistent with reports in the literature (Doolan et al., 1967). One should note that renal concentrations of Ca and K were opposite to cardiac concentrations of these two elements. The reason is not known unless the Ca mobilized from bone (Figure 6), in a compensatory effort to correct hypocalcemia, precipitated out

in the kidneys, and the aldosterone inhibitor also altered the renal Na-K balance.

EDTA has been administered to a large number of patients in order to lessen the body burden of various elements. In some patients with hypercalcemia, serious renal complications have been observed with total doses ranging from 3 to 210 grams (Holland, Danielson, and Schagian-Edwards, 1953; Dudley et al., 1955; Reuber and Bradley, 1960). Period of administration of dosages varied from minutes to days. In all the fatal cases there occurred a progressive azotemia and the changes in the kidney tubule have been described as sucrose nephrosis (Holland, Danielson, and Schagian-Edwards, 1953), acute tubular necrosis, and kaliopenic nephropathy (Dudley et al., 1955). The variability in renal pathology in these literature reports is not surprising since they were complicated cases in which necropsies were performed at varying intervals following EDTA therapy and the quantity and frequency of administration of EDTA varied. Extensive vacuolization can, however, be produced with acceptable predictability in the tubular epithelium of the rat by administration of large amounts of EDTA (Foreman, Finnegan, and Lushbaugh, 1956; Altman, Wakin, and Winkelmann, 1962). Such experimental evidence has generally been accepted as further proof of the nephrotoxicity of this agent. This extrapolation may have been excessive, not only on a basis of species differences (Seven, 1960), but also because the functional significance of the morphologic changes had never been established.

Doolan et al. (1967) further investigated the problem of the nephrotoxocity of EDTA. It was found that after 10 days treatment of Na_2-EDTA (523 mg/kg) to rats, the kidney ash contained nearly twice as much

Ca as did untreated rat kidney. The values were 3.72 mg/gram and 6.31 mg/gram of ash for untreated and treated rats, respectively. They found a consistent vacuolization in the tubular epithelium of the kidneys of EDTA-treated rats. However, these changes were not accompanied by any significant decrease in excretory function.

The localization of Ca-EDTA in the kidney of rats was further studied by Schwartz et al. (1967). They used Ca-EDTA labeled with carbon-14 in the carboxyl positions, and found that the vacuolization was present in rats receiving three doses of the drug totaling 1,000 mg/kg. The Ca-EDTA was found to be located in the proximal tubular cell of the kidney.

Possibly, this same thing happened in the calves used in this study. The Ca concentration increased greatly in the renal tissues of calves receiving EDTA. A histologic study will be made, but will not be a part of this dissertation.

In Figure 6 is depicted the comparative effects of hypoxia and Ca-K imbalance on the composition of osseous tissue (metatarsal bone). The decreased Ca concentration in bone of treated calves was induced by the removal of Ca from the skeleton to replace the Ca in blood that was chelated by EDTA and eliminated from the circulation.

If the elements for the treated groups (groups B and D, Table 70) are added, and the sum compared to the sum of the same elements for the untreated groups (groups A and C), the total is considerably less for the treated calves (43.6 percent of total ash) than for the untreated calves (53.6 percent of total ash). In the bones of the treated calves, all the elements were less concentrated except K and Zn. Even though the K and Zn concentrations were considerably higher in the treated

calves than in the untreated calves, the proportion of these two minerals in bone is small compared to most of the other elements. When one compares percent ash of the four groups of calves (Table 70), it is evident that little difference existed between treated and untreated calves. The profound decreases in major mineral elements, with only small differences in percent ash, could be explained by a substitution of some other ion for the decreased ions.

Neuman and Neuman (1958) have published evidence of ion exchange in bone mineral. One substitutional cation exchange is two hydronium ions for one Ca ion. They report that 20 percent of the Ca ions can be replaced by hydronium ions. If such a substitution took place with the treated calves, the total weight would be changed little, since the combined weight of two hydronium ions is approximately equal to the weight of one Ca ion; yet, the Ca concentration would be decreased due to the substitution. They state further that: "Of all the questions in the crystallography and surface chemistry of the apatite minerals, hydronium ion substitution is the most perplexing and most difficult to resolve" (Neuman and Neuman, 1958, p. 94). Anion exchanges also occur, such as the substitution of carbonate for phosphate and the formation of oxides. These changes could conceivably alter the concentration of mineral elements, yet not change the percent ash.

This phenomenon has been a concern of investigators for some time. Estremera and Armstrong (1948) reported a similar finding. They found that by feeding a low protein diet, an 8.6 percent decrease in the Ca and P concentrations in the rat femur could be provoked without any reduction in percent ash. The 8.6 percent decrease in Ca and P

concentrations is quite comparable to the 9.8 percent combined decrease of Ca and P observed in this study (Table 70).

Duckworth and Hill (1953) have reviewed the topic and report that several investigators have found a reduction in percent ash of dry, fat-free bone when a loss of minerals has occurred, but that the fall in percent ash did not accurately represent the loss of minerals.

When mineral matter is lost from bone, resorption occurs--not just demineralization (Bloom, Bloom, and McLean, 1941). Since both matrix and mineral matter are removed, it may be that ash remains proportionate to total bone weight, yet mineral content is reduced.

An increase of Zn concentration in bone ash, as was exhibited by EDTA-treated calves (Table 70), is in agreement with reported findings for rats (Forbes, 1961). By feeding Na_2-EDTA in the diet at the level of 230 ppm, the Zn concentration of the rat femur ash increased from a normal of 140 ppm to 195 ppm.

The control calves had a slightly higher concentration of Zn in bone ash (227 ppm) than is reported for cattle in the literature (196 ppm) by Blincoe and Bohman (1966). However, the EDTA-treated calves exhibited a great increase to 319 ppm. The increase is greater than was observed by Forbes (1961), but the calves received larger dosages and for a longer period of time.

SUMMARY

To determine the effects of hypoxia and a Ca-K imbalance on the mineral composition of bovine tissues, 40 Hereford calves were randomized into four groups arranged in a 2 x 2 factorial design. Two groups were placed at 2,745 meters and two groups remained at 1,372 meters for 16 weeks. One group at each elevation received the basal ration with Ca and K balanced to nutritional requirements and the other group (designated treated) at each elevation received an imbalanced ration. It contained one-fourth the Ca and 10 times the K needed to satisfy nutritional requirements. In addition, treated calves received K_2-EDTA at two-day intervals to further decrease hemic Ca concentrations, and an aldosterone inhibitor (SC-14266) and KCl to further increase hemic K concentrations. Small doses of K_2-EDTA and SC-14266 were given the first 10 weeks (period II) and then larger doses the last six weeks (period III).

Samples of whole blood and blood serum were collected initially (period I, while all 40 calves were at 1,372 meters and yet untreated); and subsequently, at 2-week intervals during experimental periods II and III, for a total of eight additional blood samplings. At the termination of the experiment, the calves were killed, necropsied, and tissue samples of heart, liver, kidney, and bone taken for mineral analyses. The K and Na concentrations in whole blood and blood serum; the Fe concentration in whole blood; and the Ca, P, Mg, Cl, Zn, and Cu concentrations in blood serum were determined as indices of effects of hypoxia and induced Ca-K imbalance on hemic mineral balances. As

additional indices, Ca, K, Na, P, and Mg concentration in cardiac, hepatic, renal, and osseous (metatarsal) tissues; Fe, Zn, and Cu concentrations in cardiac and hepatic tissues; and Zn concentrations in osseous tissues were determined. Absolute dry matter and percent ash were also determined for tissues other than blood.

Effects of Hypoxia (Altitude)

In blood, the serum K concentration increased ($P < 0.05$) during period III and the whole blood Fe concentration increased ($P < 0.05$) during period II and increased still further ($P < 0.05$) during period III. The concentrations of Ca, Na, P, Mg, Cl, Zn, and Cu were not significantly altered by residence at high altitude.

The concentration of Ca, and the Ca/K and Ca/Mg ratios in cardiac tissue were greatly decreased ($P < 0.01$) in calves at high elevation. Concentrations of K, Na, P, Mg, Fe, Zn, and Cu; and the ash and dry matter contents were not significantly influenced.

In hepatic tissue, decreased Na concentration ($P < 0.01$) was the only change due to hypoxia.

The concentrations of Na and P ($P < 0.05$), and Ca and the Na/K ratio ($P < 0.01$) were increased in renal tissues of calves at high elevation. The K and Mg concentrations and the dry matter and ash contents were little influenced. In osseous tissue, concentrations of K ($P < 0.01$), and Na, Mg, and Zn ($P < 0.05$) were decreased, and P ($P < 0.01$) increased in calves at high elevation. The Ca and ash contents, sum of mineral content, and the Ca/P ratio were little influenced.

Effects of Ca-K Imbalance

As a generalization, the effects of the Ca-K imbalance were more profound than those due to hypoxia. All of the effects summarized in this section are statistically highly significant ($P < 0.01$) except as indicated otherwise. In blood serum, concentrations of Ca and Na were decreased, and Cl increased during period III and Cu decreased during periods II and III. The concentration of Fe in whole blood was decreased both treatment periods. Only a small increase ($P < 0.10$) in K concentration in whole blood could be attained. Serum K, P, Mg, and Zn, and whole blood Na were not significantly altered. In cardiac tissue the concentrations of Ca, Na, Zn, and Cu decreased; and concentrations of K, P, and Mg increased. The contents of Fe, dry matter, and ash were little affected. Changes in mineral composition of hepatic tissue due to the Ca-K imbalance are as follows: Ca, Na, P, Fe, Zn, Cu, and dry matter decreased; K content also decreased, but to a lesser extent ($P < 0.05$); and Mg increased ($P < 0.05$). Total ash content was unaffected.

The Ca-K imbalance also had profound effects on renal tissue composition. Increased were Ca and P, and decreased were K, Na, Mg, dry matter, ash, and Na/K ratio. The Ca, P, Mg, and sum of minerals in osseous tissue decreased; and K and Zn increased. Since the Ca decrease was greater than the P decrease, the Ca/P ratio also decreased, but to a lesser extent ($P < 0.05$). The Na and total ash of bone remained unaffected.

CONCLUSIONS

1. Parenteral inoculation of calves with K_2-EDTA, an aldosterone inhibitor, and KCl causes drastic imbalances in tissue minerals, such as:

   a. Decreased concentrations of Ca, Na, and Cu and increased concentration of Cl in blood serum; decreased concentration of Fe, and increased concentration of K in whole blood. The Ca and Fe changes are most profound.

   b. Decreased concentrations of Ca, Na, Zn, and Cu; and increased concentrations of K, P, and Mg in cardiac tissue.

   c. Decreased concentrations of Ca, K, Na, P, Fe, Zn, and Cu; and increased concentration of Mg in hepatic tissue.

   d. Increased concentrations of Ca and P; and decreased concentrations of K, Na, and Mg in renal tissue.

   e. Decreased concentrations of Ca, P, and Mg; and increased concentrations of K and Zn in osseous tissue.

2. Exposure of calves to hypoxia at high elevation may cause alterations in body mineral composition, such as:

   a. Increased concentration of K in blood serum and of Fe in whole blood. The Fe increase is related to length of residence.

   b. Increased Ca concentration in cardiac tissue and decreased Na concentration in hepatic tissue.

   c. Increased renal concentrations of Ca, Na, and P.

   d. Decreased concentrations of K, Na, Mg, and Zn; and increased concentrations of P in osseous tissue.

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APPENDIXES

Appendix A

Tables

Preface to tables

Table 1. Experimental protocol: Groups of calves

Group identification	Treatment	Elevation (meters)
A (Low elevation, ion balanced)	None	1,372
B (Low elevation, ion imbalanced)	Imbalanced diet; and repeated injections of K_2-EDTA, aldosterone inhibitor, and KCl	1,372
C (High elevation, ion balanced)	None	2,745
D (High elevation, ion imbalanced)	Imbalanced diet; and repeated injections of K_2-EDTA, aldosterone inhibitor, and KCl	2,745

Table 2. Composition of rations, and comparison of amounts of electrolytes consumed and required by calves

Electrolyte	Composition of rations (% dry matter)			Minimal dietary requirement (grams/45 kg body weight per day)	Amount consumed (grams/45 kg body weight per day	
	Oat hay	Treatment pellets	Basal pellets		Treated calves	Nontreated calves
Ca	0.18	0.06	0.36	4.2	1.0	4.9
K	1.90	2.86	1.79	4.2	40.4	26.2
Na	0.02	0.10	0.58	2.8	1.2	7.5
P	0.061	0.75	1.13	3.8	11.4	16.6
Mg	0.031	0.29	0.27	0.076	0.45	0.40
Fe	0.0038	0.0078	0.03	0.095	0.12	0.44
Cu	0.000025	0.00029	0.00055	0.004	0.004	0.008

Table 3. Experimental protocol: Quantities of dipotassium ethylenediaminetetraacetate (K_2-EDTA), aldosterone inhibitor (SC-14266), and potassium chloride (KCl) administered to calves[a]

Route	Dose rate (mg/45 kg of body weight per injection)	Number of injections	Total amount injected (grams/calf)
	K_2-EDTA (periods II and III)		
Intramuscular (period II)	400	28	28
Intraperitoneal (period III)	1,700	16	99
Total		44	127
	SC-14266 (period II)		
Intramuscular	455	10	10
Intramuscular	910	18	25
	SC-14266 (period III)		
Intraperitoneal	1,365	16	72
Total		44	107
	KCl (period III)		
Intraperitoneal	400	11	29

[a]The SC-14266 and KCl were administered three times a week (Tuesday, Thursday, and Saturday), on alternate days to the K_2-EDTA (Monday, Wednesday, and Friday). Period II was the first 10 weeks after start of treatment and period III was from 10 to 16 weeks after start of treatment.

Table 4. Mineral elements quantitatively determined in blood, heart, liver, kidney, and bone of calves[a]

Mineral element	Serum	Whole blood	Heart	Liver	Kidney	Bone
Ca	+	-	+	+	+	+
K	+	+	+	+	+	+
Na	+	+	+	+	+	+
P	+	-	+	+	+	+
Mg	+	-	+	+	+	+
Cl	+	-	-	-	-	-
Fe	-	+	+	+	-	-
Zn	+	-	+	+	-	+
Cu	+	-	+	+	-	-

[a]+ indicates determination was made; - indicates it was not.

Table 5. Methods and dilutions used for analyses of mineral elements in whole blood and serum

Mineral element	Whole blood		Blood serum	
	Method	Dilution	Method	Dilution
Ca	---	---	Spinco[b]	None
K	Spectrophotometric[a]	1:200	Spectrophotometric[a]	1:50
Na	Spectrophotometric[a]	1:4000	Spectrophotometric[a]	1:5000
P	---	---	Colorimetric[c]	None
Mg	---	---	Spectrophotometric[a]	1:50
Cl	---	---	Spinco[c]	None
Fe	Spectrophotometric[a]	1:40	---	---
Zn	---	---	Spectrophotometric[a]	1:10
Cu	---	---	Spectrophotometric[a]	1:5

[a]Perkin-Elmer Model 303, atomic absorption spectrophotometer.
[b]Beckman/Spinco Model 150, Ultramicro analytical system, Beckman Instruments, Inc., Fullerton, California.
[c]Bausch and Lomb Spectronic 20, Type 33-29-40, Bausch and Lomb, Inc., Rochester, New York.

Table 6. Secondary dilutions of wet ashed samples of heart, liver, kidney, and bone necessary for the quantitative determination of mineral elements

Mineral element	Secondary dilution[a]			
	Heart	Liver	Kidney	Bone
Ca	None	1:10	None	1:1000
K	1:50	1:50	1:50	1:10
Na	1:1000	1:500	1:500	1:1000
P	None	None	None	1:80
Mg	1:50	1:50	1:50	1:1000
Fe	None	None	--	--
Zn	None	None	--	None
Cu	None	1:10	--	--

[a]Tissue samples of 2 grams were wet ashed in concentrated HNO_3 and 30% H_2O_2, then diluted (primary) to 25 ml volume before the above (secondary) dilutions were made.

Table 7. Calcium concentration in blood serum[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

Calf number	Period I	Period II				Period III			
		Time interval – weeks				Time interval – weeks			
	Initial	2	4	6	8	10	12	14	16
Group[b] A					(mg/100 ml)				
1	9.7	9.3	10.6	9.6	9.7	9.0	9.6	9.8	8.7
2	10.8	8.9	8.5	11.5	9.1	9.1	10.0	9.6	9.2
3	8.1	10.9	9.2	6.6	9.6	8.6	10.6	10.0	8.7
4	9.6	8.8	9.3	10.2	9.9	8.8	10.0	9.9	8.7
5	7.8	7.6	10.7	9.9	9.3	10.5	9.8	10.0	8.9
6	8.9	10.4	10.4	10.1	9.2	9.1	10.4	9.6	8.6
7	9.9	9.2	11.3	9.0	9.6	8.7	10.6	9.8	8.6
8	10.7	10.1	10.1	10.9	9.4	9.3	10.5	10.0	8.8
9	9.4	9.3	9.0	10.4	9.7	9.3	10.8	9.7	8.7
10	9.9	9.6	11.1	10.1	9.6	9.0	10.5	9.8	8.8
Group B									
1	9.4	9.4	10.2	11.7	8.0	7.1	7.5	7.9	7.5
2	8.8	9.7	10.6	9.4	10.4	7.7	7.8	8.1	7.9
3	8.9	9.2	7.0	9.5	8.3	7.7	7.5	7.6	7.7
4	8.9	10.9	9.9	10.6	9.1	7.5	7.8	8.1	7.8
5	9.6	9.1	7.9	11.0	8.5	7.0	8.1	7.8	7.0
6	9.1	10.9	8.5	10.8	10.6	7.9	8.4	8.2	8.1
7	8.1	9.6	9.0	11.0	9.4	6.2	8.7	8.3	8.0
8	9.4	9.3	9.5	8.8	8.5	7.6	8.5	8.3	8.3
9	10.9	10.0	9.9	6.8	10.2	8.1	6.8	8.4	7.5
10	10.6	8.6	9.9	10.9	11.6	7.7	7.1	7.8	7.7
Group C									
1	10.2	9.6	8.6	9.6	9.3	8.9	10.2	10.0	10.0
2	7.6	9.7	9.6	9.6	8.5	8.7	9.6	9.4	9.4
3	10.2	10.1	9.5	8.9	10.2	9.5	9.4	9.5	9.5
4	8.9	10.9	9.3	7.2	9.7	9.0	9.3	9.6	9.6
5	9.5	9.1	9.7	7.0	10.4	9.5	10.3	9.4	9.4
6	7.1	9.3	9.1	9.7	9.1	9.5	9.5	9.3	9.3
7	10.1	9.4	9.6	10.3	9.2	9.0	9.1	9.7	9.7
8	10.1	9.2	9.0	10.5	9.5	9.6	10.5	9.3	9.3
9	9.2	10.8	9.9	10.4	9.4	9.5	9.8	9.2	9.2
10	8.8	9.0	9.8	8.0	10.9	9.9	10.4	9.5	9.5
Group D									
1	9.6	8.4	9.4	9.9	9.5	7.6	8.5	8.1	8.4
2	8.6	8.7	9.5	9.9	9.2	8.4	8.5	7.5	8.1
3	9.7	9.0	9.4	7.5	9.8	7.2	7.9	7.2	8.5
4	9.7	8.3	9.5	11.3	8.5	7.0	7.2	7.7	8.3
5	9.5	9.3	9.9	10.3	8.7	7.7	7.5	8.6	8.0
6	9.9	9.1	7.4	9.6	9.1	7.3	7.4	7.7	7.8
7	8.6	9.4	10.1	9.7	8.0	8.4	8.2	6.4	8.4
8	7.9	9.7	10.9	9.4	8.2	7.8	8.2	7.7	8.1
9	11.4	9.5	9.8	8.7	9.6	8.4	7.2	7.4	7.5
10	10.1	9.2	9.7	9.3	8.3	7.7	7.2	7.6	7.6

[a]Blood drawn four hours after treatment.

[b]See Table 1, page 96 for explanation of groups and Table 3, page 97 for explanation of treatment periods, chemicals, and doses.

Table 8. Comparison of Ca concentration in blood serum at 4 hours and 24 hours after treatment with K_2-EDTA[a]

Group[a]	Calf number	Time interval (period III)					
		10 weeks		12 weeks		14 weeks	
		4 hours	24 hours	4 hours	24 hours	4 hours	24 hours
		(mg/100 ml)					
B	1	7.7	8.4	7.5	8.3	7.9	8.4
	2	7.7	8.2	7.8	7.2	8.1	8.3
	3	7.1	7.5	7.5	7.7	7.6	8.1
	4	7.5	7.9	7.8	8.1	8.1	8.3
	5	7.0	7.0	8.1	8.2	7.8	8.4
	6	6.9	7.5	6.8	7.3	8.4	8.9
	7	7.7	8.3	8.7	8.1	8.3	8.5
	8	7.6	8.1	8.5	8.7	8.3	9.1
	9	8.1	7.5	7.1	7.8	8.2	8.2
	10	7.2	7.6	8.4	8.0	7.8	8.1
	Mean	7.5	7.8	7.8	7.9	8.0	8.4
D	1	7.6	7.9	8.5	8.8	8.1	8.9
	2	8.4	8.6	8.5	8.5	7.5	8.5
	3	7.2	8.2	7.9	8.8	8.2	8.9
	4	7.0	8.6	7.2	8.4	7.7	8.6
	5	7.7	8.1	7.5	8.7	7.6	8.2
	6	8.4	8.5	7.4	7.9	7.7	8.6
	7	7.7	8.2	8.2	8.8	7.4	8.2
	8	7.8	8.4	8.2	8.9	7.7	8.3
	9	7.3	7.6	7.2	8.2	7.4	8.7
	10	8.4	8.5	7.2	8.4	6.6	7.5
	Mean	7.7	8.2	7.8	8.7	7.6	8.5

[a]See Table 1, page 96, and Table 3, page 97, for explanation of groups and treatment.

Table 9. Potassium concentration in blood serum[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

Calf number	Period I	Period II				Period III			
		Time interval – weeks				Time interval – weeks			
	Initial	2	4	6	8	10	12	14	16
Group[b] A					(mEq/L)				
1	6.4	5.0	6.0	6.2	6.7	6.8	8.2	5.5	7.1
2	8.1	6.2	6.3	6.8	6.9	7.0	8.1	5.5	8.3
3	5.8	6.3	5.3	6.9	6.7	6.8	8.0	6.0	8.0
4	7.8	7.0	6.3	8.0	8.1	7.2	7.7	5.5	8.0
5	7.4	8.7	5.4	6.9	8.0	7.1	7.8	5.7	7.2
6	6.2	8.7	5.6	9.6	8.3	7.2	8.7	4.6	7.3
7	6.4	6.6	5.8	8.2	6.3	6.8	9.3	6.4	7.5
8	3.9	5.1	5.8	8.4	7.6	7.6	8.1	5.4	7.1
9	6.5	6.5	5.8	8.9	6.7	6.8	8.0	6.2	9.2
10	7.4	6.4	4.9	7.7	8.8	6.5	7.0	5.7	9.0
Group B									
1	5.3	7.9	6.4	9.0	6.4	7.6	9.1	6.8	8.8
2	5.8	7.7	5.9	7.2	8.3	6.4	8.9	6.1	8.7
3	5.2	7.4	7.2	7.9	6.4	7.0	8.1	5.8	8.7
4	7.0	7.9	6.3	8.8	6.7	7.1	8.9	6.4	9.4
5	5.6	7.2	6.0	7.6	6.4	8.9	8.4	5.3	7.5
6	8.3	7.6	5.2	8.0	9.6	7.1	8.2	6.3	8.5
7	5.5	8.3	5.5	8.9	7.9	7.7	7.8	7.0	7.6
8	6.4	6.4	5.1	8.4	5.4	8.1	8.5	5.5	7.7
9	7.6	8.2	6.1	9.2	7.6	9.1	7.9	6.2	7.4
10	8.2	8.2	5.4	7.6	5.4	6.6	7.8	6.6	8.8
Group C									
1	6.8	6.6	7.3	8.3	5.9	8.4	7.3	6.8	9.0
2	5.8	5.8	6.0	8.9	6.4	7.0	7.9	6.3	9.3
3	5.7	5.4	8.7	7.2	6.4	8.8	8.7	7.8	8.8
4	5.5	5.3	6.3	6.4	6.6	6.5	8.0	6.0	8.7
5	7.0	5.6	9.8	8.3	7.3	9.1	8.5	5.4	6.3
6	5.2	6.4	7.1	7.1	6.7	7.8	9.5	5.7	6.7
7	6.6	6.9	7.1	8.3	7.2	7.7	9.4	6.4	7.8
8	6.6	6.2	6.4	9.0	7.1	8.1	9.0	8.1	7.7
9	5.6	5.6	6.3	8.3	6.3	6.5	8.0	7.2	9.2
10	6.4	5.6	5.4	7.6	6.5	8.4	8.8	6.4	9.5
Group D									
1	5.3	5.6	7.4	9.1	6.7	6.3	8.3	5.9	5.9
2	6.6	5.7	7.4	8.9	7.4	9.2	6.9	6.6	8.3
3	6.3	7.2	7.4	7.1	7.2	9.4	8.5	6.6	6.6
4	5.2	6.4	6.7	7.2	8.0	7.8	8.2	7.5	8.8
5	7.4	5.1	8.1	7.3	7.6	8.7	8.5	7.5	8.1
6	5.5	5.7	8.9	6.5	6.4	7.4	8.5	6.7	8.6
7	8.0	5.6	7.2	8.0	7.7	8.5	8.0	7.2	8.4
8	6.4	6.5	7.2	8.9	7.4	9.0	9.6	6.1	9.0
9	6.8	5.5	6.2	8.0	7.1	7.6	9.4	7.2	8.7
10	5.1	6.0	8.5	7.2	5.6	8.0	8.3	6.5	8.4

[a]Blood drawn four hours after treatment.

[b]See Table 1, page 96 for explanation of groups, and Table 3, page 97 for explanation of treatment periods, chemicals, and doses.

Table 10. Potassium concentration in whole blood[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

Calf number	Period I	Period II				Period III			
		Time interval – weeks				Time interval – weeks			
	Initial	2	4	6	8	10	12	14	16
					(mEq/L)				
Group[b] A									
1	12.6	13.7	11.3	13.9	12.3	14.1	17.7	12.7	13.0
2	13.1	11.3	11.3	14.9	12.6	14.3	17.8	12.0	15.1
3	12.1	13.6	8.9	12.8	10.8	13.1	14.4	10.1	11.8
4	10.8	16.2	8.7	11.3	12.6	13.1	15.5	11.1	12.5
5	15.9	15.1	15.6	16.9	13.9	17.0	20.6	15.5	18.5
6	12.3	8.7	11.3	13.3	12.8	14.6	16.7	11.3	13.8
7	10.5	9.7	9.5	12.3	12.6	12.8	14.9	11.7	15.1
8	10.4	8.7	9.5	19.0	24.6	11.5	12.7	10.5	12.0
9	10.8	9.7	8.5	13.6	10.0	10.0	12.7	10.3	12.0
10	10.8	11.8	8.7	25.6	10.3	12.4	12.2	10.1	11.3
Group B									
1	17.7	21.5	15.4	15.9	18.0	14.3	21.0	15.7	18.5
2	10.0	6.9	8.5	11.8	7.2	10.7	14.1	9.8	10.3
3	11.8	9.2	10.8	17.7	11.5	15.0	15.4	10.8	12.3
4	16.7	15.1	16.2	18.8	13.1	12.2	22.7	12.2	13.5
5	12.7	9.2	9.7	14.1	11.5	12.2	16.8	10.5	11.9
6	10.8	12.3	9.7	12.6	12.6	13.1	12.6	10.4	11.8
7	10.8	9.7	8.5	10.8	11.0	13.2	12.5	10.8	11.6
8	10.8	7.7	11.3	12.6	10.5	13.5	14.0	11.3	12.3
9	15.7	11.5	10.8	14.6	12.1	13.4	14.2	10.5	12.3
10	10.8	17.7	19.0	23.6	19.2	24.0	24.2	15.5	20.5
Group C									
1	10.0	10.0	15.8	11.8	11.0	13.1	14.1	12.1	12.9
2	10.6	11.3	13.0	13.9	9.7	13.1	13.3	11.0	12.9
3	11.3	9.0	14.3	11.8	13.9	15.4	15.4	14.3	16.0
4	10.8	10.8	15.4	17.7	11.5	11.9	12.7	11.0	12.8
5	20.0	20.5	13.3	25.1	21.5	18.5	24.4	16.2	22.0
6	13.9	11.8	18.0	12.3	13.3	12.0	17.1	12.2	14.1
7	17.4	12.2	19.8	19.5	16.7	15.4	17.4	16.5	19.4
8	13.6	11.5	17.9	16.9	15.4	16.4	17.2	16.4	18.2
9	12.1	10.8	13.9	12.6	12.6	10.4	12.1	10.8	10.3
10	9.0	9.2	12.6	10.8	11.3	12.6	14.2	10.3	10.0
Group D									
1	11.0	9.2	13.4	11.3	11.5	11.8	12.7	11.3	11.3
2	9.7	8.5	13.8	10.8	11.5	13.9	12.0	10.5	11.3
3	16.2	15.1	17.7	17.2	13.6	19.4	20.0	14.8	14.8
4	10.5	9.5	13.6	13.9	11.8	14.1	12.8	11.4	12.8
5	12.1	9.5	15.2	11.5	9.7	13.2	14.1	10.9	14.2
6	10.2	9.2	14.6	11.0	11.3	12.2	12.3	11.3	12.4
7	11.4	11.8	13.6	12.6	11.5	14.5	13.9	11.8	13.8
8	13.1	12.1	14.4	13.6	14.4	15.6	19.1	12.9	13.8
9	16.1	12.8	17.4	12.8	13.3	15.0	17.4	14.1	15.4
10	10.1	9.2	15.6	10.8	10.5	13.3	13.9	10.1	11.9

[a]Blood drawn four hours after treatment.

[b]See Table 1, page 96 for explanation of groups, and Table 3, page 97 for explanation of treatment periods, chemicals, and doses.

Table 11. Sodium concentration in blood serum[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

Calf number	Period I	Period II				Period III			
		Time interval – weeks				Time interval – weeks			
	Initial	2	4	6	8	10	12	14	16
					(mEq/L)				
Group[b] A									
1	113.0	189.1	190.2	178.3	132.6	187.0	132.6	139.1	137.0
2	111.5	157.0	198.9	188.0	129.4	171.8	152.2	110.9	143.5
3	112.0	158.3	170.7	180.4	118.5	176.1	128.3	163.1	137.0
4	162.2	173.9	141.3	159.8	129.4	189.1	132.6	139.1	141.3
5	147.4	134.8	177.2	162.0	129.4	195.7	143.5	158.7	119.4
6	126.7	195.2	122.8	182.6	180.4	182.6	158.7	132.6	130.4
7	129.4	152.2	113.0	162.0	151.1	173.9	165.2	141.3	128.3
8	130.0	181.5	121.7	146.7	130.4	130.4	182.6	165.2	128.3
9	119.1	145.7	191.3	143.5	130.4	130.4	132.6	139.1	147.8
10	156.5	198.7	156.5	152.2	129.4	182.6	147.8	154.4	154.4
Group B									
1	142.4	168.7	91.3	162.0	150.0	182.6	160.9	158.7	143.5
2	129.4	136.1	145.7	164.4	143.5	197.8	160.9	158.7	137.0
3	127.2	183.7	189.1	166.3	147.8	152.2	158.7	154.4	160.9
4	158.7	159.4	163.0	163.0	130.4	182.6	115.2	154.4	139.1
5	165.2	162.0	189.1	159.8	129.4	178.3	134.8	167.4	147.8
6	179.4	189.6	154.4	139.1	152.2	119.6	158.7	141.3	134.8
7	148.9	176.7	158.7	171.7	132.6	176.1	118.3	160.9	126.1
8	167.4	183.1	179.4	166.3	139.1	158.7	95.7	158.7	139.1
9	155.0	179.4	173.9	164.1	146.7	128.3	89.1	132.6	152.2
10	135.9	162.0	192.4	171.7	139.1	123.9	103.3	110.9	117.4
Group C									
1	121.7	163.1	125.0	171.7	145.7	180.4	143.5	160.9	123.9
2	155.2	113.0	129.4	171.7	152.2	176.1	108.7	160.9	134.8
3	130.4	121.7	133.3	172.8	147.8	126.1	158.7	158.7	150.0
4	162.2	155.4	109.8	117.4	189.1	182.6	160.9	167.4	145.6
5	136.1	104.4	143.5	147.8	167.4	193.5	163.1	154.4	130.4
6	160.9	104.4	118.5	154.4	133.9	189.1	150.0	156.5	119.4
7	136.3	123.9	110.9	141.3	194.6	189.1	160.9	154.4	152.2
8	131.5	104.4	182.6	182.6	106.5	189.1	163.1	189.1	158.7
9	156.1	114.1	145.7	137.0	189.1	189.1	106.5	171.8	128.3
10	126.7	190.2	126.1	147.8	190.2	169.6	110.9	171.8	152.2
Group D									
1	149.1	180.9	141.3	167.4	130.5	195.7	158.7	154.4	154.4
2	113.0	158.7	113.0	110.9	175.0	123.9	160.9	173.9	110.9
3	142.4	142.4	117.4	195.7	179.4	126.1	121.7	121.7	158.7
4	159.8	187.0	123.9	184.8	165.2	176.1	158.7	113.0	145.6
5	154.8	144.6	173.9	110.9	165.2	154.4	165.2	154.4	147.8
6	165.2	190.2	108.9	175.0	152.2	182.6	160.9	152.2	154.4
7	169.1	143.3	122.8	157.6	132.6	187.0	113.0	126.3	158.7
8	167.0	173.9	160.9	170.7	179.4	167.4	113.0	130.7	119.6
9	159.1	106.5	134.8	162.0	162.0	128.3	113.0	139.5	158.7
10	135.9	91.3	132.6	188.0	162.0	171.8	128.3	191.3	115.2

[a]Blood drawn four hours after treatment.

[b]See Table 1, page 96 for explanation of groups, and Table 3, page 97 for explanation of treatment periods, chemicals, and doses.

Table 12. Sodium concentration in whole blood[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

Calf number	Period I	Period II				Period III			
		Time interval – weeks				Time interval – weeks			
	Initial	2	4	6	8	10	12	14	16
					(mEq/L)				
Group[b] A									
1	123.5	116.5	119.1	91.3	129.6	118.2	105.2	130.4	135.6
2	144.4	86.1	139.1	90.4	129.6	109.6	99.1	127.0	144.3
3	138.3	101.7	89.6	93.0	133.9	107.8	124.4	132.2	81.7
4	107.8	90.4	119.1	93.9	144.4	107.8	121.7	127.0	133.9
5	113.0	120.9	101.7	97.4	139.1	137.4	97.4	128.7	140.1
6	83.5	117.4	108.7	127.8	121.7	99.1	102.6	127.0	140.1
7	87.0	96.4	113.0	106.1	135.7	149.6	121.7	130.4	76.5
8	81.0	96.4	130.4	97.4	104.3	93.9	129.6	128.7	144.3
9	120.0	140.9	123.5	91.3	107.8	109.6	122.6	130.4	83.5
10	81.7	103.5	130.4	113.9	130.4	142.6	122.6	127.0	133.9
Group B									
1	85.2	98.3	110.4	106.1	113.0	149.5	93.9	128.7	125.2
2	179.1	90.4	107.8	87.0	115.7	153.0	118.3	130.4	133.9
3	153.0	127.8	116.5	107.8	107.7	107.8	106.1	127.0	133.9
4	147.8	175.7	104.3	100.0	102.9	146.1	104.3	130.4	141.0
5	87.0	86.1	113.0	96.5	121.7	142.6	113.0	125.2	144.3
6	111.3	118.3	119.1	88.7	130.4	147.8	113.0	130.4	135.6
7	117.4	108.7	79.1	101.7	124.4	147.8	118.3	130.4	73.0
8	147.8	116.5	100.0	72.2	102.6	137.4	118.3	137.4	156.5
9	107.0	91.5	144.4	78.3	133.0	113.0	129.6	130.4	81.7
10	70.4	83.5	105.2	83.5	121.7	140.9	93.9	127.0	147.8
Group C									
1	146.1	84.4	142.6	106.1	127.0	144.3	147.8	127.0	125.2
2	121.7	80.9	123.5	93.9	111.3	140.9	127.8	127.0	142.6
3	87.0	112.6	123.5	100.9	109.6	123.5	121.7	123.5	144.3
4	114.8	85.2	139.1	124.4	129.6	114.8	124.2	120.0	133.9
5	89.6	97.4	109.6	92.2	96.5	113.0	114.8	132.2	135.6
6	109.6	97.4	113.0	121.7	103.5	123.5	125.2	121.7	142.6
7	117.4	89.6	123.5	91.3	108.7	128.7	134.8	113.0	120.0
8	113.9	78.3	102.6	99.1	127.0	137.4	130.4	137.4	141.0
9	91.3	115.7	83.5	81.7	104.4	132.2	124.4	123.5	125.2
10	126.1	135.7	86.1	81.7	116.5	132.2	132.2	121.7	139.1
Group D									
1	104.4	88.7	80.9	99.1	107.0	92.2	123.5	123.5	123.5
2	134.8	106.1	79.1	77.4	108.7	102.6	123.5	113.0	123.5
3	133.9	96.5	75.7	87.0	113.0	97.4	118.3	125.2	125.2
4	96.5	80.0	83.5	90.4	123.5	106.1	133.0	128.7	88.7
5	134.8	84.3	158.3	83.5	98.3	95.7	131.3	121.7	123.5
6	138.3	118.3	86.1	85.2	100.9	147.8	123.5	121.7	128.7
7	123.5	80.0	120.0	90.4	102.6	90.4	123.5	120.0	135.6
8	105.2	91.3	126.9	83.5	117.4	146.1	123.5	113.0	146.1
9	149.9	75.7	147.8	111.3	103.5	135.6	123.5	127.0	123.5
10	137.4	83.5	133.9	97.4	120.0	151.3	133.9	123.5	149.6

[a]Blood drawn four hours after treatment.

[b]See Table 1, page 96 for explanation of groups, and Table 3, page 97 for explanation of treatment periods, chemicals, and doses.

Table 13. Iron concentration in whole blood[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

Calf number	Period I	Period II				Period III			
		Time interval – weeks				Time interval – weeks			
	Initial	2	4	6	8	10	12	14	16
		(mg/100 ml)							
Group[b] A									
1	60.0	46.8	56.0	57.6	66.4	54.0	52.4	52.0	52.4
2	58.0	61.6	51.6	55.2	56.0	52.4	54.4	47.2	52.0
3	52.0	49.6	51.6	54.0	49.2	51.6	48.0	45.6	48.8
4	52.0	54.0	47.6	51.2	43.6	52.4	50.4	46.4	47.2
5	52.4	48.4	55.6	52.0	43.2	49.2	50.1	47.2	52.8
6	51.6	59.6	57.4	55.6	58.0	50.2	56.0	49.4	52.0
7	62.0	64.4	53.2	52.0	50.4	57.6	51.6	53.6	46.8
8	57.6	47.6	48.8	43.2	44.8	45.6	43.2	52.8	48.0
9	54.8	51.6	37.6	40.0	39.2	45.6	44.4	47.2	48.8
10	58.0	48.0	47.6	45.2	43.6	51.2	50.4	60.4	51.6
Group B									
1	55.6	54.0	50.0	53.2	54.4	57.2	54.0	44.0	46.0
2	67.6	47.6	59.6	60.8	52.4	52.8	56.4	46.4	42.8
3	40.4	47.6	51.6	50.8	48.0	56.4	46.4	48.4	45.2
4	64.4	45.6	49.6	50.4	49.6	51.2	46.0	41.2	44.0
5	49.2	48.8	48.0	55.2	46.4	50.0	46.0	46.0	46.8
6	47.6	51.6	48.0	46.0	46.4	42.4	42.4	40.0	36.0
7	48.8	57.6	44.8	46.8	46.4	45.2	45.2	42.0	37.2
8	60.4	46.8	58.4	49.6	52.0	46.4	44.8	52.8	41.2
9	65.4	57.2	45.6	53.2	47.6	42.0	42.8	44.8	35.6
10	56.0	45.6	52.0	56.0	52.4	46.8	53.2	42.8	45.2
Group C									
1	54.8	56.0	55.2	52.0	57.2	52.4	52.8	52.0	53.2
2	56.0	55.6	52.4	55.2	54.4	52.0	52.8	52.0	54.8
3	59.8	52.4	57.6	49.2	53.6	52.0	50.4	50.4	52.4
4	63.2	54.4	55.6	60.8	55.6	56.8	53.2	54.4	50.0
5	60.0	56.0	55.6	62.0	52.4	56.8	57.2	50.2	51.2
6	57.6	60.8	57.2	55.2	56.8	55.6	55.2	54.0	54.4
7	52.8	59.6	53.2	52.0	48.0	53.1	50.4	52.8	51.0
8	59.8	58.4	49.2	50.8	57.2	50.0	47.6	55.2	53.2
9	49.2	50.2	50.8	48.0	50.0	55.2	53.2	53.2	52.0
10	48.8	57.6	55.2	59.2	51.6	52.4	56.4	53.6	53.2
Group D									
1	52.8	58.0	50.8	48.1	46.8	44.4	49.6	52.0	43.2
2	50.0	50.8	45.8	45.2	47.2	53.6	54.4	48.4	43.6
3	46.0	50.4	54.0	50.8	49.6	52.8	48.4	42.0	41.6
4	60.4	44.8	54.8	46.8	47.2	46.8	53.2	37.2	43.6
5	52.8	47.2	43.6	48.0	46.8	48.8	44.8	45.2	39.6
6	53.2	49.6	49.6	48.0	47.6	47.6	56.8	45.6	44.8
7	66.8	50.8	45.6	50.8	50.4	46.8	43.6	43.2	38.0
8	63.2	47.6	47.2	49.2	46.4	54.4	47.5	49.2	49.6
9	48.0	49.6	53.2	46.4	48.4	47.9	42.8	44.4	42.4
10	58.4	46.0	48.0	47.6	50.8	43.2	54.4	43.2	43.6

[a]Blood drawn four hours after treatment.

[b]See Table 1, page 96 for explanation of groups, and Table 3, page 97 for explanation of treatment periods, chemicals, and doses.

Table 14. Phosphorus concentration in blood serum[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

	Calf number	Group[b] A	B	C	D
			(mg/100 ml)		
Pretreatment					
Period I	1	4.80	5.25	5.31	4.19
	2	6.41	5.15	4.40	5.14
	3	4.25	4.25	5.61	4.78
	4	6.00	6.25	6.65	4.67
	5	4.78	4.15	4.40	4.17
	6	5.80	5.25	7.05	5.80
	7	5.67	4.28	4.25	4.23
	8	4.20	4.21	4.21	6.47
	9	5.04	6.55	6.34	6.31
	10	4.20	4.15	4.35	4.25
Treatment[b]					
Period II	1	4.60	5.14	5.26	4.25
	2	6.13	5.25	4.88	4.88
	3	4.47	4.19	5.31	4.52
	4	5.80	6.13	6.41	4.82
	5	5.04	4.35	4.67	4.25
	6	5.14	4.88	6.55	4.92
	7	5.31	4.40	4.35	4.40
	8	5.00	4.25	4.23	6.13
	9	5.25	6.47	6.13	6.25
	10	4.56	4.82	4.47	4.67
Period III	1	4.60	5.04	5.25	4.78
	2	5.96	4.82	4.56	4.82
	3	4.61	4.71	5.26	4.56
	4	5.67	5.91	6.47	4.60
	5	5.14	4.35	4.19	4.37
	6	5.26	5.31	6.52	5.26
	7	5.25	4.47	4.15	4.61
	8	4.60	4.40	4.78	6.13
	9	4.88	6.48	5.96	6.08
	10	4.40	4.82	5.67	4.82

[a]Blood drawn once during each period, at 4 hours after treatment (eighth week period II; fourteenth week period III).
[b]See Table 1, page 96, for explanation of groups; and Table 3, page 97, for explanation of periods, chemicals, and doses.

Table 15. Magnesium concentration in blood serum[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

	Calf number	Group[b] A	B	C	D
		(mg/100 ml)			
Pretreatment					
Period I	1	2.80	2.85	2.18	2.25
	2	2.35	2.83	2.13	2.90
	3	2.60	2.25	2.30	2.23
	4	2.27	2.21	2.32	1.98
	5	2.26	2.88	2.15	2.80
	6	2.63	2.30	3.20	2.33
	7	2.68	2.34	2.15	2.25
	8	2.45	2.17	2.30	2.90
	9	2.60	2.18	2.25	1.68
	10	1.95	2.70	2.28	2.30
Treatment[b]					
Period II	1	2.38	2.50	2.18	2.30
	2	2.70	1.80	2.58	2.60
	3	2.13	2.43	2.45	2.18
	4	2.02	2.23	2.75	2.18
	5	2.02	3.15	2.60	2.23
	6	2.00	2.35	2.48	2.03
	7	1.93	2.50	2.58	3.43
	8	2.20	2.13	2.65	2.30
	9	1.75	2.73	2.28	2.28
	10	2.33	2.18	2.63	2.03
Period III	1	2.73	2.35	2.30	1.95
	2	3.00	2.10	2.78	2.23
	3	2.23	2.63	2.85	2.13
	4	1.88	2.13	2.50	1.83
	5	2.50	2.23	2.45	2.08
	6	2.80	2.35	2.31	2.13
	7	2.78	2.13	2.58	2.58
	8	2.80	2.58	2.58	1.75
	9	2.63	2.23	2.43	2.23
	10	3.08	2.50	2.63	2.65

[a]Blood drawn once during each period, at 4 hours after treatment (eighth week period II; fourteenth week period III).
[b]See Table 1, page 96, for explanation of groups; and Table 3, page 97, for explanation of periods, chemicals, and doses.

Table 16. Chloride concentration in blood serum[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

	Calf number	Group[b] A	B	C	D
		(mg/100 ml)			
Pretreatment Period I	1	383.7	376.7	387.6	392.0
	2	382.5	389.5	393.3	388.8
	3	401.6	394.6	382.5	386.9
	4	374.8	378.8	391.4	386.9
	5	397.8	390.7	380.6	384.4
	6	375.5	379.9	390.1	386.3
	7	385.7	391.4	379.3	383.1
	8	388.2	382.5	396.5	392.7
	9	388.8	393.3	387.6	391.4
	10	393.3	387.6	379.3	375.5
Treatment[b] Period II	1	390.1	385.0	387.6	395.2
	2	389.5	396.5	392.7	391.4
	3	400.3	397.1	392.0	388.8
	4	386.3	380.6	390.7	393.9
	5	399.7	392.0	391.4	392.0
	6	384.4	380.6	395.2	393.3
	7	394.6	396.5	386.3	392.0
	8	389.5	390.1	397.1	393.3
	9	391.4	395.2	393.9	395.2
	10	395.2	393.9	385.0	381.8
Period III	1	389.4	427.0	397.8	440.4
	2	392.7	436.6	403.5	435.9
	3	411.8	441.7	392.7	434.7
	4	385.0	427.7	401.6	437.2
	5	407.9	435.9	390.7	435.3
	6	385.7	432.8	400.3	436.6
	7	395.8	440.4	389.5	431.5
	8	398.4	434.7	406.7	439.1
	9	399.0	441.0	397.8	439.8
	10	403.5	434.7	389.5	427.7

[a]Blood drawn once during each period, at 4 hours after treatment (eighth week period II; fourteenth week period III).
[b]See Table 1, page 96, for explanation of groups; and Table 3, page 97, for explanation of periods, chemicals, and doses.

Table 17. Zinc concentration in blood serum[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

	Calf number	Group[b] A	B	C	D
			(µg/100 ml)		
Pretreatment					
Period I	1	210	196	325	208
	2	191	298	217	256
	3	219	286	267	230
	4	188	330	164	196
	5	186	185	188	197
	6	197	171	188	197
	7	314	202	187	196
	8	239	205	200	241
	9	236	160	195	206
	10	236	161	176	204
Treatment[b]					
Period II	1	177	166	197	204
	2	176	181	189	204
	3	191	184	206	203
	4	181	186	196	205
	5	181	198	171	187
	6	186	182	172	186
	7	181	176	192	175
	8	208	183	165	149
	9	181	196	202	155
	10	173	203	151	147
Period III	1	181	155	185	144
	2	181	140	184	131
	3	164	151	186	137
	4	180	143	174	140
	5	182	143	160	135
	6	181	143	185	127
	7	206	127	188	128
	8	168	137	192	128
	9	204	135	192	128
	10	183	142	187	132

[a]Blood drawn once during each period, at 4 hours after treatment (eighth week period II; fourteenth week period III).
[b]See Table 1, page 96, for explanation of groups; and Table 3, page 97, for explanation of periods, chemicals, and doses.

Table 18. Copper concentration in blood serum[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

	Calf number	Group[c] A	B	C	D
			(µg/100 ml)		
Pretreatment					
Period I (initial)	1	113	93	95	93
	2	115	95	95	123
	3	108	95	108	115
	4	118	115	118	128
	5	113	90	95	103
	6	91	103	95	110
	7	95	120	113	100
	8	118	119	90	98
	9	108	95	113	103
	10	115	118	103	108
Treatment[b]					
Period II	1	100	98	103	97
	2	103	97	107	93
	3	110	93	103	83
	4	100	102	120	82
	5	98	93	110	95
	6	107	82	85	93
	7	98	87	98	97
	8	100	90	110	93
	9	98	90	97	87
	10	102	85	100	86
Period III	1	110	83	120	83
	2	95	95	105	110
	3	98	102	98	100
	4	103	102	110	85
	5	117	88	105	83
	6	117	93	117	80
	7	116	90	116	95
	8	120	82	103	95
	9	113	83	102	87
	10	120	105	100	83

[a]Blood drawn once during each period, at 4 hours after treatment (eighth week period II; fourteenth week period III).
[b]See Table 1, page 96, for explanation of groups; and Table 3, page 97, for explanation of periods, chemicals, and doses.

Table 19. Calcium concentration (duplicate samples) in cardiac tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppm, dry matter basis)			
1	1	260.2	259.8	228.7	191.5
	2	265.0	260.4	220.3	201.6
2	1	292.7	244.8	233.2	198.1
	2	311.6	259.2	229.1	199.3
3	1	293.7	233.6	254.9	191.6
	2	305.2	232.0	257.6	190.7
4	1	247.9	234.5	249.9	246.9
	2	233.5	235.1	243.5	242.4
5	1	298.3	233.4	239.2	235.9
	2	279.9	234.0	249.5	231.5
6	1	250.1	223.6	220.6	227.6
	2	246.5	224.3	236.2	226.1
7	1	298.3	241.6	213.9	225.2
	2	252.3	239.0	215.1	222.2
8	1	239.7	202.4	233.3	230.1
	2	251.5	209.8	232.4	223.6
9	1	253.9	220.6	249.2	225.0
	2	247.2	221.4	246.9	227.3
10	1	249.1	206.1	218.7	224.9
	2	244.6	205.9	227.7	223.6

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 20. Potassium concentration (duplicate samples) in cardiac tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppt, dry matter basis)			
1	1	4.856	5.944	5.140	5.484
	2	4.900	5.976	5.120	5.520
2	1	5.099	5.876	5.520	5.760
	2	5.080	5.820	5.464	5.756
3	1	5.176	5.752	5.248	5.796
	2	5.044	5.840	5.206	5.760
4	1	5.072	6.028	5.228	5.744
	2	4.999	5.972	5.240	5.748
5	1	5.012	6.032	5.308	5.964
	2	4.968	5.992	5.352	5.944
6	1	4.840	5.984	5.244	5.632
	2	4.864	5.952	5.292	5.676
7	1	4.956	5.728	5.124	5.656
	2	4.988	5.684	5.056	5.592
8	1	5.040	5.924	5.276	5.636
	2	5.060	6.012	5.224	5.660
9	1	4.928	5.912	5.180	5.788
	2	4.956	5.920	5.216	5.748
10	1	5.092	6.012	5.088	5.372
	2	5.064	5.992	5.176	5.442

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 21. Sodium concentration (duplicate samples) in cardiac tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppt, dry matter basis)			
1	1	3.942	2.946	3.420	2.847
	2	3.940	2.874	3.426	2.835
2	1	3.450	2.979	3.403	2.847
	2	3.461	2.929	3.411	2.849
3	1	3.749	2.955	3.975	2.837
	2	3.720	2.904	3.991	2.826
4	1	3.978	2.904	3.424	2.826
	2	3.946	2.899	3.487	2.850
5	1	3.491	2.955	3.416	2.874
	2	3.479	2.967	3.404	2.853
6	1	3.411	2.904	3.437	2.943
	2	3.426	2.903	3.444	2.904
7	1	3.397	2.886	3.416	2.865
	2	3.389	2.880	3.433	2.846
8	1	3.455	2.949	3.376	2.858
	2	3.443	2.943	3.391	2.861
9	1	3.401	2.866	3.447	2.811
	2	3.416	2.865	3.477	2.805
10	1	3.461	2.867	3.382	2.934
	2	3.470	2.868	3.396	2.937

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 22. Phosphorus concentration (duplicate samples) in cardiac tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppt, dry matter basis)			
1	1	8.342	8.806	8.334	8.753
	2	8.438	8.807	8.286	8.718
2	1	8.405	8.725	8.309	8.770
	2	8.463	8.884	8.419	8.658
3	1	8.345	8.930	8.418	8.796
	2	8.253	8.892	8.405	8.748
4	1	8.269	8.995	8.399	8.886
	2	8.315	9.060	8.287	8.770
5	1	8.171	8.821	8.413	8.724
	2	8.203	8.851	8.454	8.825
6	1	8.212	8.748	8.488	8.846
	2	8.178	8.887	8.434	8.879
7	1	8.122	8.864	8.477	8.870
	2	8.114	8.968	8.468	8.851
8	1	8.380	8.843	8.449	8.747
	2	8.403	8.849	8.494	8.726
9	1	8.405	8.876	8.452	8.983
	2	8.398	8.843	8.445	9.091
10	1	8.349	8.893	8.404	8.866
	2	8.355	8.848	8.489	8.825

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 23. Magnesium concentration (duplicate samples) in cardiac tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
1	1	0.885	1.199	0.891	1.121
	2	0.899	1.134	0.880	1.156
2	1	0.924	1.091	0.945	1.031
	2	0.915	1.035	0.955	1.039
3	1	0.921	1.083	0.975	1.087
	2	0.946	1.061	0.964	1.082
4	1	0.966	1.117	0.864	1.153
	2	0.926	1.146	0.891	1.168
5	1	0.904	1.073	0.901	1.102
	2	0.895	1.043	0.889	1.082
6	1	0.928	1.033	0.954	1.161
	2	0.894	1.003	0.961	1.121
7	1	0.936	1.138	0.945	1.092
	2	0.908	1.116	0.940	1.091
8	1	0.896	1.129	0.950	1.178
	2	0.862	1.137	0.946	1.190
9	1	0.909	1.144	0.942	1.022
	2	0.896	1.135	0.936	1.049
10	1	0.941	1.079	0.936	1.111
	2	0.946	1.052	0.929	1.161

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 24. Iron concentration (duplicate samples) in cardiac tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppm, dry matter basis)			
1	1	210.2	232.5	209.2	188.9
	2	209.9	225.2	209.6	190.5
2	1	209.1	207.1	228.9	191.4
	2	217.7	200.9	228.3	203.3
3	1	202.4	218.9	207.5	200.2
	2	219.2	206.2	203.2	189.8
4	1	227.8	193.4	212.3	207.9
		231.0	205.4	211.8	203.8
5	1	210.8	231.9	215.2	231.4
	2	208.9	228.1	218.9	225.6
6	1	207.4	172.6	215.8	220.1
	2	198.6	174.2	216.4	230.6
7	1	217.7	181.2	209.0	227.4
	2	217.3	181.9	206.2	232.5
8	1	213.7	210.0	208.0	213.7
	2	215.3	207.4	203.5	218.2
9	1	211.3	193.4	208.6	229.5
	2	203.7	207.6	206.9	220.0
10	1	216.7	190.9	222.6	235.2
	2	218.4	194.1	213.3	219.6

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 25. Zinc concentration (duplicate samples) in cardiac tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppm, dry matter basis)			
1	1	129.06	89.60	116.87	95.41
	2	131.14	88.41	115.41	96.87
2	1	127.79	82.42	127.45	87.45
	2	126.14	84.16	128.29	86.29
3	1	104.79	94.97	105.18	95.81
	2	104.70	93.61	105.07	95.20
4	1	112.06	92.61	101.88	91.58
	2	111.03	91.30	103.58	93.88
5	1	124.06	90.85	107.45	97.54
	2	126.32	92.01	107.49	96.94
6	1	113.51	97.56	125.98	95.89
	2	112.04	98.71	125.92	94.27
7	1	112.16	97.16	146.07	86.07
	2	112.34	95.93	142.42	86.42
8	1	124.38	92.38	110.78	90.87
	2	125.20	94.02	109.84	89.84
9	1	104.00	94.00	115.04	94.04
	2	102.55	92.91	113.32	93.23
10	1	103.20	90.32	107.58	91.65
	2	102.91	91.07	111.56	90.58

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 26. Copper concentration (duplicate samples) in cardiac tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppm, dry matter basis)			
1	1	21.60	16.56	28.26	16.71
	2	22.76	15.72	29.68	16.57
2	1	18.29	15.01	23.22	15.22
	2	18.80	14.97	23.91	14.76
3	1	21.76	17.63	23.76	17.42
	2	22.64	16.99	22.40	17.20
4	1	20.06	17.00	23.71	17.22
	2	19.94	16.73	22.92	17.01
5	1	21.08	14.89	25.56	15.00
	2	21.51	15.23	25.55	15.44
6	1	19.61	18.01	18.36	17.83
	2	20.40	17.61	18.89	17.47
7	1	20.35	14.85	21.15	14.96
	2	20.19	14.78	22.30	15.66
8	1	20.95	15.86	18.66	16.16
	2	20.96	16.11	18.09	15.98
9	1	19.92	15.83	18.38	15.74
	2	18.55	15.47	19.03	15.38
10	1	18.38	16.38	21.59	16.27
	2	18.02	16.69	22.22	16.19

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 27. Ca/K ratio (duplicate samples) in cardiac tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
1	1	0.0536	0.0437	0.0445	0.0349
	2	0.0541	0.0436	0.0430	0.0365
2	1	0.0575	0.0417	0.0422	0.0344
	2	0.0613	0.0445	0.0419	0.0346
3	1	0.0567	0.0406	0.0486	0.0331
	2	0.0605	0.0397	0.0505	0.0331
4	1	0.0489	0.0389	0.0478	0.0430
	2	0.0467	0.0394	0.0465	0.0422
5	1	0.0595	0.0387	0.0451	0.0396
	2	0.0563	0.0391	0.0466	0.0389
6	1	0.0517	0.0374	0.0421	0.0404
	2	0.0507	0.0377	0.0446	0.0398
7	1	0.0602	0.0422	0.0417	0.0398
	2	0.0506	0.0520	0.0425	0.0397
8	1	0.0476	0.0342	0.0442	0.0408
	2	0.0497	0.0349	0.0445	0.0395
9	1	0.0515	0.0373	0.0481	0.0389
	2	0.0499	0.0374	0.0473	0.0395
10	1	0.0489	0.0343	0.0429	0.0418
	2	0.0483	0.0344	0.0439	0.0411

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 28. Ca/Mg ratio (duplicate samples) in cardiac tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
1	1	0.294	0.216	0.256	0.170
	2	0.294	0.229	0.250	0.174
2	1	0.316	0.224	0.246	0.192
	2	0.340	0.250	0.239	0.191
3	1	0.318	0.215	0.261	0.176
	2	0.322	0.218	0.267	0.176
4	1	0.256	0.209	0.289	0.214
	2	0.252	0.205	0.273	0.207
5	1	0.329	0.217	0.265	0.214
	2	0.312	0.224	0.280	0.213
6	1	0.269	0.216	0.231	0.196
	2	0.275	0.223	0.245	0.201
7	1	0.318	0.212	0.226	0.206
	2	0.277	0.214	0.228	0.203
8	1	0.267	0.179	0.245	0.195
	2	0.291	0.184	0.245	0.187
9	1	0.279	0.192	0.264	0.220
	2	0.275	0.195	0.263	0.216
10	1	0.264	0.191	0.233	0.202
	2	0.258	0.195	0.245	0.192

[a]See Table 3, page 97 for explanation of treatments.
[b]See Table 1, page 96 for explanation of groups.

Table 29. Dry matter content (duplicate samples) of cardiac tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(percent)			
1	1	22.01	23.32	23.39	21.44
	2	21.74	23.37	23.22	21.76
2	1	21.21	23.35	22.33	21.36
	2	21.28	23.75	22.75	21.44
3	1	21.17	22.58	22.66	23.46
	2	21.21	22.88	22.68	23.41
4	1	21.40	22.95	21.12	21.99
	2	21.57	22.62	21.48	21.71
5	1	25.13	23.22	21.25	22.63
	2	24.57	23.85	21.33	22.75
6	1	22.85	23.41	21.36	21.73
	2	22.49	23.36	21.58	21.95
7	1	22.98	22.19	22.43	22.64
	2	22.99	21.98	22.36	22.58
8	1	23.06	23.04	22.49	22.55
	2	23.17	22.98	22.74	22.02
9	1	21.94	22.87	22.93	22.09
	2	22.00	22.95	22.84	22.08
10	1	21.65	22.53	22.22	21.10
	2	21.54	22.63	21.98	21.26

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 30. Ash content (duplicate samples) of cardiac tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(percent)			
1	1	1.01	1.04	1.05	0.98
	2	1.03	1.04	1.05	0.93
2	1	1.02	1.08	1.05	1.01
	2	1.01	1.09	1.06	0.99
3	1	1.03	1.04	1.08	1.05
	2	0.99	1.01	1.07	1.01
4	1	1.01	0.99	1.02	0.95
	2	1.04	0.99	0.99	0.96
5	1	1.09	1.03	0.97	1.06
	2	1.09	1.05	0.96	1.07
6	1	0.97	1.02	1.05	1.03
	2	1.00	1.01	1.05	1.04
7	1	1.04	1.00	0.99	1.01
	2	1.05	1.00	1.01	1.01
8	1	1.06	1.04	1.05	0.99
	2	1.04	1.01	1.01	0.98
9	1	1.08	1.02	1.01	1.00
	2	1.06	1.00	1.02	0.99
10	1	1.03	0.98	0.98	0.99
	2	1.02	0.97	1.03	1.00

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 31. Calcium concentration (duplicate samples) in hepatic tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppm, dry matter basis)			
1	1	283.2	243.8	288.6	247.4
	2	285.6	243.6	289.4	241.2
2	1	274.8	239.7	288.8	241.3
	2	272.2	240.4	286.9	240.6
3	1	281.7	244.6	287.4	246.1
	2	280.0	245.8	286.2	247.6
4	1	288.9	244.2	288.8	243.6
	2	288.6	243.8	289.3	244.7
5	1	289.7	247.2	285.8	247.4
	2	289.0	243.6	284.9	248.5
6	1	282.0	245.8	286.2	246.6
	2	283.0	244.2	287.7	245.9
7	1	282.8	245.4	286.3	256.4
	2	281.9	244.0	285.9	252.8
8	1	286.6	254.6	284.2	246.7
	2	286.4	251.4	282.8	245.0
9	1	285.4	263.4	288.8	249.3
	2	286.2	261.8	288.1	249.1
10	1	280.7	244.6	287.6	251.3
	2	281.2	244.2	286.1	249.8

[a]See Table 3, page 97 for explanation of treatments.
[b]See Table 1, page 96 for explanation of groups.

Table 32. Potassium concentration (duplicate samples) in hepatic tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppt, dry matter basis)			
1	1	15.68	15.48	14.10	12.74
	2	15.28	14.74	14.02	12.91
2	1	15.66	14.93	15.25	14.00
	2	16.07	15.38	14.94	13.96
3	1	14.48	11.88	15.56	12.73
	2	14.10	11.87	14.99	12.90
4	1	16.73	13.65	14.81	15.13
	2	17.03	13.91	15.02	15.35
5	1	14.24	15.14	15.61	14.40
	2	14.33	15.51	15.69	14.78
6	1	13.34	12.95	13.13	12.62
	2	13.41	13.00	13.11	13.08
7	1	13.21	13.56	13.29	13.72
	2	13.50	13.59	13.38	14.05
8	1	14.20	12.72	15.67	14.58
	2	14.78	12.98	15.96	14.55
9	1	13.13	12.74	14.01	13.45
	2	13.24	13.18	14.28	13.36
10	1	13.65	13.44	13.37	12.68
	2	13.84	13.65	13.69	12.14

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 33. Sodium concentration (duplicate samples) in hepatic tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppt, dry matter basis)			
1	1	5.156	4.908	4.376	4.596
	2	5.004	5.004	4.404	4.604
2	1	4.936	4.276	4.928	4.652
	2	5.006	4.328	4.892	4.672
3	1	4.824	4.704	4.592	4.044
	2	4.906	4.648	4.576	4.084
4	1	5.032	4.436	4.596	5.088
	2	5.020	4.420	4.732	4.996
5	1	4.996	4.308	5.300	4.264
	2	4.924	4.212	5.236	4.212
6	1	4.860	4.252	4.428	4.036
	2	4.844	4.224	4.396	4.094
7	1	5.164	4.388	4.672	4.216
	2	5.092	4.308	4.672	4.244
8	1	5.156	4.060	4.468	4.124
	2	5.204	4.094	4.471	4.068
9	1	4.872	4.508	4.348	4.304
	2	4.852	4.532	4.294	4.286
10	1	4.852	4.488	4.216	4.384
	2	4.908	4.506	4.176	4.408

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 34. Phosphorus concentration (duplicate samples) in hepatic tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppt, dry matter basis)			
1	1	16.56	15.68	16.10	15.51
	2	15.95	16.07	16.26	15.53
2	1	17.19	15.79	17.13	16.79
	2	16.76	15.82	16.98	16.58
3	1	16.06	15.30	16.58	15.90
	2	15.88	15.01	16.46	15.61
4	1	16.73	15.90	15.97	16.82
	2	16.99	16.06	15.65	17.16
5	1	15.87	15.52	15.78	15.88
	2	15.96	15.75	15.43	15.93
6	1	16.68	15.55	15.68	15.49
	2	16.76	15.54	16.07	15.32
7	1	16.61	15.10	16.74	15.86
	2	16.75	15.38	16.99	15.92
8	1	16.09	16.04	16.41	17.12
	2	16.47	15.70	15.95	16.97
9	1	16.79	15.36	15.66	16.40
	2	16.38	15.00	15.54	16.33
10	1	15.78	14.98	15.58	17.08
	2	15.87	15.30	15.94	16.85

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 35. Magnesium concentration (duplicate samples) in hepatic tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppm, dry matter basis)			
1	1	723.8	773.7	737.0	670.4
	2	734.5	771.8	731.1	677.9
2	1	737.2	721.5	742.5	756.9
	2	732.2	722.5	745.5	756.1
3	1	701.7	755.1	686.1	691.2
	2	694.0	756.4	696.5	704.4
4	1	760.9	785.4	795.8	841.1
	2	768.0	783.2	789.4	846.6
5	1	690.2	753.2	688.9	773.8
	2	701.1	754.0	697.2	757.6
6	1	738.8	755.4	637.1	884.2
	2	737.6	761.6	649.4	878.9
7	1	731.1	761.0	795.8	765.5
	2	731.5	775.1	790.3	764.3
8	1	747.4	773.7	718.8	819.5
	2	745.3	762.4	720.7	818.0
9	1	693.4	762.1	738.6	777.2
	2	692.8	762.4	727.1	775.8
10	1	756.3	801.7	769.4	664.6
	2	754.4	798.7	779.9	666.9

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 36. Iron concentration (duplicate samples) in hepatic tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppm, dry matter basis)			
1	1	251.1	197.2	242.4	207.3
	2	250.4	196.1	243.6	206.9
2	1	256.7	201.2	243.4	191.3
	2	257.0	202.9	242.9	192.7
3	1	248.9	192.8	245.2	201.1
	2	249.1	192.2	244.7	202.9
4	1	248.2	196.7	251.1	201.5
	2	247.8	197.2	252.0	200.1
5	1	249.8	197.6	262.9	198.1
	2	249.2	196.0	261.7	199.6
6	1	246.3	192.5	253.6	203.4
	2	247.2	191.7	254.4	204.1
7	1	249.2	194.6	260.3	201.1
	2	249.1	196.2	261.4	202.5
8	1	251.6	208.1	241.5	204.1
	2	250.9	205.7	240.7	205.7
9	1	257.7	196.6	264.5	195.8
	2	256.8	197.4	265.5	196.7
10	1	246.8	205.0	257.9	199.8
	2	245.7	206.9	258.4	197.9

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 37. Zinc concentration (duplicate samples) in hepatic tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppm, dry matter basis)			
1	1	194.5	157.4	255.0	185.6
	2	195.9	156.9	254.6	186.7
2	1	242.8	153.8	223.7	155.7
	2	241.0	153.6	224.8	158.8
3	1	249.1	181.7	235.6	152.0
	2	250.2	182.2	233.9	154.3
4	1	242.7	163.5	236.7	169.7
	2	243.9	162.1	238.3	168.2
5	1	243.8	166.1	218.8	157.9
	2	241.6	168.8	220.0	158.7
6	1	221.2	153.9	240.6	159.0
	2	220.9	152.1	241.2	158.6
7	1	228.9	156.6	215.0	153.7
	2	226.7	156.1	217.9	154.0
8	1	249.1	155.7	246.2	166.7
	2	248.6	155.2	246.8	167.9
9	1	227.4	158.5	215.8	173.5
	2	225.2	159.2	218.0	171.6
10	1	201.1	158.1	210.6	174.8
	2	203.6	156.3	212.5	172.3

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 38. Copper concentration (duplicate samples) in hepatic tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppm, dry matter basis)			
1	1	86.50	47.37	81.70	39.79
	2	86.25	47.77	82.91	39.16
2	1	91.63	46.35	88.96	39.91
	2	89.28	46.12	88.11	40.10
3	1	75.99	47.87	77.07	38.84
	2	76.90	48.80	79.12	38.38
4	1	81.70	44.94	86.11	47.79
	2	82.23	44.76	86.03	47.18
5	1	75.40	39.61	78.73	46.95
	2	75.84	39.40	78.44	47.69
6	1	83.30	49.65	83.34	46.77
	2	82.84	49.76	84.74	46.53
7	1	81.61	47.97	84.31	43.56
	2	82.77	47.81	83.51	42.97
8	1	83.47	38.83	83.71	44.49
	2	83.85	38.48	82.01	44.67
9	1	80.10	39.19	87.26	39.16
	2	79.84	40.01	86.22	38.81
10	1	81.85	39.61	77.98	45.69
	2	80.16	38.97	77.00	46.27

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 39. Dry matter content (duplicate samples) of hepatic tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(percent)			
1	1	26.13	25.94	26.94	24.38
	2	26.32	25.86	26.86	24.29
2	1	26.58	25.46	26.93	24.00
	2	26.57	25.25	26.79	23.98
3	1	25.73	25.92	27.33	25.14
	2	25.55	25.80	27.62	25.11
4	1	25.42	25.97	27.17	24.52
	2	25.42	25.99	27.09	24.56
5	1	27.18	25.44	26.39	24.90
	2	27.19	25.47	26.48	24.94
6	1	26.64	26.04	24.96	25.37
	2	26.84	26.16	25.00	25.32
7	1	26.04	25.05	26.43	25.67
	2	25.98	25.02	26.21	25.65
8	1	27.60	24.05	26.33	25.07
	2	27.54	23.93	26.33	25.04
9	1	27.75	26.10	27.29	25.73
	2	27.87	26.21	27.40	25.46
10	1	27.55	25.98	28.43	25.69
	2	27.71	25.94	28.48	25.82

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 40. Ash content (duplicate samples) of hepatic tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(percent, fresh basis)			
1	1	1.23	1.34	1.29	1.20
	2	1.27	1.29	1.32	1.20
2	1	1.32	1.31	1.39	1.22
	2	1.31	1.28	1.42	1.20
3	1	1.26	1.35	1.33	1.31
	2	1.22	1.30	1.39	1.28
4	1	1.32	1.31	1.33	1.27
	2	1.31	1.32	1.34	1.27
5	1	1.35	1.32	1.22	1.31
	2	1.32	1.33	1.21	1.29
6	1	1.23	1.29	1.21	1.33
	2	1.28	1.29	1.21	1.31
7	1	1.28	1.37	1.27	1.22
	2	1.29	1.39	1.30	1.24
8	1	1.28	1.28	1.29	1.30
	2	1.33	1.24	1.25	1.30
9	1	1.35	1.26	1.28	1.25
	2	1.34	1.25	1.21	1.26
10	1	1.30	1.25	1.35	1.23
	2	1.33	1.24	1.34	1.28

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 41. Calcium concentration (duplicate samples) in renal tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppm, dry matter basis)			
1	1	274.6	455.8	293.4	613.8
	2	263.2	457.2	291.8	607.4
2	1	269.8	489.0	292.6	609.6
	2	273.6	493.0	296.4	606.2
3	1	292.6	442.8	294.2	605.2
	2	292.6	449.8	289.0	610.6
4	1	286.8	574.0	291.4	610.6
	2	299.4	574.2	290.8	605.2
5	1	256.8	478.8	306.6	581.2
	2	255.8	479.8	300.8	587.8
6	1	252.6	549.2	264.4	587.6
	2	253.0	540.2	265.6	582.0
7	1	296.4	501.4	266.8	518.6
	2	298.2	499.6	269.0	516.2
8	1	275.6	478.8	281.0	491.6
	2	277.8	480.2	282.6	498.8
9	1	277.0	554.4	287.2	603.8
	2	279.4	549.4	293.8	600.6
10	1	260.2	435.0	289.2	577.0
	2	262.4	441.2	283.6	586.2

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 42. Potassium concentration (duplicate samples) in renal tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppt, dry matter basis)			
1	1	10.31	10.44	10.18	9.19
	2	10.21	10.50	10.20	9.21
2	1	10.41	10.60	10.89	8.98
	2	10.48	10.52	10.91	9.02
3	1	10.00	10.13	10.21	9.45
	2	10.02	10.12	10.21	9.42
4	1	10.72	9.31	10.15	9.81
	2	10.99	9.26	10.22	9.83
5	1	10.68	9.66	10.71	9.98
	2	10.57	9.67	10.22	9.87
6	1	10.55	9.41	10.08	9.59
	2	10.62	9.37	9.85	9.62
7	1	10.00	9.72	10.06	10.35
	2	9.96	9.70	10.00	10.02
8	1	10.63	9.29	10.30	9.60
	2	10.29	9.35	10.29	9.30
9	1	10.03	10.42	10.56	8.66
	2	10.15	10.61	10.56	8.69
10	1	10.21	9.65	10.08	10.43
	2	10.19	9.80	9.96	10.34

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 43. Sodium concentration (duplicate samples) in renal tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppt, dry matter basis)			
1	1	10.05	8.69	10.53	8.96
	2	10.18	8.74	10.99	8.95
2	1	11.01	8.51	10.91	8.71
	2	10.95	8.54	10.60	8.59
3	1	11.57	8.80	10.17	9.97
	2	11.48	8.82	10.16	9.99
4	1	11.57	8.86	9.52	9.49
	2	11.47	8.73	9.49	9.46
5	1	9.72	8.66	11.32	9.96
	2	9.98	8.80	11.41	10.00
6	1	9.97	9.09	10.23	9.24
	2	9.93	8.98	10.16	9.33
7	1	10.94	8.90	10.99	10.11
	2	10.95	8.81	10.71	10.03
8	1	10.25	9.23	11.11	8.88
	2	10.15	9.25	11.16	8.92
9	1	10.08	8.64	10.80	9.17
	2	10.34	8.72	10.69	9.34
10	1	10.31	9.11	10.52	9.53
	2	10.29	9.19	10.57	9.77

[a]See table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 44. Phosphorus concentration (duplicate samples) in renal tissue of calves as influenced by treatments[a]

Calf number	Determ-nation	Group[b] A	B	C	D
		(ppt, dry matter basis)			
1	1	7.418	8.803	7.178	9.457
	2	7.548	8.991	7.234	9.680
2	1	7.891	8.396	7.442	9.314
	2	7.826	8.486	7.250	9.089
3	1	7.361	8.824	7.026	9.067
	2	7.229	8.857	7.067	8.990
4	1	7.526	8.935	7.852	8.972
	2	7.596	8.915	7.739	9.085
5	1	7.661	9.037	7.573	9.192
	2	7.587	8.959	7.496	9.276
6	1	7.515	8.989	7.926	8.820
	2	7.581	8.856	7.842	8.969
7	1	7.460	9.076	7.650	9.122
	2	7.436	8.940	7.561	9.231
8	1	7.586	9.139	7.632	9.436
	2	7.409	8.928	7.750	9.436
9	1	7.257	9.402	7.859	8.939
	2	7.196	9.346	7.718	9.028
10	1	7.307	9.044	7.690	9.149
	2	7.240	8.963	7.584	9.204

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 45. Magnesium concentration (duplicate samples) in renal tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppm, dry matter basis)			
1	1	503.9	477.6	498.7	495.0
	2	504.1	478.2	500.2	493.6
2	1	532.1	474.8	516.0	440.1
	2	528.8	475.3	521.1	439.8
3	1	597.0	480.1	501.6	409.1
	2	591.7	479.3	504.2	406.7
4	1	590.8	466.9	526.8	460.8
	2	595.1	469.1	531.1	461.2
5	1	557.1	445.6	530.0	422.6
	2	556.8	444.3	529.6	424.1
6	1	557.2	481.7	541.7	498.8
	2	551.9	483.6	542.9	499.0
7	1	513.8	432.1	541.2	568.3
	2	516.1	427.9	539.8	571.2
8	1	542.6	487.6	499.9	480.9
	2	544.1	486.7	501.6	481.2
9	1	521.3	565.2	511.7	437.0
	2	523.0	567.1	512.6	436.7
10	1	543.2	450.0	498.7	476.9
	2	541.6	449.8	503.2	480.1

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 46. Na/K ratio (duplicate samples) in renal tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
1	1	0.97	0.83	1.03	0.97
	2	0.99	0.83	1.07	0.97
2	1	1.05	0.80	1.00	0.96
	2	1.04	0.81	0.97	0.95
3	1	1.15	0.86	0.99	1.05
	2	1.14	0.87	0.99	1.06
4	1	1.07	0.95	0.93	0.96
	2	1.04	0.94	0.92	0.96
5	1	0.91	0.89	1.05	0.99
	2	0.94	0.91	1.11	1.01
6	1	0.94	0.96	1.01	0.96
	2	0.93	0.95	1.03	0.96
7	1	1.09	0.91	1.09	0.97
	2	1.09	0.90	1.07	1.00
8	1	0.96	0.99	1.07	0.92
	2	0.98	0.98	1.08	0.95
9	1	1.00	0.82	1.02	1.05
	2	1.01	0.82	1.01	1.07
10	1	1.00	0.94	1.04	0.91
	2	1.00	0.93	1.06	0.94

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 47. Dry matter content (duplicate samples) of renal tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(percent)			
1	1	20.14	18.22	19.27	17.67
	2	20.15	17.98	19.38	17.64
2	1	20.66	18.69	20.91	17.66
	2	20.70	18.57	21.09	17.77
3	1	18.88	17.25	21.79	17.67
	2	19.11	17.17	21.81	17.60
4	1	19.68	17.73	19.74	17.97
	2	19.67	17.90	19.74	17.97
5	1	20.42	17.69	20.22	17.96
	2	20.44	17.63	20.19	18.06
6	1	21.88	16.87	20.08	17.64
	2	22.16	16.93	20.14	17.67
7	1	20.91	17.40	19.70	17.98
	2	20.84	17.38	19.67	18.02
8	1	21.04	17.50	18.95	17.82
	2	21.04	17.47	19.08	17.96
9	1	20.68	18.27	20.03	17.80
	2	20.68	18.30	20.04	17.69
10	1	21.77	17.61	20.30	18.50
	2	21.76	17.57	20.42	18.56

[a]See Table 3, page 97, for explanation of treatments
[b]See Table 1, page 96, for explanation of groups.

Table 48. Ash content (duplicate samples) of renal tissue of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(Percent)			
1	1	1.15	1.12	1.25	1.13
	2	1.15	1.11	1.26	1.12
2	1	1.22	1.19	1.31	1.17
	2	1.20	1.19	1.34	1.18
3	1	1.24	1.11	1.31	1.24
	2	1.22	1.10	1.33	1.16
4	1	1.30	1.12	1.27	1.21
	2	1.23	1.15	1.24	1.26
5	1	1.34	1.14	1.18	1.17
	2	1.32	1.10	1.16	1.18
6	1	1.28	1.11	1.15	1.14
	2	1.34	1.09	1.15	1.15
7	1	1.32	1.09	1.12	1.28
	2	1.32	1.03	1.13	1.26
8	1	1.19	1.11	1.29	1.21
	2	1.18	1.17	1.32	1.22
9	1	1.24	1.26	1.23	1.10
	2	1.21	1.22	1.25	1.11
10	1	1.22	1.06	1.25	1.07
	2	1.21	1.04	1.24	1.10

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 49. Calcium concentration (duplicate samples) in the right metatarsal bone of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
			(percent of ash)		
1	1	32.30	25.96	34.33	25.55
	2	31.25	26.34	34.05	26.30
2	1	32.79	26.95	33.41	25.68
	2	32.58	26.61	33.22	25.86
3	1	31.52	24.29	30.34	23.88
	2	31.68	24.89	30.50	24.05
4	1	37.19	25.44	30.06	25.10
	2	36.96	25.10	29.90	24.96
5	1	34.40	24.57	32.34	26.23
	2	34.01	24.09	32.07	25.76
6	1	30.23	25.17	33.60	24.44
	2	31.09	25.58	33.85	24.42
7	1	32.23	25.93	33.57	25.72
	2	31.78	25.26	34.10	26.05
8	1	32.91	25.61	31.87	25.21
	2	32.48	25.40	31.19	25.20
9	1	31.28	23.31	33.15	26.12
	2	30.97	23.72	32.92	26.09
10	1	32.23	24.76	32.68	25.13
	2	32.79	25.01	32.21	25.05

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 50. Potassium concentration (duplicate samples) in the right metatarsal bone of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(percent of ash)			
1	1	0.0895	0.1972	0.0673	0.1228
	2	0.0850	0.1979	0.0690	0.1300
2	1	0.0967	0.1880	0.0818	0.1228
	2	0.0928	0.1892	0.0845	0.1246
3	1	0.0813	0.1800	0.0845	0.1290
	2	0.0868	0.1760	0.0881	0.1357
4	1	0.0927	0.1700	0.0761	0.1438
	2	0.0948	0.1639	0.0778	0.1391
5	1	0.0879	0.1841	0.0626	0.1403
	2	0.0862	0.1858	0.0627	0.1385
6	1	0.0806	0.1291	0.0768	0.1416
	2	0.0844	0.1272	0.0725	0.1448
7	1	0.0833	0.1734	0.0523	0.1564
	2	0.0846	0.1653	0.0523	0.1517
8	1	0.0826	0.1592	0.0805	0.1591
	2	0.0813	0.1672	0.0816	0.1568
9	1	0.1041	0.1997	0.0853	0.1891
	2	0.1023	0.1888	0.0870	0.1918
10	1	0.0747	0.1471	0.0628	0.1963
	2	0.0732	0.1489	0.0621	0.2009

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 51. Sodium concentration (duplicate samples) in the right metatarsal bone of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(percent of ash)			
1	1	1.028	1.288	1.038	1.064
	2	1.133	1.343	0.998	1.067
2	1	1.282	0.929	1.033	1.082
	2	1.295	0.985	1.086	1.001
3	1	1.106	1.367	0.922	1.253
	2	1.240	1.289	0.954	1.276
4	1	1.184	0.995	1.176	1.173
	2	1.188	0.964	1.167	1.134
5	1	1.137	1.044	1.138	1.346
	2	1.155	1.106	1.029	1.274
6	1	1.083	1.342	1.220	1.040
	2	1.141	1.272	1.135	1.143
7	1	1.294	1.235	0.960	0.988
	2	1.203	1.150	0.914	1.053
8	1	1.235	1.001	1.062	1.068
	2	1.184	1.074	0.979	1.021
9	1	1.066	1.112	0.911	1.135
	2	1.123	1.167	1.014	1.267
10	1	1.092	1.331	0.924	1.264
	2	1.204	1.274	0.899	1.384

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups

Table 52. Phosphorus concentration (duplicate samples) in the right metatarsal bone of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(percent of ash)			
1	1	18.23	16.45	19.13	16.22
	2	17.99	17.10	19.06	16.46
2	1	18.77	15.88	19.71	15.83
	2	18.91	16.61	20.04	15.90
3	1	19.25	15.94	18.69	16.76
	2	18.02	15.85	19.12	16.91
4	1	19.22	16.09	19.57	17.08
	2	18.46	15.99	19.27	16.56
5	1	17.46	16.00	19.51	17.58
	2	18.10	16.32	19.35	17.02
6	1	19.22	16.94	19.59	16.91
	2	20.08	16.05	19.87	16.68
7	1	19.24	15.97	19.75	16.04
	2	18.39	15.88	18.97	16.80
8	1	19.46	16.56	19.10	17.06
	2	18.58	16.67	19.05	16.93
9	1	17.50	16.32	19.95	16.45
	2	17.11	16.65	18.96	16.40
10	1	19.45	16.20	19.03	16.60
	2	19.27	16.46	19.92	16.74

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 53. Magnesium concentration (duplicate samples) in the right metatarsal bone of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(percent of ash)			
1	1	1.214	0.558	0.761	0.532
	2	1.186	0.505	0.714	0.529
2	1	0.949	0.512	0.831	0.485
	2	0.889	0.518	0.881	0.483
3	1	0.744	0.502	0.821	0.516
	2	0.764	0.499	0.815	0.553
4	1	0.837	0.516	0.732	0.515
	2	0.872	0.503	0.708	0.452
5	1	0.899	0.532	0.760	0.511
	2	0.916	0.553	0.779	0.517
6	1	0.907	0.558	0.796	0.486
	2	0.878	0.531	0.824	0.510
7	1	0.851	0.460	0.687	0.535
	2	0.850	0.439	0.683	0.519
8	1	0.817	0.541	0.713	0.467
	2	0.828	0.521	0.711	0.479
9	1	0.771	0.494	0.839	0.519
	2	0.775	0.479	0.843	0.515
10	1	0.816	0.514	0.801	0.475
	2	0.853	0.502	0.815	0.492

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 54. Zinc concentration (duplicate samples) in the right metatarsal bone of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(ppm of ash)			
1	1	247.2	297.6	226.8	326.0
	2	249.8	289.8	226.5	321.7
2	1	211.0	352.2	226.9	300.6
	2	213.8	351.0	222.2	299.2
3	1	241.1	336.3	202.6	320.9
	2	245.6	337.0	212.8	323.1
4	1	240.7	349.7	192.1	320.1
	2	240.7	349.3	188.8	325.9
5	1	226.4	348.5	228.8	318.8
	2	223.7	347.1	225.0	322.8
6	1	243.5	292.4	218.2	320.2
	2	238.6	295.2	216.7	320.4
7	1	239.8	297.8	212.0	288.9
	2	238.1	299.3	217.5	293.8
8	1	237.7	305.7	244.1	327.8
	2	240.5	308.8	245.6	320.5
9	1	249.4	320.5	205.2	288.8
	2	249.2	321.8	203.5	286.1
10	1	212.1	342.6	224.8	322.9
	2	210.9	340.3	229.8	324.2

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 55. Ca/K ratio (duplicate samples) in the right metatarsal bone of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
1	1	1.755	1.578	1.795	1.575
	2	1.737	1.540	1.786	1.598
2	1	1.747	1.697	1.695	1.622
	2	1.723	1.602	1.657	1.626
3	1	1.637	1.524	1.623	1.425
	2	1.758	1.570	1.595	1.422
4	1	1.935	1.581	1.536	1.469
	2	2.002	1.569	1.552	1.507
5	1	1.970	1.536	1.658	1.492
	2	1.879	1.476	1.657	1.514
6	1	1.573	1.486	1.715	1.445
	2	1.548	1.594	1.704	1.464
7	1	1.675	1.624	1.699	1.603
	2	1.728	1.591	1.797	1.551
8	1	1.691	1.546	1.668	1.478
	2	1.748	1.524	1.637	1.488
9	1	1.787	1.428	1.662	1.588
	2	1.810	1.425	1.736	1.591
10	1	1.657	1.528	1.717	1.514
	2	1.702	1.519	1.617	1.496

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 56. Dry matter content of the right metatarsal bone of calves as influenced by treatments[a]

Calf number	Group[b]			
	A	B	C	D
	(percent)			
1	90.42	90.80	91.38	88.96
2	90.11	90.62	89.62	89.13
3	90.61	90.52	91.31	89.36
4	89.86	90.70	89.89	88.57
5	88.17	87.87	89.58	88.75
6	86.22	88.87	89.59	90.95
7	89.75	89.52	89.15	90.85
8	89.76	86.35	88.69	93.88
9	88.40	89.62	87.61	90.70
10	88.41	90.43	88.47	90.67

[a]See Table 3, page 97, for explanation of treatments
[b]See Table 1, page 96, for explanation of groups.

Table 57. Ash content (duplicate samples) of the fresh metatarsal bone of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(percent, fresh basis)			
1	1	60.31	58.21	61.88	57.98
	2	60.11	57.90	62.06	57.92
2	1	58.41	58.04	59.21	57.96
	2	58.45	57.78	59.16	57.94
3	1	60.62	59.49	60.59	58.28
	2	60.67	58.91	60.59	58.14
4	1	57.02	58.54	61.06	55.59
	2	57.10	58.48	61.28	55.24
5	1	57.11	55.53	58.68	55.61
	2	57.14	55.18	58.70	55.57
6	1	55.16	55.50	57.91	60.53
	2	55.05	55.71	58.13	60.44
7	1	54.51	55.56	57.47	58.19
	2	54.39	55.03	57.34	58.22
8	1	60.88	55.14	58.24	60.04
	2	60.86	54.88	58.30	60.05
9	1	56.40	59.60	57.99	57.99
	2	56.19	59.54	58.00	57.91
10	1	58.18	59.80	57.21	58.05
	2	58.08	59.76	57.23	58.04

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 58. Ash content (duplicate samples) of the dried metatarsal bone of calves as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(percent, dry fat-free basis)			
1	1	66.70	64.11	67.72	65.18
	2	66.48	63.77	67.91	65.11
2	1	64.82	64.05	66.07	65.03
	2	64.87	63.76	66.01	65.01
3	1	66.90	65.72	66.36	65.22
	2	66.96	65.08	66.36	65.06
4	1	63.45	64.54	67.93	62.76
	2	63.54	64.48	68.17	62.36
5	1	64.77	63.20	65.51	62.66
	2	64.81	62.80	65.53	62.61
6	1	63.98	62.45	64.64	66.55
	2	63.85	62.69	64.88	66.45
7	1	63.79	62.06	64.46	64.05
	2	63.65	61.47	64.32	64.08
8	1	64.72	63.86	65.67	63.95
	2	64.70	63.56	65.74	63.96
9	1	63.80	66.50	66.19	63.94
	2	63.56	66.44	66.20	63.85
10	1	65.81	66.13	64.67	64.02
	2	65.69	66.08	64.69	64.01

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 59. Summation of Ca, K, Na, P, Mg, and Zn contents, in relation to total ash content of bone (right metatarsal) of calves, as influenced by treatments[a]

Calf number	Determination	Group[b] A	B	C	D
		(minerals, as percent of total bone ash)			
1	1	52.56	44.45	55.33	43.49
	2	51.64	45.47	54.89	44.48
2	1	53.88	44.46	55.07	43.20
	2	53.77	44.91	55.33	43.37
3	1	52.70	42.28	50.86	42.54
	2	51.79	42.70	51.47	42.92
4	1	58.52	43.21	51.61	44.02
	2	57.57	42.72	51.12	43.25
5	1	53.98	42.33	53.81	45.81
	2	54.26	42.25	53.29	44.71
6	1	51.52	44.13	55.28	43.02
	2	53.27	43.56	55.75	42.90
7	1	53.69	43.77	55.02	43.44
	2	52.31	42.89	54.72	44.57
8	1	54.50	43.87	52.83	43.96
	2	53.15	43.83	52.01	43.79
9	1	50.72	41.44	54.94	44.41
	2	50.08	42.20	53.82	44.46
10	1	53.66	42.94	53.50	43.67
	2	54.19	43.39	53.90	43.87

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 60. Group means of Ca concentration in blood serum[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

	Group[b]			
	A	B	C	D
	(mg/100 ml)			
Pretreatment				
Period I (Initial)	9.5	9.2	9.2	9.5
Treatment				
Period II				
Time interval (weeks)				
2	9.4	9.7	9.7	9.1
4	10.0	9.3	9.4	9.6
6	9.8	10.1	9.1	9.6
8	9.5	9.5	9.6	8.9
Mean	9.7	9.7	9.5	9.3
Period III				
Time interval (weeks)				
10	9.1	7.5	9.3	7.7
12	10.3	7.8	9.8	7.8
14	9.8	8.0	9.5	7.6
16	8.8	7.8	9.5	8.1
Mean	9.5	7.8	9.5	7.8

[a]Blood drawn 4 hours after treatment.
[b]See Table 1, page 96, for explanation of groups; and Table 3, page 97, for explanation of periods, chemicals, and doses.

Table 61. Group means of K concentration in blood serum[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

	Group[b]			
	A	B	C	D
	(mEq/L)			
Pretreatment				
Period I (Initial)	6.6	6.4	6.1	6.3
Treatment				
Period II				
Time interval (weeks)				
2	6.7	7.7	5.9	6.0
4	5.7	5.9	7.1	7.5
6	7.9	8.3	7.9	7.8
8	7.4	7.0	6.6	7.1
Mean	6.9	7.2	6.9	7.1
Period III				
Time interval (weeks)				
10	7.0	7.6	7.8	8.2
12	8.1	8.4	8.5	8.5
14	5.7	6.2	6.6	6.8
16	7.9	8.3	8.3	8.1
Mean	7.4	7.6	7.8	7.9

[a]Blood drawn 4 hours after treatment.
[b]See Table 1, page 96, for explanation of groups; and Table 3, page 97, for explanation of periods, chemicals, and doses.

Table 62. Group means of K concentration in whole blood[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

	Group[b]			
	A	B	C	D
	(mEq/L)			
Pretreatment				
Period I (Initial)	11.9	12.8	12.9	12.0
Treatment				
Period II				
Time interval (weeks)				
2	11.9	12.1	11.7	10.7
4	10.3	12.0	15.4	14.9
6	15.4	15.3	15.2	12.6
8	13.5	12.7	13.7	11.9
Mean	12.8	13.0	14.0	12.5
Period III				
Time interval (weeks)				
10	13.3	17.2	18.9	14.3
12	15.5	16.8	15.8	14.8
14	11.5	11.8	13.1	11.9
16	13.5	13.5	14.9	13.2
Mean	13.4	14.8	14.4	13.5

[a]Blood drawn 4 hours after treatment.
[b]See Table 1, page 96, for explanation of groups; and Table 3, page 97, for explanation of periods, chemicals, and doses.

Table 63. Group means of Na concentration in blood serum[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

	Group[b]			
	A	B	C	D
	(mEq/L)			
Pretreatment				
Period I (Initial)	130.8	152.3	141.8	151.5
Treatment				
Period II				
Time interval (weeks)				
2	168.6	170.1	129.5	151.9
4	148.4	163.7	132.5	133.0
6	165.5	162.8	154.5	162.3
8	136.1	141.5	161.7	160.4
Mean	154.7	159.5	144.6	151.9
Period III				
Time interval (weeks)				
10	155.2	160.1	178.5	161.3
12	147.6	139.6	140.6	139.3
14	144.4	149.8	164.6	144.5
16	136.7	139.8	139.6	142.4
Mean	145.8	147.3	156.3	147.0

[a]Blood drawn 4 hours after treatment.
[b]See Table 1, page 96, for explanation of groups; and Table 3, page 97, for explanation of periods, chemicals, and doses.

Table 64. Group means of Na concentration in whole blood[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

	Group[b]			
	A	B	C	D
	(mEq/L)			
Pretreatment				
Period I (Initial)	108.0	108.4	111.8	125.9
Treatment				
Period II				
Time interval (weeks)				
2	107.0	109.7	97.7	90.4
4	117.5	110.0	114.7	109.2
6	100.3	92.2	99.3	90.5
8	127.7	117.3	113.4	109.5
Mean	113.1	107.3	106.3	99.9
Period III				
Time interval (weeks)				
10	117.6	138.6	129.1	116.5
12	114.7	108.8	128.4	125.8
14	128.9	129.7	124.7	121.7
16	121.4	127.3	135.0	126.8
Mean	120.7	126.1	129.3	122.7

[a]Blood drawn 4 hours after treatment.
[b]See Table 1, page 96, for explanation of groups; and Table 3, page 97, for explanation of periods, chemicals, and doses.

Table 65. Group means of Fe concentration in whole blood[a] of calves before treatment (period I) and during parenteral injections of chemicals through the treatment periods II and III[b]

	Group[b]			
	A	B	C	D
	(mg/100 ml)			
Pretreatment				
Period I (Initial)	55.84	55.54	55.20	55.16
Treatment				
Period II				
Time interval (weeks)				
2	53.16	50.44	56.10	49.48
4	52.27	50.76	54.04	49.26
6	50.60	50.00	53.52	48.09
8	50.44	49.56	53.48	48.12
Mean	51.62	50.19	54.29	48.74
Period III				
Time interval (weeks)				
10	50.17	49.04	53.11	47.88
12	50.09	48.72	53.12	47.53
14	50.18	44.82	52.98	45.04
16	50.04	42.00	52.64	43.00
Mean	50.12	46.15	52.96	45.86

[a]Blood drawn 4 hours after treatment.
[b]See Table 1, page 96, for explanation of groups; and Table 3, page 97, for explanation of periods, chemicals, and doses.

Table 66. Group means of P, Mg, Cl, Zn, and Cu concentrations in blood serum[a] of calves before treatment (period I) and during parenteral injections of chemicals through treatment periods II and III[b]

	Group[b]			
	A	B	C	D
P (mg/100 ml)				
Pretreatment (I)	5.12	4.95	5.26	5.00
Treatment (II)	5.13	4.99	5.23	4.91
(III)	5.04	5.03	5.28	5.00
Mg (mg/100 ml)				
Pretreatment (I)	2.46	2.47	2.33	2.36
Treatment (II)	2.14	2.40	2.52	2.35
(III)	2.64	2.32	2.54	2.09
Cl (mg/100 ml)				
Pretreatment (I)	387.2	386.5	386.8	386.8
Treatment (II)	392.1	390.8	391.2	391.7
(III)	386.9	435.3	397.0	435.8
Zn (µg/100 ml)				
Pretreatment (I)	211.6	219.4	210.7	213.1
Treatment (II)	183.5	185.5	184.1	181.5
(III)	183.0	141.6	183.3	133.0
Cu (µg/100 ml)				
Pretreatment (I)	109.4	104.3	102.5	108.1
Treatment (II)	101.5	91.8	103.3	90.5
(III)	110.8	92.3	107.5	90.0

[a]Blood drawn once during each period, at 4 hours after treatment (eighth week period II; fourteenth week period III).
[b]See Table 1, page 96, for explanation of groups; and Table 3, page 97, for explanation of periods, chemicals, and doses.

Table 67. Mean composition, by group, of 10 constituents of cardiac tissue

Constituent	Group[a]			
	A	B	C	D
		(dry matter basis)		
Ca (ppm)	266.1	231.1	235.0	219.1
K (ppt)	4.999	5.918	5.230	5.684
Na (ppt)	3.571	2.911	3.478	2.860
P (ppt)	8.306	8.869	8.415	8.819
Mg (ppt)	0.915	1.097	0.930	1.109
Fe (ppm)	213.4	203.2	212.8	214.0
Zn (ppm)	115.5	92.2	116.4	92.5
Cu (ppm)	20.29	16.17	22.38	16.21
Ca/K ratio	0.053	0.039	0.045	0.039
Ca/Mg ratio	0.291	0.211	0.253	0.198
		(fresh basis)		
Dry matter (%)	22.31	22.99	22.25	22.09
Ash (%)	1.033	1.020	1.025	1.003

[a]See Table 1, page 96, for explanation of groups.

Table 68. Mean composition, by group, of 10 constituents of hepatic tissue

Constituent	Group[a]			
	A	B	C	D
		(dry matter basis)		
Ca (ppm)	285.5	246.8	286.9	247.1
K (ppt)	14.50	13.72	14.49	13.66
Na (ppt)	4.980	4.430	4.589	4.369
P (ppt)	16.41	15.54	16.14	16.25
Mg (ppm)	728.6	764.9	731.9	764.5
Fe (ppm)	250.5	198.2	252.4	200.6
Zn (ppm)	229.9	160.4	230.3	165.0
Cu (ppm)	82.08	44.16	82.86	43.24
		(fresh basis)		
Dry matter (%)	26.68	25.58	26.82	25.03
Ash (%)	1.296	1.300	1.297	1.263

[a]See Table 1, page 96, for explanation of groups.

Table 69. Mean composition, by group, of seven constituents of renal tissue

Constituent	Group[a]			
	A	B	C	D
		(dry matter basis)		
Ca (ppm)	275.4	496.2	286.5	580.0
K (ppt)	10.35	9.88	10.28	9.57
Na (ppt)	10.56	8.85	10.60	9.42
P (ppt)	7.481	8.944	7.553	9.172
Mg (ppm)	545.6	476.1	517.6	469.1
Na/K ratio	1.015	0.895	1.027	0.980
		(fresh basis)		
Dry matter (%)	20.63	17.71	20.13	17.88
Ash (%)	1.244	1.125	1.238	1.178

[a]See Table 1, page 96, for explanation of groups.

Table 70. Mean composition, by group, of eight constituents of osseous tissue (metatarsal bone)

Constituent	Group[a]			
	A	B	C	D
		(ash basis)		
Ca (%)	32.62	25.20	32.47	25.34
K (%)	0.087	0.171	0.073	0.151
Na (%)	1.169	1.163	1.028	1.152
P (%)	18.63	16.29	19.38	16.64
Mg (%)	0.880	0.512	0.776	0.505
Zn (ppm)	235.0	324.1	218.5	313.6
Ca/P ratio	1.753	1.547	1.675	1.523
Sum of minerals (%)	53.39	43.34	53.73	43.79
		(dry, fat-free basis)		
Ash (%)	64.84	64.14	65.95	64.29
		(fresh basis)		
Dry matter (%)	89.17	89.53	89.53	90.23
Ash (%)	57.83	57.42	59.05	57.98

[a]See Table 1, page 96, for explanation of groups.

Table 71. Mean squares and variance ratios for minerals in whole blood and blood serum as influenced by treatments[a] during period II: A comparison of period II to period I values

Source of variation	Degree of freedom	Serum Ca		Serum K		Whole blood K	
		Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	455	1.19	3	0.01	325	0.18
Drug (D)	1	46	0.12	1,045	2.74	1,265	0.71
E x D	1	705	1.85	796	2.09	1	0.01
Error (A)	36	382	--	382	--	1,792	--
Date (Da)	3	54	0.56	2,041	16.43**	5,826	10.49**
E x Da	3	46	0.48	1,713	13.79**	6,168	11.11**
D x Da	3	105	1.09	157	1.26	740	1.33
E x D x Da	3	179	1.85	111	0.89	81	0.15
Error (B)	108	97	--	124	--	555	--

Source of variation	Degree of freedom	Serum Na		Whole blood Na		Whole blood Fe	
		Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	1,049,590	5.93*	448,260	1.95	411,162	5.03*
Drug (D)	1	443,200	2.51	1,691,380	7.26*	547,671	6.70*
E x D	1	259,940	1.47	10	0.001	474,104	5.80*
Error (A)	36	176,919	--	230,099	--	81,742	--
Date (Da)	3	175,377	2.93*	396,393	12.51**	535,282	9.13**
E x Da	3	580,610	9.71**	42,893	1.35	161,229	2.75*
D x Da	3	25,233	0.42	6,940	0.22	187,027	3.19*
E x D x Da	3	45,560	0.76	11,867	0.37	110,222	1.88
Error (B)	108	59,824	--	31,695	--	58,629	--

[a]See Table 1, page 96, and Table 3, page 97, for explanation of treatments and periods.

*Statistical significance at P = 0.05; F(1,36) = 4.11; F(3,108) = 2.68.
**Statistical significance at P = 0.01; F(1,36) = 7.39; F(3,108) = 3.95.

Table 72. Mean squares and variance ratios for minerals in whole blood and blood serum as influenced by treatments[a] during period III: A comparison of period III to period I values

Source of variation	Degree of freedom	Serum Ca		Serum K		Whole blood K	
		Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	788	0.81	3,258	6.50*	214	0.14
Drug (D)	1	9,378	9.66**	102	0.20	191	0.12
E x D	1	1,108	1.14	216	0.43	214	0.14
Error (A)	36	971	--	501	--	1,546	--
Date (Da)	3	494	1.19	3,166	31.70**	9,235	12.87**
E x Da	3	196	0.47	60	0.60	784	1.09
D x Da	3	1,245	2.99*	95	0.95	271	0.38
E x D x Da	3	755	1.81	7	0.07	777	1.08
Error (B)	108	417	--	100	--	717	--

Source of variation	Degree of freedom	Serum Na		Whole blood Na		Whole blood Fe	
		Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	36,410	0.23	6,390	0.03	961,459	10.87**
Drug (D)	1	1,810,060	11.22**	873,490	3.85	2,213,244	25.02**
E x D	1	16,300	0.10	153,150	0.68	290,145	3.28
Error (A)	36	161,389	--	226,826	--	88,459	--
Date (Da)	3	647,690	15.08**	48,863	1.98	219,554	7.34**
E x Da	3	19,280	0.45	92,633	3.75*	38,287	1.28
D x Da	3	51,547	1.20	11,313	0.46	53,542	1.79
E x D x Da	3	46,210	1.08	56,626	2.29	32,176	1.41
Error (B)	108	42,939	--	24,707	--	29,912	--

[a]See Table 1, page 96, and Table 3, page 97, for explanation of treatments and periods.

*Statistical significance at P = 0.05; F(1,36) = 4.11; F(3,108) = 2.68.

**Statistical significance at P = 0.01; F(1,36) = 7.39; F(3,108) = 3.95.

Table 73. Mean squares and variance ratios for minerals in whole blood and blood serum as influenced by treatments[a] during periods II and III: A comparison of period III to period II values

Source of variation	Degree of freedom	Serum Ca		Serum K		Whole blood K	
		Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	2,984	3.25	63	0.13	36	0.02
Drug (D)	1	7,439	8.09**	233	0.46	7,812	3.24
E x D	1	45	0.49	1,183	2.35	540	0.22
Error (A)	36	920	--	504	--	2,408	--
Date (Da)	3	316	0.81	419	2.66	2,849	3.72*
E x Da	3	378	0.97	534	3.40*	1,121	1.46
D x Da	3	729	1.87	2,094	13.31**	5,390	7.03**
E x D x Da	3	235	0.60	839	5.33**	3,177	4.14**
Error (B)	108	391	--	157	--	767	--

Source of variation	Degree of freedom	Serum Na		Whole blood Na		Whole blood Fe	
		Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	83,480	0.51	326,970	1.26	645,568	8.00**
Drug (D)	1	292,990	1.79	1,752,470	6.74*	1,810,818	22.44**
E x D	1	50,960	0.31	10,100	0.04	271,446	3.37
Error (A)	36	163,482	--	259,872	--	80,696	--
Date (Da)	3	249,793	4.80**	91,487	2.89*	410,057	19.10**
E x Da	3	125,360	2.41	125,333	3.95**	22,972	1.07
D x Da	3	38,833	0.75	146,573	4.62**	40,791	1.90
E x D x Da	3	117,260	2.25	22,880	0.72	32,204	1.50
Error (B)	108	52,076	--	31,715	--	21,469	--

[a]See Table 1, page 96, and Table 3, page 97, for explanation of treatments and periods.

*Statistical significance at P = 0.05; F(1,36) = 4.11; F(3,108) = 2.68.

**Statistical significance at P = 0.01; F(1,36) = 7.39; F(3,108) = 3.95.

Table 74. Mean squares and variance ratios for the P, Mg, Cl, Zn, and Cu concentrations in blood serum as influenced by treatments[a] during periods II and III.

Source of variation	Degree of freedom	P		Mg		Cl		Zn		Cu	
		Mean square	F value	Mean square	F value	Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	0.02	0.08	8	0.01	1	0.9	959	0.24	0.1	0.01
Drug (D)	1	0.02	0.08	10,927	3.74	7,552	848.6**	11,931	2.98	4,277.9	15.47**
E x D	1	0.11	0.46	5,968	2.04	1	0.1	800	0.20	627.2	2.27
Error (A)	36	0.24	---	2,924	---	9	---	3,997	---	276.6	---
Date (Da)	1	0.01	0.21	308	0.24	12,318	1,785.2**	12,276	75.31**	195.3	2.61
E x Da	1	0.05	0.97	4,277	3.35	1	0.1	177	1.09	11.3	0.15
D x Da	1	0.04	0.74	14,499	11.36**	7,603	110.4**	9,702	59.48**	195.3	2.61
E x D x Da	1	0.01	0.25	7,585	5.94*	2	0.3	1	0.01	31.3	0.42
Error (B)	36	0.05	---	1,276	---	7	---	163	---	74.9	---

[a]See Table 1, page 96, and Table 3, page 97, for explanation of treatments and periods.
*Statistical significance at P = 0.05; F(1,36) = 4.11.
**Statistical significance at P = 0.01; F(1,36) = 7.39.

Table 75. Mean squares and variance ratios for the Ca, K, Na, and Mg concentrations in cardiac tissue as influenced by treatments[a]

Source of variation	Degree of freedom	Ca		K		Na		Mg	
		Mean square	F value	Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	9,195.5	13.57**	0.01	0.001	0.105	0.25	37.26	1.17
Drug (D)	1	12,869.9	18.99**	941.50	337.9**	8.154	19.32**	6,577.51	207.02**
E x D	1	1,851.9	2.73	107.93	38.75**	0.009	0.02	0.34	0.01
Sampling	40	55.5	---	0.15	---	0.002	---	3.25	---
Error	36	677.5	---	2.79	---	0.422	---	31.77	---

[a]See Table 1, page 96, and Table 3, page 97, for explanation of treatments.
*Statistical significance at P = 0.05; F(1,36) = 4.11.
**Statistical significance at P = 0.01; F(1,36) = 7.39.

Table 76. Mean squares and variance ratios for the P, Fe, Zn, and Cu concentrations in cardiac tissue as influenced by treatments[a]

Source of variation	Degree of freedom	P		Fe		Zn		Cu	
		Mean square	F value	Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	1.708	1.21	524.29	1.54	7.0	0.05	22.83	3.03
Drug (D)	1	468.272	331.87**	404.10	1.14	11,109.1	72.91**	529.93	70.38**
E x D	1	12.683	8.99**	653.23	1.92	1.8	0.01	21.01	2.79
Sampling	40	0.250	---	26.10	---	1.1	---	0.19	---
Error	36	1.411	---	339.71	---	152.4	---	7.53	---

[a]See Table 1, page 96, and Table 3, page 97 for explanation of treatments.
*Statistical significance at P = 0.05; F(1,36) = 4.11.
**Statistical significance at P = 0.01; F(1,36) - 7.39.

Table 77. Mean squares and variance ratios for the Ca/K ratios, Ca/Mg ratios, dry matter, and ash content of cardiac tissue as influenced by treatments[a]

Source of variation	Degree of freedom	Ca/K ratio		Ca/Mg ratio		Dry matter		Ash	
		Mean square	F value	Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	3.836	16.58**	1.303	17.52**	4,647	3.74	0.00351	1.68
Drug (D)	1	20.981	90.65**	9.171	123.33**	1.347	1.09	0.00630	3.02
E x D	1	3.036	13.10**	0.304	4.10	3.494	2.82	0.00045	0.22
Sampling	40	0.023	---	0.007	---	0.029	---	0.00023	---
Error	36	0.231	---	0.074	---	1.241	---	0.00209	---

[a]See Table 1, page 96, and Table 3, page 97, for explanation of treatments.
*Statistical significance at P = 0.05; F(1,36) = 4.11.
**Statistical significance at P = 0.01; F(1,36) = 7.39.

Table 78. Mean squares and variance ratios for the Ca, K, Na, and P concentrations in hepatic tissue of calves as influenced by treatments[a]

Source of variation	Degree of freedom	Ca		K		Na		P	
		Mean square	F value	Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	70.5	1.77	0.018	0.08	1.026	7.47**	1.006	2.10
Drug (D)	1	29,349.0	735.11**	13.084	5.59*	2.965	21.57**	2.861	5.98*
E x D	1	52.4	1.31	0.017	0.07	0.546	3.97	4.719	9.87**
Sampling	40	1.6	---	0.049	---	0.002	---	0.037	---
Error	36	39.9	---	2.340	---	0.137	---	0.478	---

[a]See Table 1, page 96, and Table 3, page 97 for explanation of treatments.
*Statistical significance at P = 0.05; F(1,36) = 4.11.
**Statistical significance at P = 0.01; F(1,36) = 7.39.

Table 79. Mean squares and variance ratios for the Mg, Fe, and Zn concentrations in hepatic tissue of calves as influenced by treatments[a]

Source of variation	Degree of freedom	Mg		Fe		Zn	
		Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	52.6	0.001	93.7	1.34	124.2	0.32
Drug (D)	1	23,546.9	5.60*	54,100.4	772.75**	90,901.8	236.78**
E x D	1	52.6	0.001	1.2	0.18	88.5	0.23
Sampling	40	25.2	---	0.7	---	1.3	---
Error	36	4,201.5	---	70.0	---	383.9	---

[a]See Table 1, page 96, and Table 3, page 97 for explanation of treatments.
*Statistical significance at P = 0.05; F(1,36) = 4.11.
**Statistical significance at P = 0.01; F(1,36) = 7.39.

Table 80. Mean squares and variance ratios for the Cu, ash, and dry matter content of hepatic tissue of calves as influenced by treatments[a]

Source of variation	Degree of freedom	Cu		Ash		Dry Matter	
		Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	0.1	0.03	0.00630	1.43	0.82	0.69
Drug (D)	1	30,061.6	907.66**	0.00435	0.99	41.83	35.49**
E x D	1	14.8	0.45	0.00741	1.68	2.38	2.02
Sampling	40	0.4	---	0.00046	---	0.01	---
Error	36	33.1	---	0.00441	---	1.18	---

[a]See Table 1, page 96, and Table 3, page 97, for explanation of treatments.
*Statistical significance at P = 0.05; F(1,36) = 4.11.
**Statistical significance at P = 0.01; F(1,36) = 7.39.

Table 81. Mean squares and variance ratios for the Ca, K, and Na concentration, and the Na/K ratio of renal tissue of calves as influenced by treatments[a]

Source of variation	Degree of freedom	Ca		K		Na		Na/K ratio	
		Mean square	F value	Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	45,058	21.22**	0.713	2.03	0.0280	5.67*	0.0480	7.23*
Drug (D)	1	1,322,463	622.95**	0.063	20.13**	0.4260	86.06**	0.1394	20.99**
E x D	1	26,425	12.45**	0.287	0.82	0.0044	0.89	0.0274	4.13*
Sampling	40	11	---	0.011	---	0.0001	---	0.0002	---
Error	36	2,123	---	0.351	---	0.0049	---	0.0066	---

[a]See Table 1, page 96, and Table 3, page 97 for explanation of treatments.
*Statistical significance at P = 0.05; F(1,36) = 4.11.
**Statistical significance at P = 0.01; F(1,36) = 7.39.

Table 82. Mean squares and variance ratios for the P and Mg concentration, and dry matter, and ash content of renal tissues of calves as influenced by treatments[a]

Source of variation	Degree of freedom	P		Mg		Ash		Dry matter	
		Mean square	F value	Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	45.13	4.40*	6,112.7	2.58	0.0112	1.47	0.543	0.60
Drug (D)	1	4,749.80	462.94**	69,543.3	29.34**	0.1611	21.20**	133.721	148.08**
E x D	1	12.29	1.20	2,201.8	0.93	0.0165	2.17	2.288	2.53
Sampling	40	0.66	---	3.0	---	0.0006	---	0.005	---
Error	36	10.26	---	2,370.0	---	0.0076	---	0.903	---

[a]See Table 1, page 96, and Table 3, page 97, for explanation of treatments.
*Statistical significance at P = 0.05; F(1,36) = 4.11.
**Statistical significance at P = 0.01; F(1,36) = 7.39.

Table 83. Mean squares and variance ratios for the Ca, K, Na, and P concentration and the Ca/P ratio of osseous tissue (metatarsal bone) of calves as influenced by treatments[a]

Source of variation	Degree of freedom	Ca		K		Na		P		Ca/P ratio	
		Mean square	F value	Mean square	F value	Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	0.001	0.00001	0.0058	8.71**	0.115	4.77*	6.012	14.21**	0.0514	3.81
Drug (D)	1	1,058.117	306.51**	0.1291	195.14**	0.069	2.88	128.801	304.26**	0.6416	4.76*
E x D	1	0.422	0.12	0.0003	0.28	0.083	3.41	0.786	1.86	0.0149	1.11
Sampling	40	0.081	---	0.0001	---	0.003	---	0.140	---	0.0013	---
Error	36	3.452	---	0.0007	---	0.024	---	0.423	---	0.0134	---

[a]See Table 1, page 96, and Table 3, page 97, for explanation of treatments.
*Statistical significance at P = 0.05; F(1,36) = 4.11.
**Statistical significance at P = 0.01; F(1,36) = 7.39.

Table 84. Mean squares and variance ratios for the Mg, Zn, sum of minerals, and ash content of osseous tissue (metatarsal bone) of calves as influenced by treatments[a]

Source of variation	Degree of freedom	Mg		Zn		Sum of minerals		Ash	
		Mean square	F value	Mean square	F value	Mean square	F value	Mean square	F value
Elevation (E)	1	0.0627	6.19*	3,646.4	6.00*	3.11	0.77	15.753	2.25
Drug (D)	1	2.0461	201.87**	169,823.1	279.65**	1,996.43	493.36**	10.804	1.54
E x D	1	0.0473	4.67*	178.9	0.29	0.07	0.16	2.204	0.32
Sampling	40	0.0003	---	6.8	---	0.27	---	0.018	---
Error	36	0.0101	---	607.3	---	4.05	---	6.988	---

[a]See Table 1, page 96, and Table 3, page 97, for explanation of treatments.
*Statistical significance at P = 0.05; F(1,36) = 4.11.
**Statistical significance at P = 0.01; F(1,36) = 7.39.

Table 85. Summary of the Ca concentrations in the tissues of calves as influenced by treatments[a]

Tissue	Group[b]			
	A	B	C	D
	(mg/100 ml)			
Blood serum				
Period I	9.5	9.2	9.2	9.5
Period II	9.7	9.7	9.5	9.3
Period III	9.5	7.8	9.5	7.8
	(ppm, dry matter basis)			
Heart	266.1	231.1	235.0	219.1
Liver	285.5	246.8	286.9	247.1
Kidney	275.4	496.2	286.5	580.0
	(percent of ash)			
Bone	32.62	25.20	32.47	25.34

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 86. Summary of the K concentrations in the tissues of calves as influenced by treatments[a]

Tissue	Groub[b] A	B	C	D
			(mEq/L)	
Blood serum				
Period I	6.6	6.4	6.1	6.3
Period II	6.9	7.2	6.9	7.1
Period III	7.4	7.6	7.8	7.9
			(mEq/L)	
Whole Blood				
Period I	11.9	12.8	12.9	12.0
Period II	12.8	13.0	14.0	12.5
Period III	13.4	14.8	14.4	13.5
		(ppt, dry matter basis)		
Heart	5.00	5.92	5.23	5.68
Liver	14.50	13.72	14.49	13.66
Kidney	10.35	9.88	10.28	9.57
		(percent of ash)		
Bone	0.0872	0.1708	0.0733	0.1508

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 87. Summary of the Na concentrations in the tissues of calves as influenced by treatments[a]

Tissue	Group[b] A	B	C	D
			(mEq/L)	
Blood serum				
Period I	130.8	152.3	141.8	151.5
Period II	154.7	159.5	144.6	151.9
Period III	145.8	147.3	156.3	147.0
			(mEq/L)	
Whole Blood				
Period I	108.0	108.4	111.8	125.9
Period II	113.1	107.3	106.3	99.9
Period III	120.7	126.1	129.3	122.7
		(ppt, dry matter basis)		
Heart	3.571	2.911	3.478	2.860
Liver	4.980	4.430	4.589	4.369
Kidney	10.56	8.85	10.60	9.42
		(percent of ash)		
Bone	1.169	1.163	1.028	1.152

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 88. Summary of the P concentrations in the tissues of calves as influenced by treatments[a]

Tissue	Group[b]			
	A	B	C	D
	(mg/100 ml)			
Blood serum				
Period I	5.12	4.95	5.26	5.00
Period II	5.13	4.99	5.23	4.91
Period III	5.04	5.03	5.28	5.00
	(ppt, dry matter basis)			
Heart	8.306	8.869	8.415	8.819
Liver	16.41	15.54	16.14	16.25
Kidney	7.48	8.94	7.55	9.17
	(percent of ash)			
Bone	18.63	16.29	19.38	16.64

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 89. Summary of the Mg concentrations in the tissues of calves as influenced by treatments[a]

Tissue	Group[b] A	B	C	D
		(mg/100 ml)		
Blood serum				
Period I	2.5	2.4	2.5	2.4
Period II	2.2	2.3	2.5	2.4
Period III	2.6	2.3	2.4	2.1
		(ppm, dry matter basis)		
Heart	915.2	1,097.4	930.3	1,109.7
Liver	728.6	764.9	731.9	764.5
Kidney	545.6	476.1	517.6	469.1
		(percent of ash)		
Bone	0.880	0.512	0.776	0.505

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 90. Summary of the Fe concentrations in the tissues of calves as influenced by treatments[a]

Tissue	Group[b] A	B	C	D
		(mg/100 ml)		
Whole Blood				
Period I	55.84	55.54	55.20	55.16
Period II	51.62	50.19	54.29	48.74
Period III	50.12	46.15	52.96	45.86
		(ppm, dry matter basis)		
Heart	213.36	203.15	212.76	213.98
Liver	250.52	198.24	252.47	200.60

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 91. Summary of the Zn concentration in the tissues of calves as influenced by treatments[a]

Tissue	Group[b] A	B	C	D
		(µg/100 ml)		
Blood serum				
Period I	211.6	219.4	210.7	213.1
Period II	183.5	185.5	184.1	181.5
Period III	183.0	141.6	183.3	133.0
		(ppm, dry matter basis)		
Heart	115.5	92.2	116.4	92.5
Liver	229.9	160.4	230.3	165.0
		(ppm of ash)		
Bone	235.0	324.1	218.5	313.6

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Table 92. Summary of the Cu concentration in the tissues of calves as influenced by treatments[a]

Tissue	Group[b]			
	A	B	C	D
	(µg/100 ml)			
Blood serum				
Period I	109.4	104.3	102.5	108.1
Period II	101.5	91.8	103.3	90.5
Period III	110.8	92.3	107.5	90.0
	(ppm, dry matter basis)			
Heart	20.29	16.17	22.38	16.21
Liver	82.08	44.16	82.86	43.24

[a]See Table 3, page 97, for explanation of treatments.
[b]See Table 1, page 96, for explanation of groups.

Appendix B

Figures

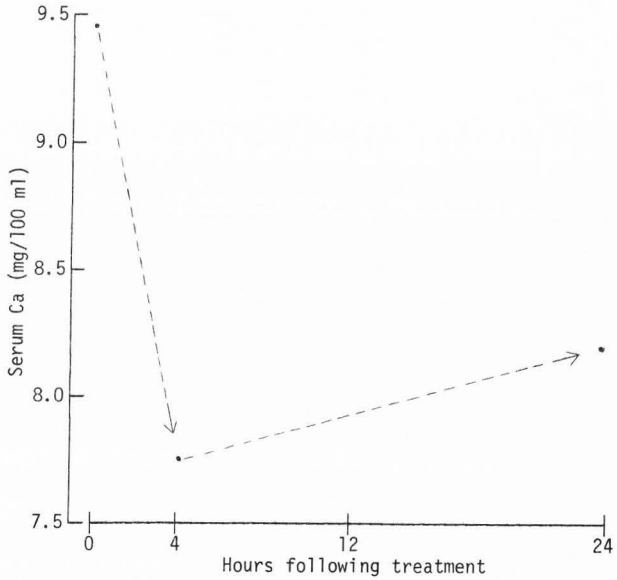

Figure 1. Sustained influence of parenterally administered K_2-EDTA on levels of Ca in blood serum of calves.

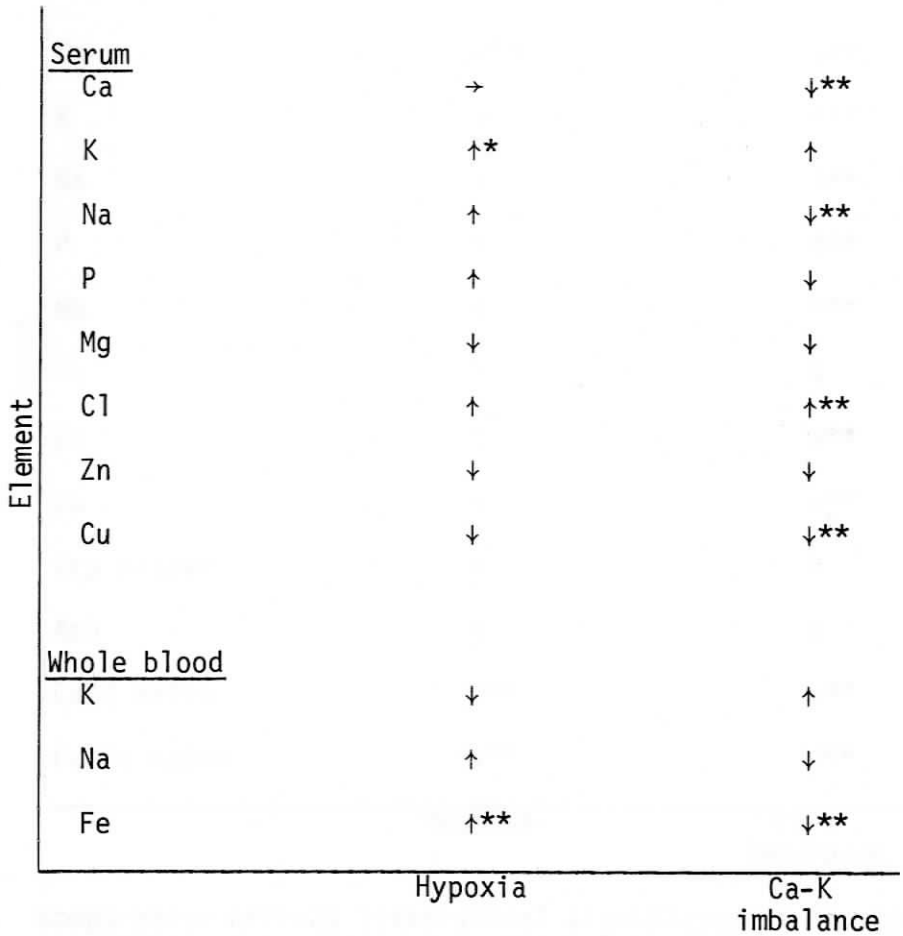

Figure 2. Comparative effects (statistical significance indicated by asterisks) of hypoxia and Ca-K imbalance on the elemental composition of blood (period III).

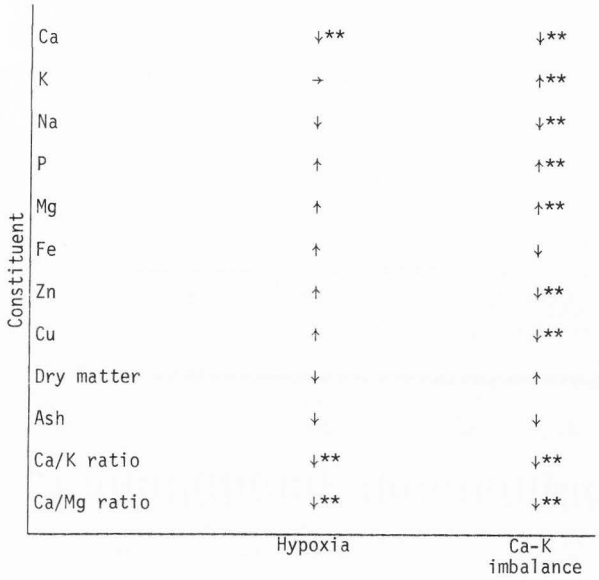

Figure 3. Comparative effects (statistical significance indicated by asterisks) of hypoxia and Ca-K imbalance on the composition of cardiac tissue.

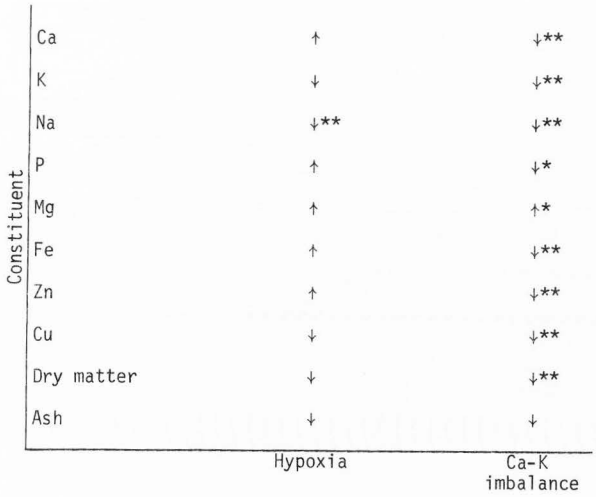

Figure 4. Comparative effects (statistical significance indicated by asterisks) of hypoxia and Ca-K imbalance on the composition of hepatic tissue.

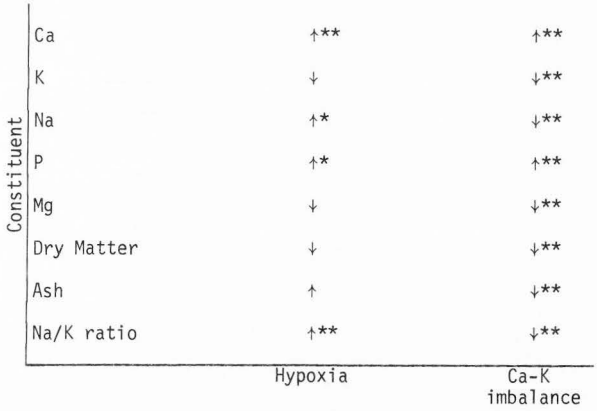

Figure 5. Comparative effects (statistical significance indicated by asterisks) of hypoxia and Ca-K imbalance on the composition of renal tissue.

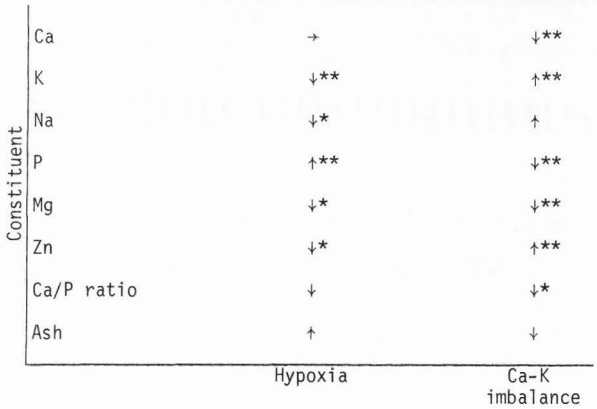

Figure 6. Comparative effects (statistical significance indicated by asterisks) of hypoxia and Ca-K imbalance on the composition of osseous tissue (metatarsal bone).

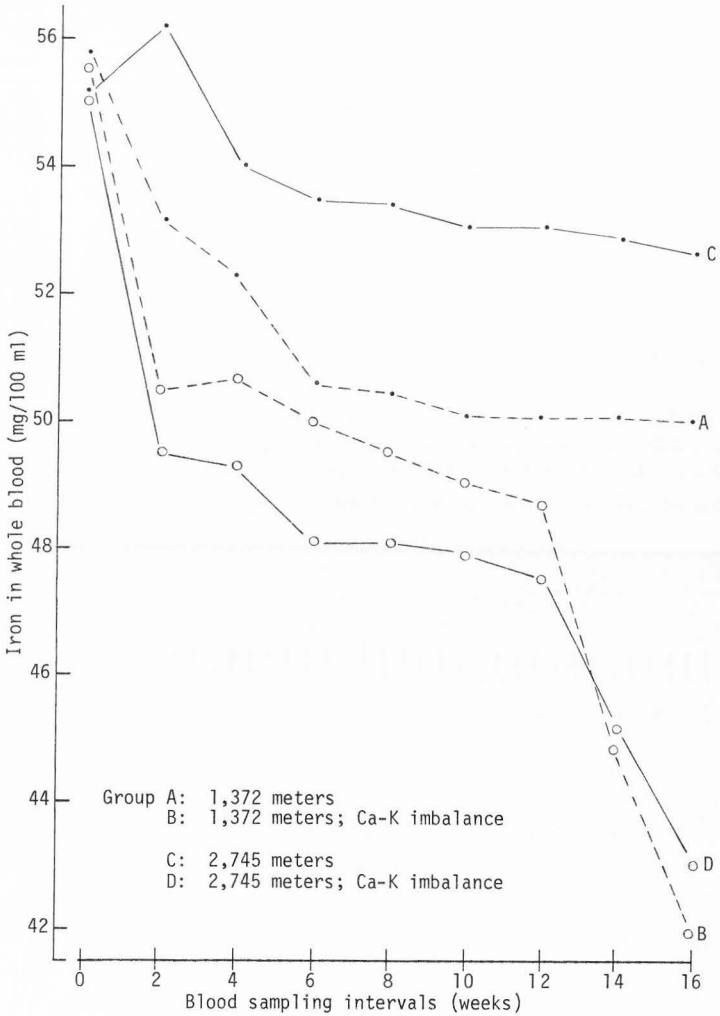

Figure 7. Changes in blood Fe concentration in cattle with time, following subjection of animals to stresses of hypoxia and Ca-K imbalance.

VITA

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Doctor of Philosophy

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