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THE EFFECT OF ENDOTOXIN SHOCK ON BLOOD ZINC LEVELS AND PLASMA PROTEIN CONCENTRATIONS

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BOBBY JACK GUNTER

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THE EFFECT OF ENDOTOXIN SHOCK ON BLOOD ZINC LEVELS

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CHAPTER I

INTRODUCTION

For many years the absorption of gram-negative bacteria into the blood stream has been known to produce shock. Therapeutic amelioration of gram-negative infections has progressed very little, despite the many advances in medical research. Endotoxin, a multimolecular lipopolysaccharide, located within the cell wall of gram-negative bacteria is the agent responsible for the lethality of such organisms (1).

The coliform group includes all aerobic and facultative anaerobic gram-negative nonspore-forming bacilli which ferment lactose with gas formation (2). Escherichia coli (E. coli) and other bacteria of the coliform group constitute one of the predominating organisms in the intestinal contents of man and animal and are only occasionally found in localities not showing recent fecal pollution (3). As a result of this characteristic, the absence of coliform bacteria has been established as an index of sanitation in environmental conditions such as potable water, bathing and swimming facilities, restaurant accommodations, and hospital care units. An example of using the presence of coliform organisms as an indicator of contamination is demonstrated in the bacteriological ex-

amination of municipal water supplies; if the examination is positive for coliform, contamination is assumed and preventive measures are taken to avoid the potential coexisting communicable diseases.

Every individual is exposed to minute quantities of endotoxin released from degenerating colon bacilli (E. coli and other coliform organisms) in the intestinal tract which causes the body to develop minute quantities of antibodies (1). When pathological conditions permit large amounts of endotoxin to enter the circulatory system, an anaphylactic reaction occurs, often resulting in severe shock which frequently leads to death (1). The association of circulatory failure with severe bacterial infections has been recognized for some time. Certain coliform serotypes as well as the pathogenic strains which are hemolytic are the cause of hemorrhagic septicemia one of the most frequently encountered causes of urinary tract infections and epidemic diarrheal diseases encountered in children. According to Gilbert (4), Laennec (1831) described the weak heart sounds present in circulatory failure which was caused by acute bacterial infections. Development of an infection in a surgical or a postoperative wound may be revealed by the appearance of acute circulatory failure. Identical circulatory failure may develop suddenly in a patient suffering from a severe bacterial infection for a period of several weeks or even months. In both situations the toxic reactions produced by endotoxin released from the degenerating gram-negative bacteria may ultimately result in death (4).

The shock state which occurs in human patients during the course of bacterial infections of the blood stream caused by gram-negative microorganisms is characterized by acute hypotension and generalized circula-

tory failure and presents a very serious clinical problem (5). Shock produced by gram-negative bacteria was initially regarded as a rare occurrence, but clinical reports of more than 200 cases in the past decade indicate that this is a relatively common complication in both medical and surgical patients. Weil (5) observed death in 138 out of 169 patients treated for bacteremic shock.

Many hypotheses and postulates have been made concerning the endotoxin reaction in man and other species; however, the underlying mechanism has not been discovered. All efforts dedicated to uncovering and comprehending this mechanism will be well spent.

CHAPTER II

LITERATURE REVIEW

Mechanisms producing bacteremic shock are constantly under observation by clinical investigators due to the prevalence of bacterial shock associated with surgery, peritonitis, pneumonia, and countless other traumatic conditions. Since bacteremic shock is caused by endotoxin released from degenerating gram-negative bacteria, researchers have attempted to relate the chemical composition of endotoxin to its biological activity. According to Nowotny (6), Boivin et al. hydrolyzed E. coli with dilute acetic acid and observed the dissociation of a slightly toxic phosphorus-containing lipid and a toxic polysaccharide. Brinkley et al. (7) subjected endotoxin to acid and alkaline hydrolysis then analyzed all fractions chemically and biologically and suggested the existence of a toxic "T" factor which was neither protein, lipid, nor polysaccharide. A substance referred to as "Lipid A" which was one-tenth as toxic as the original endotoxin has also been obtained from endotoxin treated with acid and alkaline hydrolysis (6). The decreased toxicity was attributed to loss of lipophilic polysaccharides that give endotoxin its water soluble characteristic and indicated that the entire molecule must be present in order for endotoxin to attain its characteristic physiological response (6). Ribi et al. (8) found the chloroform soluble fraction of

endotoxin to be much less toxic than the entire "Lipid A" precipitate. A macromolecular complex, preferably the entire endotoxin molecule, was shown by Haskins et al. (9) to be one of the major requirements for endotoxin to elicit its characteristic response.

Biochemical, hemodynamic, and pathological complications occurring as a result of experimentally induced endotoxin shock have been observed in a large variety of laboratory animals. The canine response to endotoxin appears to be the most popular experimental model. MacLean and Weil (10) were among the first investigators to report the response of dog to an intravenous injection of E. coli endotoxin. Their observations showed a precipitous drop in blood pressure with hemorrhagic lesions of the intestine. They suggested that increased resistance to outflow through the liver and intestine promoted stasis and loss of blood volume which supported the decreased venous return, hypotension, and increased portal pressure reported in their experiments. These observations were also supported by Weil et al. (11). They intravenously injected dogs with E. coli endotoxin and observed hypotension and increased portal vein pressure. Hypotension was produced by a fall in cardiac output without a decrease in peripheral resistance. When cardiac filling was kept constant by infusion, the arterial pressure remained constant; however, the portal pressure elevation was intensified. Weil suggested that localized venous spasm in the hepatic venous system and possibly elsewhere produced pooling of large quantities of blood. The total venous return was thereby critically reduced and a fall in cardiac output and arterial pressure was the inevitable end result which accounted for the hypotension in endotoxin shock.

Changes produced by endotoxin vary from one species to another and these changes are best considered separately by species (4). Hinshaw and Brake (12) investigated the hemodynamic effects of lethal injections of E. coli endotoxin on the dog and monkey. Their results are summarized in Table 1.

TABLE 1

THE EFFECT OF ENDOTOXIN IN THE DOG AND MONKEY

Post-endotoxin Measurement	Dog	Monkey
Systemic arterial pressure	Rapid drop	Gradual decline
Cardiac output	Decrease	Decrease
Venous return	Decrease	Decrease
Total peripheral resistance	Variable; decrease	Decrease
Portal vein pressure	Marked early decrease	Negligible decrease
Visceral pooling	Extensive	Absent
Visceral lesions	Extensive	Absent or minimal
Foreleg vascular resistance	Decrease to sustained rise	Decrease, increase
Heart rate	Early decrease	Variable
Hematocrit	Marked increase	No change or fall
Plasma hemolysis	Extensive	Absent
White cell count	Marked decrease	Marked decrease

Similar post-endotoxin responses of man and monkey have been suggested (12).

The differences observed in dog and monkey during the post-endotoxin period should not be overlooked because of the relative phylogenetic proximity of monkey and man (13). Kuida et al. (14) reported a difference in the early hemodynamic effects of endotoxin in the monkey, cat and rabbit compared with those which occurred in the dog. One possible explanation of this was the minimum and inconsistent changes in the weight of short segments of gut in the monkey, rabbit, and cat combined with the slight to moderate increment in portal venous pressure. Another explanation was the markedly lesser degree of pooling which was observed in cat venous return experiments as compared to the extensive pooling which occurred in the dog. Complete pathological examination of monkeys, rabbits, and cats failed to reveal edema or congestion of the abdominal viscera which occurred in the dog (10) (11). Morphologic changes in the monkey liver were those of ischemic necrosis while the intestines of monkeys, cats, and rabbits were pale suggesting significant ischemia. Pulmonary hemorrhage and edema were present in both the monkey and the rabbit. The pulmonary hypertensive response in the monkey and rabbit was consistently present and, although pulmonary artery pressure increased to levels averaging 258 per cent and 159 per cent of control, respectively, this effect was transient and did not appear to be the only factor leading to systemic hypotension. These investigators concluded that hepatic vein constriction with splanchnic pooling and reduced venous return could not be responsible for the early hypotension in these species (14). Although species responses vary, endotoxin caused hypotension in all mammalian species studied (4). According to Fine (15), endotoxin shock has detrimental effects on most of the organs in the body. The

effect of endotoxin shock on kidneys was demonstrated by an abrupt decline in urine secretion. When arterial blood pressure drops to 80 mm Hg or less, severe vasoconstriction of the afferent arterioles develops. At this level of blood pressure the kidney receives 1 per cent of the cardiac output, whereas in health it receives about 19 per cent. Every measurable function performed by the liver is impaired in endotoxin shock; the secretion of bile, the conjugation of bilirubin, the clearance of bromsulfalein (BSP), the deamination of amino acids, the ability to synthesize plasma protein, fibrinogen, prothrombin and urea, and the conversion of pyruvate to glucose, are all defective (15). The hemodynamic findings concerning endotoxin shock in various species provide no evidence for an important effect on myocardial function (1).

At present, isolated and perfused organs of various species are under observation by many investigators in an effort to discover the mechanisms responsible for endotoxin's ability to produce irreversible shock. In the dog, liver and small intestine increased in weight, due to pooling of blood (16). The liver weight tended to return to control levels, but the small intestine showed a steady gain in weight, which amounted to an average of 377 grams after 1 hour, indicating a significant amount of pooling. Lillehi and MacLean (17) indicated that the bowel was the prime organ in development of irreversible endotoxin shock in the dog. Vick et al. (18) administered endotoxin to eviscerated dogs and found that survival time was shortened, which indicated that the intestine provides a defense mechanism against the detrimental effects of endotoxin. Hinshaw and Nelson (19) reported an increase in weight of dog intestine after endotoxin injection with an increase in total splanchnic resistance

and arterial pressure. Hinshaw et al. (20) observed almost opposite post-endotoxin responses in the monkey intestine. Isolated sections of monkey small intestine were continuously perfused with the animals own blood and it was found that the weight of the intestine remained relatively constant and that no marked increase in intestinal arterial pressure was observed. Chien et al. (21), using splenectomized dogs and dogs with spleen intact, observed changes in blood volume and its distribution after an intravenous injection of E. coli endotoxin. The plasma volume decreased after endotoxin in dogs with spleens intact. A total reduction of plasma volume by an average of 36 per cent was observed 2.5 hours after treatment with endotoxin. Loss of plasma volume became progressively greater with time and was significantly larger in dogs that died within 200 minutes. Splenectomized dogs did not show a significant decrease in plasma volume following endotoxin shock. The total circulating plasma protein concentration decreased in all dogs after endotoxin; however, a greater decrease was observed in dogs with intact spleens. Total red cell volume which was determined with Cr^{51} tagged erythrocytes, showed a slight increase in dogs with intact spleens and a slight decrease in splenectomized dogs. Total blood volumes in dogs with spleens intact, decreased progressively, declining to an average value of 73 per cent of the control values within 2.5 hours after administration of the endotoxin. The slight decrease in total blood volume observed in splenectomized dogs was not significant (21). Chien et al. (22) did not observe a decrease in red cell volume in dogs that were sympathectomized and splenectomized and consequently he suggested that the slight decrease in total red cell volume in splenectomized dogs was a result of seques-

tration of red blood cells due to sympathetic vasoconstriction. Emerson et al. (23) reported the effect of splanchnic sympathetic denervation on survival during endotoxin shock in the dog. Hemodynamic parameters such as increased hematocrit, decreased pH, and drop in mean systemic arterial pressure were very near the same in adrenalectomized, splenectomized, and intact animals. Splanchnic sympathectomy did not enhance survival during endotoxin shock.

Almost every investigator in the field of endotoxin shock has created his own definition of shock. White, Handler, and Smith (24) define shock as circulatory failure resulting from loss of fluid from the vascular compartment. Fluid loss may occur by hemorrhage, pooling in various organs, or through an increase in capillary permeability. Fluid loss is controlled in plasma by plasma proteins whose prime function is regulation of osmotic balance between interstitial fluid and plasma. At ordinary plasma flow rates, proteins do not cross the capillaries, but when plasma flow falls the diffusion of protein becomes very significant (24). Decreased venous return and reduced cardiac output followed by a drop in blood pressure are consistent observations during endotoxin shock, thus indicating a possible mechanism promoting the decreased plasma protein concentration observed in dogs during the post-endotoxin shock period (10) (11) (22). In all shocked states the capillaries appear to become permeable to plasma protein and large volumes of protein and albumin rich fluid enter extravascular spaces, thereby reducing both plasma protein concentration and blood volume (24). The normal total plasma protein concentrations for the human, monkey, and dog are approximately 7.5, 7.3, and 5.5 g/100 ml, respectively (24) (25). Post-endotoxin shock

responses may be partially caused by an accumulation of plasma protein in various organs. This was suggested by Aust *et al.* (26) who observed an accumulation of I^{131} tagged albumin in the intestinal wall of dogs during the post-endotoxin shock period. Hinshaw *et al.* (27) did not observe a decrease in total plasma protein concentration in dogs within 4 hours after administering a LD/80 *E. coli* endotoxin.

Endotoxins possess a wide range of biological activity. They can affect structure and function of numerous enzymes, organs, and cells, change tissue and blood levels of many enzymes, modify carbohydrate, fat, and protein metabolism, raise or lower body temperature and increase or decrease resistance to bacterial infection (28). This represents only a fraction of the broad spectrum of endotoxin's activity. According to Bennett (28) an investigator in almost any biological field is likely to obtain a "positive" result if he tries endotoxin in the experimental system he is studying.

The relation of specific metallic ions to various disease states has been shown to have diagnostic value (29). For example, in the presence of clinical symptoms, the finding of chloride concentration in sweat above 60 mEq/liter or sodium above 70 mEq/liter is consistent with the diagnosis of cystic fibrosis (29). A single injection of 0.0001 μg of *E. coli* endotoxin produced a hypoferremia in rats with maximum decreases in plasma iron 8 to 16 hours after injection (30). The recent discovery of zinc in many purified enzymes has revealed the diversity of its function in protein and carbohydrate metabolism (31). Zinc is present in a peptidase, a phosphatase, and several dehydrogenases of many species which jointly cover the entire evolutionary spectrum (31). The following zinc

metalloenzymes have been characterized thus far: carbonic anhydrase, carboxypeptidase, alcohol dehydrogenase, glutamic acid dehydrogenase, muscle lactic dehydrogenase, and kidney alkaline phosphatase (31). Zinc activates a number of enzymes important in the metabolism of protein, including arginase, glyclyglycine dipeptidase, dehydropeptidase, tripeptidase, carnosinase, histidine deaminase, as well as oxaloacetic carboxylase, various lecithinases and enolases (31) (32) (33). The total amount of zinc in the human body has been estimated to range from 1.3 to 2.3 grams (34). Apparently no tissue stores zinc preferentially. Injected radioactive zinc disappears rapidly from the blood plasma and was concentrated initially in the liver, with the liver still containing about 40 per cent of the injected zinc at the end of several hours. There was considerable and rapid uptake of zinc by the pancreas, kidneys, and pituitary; as the radioactive zinc concentration gradually decreased in these organs, it slowly increased in skeletal muscle, skin, hair, red blood cells and bones. Attempts have been made to correlate clinical entities with either an increase or decrease in blood zinc concentration. An above average concentration of zinc was found in patients suffering from hyperthyroidism, hypertension, polycythemia, eosinophilia, in patients administered adrenaline, thyroxine, and in patients receiving X-ray irradiation. Lower than normal levels of plasma zinc have been found in certain infections, myocardial infarction, and prenicious anemia. In normal individuals, patients with polycythemia and most anemias, the erythrocyte zinc and carbonic anhydrase activity vary with the hematocrit (34). Wohl and Goodhart (35) observed elevated erythrocyte zinc concentration in patients with sickle cell anemia and pernicious anemia. Decreased serum

zinc concentrations have been observed in patients with untreated pernicious anemia. The zinc content in leukocytes of patients with various chronic and acute leukemias was found to be less than normal and the lowest values occurred in patients with chronic leukemia and acute lymphocytic leukemia. The low erythrocyte zinc levels were not observed when a remission was induced by appropriate therapy. The condition of these patients was not altered by intravenous injections of zinc (35). The normal daily diet of humans contains 10 to 15 milligrams of zinc and the normal person excretes from 0.4 to 0.5 mg of zinc in their urine per day; however, patients with proteinuria may excrete 2.1 mg of zinc in their urine daily.

The potent effects of zinc on growth are recognized and are often utilized in animal husbandry. Accelerated growth of pullets, swine, cattle, and other livestock has been demonstrated by adding small amounts of zinc to their diets (36) (37) (38). Evidence supporting zinc deficiency in human patients has been published by several investigators. Parsad et al. (39) (40) (41) examined people in Iran and Egypt suffering from a syndrome consisting of iron-deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism. Evidence that these patients were zinc deficient was as follows: the plasma zinc was low, plasma zinc turnover rate was greater than normal, and the excretion of zinc in stool and urine following radioactive zinc administration was less than in control subjects. The zinc content in the hair of these dwarfs was low and all patients that were studied excreted less zinc in their urines than did normal subjects. Addition of zinc to the diets of these patients did not appear to improve their clinical symptoms.

Strain et al. (42) demonstrated zinc accumulation around the edge of surgical wounds. The demand for zinc in wound healing has been shown indirectly in patients with severe thermal burns. These patients showed a striking decrease in hair zinc levels; however, these levels progressively increased and reached normal values by the time the wound completely healed. Atherosclerotic patients were found to have low zinc levels by Volkov (43), whose patients demonstrated a significant decrease in the zinc content of plasma, aorta, liver, myocardium, and pancreas with an increased zinc content in the erythrocytes. Wacker et al. (44) observed values of 67 $\pm$ 13.8 µg/100 ml in patients with acute myocardial infarction compared to a normal mean of 120 $\pm$ 19 µg/100 ml for normal plasma. Underwood (45) reported a decreased zinc content in malignant prostate glands. Vallee (31) states, "it may be predicted safely that this element, which plays such a cardinal role in significant aspects of metabolism, will be shown to be involved in many other aspects of human and animal pathology."

CHAPTER III

PURPOSE AND SCOPE

The literature seems to indicate that extensive work has been done in the field of shock produced by E. coli endotoxin. It also indicates that considerable work has been done on zinc alterations associated with similar traumatic conditions; however, no attempt has been made by previous invetigators to observe the effect of endotoxin shock on zinc concentrations in plasma or whole blood. The importance of zinc in the hemopoetic system is evidenced by the role of this trace metal in several enzyme systems and disease states related to metabolic disorders of the blood. For example, metabolic acidosis is known to decrease the activity of carbonic anhydrase, an enzyme identified as a zinc protein complex, which contains 0.33 per cent zinc as part of its molecule, and is responsible for the interconversion of CO_2 and H_2CO_3 (46). Metabolic acidosis has also been a consistent observation in patients suffering from leukemia, diabetes, and cirrhosis. These patients were observed to have lower blood zinc concentrations than normal people (46). It is interesting to note that the pancreas of diabetics contains only one-half the normal amount of zinc (46). Because of the importance of zinc in the activity of certain enzymes, as mentioned previously, it may be supposed that a zinc deficiency would cause significant biochemical changes

in the metabolism of those substrates affected by the enzymes concerned. For example, alcohol, a substrate, whose role in the etiology and exacerbation of cirrhosis has not yet been fully explained. Alcohol dehydrogenase, a zinc activated enzyme, is partially responsible for the detoxification of alcohol.

This investigation was performed in an effort to expand and illustrate the importance of increased knowledge and understanding of trace metal alterations induced by traumatic conditions, and to investigate the biochemical aspects of shock produced by gram-negative microorganisms. Specifically the purposes of the present investigation were to observe alterations in plasma zinc, total blood zinc, plasma protein, and hematocrit values in dogs intravenously injected with 0.4 mg/kg (LD/80) E. coli endotoxin and to observe the effect of indomethacin and acetylsalicylic acid on these parameters during endotoxin shock. The ultimate goal of this investigation is to provide a foundation for a diagnostic tool utilizing the role of zinc during E. coli endotoxin shock. If the development and application of such a diagnostic tool appears to be of little usefullness, it must be remembered that 10 years ago few investigators comprehended the deleterious effect of 100 μg/ml sodium in potable water when ingested by individuals under a physician's care for medical conditions such as congestive heart failure, chronic renal disease with edema, toxemia of pregnancy, cirrhosis with ascites, and hypertension (47).

CHAPTER IV

EXPERIMENTAL EQUIPMENT

Introduction

Equipment and laboratory facilities used in this investigation were located at the Institute of Environmental Health, Department of Preventive Medicine and Public Health, and the Veterans Administration Hospital, both located at the University of Oklahoma Medical Center, Oklahoma City, Oklahoma. The Institute of Environmental Health is concerned with chronic health problems provoked by environmental conditions, while medical research is the major concern of the research group at the Veterans Administration Hospital. Experimental data was collected at the Veterans Hospital and sample analysis was performed at the Institute of Environmental Health.

Experimental Animal

The experimental animals consisted of adult mongrel dogs, weighing from 10 to 20 kg each, purchased from Alexander Kennels, Lexington, Oklahoma. Before being randomly assigned for experimental use animals were checked by the staff veterinarian to eliminate animals having bacterial and parasitic infections.

Housing and Eating Facilities

Animal quarters used throughout this investigation consisted of

concrete cages with galvanized iron doors and stainless steel feeders. Since all animals were housed under identical conditions and fed a uniform diet composed of Kasco Meal supplied by Purina and Horsemeat and Gravy purchased from Hill Dog Food Company, the small amount of uncontrolled zinc ingestion resulting from oral contact with the galvanized doors was the same for the exposure and the control animals.

Pharmaceutical and Bacterial Preparations

The following preparations were used independently and conjunctively throughout various phases of this investigation.

E. coli Endotoxin

This preparation was purchased from Difco, Detroit, Michigan, and was made up in the laboratory in 1.0 mg/ml concentration by dissolving in distilled water.

Acetylsalicylic Acid (Aspirin)

This reagent was purchased from Merck, Chicago, Illinois, and was prepared in our laboratory in 150 mg/ml concentration by dissolving in 90 per cent ethanol.

Ethanol, 90 Per Cent U.S.P.N.F. Reagent Quality

Ethyl alcohol was purchased from U. S. Industrial Chemicals Company, New York City, New York, and was used as a solvent for aspirin.

Indomethacin (1-p-Chlorobenzoyl)-Methoxy-2-Methylindole-3-Acetic Acid)

This pharmaceutical was donated by Merck, West Point, Pennsylvania, and was used in 20 mg/ml concentration.

Nembutal (Pentobarbital) Sodium Solution

The anesthetic was purchased from Abbott Inc., North Chicago, Illinois, and contained 50 mg/ml.

Heparin Sodium Solution

This solution was purchased from Oragon Inc., West Orange, New Jersey, and contained 1000 USP units of heparin per ml of solution.

Isotonic Saline Solution

This solution was prepared in our laboratory by dissolving 225 grams of sodium chloride in 25 liters of distilled water.

Deionized Water

The water used for all aqueous solutions was prepared in our laboratory by passing distilled water through two ion-exchange resin columns. After the preceding treatment the water was analyzed by atomic absorption and it was found to be zinc free.

Zinc Reference Solution

A standard zinc solution, which contained 10,000 ppm zinc, was purchased from Fisher Scientific Company, Fairlawn, New Jersey, and was diluted with deionized water to meet our specifications.

Phenol Reagent

This reagent was purchased from Harelco Chemical Company, Philadelphia, Pennsylvania, and was used for making protein determinations.

Infusion Apparatus

A Harvard infusion pump was used throughout this study for ad-

ministering various substances. The pump controlled the rate of infusion and eliminated physical impairments such as blood clotting and lung damage both of which are consistently observed in animals receiving direct injections of alcohol or aspirin or a mixture of the two.

Sample Collection and Dilution

Blood samples were secured in 10 milliliter quantities and centrifuged at 5,000 x g for 15 minutes following which the plasma was withdrawn and placed in 10 milliliter test tubes which were sealed with parafilm and refrigerated until analyzed. Whole blood samples were collected in 10 milliliter quantities and frozen until analyzed. All equipment used for securing samples and for making the final analyses was checked periodically for zinc contamination. At the time of sample collection blood pH and hematocrit ratios were determined. Hematocrit values were duplicately determined in capillary tubes centrifuged at 15,000 x g for 5 minutes and blood pH was determined on an electric, expanded scale meter, manufactured by Dallas Radionics.

Several methods have been reported for the determination of zinc by atomic absorption; however, it was found that the procedure described for serum analyses was not applicable for determining plasma and whole blood zinc (29) (39) (48). The technique developed during the present investigation involved the dilution of each plasma sample with 5 parts deionized water and the dilution of each whole blood sample with 5 parts 0.12 N HCl. Direct aspiration of these samples into the flame gave accurate and reproducible recovery values and minimized the mechanical difficulties encountered when concentrated whole blood and plasma were analyzed.

Zinc Analysis

A Jarrell-Ash Atomic Absorption Spectrophotometer Model 82-362 was the instrument used for zinc analysis. This instrument utilized a 0.5 meter Ebert mount monochromator, a grating of 30,000 lines per inch in the ultraviolet range (Figure 1) and incorporated a five pass optical system (Figure 2) which consisted of Corning optics and permitted almost 100 per cent transmission of ultraviolet light. The photon source was a zinc hollow cathode tube manufactured by Westinghouse and operated at 10 milliamperes. Sample excitation was provided by a direct aspiration Hecto burner using hydrogen at 8 psi and air at 21 psi. An RCA photomultiplier tube Model IR 106 was operated at 900 volts and the analytical line used for zinc analysis at maximum sensitivity was 2139 A.

Atomic absorption spectroscopy is a sensitive method for the analysis of metals in solution and is based on the capability of atoms to absorb radiation of well-defined characteristic wavelengths. When resonant radiation is passed through a dense population of neutral atoms in a flame, absorption occurs and traversing radiation is diminished. This intensity decrease of incident radiation is proportional to the concentration of absorbing atoms in the flame and thus proportional to the concentration in the dissolved material (49). As exemplified in Figure 3 working curves (Beer's Law Curves) were prepared for each series of samples.

Protein Determination

Total plasma proteins were determined by a method described by Lowry *et al.* (50). This method measures the color produced by a combination of the biuret reaction of protein with copper in alkali and the

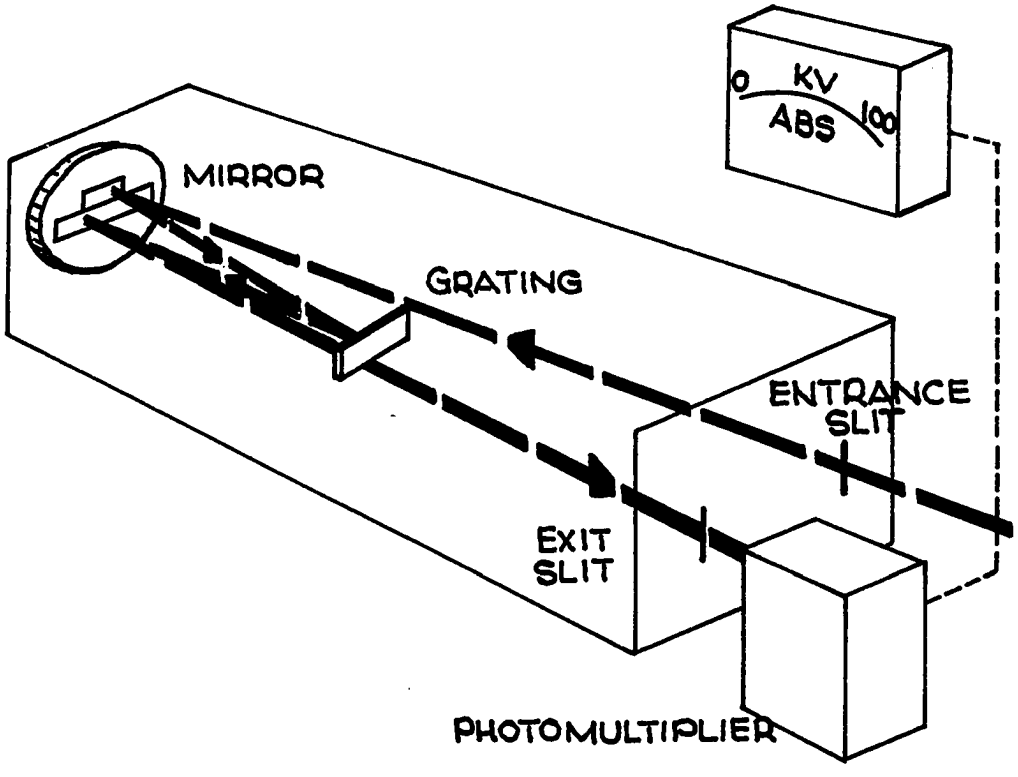


Figure 1. Optical design of the Jarrell-Ash Ebert spectograph, with photomultiplier and recorder attachments.

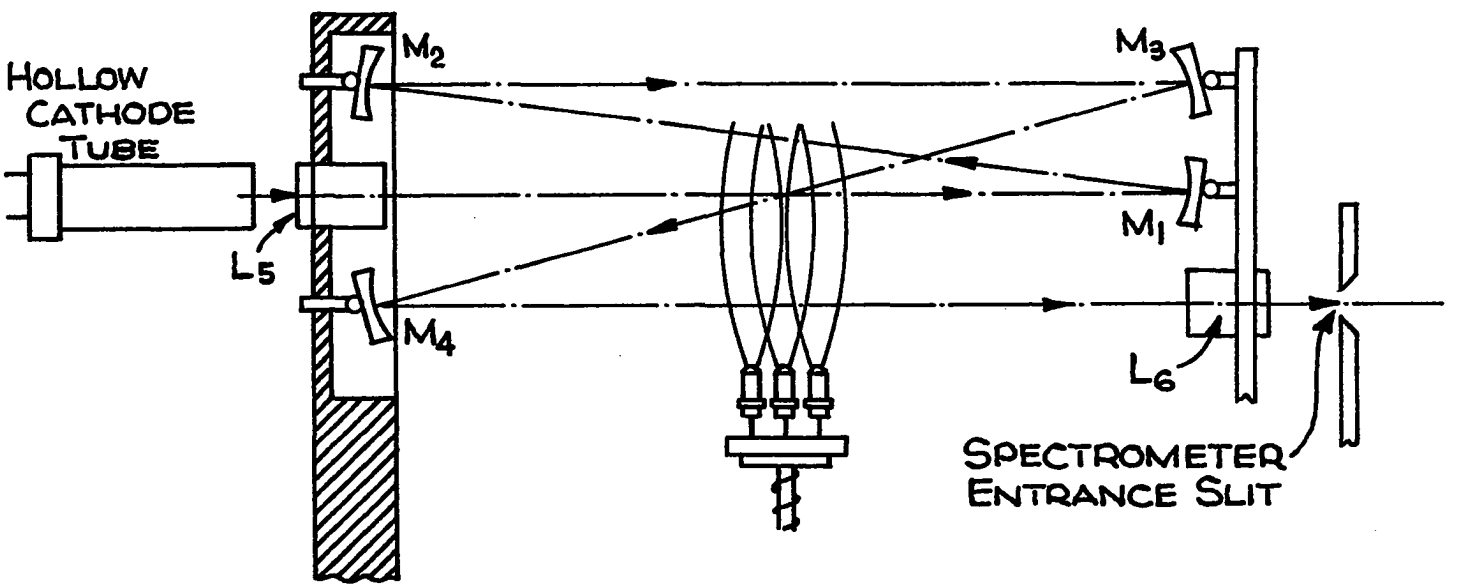


Figure 2. Multi-pass optical system composed of the new Corning optics.

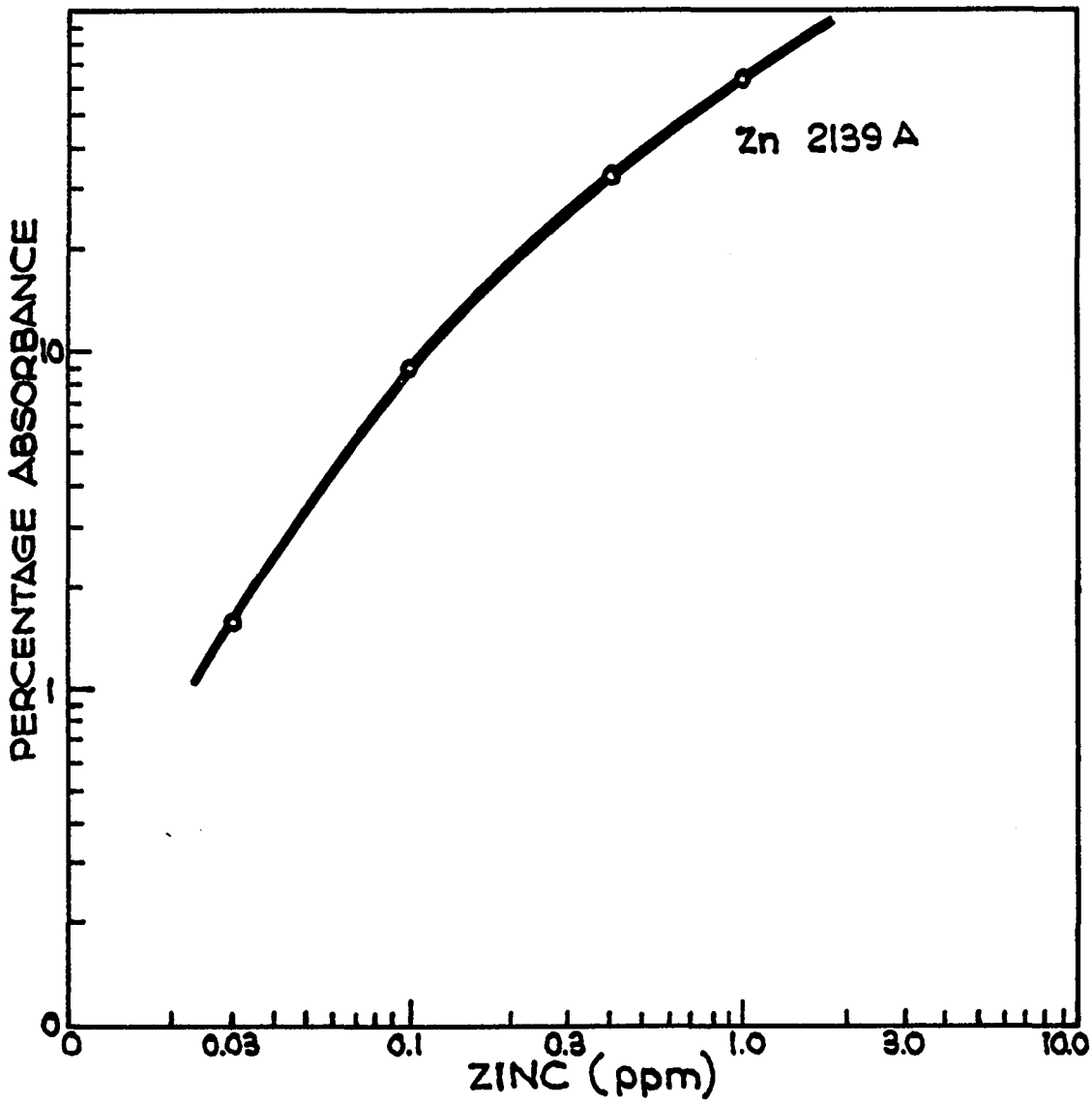


Figure 3. Standard zinc curve.

reduction of phosphomolybdic-phosphotungstic reagent by the tyrosine and tryptophan present in the treated protein. The final blue color was measured on a Beckman DU Spectrophotometer at 500 mμ. Protein fractions were qualitatively separated by paper electrophoresis and quantitated on a Beckman Analytrol which measures the density of the stained electrophoretic strips.

CHAPTER V

EXPERIMENTAL PROCEDURE

A total of 93 animals, divided into two experimental groups, were used in this investigation. Animals in one group were observed for a maximum of 144 hours unless terminated by death while animals in the other group were observed for a maximum of 2 hours.

Survival Animals

The initial portion of this study evaluated the LD value of the endotoxin employed which had been previously established as LD/80 at 0.4 mg/kg body weight. Twenty-five animals were anesthetized with sodium pentobarbital given intravenously, 25 to 35 mg/kg body weight, followed by an intravenous injection of E. coli endotoxin, 0.4 mg/kg body weight. These intact animals offered an excellent opportunity to observe plasma zinc concentrations before and after endotoxin injection. Blood samples were secured for zinc analysis from the cephalic vein of the left forelimb immediately after the animals were anesthetized and before endotoxin injection. Additional samples were collected every 24 hours after endotoxin injection for a maximum of 144 hours unless interrupted by death of the animal. Animals that survived 144 hours were listed as permanent survivors. Plasma zinc was the only measurement possible on this group. Observing other parameters would have been useful; however,

circulatory collapse made it difficult to secure sufficient blood sample for zinc analysis and in most cases impossible to secure enough sample to make additional measurements.

Two control groups were observed throughout this phase of the study. One group was composed of six animals, anesthetized with sodium pentobarbital intravenously, 25 to 35 mg/kg body weight while the other group was composed of nine unanesthetized animals. Plasma zinc was measured on all controls and hematocrits were measured on anesthetized controls.

In the preceding study most of the animals that were given endotoxin survived at least 2 but less than 12 hours, therefore the following experimental groups were limited to observation periods of 2 hours each.

Two-Hour Post-Endotoxin Shock

This group was composed of five animals each anesthetized with sodium pentobarbital intravenously, 30 mg/kg body weight, followed by an intravenous injection of *E. coli* endotoxin, 0.4 mg/kg body weight. One femoral artery and vein were exposed; the artery was used to measure pressure while the vein was used for sampling and administration of endotoxin. Plasma zinc, total blood zinc, hematocrit, and total plasma protein concentrations were measured before endotoxin injection and again 120 minutes after endotoxin administration. This group showed alterations in all parameters that were observed; therefore, the following study was devised to see if aspirin or indomethacin, two anti-inflammatory agents, would effect these changes.

Indomethacin Therapy

This experimental group consisted of 12 dogs each subjected to indomethacin treatment prior to E. coli endotoxin injection. The animals were anesthetized with sodium pentobarbital intravenously, 30 mg/kg body weight, then given an intravenous injection of indomethacin, 20 mg/kg body weight, followed by an intravenous injection of E. coli endotoxin, 0.4 mg/kg body weight. One femoral artery and vein were exposed; the artery was used to measure pressure while the vein was used for securing blood samples and administering drugs. Blood samples were taken before anesthesia and again 120 minutes after endotoxin administration. Plasma zinc, total blood zinc, total plasma protein, plasma protein electrophoretic separations, and hematocrit values were determined on all samples.

Aspirin (Acetylsalicylic Acid) Therapy

The pharmacological actions of salicylates are complex and appear to be at least in part, central and part peripheral (51). The analgesic action of aspirin is much more effective in relieving skeletal pain and headache than visceral pain. The analgesic or anti-nociceptive action has generally been assumed to be central because of an interference in the transmission of pain impulses between the hypothalmus and the sensory cortex which alters the interpretation of the pain. The antipyretic action of aspirin probably results from an interference with the heat regulating centers in the hypothalmus with a resulting dissipation of heat by cutaneous vasodilation. The anti-inflammatory reaction of aspirin is similar to that of cortisone, aminopyrine, and certain cinchonic acid derivatives whose actions are mostly due to their influence on carbohydrate metabolism (51).

This phase of the investigation involved 36 animals divided into four groups with eight to ten animals in each group. Surgical procedures as described by Hinshaw et al. (52) were followed throughout this study. The aspirin employed was dissolved in 90 per cent ethanol and contained 150 mg aspirin per milliliter of solution.

Group One

The animals were anesthetized with sodium pentobarbital intravenously, 30 mg/kg body weight, followed by an intravenous infusion of the aspirin solution, 30 mg/kg body weight, administered at the rate of 0.33 ml/minute, which was flushed into the animal with an isotonic saline flush, administered at the rate of 2.5 ml/minute. Approximately 5 minutes after the aspirin was given an intravenous injection of E. coli endotoxin, 0.4 mg/kg body weight was administered. Plasma zinc, pH and hematocrit values were determined after the animal had been anesthetized and again 120 minutes after endotoxin.

Group Two

The animals were anesthetized with sodium pentobarbital intravenously, 30 mg/kg body weight, and given an intravenous infusion of the aspirin solution, 30 mg/kg body weight, which was flushed at the rate of 2.5 ml/minute with an isotonic saline solution. Plasma zinc, pH and hematocrit values were determined after the animal had been anesthetized and again 120 minutes after aspirin infusion.

Group Three

Animals in this group were anesthetized with sodium pentobarbital intravenously, 30 mg/kg body weight, and given an intravenous in-

fusion of 90 per cent ethanol, 0.2 ml/kg body weight, which was flushed at the rate of 2.5 ml/minute with an isotonic saline solution. Plasma zinc, pH and hematocrit values were determined after the animal had been anesthetized and again 120 minutes after the alcohol infusion.

Group Four

This group was anesthetized with sodium pentobarbital intravenously, 30 mg/kg body weight, and given an intravenous injection of E. coli endotoxin, 0.4 mg/kg body weight. Plasma zinc, pH and hematocrit values were determined after the animal had been anesthetized and again 120 minutes after endotoxin injection.

CHAPTER VI

RESULTS

The paired "t" test was used for making a comparative analysis of data obtained from animals observed for 2 hours. Mean zinc concentrations with the appropriate standard errors were used to compare animals in the long term "survival" group. Paired observations on the same animal minimized the disadvantage of using many varieties of dogs and also helped to eliminate any experimental error which could have been caused by the difference in the state of health of the dogs.

Long Term "Survival" Observations

Data from the series of animals employed in this investigation are illustrated in Figures 4 and 5. Inspection of this data permits several general observations to be made. One of the most interesting is that the animal population decreased over 50 per cent within 24 hours after administering E. coli endotoxin intravenously, 0.4 mg/kg body weight. The calculated LD/80 value of the endotoxin preparation was verified by an 80 per cent reduction of our animal population 72 hours post-endotoxin injection. Another interesting observation is that the plasma zinc concentration decreased from 68 μg/100 ml the first 24 hours after endotoxin injection but returned to near pre-endotoxin values at the end of 144 hours. The increase in plasma zinc occurring after the

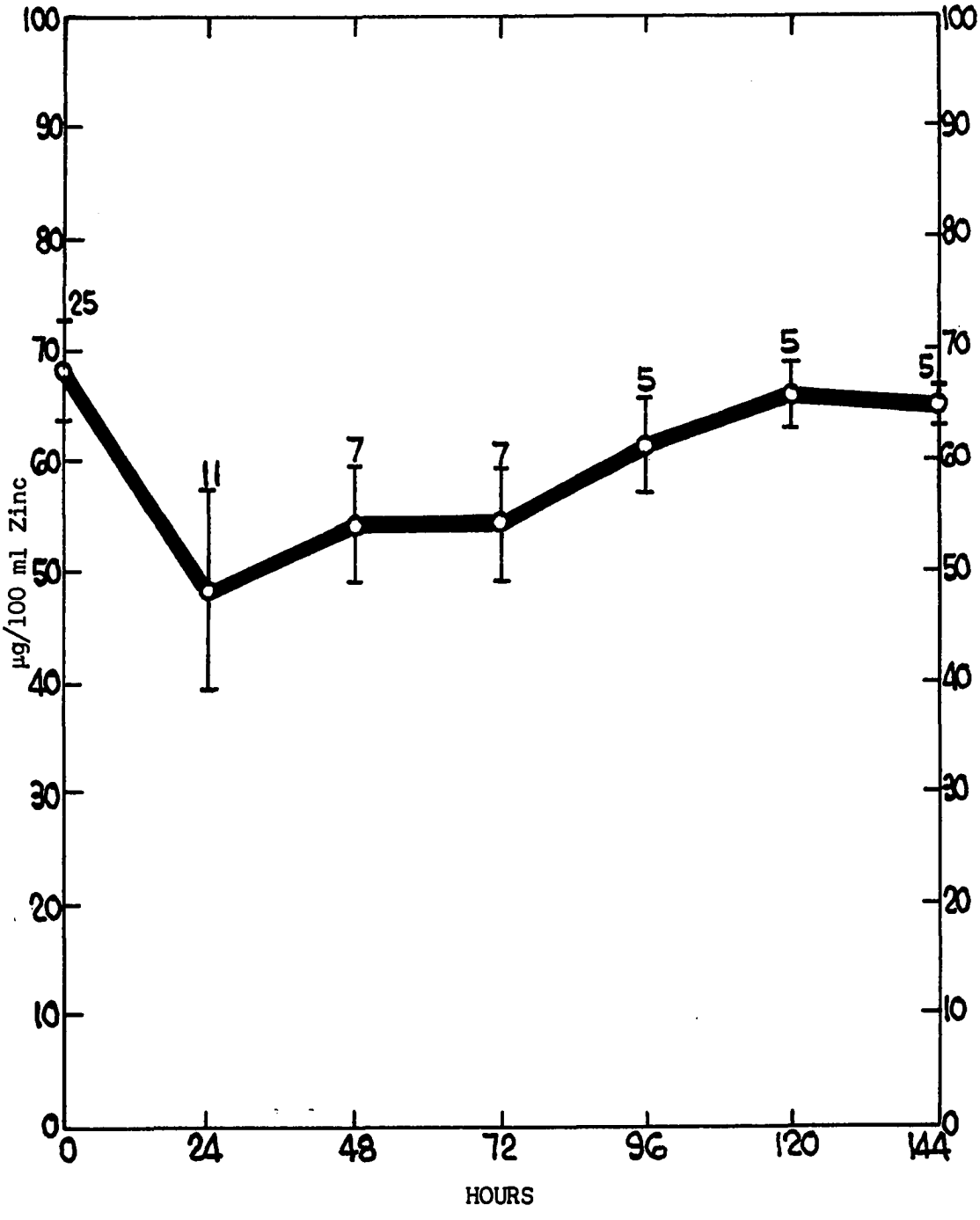


Figure 4. Plasma Zinc. Dogs surviving 0.4 mg/kg E. coli endotoxin.

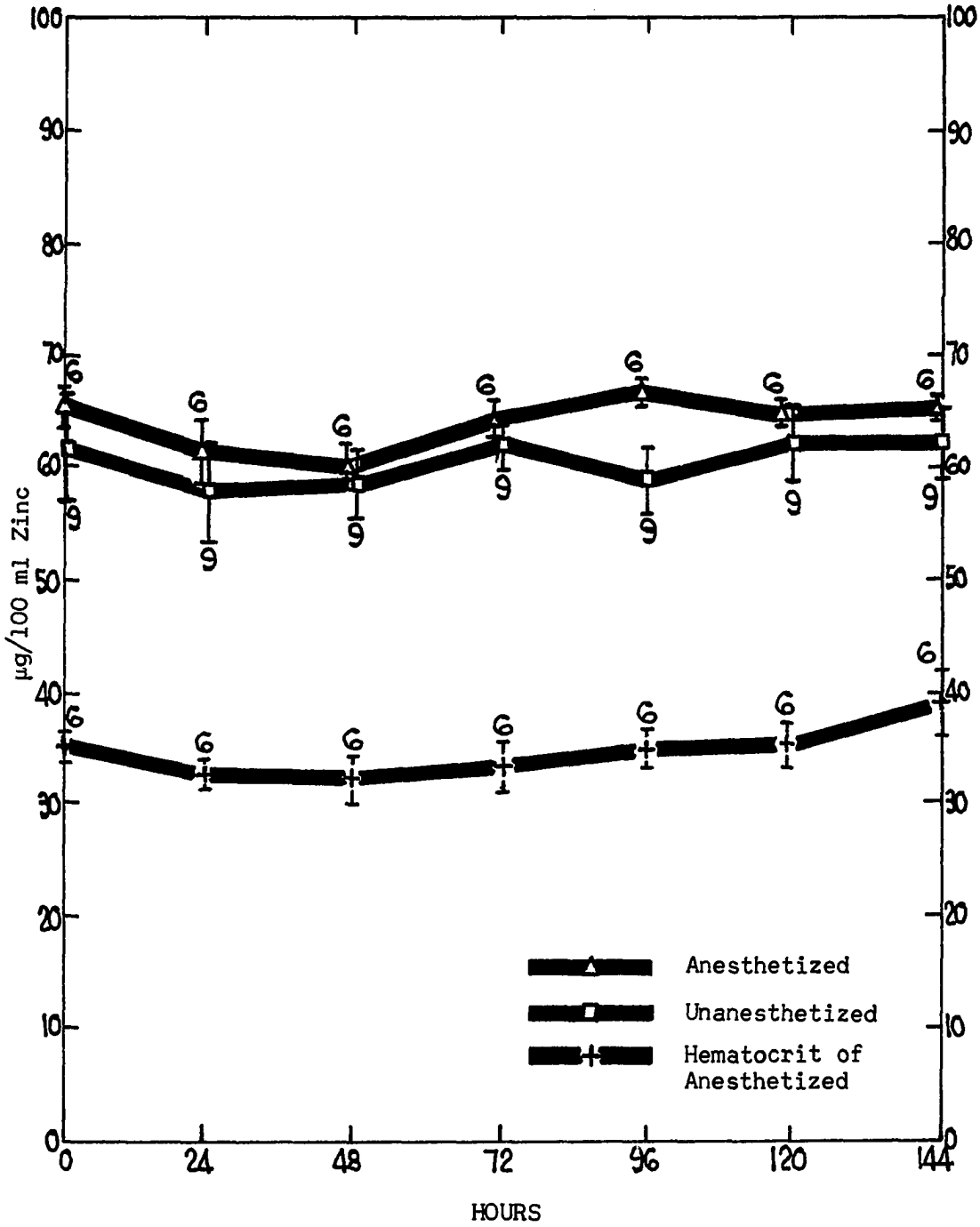


Figure 5. "Control Dogs" mean plasma zinc concentrations with standard errors and mean hematocrit values with standard errors.

24-hour post-endotoxin period was progressive and appeared to occur as the physical appearance of the animal improved. Blood samples were very hard to withdraw during this 24-72 hour period. Plasma separated from this blood had a yellowish green color indicating the possibility of liver dysfunction. The vascular collapse and the metabolic disorder producing the yellowish green color had been reversed by the end of 144 hours.

Figure 5 represents two series of control animals; one group was anesthetized with sodium pentobarbital intravenously, 30 mg/kg body weight while the other group was not anesthetized. Plasma zinc concentrations were nearly the same in all controls and the hematocrit values, taken only on anesthetized controls, remained relatively constant throughout the 144-hour period.

Two-Hour Post-Endotoxin Shock Observations

Data presented in Tables 2 through 5 (APPENDIX) illustrate changes observed 2 hours after an intravenous injection of E. coli endotoxin, 0.4 mg/kg body weight. Careful examination of the data permits several general statements to be made. Significant increases (at a confidence level of 95 per cent) were observed in hematocrit values and total blood zinc concentration while a significant decrease (at a confidence level of 95 per cent) was observed in total plasma protein concentration; however, changes in plasma zinc were not significant.

Observations Concerning Indomethacin Therapy

The data from this phase of the investigation are presented in Tables 6 through 9 (APPENDIX) and in Figures 6 through 9. Changes were

observed before and after intravenous injections of indomethacin, 20 mg/kg body weight, and intravenous injections of E. coli endotoxin, 0.4 mg/kg body weight. Significant increases (at a confidence level of 95 per cent) were observed in total blood zinc, and hematocrit while a significant decrease (at a confidence level of 95 per cent) was observed in total plasma protein concentration. Changes in plasma zinc concentration were not significant. Figures 6 through 9 illustrate the decrease in individual plasma protein fractions at the end of 2 hours. The albumin fraction decreased as shown in Figures 7 and 8. Figures 6 and 9 do not present such drastic reductions in albumin but do indicate a more general decrease in all plasma protein fractions.

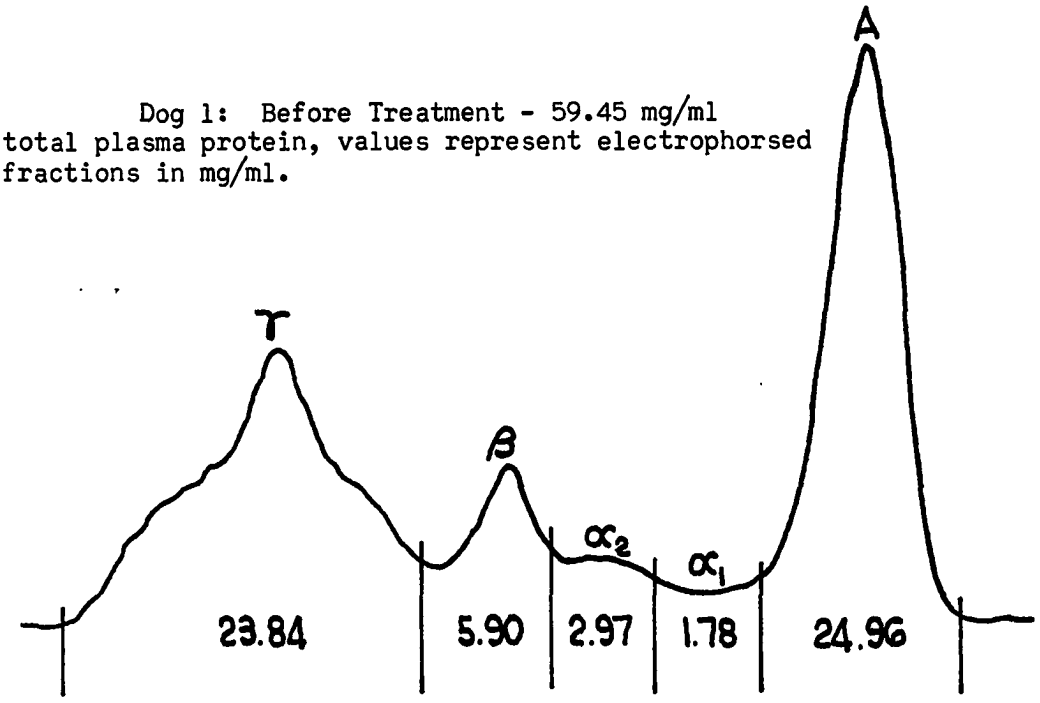
Observations Concerning Aspirin (Acetylsalicylic Acid) and Ethanol Infusion

Data presented in Tables 10 through 12 (APPENDIX) were collected before and after intravenous infusions of acetylsalicylic acid dissolved in 90 per cent ethanol, 30 mg/kg body weight, and intravenous injections of E. coli endotoxin, 0.4 mg/kg body weight. A study of this data permits limited conclusions to be made. Plasma zinc concentration and pH appeared to decrease while the hematocrit values appeared to increase. None of these changes were significant. The possibility of aspirin and alcohol infusion to assist in E. coli endotoxin shock therapy appeared to be quite questionable and as a result additional investigations were conducted. The results of these successive studies are summarized in the following sections.

Aspirin (Acetylsalicylic Acid) and Ethanol Infusion Observations

Tables 13 through 15 (APPENDIX) represent data obtained from

Dog 1: Before Treatment - 59.45 mg/ml total plasma protein, values represent electrophorsed fractions in mg/ml.



Dog 1: 2 Hours Post Treatment - 54.55 mg/ml total plasma protein, values represent electrophoresed fractions in mg/ml.

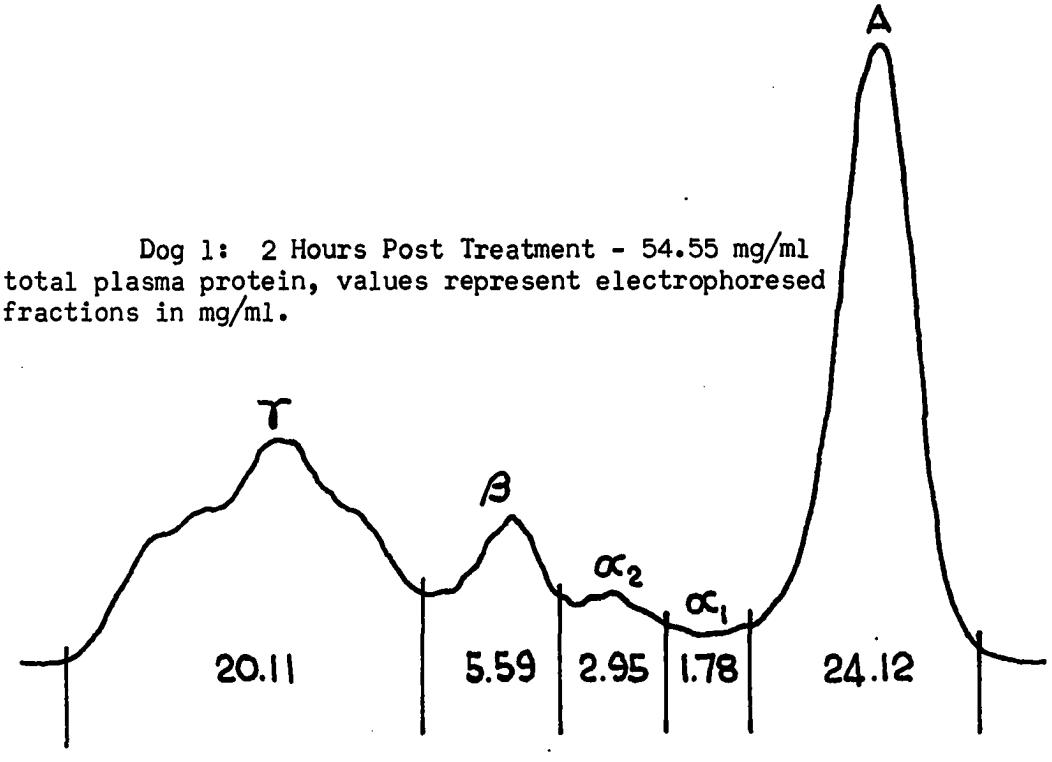
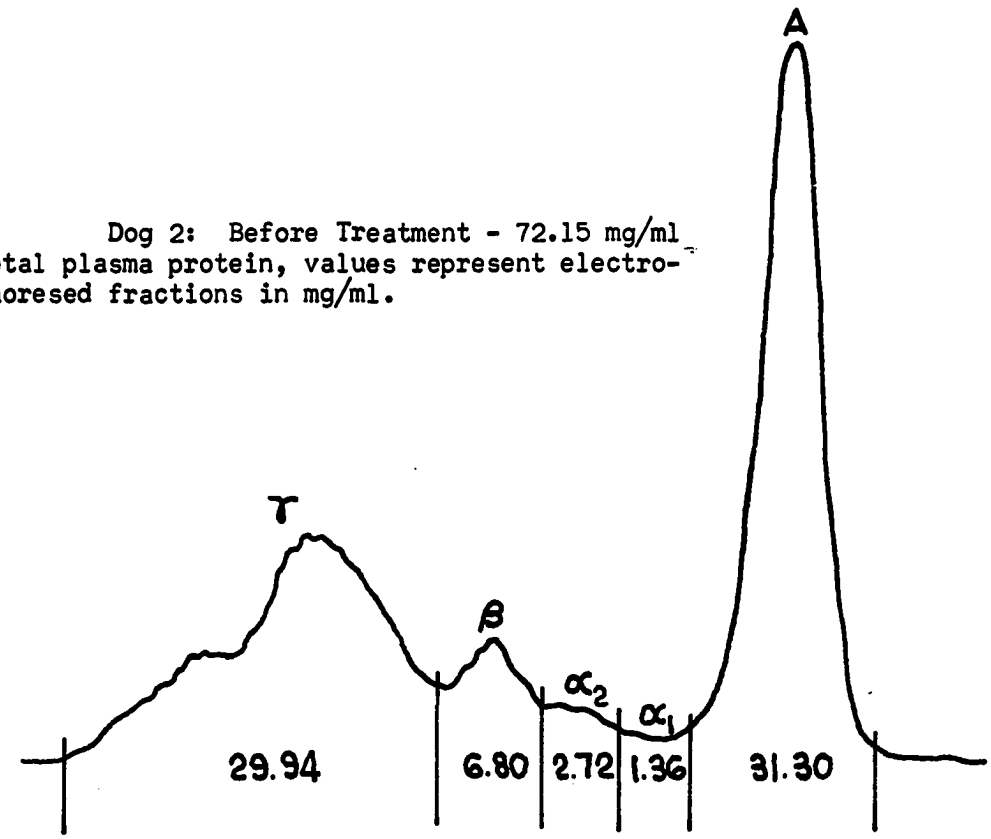


Figure 6. Electrophoretic patterns of dog 1 pretreated with indomethacin administered I.V., followed by I.V. injection of E. coli endotoxin (LD_{80}).

Dog 2: Before Treatment - 72.15 mg/ml total plasma protein, values represent electrophoresed fractions in mg/ml.



Dog 2: 2 Hours Post Treatment - 40.91 mg/ml total plasma protein, values represent electrophoresed fractions in mg/ml

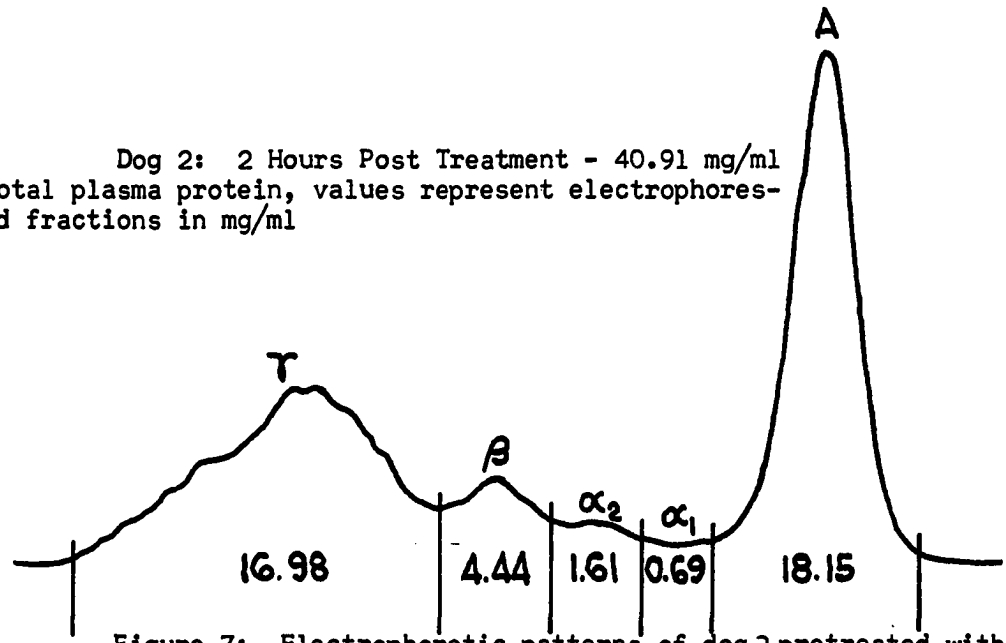
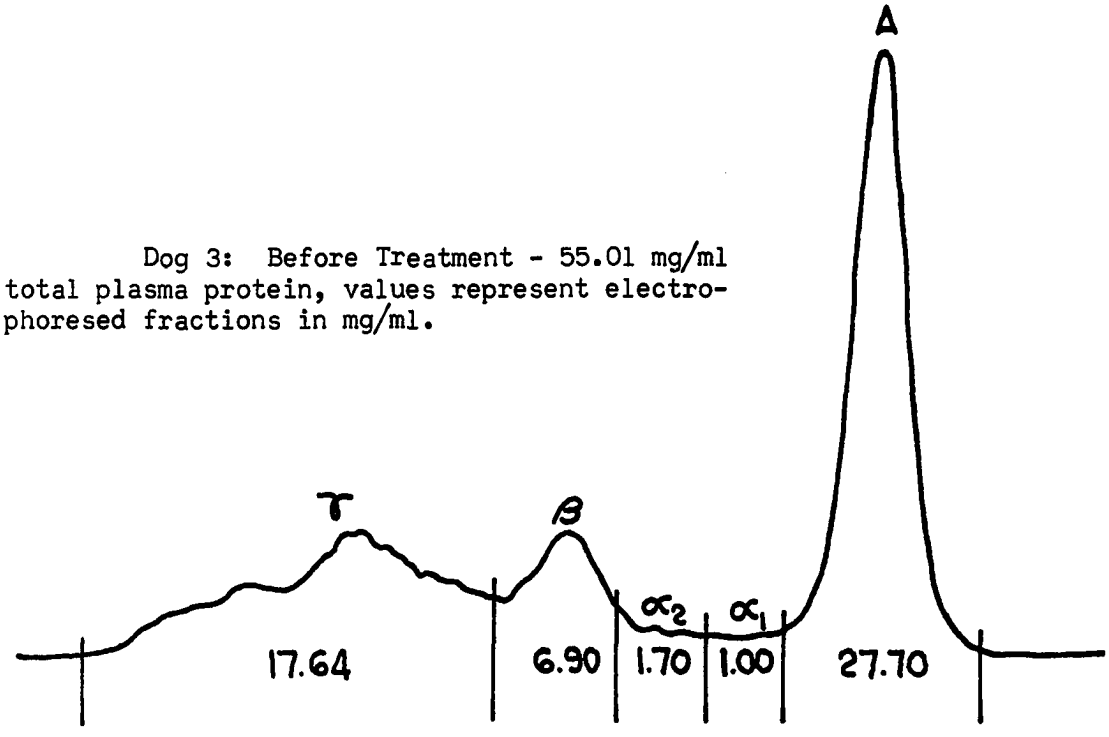
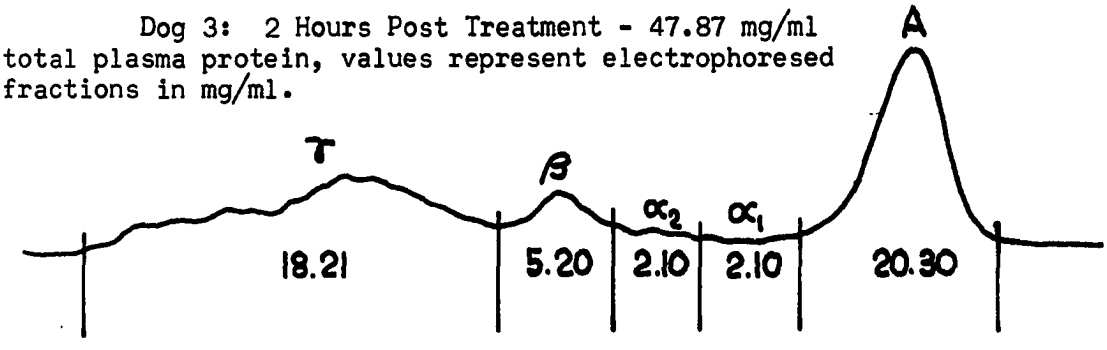


Figure 7: Electrophoretic patterns of dog 2 pretreated with indomethacin administered I.V., followed by I.V. injection of E. coli endotoxin (LD_{80}).



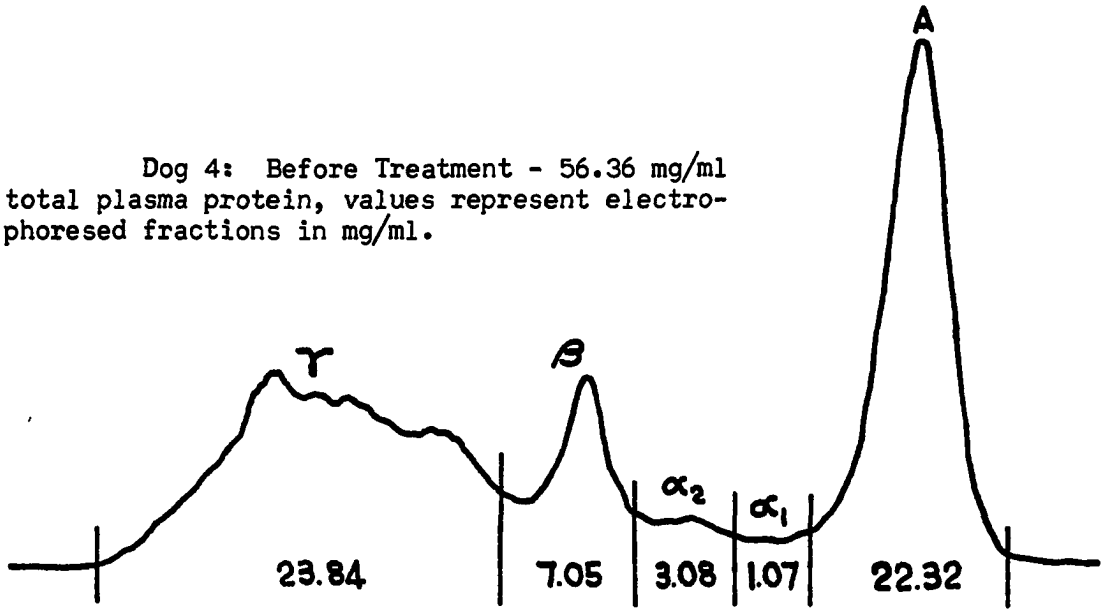
Dog 3: Before Treatment - 55.01 mg/ml total plasma protein, values represent electrophoresed fractions in mg/ml.



Dog 3: 2 Hours Post Treatment - 47.87 mg/ml total plasma protein, values represent electrophoresed fractions in mg/ml.

Figure 8. Electrophoretic patterns of dog 3 pretreated with indomethacin administered I.V., followed by I.V. injection of E. coli endotoxin (LD_{80}).

Dog 4: Before Treatment - 56.36 mg/ml total plasma protein, values represent electrophoresed fractions in mg/ml.



Dog 4: 2 Hours Post Treatment - 44.82 mg/ml total plasma protein, values represent electrophoresed fractions in mg/ml.

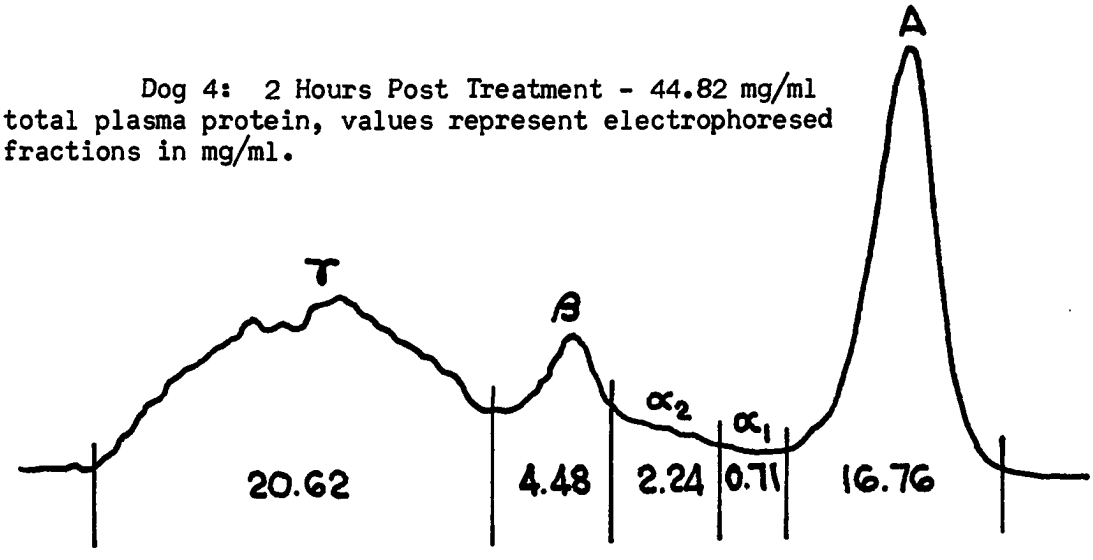


Figure 9. Electrophoretic patterns of dog 4 pretreated with indomethacin administered I.V., followed by I.V. injection of E. coli endotoxin (LD_{80}).

animals before and after an intravenous infusion of aspirin dissolved in 90 per cent ethanol, 30 mg/kg body weight. Hematocrit values, pH, and plasma zinc concentrations were measured and were found to remain constant throughout the experimental group.

Ethanol Infusion Observations

Tables 16 through 18 (APPENDIX) represent data obtained from animals before and after an intravenous infusion of 90 per cent ethanol, 0.2 ml/kg body weight. Hematocrit values, pH, and plasma zinc concentrations were not altered throughout this experimental group.

E. coli Endotoxin Shock Observations

Data representing this experimental group may be found in Tables 19 through 21 (APPENDIX). As in the three preceding groups, these data represent measurements taken during the pre- and post-endotoxin shock periods. These animals showed a significant decrease in plasma zinc (at a confidence level of 95 per cent) within 120 minutes after *E. coli* endotoxin injection. Changes in pH and hematocrit values were not significant.

CHAPTER VII

DISCUSSION

Alterations observed during the endotoxin reaction consisted of decreased plasma zinc and plasma protein concentrations with elevated total blood zinc concentration and increased hematocrit values.

Decreased plasma protein concentrations were observed in animals receiving a LD/80 E. coli endotoxin, administered intravenously. Liver congestion and malfunction, both common during endotoxin shock, appeared to be the metabolic disorders producing the decreases in plasma protein (10) (11) (54). Fine (15) states that even though increased capillary permeability exists during the endotoxin shock reaction, significant leakage from the capillary bed does not exist, thus, plasma protein decreases during endotoxin shock appeared to result from improper synthesis of such proteins by the liver. Decreased plasma zinc concentrations were observed in most of the animals that exhibited decreased plasma protein concentrations. This observation was expected since 12 to 22 per cent of the total blood zinc was bound to the serum albumin and globulin fractions (45). Since 75 to 85 per cent of the total blood zinc was found in the erythrocytes (34), the increased total blood zinc which was accompanied by increased hematocrit values throughout this study was expected.

Anti-inflammatory treatment prior to endotoxin injection was included in this study. Indomethacin, an anti-inflammatory agent, was intravenously injected, 20 mg/kg body weight, then the animal was given an intravenous injection of E. coli endotoxin, 0.4 mg/kg body weight. A significant decrease in plasma protein concentration and significant increases in total blood zinc concentration and hematocrit values were similar to those observed in the animals that received only endotoxin. Hypotension produced by E. coli endotoxin was not altered by indomethacin. Based on this study, indomethacin was essentially useless in the therapy or prevention of shock produced by E. coli endotoxin.

Aspirin dissolved in 90 per cent ethanol was intravenously infused, 30 mg/kg body weight, then the animal was given an intravenous injection of E. coli endotoxin, 0.4 mg/kg body weight. Plasma zinc, pH, and hematocrit values were determined and no significant changes from normal were found. Thus, from our observations, it was assumed that aspirin should be considered as a potential therapeutic agent for shock produced by E. coli endotoxin.

Animals not receiving endotoxin but receiving an intravenous infusion of 90 per cent ethanol, 0.2 ml/kg body weight, and animals intravenously infused with aspirin dissolved in 90 per cent ethanol, 30 mg/kg body weight, showed no significant alterations in plasma zinc, pH and hematocrit values.

Figure 10 is a proposed schema illustrating the biochemical and hemodynamic alterations leading to the results reported in this study. Each of these physiological impairments appears to be partially responsible for the death of the animal. Reduced plasma protein and plasma

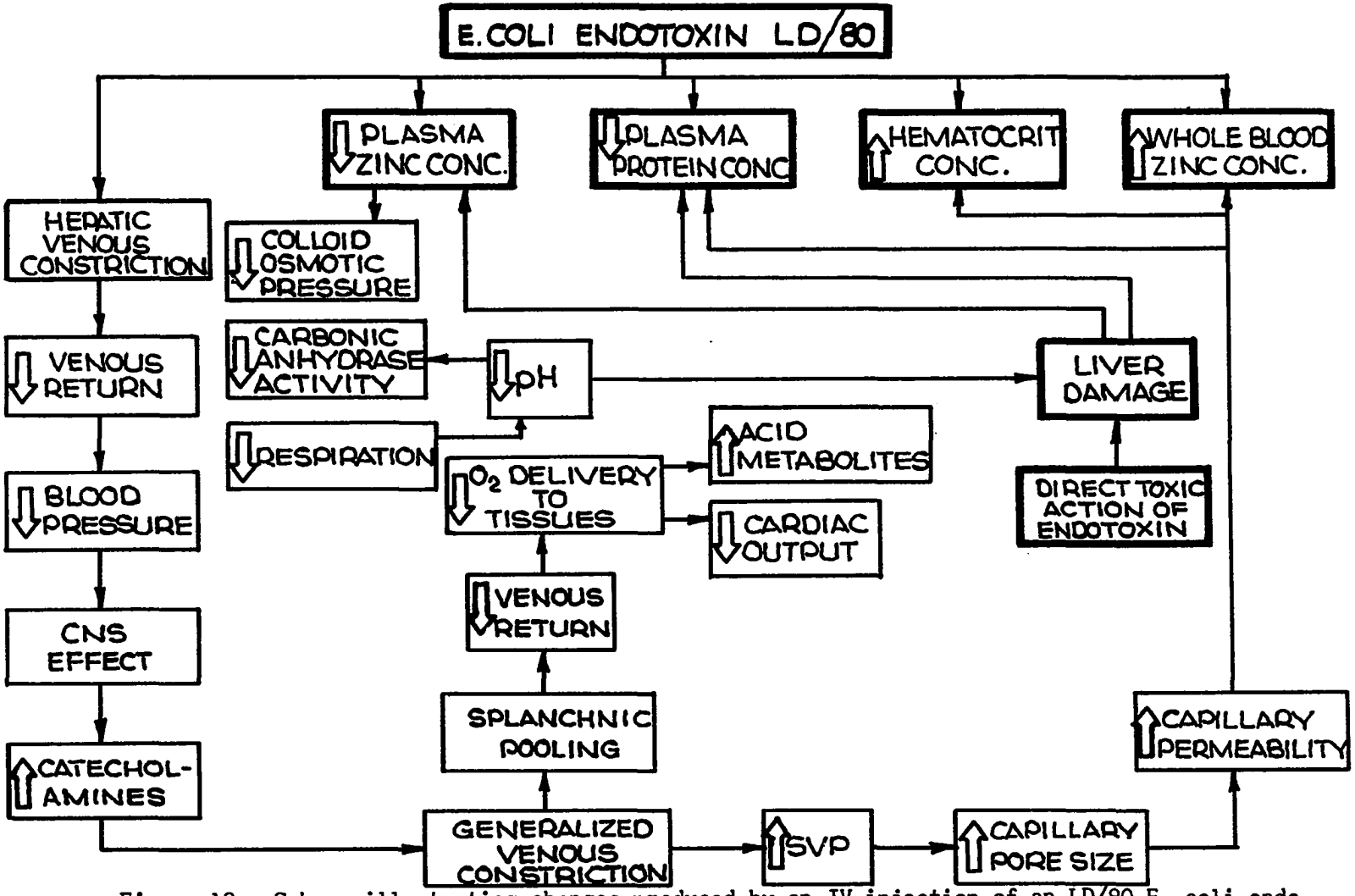


Figure 10. Schema illustrating changes produced by an IV injection of an LD/80 E. coli endotoxin.

zinc concentrations were consistently accompanied by increased hematocrit values and total blood zinc concentrations. A significant alteration in one of the four parameters was usually accompanied by significant alterations in the remaining three. The only group of animals deviating from the preceding statement were those infused with aspirin prior to endotoxin injection. Additional investigation concerning aspirin's potential as a therapeutic agent for shock produced by E. coli endotoxin is now in progress.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

This investigation was concerned with changes in total blood zinc, plasma zinc, hematocrit ratios, pH and total plasma protein concentration resulting from experimentally induced E. coli endotoxin shock. A total of 93 adult mongrel dogs was divided into two groups; the dogs in one group were intravenously injected with E. coli endotoxin (0.4 mg/kg body weight) while those in the other groups received identical treatment preceded by an intravenous injection of indomethacin (20 mg/kg body weight) or aspirin (30 mg/kg body weight).

Based on the results of various chemical and biochemical determinations made during this investigation, the following conclusions have been drawn:

1. Intravenous administration of E. coli endotoxin, 0.4 mg/kg body weight, resulted in a LD/80 for the adult mongrel dogs employed in this investigation.
2. An intravenous injection of E. coli endotoxin, 0.4 mg/kg body weight produced significant decreases in plasma zinc and total plasma protein concentrations and significant increases in the hematocrit values and total blood zinc concentrations.

3. Indomethacin was observed to have no effect on the alterations in total blood zinc, hematocrit values and total plasma protein concentrations induced by an intravenous injection of E. coli endotoxin, 0.4 mg/kg body weight.
4. Intravenous infusion of aspirin prior to an intravenous injection of a LD/80 E. coli endotoxin was observed to protect the experimental animal from significant changes in pH, hematocrit ratios, and plasma zinc concentration.
5. A drastic decrease in plasma zinc concentration occurred 24 hours after an intravenous injection of E. coli endotoxin, 0.4 mg/kg body weight; however, plasma zinc concentrations returned to approximately pre-endotoxin levels in animals surviving 144 hours or longer.

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APPENDIX

TABLE 2

WHOLE BLOOD ZINC CONCENTRATION IN DOGS INTRAVENOUSLY INJECTED WITH 0.4 mg/kg (LD/80) *E. coli* ENDOTOXIN

Zinc µg/100 ml		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
192	240	- 48	2,304
162	222	- 60	3,600
264	396	-130	16,900
262	324	- 62	3,844
180	204	- 24	576
ΣX 1,060	1,386	-324	27,224
$\bar{x}$ 212	277.2	- 64.8	

$$s_{\bar{d}}^2 = \frac{\Sigma(X_1-X_2)^2 - [\Sigma(X_1-X_2)]^2/n}{n(n-1)} = 310.45$$

$s_d = \underline{17.61}$ $\quad t = \frac{\bar{d}}{s_{\bar{d}}} = \frac{65}{17.6} = 3.82^*$, for 4 df

TABLE 3

PLASMA ZINC CONCENTRATION IN DOGS INTRAVENOUSLY INJECTED WITH 0.4 mg/kg (LD/80) *E. coli* ENDOTOXIN

Zinc µg/100 ml		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
71.00	60.00	11.00	121.00
54.00	46.00	8.00	64.00
61.20	54.00	7.20	51.84
96.00	90.00	6.00	36.00
39.00	46.80	- 7.80	60.84
ΣX 321.20	296.80	24.40	333.68
$\bar{x}$ 64.25	59.36	4.88	

$s_{\bar{d}}$ = 3.27

t = 1.49 for 4 df

TABLE 4

HEMATOCRIT VALUES IN DOGS INTRAVENOUSLY INJECTED WITH 0.4 mg/kg (LD/80) *E. coli* ENDOTOXIN

Hematocrit		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
33	46	- 13	169
28	34	- 6	36
43	62	- 19	361
42	58	- 16	256
32	38	- 6	36
ΣX 178	238	- 60	858
$\bar{x}$ 35.6	47.6	- 12	

$s_{\bar{d}} = 2.62$

$t = 4.6^{*}$ for 4 df

TABLE 5

PLASMA PROTEINS IN DOGS INTRAVENOUSLY INJECTED WITH 0.4 mg/kg (LD/80) *E. coli* ENDOTOXIN

Plasma Proteins		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
55.33	46.85	8.48	71.91
51.65	44.65	7.00	49.00
62.00	60.00	2.00	4.00
65.97	60.95	5.02	25.20
74.40	58.03	16.37	108.16
ΣX 307.35	270.48	38.87	258.27
$\bar{x}$ 61.47	54.10	7.77	

$s_{\bar{d}} = 1.46$

$t = 5.05^*$ for 4 df

TABLE 6

WHOLE BLOOD ZINC CONCENTRATION IN DOGS INTRAVENOUSLY INJECTED WITH 20 mg/kg INDOMETHACIN, FOLLOWED BY INTRAVENOUS INJECTION OF 0.4 mg/kg (LD/80) *E. coli* ENDOTOXIN

Zinc µg/100 ml		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
300	364	- 64	4,096
234	252	- 18	324
192	240	- 48	2,304
244	244	0	00
222	264	- 42	1,764
234	264	- 30	900
276	324	- 48	2,304
252	276	- 24	576
252	324	- 72	5,184
180	228	- 48	2,304
264	328	- 64	4,096
300	364	- 64	4,096
ΣX 2,950	3,472	-522	27,948
$\bar{x}$ 245.83	289.50	- 43.50	

$s_{\bar{d}} = 6.4$

$t = 6.79^{*}$, for 11 df

TABLE 7

PLASMA ZINC CONCENTRATION IN DOGS INTRAVENOUSLY INJECTED WITH 20 mg/kg INDOMETHACIN, FOLLOWED BY INTRAVENOUS INJECTION OF 0.4 mg/kg (LD/80) E. coli ENDOTOXIN

Zinc µg/100 ml		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
108.00	69.00	39.00	1,521.00
79.20	90.00	- 10.80	117.00
69.60	55.80	13.80	190.44
69.60	80.00	- 10.40	108.16
71.00	92.00	- 21.00	441.00
60.00	70.80	- 10.80	117.00
70.80	78.00	- 7.20	51.84
70.80	54.00	16.80	282.24
79.60	69.60	10.00	100.00
60.00	70.80	- 10.80	117.00
60.00	61.10	- 1.10	1.21
72.00	62.60	7.40	54.76
ΣX 870.60	853.70	15.10	3,101.65
$\bar{x}$ 72.50	71.14	1.26	

$s_{\bar{d}} = 15.2$

$t = 0.83$, for 11 df

TABLE 8

HEMATOCRIT VALUES IN DOGS INTRAVENOUSLY INJECTED WITH 20 mg/kg INDOMETHACIN, FOLLOWED BY INTRAVENOUS INJECTION OF 0.4 mg/kg (LD/80) *E. coli* ENDOTOXIN

Hematocrit change %		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
29.0	44.0	- 15.0	225.00
35.0	32.0	3.0	9.00
33.0	34.0	- 1.0	1.00
39.0	66.5	- 27.5	756.25
38.5	53.0	- 14.5	210.25
35.0	52.0	- 17.0	289.00
35.0	44.0	- 9.0	81.00
35.0	46.0	- 11.0	121.00
30.0	53.0	- 23.0	529.00
37.0	38.0	- 1.0	1.00
31.0	37.0	- 6.0	36.00
36.0	47.0	- 11.0	121.00
ΣX 413.5	546.5	-133.00	2,379.50
$\bar{x}$ 34.46	45.54	- 11.10	

$s_{\bar{d}} = 2.62$

$t = 4.19^*$, for 11 df

TABLE 9

PLASMA PROTEINS IN DOGS INTRAVENOUSLY INJECTED WITH 20 mg/kg INDOMETHACIN, FOLLOWED BY INTRAVENOUS INJECTION OF 0.4 mg/kg (LD/80) *E. coli* ENDOTOXIN

Plasma proteins mg/ml		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
62.41	49.12	15.29	176.62
59.45	55.11	4.34	18.83
55.01	47.87	7.14	50.98
63.86	49.11	14.75	217.56
58.40	47.03	11.37	129.28
56.36	44.82	11.54	133.17
72.15	40.91	31.24	975.93
68.20	57.21	10.99	120.78
61.12	55.17	15.95	254.40
60.17	49.92	10.25	105.06
62.50	49.57	12.93	167.18
63.14	59.51	3.63	13.17
ΣX 742.77	605.35	147.42	2,362.96
$\bar{x}$ 61.85	50.44	12.28	

$s_{\bar{d}} = 4.09$

$t = 3.0^*$, for 11 df

TABLE 10

PLASMA ZINC CONCENTRATION IN DOGS INTRAVENOUSLY INFUSED WITH 30 mg/kg ASPIRIN DISSOLVED IN 90 PER CENT ETHYL ALCOHOL, FOLLOWED BY INTRAVENOUS INJECTION OF 0.4 mg/kg (LD/80) *E. coli* ENDOTOXIN

Zinc µg/100 ml		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
60.0	60.0	0.0	0.00
37.2	30.0	7.2	51.84
63.0	36.0	27.0	629.00
60.0	60.0	0.0	0.00
64.0	64.0	0.0	0.00
78.0	64.0	14.0	196.00
96.0	96.0	0.0	0.00
54.0	54.0	0.0	0.00
39.0	30.0	9.0	81.00
ΣX 551.2	494.0	57.2	957.84
$\bar{x}$ 61.24	56.0	6.35	

$s_{\bar{d}} = 2.87$

$t = 2.21$, for 8 df

TABLE 11

HEMATOCRIT VALUES IN DOGS INTRAVENOUSLY INFUSED WITH 30 mk/kg ASPIRIN DISSOLVED IN 90 PER CENT ETHYL ALCOHOL, FOLLOWED BY INTRAVENOUS INJECTION OF 0.4 mg/kg (LD/80) *E. coli* ENDOTOXIN

Hematocrit change, percentage		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
29.00	33.50	- 4.5	20.25
20.00	24.50	- 4.5	20.25
36.00	38.00	- 2.0	4.00
27.00	26.50	0.5	.25
37.00	37.00	0.0	0.00
38.00	44.00	- 6.0	36.00
31.00	35.00	- 4.0	16.00
35.00	52.00	-17.0	289.00
30.00	28.50	1.5	2.25
ΣX 283.00	319.00	-36.0	388.00
$\bar{x}$ 31.44	35.44	4.0	

$s_{\bar{d}} = 1.84$

$t = 2.18$, for 8 df

TABLE 12

BLOOD pH IN DOGS INTRAVENOUSLY INFUSED WITH 30 mg/kg ASPIRIN DISSOLVED IN 90 PER CENT ETHYL ALCOHOL, FOLLOWED BY INTRAVENOUS INJECTION OF 0.4 mg/kg (LD/80) *E. coli* ENDOTOXIN

pH change		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
7.27	7.25	0.02	0.0004
7.32	7.15	0.17	0.0029
7.24	7.22	0.02	0.0004
7.37	7.31	0.06	0.0036
7.33	7.36	- 0.03	0.0009
7.29	7.30	- 0.01	0.0001
7.33	7.34	- 0.01	0.0001
7.27	6.97	0.30	0.0900
7.35	7.30	0.05	0.0025
ΣX 65.77	65.20	0.57	0.1009
$\bar{x}$ 7.31	7.24	0.063	

$s_{\bar{d}} = 3.25$

$t = 0.016$, for 8 df

TABLE 13

PLASMA ZINC CONCENTRATION IN DOGS INTRAVENOUSLY INFUSED WITH 30 mg/kg ASPIRIN, DISSOLVED IN 90 PER CENT ETHYL ALCOHOL

Zinc µg/100 ml		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
64.00	64.00	0.00	0.00
32.40	32.40	0.00	0.00
60.00	45.00	15.00	225.00
63.00	51.60	11.40	129.96
51.60	51.60	0.00	0.00
39.00	27.00	12.00	144.00
39.00	39.00	0.00	0.00
46.80	46.80	0.00	0.00
65.80	65.80	0.00	0.00
ΣX 461.60	432.20	38.40	498.96
$\bar{x}$ 52.28	47.02	4.26	

$s_{\bar{d}} = 2.15$

$t = 1.981$, for 8 df

TABLE 14

HEMATOCRIT VALUES IN DOGS INTRAVENOUSLY INFUSED WITH 30 mg/kg ASPIRIN, DISSOLVED IN 90 PER CENT ETHYL ALCOHOL

Hematocrit change, percentage		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
37.00	37.00	0.00	0.00
25.00	24.50	0.50	0.25
36.50	33.00	3.50	12.25
31.00	31.00	0.00	0.00
35.00	34.00	1.00	1.00
30.00	33.00	- 3.00	9.00
35.00	35.00	0.00	0.00
31.00	33.50	- 2.50	6.25
38.00	32.00	6.00	36.00
ΣX 298.50	293.00	5.50	64.75
$\bar{x}$ 33.16	32.59	0.61	

$s_{\bar{d}} = 0.94$

$t = 0.1$, for 8 df

TABLE 15

BLOOD pH IN DOGS INTRAVENOUSLY INFUSED WITH 30 mg/kg ASPIRIN, DISSOLVED IN 90 PER CENT ETHYL ALCOHOL

pH change		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
7.12	6.97	0.15	0.0225
7.31	7.40	- 0.09	0.0081
7.29	7.36	- 0.07	0.0049
7.20	7.25	- 0.05	0.0025
7.33	7.37	- 0.04	0.0016
7.35	7.29	0.06	0.0036
7.27	7.35	- 0.08	0.0064
7.29	7.28	0.01	0.0001
7.35	7.38	- 0.03	0.0009
ΣX 65.51	65.65	- 0.14	0.0506
$\bar{x}$ 7.28	7.29	0.015	

$s_{\bar{d}} = 0.02588$

$t = 0.60$, for 8 df

TABLE 16

PLASMA ZINC CONCENTRATION IN DOGS INTRAVENOUSLY INFUSED WITH 90 PER CENT ETHANOL 0.2 ml/kg BODY WEIGHT

Zinc µg/100 ml		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
62.00	62.00	0.00	0.00
99.00	60.00	39.00	1,521.00
79.80	79.80	0.00	0.00
43.00	43.00	0.00	0.00
60.00	60.00	0.00	0.00
52.00	37.20	14.80	219.04
60.00	49.50	10.50	110.25
39.00	39.00	0.00	0.00
54.00	54.00	0.00	0.00
46.20	46.20	0.00	0.00
ΣX 595.00	530.70	64.30	1,850.29
$\bar{x}$ 59.50	53.07	6.43	

$s_{\bar{d}} = 4$

$t = 1.61$, for 9 df

TABLE 17

HEMATOCRIT VALUES IN DOGS INTRAVENOUSLY INFUSED WITH 90 PER CENT ETHANOL 0.2 ml/kg BODY WEIGHT

Hematocrit change, percentage		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
35.50	32.00	3.5	12.25
40.00	39.00	1.0	1.00
33.00	31.00	2.0	4.00
30.00	28.00	2.0	4.00
33.00	32.00	1.0	1.00
25.50	22.00	3.5	12.25
40.50	38.00	2.5	6.25
31.00	31.00	0.0	0.00
34.50	33.50	0.5	0.25
32.00	35.00	- 3.0	9.00
ΣX 335.00	321.50	13.0	50.00
$\bar{x}$ 33.50	32.15	1.3	

$s_{\bar{d}} = 0.5941$

$t = 2.19$, for 9 df

TABLE 18

BLOOD pH IN DOGS INTRAVENOUSLY INFUSED WITH 90 PER CENT ETHANOL, 0.2 ml/kg BODY WEIGHT

pH change		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
7.24	7.30	- 0.06	0.0036
7.31	7.37	- 0.06	0.0036
7.30	7.34	- 0.04	0.0016
7.30	7.31	- 0.01	0.0001
7.35	7.40	- 0.05	0.0025
7.29	7.28	0.01	0.0001
7.38	7.35	0.03	0.0009
7.36	7.35	0.01	0.0001
7.29	7.29	0.00	0.0000
7.32	7.32	0.00	0.0000
ΣX 73.14	73.31	0.17	0.0125
$\bar{x}$ 7.314	7.331	0.017	

$s_{\bar{d}} = 0.01$

$t = 1.7$, for 9 df

TABLE 19

PLASMA ZINC CONCENTRATION IN DOGS INTRAVENOUSLY INJECTED WITH *E. coli* ENDOTOXIN, 0.4 mg/kg BODY WEIGHT

Zinc µg/100 ml		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
80.00	56.00	24.00	576.00
87.00	84.00	3.00	9.00
52.00	37.00	15.00	225.00
60.00	52.00	8.00	64.00
105.00	96.00	9.00	81.00
108.00	60.00	48.00	2,340.00
60.00	52.00	8.00	64.00
46.00	46.00	0.00	0.00
ΣX 598.00	483.00	115.00	3,349.00
$\bar{x}$ 74.75	60.37	14.37	

$s_{\bar{d}} = 5.50$

$t = 2.67^{*}$, for 7 df

TABLE 20

HEMATOCRIT VALUES IN DOGS INTRAVENOUSLY INJECTED WITH 0.4 mg/kg (LD/80) E. coli ENDOTOXIN

Hematocrit change, percentage		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
32.00	31.00	1.00	1.00
35.00	46.00	- 9.00	81.00
35.00	35.00	0.00	0.00
33.00	32.00	1.00	1.00
35.00	40.00	- 5.00	25.00
28.00	31.00	- 3.00	9.00
30.00	28.50	1.50	2.25
38.00	41.50	- 3.50	12.25
ΣX 266.00	285.00	-17.0	132.50
$\bar{x}$ 33.25	35.62	2.12	

$s_{\bar{d}} = 1.31$

$t = 1.62$, for 7 df

TABLE 21

BLOOD pH IN DOGS INTRAVENOUSLY INJECTED WITH 0.4 mg/kg (LD/80) E. coli ENDOTOXIN

pH change		X_1-X_2	$(X_1-X_2)^2$
Before treatment X_1	2 hr post-treatment X_2		
7.42	7.17	0.25	0.0625
7.20	7.17	0.03	0.0009
7.34	7.14	0.20	0.0400
7.29	7.19	0.10	0.0100
7.31	7.34	- 0.03	0.0009
7.22	7.24	0.02	0.0004
7.35	6.99	0.36	0.1296
7.29	7.15	0.14	0.0196
7.18	7.15	0.03	0.0009
ΣX 66.50	64.54	1.10	0.2648
$\bar{x}$ 7.38	7.17	0.122	

$s_{\bar{d}} = 0.191$

$t = 0.64$, for 8 df