A POSSIBLE ANIMAL MODEL FOR ACUTE CANTHARIDIN POISONING IN THE EQUINE

By

ERNESTINA SEIBOLD BUCHANAN

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1977

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1979

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Thesis Approved:

Lester L. Rolf, Jr.
Thesis Advisor

Arthur R. Craigmill

Louis G. Stratton

Norman N. Durham
Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to Dr. Lester Rolf, my major advisor, for his help, guidance and patience without which I would not have completed this project. I am also grateful to him for the many hours he spent working in my behalf and for kindling in me an interest in research.

To Dr. Louie Stratton and Dr. A.L. Craigmill, I would like to extend my thanks for their efforts as members of my committee and for their guidance in preparing this thesis. My thanks also go to Dr. Roger Panciera for his advice in developing this study and to Dr. William Saunders for his aid in identifying and evaluating microscopic lesions. Dr. Jerry Hurst, Dr. Richard Orts, Dr. Charlotte Ownby and Dr. Ted Staley all provided me with access to equipment and facilities in their labs. For this I am truly grateful.

A special thanks goes to my parents, Mr. and Mrs. Max R. Seibold, and the rest of my family for their encouragement and for being there whenever I needed them.

Finally, I would like to express my appreciation to my husband, Henry, and son, Scott, for making it all worthwhile.

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CHAPTER I

INTRODUCTION

Cantharidin has known a variety of uses both therapeutic and malicious. As the active principle derived from dried blister beetles, cantharidin has been used in veterinary medicine as a blistering agent and counter-irritant. Its application externally causes an intense irritation of the skin resulting in blistering with slight penetration. Red Mercuric Iodide is sometimes combined with cantharidin to promote deeper penetration (Daykin, 1960). As a counter-irritant, this compound has been used in cases of rheumatism, for aid in closing open joints and as a stimulant of hair growth in alopecia (Milks, 1949).

Although cantharidin is at times used therapeutically, it also possesses tremendous toxic properties. Once widely used as an aphrodisiac, commonly termed Spanish Fly, it is now used in this manner only by the uninformed. Its administration internally results in no increase in sexual desire although engorgement of erectile tissue may occur in both males and females (Daykin, 1960).

Cantharidin is present in the striped blister beetles of the family Meloidae. These beetles of the genus Epicauta are known to cause severe toxic problems in

horses that have ingested them along with their hay (Panciera, 1972). A colic type syndrome develops in the affected animal which often progresses through a fatal course. Diagnosis at the present time is based primarily on finding evidence of the ingested beetle in the stomach on post mortem exam or by finding the actual blister beetles in the animal's hay.

A total of 66 species and subspecies of blister beetles are known to exist in Oklahoma (Arnold, 1964). These insects are common and severe pests that feed upon a variety of plants. Potatoes, tomatoes, beets, ironweed and legumes are among the more common plants affected (Edwards, 1949). It is the legumes that are responsible for the problem seen in veterinary medicine. The blister beetles often feed in great hordes, overrunning vast acreages (Gilbertson and Horsfall, 1940). Modern methods of cutting and baling alfalfa results in blocks or bales of hay that may contain large numbers of beetles. Animals that later consume this hay are able to ingest fatal levels of cantharidin.

Although the first case of cantharidin poisoning in the United States was reported in 1963, a method to diagnose the problem antemortem without the actual identification of blister beetles in hay has not been developed. Without a definite diagnosis, treatment is often purely symptomatic. It is then the purpose of this research to identify physiological, biochemical and histopathological

alterations occuring in a rabbit model of cantharidin toxicity. By developing an animal model, it is possible that a greater insight into the pathogenesis of the problem could be found leading to more accurate diagnosis and treatment of such cases.

CHAPTER II

LITERATURE REVIEW

Physio-chemical Properties of Cantharidin

Cantharidin is an anhydride of canthardic acid which is crystalline in nature and forms soluble salts with alkalies (Milks, 1949). This compound is colorless and odorless causing intense irritation and rubefacient action when dilute and blistering when stronger (Daykin, 1971). This vesicant is soluble in acetone, chloroform, ether, ethyl acetate and oils (Swinyard, 1970). Cantharidin is absorbed through the skin and gastrointestinal tract and excreted almost exclusively via the kidneys (Stecker et al., 1960).

Equine Poisonings

Cantharidin poisoning in the equine can be illustrated by several case reports. The first documented case of cantharidin poisoning was reported in 1963 by Moore. This instance involved an eight-year-old mare with a history of ingesting ground alfalfa hay containing blister beetles identified as Epicuata vittata. The animal devel-

oped an elevated temperature, rapid respirations and pulse along with congested cyanotic mucus membranes. She exhibited muscle rigidity and soon became unconscious. After regaining consciousness the mare voided large volumes of urine, was polydipsic and had developed ulcers of the cheeks, tongue and gums. Bloody mucus was present in the feces. The muscular rigidity was still present and treated with intravenous calcium. The horse recovered in two weeks.

Lee (1968) reported the acute death of both a horse and a mule following the ingestion of dead blister beetles in their feed. Each animal exhibited intense abdominal pain and an increase in hematocrit to 60 per cent prior to death. The horse passed a thin bloody fluid rectally and developed an elevated temperature. Necropsy showed a perforated stomach and fluid filled intestines. The mule died with similar symptoms. Dead blister beetles found in the animals' hay were ground and administered to a Shetland pony. The pony was given a total of 1.4 grams of the ground beetles over a two-day period. He subsequently died and was necropsied. The results of the post mortem included red, hemorrhagic and edematous ureters and bladder along with a reddened gastric mucosa. Sloughing of the duodenal mucosa and blood tinged jejunal fluid were also observed.

A group of 21 naturally occurring cases of cantharidin poisonings was compiled by Schoeb (1977). The clin-

ical signs most frequently observed in this retrospective study were abdominal pain, increased heart rate and respirations, congested mucous membranes and a slowed capillary refill time. Occasionally sweating, soft stools or decreased intestinal motility were noted. In fatal cases muscle spasms were often observed and the course of the disease was frequently two days in length or less. Frequent urination and urinary tract abnormalities such as hematuria were seen more commonly in animals affected for two days or more. Milder but similar clinical signs were observed in non-fatal cases. Frequent urination was a more common observation in the non-fatal cases than was abdominal pain.

Laboratory data compiled from this group included an increase in packed cell volume up to 59 per cent and an increase in serum protein content. The majority of cases had elevated white cell counts up to 17,000/mm^3. Band cell and monocyte numbers were also elevated in some cases. All white blood cell counts returned to normal when the animal recovered. Blood urea nitrogen levels were increased up to 50 mg/dl especially during the early phase of the illness. Serum potassium, sodium and chloride were not significantly affected but calcium levels dropped in four of six horses monitored. The hypocalcemia was most pronounced on the first two days of illness and returned to normal in three to four days. All horses

developed increased serum glucose levels in the early stage of the disease.

Urinalysis was performed on several of the affected horses. The urine specific gravity was usually low whether or not the animals had been given intravenous fluids. Epithelial cells and red blood cells were seen in five of the cases. There was no evidence of casts in any of the monitored animals. A mild proteinuria was observed in one case.

Gross pathological studies of the fatal cases were also obtained. The esophagus was normal in the majority of cases but several animals exhibited evidence of mucosal detachment. The stomachs and intestines of most cases were reddened and some developed a pseudomembranous inflammation. Submucosal edema and fibrin strands were evident in many of the intestinal tracts. Although no lesions of the cecum were noted, the large intestine in over half the cases contained abnormally watery contents. Mucosal hyperemia in this area was also observed.

In horses living at least five days, kidneys were slightly enlarged, pale and some had single or multiple infarcts. Renal pelvises often contained an increased amount of mucus. Ureters were normal in some of the animals and petechiated in others. All animals necropsied showed abnormalities of the bladder. The lesions described ranged from hyperemia to hemmorhagic. Myocar-

dial damage was observed in five horses. The internal and external surfaces of the ventricles were most commonly affected. Earlier lesions were observed in an acutely ill animal that died within seven hours of onset of clinical signs.

Microscopic lesions were also presented in this study. More common and significant lesions described included necrosis and separation of esophageal epithelium and vascular engorgement of the gastric mucosa. Intestinal lesions were mostly confined to the villous tips where necrosis was observed. In some cases lamina propria and submucosa were also involved. In the kidney degenerative changes of the tubules were observed although no necrosis was evident. More commonly the collecting ducts were more severely affected. Lesions in all kidneys were mild with the exception of one case. Denuded epithelium, hemmorhagic areas and acute inflammation were common observations in the bladder. Focal and some diffuse areas of myocardial necrosis was observed in all animals except the most acute death. Edema of the muscle fibers along with some mineralization was observed. Lung tissue from seven horses were edematous and hyperemic.

Human Poisonings

Cases of human toxicity attributed to cantharidin are often the result of the erroneous idea that the chemical has aphrodisiac properties. Although the majority of

reports are due to this misuse, a number of cases have been attributed to the ingestion of blister beetles by young children. In one such case reported by Wertelecki et al. (1967), a 10-month-old child ingested a blister beetle and then shortly after vomited the insect along with a volume of blood. Blood and clots of blood appeared in the child's urine. Urinalysis revealed hematuria, glucosuria, 3+ protein and a specific gravity of 1.010. The child also developed polyuria and hemoconcentration. An elevated white cell count of 30,302/mm^3 was present early in the course but dropped to 10,447 by the seventh day after clinical signs first appeared. The child made an uneventful recovery.

Toxicity in such cases can be local or systemic in nature as illustrated by the Wertlecki case and two others reported by Oaks et al. (1960). Vesication of mucous membranes and epithelial surfaces was noted in two of the cases along with hemorrhage and ulceration of mucosa. Other tissue alterations reported were degeneration of proximal and distal renal tubules, subendocardial hemorrhages, and liver cell damage. Common to all three cases were the classical signs of hematuria, proteinuria, increased packed cell volume and decreased serum calcium.

The two cases reported by Oaks involved a 45-year-old woman and a 17-year-old boy. Each had ingested cantharidin in a cocktail. Acid-base studies on the woman indicated the presence of acidosis which was treated with

sodium lactate. Both patients required tranquilization and the woman also received diphenylhydantoin for treatment of convulsions.

Experimental Studies

Bagatell et al. (1969) has reported one of the few experimental studies of cantharidin effects. In this experiment 10 mg of the drug was administered to albino rats intraperitoneally. Tissue sections for histologic examination were taken at 10 minute intervals. Damage to epithelial cells of the esophagus, stomach, small and large intestines, bladder and ureters was observed. Damage to these cells included cytolysis, separation of cells and disruption of cell structures.

In the kidney early samples exhibited glomerular congestion, and capillary engorgement. The damage progressed to vacuolation of distal tubule cells and cytolysis of tubular epithelial cells. Damage in the liver consisted of loss of cellular detail, massive hyperemia, extensive cytolysis and cytoplasmic clumping.

Tissue sections were also studies with the electron microscope. At this level there was extensive disruption of cell membranes and the nuclear envelope. Also evident was lysis of vessels and tubules of the endoplasmic reticulum. Mitochondrial swelling followed by shrinkage was observed.

In a study by Schoeb and Panciera (1978), five horses

were experimentally poisoned with ground blister beetles. The dosage varied from three to six grams of the ground beetles. The actual cantharidin content of blister beetles is somewhat variable (Carrel and Eisner, 1974) but is in the range of 0.6 per cent to five per cent of body weight (Wertlecki et al., 1967).

Of the five horses experimentally poisoned, four died. All five animals showed signs of abdominal pain and depression. Some animals exhibited frequent urination or erections. The course of illness ranged from four hours to six days with the sole survivor exhibiting clinical signs a total of four days.

With the exception of the horse that died in four hours, blood samples were taken before administering the drug and then again one day after. All four horses had an increase in packed cell volume, some as much as 18 per cent. Three of the four developed a leukocytosis with an increase in nonsegmented neutrophils, a neutrophilia and a lymphopenia. Blood urea nitrogen levels were elevated as was the serum glutamic oxalic transaminase. Calcium levels dropped appreciably in all cases. Post mortem examination of the four horses showed lesions very similar to those described in the natural disease.

CHAPTER III

MATERIALS AND METHODS

Animal Aquisition and Care

White laboratory rabbits between two and four kg of either sex were obtained from one distributor. The rabbits were housed in the temperature-controled quarters of the Department of Physiological Sciences. Normal laboratory chow was provided along with water ad libitum.

Surgical Technique Including Anesthesia

Sodium pentobarbital was given at a dosage of 30 mg/kg via the ear vein in order to induce anesthesia. The animals were then maintained in a surgical plane of anesthesia by administering additional doses of pentobarbital to effect. Certain animals were induced by an ether drip when attempts to utilize the ear vein failed. This procedure was undesirable in that the ether produced an excessive amount of salivation and was discontinued.

Once anesthesized, the animals were placed in dorsal recumbancy and the fur clipped from the rami of the mandible caudal approximately six centimeters. An incision one-and one-half centimeters in length was made over the

trachea caudal to the larynx. Ventral neck muscles were then bluntly dissected in order to isolate the left carotid artery. Three silk ligatures were placed around the carotid being careful to avoid ligation or excessive manipulation of the vagus nerve. The most cranial ligature was tied permanently in order to occlude the carotid and prevent any backflow of blood. The central and caudal ligatures were used to temporarily inhibit blood flow through the carotid until PE 160 tubing could be inserted into a nick in the artery. The tubing was then threaded into the left ventricle. Once in the ventricle the ligatures were secured around the carotid and tubing insuring that no hemorrhage would occur. The indwelling catheter was then sutured to the animal's neck and the skin incision closed.

Attempts were made in the earlier experimental studies to place a urethral catheter just inside the urinary bladder. This was accomplished but due to the lack of suitable method of preventing the animal from removing the catheter this procedure was eliminated and the analysis of urine electrolytes omitted from the study.

Protocol for the Acute Study

Animals were allowed 24 hours to recover from the surgery before being used in the experiment. Each animal was placed in a rabbit restraint box and the indwelling catheter attached to the physiograph for measurement of

left ventricular pressure. Electrocardiogram recordings were made using the limb lead II configuration. Electrodes (25 gauge needles) were placed under the skin of all four limbs. Heart rate was also monitored using the NARCO® Physiograph. Recordings from the physiograph were made at 15-minute intervals until the conclusion of the experiment.

The animals were allowed one hour of time to accommodate to the restraint box prior to administering the cantharidin. During this time recordings of the left ventricular pressure, heart rate and electrocardiogram tracings were made at the specified 15-minute intervals. One milliliter of heparinized blood was removed anaerobically from the catheter at 30-minute intervals and used for determination of packed cell volume, plasma electrolytes and hemoglobin analysis. The catheter was flushed with normal saline following collection of the sample to help prevent clot formations. Samples taken during this period were used as controls for the data accumulated during the rest of the study.

At the conclusion of the one hour control period, the animals were administered cantharidin dissolved in five milliliters of saline and three milliliters of propylene glycol. Tubing was passed into the stomach through a plastic speculum and used to administer the drug. The tube and solution container were flushed using a small volume of saline. The dose was calculated based on the

LD_{50} for cantharidin given in the Merck Index. For rabbits, the LD_{50} is listed as 100 mg/kg of drug given by the subcutaneous route (Stecker, 1960). The first rabbit was given 40 mg/kg and died due to the effects of the cantharidin in 40 minutes. Since the intent of the study was to collect data from each rabbit over a period of several hours, the dose was decreased on each successive animal until an acceptable time span was reached. A dosage of 10 mg/kg resulted in death of the rabbit in approximately six hours. This dosage was then administered to succeeding animals.

Following the death of each animal a post mortem examination was performed and tissue samples from 10 organs were removed. The tissue samples were taken from the liver, lung, muscle, esophagus, spleen, kidney, heart, intestine, stomach and bladder. Stomach and bladder tissues were flushed with distilled water in order to decrease contamination with urine and gastric contents. Two sections of each organ were obtained. One section of each organ was placed in 10 per cent buffered formalin as a fixative for later histologic study. The second section of each tissue was weighed, dried 24 hours, and reweighed. The weights were then used to calculate the water content of that tissue. The dried tissue was then prepared for determination of its calcium content. This procedure will be described in detail under a later section. All animals not given cantharidin were treated similarly and used as

controls.

Recordings from the physiograph were compiled for each rabbit and used to determine the changes in left ventricular pressure, electrocardiogram and heart rate. The heparinized blood samples were first utilized to determine the packed cell volume and hemoglobin content. The blood was then centrifuged and the plasma separated and stored in the refrigerator for later electrolyte determination.

Hematocrit and Hemoglobin Determination

Packed cell volumes of each blood sample were determined by the microhematocrit method. After the sample was agitated to insure adequate mixing, two heparinized capillary tubes were filled with the whole blood. The tubes were placed in a centrifuge in order to separate the red cells from the plasma. The hematocrits were then read from an appropriate scale and recorded for each time period. Hemoglobin content of the whole blood was determined by the acid hematin method.

Plasma Electrolyte Analysis

Determination of plasma calcium, sodium and potassium values were made using the Perkin-Elmer Atomic Absorption Spectrometer. For plasma calcium analysis standard solutions of two, five, eight and 10 ug/ml calcium were prepared using calcium stock solution, La_2O_3, and distilled water. The spectrometer was set to the appropriate wave-

length for calcium and readings made of each of the standard solutions. Once these readings stabilized six readings were recorded for each solution and averaged. The means of the four standard solutions were used to plot the standard curve for that day's calcium analysis.

Plasma samples were then diluted by adding 0.2 ml La_2O_3 to 0.1 ml plasma and adding distilled water to bring the volume to 2 ml. The diluted samples were then run through the spectrometer and the absorbance recorded. The plasma calcium in ug/ml values could then be obtained by finding the absorbance on the standard curve. By multiplying the resultant calcium value by the dilution factor, in this case 20, and dividing by the number of micromoles of calcium per microgram the total calcium content in millimoles was calculated.

Sodium and potassium values were determined in much the same manner. The standard solutions for these electrolytes were prepared in concentrations of one, two, three and four ug/ml. The dilution factors for sodium and potassium were 2,000 and 100 respectively. New standard solutions and standard curves were prepared prior to each day's use. A sample of normal dog plasma was appropriately diluted and run in the analysis along with the experimental samples. This served as a check on the functioning of the spectrometer.

Tissue Calcium Analysis

The dried and weighed tissue sections were prepared for spectrometeric analysis of calcium content according to the method of Mayer and Kowalczyk (1972). In this procedure the tissue samples were placed in polypropylene test tubes and crushed with a glass rod. Ten ml of 0.5 N nitric acid was added to each sample, the tube covered tightly and placed on an agitating tray at room temperature. At the end of 24 hours the samples were then placed in the refrigerator until diluted and analyzed.

One ml of La_2O_3 was added to four ml of each nitric digest and this solution used to determine calcium content as described under plasma electrolytes. It should be noted that the tissues from the first five rabbits were prepared by ashing rather than nitric digestion. The ashing, however, is thought to result in an abnormally high value due to absorption of calcium ions from the crucibles used in this process. These samples were thus discarded and are not listed in the tabulated results. Tissues from all other experimental animals were analyzed for calcium ions whether or not they were treated with the cantharidin. The animals not treated thus serve as controls for the treated animals.

Histologic Studies

Tissue samples were placed in a beaker of 10 per cent

neutral buffered formalin for fixation and then changed to a fresh formalin solution after several days. Prior to imbedding and sectioning, the tissues were rinsed in distilled water. The samples were then placed in an automatic processor and imbedded in wax. Eight to 10 angstrom sections of each tissue were then made and routinely prepared. An overview of liver, kidney, ureter, stomach, bladder and intestinal sections at the light microscopic level was prepared.

Physiograph Data

As described under the protocol for the acute study, left ventricular pressure, heart rate and electrocardiogram tracings were recorded for each animal. These tracings were first recorded during the one hour acclimation period and continued at 15-minute intervals throughout the experiment. Thus the tracings made prior to drug administration serve as control values for the animal after treatment.

In order to better analyze the data, each tracing was made with the paper pulled rapidly by hand through the physiograph at a near constant speed. This procedure helped to spread the tracings over a longer paper area thus facilitating more accurate interpretation of electrocardiogram changes and aiding in the measurement of pressure differences. Each 15-minute tracing period was found and the eight central recordings used for analysis of

changes in left ventricular pressure and heart rate. The electrocardiogram was observed along its entire length.

Protocol for the Acid-Base Study

A total of six laboratory rabbits were used in this portion of the study. Each rabbit was anesthesized and prepared surgically as described under surgical technique section. These animals were also allowed 24 hours to recover and given a one-hour acclamation period in the restraint box prior to drug administration. During this period anerobic, heparinized samples of arterial blood were taken from the carotid catheter and stored in an ice water bath. The sampling was done at 30-minute intervals beginning with the acclamation period and continuing through the experiment. The samples taken during the two periods prior to drug administration served as controls for each individual rabbit.

Each animal received a dose of cantharidin equal to 20 mg/kg. As described previously, the animals were not disturbed except to withdraw the blood samples. Once the animals expired, a post mortem examination was performed immediately and tissue samples recovered as described in the acute protocol.

Anaerobic blood samples were then placed in a Beckman pH-blood gas analyzer and the data recorded. From this study the P_{CO_2}, HCO_3^-, pH, and P_{O_2} of each sample was obtained. By observing the changes in these values the

animal's physiologic state could be more accurately assessed.

Statistical Analysis

All data presented in this study reflect the mean plus or minus one standard error unless otherwise indicated. The programable Olivetti calculator was used to obtain means and standard errors. With the exception of the tissue calcium and water contents, results were evaluated by the paired Student's "t" test. "T" values giving $P \leq .05$ were considered statistically significant. Non-paired Student's "t" test was employed to evaluate tissue calcium and water content results. A programable Texas Instruments calculator was utilized to compare groups of unequal numbers and variance by standard formulae.

CHAPTER IV

RESULTS

Introduction

A total of 25 rabbits were used in this study. From this group, 12 animals received no cantharidin nor any of the saline-propylene glycol vehicle used to administer the drug. These control animals were of varying weights and of both sexes (Table I). The remaining 13 rabbits, also of a variety of weights and sexes, received cantharidin (Table II). For statistical purposes, treated rabbits were grouped together according to the dosage of cantharidin they received. Rabbits receiving dosages of five to 10 mg/kg, 25 mg/kg and 40 mg/kg were used in the protocol measuring cardiovascular pressures. Six rabbits in the 20 mg/kg group were used to evaluate the change in acid-base status during acute cantharidiasis.

Appearance and Clinical Signs Observed in Cantharidin Treated Animals

Certain clinical signs were noted in rabbits treated with cantharidin regardless of the dosage administered. However, the time span between the drug administration and

TABLE I

SUMMARY OF SIGNALMENT OF CONTROL RABBITS

Rabbit No.	Date	Sex	Body Wt. (kg)
R_1	6/ 7/77	M	2.89
R_2	6/10/77	M	2.71
R_3	6/13/77	M	3.03
R_4	6/16/77	M	2.96
R_5	6/20/77	M	2.74
R_6	6/23/77	F	3/18
R_7	6/27/77	F	2.61
R_9	7/ 6/77	M	2.47
R_{12}	7/14/77	M	2.70
R_{14}	7/22/77	M	2.70
R_{16}	7/30/77	M	3.20
R_{17}	7/31/77	M	3.00

TABLE II

SUMMARY OF SIGNALMENT & TREATMENT OF CANTHARIDIN TREATED RABBITS

Rabbit No.	Date	Sex	Body Weight (kg)	Dosage (mg/kg)	Time to Death (minutes)	Euthanized	Anesthetized During Part or all of Experiment
R_8	7/ 1/77	M	3.10	40	40		
R_{10}	7/11/77	M	2.69	40	90		
R_{11}	7/13/77	M	2.70	25	180	*	
R_{13}	7/20/77	M	3.00	25	180	*	*
R_{15}	7/27/77	M	2.81	10	180	*	
R_{18}	8/ 9/77	M	3.30	10	210		
R_{19}	8/11/77	F	2.80	5	345		
R_{20}	5/25/78	M	3.01	20	60		
R_{21}	5/30/78	F	3.10	20	180		
R_{22}	6/14/78	F	2.45	20	120		
R_{23}	6/20/78	M	2.76	20	150		*
R_{24}	6/21/78	M	2.70	20	120		
R_{25}	6/22/78	F	2.80	20	180		

onset of these signs seemed to vary with the dosage. The most common and notable of these signs included depression, an increase in the number of respirations per minute, a transition to abdominal breathing, cyanosis, and an increase in capillary refill time.

The progression of clinical signs took a similar course in all cases. The first change from the pre-treatment state was the gradual onset of depression. This sign was exhibited by all treated animals and was usually first noted about midway between drug administration and death.

Generally, the depression was progressive in nature and culminated in a coma-like state immediately preceeding death. This depression was not as marked in those rabbits receiving the largest dose of cantharidin. The two rabbits receiving 40 mg/kg exhibited a pronounced depression but not to the level of the other groups. Both rabbits in this group went through an excitatory phase seconds prior to death rather than slipping into the comatose state of the other groups. These two animals began a series of thrashing movements similar to convulsions inside the restraint box. The duration of this activity was short, and invariable ended with death.

Another clinical sign frequently observed was rapid respiration which became abdominal in nature as the experiment progressed. These changes in respiratory rate and character often coincided with the development of depression. The rate of respiration increased progressively and

then leveled off to be maintained near that point throughout the remainder of the study. This alteration of respiratory rate was observed in all treated animals except the one rabbit maintained under a general anesthetic during the bulk of the experiment. This rabbit could not alter his respiratory pattern while under the effects of the anesthetic.

Clinical signs observed in the more terminal stages of each experiment included cyanosis and an increased capillary refill time. These, too, were observed in all treated rabbits. The cyanosis was very evident in the oral mucous membranes, eyes and the thin skin of the ears. The capillary refill times of approximately one second duration prior to treatment, increased to four seconds in the terminal stages.

Hematocrit and Hemoglobin

Packed cell volumes were recorded for all rabbits except those in the acid-base study. Although no statistically significant difference was shown between the control values in each group and the values at 180 minutes, nor between the three treatment groups, a possible trend appeared to be developing (Figure 1). Group A (5-10 mg/kg) had a nine per cent increase in packed cell volume at 180 minutes post treatment. Groups B (25 mg/kg) and C (40 mg/kg) had smaller per cent increases, but mean values did increase progressively.

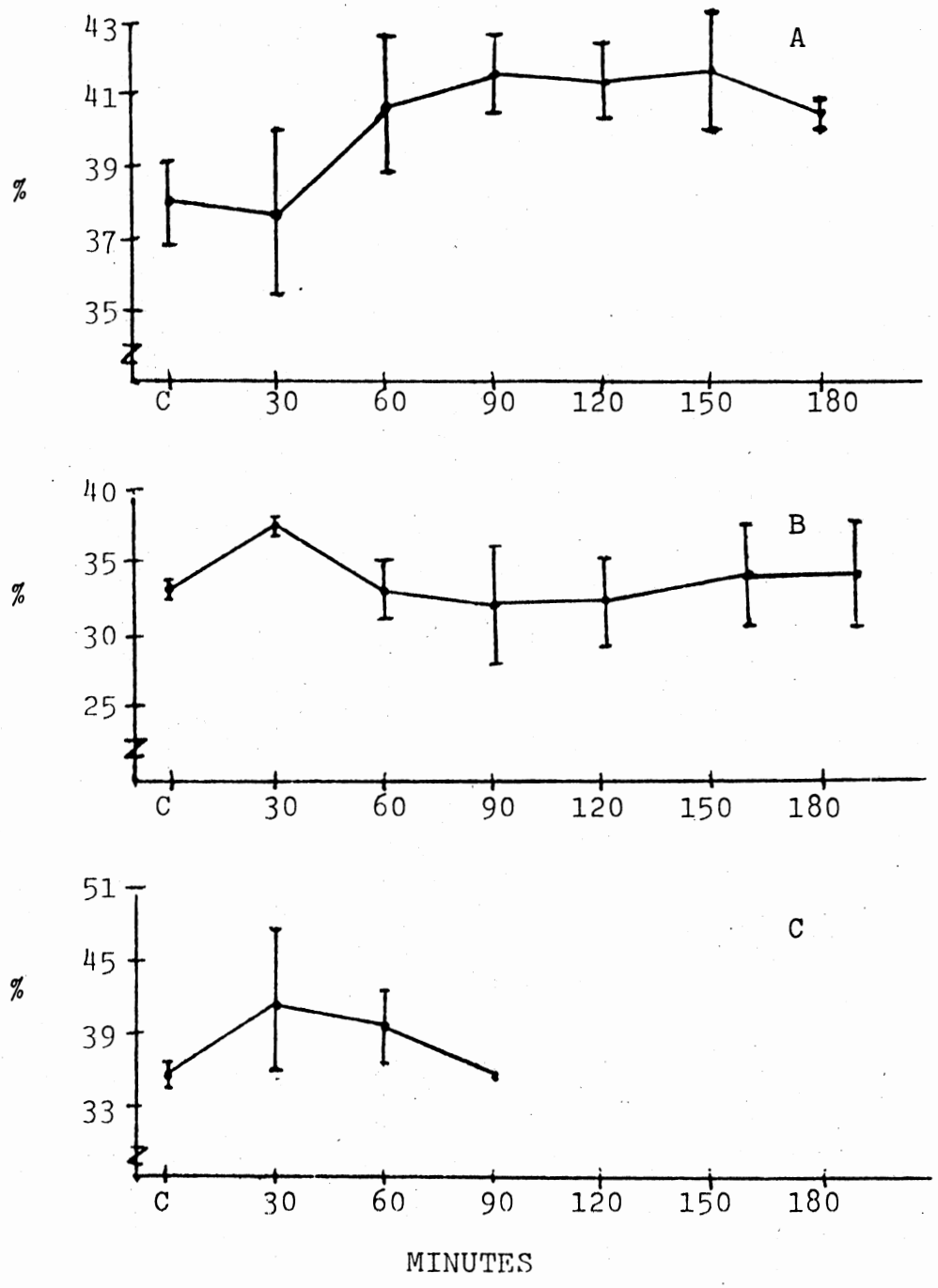

Figure 1. Time Related Changes in the Packed Cell Volume of Cantharidin Treated Animals. For control values taken from each rabbit three times (at 30 minute intervals) prior to treatment n=6-9 while there were 3 post-treatment samples in group A (5-10 mg/kg). For group B (25 mg/kg) n=2 while n=1-2 in group C (40 mg/kg) post-treatment. Values represent means ± 1 S.E.M.

The hemoglobin content of each blood sample was determined and mean values of each time period recorded for the different dose of cantharidin administered. As in the case of packed cell volumes, there was no statistical significance between the control and post-treatment values. Here again a trend of increasing hemoglobin values may have been developing (Figure 2).

Plasma Electrolytes

Arterial blood samples were obtained from cantharidin treated rabbits at 30-minute intervals beginning one hour prior to drug administration. This sampling continued throughout the experiment until the rabbit died or was euthanized. These samples were analyzed and the concentrations of plasma calcium, sodium and potassium were determined for each animal. The mean of the values from the three pre-treatment samples served as the control value for each individual rabbit. Data from each treated rabbit was grouped according to the dosage of cantharidin received and the differences between the various dosage groups evaluated. Differences between control values and values obtained post-treatment were also evaluated.

Plasma calcium concentrations exhibited no statistical significance between the various dosage groups (Figure 3). Likewise there was no statistical significance between control values and post-treatment values on an intragroup basis.

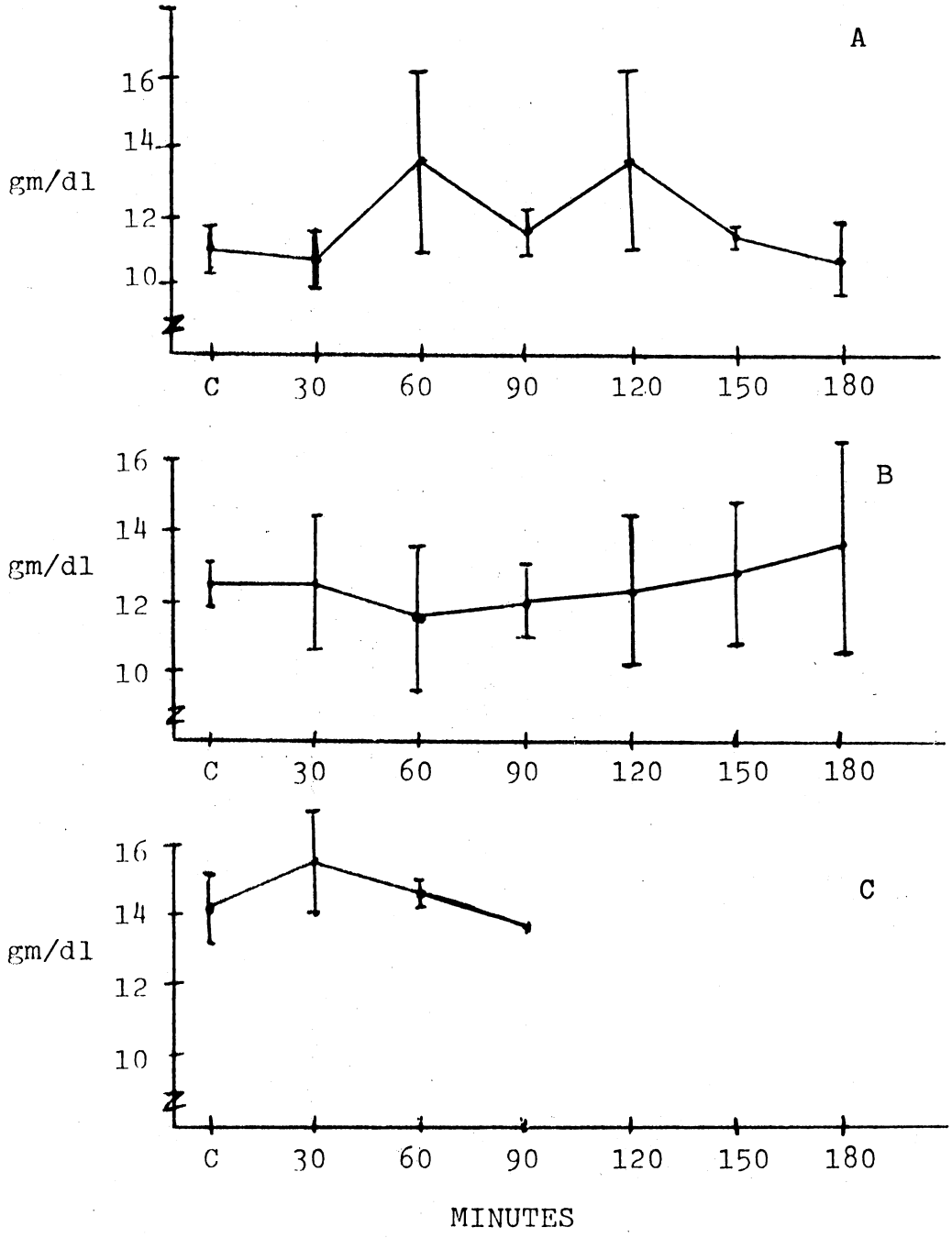

Figure 2. Time Related Changes in Hemoglobin Content of Arterial Blood from Rabbits Treated with Cantharidin. For control values taken from each rabbit three times (at 30 minute intervals) prior to treatment n=6-9 while there were three post-treatment samples in group A (5-10 mg/kg). For group B (25 mg/kg) n=2 while n=1-2 in group C (40 mg/kg) post-treatment. Values represent mean ± 1 S.E.M.

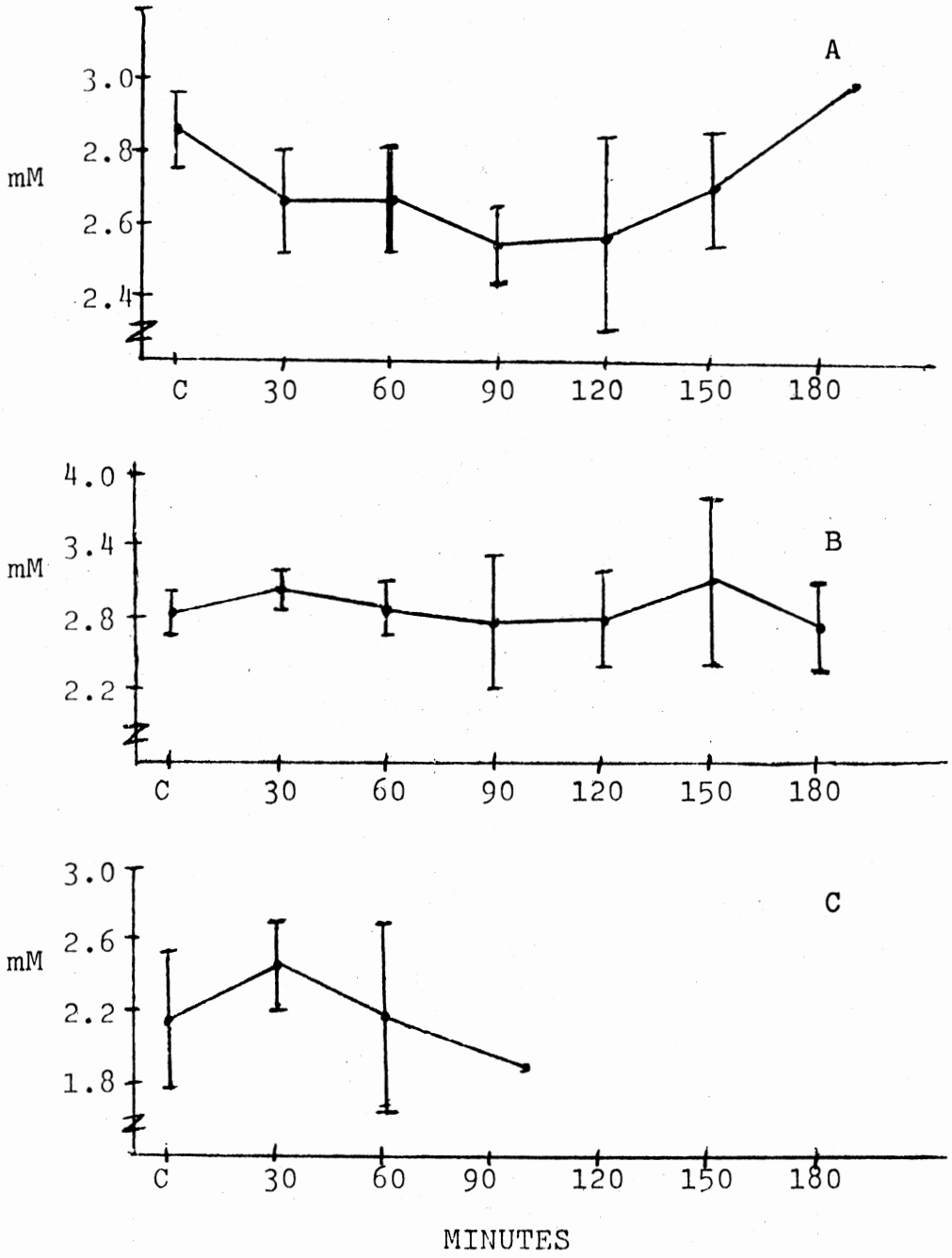

Figure 3. Time Related Changes in Plasma Calcium Concentration in Cantharidin Treated Rabbits. For control values taken from each rabbit three times (at 30 minute intervals) prior to treatment n=6-9 while there were 3 post-treatment samples in group A (5-10 mg/kg). For group B (25 mg/kg) n=2 while n=1-2 for group C (40 mg/kg) post-treatment. Values are means ± 1 S.E.M.

No statistically significant difference was observed in plasma sodium concentrations either between the different groups or within individual groups (Figure 4). Similarly, plasma potassium concentrations were not statistically different on an inter-or intragroup basis (Figure 5).

Tissue Calcium

All rabbits involved in the acute protocol and the acid-base experiment were necropsied and 10 different body tissues removed for calcium analysis. This was done in order to determine if any of these areas were sites of calcium deposition or sequestration. Tissues from control rabbits were also obtained and analyzed. The differences between the groups and the control animals were evaluated (Figures 6 and 7) by grouping the rabbits according to the dosage of cantharidin administered.

Tissues in which a statistical difference in calcium concentration was observed were the heart, intestine, kidney, bladder and stomach. The direction of change in calcium levels was often different among the dosage groups. Animals utilized in the acid-base protocol had significantly lower calcium levels in the heart, kidney, intestine and bladder. An increase in tissue calcium content was observed in the heart and intestine of the five to 10 mg/kg, 25 mg/kg and the 40 mg/kg groups. The 40 mg/kg dosage group exhibited a 30 per cent increase in calcium

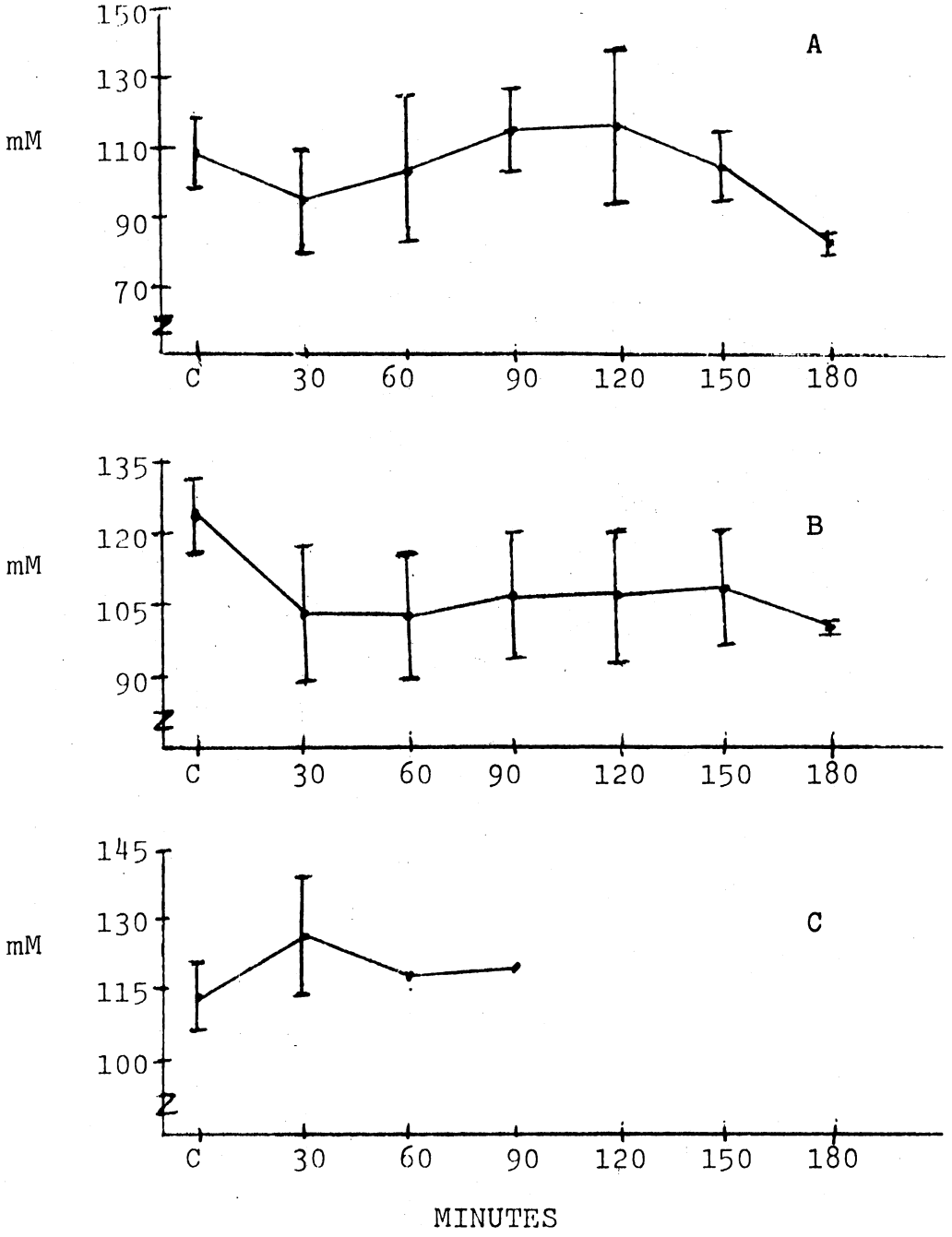

Figure 4. Time Related Changes in Plasma Sodium Concentration in Cantharidin Treated Rabbits. For control values taken from each rabbit three times (at 30 minute intervals) prior to treatment n=6-9 while there were 3 post-treatment samples in group A (5-10 mg/kg). For group B (25 mg/kg) n=2 while n=1-2 for group C (40 mg/kg) post-treatment. Values are means ± 1 S.E.M.

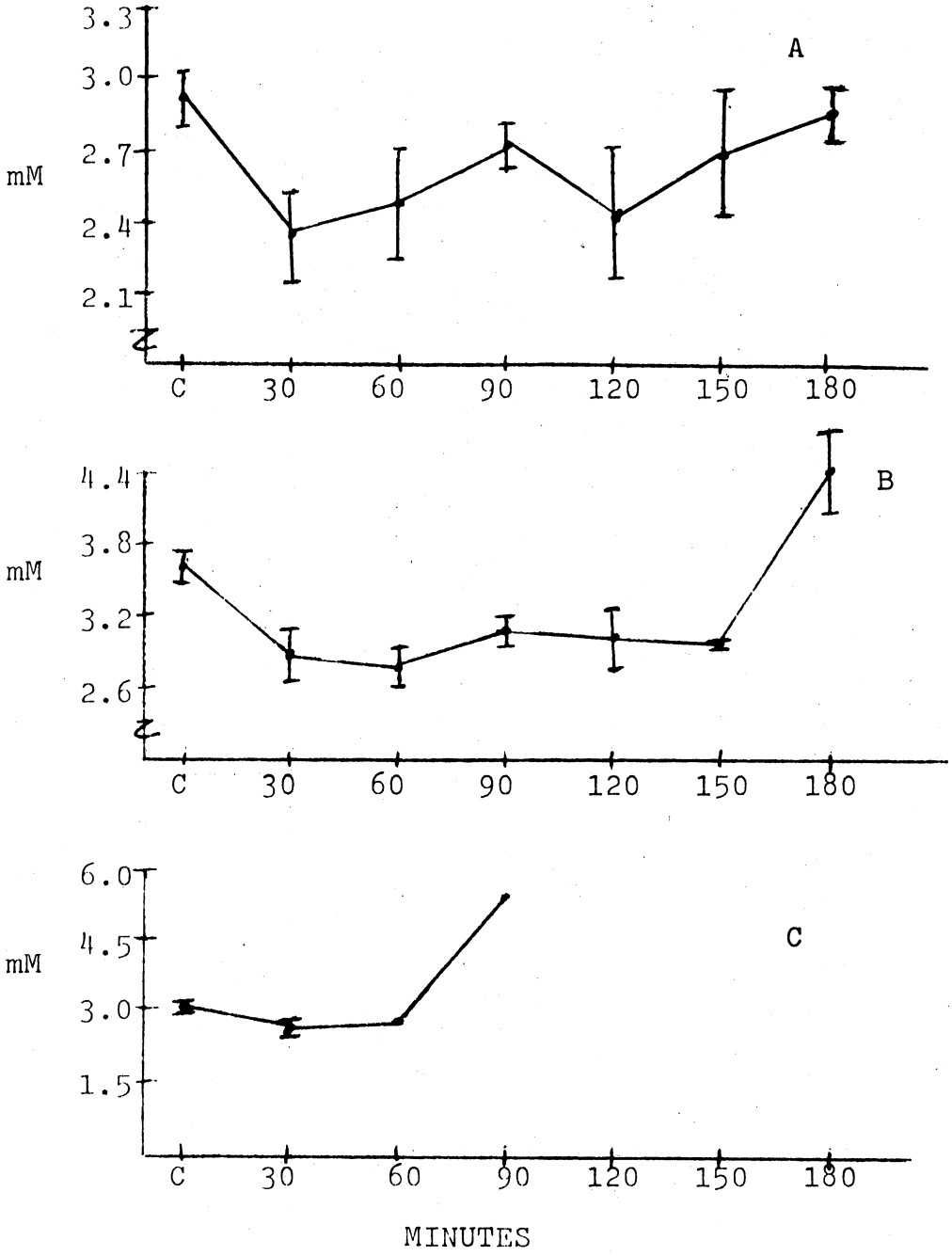

Figure 5. Time Related Changes in Plasma Potassium Concentration in Cantharidin Treated Rabbits. For control values taken from each rabbit three times (at 30 minute intervals) prior to treatment n=6-9 while there were 3 post-treatment samples in group A (5-10 mg/kg). For group B (25 mg/kg) n=2 while n=1-2 for group C (40 mg/kg) post-treatment. Values represent means ± 1 S.E.M.

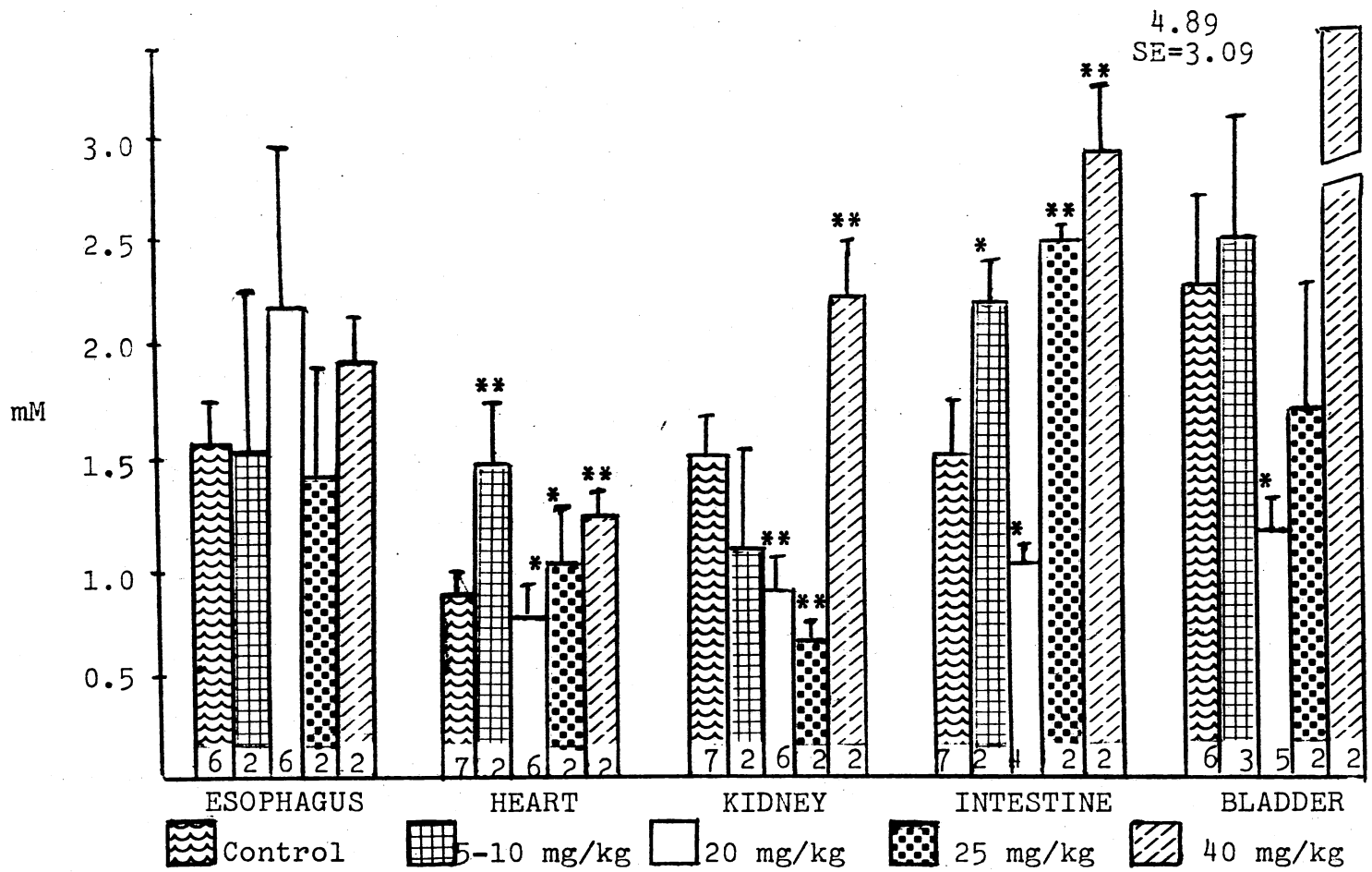

Figure 6. Calcium Concentration in Control and Cantharidin Treated Rabbits. One asterisk (*) indicates significance between control group and treated groups at P=.05, while two asterisks (**) indicate significance at P=.01. Portions not marked have no statistical significance. Values represent means ± 1 S.E.M.

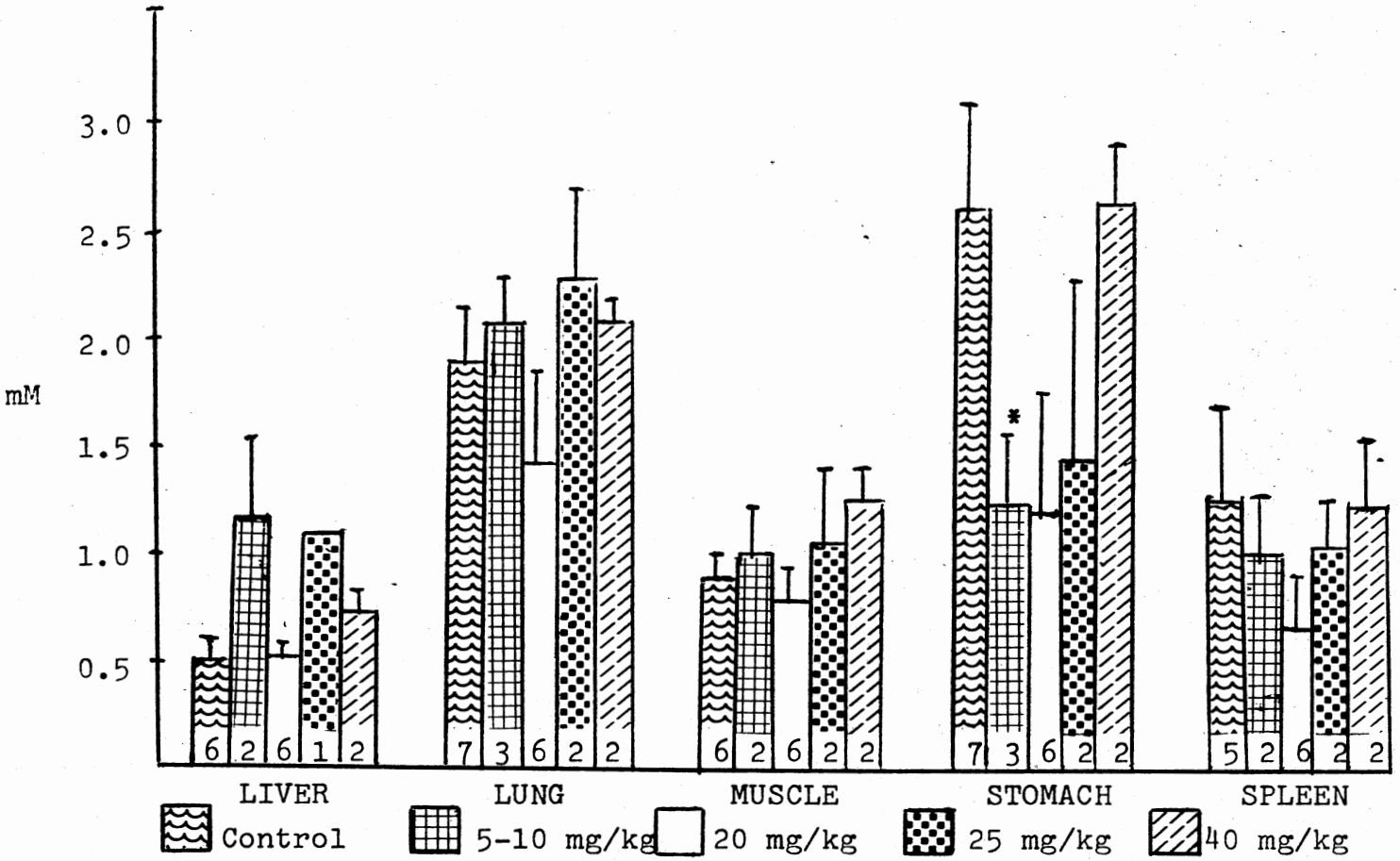

Figure 7. Calcium Concentration in Control and Cantharidin Treated Rabbits. One asterisk (*) indicates significance between control group and treated groups at P=.05, while two asterisks (**) indicate significance at P=.01. Portions not marked have no statistical significance. Values represent means ± 1 S.E.M.

level in the kidney. In the 25 mg/kg group, however, the kidney calcium level declined by 30 per cent. Calcium levels in gastric tissue were statistically significant in only the five to 10 mg/kg group. The calcium content in this case declined by approximately 50 per cent.

A second set of tissue samples were obtained from each animal. These tissues were used to determine the water content. There was no significant difference between the water content of tissues taken from the control rabbits and those taken from the rabbits receiving 20 mg/kg of cantharidin (Table III). This would indicate that little change occurred in the distribution of body water at this acute stage.

Post Mortem and Histopathology

Each rabbit treated with cantharidin was necropsied at the conclusion of the study. Tissues from various organs and structures were placed in phosphate-buffered formalin for later histologic examination. Sections were taken from areas of gross pathological lesions as well as more normal appearing structures.

In all cantharidin treated rabbits, gross lesions were observed in the gastrointestinal tract. The stomachs of all these animals were altered in color from a red to a near-purple blue. The stomach lesions covered broad patches and were most frequently found along the greater curvature and in the pyloric region. In no case was the

TABLE III

COMPARISON OF TISSUE WATER IN CANTHARIDIN TREATED AND UNTREATED RABBITS

Tissue	Control	Treated	Significance
	(gm H_2O/gm tissue)		
Liver	.6901 ± .0328	.7192 ± .0054	n.s.
Lung	.7513 ± .0177	.7590 ± .0071	n.s.
Muscle	.6921 ± .0301	.7136 ± .0127	n.s.
Spleen	.7503 ± .0115	.7470 ± .0090	n.s.
Stomach	.7878 ± .0096	.8026 ± .0066	n.s.
Esophagus	.6856 ± .0520	.7202 ± .0259	n.s.
Heart	.7753 ± .0098	.7530 ± .0068	n.s.
Kidney	.7532 ± .0156	.7996 ± .0081	n.s.
Intestine	.7475 ± .0239	.7760 ± .0271	n.s.
Bladder	.8108 ± .0255	.7676 ± .0462	n.s.

--For control group n = 6 whereas n = 5 for cantharidin treated rabbits.

--Significance established by the Student's "t" test as described in methods section. "n.s." indicates no statistical significance.

--Values reported indicate mean plus or minus one standard error.

--Treated rabbits received 20 mg/kg cantharidin.

--Water content reported on a wet weight basis.

animal held off feed, so the stomachs were generally full of ingesta. Often large patches of gastric mucosa were readily pealed from tissue.

Lesions in the upper intestines were very similar to those observed in the stomach. Intestinal lesions were a frequent finding. The lesions consisted of the same red to purple discoloration along with increased fluid in some cases. Intestinal lesions were confined to the duodenum and jejunum. Sections of intestine posterior to this area were normal on gross examination.

The urinary tracts of many treated animals exhibited evidence of damage to some degree. Kidneys were for the most part normal in size and shape. However, some were petechiated in the outer cortex. The bladders of these rabbits ranged from normal to having focal areas of hemorrhage scattered through the mucosal lining. No lesions were observed grossly in the ureters and the urethras were not examined.

Little change was observed in the heart or lungs. The lungs in some cases appeared wet and edematous, but as noted earlier no increase in content was observed. Liver, adrenals, muscle, esophagus and spleen appeared normal.

Tissues fixed in formalin were imbedded in parafin, sectioned and stained. For the purpose of this study tissues from the kidney, stomach, intestine, ureter and urinary bladder were examined for evidence of histologic

alterations. Nine cantharidin treated animals and four control animals were picked at random for study. The dosage of cantharidin administered to these rabbits ranged from five mg/kg to 25 mg/kg. Lesions were observed in all dosage groups and severity did not appear to be dose related.

Microscopic lesions were similar in nature in all five tissue types examined. The most frequent observation was sloughing of epithelial surfaces and capillary congestion. This was especially true of the stomach, intestines, bladder and ureters. Gastric mucosa often had a loss of both epithelial cells and gastric glands. In some of the worst cases, areas of extensive hemorrhage were observed. There was evidence of some inflammatory cells in several tissue sections.

Intestinal sections were similar to the stomach in type and severity of lesions. Depending on the individual rabbit involved, intestinal lesions ranged from a loss of a few epithelial cells to sloughing of villous tips to necrosis of most of the villi. Other changes included an increase in cellularity, capillary congestion, edema of the lamina propria and, in one case, numerous areas of hemorrhage. Eosinphils were observed in lamina propria of some sections.

The bladder and ureters exhibited similar lesions, those primarily being loss or disruption of the transitional epithelium and, in some instances, capillary con-

gestion or hemorrhage. The kidney sections ranged from near normal to sections with marked tubular disruption. In the most severely affected animals, renal tubules were grossly distorted due to epithelial cell death and/or destruction. In these same individuals, Bowman's space appeared to be significantly enlarged and the glomeruli congested. In less severely involved individuals, glomeruli appeared normal and tubular cells merely swollen. Proteinic material was present in tubular lumens and in the area of Bowman's space.

Two abnormalities not related to the cantharidin were found. In one control animal a relatively recent infarct was observed. This may have been the result of emboli generated in response to the left ventricular catheter. The other lesion involved a cantharidin treated animal. This rabbit's kidneys had evidence of chronic renal disease including stromal fibrosis and dialated tubules.

Physiograph Data

Measurements of heart rate and electrocardiogram tracings were obtained from six cantharidin treated animals in three different dosage groups. An increase in heart rate by 60 beats per minute was observed in all six rabbits. The onset of this tachycardia was closely related to the onset of respiratory changes.

Similar changes in electrocardiogram tracings were observed regardless of the dose of cantharidin administer-

ed. Common findings in all dosage groups were a gradual increase in height of the T wave and ventricular fibrillation immediately prior to death. Two of the five to 10 mg.kg group rabbits did not fibrillate. These animals, however, were euthanized. Rabbits in the 25 mg/kg and five to 10 mg/kg groups all exhibited a gradual decline in the height of the R wave. One rabbit in the five to 10 mg/kg group also developed a notched R wave.

Left ventricular pressures were available from so few rabbits on a consecutive basis that no conclusions could be drawn from the data. Thus the data will be omitted from this section.

Acid-Base Study

Six rabbits were utilized in this section of the study. Anaerobic arterial samples were taken from these rabbits during a pre-treatment control period and then again at 30-minute intervals. Post treatment (20 mg/kg) analysis demonstrated a marked alteration in blood gas composition (Figure 8). Although not all values were statistically significant, the drop in blood pH from a control normal of 7.44 to a low of 7.10 after treatment must be significant in terms of change in rate of cellular reactions and effect on the organism.

Arterial blood samples obtained from these six rabbits were analyzed for P_{CO_2} and the bicarbonate calculated. By 90 minutes post treatment, a statistically sig-

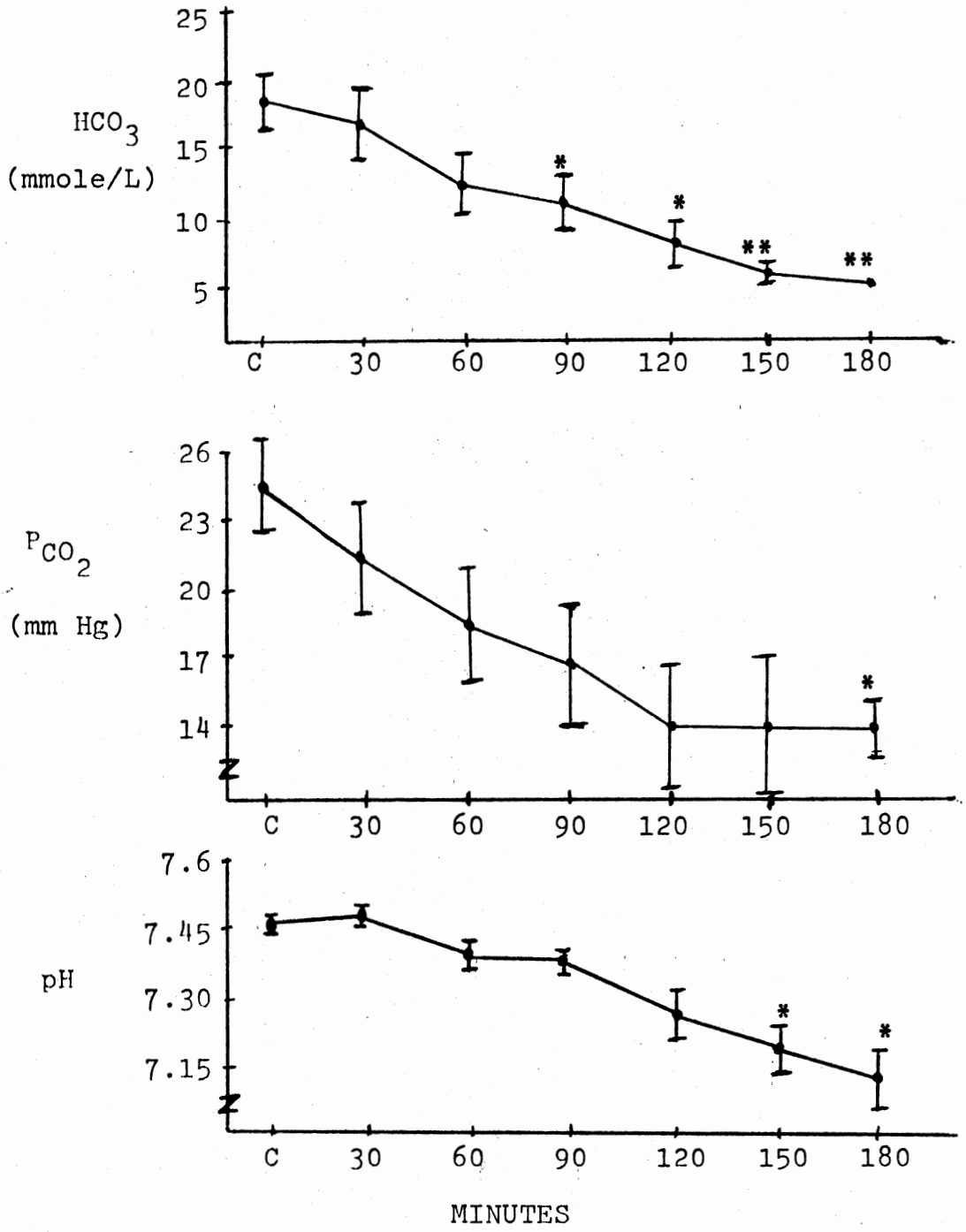

Figure 8. Time Related Changes in Arterial Blood pH, P_{CO_2} and HCO_3^-. All rabbits (n=6) treated with cantharidin at 20 mg/kg level. One asterisk (*) indicates significance at P=0.05 whereas two asterisks (**) indicate significance at P=0.01.

nificant decline in blood bicarbonate level was observed. Five of these rabbits also exhibited a significant decline in arterial P_{CO_2}. The only animal not demonstrating a lowered P_{CO_2} was under barbiturate anesthesia during the last two-thirds of the experiment. Since this animal was not treated in the same manner, the data obtained from it was deleted from the results.

CHAPTER V

DISCUSSION

Few studies have been made on the effects of purified cantharidin in animals. Although several cases of human poisonings with cantharidin in the form of Spanish Fly have been documented, animal poisonings have been limited to clinical cases and one experimental study. In these situations blister beetles were involved and cantharidin contained within them assumed to be the toxic principal. Results from this study tend to substantiate this assumption.

Reports of cantharidin poisonings in humans, whether due to the ingestion of blister beetles or a purified form of the drug, generally included very similar clinical signs. Commonly hemoconcentration, gastric hemorrhage and erosion, increased respiratory rates, convulsions and coma developed (Oaks, 1960; Wertelecki et al., 1967). Clinical signs observed in cantharidin treated rabbits correspond to the human case reports and those reported by Schoeb and Panciera (1978) in their retrospective and experimental studies. Cases documented by Moore (1963) and Lee (1968) reported coma, depression, cyanosis and rapid respirations. Although reported in these investigations,

Increased packed cell volumes were not observed in this study.

The small change in packed cell volume observed in this study could have been an accurate assessment of the situation and the trend toward hemoconcentration real in spite of the lack of statistical significance. In this study the course of clinical illness was much shorter than that of naturally occurring cases in the equine. Thus the data presented here may have been a reflection of the normal clinical picture in an acute crisis. Had the situation progressed to a chronic stage, the packed cell volumes might have continued increasing as the animal had more time to sequester fluids and hemoconcentrate.

Although a trend could have been developing, it is also possible that other factors could have caused a masking of the true picture. The primary factor involved here is one introduced experimentally in flushing the carotid catheter post sampling. Also flushing the catheter in an attempt to remove clotted blood occurred in several animals in these groups.

It is unlikely that enough fluid was administered via the catheter to significantly alter the packed cell volume. Approximately three milliliters of five per cent dextrose was used to flush the catheter following each 30-minute sampling. During the average four-hour period, approximately 24 milliliters of fluid was administered to each animal. Assuming a total body water content equal to

0.6 body weight the water content of the average experimental rabbit would be 1.8 liters. The amount of fluid administered was approximately one per cent of the normal content. This should not have had any significant effect upon the packed cell volume due to the small amount administered, the rapid equilibration of fluids between body compartments and the speedy elimination of excess fluids by the kidneys (Pitts, 1974).

Although hypocalcemia was reported in several equine blister beetle poisonings (Schoeb, 1977; Moore, 1963). it was not observed in this study. Hypocalcemia has also been observed in human poisonings. In the Oaks case, plasma potassium, calcium and sodium levels were abnormal and required fluid therapy.

The absence of hypocalcemia in this study may again be related to the difference in duration of the disease. Prolonged cases in the equine allow time for a great number of physiologic changes to occur. Anorexia, intestinal damage leading to decreased absorption ability, renal damage and possibly calcium regulating hormones may all play a part in the hypocalcemia syndrome associated with cantharidin poisoned horses. In this study the duration of the disease was very short and probably did not allow sufficient time for hypocalcemia to occur.

However, one might expect to have seen plasma potassium values increase slightly if the animals were acidotic. In such cases intracellular potassium exchanges for

extracellular hydrogen. The kidneys generally excrete excess quantities of potassium but with renal shutdown due to lowered blood pressure or renal disease, the potassium values could rise to a dangerous level in the plasma. Clinical signs presented previously along with acid-base studies indicate acidosis occurs in cantharidin treated animals.

The calcium content of various tissues in cantharidin poisoned animals has not been reported prior to this acute study. Of the 10 tissue areas sampled the kidney, heart, intestine, bladder and stomach were the only ones to exhibit any alteration in calcium content. Since these tissues are the ones in which gross and microscopic damages are frequently observed, both in this study and documented cantharidin cases, one might conclude that tissue damage and calcium content are related.

It should be noted that the changes in calcium content are not consistant in direction of change between the various dosage groups. Tissues from rabbits in the 20 mg/kg dosage group were consistently lower in calcium content than those from rabbits in other groups. A period of approximately one year separated the study involving the acute protocol and the acid-base study. During this time several changes in personnel and in brand of laboratory chow were made. Different handling of this particular group of rabbits, such as altered calcium content in the feed at the laboratory or in the supplier's ration could

account for the difference.

Changes in calcium content of tissues from rabbits in other dosage groups may not be accurate reflections of the clinical picture. The small number of rabbits involved in these dosage groups are not sufficient to draw accurate conclusions.

Many of the cases in the literature documented as cantharidiasis had evidence of gross and microscopic lesions similar to those observed in this acute study. Lee (1968) reported reddened gastric mucosa, fluid filled small bowel, and hemorrhagic ureters and urinary bladder. Renal biopsies performed on two people known to have ingested cantharidin were very similar histologically to the damage observed in this study (Oaks et al., 1960). The damage reported by Oaks (1960) included swelling of tubular epithelium, degeneration, sloughing of epithelial cells and congestion of the glomeruli. An amorphous eosinophilic material similar to that seen in some of the rabbit sections was described. No evidence of an inflammatory response was observed.

Gastrointestinal lesions observed in these cantharidin treated rabbits correspond for the most part to those reported by Schoeb and Panciera (1979). Separation of gastric mucosa could have been interpreted as autolytic changes rather than damage by cantharidin. However, necropsies were performed immediately after death in order to keep autolysis to a minimum. Eosinophils observed in

the lamina propria of some intestinal sections were probably the result of the heavy parasitism existing in several of the animals.

In general, the gross lesions observed in this study tend to support the clinical signs observed in both human and animal cases. Gastrointestinal and urinary lesions present in these cantharidin treated rabbits certainly correspond with the abdominal pain observed in the equine (Lee, 1968; Moore, 1963; Schoeb, 1977). Glomerular damage would account for the proteinuria reported in both human and equine cases. Hematuria can easily be explained by the massive destruction of transitional cells in the ureters and bladder. It should be noted that the effect of the propylene glycol and saline vehicle on these tissues is not known. Control rabbits should have been given the vehicle alone in order to accurately compare the cantharidin treated groups with the non-treated group.

Electrocardiogram alterations were consistant in four of the six recorded tracings. The ventricular fibrillation observed indicates that cardiac irritation has occurred and is probably the direct cause of death in these cases. The two rabbits that did not fibrillate prior to euthanasia may have in fact fibrillated if allowed to die from the effects of cantharidin. The frequency of fibrillation in these rabbits indicates the need for further studies. The possibility of using antiarrythmic drugs such as Lidocaine as well as cardiac glycosides to

increase cardiac output and decrease heart rate should be explored. The increased size of the T wave is indicative of acidosis or high potassium concentrations. In this study, the presence of acidosis was clearly documented, and in view of the absence of hyperkalemia, was most likely the cause of this alteration.

Acid-base studies have not been performed on an experimental basis. Oaks et al. (1960) did, however, report the presence of metabolic acidosis in one cantharidin poisoned patient. Although there has been no documentation of acid-base disturbances in equine clinical situations, the signs reported are indicative of an acidotic state. Rapid respirations, abdominal breathing, depression and eventual coma all are symptoms of acidosis.

The drop in arterial pH demonstrated in this study explains many of the clinical signs observed in cantharidin poisoned animals. Acidosis generally results in a depression of the central nervous system and at levels near 7.0 can result in coma (Guyton, 1971). Another clinical sign related to the development of an acidotic state is an increase in rate and depth of respiration. These hyperventilating efforts are an attempt to excrete excess CO_2, shifting the extracellular pH back into a more normal range. The alteration in ventilation is the result of the direct action of the hydrogen ions on the respiratory center in the medulla oblongata as well as peripheral receptors.

The P_{CO_2} values obtained in this study are indicative of a partial respiratory compensation of the acidotic state. They also tend to substantiate the fact the acidosis is metabolic rather than respiratory. Had the P_{CO_2} been elevated one would have suspected a respiratory acidosis.

Metabolic acidosis can result from several mechanisms including the formation of metabolic acids in the body, intravenous administration of metabolic acids, oral intake of metabolic acids or loss of alkali from body fluids (Guyton, 1971). In this situation the acidosis most likely resulted from the formation of metabolic acids such as lactic acid since no intravenous or oral source was provided. The loss of alkali can also be eliminated from the list of possible causes since the common ways alkali is lost from the body, vomiting and diarrhea, were not present in these animals. In severe renal disease damage can be severe enough to prevent elimination of metabolic acids from the body but this requires a longer time period than this study allowed.

CHAPTER VI

CONCLUSION

Although the course of disease in most clinical cases of cantharidiasis is of a longer duration, the findings in this acute study are very similar to those observed in the naturally occurring disease. Most of the differences between the results in this study and case reports relate to the very acute nature of this situation. Therefore, this study is probably an accurate reflection of alterations one would see in acute cantharidiasis and, as such, constitutes a legitimate model for the acute crisis.

In spite of data compiled from both experimental studies and naturally occurring clinical cases, a definite method for diagnosing cantharidin poisoning antemortem does not exist. Further studies will be required to solve this problem and investigate efficacy of possible treatment regimes. At the present time horses exhibiting signs of gastrointestinal and urinary tract damage should be considered as prime suspects especially when the diet includes alfalfa hay. Treatment in such cases may be aimed at correcting acidosis along with supporting gastrointestinal and urinary function.

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VITA[2]

Ernestina Seibold Buchanan

Candidate for the Degree of

Master of Science

Thesis: A POSSIBLE ANIMAL MODEL FOR ACUTE CANTHARIDIN POISONING IN THE EQUINE

Major Field: Physiological Sciences

Biographical:

Personal Data: Born in Lawton, Oklahoma, February 18, 1954, the daughter of Mr. and Mrs. Max R. Seibold.

Education: Graduated from Eisenhower Senior High School, Lawton, Oklahoma, in June, 1972; received Bachelor of Science degree in Agricultural Economics from Oklahoma State University in December, 1977; completed requirements for the Doctor of Veterinary Medicine degree at Oklahoma State University in May, 1979; completed requirements for the Master of Science degree in Physiological Sciences in May, 1979.