EFFECTS OF LOW AND HIGH SODIUM CHLORIDE DIETS AND FUROSEMIDE ADMINISTRATION, ON CARDIAC FUNCTION, PLASMA ELECTROLYTE CONCENTRATIONS, AND THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

BY

Cindy Marie Swancott

Thesis submitted to the faculty of the Virginia
Polytechnic Institute and State University in partial
fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Veterinary Medical Sciences

APPROVED:

W. S. Swecker

J.C. Lee

M. L. Moon

May 1998

Blacksburg, Virginia

EFFECTS OF LOW AND HIGH SODIUM CHLORIDE DIETS AND FUROSEMIDE ADMINISTRATION ON CARDIAC FUNCTION, PLASMA ELECTROLYTE CONCENTRATIONS, AND THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

CINDY M. SWANCOTT

(ABSTRACT)

Congestive heart failure is commonly treated with a low sodium diet and diuretic. The purpose of this treatment is the reduction of preload, or blood volume presented to the diseased cardiac muscle. The purpose of this study was to assess the roles of dietary sodium and furosemide on cardiac function, plasma electrolyte concentrations, and the renin-angiotensin-aldosterone system, in healthy canines. Twenty mixed-breed canines were allotted to one of four groups, Group I - Dogs fed low sodium diet (0.08% sodium), Group II - Dogs fed high sodium diet (1.0% sodium), Group III - Dogs fed low sodium (0.08%) and treated with furosemide (2 mg/kg orally (PO) every twelve hours (BID)), and Group IV - Dogs fed high sodium (1.0%) and furosemide (2 mg/kg PO BID). Cardiac function was assessed via echocardiography on days 0, 21,and 53. Plasma electrolyte concentrations were measured on days 0, 21, and 35. Activation of the renin-angiotensin-aldosterone system was evaluated on days 0, 21, 35, and 53. Low and high sodium diet with and without furosemide treatment did not alter cardiac function, plasma sodium, or plasma potassium concentrations. However, furosemide treatment combined with a low sodium diet resulted in the lowest plasma chloride concentrations, on days 21 and 35 ($p<0.05$). Furthermore, furosemide treatment resulted in significant alterations in the renin-angiotensin-aldosterone system, on days 21, 35, and 53, ($p < 0.0001$). Furosemide treatment significantly increased renin activity and aldosterone concentration. The interaction between furosemide and the low sodium diet yielded a greater increase in plasma renin activity and plasma aldosterone concentrations than furosemide administration with the high sodium diet. These results suggest direct activation of the renin-angiotensin-aldosterone system by furosemide. Future research is warranted in congestive heart failure subjects, due to the adverse affects of decreased plasma chloride concentrations and activation of the renin-angiotensin-aldosterone system.

ACKNOWLEDGMENTS:

Special thanks to:

Virginia Tech for the graduate stipend that made this thesis possible

Mark Morris Institute for the funding to make this project possible

Dr. Manis Smith at Mississippi Medical Center for analyzing the renin activities

Doctors WS Swecker, JC Lee, and ML Moon for all of their time and hard work

The VA-MD Regional College of Veterinary Medicine for supplying the animals, testing facilities and equipment

Megan Irby and Rachel Bethard for their many hours of hard work during the data collection

Chris Wakely for the animals, housing facility, and food for the project

Kevin Lovern for putting up with me during all the long hours and trying times, while I completed my thesis. I appreciate his love and support more than he will ever know

And a special thanks to Dr. WS Swecker for all the help in planning and executing the successful finish of this thesis

TABLE OF CONTENTS

APPENDICES

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Dietary alterations and drug combinations are used in the management of many disease conditions. The effects of diet and drug use individually may be known, however the interaction between diet and drug may be unknown. Interactions between diet and drug may result in physiological changes that are different and undesirable from changes caused by diet or drug alone. Physiological parameters of combination diet and drug therapy need to be evaluated prior to treatment of the patient. The changes induced by combination therapy should be known in order to accurately treat any disease condition.

The management of congestive heart failure (CHF) includes the use of diet and drug combinations, such as low sodium diets and diuretics, like furosemide. Sodium restriction in the diet often accompanies diuretic therapy to decrease blood volume, by decreasing renal water retention. Diminished blood volume decreases preload, the volume of blood presented to the heart, and vascular resistance encountered by the heart. Decreased preload and vascular resistance will decrease the effort needed to be expended by the heart to maintain cardiac output; the blood volume pumped to the tissues and organs. However, as sodium is restricted or lost due to diuretic therapy, compensatory mechanisms counteract the sodium depletion and conserve sodium, thereby maintaining sodium concentrations within normal ranges. Two components that help maintain electrolyte concentrations within normal ranges are the renal tubules and the renin-angiotensin-aldosterone system (RAS). Severe sodium restriction causes renin release, which increases aldosterone concentrations and results in increased sodium and water absorption from the distal convoluted tubule and collecting duct of the kidneys. Angiotensin II, another component of the RAS may also be harmful to a patient in CHF. Angiotensin II stimulates aldosterone release and is a potent vasoconstrictor.[9] This vasoconstriction can cause increased afterload on the cardiac muscle. Afterload is any additional wall stress on the left ventricle and may be a result of increased arterial pressure, vasoconstriction, or increased blood volume, the increased pressure in the vessels exiting the left ventricle makes it harder for blood to exit the left ventricle. In the cardiac patient with left heart failure, increased afterload likely decreases cardiac output and often causes fluid to pool in the lungs (pulmonary edema), around the lungs (pleural effusion), or in the abdomen (ascites). The aforementioned side effects can be devastating to a patient in CHF. [19]

To the author's knowledge, the effects of a combination of sodium restriction and furosemide treatment has not been evaluated in canines. In order to gain further understanding of the combination therapy used most commonly in CHF, this study investigated the effects of low and high sodium diets with and without furosemide administration, in healthy adult dogs.

CHAPTER I

LITERATURE REVIEW

Therapy for heart failure in canines is aimed at relieving fluid accumulation due to excessive cardiac filling pressures as needed to improve tissue oxygenation and patient comfort. This may be accomplished by restricting sodium intake and/or administering a diuretic. Diuretic treatment decreases blood volume by increasing sodium chloride and water excretion via the renal tubules. The body, regardless of species, regulates plasma sodium concentrations by the release of renin and aldosterone. A desired therapeutic goal in heart disease, reduction of ventricular filling pressure, may be counteracted if the renin-angiotensin-aldosterone system (RAS) is activated. Sodium and other electrolytes are known to affect RAS activity. Diuretics, in addition to increasing sodium chloride excretion, may also directly affect RAS activity. [14] Combination therapy of sodium restriction and furosemide administration needs to be evaluated in healthy adult dogs, so that studies on dogs with cardiac disease may follow.

Effects of Electrolyte Alterations on the RAS

Electrolytes exist intracellularly and extracellularly. Maintenance of all electrolytes within normal ranges maintains health. The kidney is one of the first organs to detect changes in body electrolytes. Macula densa cells located in the juxtaglomerular apparatus of the renal nephron release renin in response to decreased solute concentrations of the distal tubular fluid.[12] Alterations in any plasma electrolyte will alter renal solute load, therefore all body electrolytes play a role in activation of the RAS system.

Abboud et al [1] concluded that chloride depletion is a potent stimulus for renin release in rats that were selectively chloride depleted without being sodium depleted. Their experiments demonstrated a significant increase in renin activity in the face of chloride depletion without changes in arterial blood pressure, glomerular filtration rate, filtered sodium load, arterial pH, or plasma bicarbonate. Chloride depletion stimulated renin release via a renal tubular (macula densa) mechanism, due to decreased solute in the tubular fluid.

Similar to Abboud, Shade et al showed[22] that potassium depletion increased plasma renin activity, which increased aldosterone, in normal and hypertensive dogs. In addition, direct intrarenal arterial infusion of potassium chloride in normal or acutely sodium-depleted anesthetized dogs decreases renin activity, which decreases aldosterone. The decrease in renin activity was most likely due to the presence of increased solute in the tubules. The role of potassium supplementation in the treatment of congestive heart failure (CHF) is unknown. Potassium supplementation may decrease plasma renin levels, which will lead to decreased AgII. Angiotensin II causes cardiac hypertrophy and has been shown to cause myocyte injury.[19] The authors make the case that cardioprotective effects might be obtained by feeding dogs with heart failure a diet high in potassium. A more obvious advantage of such a diet is the reduced risk of hypokalemia in patients treated with diuretics. [18]

Sodium Requirements and Affects on RAS

The Association of American Feed Control Officials (AAFCO) recommends the minimum sodium requirement for adult dogs to be 0.06% of dry matter or 170 mg/ 1000 Kcal metabolizable energy. Most commercial dog foods contain 0.2 - 1.0% sodium. Thus, canine sodium requirements are exceeded 3 to 16 fold under normal feeding conditions. Healthy dogs can compensate for dietary sodium changes by increasing or decreasing sodium excretion in both urine and feces.[12] In healthy canines, total sodium output correlates with total sodium input. [10]

Animals fed sodium restricted diets experienced changes within juxtaglomerular cells. Spangler demonstrated that when dogs were fed a low sodium diet (<1mEq Na+/dog/day), the juxtaglomerular cell numbers increased in order to store increased amounts of renin. [23] The number of cell nuclei associated with each juxtaglomerular apparatus increased significantly. In some cases, the juxtaglomerular cells contained such numerous numbers of renin secretory granules that many of the organelles were obscured. The increased granularity was attributed to hyperplasia under the stimulus of sodium restriction. The result is increased renin secretion by the macula densa specialized cells. The elevated cytoplasmic granules were rapidly diminished in the canine juxtaglomerular cells, after eight days on the sodium restricted diet. Increased demand for the renin being stored in the granules became apparent as time passed for the dogs that consumed restricted sodium diets. The small amount of granularity in the face of large amounts of plasma renin activity indicate that renin was being depleted from the secretory granules and being produced at an increased rate by hyperplastic organelles. As time passed, the organelles were elaborating the renin directly, bypassing a cytoplasmic storage phase in the secretory granules, as was seen at the beginning of the study. Juxtaglomerular cells in the dogs fed normal sodium concentrations (40mEq Na+/ dog/day), rarely contained renin secretory granules because renin is not released when sodium intake is adequate. [24] Renin is released due to decreased renal perfusion and decreased solute concentrations in the renal tubules (Figure 1). Decreased plasma potassium, chloride, and sodium all activate renin release.

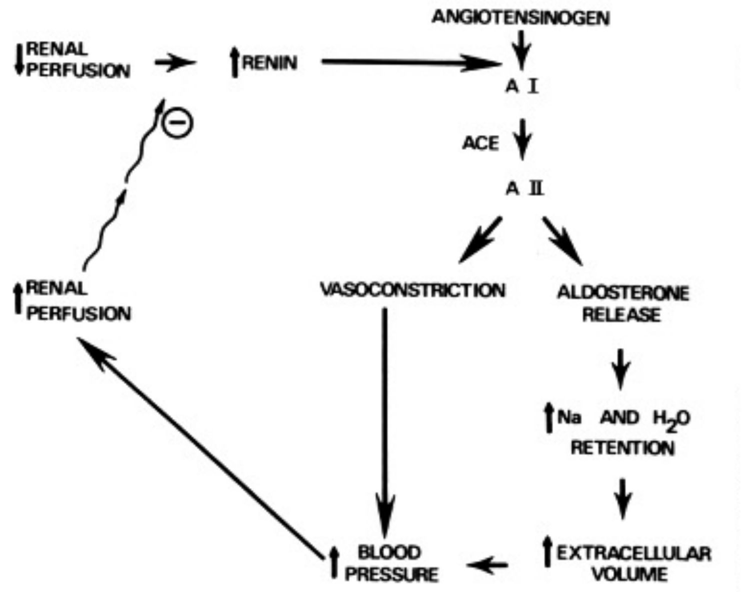

Figure 1- The Renin-Angiotensin-Aldosterone System. [7]

Furosemide: Biological Action and Role with Electrolytes

Furosemide is a loop diuretic. The term loop diuretic encompasses pharmacologic compounds that exert their primary action on the thick ascending loop of Henle.[4]

Oral furosemide has 40% bioavailability and is extensively bound to plasma proteins. The diuretic effect is apparent within 30 minutes after oral administration and peaks in 1-2 hours. Furosemide is secreted into the proximal tubule of the renal nephron (Figure 2). Once in the lumen, furosemide inhibits active reabsorption of chloride in both the medullary and cortical portions of the loop of Henle. Chloride is cotransported with sodium and potassium, therefore concentrations of all three plasma electrolytes are believed to decrease during furosemide administration. Electrolyte disorders are the most common adverse effects of all diuretics. [4]

Furosemide treatment of dogs with heart failure has been reported to reduce plasma concentrations of potassium, magnesium, sodium, and chloride as compared to healthy control dogs and untreated dogs with dysrhythmias. [3] The authors reported that the reductions in sodium and chloride seem too slight to be clinically significant but reductions in magnesium and potassium could potentially have harmful effects which include the induction of cardiac dysrhythmias. Diuretic-treated dogs with ventricular ectopic beats had significantly lower plasma potassium concentrations than other dogs undergoing diuretic treatment. [3] Potassium and magnesium are the predominant intracellular cations, thus a decrease in plasma concentration may well be associated with substantial deficits within cells, including those of the myocardium. Significant risks are associated with hypokalemia, and hypomagnesemia, therefore careful monitoring of plasma electrolyte concentrations is justified for those patients treated with furosemide. [3]

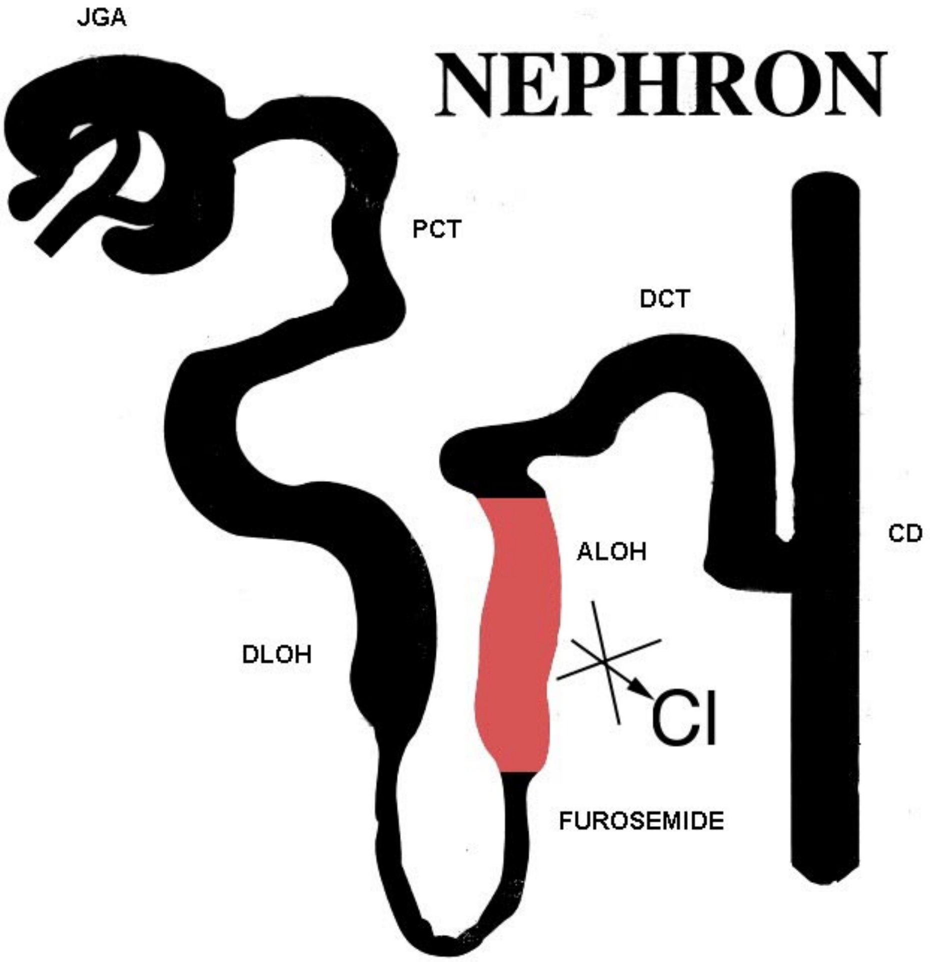

Figure 2: The Nephron: JGA-Juxtaglomerular Appartus surrounded by Bowman's capsule, PCT-Proximal Convoluted Tubule, DLOH-Descending limb of the Loop of Henle, ALOH-Ascending limb of the Loop of Henle, DCT-Distal Convoluted Tubule, CD-Collecting Duct. Furosemide inhibits the active chloride ion pump in the thick portion of the Ascending limb of the Loop of Henle resulting in decreased solute and water retention and diuresis.[26]

Furosemide: Role with RAS

Kelly et al demonstrated that furosemide administration in humans results in an immediate increase in plasma renin activity, accompanied by a 130% increase in plasma AgII and a 71% increase in plasma aldosterone. Furosemide activated renin despite a liberal dietary sodium intake. This may have occurred due to decreased extra-cellular volume, but loop diuretics have been shown to release renin independently of extra-cellular volume depletion.[14] Increases in plasma renin activity after furosemide administration have been documented in resting rats, humans, and horses. [6]

Contrary to Kelly et al, Ikram et al demonstrated an initial decrease in plasma renin activity when furosemide was first administered to humans with CHF, and then a subsequent rise in the RAS after continued use of the diuretic. The initial fall probably coincides with maximum natriuresis dictated by a combination of increased delivery of sodium and chloride to the macula densa, and improved renal perfusion which would diminish renal arteriolar baroreceptor input to release renin. With continued diuretic therapy, increased RAS activation occurs once edema fluid has been absorbed and excreted, and delivery of sodium and chloride to the macula densa decreases. [11]

Activation of the Renin-Angiotensin-Aldosterone System

The RAS is an intricate system of enzymes and hormones, that work together to keep electrolyte concentrations and blood volume within normal physiologic range. The components of the RAS system are derived from general circulation, the kidneys, the lungs, the liver, and the adrenal glands (Figure 3). [12] Renin is an acid protease that is stored in the epithelioid granular cells of the juxtaglomerular apparatus of the kidney. Angiotensinogen, a glycoprotein (molecular weight 55 to 65 kDa) synthesized mainly in the liver, is

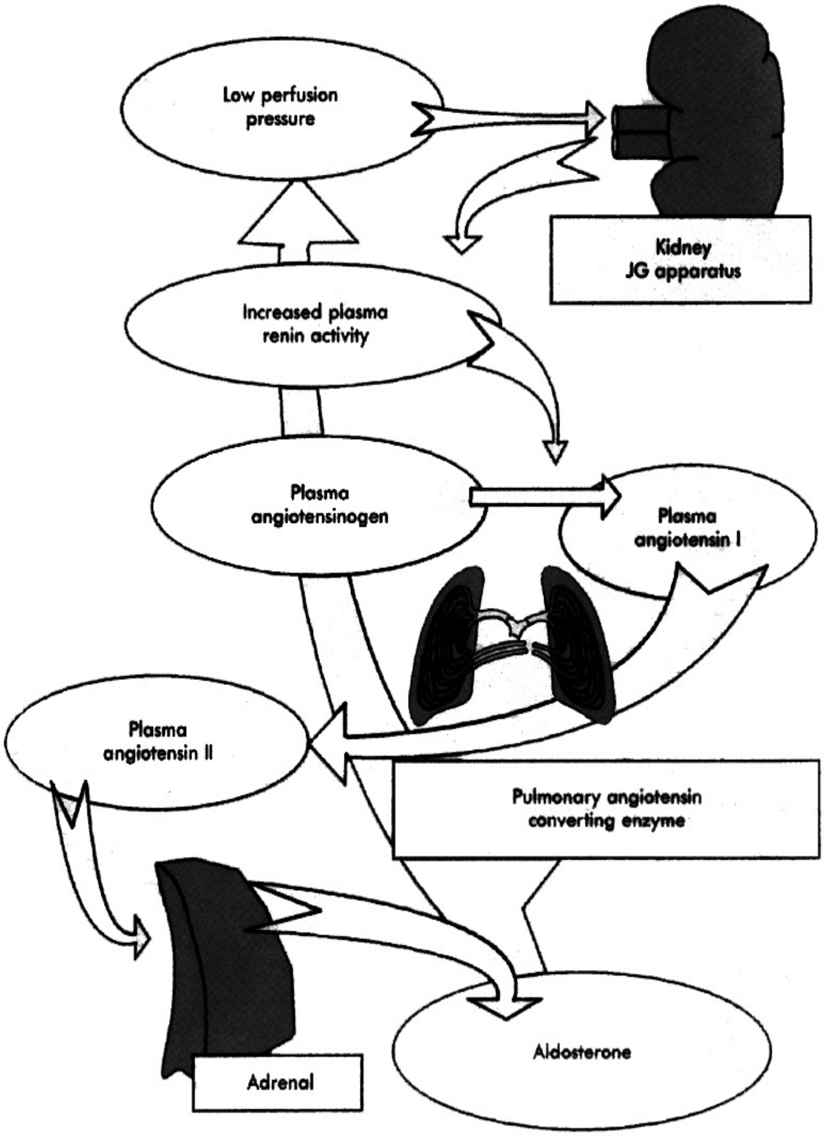

Figure 3- Regulation of Aldosterone Production. [16]

transformed in the plasma by renin into the biologically inactive decapeptide angiotensin I (AgI). In turn, AgI is converted by Angiotensin Converting Enzyme (ACE), a dipeptidyl carboxypeptidase, to the physiologically active octapeptide Angiotensin II (AgII). AgII is subsequently converted by aminopeptidase to another physiologically active though less potent peptide, Angiotensin III (AgIII). A large portion of ACE activity (20%-40%) occurs during a single pass through the pulmonary circulation. Angiotensin II increases blood pressure through potent vasoconstriction. [9] In addition, Ag II stimulates aldosterone secretion by the zona glomerulosa of the adrenal gland. [12]

Plasma Renin Activity Assays

Plasma renin exists in two forms, as renin and as prorenin. More that 90% of the total renin in plasma is prorenin. Plasma prorenin is not a metabolic source of plasma renin *in vivo*, however it may be cryoactivated causing falsely high renin activity when measured. Cryoactivated prorenin is indistinguishable from renin, therefore collection technique is crucially important for accurate plasma renin activity results. [21] Irreversible cryoactivation of plasma prorenin (which is present in 10-fold greater concentrations than renin) can be avoided by processing the plasma samples in chilled EDTA tubes and then rapidly freezing the samples. (M. Smith- personal communication, 1995) Cryoactivation occurs when plasma is liquid at temperatures <6∞C. Plasma renin activity (PRA) is commonly measured by radioimmunoassay in which AgI is formed by the reaction of plasma renin with endogenous angiotensinogen. The PRA is an expression of the hourly rate of AgI formation from the action of renin on angiotensinogen. [21]

The Role of the RAS in Congestive Heart Failure

Increased concentrations of plasma renin and aldosterone are found in humans and experimental animals with various forms of heart failure.[8] One of the factors that contributes directly, or in concert with elevated blood pressure, to the development of cardiac hypertrophy appears to be the RAS.[18] AgII may play a significant role in the development of left ventricular hypertrophy (LVH). It exerts mitogenic effects on fibroblast cells in vitro and human vascular smooth-muscle cells. Initial experiments demonstrated that the administration of ACE inhibitors to spontaneously hypertensive rats prevented and reversed the development of LVH, by halting the conversion of AgI to AgII.[9]

In the early stages of congestive heart failure (CHF), compensatory mechanisms at the renal tubules are evoked to maintain cardiac output and tissue perfusion. The RAS is stimulated and increases preload to maintain cardiac output through angiotensin II and aldosterone release. Angiotensin II results in vasoconstriction and increased venous return to the right atrium (preload). In addition to preload, afterload (arterial pressure) is subsequently elevated as well. Increased preload and afterload eventually worsen heart failure by increasing ventricular wall stress which may result in ventricular hypertrophy. Aldosterone increases sodium and water reabsorption from the distal convoluted tubule and collecting duct in the renal nephron and this results in increased blood volume. By blocking the RAS, ACE inhibitors decrease systemic vascular resistance and increase venous compliance, thereby decreasing left ventricular end diastolic pressure allowing blood to exit the heart easier.[13]

Congestive Heart Failure: Considerations with Furosemide Treatment

Patients with CHF probably represent the most common circumstance in which resistance to furosemide is encountered clinically. Congestive heart failure is characterized by decreased left ventricular systolic function and, in some cases, abnormal diastolic function. Decreased cardiac output results in decreased tissue and organ blood flow. This relationship is clearly demonstrated in the kidney, where the magnitude of renal blood flow reduction is correlated with decreased cardiac output. The pharmacokinetics of oral furosemide can be altered by heart failure; absorption may be prolonged, thereby delaying the time to appearance and peak concentration of furosemide within the urine. The absorption of oral furosemide is prolonged 0.5-1.0 hour longer in patients with CHF, compared to healthy subjects. The time course but not the extent of absorption is altered with oral diuretic administration. The delay in absorption and concomitant decreased peak concentrations of diuretic attained, albeit with normal total absorption of drug, could contribute to the diuretic resistance that occurs with oral administration in patients with CHF. To circumvent the problem, the drug can be administered intravenously (IV). The pharmacodynamics of IV furosemide administration are relatively normal in human patients with CHF as compared to healthy subjects. If IV administration is not possible, a large oral dose should eventually result in delayed attainment of drug concentrations in the urine comparable to those after IV administration. Peak concentrations are approximately one-half normal in this circumstance, and bioavailability of furosemide is 40%. Therefore, an oral dose of furosemide 2-4 times larger than an effective IV dose is necessary. Frequent administration of smaller doses, or continuous infusion of the diuretic can be employed. [2] Furosemide administration in acute CHF benefits the patient due to its hemodynamic effects on pulmonary and systemic vasculature. Furosemide treatment results in the removal of edema fluid and excess vascular fluid; eleviating dyspnea and decreasing preload to the heart, prior to any diuretic effects. However, long term furosemide use may potentiate dehydration, hypokalemia and azotemia due to the continued electrolyte and water loss from the body.[3]

Summary

Furosemide, directly or indirectly activates the RAS, however the mechanism of activation is unknown. In addition, published research on the interactions between furosemide and the RAS is limited in the medical and veterinary literature. Studies are needed to determine if renin activity increases as a direct or indirect effect of furosemide administration. In addition, studies investigating physiological effects of low and high sodium diets and diuretic combinations are needed. The affect of each component separately is important, but more importantly is how the medical alterations work synergistically or antagonistically toward the therapeutic goal.

Hypothesis

The hypotheses of this study were 1) furosemide administration would result in decreased plasma concentrations of sodium, chloride and potassium; 2) alteration of sodium concentrations in the diet and administration of furosemide would alter cardiac function by changing the parameters of ejection fraction, fractional shortening, cardiac output, and heart rate; 3) low sodium diet and furosemide administration would increase plasma renin activity and aldosterone concentrations; and 4) the combination therapy of a low sodium diet and furosemide administration would not have an additive effect on plasma renin activity or plasma aldosterone concentrations.

CHAPTER III

MATERIALS AND METHODS

Dogs, Facilities and Feed - Adult, mixed breed dogs were used for this experiment. The dogs were housed at the Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM) research facility, in indoor or outdoor runs. Prior to the experiment, dogs were fed Hills® Canine Maintenance dry dog food twice daily, based on their individual caloric requirements. For the purposes of the experiment, the type of food, sodium concentration in the food, and furosemide ingestion were altered. Free choice water was available to all dogs during the entire study. At the end of the experiment, all dogs were again fed Hill's Canine Maintenance dog food. The protocol was approved by the Animal Care Committee, Virginia Tech.

Experimental Design - Twenty dogs, average body weight of 17 kilograms (Appendix I), were allotted to one of four groups. One dog would not eat the desired diet. Final allotment was, Group I- Four dogs fed a commercially available low sodium diet (Appendix V) (Low sodium .08%). Group II-Five dogs fed the same low sodium diet plus sodium chloride to establish a high sodium diet (1.0% sodium). Group III-Five dogs fed the same low sodium diet, plus furosemide (dosage 2.0 mg/kg) by mouth (PO) every twelve hours (BID) (Appendix IV). Group IV- Five dogs fed the same low sodium diet plus sodium chloride (1.0% sodium) and furosemide (2 mg/kg PO BID). Energy requirements were determined by the body weight in kilograms, and the following equation

$$\text{Metabolizable Kcal/day} = 2[70\ (\text{body weight kg}^{.75})\ ] \times 0.8$$

The 0.8 accounts for inactivity due to the housing arrangement for the dogs. Each dog was fed the required amount to meet his or her metabolic needs. (Appendix II) Sodium chloride was added to the diets to achieve 1.0% sodium. (Appendix III) Diets were fed for 53 days.

Blood was collected from dogs into heparinized collection tubes, via jugular venipuncture, on days 0, 21 and 35. Plasma was obtained after centrifugation and plasma concentrations of electrolytes were determined on an automated analyzer (Ektochem 700 by Kodak).

Cardiac function was evaluated in dogs on days 0, 21 and 53. Measurements of Left Ventricular (LV) Ejection Fraction (EF), Fractional Shortening (FS), Cardiac Output (CO), and Heart Rate (HR) were obtained on an Acuson XP ultrasound machine. Measurements from M-mode echocardiography and the following equations were used to calculate EF, FS, and CO.

$$\text{EF} = \frac{\text{LV end diastolic volume (LVEDV) - LV end systolic volume (LVESV)}}{\text{LVEDV}} \times 100$$

$$\text{FS} = \frac{\text{LV end diastolic dimension (LVEDD) - LV end systolic dimension}}{\text{LVEDD}} \times 100$$

$$\text{CO} = \text{Stroke volume (SV)} \times \text{Heart rate}$$

$$\text{SV} = \text{LVEDV} - \text{LVESV}$$

Blood collected from dogs via jugular venipuncture on days 0, 21, 35 and 53, was evaluated for plasma renin activity and plasma aldosterone concentration. Blood was collected in cold tubes containing disodium EDTAate (Vacutainer, Becton-Dickinson, Rutherford, New Jersey), centrifuged in a cold centrifuge (Beckman table top) for 10 minutes at 1000x g to pack the cells, and the supernatant was then pipetted into polystyrene tubes and frozen at - 20∞C or below, pending radioimmunoassay (Appendix VI).

Statistical Analysis- The general linear model procedure was used to analyze data. The model for cardiac output, plasma electrolyte concentrations, plasma renin activity, and plasma aldosterone concentrations included furosemide treatment, low and high sodium diet, and the furosemide treatment and diet interaction. The effects of treatment, diet, and their interaction, were considered significant if the p value was less than 0.05. When the interaction of diet and furosemide treatment was significant, group means within furosemide treatment or diet were separated by a t-test ($P<0.05$).

CHAPTER IV

RESULTS

Cardiac function- The effects of low and high sodium diet, with and without concurrent furosemide treatment are summarized in Table I. It may be noted that following 53 days of treatment, cardiac function was not altered between all four groups. The mean values of ejection fraction, fractional shortening, cardiac output and heart rate did not differ significantly between the four groups. (Figures IV - VII)

Plasma electrolytes- Dogs fed low and high sodium diets and treated with furosemide did not have significantly different plasma sodium or plasma potassium concentrations on days 21 and 35. Furosemide treatment and the interaction between a low sodium diet and furosemide treatment significantly lowered plasma chloride concentrations on days 21 and 35 ($p<.05$). Dogs fed low sodium diets and given furosemide had the lowest plasma chloride means. Results are summarized in Table II. (Figures VIII - X)

Plasma renin activity-Diet, treatment, and their interaction had a significant effect on plasma renin activity on days 21, 35 and 53 ($p<0.05$). The data were transformed on a log base 10 scale so as not to violate the homogeneity of variance. On all three test days, the groups of dogs fed the low sodium diet had significantly higher plasma renin activities (12.3-15.9 nG AI/ml/hr) than dogs fed the high sodium diet (PRA = 2.6-3.6 nG AI/ml/hr). On all three test days dogs given furosemide had significantly higher PRA (12.7-17.86 nG AI/ml/hr) than dogs not given furosemide (1.6-2.1 nG AI/ml/hr). The interaction between furosemide and the low sodium diet produced the highest plasma renin activities, and demonstrates an additive affect between treatment and diet ($p<0.05$), results are summarized in Table III. (Figure XI)

Plasma aldosterone concentrations- Diet, treatment, and their interaction had a significant effect on plasma aldosterone concentrations on days 21, 35 and 53 ($p<0.05$). The data were transformed on a log base 10 scale so as not to violate the homogeneity of variance. On all three test days, the groups of dogs fed the low sodium diet had significantly higher plasma aldosterone concentrations (165.7-237.8 nG/dl) than the dogs fed the high sodium diet (6.7-13.23 nG/dl), ($p<0.0001$). On all three test days, the dogs given furosemide had higher plasma aldosterone concentrations (163.6-240.8 nG/dl) than dogs not given furosemide (9.0-11.2 nG/dl). The interaction between treatment and diet was significant, the dogs fed the low sodium diet and given furosemide experienced the highest increase in plasma aldosterone concentrations ($p<0.05$), results are summarized in Table III. (Figure XII)

TABLE I. Mean Ejection Fraction (EF%), Mean Fractional Shortening (FS%), Mean Cardiac Output (CO ml/kg/min), in dogs fed low and high sodium diets, with and without concurrent furosemide treatment.

	DIET					CONTRAST		
DAY 0	LNA[1]	HNA[2]	LNAF[3]	HNAF[4]	SE[5]	NA[6]	F[7]	NA*F[8]
EF	61.3	56.6	63.8	60.6	2.9	NS[9]	NS	NS
FS	32.3	28.8	33.6	31.6	2.1	NS	NS	NS
CO	177.3	169.4	161.4	246.3	32.0	NS	NS	NS
DAY 21								
EF	56.5	55.0	55.8	54.8	3.8	NS	NS	NS
FS	28.8	28.0	27.8	28.4	2.4	NS	NS	NS
CO	192.8	262.0	161.3	208.8	37.0	NS	NS	NS
DAY 53								
EF	58.5	54.6	52.6	54.0	3.8	NS	NS	NS
FS	30.8	28.0	26.2	27.2	2.5	NS	NS	NS
CO	182.6	183.9	166.0	196.8	33.0	NS	NS	NS

[1] Low sodium diet (0.08%)

[2] High sodium diet (1.0%)

[3] Low sodium diet plus furosemide (2 mg/kg orally (PO) twice daily (BID))

[4] High sodium diet plus furosemide

[5] Pooled standard error of the mean

[6] P-value for diet effect

[7] P-value for furosemide effect

[8] P-value for the interaction between diet and drug

[9] P>0.05

TABLE II. Mean plasma electrolyte concentrations (mEq/L) in dogs fed low and high sodium diets, with and without the concurrent administration of furosemide.

	DIET					CONTRAST		
DAY 0	LNA[1]	HNA[2]	LNAF[3]	HNAF[4]	SE[5]	NA[6]	F[7]	NA*F[8]
K	3.9	3.8	3.9	4.1	0.1	NS[9]	NS	NS
Na	148.5	143.0	144.6	143.4	2.0	NS	NS	NS
Cl	111.8	110.0	112.2	110.0	1.0	NS	NS	NS
DAY 21								
K	4.1	3.8	4.4	4.0	0.2	NS	NS	NS
Na	140.5	141.0	136.8	139.6	1.3	NS	NS	NS
Cl	114.3^{a}	112.4^{ab}	105.4^{c}	109.6^{b}	1.3	NS	0.001	0.04
DAY 35								
K	4.1	4.2	4.5	4.0	0.2	NS	NS	NS
Na	141.8	140.4	137.6	140.2	1.3	NS	NS	NS
Cl	116.3^{a}	113.8^{ab}	110.6^{b}	113.8^{ab}	1.3	NS	0.046	0.046

---

[1] Low sodium diet (0.08%)

[2] High sodium diet (1.0%)

[3] Low sodium diet plus furosemide (2 mg/kg orally (PO) twice daily (BID))

[4] High sodium diet plus furosemide

[5] Pooled standard error of the mean

[6] P-value for sodium effect

[7] P-value for furosemide effect

[8] P-value for the interaction between diet and drug

[9] $P>0.05$

[a,b,c] Means without similar superscripts are different ($p<0.05$)

TABLE III. Mean plasma renin activity (nG AI/ml/hr) and mean plasma aldosterone concentration (nG/dl), in dogs fed low and high sodium diets, with and without the concurrent administration of furosemide. [10]

	DIET					CONTRAST		
DAY 0	LNA[1]	HNA[2]	LNAF[3]	HNAF[4]	SE[5]	NA[6]	F[7]	NA*F[8]
PRA	0.2	0.1	0.4	0.4	0.2	NS[9]	NS	NS
ALDO	0.9	0.8	1.0	0.8	0.1	NS	NS	NS
DAY 21								
PRA	0.2^{c}	0.2^{c}	1.4^{a}	0.6^{b}	0.1	0.008	0.0001	0.01
ALDO	$1.1^{c,d}$	0.9^{d}	2.5^{a}	$1.2^{b,c}$	0.1	0.0001	0.0001	0.0001
DAY 35								
PRA	0.3^{b}	0.1^{b}	1.3^{a}	0.5^{b}	0.2	0.003	0.0001	0.047
ALDO	1.1^{b}	0.6^{c}	2.5^{a}	$0.9^{b,c}$	0.1	0.0001	0.0001	0.0001
DAY 53								
PRA	0.2	-0.005	1.5	0.7	0.1	0.002	0.0001	0.06
ALDO	1.1^{c}	0.9^{d}	2.7^{a}	$1.3^{b,c}$	0.1	0.0001	0.0001	0.0001

[1] Low sodium diet (0.07%)

[2] High sodium diet (1.0%)

[3] Low sodium diet plus furosemide (2mg/kg orally (PO) twice daily (BID))

[4] High sodium diet plus furosemide

[5] Pooled standard error of the mean

[6] P-value for sodium effect

[7] P-value for furosemide effect

[8] P-value for the interaction between diet and drug

[9] $P>0.05$

[10] Values expressed as $\log_{10}$

[a,b,c,d] Means without similar superscripts are different ($p<0.05$)

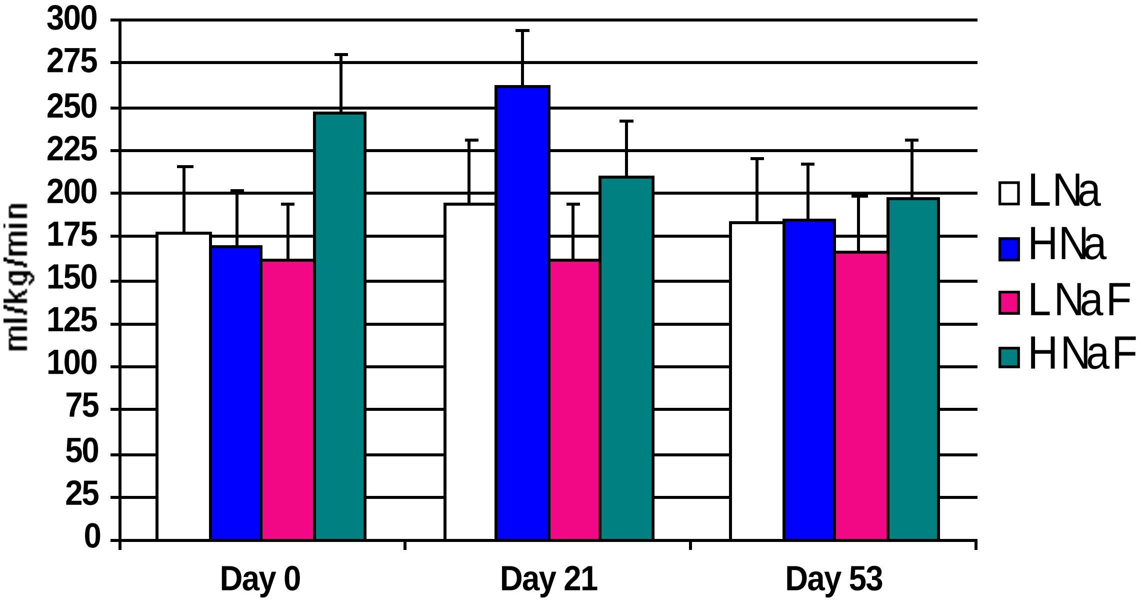

Figure IV: Cardiac output measured in healthy dogs fed low or high sodium diets, with or without furosemide administration, on days 0, 21 and 53 L Na=low sodium diet (0.08%), H Na=high sodium diet (1.0%), L Na F=low sodium diet and furosemide (2 mg/kg PO BID), H Na F=high sodium diet and furosemide Data are presented as mean and standard error of the mean.

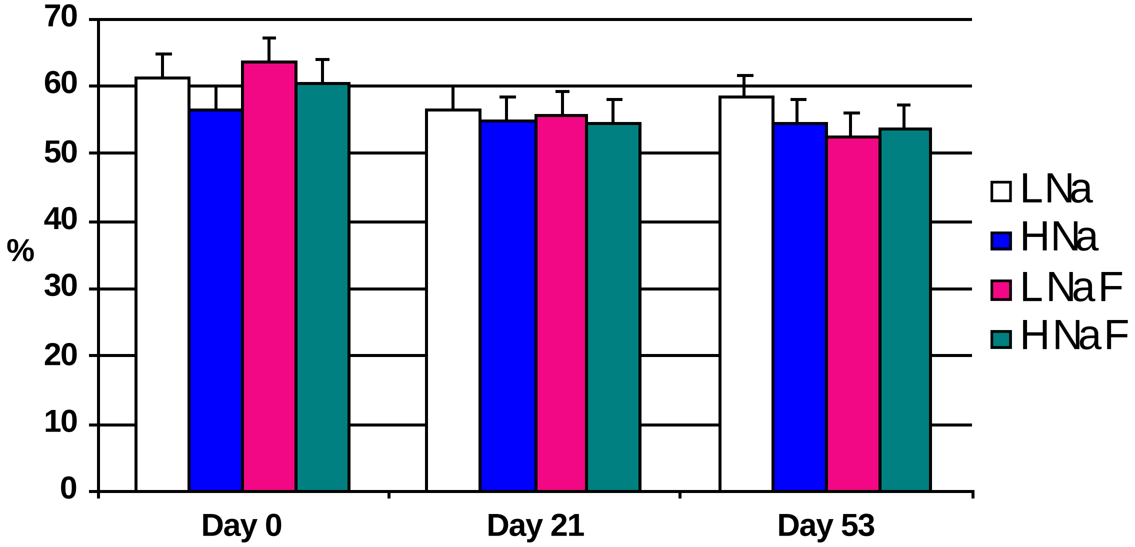

Figure V: Cardiac Ejection Fraction measured in healthy dogs fed a low or high sodium diet, with and without furosemide administration on days 0, 21 and 53 L Na=low sodium diet (0.08%), H Na=high sodium diet (1.0%), L Na F=low sodium diet and furosemide (2 mg/kg PO BID), H Na F=high sodium diet and furosemide Data are presented as mean and standard error of the mean.

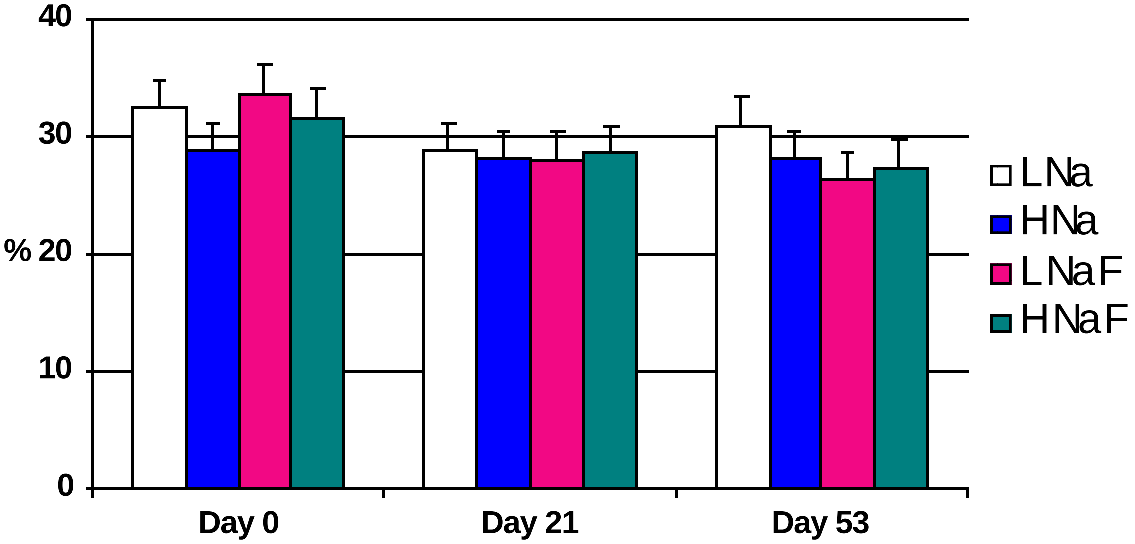

Figure VI: Cardiac Fractional Shortening measured in healthy dogs fed a low or high sodium diet, with and without furosemide administration on days 0, 21 and 53 L Na=low sodium diet (0.08%), H Na=high sodium diet (1.0%), L Na F=low sodium diet and furosemide (2 mg/kg PO BID), H Na F=high sodium diet and furosemide Data are presented as mean and standard error of the mean.

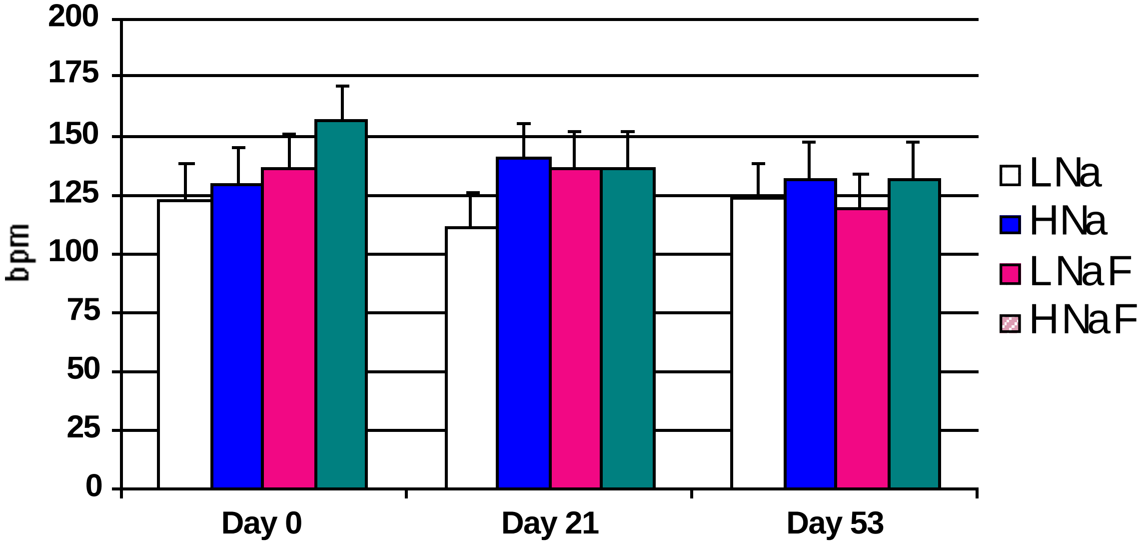

Figure VII: Pulse in beats per minute (bpm) measured in healthy dogs fed a low or high sodium diet, with and without furosemide administration on days 0, 21 and 53   L Na=low sodium diet (0.08%), H Na=high sodium diet (1.0%), L Na F=low sodium diet and furosemide (2 mg/kg PO BID), H Na F=high sodium diet and furosemide Data are presented as mean and standard error of the mean.

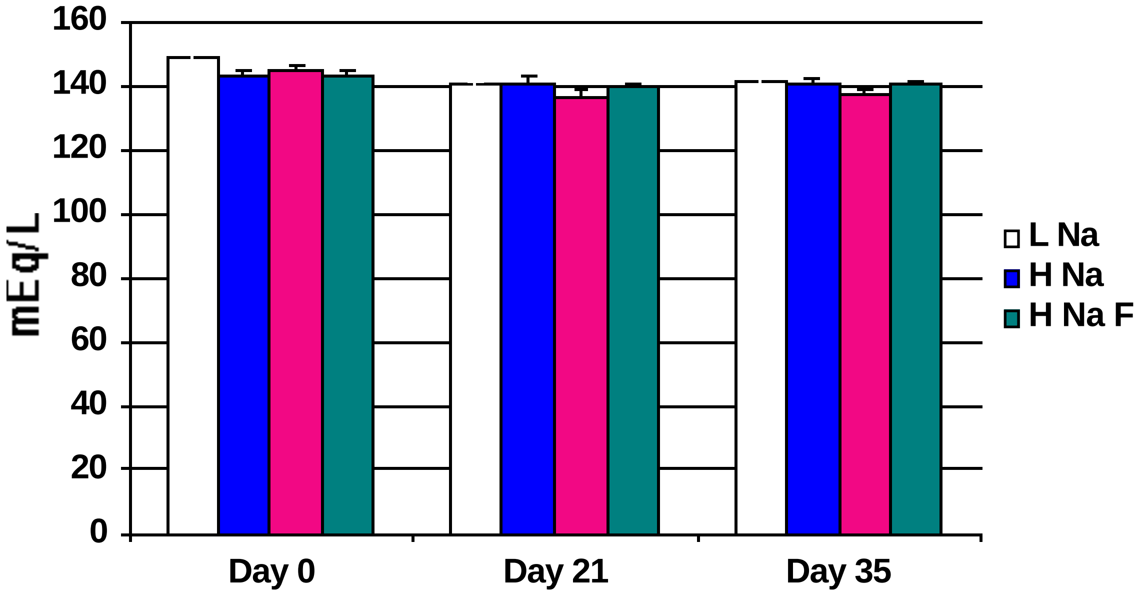

Figure VIII: Plasma sodium concentrations measured in healthy dogs fed a low or high sodium diet, with and without furosemide administration on days 0, 21 and 35 L Na=low sodium diet (0.08%), H Na=high sodium diet (1.0%), L Na F=low sodium diet and furosemide (2 mg/kg PO BID), H Na F=high sodium diet and furosemide Data are presented as mean and standard error of the mean.

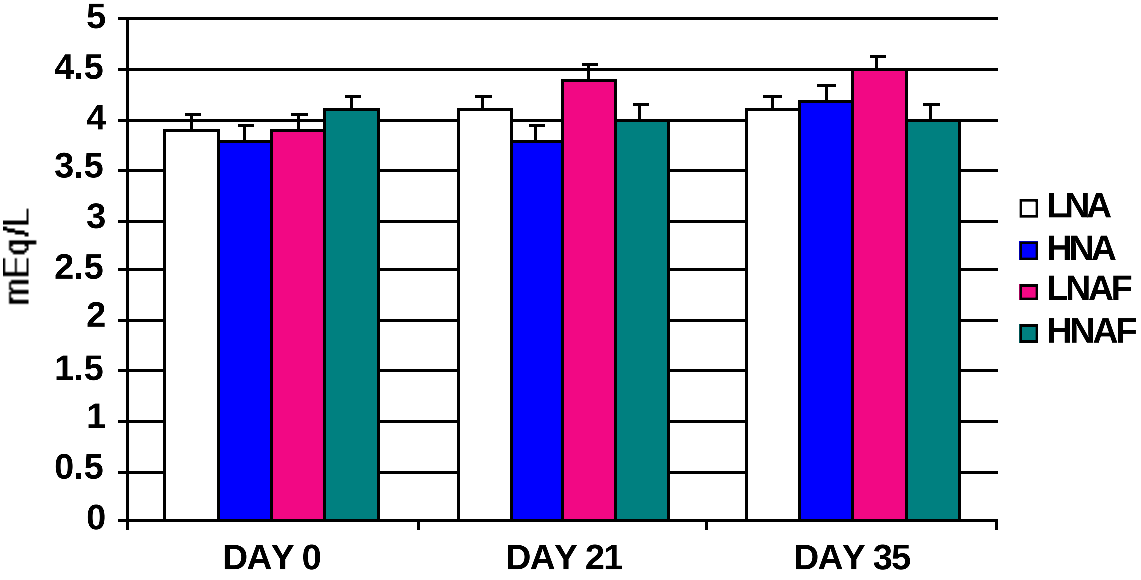

Figure IX: Plasma potassium concentrations measured in healthy dogs fed a low or high sodium diet, with and without furosemide administration on days 0, 21 and 35 L Na=low sodium diet (0.08%), H Na=high sodium diet (1.0%), L Na F=low sodium diet and furosemide (2 mg/kg PO BID), H Na F=high sodium diet and furosemide Data are presented as mean and standard error of the mean.

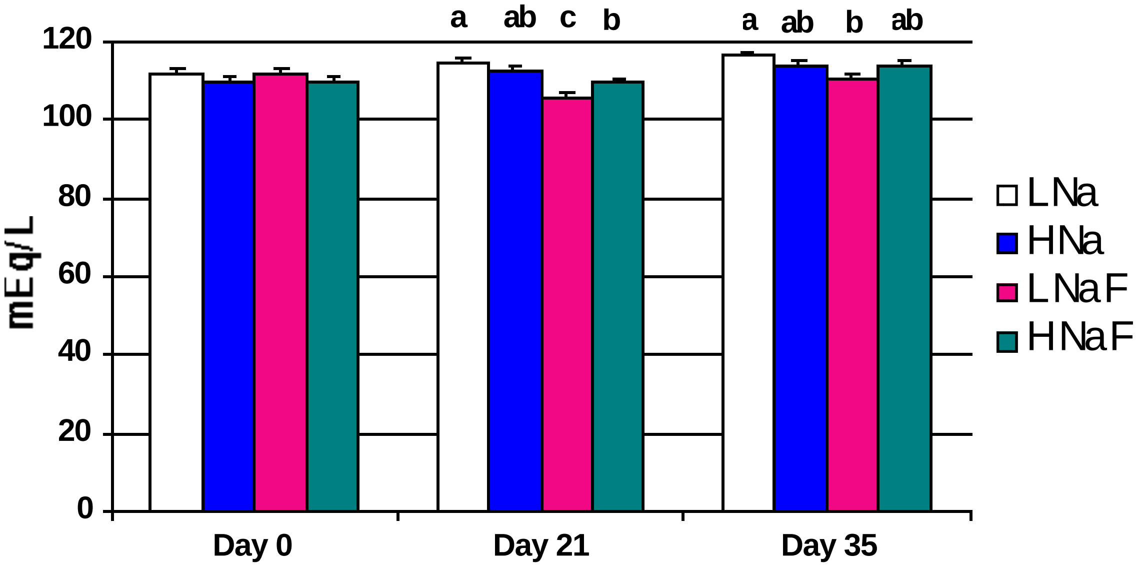

Figure X: Plasma chloride concentrations measured in healthy dogs fed a low or high sodium diet, with and without furosemide administration on days 0, 21 and 35 L Na=low sodium diet (0.08%), H Na=high sodium diet (1.0%), L Na F=low sodium diet and furosemide (2 mg/kg PO BID), H Na F=high sodium diet and furosemide Data are presented as mean and standard error of the mean Means without similar letters are different ($p<0.05$).

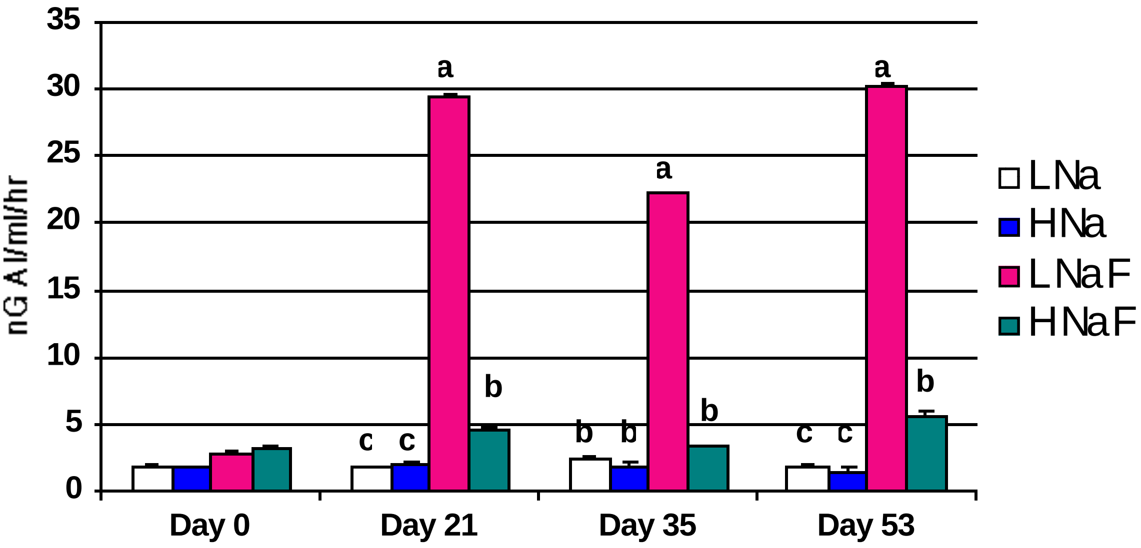

Figure XI: Plasma Renin Activity measured in healthy dogs fed a low or high sodium diet, with and without furosemide administration, in nG of Angiotensin I formation (ml/hr) on days 0, 21, 35 and 53 L Na=low sodium diet (0.08%), H Na=high sodium diet (1.0%), L Na F=low sodium diet and furosemide (2 mg/kg PO BID), H Na F=high sodium diet and furosemide Data are presented as mean and standard error of the mean Means without similar letters are different (p<0.05).

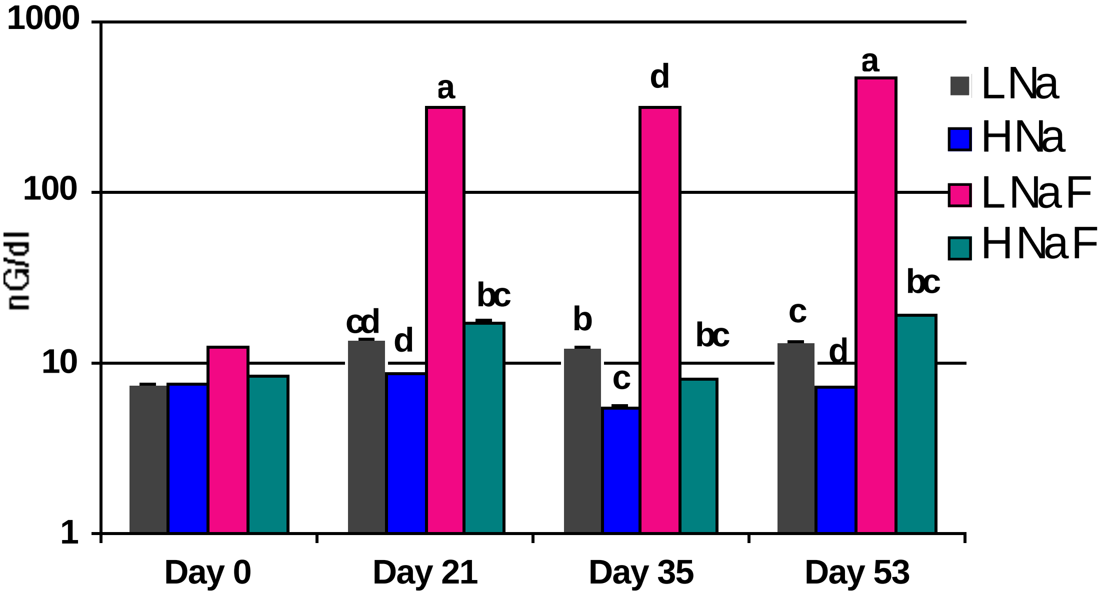

Figure XII: Plasma Aldosterone concentrations measured in healthy dogs fed a low or high sodium diet, with and without furosemide administration on days 0, 21, 35 and 53 LNa=low sodium diet (0.08%), H Na=high sodium diet (1.0%), L Na F=low sodium diet and furosemide (2 mg/kg PO BID), H Na F=high sodium diet and furosemide Y-axis transformed to $\log_{10}$ scale Data are presented as mean and standard error of the mean Means without similar letters are different ($p<0.05$).

CHAPTER V

DISCUSSION

Veterinarians commonly use a low sodium diet and diuretic to treat the symptoms congestive heart failure (CHF). The goal is to decrease sodium intake and promote diuresis resulting in decreased preload and afterload on the damaged heart.

During the 53 day study, nineteen dogs were allotted to one of four groups and fed diets that contained either 0.08% or 1.0% sodium with or without concurrent furosemide administration. Cardiac function, plasma electrolytes, plasma renin activity and plasma aldosterone concentrations were evaluated for changes induced by diet and/or drug. Results from these healthy adult dogs must be interpreted accordingly, extrapolations to CHF patients may not be accurate.

Cardiac function- Regardless of sodium alterations in the diet, with or without the addition of furosemide, cardiac function was maintained within normal limits for all groups. Fractional changes in dimensions are unitless numbers expressing the percent change in a dimension from end-diastole to end-systole, and are calculated as the difference between the systolic and diastolic dimensions divided by the diastolic dimension times 100.[17] Ejection Fraction is a measure of the percentage of end-diastolic volume that is ejected with each heart beat and should be greater than 50%. Ejection fraction of dogs in the present study was 52.6 - 63.8%. The left ventricular shortening fraction in most normal dogs is greater than 30%, with most dogs falling in the 30% to 40% range. Normal dogs with values less than 30% are usually large to giant breeds. However, reported normal ranges are broader than these guidelines, and range from 26% to 50%. These broad ranges illustrate the importance of correlating such measurements with other findings in the overall cardiac evaluation.[17] Fractional Shortening of dogs in the present study was 26% to 33%. In conscious dogs, cardiac output should be between 2-4 liters of blood/minute, and can be determined by using a cardiac index of 131ml/min/kg of body weight.[25] The mean cardiac output of dogs in the present study ranged from 161-262 ml/min/kg or 2.5 - 4.2 L/min. Cardiac output is a measure of global cardiac function, traditionally measured invasively by the Fick technique or thermodilution. However, CO is an insensitive indicator of cardiac performance because many compensatory mechanisms act to maintain normal CO even when congestive heart failure is present. CO measurement is best used together with other invasive or noninvasive parameters to obtain a more complete evaluation of cardiac performance.[17] The lack of changes in cardiac parameters demonstrates the compensatory action by a healthy cardiac system to maintain status quo within the body in the face of dietary and/or drug changes. The next logical step is to gather data on patients with compromised cardiac function.

Plasma electrolytes- Furosemide inhibits active reabsorption of chloride through inhibition of the chloride ion pump in the renal tubules of the kidney. Treatment with furosemide, and the interaction between furosemide and diet, did significantly lower plasma chloride concentrations. Chloride ions are cotransported with sodium and potassium, therefore all three plasma electrolyte concentrations may decrease during furosemide administration.[4] In the present study, plasma chloride decreased in healthy dogs treated with furosemide, whereas plasma sodium and potassium concentrations did not differ between treated and untreated dogs. It is important to note that chloride ions are excreted with one sodium or one potassium ion. Therefore, the loss of sodium or potassium should be relatively slower than the chloride loss. However, other researchers have noted significant declines in plasma electrolytes concurrently, the differences may be attributed to furosemide dose, frequency of furosemide administration, or electrolyte content of the diet.

Renin-Angiotensin-Aldosterone- Two factors activate the renin-angiotensin-aldosterone system (RAS); 1) decreased blood pressure at the juxtaglomerular cells and 2) decreased solute concentration in the glomerular filtration.[12] Loop diuretics have been shown to release renin independently of extra-cellular volume depletion. A 50% - 200% increase in renin activity and aldosterone concentration has been observed after furosemide administration.[14] In the present study, similar results were obtained with furosemide administration. Furosemide administration with a low or high sodium diet resulted in significant elevations of plasma renin activity and plasma aldosterone concentrations. The addition of furosemide to the low sodium diet resulted in the highest plasma renin activities and plasma aldosterone concentrations. These results indicate a direct correlation between furosemide administration and increased renin release. If furosemide directly increases renin activity, and not simply by indirect sodium-chloride and water depletion, the consequences could be very detrimental for a patient with cardiac disease. One of the factors that contributes directly, or in concert with elevated blood pressure, to the development of cardiac hypertrophy appears to be the RAS.[19] If furosemide does indeed stimulate the RAS, then the body will, as a result of furosemide treatment, undergo vasoconstriction and increased sodium and water resorption from the distal tubules of the kidneys, resulting in elevated blood pressure and increased preload and afterload on the heart.[12] These changes are potentially life-threatening for a patient in CHF. Congestive heart failure results in increased preload and a resultant decreased cardiac output due to the damaged myocardium. A resultant increase in afterload due to vasconstriction via the RAS could result in death of the patient. The veterinarian may simply want to utilize a low sodium diet and reconsider adding furosemide treatment in all cases of congestive heart failure. Other considerations include administration of furosemide with maintenance level sodium concentrations in the diet, or low sodium diet and furosemide with an angiotensin converting enzyme inhibitor. Consideration of the apparent additive effect of furosemide treatment and a low sodium diet may change treatment protocol for congestive heart failure in the future.

CHAPTER VI

CONCLUSION

This study was designed to evaluate the physiological effects of a low and high sodium diet, with and without furosemide administration, in healthy dogs. The results indicate that the administration of furosemide and sodium content of the diet causes significant changes in the renin-angiotensin-aldosterone system (RAS). It was shown that a low sodium diet, furosemide treatment, and the interaction between furosemide and a low sodium diet, activate the RAS in healthy dogs. In addition, furosemide administration significantly lowered plasma chloride concentrations. The interaction between furosemide and a low sodium diet resulted in the lowest plasma chloride concentrations, again illustrating the additive affect of furosemide with a low sodium diet. In the present study, furosemide treatment coupled with a low sodium diet produced the adverse side effects of decreasing plasma chloride concentrations, increasing plasma renin activity, and increasing plasma aldosterone concentrations. If the combination of a low sodium diet and furosemide produce the same results in congestive heart failure patients, these side effects may increase systolic demands on the heart by increasing preload and afterload, worsen congestive signs and heart failure, and ultimately shorten the life span of the patient.

REFERENCES

1. Abboud HE, Luke RG, Galla JH, Kotchen TA. Stimulation of renin by acute selective chloride depletion in the rat. *Circulation research* 1979;44: 815-821.
2. Brater DC. Resistance to loop diuretics. Why it happens and what to do about it. *Drugs* 1985; 30: 427-443.
3. Cobb M, Michell AR. Electrolyte concentrations in dogs receiving diuretic therapy for cardiac failure. *Journal of Small Animal Practice* 1992; 33:526-529.
4. Cody RJ, Pickworth KK. Approaches to diuretic therapy and electrolyte imbalance in congestive heart failure. *Cardiology Clinics* 1994; 12: 37-49.
5. Cody RJ. Hemodynamic responses to specific renin angiotensin inhibitors in hypertension and heart failure. *A review, Drugs* 1984; 28: 144-169.
6. Cooley JL, Hinchcliff KW, Mckeever KH, Lamb DR, Muir WW. Effect of furosemide on plasma atrial natriuretic peptide and aldosterone concentrations and renin activity in running horses. *Am J Vet Res* 1994; 55: 273-277.
7. Cunningham JG, Verlander JW. In: *Textbook of Veterinary Physiology*. Philadelphia:W.B. Saunders Co, 1992; 495.
8. Davis JO, Freeman RH. Mechanisms regulating renin release. *Physiol. Rev.* 1976; 56: 1-56.
9. Greenwald L, Becker RC. Expanding the paradigm of the renin-angiotensin system and angiotensin-converting enzyme inhibitors. *American Heart Journal* 1994; 128: 997-1009.
10. Hamlin RL, et al. Effects of a controlled electrolyte diet, low in sodium, on healthy dogs. *Veterinary Medicine/Small Animal Clinician* 1964; 748-751.
11. Ikram H, Chan W, Espiner A, Nicholls MG. Haemodynamic and hormone responses to acute and chronic furosemide therapy in congestive heart failure. *Clinical Science* 1980; 59: 443-449.

12. Itkin RJ. Effects of the renin-angiotensin system on the kidneys. *The Compendium* 1994; 16:6 753-763.

13. Kang PM, Landau AJ, Eberhardt RT, Frishman WH. Angiotensin II receptor antagonists: A new approach to blockade of the renin-angiotensin system. *American Heart Journal* 1994; 127: 1388-1401.

14. Kelly RA, et al. Response of the kidney to furosemide. Effect of captopril on sodium balance. *Kidney International* 1983; 24: 233-239.

15. Krieger JE, Liard JF, Cowley AW. Hemodynamics, fluid volume, and hormonal responses to chronic high-salt intake in dogs. *American Physiological Society* 1990; H1629-H1635.

16. Montgomery. In: *Biochemistry: a case-oriented approach*. St. Louis: Mosby Co, 1990; 818.

17. Nyland TG, Kienle RD, Thomas WP. In: Veterinary Diagnostic Ultrasound. Philadelphia: W.B. Saunders Co, 1995; 211-214.

18. Pedersen HD, Koch J, Jensen AL, Poulsen K, Flagstad A. Some effects of a low sodium diet high in potassium on the renin-angiotensin system and plasma electrolyte concentrations in normal dogs. *Acta Vet. Scand* 1994; 35: 133-140.

19. Raman VK, Lee YA, Lindpainter K. The cardiac renin-angiotensin-aldosterone system and hypertensive cardiac hypertrophy. *The American Journal of Cardiology* 1995; 76: 18D-23D.

20. SAS Institute Inc. SAS user's guide:Statistics, Version 5 Edition. Cary, NC. SAS Institute, 1987.

21. Sealy JE. Plasma renin activity and plasma prorenin assays. *Clinical Chemistry* 1991; 37: 1811-1819.

22. Shade RE, Davis JO, Johnson JA, Witty RT. Effects of renal arterial infusion of sodium and potassium on renin secretion in the dog. *Circulation Research* 1972; 31: 719-727.

23. Smith M. Verbal Communication and E-mail. University of Mississippi Medical Center 1998.

24. Spangler WL. Pathophysiologic response of the juxtaglomerular apparatus to dietary sodium restriction in the dog. *Am J Vet Res* 1979; 40: 809-819.

25. Woltmann RF, et al. Lack of beneficial effects of growth hormone treatment in conscious dogs during development of heart failure. *The American Physiological Society* 1998; 43: H456-H466.

26. Notes from Urology at Virginia-Maryland Regional College of Veterinary Medicine, 1996.

APPENDIX I: Dog identification number, and starting weight.

Dog Identification Number	Starting Weight in Kilograms (Kg)
682	12.1
683	12.5
715	15.4
716	23.1
1118	19.3
725	12.2
731	22.0
733	14.3
745	14.4
747	19.4
760	15.3
764	17.1
765	13.1
766	23.2
767	19.5
768	23.7
769	9.1
770	26.1
804	9.8

APPENDIX II: Energy requirements of the dogs in Kilocalories (Kcals), and amount of Hills HD to be fed.

Dog Number	Kcals Needed	Cans of HD to be fed[1]
682	726.6	1.25
683	744.6	1.25
715	870.7	2.00
716	1180.1	2.00
1118	1031.3	1.75
725	731.1	1.25
731	1137.7	1.75
733	823.6	1.50
745	827.9	1.50
747	1035.3	1.75
760	866.4	1.50
764	941.8	1.50
765	771.2	1.25
766	1184.0	2.00
767	1039.3	1.75
768	1203.0	2.25
769	586.8	1.00
770	1293.3	2.00
804	931.8	1.50

[1] 643 Kcals of energy in each 15.5 once can of Hills HD

APPENDIX III: Grams of sodium added to Hills HD to achieve a high sodium diet (1.0%).

Dog Number	Grams of Sodium Needed	Grams of Salt Added
682	1.43	3.58
683	1.43	3.58
715	2.28	5.70
716	2.28	5.70
768	2.28	6.43
760	1.71	4.28
804	1.71	4.28
766	2.28	4.28
764	1.71	3.58
765	1.43	5.70

APPENDIX IV: Furosemide dose for each dog in the treatment group.

Dog Number	Milligrams (Mg)[1]
725	24.4
767	39.0
1118	38.6
769	18.2
770	52.2
760	30.6
804	19.6
766	46.4
764	34.2
765	26.2

[1] Milligrams of Furosemide to be given every twelve hours, based on 2mg/kg body weight

APPENDIX V. Nutrient analysis of Hills HD canned dog food. Each 15.5 once can contains 643 Kcals metabolizable energy .

Nutrient	As Fed %	Dry Matter %
Protein	4.80	17.27
Fat	8.00	28.78
Carbohydrate	13.60	48.92
Fiber	0.20	0.72
Calcium	0.19	0.68
Phosphorous	0.13	0.47
Sodium	0.02	0.08
Potassium	0.23	0.83
Magnesium	0.04	0.13
Chloride	0.21	0.76

APPENDIX VI. Plasma Renin Activity and Plasma Aldosterone Concentration Assays. Plasma Renin Activity and Plasma Aldosterone Concentrations were measured by Dr. Manis Smith at the University of Mississippi Medical Center, and the below information provided on the radioimmunoassay procedures.

Plasma Renin Activity- Blood was collected in cold tubes containing disodium EDTAate (Vacutainer, Becton-Dickinson, Rutherford, New Jersey), centrifuged in a cold centrifuge (Beckman table top or Clay-Adams Dynac in a walk-in cold room) for 5-20 minutes at 1000 X g to pack the cells, and the supernatant was then pipetted into 12x75 polystyrene tubes and frozen at -20ºC or below pending assay.

Plasma renin activity was measured by a modification of the method of Haber, et al. Renin activity was determined by the amount of angiotensin I formation in nG/ml/hr through the interaction of renin and angiotensinogen. Angiotensin I was measured via radioimmunoassay in which 0.1ml of 4M Tris buffer was added per ml of sample to produce a sample pH of 7.35-7.4 at 37∞C.

Plasma Aldosterone Concentrations- Heparinized plasma was extracted into 7 volumes of spectrophotometric-grade dichloromethane, 5 mls of which were evaporated to dryness at 37∞C under a stream of air, and reconstituted in assay buffer (0.1M phosphate containing 0.1% gelatin and 0.1% sodium azide, pH 7.4 at 2∞C. Recovery of 3H-aldosterone (Amersham, Arlington Heights, Illinois) form recovery controls extracted along with the samples was measured, averaged 87.7% ± 8.2% (CV) of the added 3H-aldosterone, and was used to correct the assayed aldosterone concentrations.

Aldosterone concentrations in these extracts were measured by radioimmunoassay using 125I-aldosterone and a highly specific liquid-phase antibody (Diagnostic Products, Los Angeles, California). Aliquots of a buffer pool having an expected concentration of 1.78 nM were assayed in every tenth position and had observed concentrations of 1.83 nM ± 13% (CV, n=21, between-assay, O/E=102.7%). Extracted controls having expected concentrations (±95% Confidence Limit) of 108± 49.9 pM, and 1299± 216 pM had respective between-assay concentrations of 135 pM ±10.2% (CV, n=4), 633 pM ±8.3% (CV, n=4), and 1395 pM ±8.5% (CV, n=9).

E. Haber , T. Koerner, L.B. Kliman, and A. Purnode. Application of a radioimmunoassay for Angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. J. Clin. Endocr. 29: 1349-1355, 1969.

VITA

Cindy Marie Swancott was born on July 31, 1970 in Fairfax, Virginia. After graduating from high school at Broadneck Senior High, Annapolis, Maryland in June 1988, she attended Salisbury State College in Salisbury, Maryland for two years and then transferred to Towson State University in Towson, Maryland. She majored in Biology in the Department of Arts and Sciences, where she graduated cum laude with a Bachelor of Science Degree in May 1993.

In July of 1993, she enrolled in the combined Doctor of Veterinary Medicine and Veterinary Medical Science Graduate program at the Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University. She received a Doctorate of Veterinary Medicine from the Virginia-Maryland Regional College of Veterinary Medicine in May of 1997.

She is currently a member of the American Veterinary Medical Association, the Virginia Veterinary Medical Association, the Greater Roanoke Valley Veterinary Association, and the American Association of Feline Practitioners.

GRANT

The Effect of Dietay Sodium, With and Without Concurrent Diuretic Use, on Ventricular Contractility, Renin-Angiotensin-Aldosterone, and Electrolyte Concentrations W.S. Swecker, C.M. Swancott. Mark Morris Institute Grant. $5840.00. 1995 -1997

PRESENTATION

C. Swancott, W. Swecker, J. Lee, M. Moon. The effect of dietary sodium, with and without concurrent diuretic use, on plasma renin-angiotensin-aldosterone concentrations in dogs. Eighth Annual Research Symposium June, 1996. VMRCVM, Blacksburg, VA.

EXAMINATION

GRADUATE COMMITTEE

Dr. William S. Swecker, D.V.M, Ph.D., Diplomate of ACVN,
Chair
Associate Professor, Virginia-Maryland Regional College of Veterinary Medicine
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061

Dr. John C. Lee, Ph.D.,
Professor and Associate Dean, Research and Graduate Studies
Virginia-Maryland Regional College of Veterinary Medicine
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061

Dr. Martha L. Moon, D.V.M., M.S., Diplomate ACVR,
Associate Professor, Virginia-Maryland Regional College of Veterinary Medicine
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061