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THE EFFECT OF VARIOUS EXPERIMENTAL
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RELEASE IN THE WATER SNAKE, NATRIX.

The Ohio State University, Ph.D., 1976
Physiology

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THE EFFECT OF VARIOUS EXPERIMENTAL PROCEDURES ON
RENAL FUNCTION AND RENIN RELEASE
IN THE WATER SNAKE, NATRIX

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Bence David Boelcskevy, B.S., M.S.

The Ohio State University
1976

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INTRODUCTION

Life as we know it requires that the organism maintain sodium and water balance. The mammalian kidney has evolved to a point where it is highly developed in this respect. Mammals can regulate plasma sodium concentration by retaining or excreting sodium and/or water. This research was undertaken to more clearly define a hormonal mechanism regulating sodium retention or excretion in mammals. To minimize variables present in a highly developed mammalian kidney, the experimental work was carried out in a more primitive animal from the Class Reptilia, subclass Ophidia, the water snake (Natrix).

The Renin-Angiotensin-Aldosterone System

In 1898, Tigerstedt and Bergman discovered that some substance found in kidney extract could increase blood pressure. Their study used a rabbit kidney extract injected intravenously into rabbits giving a pressor effect. Tigerstedt named the pressor substance, in the extract, renin (58). Renin was rediscovered by Pickering and Printzmetal in 1938 (48) and finally characterized by Page and Helmer in 1940 (45). The product of renin enzyme action was called angiotonin or hypertensin by various groups, until 1958 when one term angiotensin was adopted.

In mammals the renin-angiotensin-aldoosterone system is part of an elaborate feedback mechanism which helps maintain proper sodium balance. The components of the system are: renin substrate (angiotensinogen),
renin, angiotensin I (AI), converting enzyme, angiotensin II (AII), angiotensinase(s), and aldosterone. These components are integrated into a negative feedback system which helps maintain sodium balance. Changes in circulating sodium levels are thought to affect the kidney, causing changes in renin release. Renin liberates the decapeptide angiotensin I from a specific circulating plasma globulin. Angiotensin I is acted upon by converting enzyme to produce a potent vasoconstrictor, AII, an octapeptide (51). Angiotensin II is inactivated by enzymes (angiotensinases) which hydrolyze bonds in the molecule, forming inactive fragments. Laragh, et al., 1960, showed that AII is a potent stimulant for aldosterone secretion by the adrenal cortex (33). It has been well documented that about 2% of sodium reabsorption by the kidney is controlled by aldosterone (6,29). Aldosterone and the direct pressor effect of AII work to maintain blood pressure and restore sodium balance. This system has been summarized in Figure 1.

Biochemical Profile

**Renin substrate.** Renin substrate or angiotensinogen is a plasma protein factor contained largely in the <2-globulin fraction. Skeggs, et al., (22) treated horse plasma substrate with trypsin from which they isolated a 14-amino acid residue polypeptide. When this tetradecapeptide was incubated with renin, AI was formed.

**Renin.** Renin is a protein enzyme. In 1953, Haas, et al., (25) used hog kidneys for the first extensive isolation and purification of renin. Peart, et al., (46) have purified renin 14 thousandfold using ammonium sulfate fractionation followed by various chromatography techniques. Renin has an estimated molecular weight of 40,000, and is a
highly stable enzyme when kept concentrated and semi-purified. Renin resists pH 2.5 for up to 1 hour at 0°C and pH 3.0 for 30 minutes at 37°C (27). Renin is resistant to trypsin, so that digestion requires prolonged incubation at a high temperature. Pig renin resists heating to 50-55°C for 15 minutes but is rapidly destroyed at temperatures above 56°C (27).

Angiotensin I. Al is a decapeptide which has no significant vasoconstrictor properties in the absence of plasma. Skeggs, et al., 1955 (52) first isolated horse Al. Elliot and Peart isolated bovine Al in 1956 (16). Bumpus, et al., subsequently isolated hog Al in 1967 (7) and finally, Arakawa, et al., isolated human Al in 1967 (3). The amino acid sequence of horse Al was determined by Lentz, et al., in 1956 (36). Hog, horse, and human Al have the same amino acid sequence; however, bovine Al has valine instead of isoleucine in the number 5 position.

Converting enzyme. The peptidases which convert the decapeptide Al to the active octapeptide AII have been collectively called converting enzyme(s). When Al is incubated with converting enzyme, the resulting mixture contains a dipeptide, hystidyleneucine, and AII. The plasma converting enzyme was first isolated by Lentz, et al., in 1956 (36) and requires the presence of monovalent anions such as chloride, bromide, nitrate or bicarbonate for activation. The enzyme is inhibited by ethylenediaminetetraacetic acid (EDTA), British Anti-Lewisite factor (BAL), or 8-hydroxyquinoline. High concentrations of converting enzyme are present in lung and liver tissue, with smaller quantities present in plasma, heart, aorta and ileum (8).
Angiotensin II: AII is an octapeptide and is by weight the most potent pressor substance known. AII causes an increase in peripheral resistance in addition to increased aldosterone secretion by the adrenal glands. AII has a very short biological half-life. Approximately 70% or more of an AII infusion is removed in one pass through the circulatory system with the largest removal occurring in the liver (38), femoral and renal vascular beds (28). More than 80 analogs of AII have been synthesized, with some being inhibitory in nature.

Ames, et al., (2) found that mildly pressor doses of AII produced marked increases in aldosterone secretion with no effects on cortisol or corticosterone secretion. During infusions of large amounts of AII, however, increased cortisol secretion can occur. When low concentrations of ACTH are infused, cortisol secretion is increased with no effect on aldosterone. When high concentrations of ACTH are infused, aldosterone secretion may also increase. These findings suggest a certain overlap exists in the control of aldosterone, cortisol, and corticosterone secretion. In animals, and in man, the acute aldosterone response to ACTH infusion is greatly amplified by prior sodium restriction (60).

Angiotensinase(s). The plasma and tissue peptidases capable of inactivating AII have collectively been called angiotensinase(s). At least 2 different types of angiotensinases have been identified. Angiotensinase A has a pH optimum of 7.5, requires Calcium ion, and is inhibited by EDTA (32). Angiotensinase B has a pH optimum of 5.5 and is inhibited by diisopropylfluorophosphate (DFP) (47).

Aldosterone: This hormone is synthesized and secreted primarily
by the adrenal zona glomerulosa. Accessory cortical tissue has been reported in some animals (63). Aldosterone contains an aldehyde grouping on the 18th carbon and exists in the hemiacetal form in solution.

Functionally, aldosterone increases distal tubular sodium reabsorption. There is also evidence that aldosterone can act on the proximal tubule as well as the ascending limb of Henle's loop (19).

Patients with Addison's Disease can lose 25-30 g of sodium per day if supportive doses of aldosterone are not given. Water balance is also affected by Addison's Disease. The sodium loss requires water excretion and can lead to circulatory collapse and death.

Investigators have used various techniques to increase or decrease renin release. These include ureteral occlusion (31), aortic constriction (5), blood volume and osmolarity changes (64), renal artery constriction (39), and renal nerve stimulation (37). These investigators have explained their results depending on the theory of renin release they supported. There are three release mechanisms proposed: the baroreceptor, the sympathetic nerve and the macula densa theories.

**Baroreceptor Theory.** Tobian, et al., (59) postulated that the juxtaglomerular cells are sensitive to stretch. A decrease in blood pressure increases renin release. Hemorrhage or dehydration are ideal stimuli for the decreased blood pressure response. Currently, this theory has stressed the role of interstitial and transmural pressure rather than intravascular pressure in renin release. During ureteral occlusion, renal vein occlusion and administration of osmotic diuretics, interstitial pressure is elevated. This decreases transmural pressure
and is thought to stimulate renin release without requiring a change in intravascular pressure.

**Sympathetic Nerve Theory.** Electrical stimulation of the renal nerves produces increased renin release. The beta adrenergic receptor in the renal arteriolar wall can be blocked pharmacologically, thereby eliminating this response. A denervated kidney, however, responds appropriately, even when transplanted to other parts of the body. Therefore it would appear that sympathetic control is not essential, but may be a "fine adjustment" of renin release (23).

**Macula Densa Theory.** Various investigators have suggested ways in which the macula densa can influence renin release. Vander and Miller (61) suggested that, in the dog, renin release is not controlled by blood pressure per se, or intrarenal pressure, but by the sodium composition of intratubular fluid, probably at the level of the macula densa. Vander and Carlson (62) on the basis of diuretic experiments suggested that decreased sodium transport by the macula densa cells increased renin release. These data suggest that the stimulus for renin release is either low concentration or reduced delivery of sodium to the macula densa. Since tubular fluid is normally hypotonic as it enters the macula densa from the loop of Henle, it would appear that some minimal level of sodium must be available to prevent renin release.

Other investigators have suggested that hypertonic solutions increase renin release. Thurau, et al., (57) carried out experiments using a retrograde micropuncture perfusion technique which presented hypertonic sodium chloride solutions to the macula densa and caused an increase in renin release. Cooke (10) has generated information, using
diuretics, which support Thurau's theory. Cooke found that ethacrynic acid, which inhibits sodium reabsorption in Henle's loop stimulates renin release. Chlorothiazide, which affects distal tubular sodium reabsorption but has no effect in the loop of Henle, does not stimulate renin release.

**Anatomy of the juxtaglomerular apparatus**

In mammals, the kidney juxtaglomerular apparatus (JGA) consists of 3 components; juxtaglomerular cells (JC), macula densa (MD), and extraglomerular mesangium (EGM). Birds, reptiles, amphibians, and non-teleost fishes lack MD and EGM structures (55).

**Juxtaglomerular cells.** The JC are composed of modified, granular, smooth muscle cells, modified, agranular, smooth muscle cells, and non-modified, smooth muscle cells. The modified granular muscle cells are epitheloid, and contain renin. When stained using the Hartroft method (26) the granules appear to be stored renin. The modified agranular muscle cells have been termed pseudomeissnerian or "lacis" cells, and were first described by Goormaghtigh in 1932 (22). These cells and components of the EGM comprise the polkissen in which the modified agranular muscle cells are the predominant type. The non-modified smooth muscle cells are indistinguishable from vascular smooth muscle cells found in arterioles throughout the body.

**Macula densa.** This accumulation of distal tubular epithelial cells was named macula densa by Zimmermann in 1933 (65). These cells are in intimate contact with the lumen of the distal convoluted tubule and possess all the characteristics of common distal tubular epithelium. The cells have a basal pole with membraneous intracytoplasmic projec-
tions and an apical pole whose surface erupts into microvilli (65).

**Extraglomerular mesangium.** The mesangial cells have much in common with the cells of the polkissen and afferent and efferent arterioles. The cells also contain fibrillar bundles and attachment bodies, suggesting derivation from smooth muscle cells. The EGM is bounded by the afferent and efferent arterioles and macula densa.

**Gross anatomy of snake kidney**

A thorough study of the anatomy of snake kidney was carried out by Bishop in 1959 (4). The snake nephron is similar to those of other reptiles and consists of 6 parts: the glomerulus, the neck segment, the proximal tubule, the intermediate segment, the distal tubule, and the collecting duct. The glomeruli of snake kidney are smaller and less numerous than in mammalian kidney. The number of glomeruli varies from 1,500 to 1,700. The external diameter of a glomerulus is approximately 60 microns.

Immediately past the glomerulus, the nephron forms the neck segment. This poorly developed segment is about 20 microns in diameter and 0.07 mm in length, and consists of small cuboidal cells with disproportionately large and densely basophilic nuclei. After an abrupt transition, the proximal tubule occurs. The proximal tubule, which is about 2.8 mm long, is lined with cuboidal epithelium which has a low brush border. The nephron proceeds to the intermediate segment, which has a length of only 0.3 mm and where the tubular diameter gradually tapers to about 25 microns. This segment is comparable in location to the loop of Henle in birds and mammals. The tubular diameter increases to about 35 microns which marks the beginning of the distal tubule,
which is about 1.9 mm in length. The distal tubule is lined with low cuboidal epithelial cells which have unmodified cell borders. The distal tubule terminates in the collecting duct.

The numerous collecting ducts empty into a common ureter located dorsally. The ureter proceeds posteriorly along with the renal portal vein and terminates in a papilla within the cloaca. Since the urinary bladder is absent in snakes, the cloaca is subdivided by folds in its walls into a coprodaeum into which the large intestine opens; the urodaeum into which the urogenital ducts open; and the posterior proctodaeum into which both urodaeum and coprodaeum empty. The urinary papilla which marks the location of the urodaeum, is located immediately inside the external opening to the cloaca.

The snake kidney receives 5 or 6 arterial branches from the dorsal aorta, each branch being a renal artery supplying several kidney lobules. The branches make several small loops within the glomerulus and continue as the efferent arteriole ultimately draining into the renal vein. Branches of the renal portal vein also supply the tubular segments, providing the nephron with a double blood supply.

Hormonal and renal function in reptiles

Of particular interest in the snake is the mechanism of renin release by the kidney. Renin-like activity has been reported in birds, reptiles, amphibians, and teleost fishes. The absence of renin-like activity has been reported in elasmobranches and cyclostomes (stingrays, lamphreys and hagfishes) (41). Johnson, et al., found renin-like activity in bullfrog post-caval blood (29). They also found an increase in plasma aldosterone production when a frog kidney extract was
infused into hypophysectomized frogs. This is especially interesting since the frog responded to the renin stimulus but does not have a macula densa.

Considerable renal function data are available on snakes (Natrix). In 1962, LeBrie and Sutherland (34) carried out a comprehensive study on snake renal function. Several species of Natrix were used in these experiments in which electrolyte and osmolar concentrations of ureteral urine and plasma were compared. They found that even after water deprivation, snakes were unable to form a hyperosmotic urine. The loop of Henle is required for hyperosmotic urine formation, a structure snakes lack. LeBrie and Sutherland (34) suggested that snakes regulate urinary solute excretion by varying the number of functioning tubules. This was the only possible explanation of a linear relationship which existed between glomerular filtration rate (GFR) and urine flow and a constant fractional osmotic reabsorption over a 10-fold increase in GFR. They further suggested that high circulating ADH levels affect GFR and distal tubular water reabsorption in snakes.

Dantzler and Schmidt-Nielson (13) carried out renal function experiments in the fresh-water turtle (Pseudemys scripta) and desert tortoise (Gopherus agassizii). Mild dehydration in fresh-water turtles decreased GFR and increased tubular reabsorption of water with urine osmolality approaching that of the blood. Severe dehydration or salt loading resulted in anuria. In desert tortoises anuria did not occur until approximately 5 times the salt load given the fresh-water turtles. In both species, an increasing water load increased GFR markedly but
had little or no effect on tubular function. These studies support LeBrie and Sutherland's hypothesis (34) that changes in GFR result from changes in numbers of functioning tubules and glomeruli.

Dantzler (11) using unanesthetized water snakes (Natrix sipedon) studied the effect of a synthetic neurohypophysial peptide during water diuresis. Arginine vasotocin (AVT) caused no change in arterial blood pressure, but caused a decrease in urine flow, GFR and relative free water clearance ($C_{H_2O}/GFR$). He also suggested that AVT increases tubular reabsorption of sodium and decreases tubular secretion of potassium. The tubular maximum for p-aminohippurate secretion ($Tm_{PAH}$) varied with GFR which is strong supporting evidence for the hypothesis that changes in GFR result from changes in the number of functioning glomeruli.

Dantzler, et al., (14) studied the inhibitory effects of probenecid infusion on arginine vasotocin treatment in unanesthetized water snakes (Natrix sipedon). Infusion of probenecid produced a diuresis and inhibited the renal response to exogenously administered AVT. Probenecid produced an increase in relative free water clearance ($C_{H_2O}/GFR$) when given intravenously to water snakes with low urine flow. Probenecid did not change ($C_{H_2O}/GFR$) in snakes that were already in a good water diuresis. These results suggested that probenecid inhibits the renal tubular effects of AVT.

Elizondo and LeBrie (15) examined adrenal-renal function in the water snake (Natrix cyclopion). These studies used functionally adrenalectomized snakes and normal snakes treated with aldactone (an aldosterone inhibitor), aldosterone, triamterene (a diuretic), and
corticosterone. In adrenalectomized snakes during water diuresis, significant decreases in plasma osmotic pressure as well as plasma sodium, chloride and potassium were observed. No change in GFR or urine flow occurred, although fractional sodium and water reabsorption were decreased. Adrenalectomy and triamterene apparently affect the proximal tubule in snakes. Administration of aldactone, aldosterone or corticosterone did not affect renal handling of either sodium or water in unoperated snakes.

LeBrie and Elizondo (35) studied the effect of saline loading and aldosterone in the water snake (*Natrix cyclopion*). When compared to water loading, saline loading produced significantly increased plasma sodium, chloride and osmotic pressure, but did not affect plasma potassium levels. Saline loading did not affect GFR but did significantly decrease fractional sodium, water and osmotic reabsorption to levels below those found in water loading. Distal water reabsorption was small or absent in either water or saline loading. The authors concluded that saline loading reduced isosmotic sodium and water reabsorption in the proximal tubule while water loading does not. They also suggested that distal sodium reabsorption was inhibited and that little or no distal water reabsorption occurred. The absence of distal water reabsorption during saline loading was attributed to an inhibition of ADH by a hypothesized volume receptor similar to that described in mammals. Saline loaded snakes, which would be expected to have low circulating levels of aldosterone, responded with decreased urinary sodium excretion during exogenous aldosterone administration. During a water load, however, when endogenous aldosterone
levels would be expected to be high, exogenous aldosterone administration had no effect on urinary sodium excretion.
PURPOSE

The purpose of this research was to test the macula densa theory. The snake model was used for several reasons.

1. As noted earlier, snakes (reptiles) do not have a macula densa but do synthesize renin. This animal, therefore, is an excellent model for studying renin response without macula densa effects. If experimental procedures which are known to increase or decrease renin release in mammals produce the same effects in snakes, some other, non-macula densa mechanism must be operative.

2. The snake has proximal and distal tubules but no loop of Henle. We were interested in determining the site of action of the diuretic furosemide. This diuretic affects principally the proximal tubule and ascending loop of Henle in mammals and is known to be a strong stimulus for renin release.
METHODS

A total of 220 water snakes, primarily female *Natrix taxispilota*, were used in this study. These animals were collected in the Gainesville, Florida area by commercial reptile dealers and shipped to us via Air Express. Transit time never exceeded 48 hours. On arrival, the animals were placed immediately into one of three 20 gallon aquariums which were partially filled with water (3-4 inches) and which contained several glass bricks so the animals could "sun themselves" under a 100 watt lamp. To minimize effects on renal function which could have been caused by variations in light periodicity, a 24 hour automatic timer maintained equal 12 hour periods of light and darkness. The temperature in the aquariums was maintained at 26° ± 0.05°C by an automatic thermostat and electric heater.

Every 10-14 days the animals were fed minnows. The snakes seemed to tolerate this diet quite well. LeBrie and Sutherland have shown that no significant differences were present in plasma electrolyte concentrations and osmotic pressure between fasted and fed snakes (34). Therefore, we did not consider feeding as having a significant effect on our results.

All renal studies were carried out on unanesthetized snakes at 27°C ± 1°C. All experiments, except for the non-diuresis control and saline groups were carried out during water diuresis. Dantzler (11) showed that the administration of arginine vasotocin (AVT) during water
diuresis increased distal tubule permeability to water. Earlier work by LeBrie and Sutherland suggested that the snake distal tubule becomes impermeable to water during water diuresis because the circulating level of antidiuretic hormone (ADH) is reduced (34). Snakes lack a loop of Henle and therefore, cannot produce a concentrated urine. If we assume that proximal reabsorption in the snake is isosmotic, as it is in other vertebrates, than per cent water reabsorption during maximum water diuresis would be an index of solute reabsorption in the proximal tubule. Also the per cent distal osmotic reabsorption can be calculated by subtracting per cent water reabsorption (proximal osmotic reabsorption) from total osmotic reabsorption. Thus during water diuresis:

(1) per cent water reabsorption = per cent proximal osmotic reabsorption

(2) per cent distal osmotic reabsorption = total osmotic reabsorption - per cent water reabsorption.

The following general protocol was used on all animals in which renal function studies were carried out. Approximately 16 hours before the experiment, a priming dose of inulin (0.02 g per 100 g body weight) was administered intraperitoneally to each snake. The animals were returned to an aquarium in which they had free access to water but not food. The following day, a second dose of 0.02 g of inulin per 100 g body weight was administered intraperitoneally along with a water load of 10 milliliters per 100 g body weight.

Approximately 90 minutes after the administration of the water load and inulin, a blood sample was obtained by direct cardiac puncture.
A one milliliter plastic disposable syringe (B-D) with a $\frac{1}{2}$ inch 20 gauge needle was used to obtain approximately 0.5 milliliters of blood. LeBrie and Sutherland have shown that this volume of blood is less than 5.0 per cent of the total blood volume in snakes of the weight we used, and has no effect on GFR and urine flow \((34)\). The fresh blood was placed in two Spinco microcentrifuge tubes which were pretreated with ammonium heparin (5 units per 500 microliters of blood). Two microhematocrit tubes pretreated with sodium heparin were also filled.

After the first plasma sample was obtained, the snake was immobilized in a plexiglass tube. The tail of the snake was taped to an 8 inch flat extension fixed to the side of the tube \((34)\). One or both of the ureters were cannulated with Intra Medic P.E. 50 polyethylene tubing \((I.D. 0.023, O.D. 0.038)\). The room was kept semi-dark and under these conditions, urine would begin to flow freely within 45 minutes after cannulation. Blood samples were not collected during clearance periods since noxious stimuli may cause depression of cardiac function, thereby altering GFR and urine flow. After one to three clearances were collected, a second blood sample was obtained, also by cardiac puncture. The time period between the two blood samples was approximately two hours. The two blood samples were centrifuged immediately, and the plasma saved for inulin, sodium, potassium, and osmotic pressure analyses. These values were used to obtain a semilog plot of plasma concentration vs collection time of each plasma sample and a line drawn through the two points. The extrapolated plasma value of inulin, sodium and potassium at the midpoint of each clearance period was used to calculate clearance values. This method had been amply documented \((34)\).
Urine was collected in previously tared test tubes. The tubes were weighed immediately after collection. One milliliter of urine was assumed to weigh one gram, since the specific gravity of urine is very close to 1.0, the specific gravity of water. After weighing, the urine was stored in a refrigerator for up to 48 hours before being analyzed for inulin, sodium, potassium, and osmotic pressure.

After the collection of the second plasma sample, the snake was euthanized with a sharp blow to the neck and skull. The kidneys were removed immediately and placed in previously tared cups to obtain kidney weight. The kidneys were weighed on a H-16 Mettler balance and then stored at -20°C until assayed for renin content.

The inulin concentration of the plasma and urine was determined by the method of Galli and Jeanmaire (18) adapted to the autoanalyzer. Plasma and urinary sodium and potassium were analyzed using a flame photometer (I.L. model 143). Osmotic pressure was determined using a freezing point osmometer (Osmette A Precision Systems, Inc.).

To evaluate kidney renin levels in snakes, it was necessary to develop a renin assay. We attempted to use an available radioimmunoassay (New England Nuclear), however, we were not successful and switched to a bioassay. As with all bioassays, our assay does not measure renin directly, but angiotensin II, the final product of renin activity.

The test animal used in our assay is the rat. Solutions of snake kidney extract are injected intravenously into the rat while the blood pressure is monitored. The assumption is that the pressor effect of the angiotensin II is proportional to the kidney renin concentration.
Renin is an intermediate in the production of angiotensin II. Kidney extracts must be processed in a manner to insure that the final concentration of angiotensin II is proportional to the unknown renin concentration.

Extract Procedure

Snake kidneys are excised, weighed and frozen at -20°C until processed. For processing, the kidneys are thawed at 4°C, finely minced with scissors, and placed in a siliconized tissue homogenizer. Ten milliliters of 0.9 per cent saline are added to each gram of kidney (44). The saline contains, 0.001 molar phenylmercuric acetate (PMA) per liter and 2 mg disodium ethylenediaminetetraacetic acetate (Na₂EDTA) to act as a bacteriostatic agent and angiotensinase inhibitor respectively. The homogenizer is placed in an ice bath and the kidney is homogenized for approximately 90 seconds with a teflon pestle. The homogenized mixture is transferred to polycarbonate test tubes and is centrifuged at 4°C for 20 minutes at 15,000 gravities. The supernatant is decanted into a second polycarbonate test tube, the tube is placed in an ice slush, and the pH is adjusted to 3.0 with 1 Normal HCl. After 30 minutes, the supernatant is then adjusted to pH 6.5-7.0 with 1 Normal NaOH and centrifuged to remove additional precipitate. The kidney extract is divided into several one milliliter aliquots and frozen at -20°C until incubation.

The renin incubation procedure used is the method of Skeggs, et al. (54) modified to optimize angiotensin generation from snake kidney extract. The incubation mixture for each sample contains 5 micro-
liters of kidney extract, 100 microliters of highly purified, angiotensinase-free hog renin substrate (kindly provided by Doctor L. T. Skeggs, Veterans Administration Hospital, Cleveland, Ohio), 1000 microliters of normal saline with PMA, and 1000 microliters of NaH$_2$PO$_4$/Na$_2$HPO$_4$ phosphate buffer (pH 7.5). A sample blank is also prepared in which the substrate volume is replaced by 100 microliters of saline. The blank and samples are treated identically. These ingredients are placed in a polycarbonate test tube which is mixed on a vortex mixer for one second before being placed in a constant temperature incubation bath. The bath is calibrated against a mercury thermometer and has a circulating pump and immersion heater that automatically holds bath temperature at 37°C. The volume of water in the bath (over 3 gallons) is a large heat sink and is adequate to prevent temperature fluctuation when samples are placed in the bath. After incubation for 10 minutes, the samples are immediately placed in boiling water to stop the reaction by denaturing renin. The boiled samples are then centrifuged at 4°C for 10 minutes at 15,000 gravities to remove any precipitate. The supernatant is transferred to an ice bath until the samples are assayed. The samples are assayed within two hours of incubation.

Rat Bioassay Procedure

The rat bioassay procedure used is based on the method of Gunnels, et al., (24). Two male 180-230 gram rats (Sprague-Dawley) are anesthetized with sodium-pentobarbital, (50 mg per kilogram of body weight). Each rat is taped to a rat board, and a midline incision is made in the ventral neck area. The left jugular vein (used for sample,
standard and supplemental anesthetic, if required) is cannulated with PE 20 tubing (filled with heparinized saline) connected to a 27 gauge needle and heparinized saline filled syringe. The trachea is cannulated with PE 240 tubing. The left carotid is cannulated using PE 50 tubing filled with heparinized saline. This tubing is connected to a Statham P23AA pressure transducer which interfaces with a Gilson polygraph CH-CBPP amplifier. The oscilloscope output from the Gilson is applied to a 741 op-amp which then applies a signal to a HeathKit Ir-18M strip chart recorder. The 741 circuitry has special filtering capabilities which decrease the diastolic component of the pressure pulse but allows the systolic peak to be recorded unchanged. The sensitivity of the equipment can monitor the range of blood pressure from 1-200 mm Hg. If required, the gain of the recorder can be adjusted to give a 1 inch deflection per mm Hg change. The equipment is calibrated against a mercury manometer. A ganglionic blocking agent, pentolinium tartrate (Ansolysen HCl) is injected (3.5 mg per kilogram body weight subcutaneously), as is atropine sulfate (0.5 mg per kilogram body weight subcutaneously). The blood pressure normally at 140-160 mm Hg is monitored until a stable plateau is reached in the 60-100 mm Hg range. At this point the preparation is ready for kidney extract assay. A two way valve switches the transducer input input from one rat to the other, allowing one recording apparatus to be used.

Lyophilized synthetic human angiotensin I standard (Beckman) is diluted in normal saline to obtain a concentration of 0.1 nanogram per 10 microliters. A series of standard injections are made at 0.3; 0.5; 0.7; and 1.0 nanograms to obtain a standard response curve. Up to two
unknown and blank samples are injected followed by a second series of standard injections. This sequence is followed until a total of four unknowns and blanks are assayed. All samples are assayed in duplicate. Angiotensin I has no pressor response. Our assay uses EDTA as an angiotensinase inhibitor which also inhibits converting enzyme. The conversion of angiotensin I to angiotensin II occurs in the rat mainly by passage through the lungs; however, the plasma also has some converting enzyme activity. Since the samples and standards contain only angiotensin I, the enzyme velocities are similar and the pressor peaks are directly comparable.

Kidney renin concentration is calculated by comparing the sample pressor response against the pressor standard curve obtained by the injection of known quantities of angiotensin I. The standard pressor response is fitted with a least squares linear regression line using a Hewlett-Packard desk top computer. This line is generated using the standard responses obtained before and after a set of samples. The line is recalculated if the rat sensitivity to standard changes.

After the equivalent values in nanograms of angiotensin I are obtained for both sample and blank, the effect of diluting the kidney extract in the preparation step is taken into account. The blank is substractioned from the sample and the answer is expressed as nanograms of renin activity per gram of kidney tissue.

In order to estimate the variability of the bioassay, duplicate samples were assayed in six different rats on four different days. The average deviation was 10.6 per cent ± S.D. 6.3 per cent. These results compare favorably to the method of Vander, et al., whose average
deviation was 15.2 per cent ± S.D. 13.4 per cent (62). In all experiments reported here, renin assays were run on at least two rats simultaneously. If the results from the two rats were inconclusive, a third rat and fresh samples were prepared and the results of all three rats were averaged.

In order for our renin assay to be considered a valid estimate of renin activity, the assay had to generate angiotensin from renin extracts, had to be angiotensinase free, and had to show that the product assayed in the rat was angiotensin generated from renin. A series of experiments were carried out as a proof of the method.

Increasing amounts of kidney extracts (5 to 500 microliters) were incubated with an excess of substrate for a constant 5 minute period of time. The results (Table 1) represent angiotensin I generated in the incubation mixture. Results are given as nanograms of angiotensin I per milligram of kidney. Column 1 represents the amount of snake extract added. Columns 2 and 3 represent duplicate assays on two different days in different rats. Column 4 was a rerun of the first two assays with 5 times more substrate added to the reaction mixture. As the amount of kidney extract in the incubation mixture is increased, the nanograms of $A_1$ generated per milligram of kidney decreases in all cases. This suggests that some substance in the kidney extract either inhibits angiotensin generation or inactivates angiotensin as extract concentration increases. These data also suggest that 5 microliters of extract is the optimum volume since the extract obviously contains some angiotensinase activity. Increasing the hog substrate concentration from 40 to 200 microliters did not significantly
increase the amount of angiotensin generated. This suggests that an
excess of substrate is present in 40 microliters; however, we used 100
microliters of substrate for all our samples as further assurance that
lack of substrate would not limit the reaction rate.

To determine whether angiotensin inhibition or inactivation was
responsible for the results described above, recovery studies of added
angiotensin I were also carried out. Angiotensin I at a concentration
of 90.7 nanograms per milliliter was added to samples of extract, sub-
strate, and a mixture of extract and substrate. These samples were in-
cubated for increasing periods of time and assayed for angiotensin
content. A sample of each extract used in the recovery study was in-
cubated normally, assayed and subtracted from the samples which had
angiotensin added. These data are presented in Table 2, and indicate
at least a 70 per cent recovery of added angiotensin I. This suggests
the angiotensinase activity of our assay is minimal for up to 10 minutes
incubation and that processing of our samples probably has little ef-
fekt on the total amount of angiotensin generated and assayed.

It is generally accepted that when renin is incubated with renin
substrate, angiotensin I is formed at a constant rate, until substrate
exhaustion causes the rate to decrease. To determine proper sample in-
cubation time for our method, we prepared duplicate samples and in-
cubated them for 0 to 20 minutes (Table 3). Sample series XT A was pre-
pared and assayed on one day. Series XT B(1) and XT B(2) were dupli-
cate samples assayed on two different days. All series show an increase
in angiotensin levels as incubation time is increased. We, there-
fore, use a 10 minute incubation for our samples since this time is on
the linear portion of the angiotensin generation curve. These data also show that the total amount of angiotensin assayed varies among extracts (XT A and XT B), as would be expected if the kidney renin concentration varies from animal to animal.

Enzyme reaction rates are affected by the pH of the incubation mixture. We carried out additional experiments to determine the optimum pH at which to incubate our samples. Samples and blanks were incubated for 10 minutes at 37°C. The pH of the various incubation mixtures was buffered to 4.5, 5.5, 6.5, and 7.5 ± 0.5 respectively. The phosphate buffer \( \text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4 \) ratio was adjusted to hold each sample at the proper pH. The data in Table 4 show that the maximum amount of angiotensin is generated at pH 7.5.

Additional studies were carried out to confirm that the substance which produced the pressor response was actually angiotensin II generated by renin activity. Renin is denatured at temperatures over 65°C. Therefore, boiling kidney extract material before incubation with substrate should eliminate the pressor response since angiotensin should not be formed. Treating kidney extract with trypsin after incubation should also reduce or eliminate the pressor response since trypsin attacks angiotensin but not renin. The data presented in Table 5 suggest that the substrate producing the pressor substance is renin, since boiling of the extract for one hour before incubation reduces the pressor response assayed; i.e., that since reduced levels of renin were present, less angiotensin was formed. The fact that trypsin treatment of the extract for 10 minutes before incubation and then during incubation decreases the pressor response suggests the pressor substance
is angiotensin since trypsin inactivates angiotensin, but not renin.

Other investigators (42, 43) have found an absence of a reaction when mammalian substrate is incubated with a non-mammalian kidney extract. A very strong possibility is that the renin and renin substrate isolation technique used by those investigators was responsible for the varied responses in that angiotensinase activity has not been completely suppressed. We have found that lowering the pH of the renin extract to 3.0 during the isolation effectively deactivates DFP sensitive angiotensinases. Those angiotensinases sensitive to EDTA are deactivated by the Na₂EDTA added during kidney homogenization. The renin substrate provided by Doctor L. T. Skeggs is also low in angiotensinase activity.

Incubation of added angiotensin with the substrate for up to 18 hours did not show a decrease in pressor activity from the added angiotensin when aliquots of this mixture were assayed. This suggests that angiotensinase activity in our assay procedure is extremely low. Furthermore, we discovered that the use of angiotensinase inhibitors alone, without the pH 3.0 treatment is not adequate to remove angiotensinase activity in snake samples. British Anti-Lewisite Factor (BAL), 8 hydroxyquinoline, and EDTA are used in the New England Nuclear (NEN) angiotensin I radioimmunoassay to block angiotensinase activity. In the plasma and kidney samples we assayed using the RIA, the 0°C samples (blanks) routinely had greater activity than the 37°C samples, suggesting deactivation of angiotensin at the elevated temperature. We ascribe this deactivation to angiotensinase activity.

Capelli, et al., carried out studies in which he incubated kidney
extracts from teleost fishes, amphibians, reptiles, birds, and mammals, with calf substrate (9). The results he obtained for the fresh water turtle (Chrysemys picta) and desert tortoise (Gopherus agassizii) are quantitatively in the same range as we have found for the water snake (Natrix)

**Experimental Groups**

Ten different experimental groups were employed during this study. Each group was compared to a control, water diuresis group. The groups were as follows:

1. **Water control**: These animals received a standard water load and inulin as described previously.

2. **Furosemide treated**: Eighteen hours before the experiment, these animals received a priming dose of Furosemide (5 mg per kilogram body weight) intraperitoneally along with the initial dose of inulin. The next day, 90 minutes before the start of the experiment, a second Furosemide dose was administered (10 mg per kilogram body weight) along with the water load and inulin.

3. **Functional adrenalectomy**: Three days before the experiment, these animals were placed in a 0°C cold room for approximately two hours. At 0°C, the snakes become dormant. A ventral midline incision was made proximally to the kidneys and the adrenals were exposed. The surrounding fascia was teased away to expose the arteries and the veins entering and leaving each adrenal. As illustrated in Figure 2, the
adrenal has one arterial connection with the aorta and numerous venous connections with the inferior vena cava and body wall. Each vessel was carefully ligated using 6-0 silk (Ethicon, cardiovascular 706 G), but the adrenal was not removed. This procedure is similar to the one reported by Elizondo and LeBrie (15). The incision was lightly sprayed with 1.0 per cent Neomycin Sulfate and closed, sutureing alternate rows of scales. The incision area was then sprayed with Aeroplast dressing (Parke, Davis and Company). The animals were placed in a clean, dry tank and allowed to recover. They would become quite active within 20 minutes after removal from the cold room. Approximately 14 hours after surgery, the animals were transferred to a second tank which contained water (3 inches) and glass bricks as described earlier. Forty-eight hours after the operation, the snakes received a priming dose of inulin and from this point on, were treated identically to the control animals for renal function studies.

(4) Total adrenalectomy: These animals were treated identically to the functional adrenalectomy group except that after the arteries and veins to each adrenal were ligated, the adrenals were removed.

(5) Sham operation: These animals followed the same surgical and experimental time table as the adrenalectomy groups except that the adrenal area was exposed and the incision closed without further surgery.
(6) **Vena caval occlusion:** These animals followed the same surgical and experimental time table as the adrenalectomy groups except that the inferior vena cava was ligated (6-0 silk) proximally to the right adrenal. A ventral midline incision was made proximally to the adrenals and the inferior vena cava exposed. The vessel was ligated (6-0 silk) and cut. The approximate site of the ligation is shown in site A, Figure 2. Seventy-two hours after the operation, renal function studies as previously described, were carried out.

(7) **Partial aortic constriction:** These animals followed the same surgical and experimental time table as the adrenalectomy groups except that the aorta was constricted proximally to the right adrenal. A ventral midline incision was made proximally to the kidneys and the aorta exposed. A curved, 27 gauge needle was placed next to the aorta, and a ligature was placed around both of them. When the needle was withdrawn, a limited amount of blood could still flow through the aorta. The approximate site of the constriction is shown in site B, Figure 2. Seventy-two hours after the operation, renal function studies, as previously described, were carried out.

(8) **Renal portal occlusion:** A ventral midline incision was made proximally to the cloaca and the renal portal veins exposed. The veins were ligated (6-0 silk) and cut. The approximate site of the ligation is shown in site C, Figure 2. The area
was lightly sprayed with 1.0 per cent Neomycin Sulfate and the incision closed. The surgical area was then sprayed with Aeroplast and the animals confined in a dry tank. Since the incision and bleeding in this procedure was small, the animals were transferred to a tank containing water two hours after the operation. Also, this group did not receive a priming dose of inulin 14 hours before the experiment as this would have required the injection of inulin during the surgical procedure. The next morning, inulin and the water load were injected intraperitoneally 90 minutes before the experiment and the renal function protocol as described earlier was followed.

(9) 1.5 per cent Saline: These animals received a priming dose of inulin 18 hours before the experiment. A 1.5 per cent load (10 milliliters per 100 g body weight) and inulin were given the next day.

(10) 2.0 per cent Saline: These animals were treated identically to the 1.5 per cent saline group except that 2.0 per cent saline was administered.
RESULTS AND DISCUSSION

Water Loaded Snakes

Water diuresis control: This work presents for the first time, hematocrit and plasma protein values for Natrix during water and saline diuresis. Table 6 shows the body weight, hematocrit and plasma values for the water control group. The $P_2$ values for these variables are not significantly lower than $P_1$ values. This suggests that urine was collected during the period of maximal water absorption from the peritoneum.

Table 7 notes the renal function data for the water control group to which all experimental water diuresis groups were compared. The hyperbolic relationship of urine flow versus fractional water reabsorption for all water diuresis groups is shown in Figure 3. All water diuresis groups were plotted since statistical analysis showed significant differences did not exist between groups for urine flow versus fractional water reabsorption. These data show that as urine flow increases, the per cent water reabsorbed decreases to a constant fraction. This decrease is due to decreasing levels of circulating Anti-Diuretic Hormone (ADH) as the animal absorbs the water load and becomes volume expanded (34). The plateau in the curve occurs when the distal tubule becomes impermeable to water with continued isosmotic reabsorption occurring in the proximal tubule. The constant per cent water reabsorp-
tion during an increasing urine flow is attributed to an increasing functioning population of nephrons in which each nephron reabsorbs a constant per cent of the total load (34). As urine flow varies, so does the total population of functioning glomeruli. Urine flows on the linear portion of the curve would then represent periods of maximal water diuresis. It should be noted that urine flow in all graphs and figures is for only one ureter. We assume that total urine flow is two times our urine flow values.

Distal water reabsorption is absent in Natrix when urine flow exceeds 0.400 ml/100g/hr and water reabsorption is relatively constant at 49 per cent. Elizondo and LeBrie (15) reported that, in Natrix, a relatively constant 40 per cent water reabsorption was present when urine flow was greater than 0.400 ml/100g/hr. LeBrie and Sutherland (34) earlier had reported a constant fractional water reabsorption (60 per cent) when urine flow exceeded 0.200 ml/100g/hr. Comparison of these earlier data shows no statistical difference between them and the data in the present experiment shown in Figure 3.

As in mammals, snakes show a hyperbolic relationship during water diuresis between urine osmolarity and urine flow (Figure 4). At low urine flow, urine osmotic pressure is isosmotic with plasma and as urine flow increases, urine osmotic pressure reaches a relatively constant hypoosmotic value. The dotted line, representing a least squares fit of the current data, was compared statistically to the solid line, representing similar data from LeBrie and Sutherland (34). No statistical difference exists between the two lines. These data indicate that urine osmolarity approximates plasma osmolarity at urine flows
below 0.200 ml/100g/hr. At higher urine flows, the urine osmolarity drops to a relatively constant value of approximately 75 mOsm/liter. The dotted line at 299 mOsm/liter indicates mean plasma osmolarity for all water diuresis groups during the experimental period.

LeBrie and Sutherland (34) and Elizondo and LeBrie (15) have shown that urine flow and GFR vary proportionally. The current data were compared statistically to the data of Elizondo and LeBrie. No significant difference between the groups of data was found.

**Furosemide:** As shown in Table 6, no significant differences from control were noted for body weight, hematocrit, plasma protein or plasma sodium, potassium and osmotic pressure. Table 7 shows urine flow increased significantly (P<.001), after Furosemide, from 0.454 ±0.034 (SE) ml/100g/hr to 0.671 ±0.036 ml/100g/hr while GFR did not. Sodium excretion increased significantly (P<.001) from 9.4 ±1.6 uEq/100g/hr to 36.6 ±3.2 uEq/100g/hr as did potassium excretion (P<.001). Osmotic excretion also increased significantly (P<.001) as would be expected since increased amounts of sodium and potassium, and their associated anions are present in the urine.

Furosemide decreased fractional sodium reabsorption significantly (P<.001) from 93.23 ±0.85 per cent to 76.30 ±2.05 per cent (Figure 5). Fractional water reabsorption decreased significantly (P<.001) from 51.26 ±1.45 per cent to 37.88 ±2.10 per cent. This change is consistent with the excretion data. Total osmotic reabsorption decreased significantly (P<.001) from 88.82 ±0.83 per cent to 71.67 ±2.27 per cent.

The renal function data suggest a decrease in proximal sodium and
water reabsorption in that per cent total osmotic reabsorption decreased but the per cent distal tubular osmotic reabsorption did not decrease significantly. This is strong evidence that Furosemide decreases proximal reabsorption in snakes. This is further reflected in increased urine flow. The GFR did not increase significantly, suggesting the filtered load was not increased. Of interest is that potassium as well as sodium excretion was increased. Furosemide had been reported to have little effect on potassium excretion in mammals (56).

Furosemide has been shown to effect the proximal tubule and loop of Henle in mammals (56). Snakes lack a loop of Henle but do have a proximal tubule and, as the results indicate, the proximal effect is the only one observed in snakes. These results are similar to those reported in snakes by Elizondo and LeBrie for another diuretic, Triamterine (15). In mammals, Triamterine reverses the sodium retaining and potassium excreting effects of aldosterone.

Functional adrenalectomy: The only significant change we observed after functional adrenalectomy was a fall in plasma sodium concentration (P<.001) from 145.4 ±0.8 mEq/liter to 135.8 ±1.1 mEq/liter. This effect was also shown by Elizondo and LeBrie (15); however, their plasma sodium values were significantly lower than ours. Elizondo and LeBrie further showed a significant decrease in fractional sodium, water and proximal osmotic reabsorption. Their fractional sodium reabsorption fell from 91.1 ±0.82 per cent to 70.6 ±1.72 per cent (15). Their work also showed a significant decrease in plasma potassium concentration after adrenalectomy. Our plasma potassium is not significantly dif-
ferent from control. Since we could not confirm the previous work we attempted to determine what may have caused the different results. To this end, we undertook total adrenalectomy experiments.

**Total adrenalectomy:** As shown in Table 6, no significant differences from control were noted for body weight, hematocrit and plasma protein. The renal function values on Table 7 show that urine flow, decreased significantly (P<.001) from 0.454 ±0.034 ml/100g/hr to 0.256 ±0.073 ml/100g/hr. However, glomerular filtration rate decreased significantly (P<.001) from 0.940 ±0.066 ml/100g/hr to 0.556 ±0.067 ml/100g/hr. This finding suggests that the decrease in urine flow was due to a decrease in filtered load. This is also confirmed by the absence of a significant difference in fractional sodium reabsorption. Distal osmotic fractional reabsorption decreased significantly (P<.02) from 37.56 ±1.25 per cent to 23.56 ±4.66 per cent, suggesting total adrenalectomy affects mainly distal, not proximal, tubular reabsorption.

**Functional adrenalectomy versus total adrenalectomy:** The plasma sodium concentration of the functionally adrenalectomized animals varied significantly (P<.001) from the totally adrenalectomized animals, the values being 135.8 ±1.1 mEq/liter and 148.2 ±3.6 mEq/liter respectively (Table 6). Comparison of renal function studies as noted in Table 7, indicates that urine flow was significantly lower (P<.02) in the totally adrenalectomized group (0.481 ±0.050 ml/100g/hr versus 0.256 ±0.073 ml/100g/hr). Glomerular filtration rate was also significantly lower (P<.005) in the totally adrenalectomized group (0.993 ±0.102 ml/100g/hr versus 0.556 ±0.067 ml/100g/hr).

Total adrenalectomy does not produce a decrease in plasma sodium
concentration as apparently functional adrenalectomy does. Further, urine flow and GFR were significantly lower than control or functional adrenalectomy values. Since there was no change in plasma sodium and since GFR decreased significantly, the filtered load was decreased. Distal tubular reabsorption also decreased, unlike the results of LeBrie and Elizondo or results from our functional adrenalectomy group, suggesting total adrenalectomy has a distal effect.

A possible explanation for the different results we obtained from Elizondo and LeBrie is suggested by the results from the sham and vena cava groups.

**Sham**: Osmotic excretion decreased significantly (P<.01) from 32±4 uOsm/100g/hr to 22±1 uOsm/100g/hr. Fractional sodium reabsorption increased significantly (P<.05) from 93.23±0.85 per cent to 95.93±0.88 per cent. Total osmotic fractional reabsorption also increased significantly (P<.005) from 88.82±0.83 per cent to 92.22±0.51 per cent (Table 7). Urine flow and GFR were not significantly different from control, while water reabsorption and presumably proximal reabsorption decreased. These data suggest that the operation, in some non-specific manner, significantly increased distal tubular sodium reabsorption, since total osmotic reabsorption increased and proximal reabsorption decreased. The net effect would then be a three per cent increase in fractional sodium reabsorption, with a significantly higher (P<.005) plasma sodium concentration (Table 6). This increase occurred, most probably, by increased adrenal steroid secretion due to surgical trauma and pain. This effect has been well documented in mammals (20).
Vena cava occlusion: Plasma sodium concentration decreased significantly ($P<.001$) from $145.4 \pm 0.8$ mEq/liter to $137.0 \pm 1.4$ mEq/liter as did plasma potassium concentration ($P<.01$). Fractional sodium reabsorption decreased significantly ($P<.025$) from $93.23 \pm 0.85$ per cent to $85.93 \pm 3.35$ per cent. Total osmotic reabsorption also decreased significantly ($P<.005$) from $88.82 \pm 0.83$ per cent to $80.08 \pm 2.61$ per cent. The fact that proximal reabsorption remained at approximately 50 per cent, indicates vena caval occlusion has primarily a distal effect since distal osmotic reabsorption is equal to total osmotic reabsorption minus proximal osmotic reabsorption. Osmotic excretion is increased because of decreased distal tubular reabsorption. The net sodium loss is further reflected in the decreased plasma sodium concentration. In terms of renal function, fractional sodium reabsorption decreased by approximately 10 per cent. Further, the vena cava occlusion group showed a decrease in plasma sodium concentration not significantly different from the functionally adrenalectomized group.

The data from the vena cava group together with data from the sham group suggest why our results for functionally adrenalectomized snakes were different from those of Elizondo and LeBrie (15). Because of the many short veins connecting the adrenal glands to the vena cava, it is extremely difficult in the functionally adrenalectomized group to avoid occluding the vena cava to some extent while ligating the adrenals. Further, the vena cava occlusion apparently has two effects; the vena cava occlusion itself and the effect of the surgery described in the sham group. The effects of the sham operation, therefore, would be additive to any other surgical procedures carried out on our snakes.
The 10 per cent decrease in fractional sodium reabsorption for the vena caval group compared to the water control group, does not take into account the fact that sham operation alone increased fractional sodium reabsorption by three per cent, compared to water control. To determine per cent fractional sodium reabsorption for vena caval occlusion only, fractional sodium reabsorption must be decreased by three per cent for a total decrease of approximately 13 per cent. In comparison, Elizondo and LeBrie obtained a decrease of 20 per cent for fractional sodium reabsorption in their functionally adrenalectomized groups. Our data suggest that they produced a partial vena cava occlusion when they ligated the adrenals in their snakes.

Aortic constriction: Referring to Table 6, aortic constriction produced the lowest plasma sodium concentration of all the groups, decreasing significantly ($P<.001$) from $145.4 \pm 0.8$ mEq/liter to $116.0 \pm 2.1$ mEq/liter. The plasma osmotic pressure also decreased significantly ($P<.02$). Urine flow decreased significantly ($P<.02$) from $0.454 \pm 0.034$ ml/100g/hr to $0.286 \pm 0.055$ ml/100g/hr. Glomerular filtration rate decreased significantly ($P<.02$). The presumed decreased arterial pressure, subsequent to aortic constriction is probably the cause of the decrease in GFR. This suggests that the filtered load should be decreased. Sodium excretion decreased significantly ($P<.005$) from $9.4 \pm 1.6$ uEq/100g/hr to $4.1 \pm 0.7$ uEq/100g/hr as did potassium excretion ($P<.001$). Osmotic excretion decreased significantly ($P<.01$) from $32 \pm 4$ uOsm/100g/hr to $14 \pm 2$ uOsm/100g/hr. Fractional sodium reabsorption increased significantly ($P<.05$) from $93.23 \pm 0.85$ per cent to $95.52 \pm 0.49$ per cent (Table 7). These data indicate that aortic constriction
increases proximal tubular sodium reabsorption. Since distal osmotic reabsorption is not different from control, but total osmotic reabsorption increases, proximal osmotic reabsorption must increase. Under these conditions of reduced GFR, proximal tubular reabsorption would normally decrease in mammals due to glomerular-tubular balance. The term glomerular-tubular balance refers to the fact that the rate of reabsorption of fluid in the proximal tubule varies directly with the rate of glomerular filtration resulting in a constant percentage sodium reabsorption.

In mammals, a decrease in arterial pressure could cause a decrease in the filtration fraction, i.e., a decrease in GFR without a reduction in renal plasma flow. The result would be a decrease in peritubular capillary oncotic pressure. As filtered sodium is actively reabsorbed out of the proximal tubule followed passively by water, the gradient between interstitial hydrostatic pressure and plasma oncotic pressure in the peritubular capillaries would be reduced. That portion of the sodium and water not reabsorbed into the capillaries could backflux through intercellular channels to the proximal tubule. The net effect would be decreased proximal reabsorption of sodium and water. The fact that the fractional sodium reabsorption during aortic constriction in snakes is greater than in control snakes may be explained by the fact that snakes possess a renal portal system which should not be affected by changes in filtration fraction and colloid osmotic pressure and can continue to reabsorb interstitial fluid when arterial pressure is decreased.

Another possible mechanism is suggested by the report of Selkurt
(50), who studied the effect of decreases in renal perfusion pressure in the in situ pump perfused dog kidney. He found that tubular sodium reabsorption increased during reduction in renal perfusion pressure. He suggested that a decreased transtubular hydrostatic pressure gradient in addition to the decreased perfusion pressure was responsible for increased electrolyte reabsorption. A similar effect may occur in snakes.

Renal portal occlusion: Glomerular filtration rate decreased significantly (P<.001) from 0.940 ±0.066 ml/100g/hr to 0.569 ±0.069 ml/100g/hr as did urine flow (P<.005). As expected, osmotic excretion also decreased significantly (P<.05) from 32 ±4 u0sm/100g/hr to 20 ±4 u0sm/100g/hr (Table 7). These data suggest that renal portal occlusion affects mainly GFR and urine flow since fractional reabsorption was not significantly different from control. Since glomerular filtration rate decreased, and there was no change in plasma sodium concentration, the filtered load must have decreased also. As the absolute amount of solute and water delivered to the tubule decreased but fractional reabsorption was unchanged, sodium and potassium excretion would be expected to decrease also. This did occur in that potassium excretion decreased. The plasma potassium concentration decreased significantly (P<.025) from 4.8 ±0.1 mEq/liter to 4.0 ±0.1 mEq/liter (Table 6).

The snake renal portal system as illustrated in Figure 2, receives venous blood from the caudal vein and enters the kidney. Approximately 75 per cent of the renal portal system branches into capillaries which supply venous blood to the peritubular network, with the remaining 25 per cent as distinct vessels which enter the post caval veins. These percentages of capillary networks and shunts can vary widely in various
species. The renal portal system of the crocodile does not branch into capillaries, with virtually all veins communicating directly with the post caval veins (30). In frogs, about 50 per cent of the renal peritubular circulation is provided by the renal portal system (17). Birds can shunt the renal portal supply away from the middle and caudal portions of the kidney to the liver (1). These data suggest that ligating the renal portal system in snakes decreases blood flow to the peritubular capillary network, which in snakes appears to decrease the GFR and consequently the filtered sodium load. This effect on GFR can be explained in that the renal portal and post glomerular vessels anastomose before entering the peritubular capillary network. The blood flow from the renal portal system effectively increases blood back pressure to the glomerulus, thereby increasing filtration pressure and GFR. When flow from the renal portal system is blocked, the back pressure in the post glomerular vessels decreases, which in turn decreases filtration pressure and GFR.

**Saline Loaded Snakes**

1.5 per cent saline: As compared to an equivolume water load, Table 8 notes that a 1.5 per cent saline load significantly decreased the P1 plasma protein concentration from 7.03 ±0.49 grams per cent to 5.27 ±0.21 grams per cent (P<.01), while plasma sodium increased significantly (P<.001) from 145.4 ±0.8 mEq/liter to 163.0 ±2.4 mEq/liter. Plasma potassium decreased significantly (P<.001), while plasma osmotic pressure increased significantly (P<.001) from 300 ±3 mOsm/liter to 329 ±6 mOsm/liter. Table 9 shows that fractional sodium reabsorption de-
creased significantly (P<.001) from 93.23 ±0.85 per cent to 79.82 ±2.54 per cent. These data are illustrated in Figure 6 where fractional sodium reabsorption is plotted against GFR. Sodium excretion increased significantly (P<.001) following 1.5 per cent saline loading. Total osmotic reabsorption was also decreased significantly (P<.001) from 88.82 ±0.83 per cent to 76.10 ±2.42 per cent. Osmotic excretion increased significantly (P<.001).

The decrease in plasma protein concentration as compared to the water control group is most likely due to movement of water from the intracellular fluid (ICF) compartment into the circulatory system, as a consequence of the 1.5 per cent saline load. The saline load is excreted by the kidney; however, snakes lack a loop of Henle and, therefore, can excrete only an isosmotic or hypoosmotic urine. In either case, water in excess of solute is excreted in an attempt to excrete the saline load. As more water is lost, the cells of the animal become progressively more dehydrated. This cellular dehydration and movement of water into the extracellular compartment is reflected in the decreased hematocrit and plasma protein values. As one might expect, plasma sodium concentration increased since the saline load was hypertonic. This is further reflected in the increased plasma osmotic pressure.

The plasma potassium concentration decreased in the 1.5 per cent saline group as compared to the water control group. This decrease in plasma potassium concentration is explained by the diluting effect of water from the saline load and the water moving into the circulatory system from the cells. When this water shift and the diluting effect of
the saline load are taken into account, the calculated values for plasma potassium concentration and plasma osmotic pressure are 4.1 mEq/liter and 333 mOsm/liter. The experimental values are 3.9 mEq/liter and 329 mOsm/liter respectively. These numbers are not significantly different from the calculated values. LeBrie and Elizondo showed a similar decrease in plasma potassium concentration during 1.5 per cent saline loading (35).

The decrease in fractional sodium reabsorption is supported by the increased sodium excretion and increased osmotic excretion. The fact that total osmotic reabsorption decreased without a significant change in distal osmotic reabsorption, suggests 1.5 per cent saline loading affects the proximal tubule in snakes. LeBrie and Elizondo reported similar findings (35).

Figure 6 illustrates a constant but decreased fractional sodium reabsorption over a wide range of GFR for 1.5 per cent saline loaded animals as compared to water control. These data confirm the results of LeBrie and Elizondo (35) which suggest an increasing functional tubular population with each tubule reabsorbing a constant but smaller per cent of the filtered sodium. These authors also showed that administration of aldosterone during 1.5 per cent saline loading returned sodium reabsorption to control values. The present data, therefore, confirm the previous work, and suggest that the difference in fractional sodium reabsorption between water and 1.5 per cent saline loaded groups is the absence of aldosterone.

2.0 per cent saline: As compared to an equivolume water load, 2.0 per cent saline significantly lowered the $P_1$ hematocrit ($P<.02$) from
26.5 ±1.2 per cent to 22.1 ±0.9 per cent (Table 8). The P₁ plasma protein concentration also decreased significantly (P<.01) from 7.03 ±0.49 grams per cent to 5.26 ±0.30 grams per cent. Because a hypertonic sodium load was administered, the plasma sodium concentration increased significantly (P<.001) from 145.4 ±0.8 mEq/liter to 176.2 ±1.2 mEq/liter. Consequently, the plasma osmotic pressure also increased significantly (P<.001) from 300 ±3 mOsm/liter to 357 ±4 mOsm/liter.

Table 9 indicates that sodium excretion increased significantly (P<.005). Consequently, fractional sodium reabsorption decreased significantly (P<.001) from 93.23 ±0.85 per cent to 76.06 ±3.84 per cent. Osmotic excretion increased significantly (P<.02) while total osmotic reabsorption decreased from 88.82 ±0.83 per cent to 73.47 ±3.88 per cent (P<.001). Fractional water reabsorption decreased significantly (P<.05) from 51.26 ±1.45 per cent to 40.55 ±4.65 per cent.

The plasma protein and hematocrit data in both saline groups suggest a movement of water from other body compartments into the circulation because of the hyperosmotic load. Plasma sodium concentration shows the predictable increase as does the plasma osmotic pressure. A shift in water balance alone cannot be used to explain the plasma potassium results obtained in the 2.0 per cent saline group. The calculated values for plasma potassium concentration and plasma osmotic pressure are 3.7 mEq/liter and 359 mOsm/liter. The experimental values are 4.4 mEq/liter and 357 mOsm/liter respectively. While it is evident that the number of osmotically active ions present in the plasma agrees with the calculated value, the calculated potassium value appears to be too low. This may indicate a shift of potassium from the cells into the
circulation.

Decreased fractional sodium reabsorption is reflected in increased sodium and osmotic excretion. Figure 7, which plots fractional sodium reabsorption versus GFR, shows that an inverse relationship exists between fractional sodium reabsorption and GFR during 2.0 per cent saline loading. As in the 1.5 per cent saline group, aldosterone secretion is also suppressed in this group. The fact that sodium reabsorption decreases with an increasing GFR would suggest the 2.0 per cent saline load was acting as an osmotic diuretic. At low glomerular filtration rates, over 90 per cent of the filtered sodium load is reabsorbed. However, as GFR increases, the sodium remaining in the tubule apparently binds osmotically equivalent volumes of water that normally would be reabsorbed and the two are excreted. This osmotic effect is reflected in the decreased fractional water reabsorption.

2.0 per cent saline versus 1.5 per cent saline: Table 8 notes that plasma sodium concentration was significantly higher (P<.001) in the 2.0 per cent saline group (176.2 ±1.2 mEq/liter) versus the 1.5 per cent saline group (163.0 ±2.4 mEq/liter). The plasma osmotic pressure was also significantly higher (P<.001) in the 2.0 per cent saline group (357 ±4 mOsm/liter) versus the 1.5 per cent saline group (329 ±6 mOsm/liter). These results were expected in that the 2.0 per cent saline group received a greater sodium load which is reflected in the higher plasma sodium and osmotic pressure concentrations.

Figure 8 illustrates that the hyperbolic relationship between fractional water reabsorption and urine flow seen during water loading becomes a straight line during 1.5 per cent and 2.0 per cent saline
loading. No significant difference was found when our data were compared to those of LeBrie and Elizondo (35). LeBrie and Sutherland (34) suggested that the decreased water reabsorption during water loading was caused by a reduction in circulating ADH. They further suggested ADH had an inhibitory effect on GFR and functioning tubular population. Since a saline load would not be expected to inhibit ADH due to osmotic effects as would be present during a water load, LeBrie and Elizondo suggested that if volume receptors similar to those proposed by Gauer and Henry (21) are present in reptiles, ADH inhibition could occur during saline loading. Such inhibition of ADH would then increase the functioning tubular population and decrease distal water reabsorption.

Figure 9 illustrates the relationship between urine flow and GFR for the 1.5 per cent and 2.0 per cent saline groups. The line for the 2.0 per cent saline group is significantly different from the 1.5 per cent saline group (P<.05). These data show that in 2.0 per cent saline loading, as GFR increases, urine flow increases to a greater extent than in 1.5 per cent saline loading. This must mean that water reabsorption is less in 2.0 per cent saline than 1.5 per cent saline; however, a significant difference in water reabsorption does not exist between the two groups (Table 9). Table 9 also illustrates the fact that mean water reabsorption was decreased in both saline groups as compared to the control water load. Since there was no significant difference between these two groups in water reabsorption and since the 2.0 per cent saline group had a significantly lower fractional water reabsorption than water control, we attribute the lack of significance to the re-
Markable variability seen in the 1.5 per cent saline group where fractional water reabsorption was 42.79 ± 4.49 per cent. This was not statistically different than the water controls, which had a 51.26 ± 1.45 per cent fractional water reabsorption. These data further suggest that 2.0 per cent saline loading produces an osmotic diuresis, in addition to the effect on aldosterone.

Dantzler found that a 50 mOsm increase of plasma osmolarity produced anuria in snakes (12). We were able to increase plasma osmolarity by 57 mOsm in the 2.0 per cent saline loaded group and maintain renal function. Dantzler, however, used mannitol in his experiments, which may account for the differences between our results. Because mannitol is non-reabsorbable, the osmotic effect of the mannitol would be much greater; consequently, greater fluid loss and circulatory collapse may have occurred.

Renin Levels in Snake Kidney

The results of renin assays on snake kidney are presented in Table 10. All groups were compared to the water control group. Functional adrenalectomy significantly increased kidney renin levels (P < .005) from 3.56 ± 0.81 ng/g kidney to 7.43 ± 0.23 ng/g kidney. Total adrenalectomy also significantly increased kidney renin levels (P < .02) from 3.56 ± 0.81 ng/g kidney to 6.81 ± 0.60 ng/g kidney. The functional and total adrenalectomy groups were not significantly different from each other. All other groups were not significantly different from control.

These results indicate that only the functional and total adrenalectomy procedures produce an increase in renal renin levels in snakes.
The administration of Furosemide in mammals produces an increase in plasma renin as does vana caval occlusion and aortic constriction. These procedures did not significantly increase kidney renin levels in snakes.

The fact that aortic constriction, vena caval and renal portal occlusion did not significantly increase renin levels may argue against the baroreceptor theory of Tobian, et al., (59) playing a significant role in control of renin release in reptiles. All of these procedures would be expected to change transmural or interstitial pressures and alter renin levels, which did not occur. The snake does not have a macula densa (55), therefore, the infusion of water or saline would be expected to have no direct affect on renin levels by changing plasma sodium and/or filtered sodium concentration. This appears to be the case, in that no significant differences exist in renal renin levels among non-diuresis, water diuresis, and 1.5 per cent and 2.0 per cent saline loaded snakes.

The question which then arises is, does a high renal renin level indicate a high circulating plasma renin level? There are at least five possible models we can consider.

1. Kidney renin production rate increases without an increase in renin release rate. Kidney renin levels will increase but the plasma renin level will remain unchanged.

2. Kidney renin production rate increases with a smaller increase in renin release rate. Kidney renin levels will increase as will plasma renin levels.

3. Kidney renin production rate remains unchanged but the renin
release rate increases. Kidney renin levels will decrease while plasma renin levels increase.

4. Kidney renin production and release rates are equal. Regardless of changes in renin production, kidney levels will remain unchanged. The plasma renin level can increase or decrease depending on changes in renin release.

5. Kidney renin production rate remains unchanged while the renin release rate decreases. Kidney renin levels will increase while plasma renin levels will decrease.

Either model 1 or 2 could explain the renin data from our adrenalectomized snakes. In mammals, adrenalectomy and the subsequent reduction of circulating aldosterone produces a dramatic increase in both kidney and plasma renin levels (6, 26). A similar feedback system involving some other adrenal hormone may be present in snakes. The conclusion that some other hormone is involved is based upon two things:

1. Saline loading has been shown to reduce circulating aldosterone in snakes and

2. In our experiments, saline loading had no affect on renal renin levels.

Therefore, in snakes, reduction in aldosterone does not appear to affect kidney renin levels. Consequently, reduction of some other adrenal steroid must be hypothesized as the stimulus for the increase in renin following adrenalectomy. The availability of an assay which measures plasma renin levels in reptiles would clarify whether model 1 or 2 applies to adrenalectomized snakes.

It must also be recognized that our adrenalectomized snakes did
not show the typical decrease in fractional sodium reabsorption seen by Elizondo and LeBrie in snakes (15) and other investigators in mammals (40). Therefore, we really have no functional evidence that a decrease in any adrenal hormone actually occurred. Why then should renal renin increase after both functional and total adrenalectomy?

As indicated above there were no significant differences between control water diuresis animals and functionally adrenalectomized animals, yet renal renin was significantly higher in the adrenalectomized snakes. Since Elizondo and LeBrie got much lower fractional sodium and water reabsorption in their adrenalectomized animals it is possible that ADH is playing a role in both fractional sodium and water reabsorption, as well as renal renin levels. Suppose that their animals had a lower arginine vasotocin (AVT) level than did the snakes reported on here. This would suggest that high levels of AVT in our animals stimulated high sodium and water reabsorption primarily in the proximal tubule since this is where adrenalectomized snakes have been reported to show a decrease in sodium reabsorption (15). It is also possible that high levels of AVT stimulate renal renin build-up, i.e., reduce the release of renin from the kidney. Model 5 would represent this case. The fall in plasma renin as proposed in this model could easily be determined if we had a plasma assay.

Concerning the absence of a change in renal renin concentration during either aortic occlusion, furosemide, or saline loading, procedures which are known to increase plasma renin in mammals, it is quite possible that these results indicate that model 4 is the usual and appropriate one for snakes. This question can not be answered however,
until a plasma renin assay is developed for reptiles.

Finally, a renin bioassay has been developed that appears to assay angiotensin generated by snake renal renin activity. The assay allows measurement of kidney renin activity with reasonable accuracy using hog renin substrate in the incubation mixture on which snake renin can act. At this time we cannot measure the specificity of the snake renin for the hog substrate or the amount of hog substrate eventually converted into angiotensin. The fact that kidney renin activity in both functional and total adrenalectomy groups was increased significantly, but was not significantly different from each other, suggests that the conversion rate of renin substrate to angiotensin is relatively constant. Further, the same batch of hog substrate was used in all of the assays presented here. This suggests that differences in kidney renin activity were not due to substrate variations, but differences in snake kidney extracts. More work is needed in this area to develop a snake substrate which can be used instead of the hog substrate as required in the current assay.
Table 1
Comparisons of Different Amounts of Snake Extract with Excess of Substrate

<table>
<thead>
<tr>
<th>snake extract (ul)</th>
<th>40 ul substrate (day 1)</th>
<th>ng A&lt;sub&gt;I&lt;/sub&gt;/mg kidney</th>
<th>40 ul substrate (day 2)</th>
<th>200 ul substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>17.12</td>
<td>30.32</td>
<td>14.31</td>
<td>12.31</td>
</tr>
<tr>
<td>10</td>
<td>3.73</td>
<td>2.85</td>
<td>7.87</td>
<td>3.89</td>
</tr>
<tr>
<td>20</td>
<td>2.27</td>
<td>1.45</td>
<td>2.00</td>
<td>1.26</td>
</tr>
<tr>
<td>50</td>
<td>1.17</td>
<td>1.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.94</td>
<td>0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Average Per Cent Recovery of Angiotensin I from Extract, Substrate, and Extract-substrate

<table>
<thead>
<tr>
<th>time incubated (minutes)</th>
<th>0</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>XT + 90.7ng of A&lt;sub&gt;I&lt;/sub&gt; Recovery, ng</td>
<td>69.3</td>
<td>63.7</td>
<td>75.5</td>
</tr>
<tr>
<td>% Recovery</td>
<td>76.4</td>
<td>70.2</td>
<td>83.2</td>
</tr>
<tr>
<td>Sub + 90.7ng of A&lt;sub&gt;I&lt;/sub&gt; Recovery, ng</td>
<td>72.3</td>
<td>75.0</td>
<td>77.7</td>
</tr>
<tr>
<td>% Recovery</td>
<td>79.8</td>
<td>82.7</td>
<td>85.6</td>
</tr>
<tr>
<td>XT + sub + 90.7ng of A&lt;sub&gt;I&lt;/sub&gt; Recovery, ng</td>
<td>67.7</td>
<td>68.0</td>
<td>68.0</td>
</tr>
<tr>
<td>% Recovery</td>
<td>74.6</td>
<td>75.0</td>
<td>75.0</td>
</tr>
</tbody>
</table>

XT = Extract
Sub = Substrate
Table 3

Amount of Angiotensin I formed in Different Extracts as Incubation Time is Increased

<table>
<thead>
<tr>
<th>Incubation time (minutes)</th>
<th>XT A</th>
<th>XT B(1)</th>
<th>XT B(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7.17</td>
</tr>
<tr>
<td>2.5</td>
<td>0.28</td>
<td>0</td>
<td>7.17</td>
</tr>
<tr>
<td>5</td>
<td>1.12</td>
<td>7.17</td>
<td>8.06</td>
</tr>
<tr>
<td>7.5</td>
<td>1.68</td>
<td>7.17</td>
<td>8.06</td>
</tr>
<tr>
<td>10</td>
<td>1.96</td>
<td>11.20</td>
<td>8.96</td>
</tr>
<tr>
<td>15</td>
<td>----</td>
<td>10.75</td>
<td>9.86</td>
</tr>
<tr>
<td>20</td>
<td>2.80</td>
<td>10.75</td>
<td>9.41</td>
</tr>
</tbody>
</table>

Table 4

Amount of Angiotensin I Formed in Three Different Samples of Extracts Incubated with Standard Hog Substrate as Incubation pH is Varied

<table>
<thead>
<tr>
<th>Sample pH</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>0</td>
<td>0</td>
<td>5.82</td>
</tr>
<tr>
<td>5.5</td>
<td>0</td>
<td>6.27</td>
<td>5.82</td>
</tr>
<tr>
<td>6.5</td>
<td>0.68</td>
<td>6.72</td>
<td>8.06</td>
</tr>
<tr>
<td>7.5</td>
<td>5.38</td>
<td>8.51</td>
<td>7.17</td>
</tr>
</tbody>
</table>
Table 5
Effect of Boiling Pretreatment of Extract and Trypsin Treatment of Incubation Mixture on Angiotensin Assayed in Different Samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng $A_I$ assayed without treatment</td>
<td>9.41</td>
<td>7.29</td>
<td>4.00</td>
<td>10.75</td>
<td>13.44</td>
</tr>
<tr>
<td>% decrease</td>
<td>100</td>
<td>93</td>
<td>87</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.15</td>
<td>10.75</td>
<td>12.99</td>
<td>9.86</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Boiled*</th>
<th>Trypsin Treated+</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng $A_I$ assayed with treatment</td>
<td>0</td>
</tr>
<tr>
<td>% decrease</td>
<td>100</td>
</tr>
</tbody>
</table>

* Extract boiled for 60 minutes before routine incubation
+ Extract incubated with trypsin (1 ng/sample) before routine incubation
Table 6
Snake Weight, Plasma Osmolality, and Electrolyte Concentration During Water Diuresis

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Weight (g)</th>
<th>Hematocrit (%)</th>
<th>Plasma Protein (%)</th>
<th>Plasma Osmotic Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P1</td>
<td>P2</td>
<td>P1</td>
</tr>
<tr>
<td>WATER</td>
<td>12</td>
<td>523(12)b</td>
<td>±11.2</td>
<td>±11.1</td>
<td>±0.49</td>
</tr>
<tr>
<td>CONTROL</td>
<td></td>
<td>26.5(11)</td>
<td>24.2(12)</td>
<td>6.72(12)</td>
<td>6.26(12)</td>
</tr>
<tr>
<td>FUROSIDE</td>
<td>10</td>
<td>531(10)</td>
<td>±1.5</td>
<td>±1.5</td>
<td>±0.58</td>
</tr>
<tr>
<td>VS CONTROL</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>FURC T ADX</td>
<td>8</td>
<td>525(8)</td>
<td>±1.2</td>
<td>±1.9</td>
<td>±0.59</td>
</tr>
<tr>
<td>VS CONTROL</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>TOTAL</td>
<td>4</td>
<td>508(4)</td>
<td>±2.6</td>
<td>±2.0</td>
<td>±0.48</td>
</tr>
<tr>
<td>ADX</td>
<td>±121</td>
<td>±2.9</td>
<td>±5.0</td>
<td>±0.64</td>
<td>±0.64</td>
</tr>
<tr>
<td>VS CONTROL</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VS FURC TADX</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SHAH</td>
<td>4</td>
<td>399(4)</td>
<td>±12.9</td>
<td>±15.0</td>
<td>±0.64</td>
</tr>
<tr>
<td>VS CONTROL</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VENA</td>
<td>3</td>
<td>636(3)</td>
<td>±2.3</td>
<td>±2.4</td>
<td>0.08</td>
</tr>
<tr>
<td>CAVA</td>
<td>±114</td>
<td>±2.3</td>
<td>±2.4</td>
<td>±0.08</td>
<td>±0.15</td>
</tr>
<tr>
<td>VS CONTROL</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>AORTIC CONSTR</td>
<td>±59</td>
<td>±0.9</td>
<td>±0.2</td>
<td>±0.13</td>
<td>±0.25</td>
</tr>
<tr>
<td>VS CONTROL</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>REJAL</td>
<td>5</td>
<td>615(5)</td>
<td>±3.4</td>
<td>±3.0</td>
<td>±0.59</td>
</tr>
<tr>
<td>PON TAL</td>
<td>±102</td>
<td>±3.4</td>
<td>±3.0</td>
<td>±0.59</td>
<td>±0.71</td>
</tr>
<tr>
<td>VS CONTROL</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

a = number of animals
b = number of determinations
c = standard error
Table 7
Renal Function in Snakes During Water Diuresis

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>V (ml/100g/hr)</th>
<th>GFR (uEq/100g/hr)</th>
<th>Na (mEq/100g/hr)</th>
<th>K (mEq/100g/hr)</th>
<th>Osmotic Pressure (Osm/lOOg/hr)</th>
<th>Fractional Reabsorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WATER</td>
<td>12</td>
<td>0.45±(26)²</td>
<td>0.94±(26)</td>
<td>9.4±(26)</td>
<td>2.1±(26)</td>
<td>93.2±(26)</td>
<td>93.2±(26)</td>
</tr>
<tr>
<td>CONTROL</td>
<td>20</td>
<td>0.09±6</td>
<td>0.06±6</td>
<td>1±6</td>
<td>2±0.2</td>
<td>5±1</td>
<td>1±1.25</td>
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<td>FURO-SEMIDE</td>
<td>10</td>
<td>0.67±(21)²</td>
<td>1.06±(21)</td>
<td>36.6±(21)</td>
<td>4.9±(21)</td>
<td>76.9±(21)</td>
<td>76.9±(21)</td>
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<tr>
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<td></td>
<td>P&lt;0.01</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>FUNCT ADX</td>
<td>8</td>
<td>0.48±(18)²</td>
<td>0.99±(18)</td>
<td>6.3±(18)</td>
<td>2.9±(18)</td>
<td>94.7±(18)</td>
<td>94.7±(18)</td>
</tr>
<tr>
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<td>NS</td>
<td>NS</td>
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<td>NS</td>
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</tr>
<tr>
<td>TOTAL ADX</td>
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<td>NS</td>
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<tr>
<td>VS FUNCT ADX</td>
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<td>NS</td>
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<td>NS</td>
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<td>NS</td>
</tr>
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<td>SHAM</td>
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<td>1.5±(5)</td>
<td>95.9±(5)</td>
<td>95.9±(5)</td>
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<tr>
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<td></td>
<td>P&lt;0.01</td>
<td>NS</td>
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<td>NS</td>
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</tr>
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<td>VENA</td>
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<td>0.91±(6)</td>
<td>23.4±(6)</td>
<td>3.2±(6)</td>
<td>84.9±(6)</td>
<td>84.9±(6)</td>
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<td>CAVA</td>
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<td>0.8±(5)</td>
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<td>73.3±(5)</td>
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<td>NS</td>
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<td>NS</td>
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<td>AORTIC</td>
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<td>95.5±(4)</td>
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<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
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<td>NS</td>
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<td>RENAL</td>
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<td>1.5±(8)</td>
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<td>93.0±(8)</td>
</tr>
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<td>0.04±(8)²</td>
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<td>1±7</td>
<td>0.4±(8)</td>
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<td>2±4</td>
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<tr>
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<td>P&lt;0.01</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
</tr>
</tbody>
</table>

a = number of animals  
b = number of determinations  
c = standard error
<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Weight (g)</th>
<th>Hct (g%)</th>
<th>Plasma Protein (g%)</th>
<th>Na (mEq/liter)</th>
<th>K (mEq/liter)</th>
<th>Osmotic Pressure (mOsm/liter H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WATER CONTROL</td>
<td>12²</td>
<td>26.3(12)²</td>
<td>7.03(12)</td>
<td>6.72(12)</td>
<td>155.4(26)</td>
<td>4.8(26)</td>
<td>300(26)</td>
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<tr>
<td>1.5% SALINE</td>
<td>5</td>
<td>26.8(5)²</td>
<td>5.27(3)</td>
<td>5.71(3)</td>
<td>163.0(11)</td>
<td>3.9(11)</td>
<td>329(11)</td>
</tr>
<tr>
<td>VS CONTROL</td>
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<td>P&lt;.01</td>
<td>NS</td>
<td>P&lt;.001</td>
<td>P&lt;.001</td>
<td>P&lt;.001</td>
<td></td>
</tr>
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<td>2.0% SALINE</td>
<td>7</td>
<td>22.1(7)²</td>
<td>5.26(7)</td>
<td>5.32(7)</td>
<td>176.2(15)</td>
<td>4.4(15)</td>
<td>357(15)</td>
</tr>
<tr>
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<td>P&lt;.02</td>
<td>NS</td>
<td>P&lt;.01</td>
<td>NS</td>
<td>P&lt;.001</td>
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<tr>
<td>VS 1.5% SALINE</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;.001</td>
<td>NS</td>
<td>P&lt;.001</td>
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</tbody>
</table>

a = number of animals  
b = number of determinations  
c = standard error
Table 9
Renal Function in Snakes During Water and Saline Diuresis

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>V (ml/100g/hr)</th>
<th>GFR</th>
<th>Excretion Na (µg/100g/hr)</th>
<th>K (µg/100g/hr)</th>
<th>Osmotic Pressure Na (µosm/100g/hr)</th>
<th>H2O (µosm/100g/hr)</th>
<th>Total Osmotic</th>
<th>Distal Osmotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>WATER</td>
<td>12</td>
<td>0.454(26)</td>
<td>0.940(26)</td>
<td>9.4(26)</td>
<td>2.1(26)</td>
<td>9(26)</td>
<td>51.26(26)</td>
<td>88.82(26)</td>
<td>37.56(26)</td>
</tr>
<tr>
<td>CONTROL</td>
<td></td>
<td>±0.034c</td>
<td>±0.066c</td>
<td>±1.6</td>
<td>±0.2</td>
<td>±4</td>
<td>±1.14c</td>
<td>±2.08c</td>
<td>±1.25</td>
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<tr>
<td>1.5% SALINE</td>
<td>5</td>
<td>0.499(11)</td>
<td>0.836(11)</td>
<td>8.2(11)</td>
<td>1.9(11)</td>
<td>8(11)</td>
<td>79.82(11)</td>
<td>76.10(11)</td>
<td>23.29(11)</td>
</tr>
<tr>
<td>VS CONTROL</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;.001</td>
<td>NS</td>
<td>P&lt;.001</td>
<td>NS</td>
<td>NS</td>
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</tr>
<tr>
<td>2.0% SALINE</td>
<td>7</td>
<td>0.491(15)</td>
<td>0.753(15)</td>
<td>8.4(15)</td>
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<td>8(15)</td>
<td>76.06(15)</td>
<td>73.47(15)</td>
<td>29.71(15)</td>
</tr>
<tr>
<td>VS CONTROL</td>
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<td>NS</td>
<td>P&lt;.005</td>
<td>NS</td>
<td>P&lt;.005</td>
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<tr>
<td>VS 1.5% SALINE</td>
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<td>NS</td>
<td>NS</td>
<td>P&lt;.001</td>
<td>NS</td>
<td>P&lt;.001</td>
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</tr>
</tbody>
</table>

a = number of animals  
b = number of determinations  
c = standard error
Table 10
Kidney Renin Levels in Snakes During Non-Diuresis, Water Diuresis, and Saline Diuresis

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Kidney renin (ng/g kidney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WATER</td>
<td>6a</td>
<td>3.56 ± 0.81b</td>
</tr>
<tr>
<td>CONTROL</td>
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<td></td>
</tr>
<tr>
<td>NON-DIURESIS</td>
<td>5</td>
<td>3.71 ± 1.29</td>
</tr>
<tr>
<td>CONTROL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VS CONTROL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>8</td>
<td>4.97 ± 0.69</td>
</tr>
<tr>
<td>VS CONTROL</td>
<td></td>
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<td>FUNCT ADX</td>
<td>4</td>
<td>7.43 ± 0.46</td>
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<td>VS CONTROL</td>
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<td>TOTAL ADX</td>
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<td>6.81 ± 0.60</td>
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<td>VS CONTROL</td>
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<tr>
<td>VS FUNCT ADX</td>
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<td></td>
</tr>
<tr>
<td>SHAM</td>
<td>4</td>
<td>3.19 ± 1.77</td>
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<tr>
<td>VS CONTROL</td>
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<td></td>
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<td>VENA CAVA</td>
<td>3</td>
<td>4.48 ± 0.45</td>
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<td>AORTIC CONSTR</td>
<td>2</td>
<td>2.63 ± 2.33</td>
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<td>VS CONTROL</td>
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<td></td>
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<td>RENAL PORTAL</td>
<td>5</td>
<td>3.59 ± 1.13</td>
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<tr>
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<td></td>
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<tr>
<td>1.5% SALINE</td>
<td>5</td>
<td>2.01 ± 0.37</td>
</tr>
<tr>
<td>VS CONTROL</td>
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<td></td>
</tr>
<tr>
<td>2.0% SALINE</td>
<td>6</td>
<td>3.95 ± 0.92</td>
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<tr>
<td>VS CONTROL</td>
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<tr>
<td>VS 1.5% SALINE</td>
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</table>

a = number of determinations
b = standard error
Figure 1

Steps in Activation and Degradation of Human Angiotensin and Effects on Adrenal Zona Glomerulosa, Aldosterone Secretion, and Sodium Reabsorption
Figure 3
Fractional Water Reabsorption and Urine Flow for all Water Diuresis Groups
Figure 4

Mean Plasma Osmolarity for all Water Diuresis Groups, Urine Osmolarity and Urine Flow for all Water Diuresis Groups Compared to Earlier Urine Osmolarity and Urine Flow Data by LeBrie and Sutherland
Figure 5
Fractional Sodium Reabsorption and GFR for Water Control and Furosemide Treated Snakes
Figure 6

Fractional Sodium Reabsorption and GFR for Water Control and 1.5 Per Cent Saline Loaded Snakes
Figure 7

Fractional Sodium Reabsorption and GFR for Water Control and 2.0 Per Cent Saline Loaded Snakes
Figure 8

Fractional Water Reabsorption and Urine Flow in Snakes During 1.5 Per Cent and 2.0 Per Cent Saline Loading
Figure 9

Urine Flow and GFR in Skeins During 1.5 Per Cent and 2.0 Per Cent Saline Loading
APPENDIX C
CALCULATIONS

(1) Per cent water reabsorption:
\[ 1 - \frac{P_{\text{inulin}}}{U_{\text{inulin}}} \cdot 100 = \% \text{ water reabsorption} \]

\( P = \text{plasma concentration} \)
\( U = \text{urine concentration} \)

(2) Per cent sodium or osmotic reabsorption:
\[ \frac{\text{GFR} \cdot P_x - U_x \cdot V}{\text{GFR} \cdot P_x} \cdot 100 = \% \text{ sodium or osmotic reabsorption} \]

\( x = \text{substance measured} \)
\( P = \text{plasma concentration} \)
\( U = \text{urine concentration} \)

(3) Per cent distal osmotic reabsorption:
\[ \% \text{ osmotic reabsorption} \text{ (2)} - \% \text{ water reabsorption} \text{ (1)} = \% \text{ distal osmotic reabsorption} \]

(4) Statistical analysis (t-test for unpaired groups):

\[ \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}} = t \]

\( \bar{x}_1 = \text{mean (group 1)} \quad \bar{x}_2 = \text{mean (group 2)} \)

\( \sigma_1^2 = \text{variance (group 1)} \quad \sigma_2^2 = \text{variance (group 2)} \)

\( n_1 = \text{sample size (group 1)} \quad n_2 = \text{sample size (group 2)} \)
BIBLIOGRAPHY


