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Participation of the Renin-Angiotensin System in the Control of Vascular Capacity during Hemorrahagic Hypotension

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PARTICIPATION OF THE RENIN-ANGIOTENSIN SYSTEM IN THE CONTROL OF VASCULAR CAPACITY DURING HEMORRHAGIC HYPOTENSION

by

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James R. Powell, II

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INTRODUCTION

Review of the Literature

Severe hypotension has long been held as being one of the most potent stimuli for activation of the mechanisms responsible for circulatory homeostasis. It has been accepted that the most immediate response to hypotension involves activation of the autonomic nervous system causing an increased vascular tone. In addition to various neurogenic mechanisms that function to cause an increase in vascular tone, several humoral mechanisms have also been implicated.

Rocha e Silva and Rosenberg (1969) described the release of vasopressin (ADH) from the posterior lobe of the pituitary in response to hemorrhage in the dog. They postulated that the stimuli for release arose from cardiovascular sensory receptors and that vasopressin was responsible for a role in blood pressure regulation during hypotension.

A homeostatic role of the kidney during hypotension was also described by Hamilton and Collins (1942). These authors found that when blood was removed from dogs in a quantity sufficient to reduce the arterial pressure to 30 mm Hg, a difference existed in the amounts of blood removed from normal as compared to nephrectomized dogs.

They reported it was necessary to remove more blood from the normal dogs than from their nephrectomized counterparts. These authors concluded that the kidney was responsible for the elaboration of some material that increased vascular tone during hypotension. Sapirstein et al. (1941) concluded from a similiar observation, that the elaboration of renin by the kidney was the renal homeostatic material.

More recently DuCharme and Beck (1971) reported that when anesthetized dogs were bled to and maintained at a fixed hypotensive blood pressure of 45 mm Hg, sham-operated dogs lost considerably more blood than did nephrectomized dogs during a two hour hypotensive period. These authors also demonstrated that the renal contribution to the blood loss was almost entirely eliminated in dogs pretreated with spinal anesthesia. They concluded the potential of the renal pressor system to reduce vascular capacity during hypotension was 60% that of the sympathetic nervous system. They also raised some doubt as to the role of the renin-angiotensin system in reducing vascular capacity during hemorrhagic hypotension.

The renin-angiotensin system

In 1898 Tigerstedt and Bergman demonstrated that crude saline extracts from kidneys caused a pressor response when injected into anesthetized rabbits. They named the sub-

stance responsible for the pressor response "renin". Kohlstaedt et al. (1938) demonstrated that no pressor response occurred when renin was injected into a rabbit's ear perfused with saline. However, when plasma was added to the perfusate, the pressor activity of renin was restored. This led them to postulate that renin was an enzyme which acted on a plasma substrate causing the formation of a pressor substance.

Page and Helmer (1940) and Braun-Menendez et al. (1940) both succeeded in isolating this pressor material formed by the action of renin with plasma. Due to the geographic as well as investigational differences between the two groups, each gave the pressor material a different name. Braun-Menendez and his group called the material "hypertensin" while Page and Helmer referred to it as "angiotonin". In what has been called a model of scientific agreement, the two parties published a joint communication (Braun-Menendez and Page, 1958) where the compromised name "angiotensin" was given to the pressor material.

Skeggs et al. (1954) using a countercurrent distribution purification procedure determined that angiotensin existed in two similiar, but different forms. These forms were identified as angiotensin I and angiotensin II. These investigators also determined that each form of angiotensin possessed a nearly equal pressor ability when injected into an animal. They concluded that angiotensin I was the pro-

duct of the action of renin on a renin-substrate (angiotensinogen) and that angiotensin II was the biologically active conversion product of angiotensin I. These investigators believed the "converting enzyme" was contained in the plasma. However, Ng and Vane (1968) using a blood bathed organ assay technique for angiotensin II determined that the primary site of conversion of angiotensin I to angiotensin II was the lungs. They also determined that the primary site of angiotensin II extraction from plasma was the peripheral vascular beds. This site of conversion was verified by Bakhle (1968) who demonstrated the conversion of angiotensin I to angiotensin II by cell-free extracts of dog lungs. Biron et al. (1968) studied the extraction of angiotensin II by several vascular beds and concluded the lung was the only major vascular bed that did not extract significant amounts of angiotensin II from the circulation. This observation agrees well with the observations of Ng and Vane and of Bakhle in that if an organ is to be responsible for the production of a hormone it seems unlikely that the same organ would be responsible for its degradation.

Although the exact chemical structures of the components of the renin-angiotensin system in the dog have not as yet been elucidated as they have for the horse, cow, pig and man; the following schema for the system in the horse, as presented by Skeggs et al. (1964), is assumed to be

similiar to the system in the dog.

H•Asp•Arg•Val•Tyr•Ile•His•Pro•Phe•His•Leu•Leu•Val•Tyr•Ser•R

angiotensinogen

| renin

H•Asp•Arg•Val•Tyr•Ile•His•Pro•Phe•His•Leu•OH

angiotensin I

| converting enzyme

H•Asp•Arg•Val•Tyr•Ile•His•Pro•Phe•OH

angiotensin II

Assay for renin

Until recently the major methods for the determination of renin involved the bioassay of pressor materials generated by the incubation of plasma. One of the more widely used bioassays for renin was described by Boucher et al. (1964). This method involves the incubation of acidified plasma at 37°C in the presence of a cation exchange resin which binds the generated angiotensin for protection from enzymatic degradation. The pressor activity of the incubated plasma in anesthetized rats is compared to the pressor response of standard amounts of angiotensin. The result of this assay is the determination of renin activity which is expressed as the rate of formation of angiotensin

per volume of the incubated plasma. Boucher originally believed that the incubation product of his assay was angiotensin II. However, Hollmans et al. (1969) identified the product as angiotensin I. This identification was accomplished by performing radioimmunoassays for both angiotensin I and angiotensin II on the incubation product.

Scornik and Paladini (1961) described a bioassay for angiotensin employing an alcoholic extraction of non-incubated plasma for the purification of the pressor material. The purified samples were then injected into anesthetized nephrectomized rats and the pressor response compared to standard injections of angiotensin. A similiar purification of incubated plasma was described by Pickens et al. (1965).

Regoli and Vane (1966) described a method for the continuous estimation of angiotensin. This method involved a superfusion of assay organs with blood. The differential contractile responses of the smooth muscle assay organs allowed the authors to distinguish angiotensin responses from responses due to other circulating substances.

Recent developments in the field of immunology have made possible the production of radioimmunoassays which are both sensitive and specific for given hormones. Haber et al. (1969) described the development of a radioimmunoassay for the determination of angiotensin I in plasma. Goodfriend et al. (1968) described a similiar assay for the de-

termination of angiotensin II. These assays have permitted the specific determination of these two components of the renin-angiotensin system and represent a significant improvement over previous bioassay methods.

Vascular actions of angiotensin

Angiotensin is the most potent vasoconstrictor agent known. Finnerty (1962) reported that on an equal weight basis angiotensin had several times the vasoconstrictor potency of norepinephrine. The primary site of vasoconstrictor activity is the arterioles. The pharmacology of angiotensin in regard to its venoconstrictor activity is not clear at the present time. Folkow et al. (1961) Haddy et al. (1962) and Abdel-Sayed (1970) have all reported that angiotensin exerted little or no effect on veins. However, Emerson et al. (1965), using a constant flow forelimb preparation found angiotensin to have a greater effect in increasing small vein pressure and decreasing limb weight than did norepinephrine. Angiotensin stimulates other smooth muscles besides that of the vasculature. For a review of these actions, the reader is referred to Braun-Menendez (1956).

A recent study by Lin and Goodfriend (1970) in which radioiodinated angiotensin was used, described binding of the polypeptide by several tissues. This binding was assumed to be at least partially attributable to combination

of angiotensin with cell receptor sites. The binding receptors were found in all tissues to which a cell response to angiotensin was known as well as to several tissues to which no action of angiotensin had been described.

The renal response to angiotensin has been well studied in recent years. Healy et al. (1965) in studies on unanesthetized dogs presented data which showed angiotensin to reduce glomerular filtration rate (GFR) and produce natriuresis. Thurau (1964) suggested that the renin-angiotensin system may be important in the auto-regulation of renal blood flow and glomerular filtration. However, Gagnon et al. (1970) demonstrated intrarenal infusions of angiotensin to have no effect on GFR. McGiff and Itskovitz (1964) demonstrated that the renal vasoconstrictor activity of angiotensin was lost during renal ischemia.

The relationship between angiotensin and the sympathetic nervous system has been explored by several investigators. Zimmerman (1962) in experiments utilizing the perfused hindquarters of the dog, found that acute sympathectomy reduced the pressor responses to injected angiotensin but not norepinephrine. Similiar results were obtained after administration of the ganglionic blocking agent, hexamethonium, and cervical spinal transection.

Sweet and Brody (1970) also employing the perfused hindquarters of the dog, demonstrated that subpressor doses of angiotensin infused into the vertebral artery re-

duced reflex dilatation in the hindquarters. The reduction in renal perfusion pressure by a clamp on the aorta placed above the renal arteries also reduced active reflex dilatation. These experiments indicated that the kidney may be involved in modification of the baroreceptor reflexes by a central inhibition.

McGiff and Fasy (1965) demonstrated that the renal vasoconstrictor response to angiotensin was abolished by drugs (guanethidine, bretylium, and hydralazine) which influence sympathetic nervous activity by reducing the release of neurotransmitter. Renal denervation or cervical spinal transection also blocked the pressor response to angiotensin in the kidney. However, the alpha adrenergic blocking agent, phentolamine, and ganglionic blocking agent, hexamethonium, had no influence on the pressor response in the kidney.

The renin-angiotensin system has also been shown to participate in the release of humoral agents from several organs. Michelakis and Horton (1970) found that there existed a high correlation between plasma renin activity (PRA) and aldosterone levels. This correlation was found to exist in several physiological variations such as assumption of an upright posture, diurnal variability and low dietary sodium intake. Blair-West *et al.* (1970) also verified this correlation but stated that some other factor in addition to plasma angiotensin levels may control aldosterone secretion.

Severs et al. (1970) demonstrated a 33 mm Hg blood pressure increase in response to a single injection of 0.5 mcg of angiotensin into the lateral ventricle of the unanesthetized rat. Concomitant with the elevated blood pressure there was a neurogenic thirst produced. These authors concluded that angiotensin when injected into the lateral ventricle caused the release of vasopressin (ADH). This contention was supported by Mouw et al. (1971) who observed that intercarotid artery infusions of subpressor doses of angiotensin in dogs caused the increased release of ADH. Similiar ADH increases were also observed as a result of intraventricular infusions of angiotensin.

McGiff et al. (1970) have reported the existence of a prostaglandin-like substance appearing in the renal venous effluent as a result of arterial angiotensin infusions. This substance resembles PGE_2 and the authors speculate that renal prostaglandins might contribute to regulate renal blood flow when the circulating levels of angiotensin are increased.

Although angiotensin II has long been thought to be the only biologically active component of the renin-angiotensin system, recent studies indicate that the decapeptide angiotensin I may possess biological activity. Carriere and Biron (1970) utilizing unilateral renal infusions of angiotensin I found a slight decrease in renal cortical blood flow without alteration of outermedullary flow. The

effect of the angiotensin I infusions were observed to occur only in the infused kidney. The authors suggest that there is either a direct action of angiotensin I on renal vasculature or that the kidney is responsible for the conversion of angiotensin I to angiotensin II. Robinson (1967) demonstrated the release of adrenal catecholamines by angiotensin II. Recently, Peach et al. (1971) have demonstrated a similiar adrenal release of catecholamines by angiotensin I and several analogues.

Stimuli for renin release

A general review of renal anatomy is presented to aid understanding of the stimuli for renin release. Renin is formed and stored in specialized areas of each nephron known as the juxtaglomerular (JG) apparatus. The JG apparatus is composed of the granular cells, macula densa, and mesangial cells. The granular cells are contained in the medial layer of the afferent arterioles to the glomerulus and are the site of renin formation, storage, and release. The macula densa is a specialized area of the tubule which marks the transition from the ascending loop of Henle to the distal tubule. The mesangial cells are interstitial cells in intimate contact with both the macula densa and granular cells.

For a general review of the theories concerning renin release the reader is referred to Vander (1967) who divides

the stimuli theories into macula densa, baroreceptor and sympathetic nervous system components.

One of the earliest postulated theories regarding renin release was renal ischemia. Divry (1951), however, demonstrated that perfusion of the kidney at normal pressure with venous blood did not increase renin activity. Kohlstaedt and Page (1940) demonstrated increased renin release following a reduction in pulse pressure to the kidney without a change in mean pressure. Tobian (1962) has suggested that since the JG cells are located within the media of the afferent arteriole a decrease in the pressure on the afferent arterial wall would facilitate the release of renin.

Vander and Luciano (1967) demonstrated increased plasma renin activity in response to acute salt depletion in the dog. The salt depletion is believed to decrease the sodium load of the tubular fluid at the level of the macula densa. In this respect the macula densa would sense a sodium decrease and respond by the release of renin which would decrease GFR and promote sodium retention. This theory has been recently challenged by Blaine et al. (1970) who demonstrated that in hemorrhaged dogs in which the ureter had been chronically ligated to prevent sodium delivery to the macula densa, a significant increase in plasma renin activity occurred. They concluded that sodium delivery to the macula densa was not essential in the release of renin.

Many investigators have presented evidence showing renin release following stimulation of the sympathetic nerves to the kidney. Vander (1965) demonstrated that catecholamine infusions and renal nerve stimulation caused increased plasma renin activity. He concluded that, although other factors cannot be ruled out, the sympathetic nervous system is involved in renin release. Bunag et al. (1966) bled dogs an amount sufficient to evoke vasomotor discharges without changes in either blood pressure or renal blood flow and demonstrated increased plasma renin activity. In six of seven dogs that exhibited renin release as a result of the mild hemorrhage the release was blocked with the ganglionic blocking agent, tetraethylammonium acetate. In another group of three dogs, infiltration of the renal hilus with a local anesthetic also prevented the previously observed renin release in response to mild hemorrhage. Carotid occlusion was observed to increase plasma renin activity without changes in renal blood flow. Bunag concluded that altered sympathetic discharge in the absence of changes in renal perfusion pressure or flow was a sufficient stimulus to cause renin release. Increased angiotensin formation occurred despite a rise in renal perfusion pressure. They concluded that the reflex activation of the renin-angiotensin system can override changes in renal arterial pressure. Mogil et al. (1970) demonstrated that chronically renal denervated dogs did not respond to acute sodium

deprivation with increased plasma renin activity as did dogs with intact renal innervation. Birbari (1971) demonstrated that in hemorrhaged rats the increase in plasma renin activity could be blunted by pretreatment with reserpine and abolished by phenoxybenzamine pretreatment. Hauger-Klevene and Brown (1970) found that increased plasma renin activity stimulated by ACTH injection in rats was inhibited by pretreatment with the ganglionic blocking agent, pentolinium. They postulated that the sympathetic nervous system might participate to amplify the signal (ACTH) which is acting on receptor sites in the JG apparatus.

Evidence for angiotensin acting as an inhibitory agent for the release of renin was presented by Bunag et al. (1967) who demonstrated renin inhibition by angiotensin infusions that did not alter renal perfusion pressure or renal flow, during a period of reduced renal perfusion pressure. They also found this same inhibition when vasopressin was infused. This led them to postulate that both angiotensin and vasopressin may exert a negative feedback control over renin secretion. Tagawa et al. (1971) have presented similiar data regarding inhibition of renin release by vasopressin in sodium-deprived dogs.

Needless to say, the question of the exact stimulus for the release of renin has not been fully resolved. The diversity of experimental methods and results suggests that possibly more than stimulus for renin release exists and

that the exact stimulus observed depends on the experimental situation.

Renin activity during hypotension

In addition to the aforementioned report of Hamilton and Collins (1944) and Sapirstein (1941), Dexter et al. (1943) also reported marked increases in plasma renin activity of hemorrhaged dogs. They found that during severe hypotension there was a progressive decrease in the level of renin-substrate (angiotensinogen). They concluded that the elaboration of renin was part of a renal homeostatic mechanism functioning during hemorrhage. However, due to the substrate depletion, they concluded the system to be inefficient.

Mikasa and Masson (1961) demonstrated an increased survival time in hemorrhaged, bilaterally nephrectomized rats to which kidneys were grafted during hemorrhage. Due to the inaccuracy of the assay methods of the time, Mikasa and Masson refrained from attributing this prolonged survival to the renin-angiotensin system but instead referred to the protective role of the renal pressor system.

Paladini and Scornik (1963) observed progressive increases in the blood angiotensin levels of dogs which were bled to over half of their normal blood volume. Similiar experiments in which the aorta was clamped above the renal arteries failed to yield similiar increases in angiotensin.

Infusion of the ganglionic blocking agent, trimethaphan, in amounts sufficient to reduce the arterial blood pressure to a level comparable to hemorrhage caused no increase in blood angiotensin levels. In a subsequent study employing a more sensitive assay for angiotensin, Scornik and Paladini (1964) observed a five-fold increase in circulating angiotensin levels in blood samples from hemorrhaged dogs as compared to pre-hemorrhage blood samples from the same dogs.

McKenzie et al. (1966) demonstrated a three-fold increase in plasma renin activity after five minutes of severe hemorrhage in the rabbit. After forty minutes, the values for plasma renin activity were five times greater than pre-hemorrhage values.

Regoli and Vane (1966) determined that after a small hemorrhage in the dog renin appears in the blood without a concomitant increase in catecholamines. However, both renin and catecholamines appeared when a greater hemorrhage occurred. Ganglionic blockade during hemorrhage prevented the appearance of catecholamines in the blood but not the appearance of renin.

Finkielman et al. (1968) demonstrated that hypertonic glucose infusions prevented the increased angiotensin levels found during hemorrhage. These data led them to believe that the renal baroreceptor theory of renin release was contradicted by the macula densa theory, where renin release would occur in response to changes in sodium load at the macula

densa and not to changes in renal perfusion pressure.

Statement of the Problem

A homeostatic role of a renal pressor system has been documented. There is also ample evidence that the renin-angiotensin system is activated by hemorrhagic hypotension. However, due to inadequate assay methods, no direct evidence that the renin-angiotensin system is the homeostatic renal pressor system has been presented.

The problem under investigation was to determine, by use of a sensitive and specific assay for angiotensin, if the renin-angiotensin system is the renal pressor system active in homeostasis during hemorrhagic hypotension. A second aspect of the study was to investigate the neurogenic control of the renin-angiotensin system.

METHODS AND MATERIALS

Animal Preparation

Adult mongrel dogs of either sex were anesthetized with pentobarbital sodium (35 mg/kg) given intravenously. A midline neck incision was made to permit the insertion of a glass T-tube into the trachea to assure a patent airway. An incision was made in the medial aspect of the upper foreleg and the brachial artery isolated. A polyethylene cannula connected to a Statham P23G transducer was inserted into the artery and blood pressure was monitored on a Grass Model 5 Polygraph. Another incision was made in the medial aspect of the thigh and the femoral artery and vein were isolated. The femoral vein was cannulated with polyethylene tubing to provide a route of administration for drugs. The femoral artery was cannulated with a polyethylene tube with an outside diameter large enough to provide a tight fit when inserted into the vessel lumen. Petroleum jelly applied to the cannula tip facilitated insertion into the artery. The cannula was advanced until the tip was well into the abdominal aorta. This cannula was connected to a blood pressure stabilizer reservoir. The blood pressure stabilizer reservoir permitted the production of controlled hypotension, at a level of over 80 mm Hg below the normal blood pressure of an

anesthetized dog. This stabilization system is described in the following section.

Controlled Hypotension

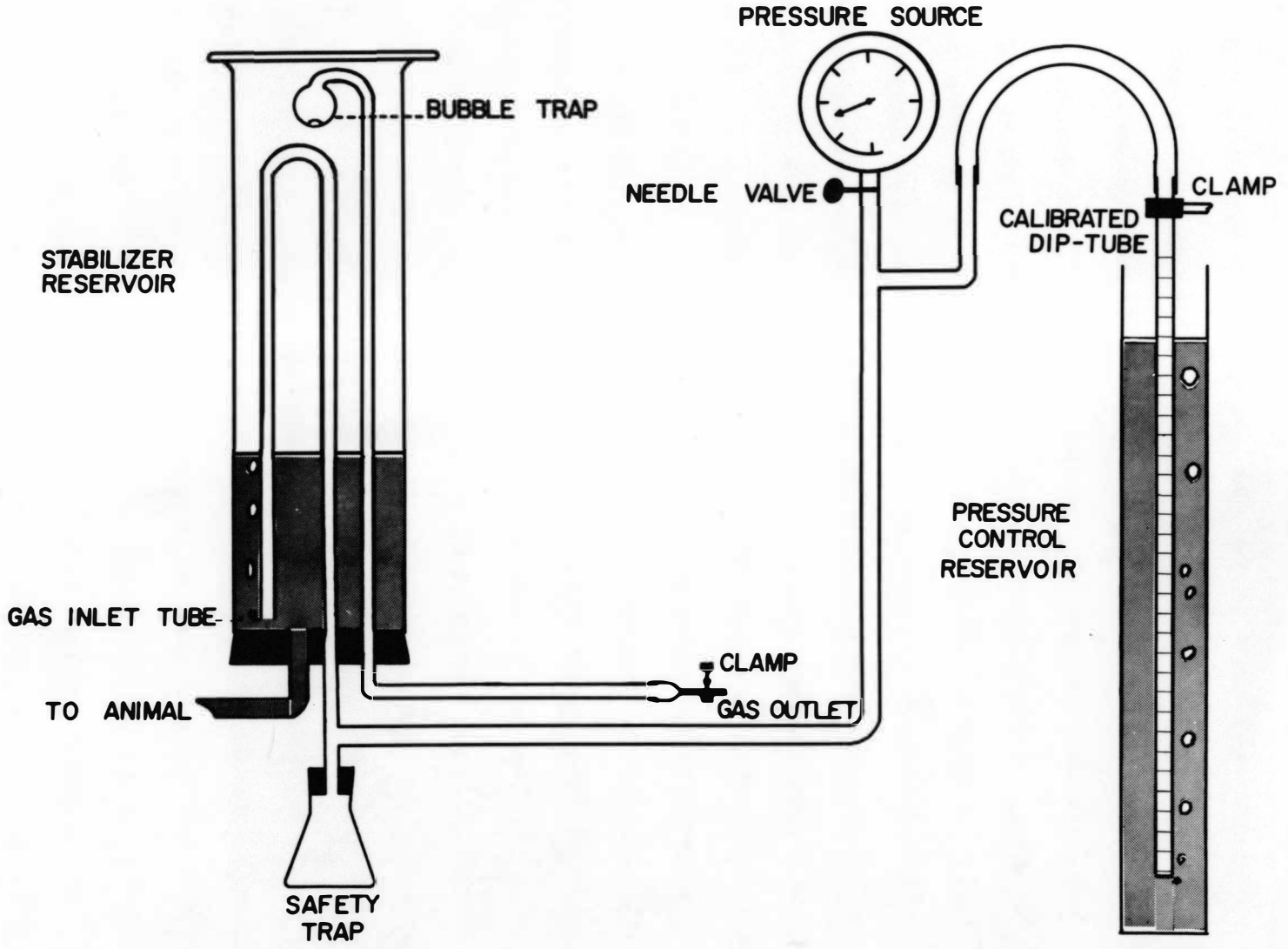
The blood pressure stabilization system used in this study was previously described by Beck (1958). The use of this system permits the rapid equilibration to and maintainance of a predetermined blood pressure in an animal. The system (Figure 1) consists of a stabilizer reservoir and pressure reservoir connected in parallel to an air pressure source. The stabilizer reservoir used in this study was an inverted 1000 ml glass mixing cylinder. The cylinder was calibrated for volume from the mouth to the base.

Air enters the stabilizer through an inverted glass tube and exits through a glass tube open at the top of the reservoir. The rate at which air passes from the reservoir is controlled by a clamp at the end of the air outlet tube. Blood enters the stabilizer reservoir from a large diameter latex tube commected to the femoral artery cannula in the animal. The pressure of the air entering the stabilizer reservoir is determined by the height of the column of liquid above the dip-tube tip in the control reservoir. In this study the liquid used was water and the dip-tube was lowered into the water to a depth resulting in a pressure of 35 mm Hg. Air entering the stabilizer

FIGURE 1

SCHEMATIC DIAGRAM OF THE SYSTEM UTILIZED

FOR BLOOD PRESSURE STABILIZATION


PRESSURE SOURCE
BUBBLE TRAP
NEEDLE VALVE
CLAMP
CALIBRATED DIP-TUBE
STABILIZER RESERVOIR
PRESSURE CONTROL RESERVOIR
GAS INLET TUBE
CLAMP
TO ANIMAL
GAS OUTLET
SAFETY TRAP

reservoir must overcome the hydrostatic pressure of the volume of blood in the reservoir. As long as air bubbles freely from the dip-tube and gas inlet tube the pressure of the air entering the stabilizer reservoir equals the sum of the hydrostatic pressure of the blood plus the pressure of the air above the blood. As the volume of blood in the stabilizer reservoir increases the hydrostatic pressure exerted by the height of blood also increases. The increased hydrostatic pressure causes an increased resistance to air entering the reservoir and therefore a decrease in the pressure of the air above the blood. The net result is a constant pressure of 35 mm Hg at the inlet tube at the bottom of the reservoir. The stabilizer reservoir is positioned at a height above the animal so that the hydrostatic pressure of the blood-filled latex tube and femoral cannula is 10 mm Hg. This 10 mm Hg hydrostatic pressure plus the 35 mm Hg pressure from the control reservoir creates a stabilization pressure in the animal of 45 mm Hg.

The rate of egress from the stabilizer reservoir controls the rate of bubbling from the air inlet tube. This bubbling prevents sedimentation of the blood as well as providing aeration. To minimize frothing of the blood, the reservoir was coated with a small amount of an antifoam emulsion (Antifoam Emulsion AF, Dow Corning Corp.). A three-way stopcock placed in the latex tubing connecting the stabilizer reservoir with the animal permitted the

sampling of arterial blood.

The volume of blood that an animal mobilizes into the stabilizer reservoir (bleeding volume) in response to the lowered pressure of 45 mm Hg is a measure of the capacity of the vascular system. A decrease in capacity will manifest itself by an increased amount of blood entering the stabilizer reservoir.

At the onset of hypotension a clamp on the latex tubing connecting the reservoir to the animal was removed and the animal allowed to bleed freely into the stabilizer reservoir. The volume of blood within the stabilizer reservoir was recorded at one minute intervals for the first fifteen minutes of hypotension, every five minutes for the next fifteen minutes, and every ten minutes for the remainder of a two-hour hypotensive period. All animals were given heparin sodium (5 mg/kg) one-half hour prior to hypotension. Hot wire cautery was used in all surgical procedures to minimize operative blood loss. Care was taken to ligate all bleeders. The hypotensive period was begun one hour after all surgical procedures were completed. At the end of experimentation all animals were sacrificed by an overdose of the anesthetic agent.

Surgical Procedures

Nephrectomy

The kidneys were approached retroperitoneally through left and right flank incisions. Each flank incision extended from the cranial aspect of the pelvis to the angle of the last rib. The incision was deepened along a fascial plane between the muscles, and the renal artery was isolated and ligated close to the aorta. The kidney was then freed from the surrounding fatty and connective tissue. The ureter was located and used to lift the kidney from the incision. A second ligature was placed around the renal artery, vein, and ureter. A cut distal to the ligature separated the kidney from the animal. The incision was packed with saline-soaked gauze sponges and the wound was closed with autoclips.

Sham-nephrectomy

The procedure used to approach the kidneys was the same as that used for nephrectomy. However, once the kidney was located the renal artery was not disturbed and no attempt was made to isolate the kidnay to avoid disturbance of either renal nerves or vasculature. The flank wounds were also packed with saline-soaked sponges and closed with autoclips.

Interruption of Sympathetic Tone

Spinal anesthesia

Spinal anesthesia was administered via a spinal needle inserted into the subarachnoid space at a level of L-4 or L-5. The appearance of clear cerebrospinal fluid indicated proper placement of the needle. A 10% solution of procaine hydrochloride was slowly injected into the subarachnoid space. During the injection, cerebrospinal fluid was periodically aspirated into the injection syringe to facilitate mixing. Total anesthesia of the cord was indicated by a cessation of respiration. This indicated anesthesia to a level of C-1 or C-2. At this time the animals were artificially ventilated with a Harvard Respirator Pump (Harvard Apparatus Co. Dover, Mass.). The artificial respiratory rate was 15 cc of room air per kilogram body weight per minute which approximated normal respiration. In these animals, a Valsalva maneuver was performed and the absence of the normally observed blood pressure overshoot following this maneuver was taken as further indication of completeness of spinal anesthesia. Spinal anesthesia was administered 30 minutes prior to the hypotensive period.

Ganglionic blockade

Ganglionic blockade was accomplished by the administration of hexamethonium bromide (5 mg/kg) intravenously.

This drug was given 30 minutes prior to hypotension.

Angiotensin Infusion

Valine^{5}- asparagine^{1}- angiotensin II (Hypertensin, Ciba) was infused into a group of nephrectomized dogs which had been subjected to a 60 minute period of hypotension. A polyethylene cannula advanced through the left jugular vein to the level of the heart was connected to an infusion syringe which was driven by a Harvard Syringe Pump (Harvard Apparatus Co.). Angiotensin was infused at the rates of 0.01 mcg, 0.10 mcg, and 1.0 mcg per kilogram per minute. Each rate of infusion was of 10 minutes duration. The infusions were given successively so that the total period of infusion was ended at 90 minutes after the onset of hypotension. Blood samples for angiotensin determination were drawn immediately prior to the onset of hypotension, one minute prior to the beginning of each infusion period, and during the tenth minute of each infusion period. A final blood sample was also taken at the end of 120 minutes of hypotension. Blood volumes were recorded at one minute intervals during the infusions.

Sampling Procedures

Blood samples for angiotensin analysis were drawn from the stopcock in the femoral artery cannula. At all times of sampling, the tubing between the stopcock and stabili-

zation reservoir was clamped to prevent drawing blood from the reservoir. Preceding each sample 5 ml of blood was drawn and discarded to remove all unmixed blood between the stopcock and cannula tip. Blood samples were drawn into polyethylene syringes and immediately placed into pre-chilled (4°C) glass test tubes containing 50 mg of disodium ethylenediaminetetra acetate (EDTA). The sample volume placed into each test tube was 10 ml. The blood volume removed for sampling was added to the bleeding volume at the various times of sampling.

An initial sample of 50 ml was taken immediately prior to hypotension. Subsequent 10 ml samples were taken at 1, 2, 5, 7, 10, 15, 30, 60, 90, and 120 minutes after the onset of hypotension. The samples were centrifuged at 1000 X g for 15 minutes at 4°C. The plasma supernatants were removed and phenylmethylsulfonylfluoride (PMSF) was added to each plasma sample so that the concentration of PMSF in the plasma was 1 mg/ml. The EDTA and PMSF were added to inhibit the enzymatic degradation of angiotensin. The plasma samples were stored frozen at -20°C until assay.

Radioimmunoassay

Generation of antibody

The techniques used in this study for the determination of angiotensin II were described in detail by Goodfriend *et al.* (1968). These techniques permit the sensi-

tive and specific determination of angiotensin II in plasma.

Angiotensin was rendered antigenic by conjugating the octapeptide with rabbit serum albumen (RSA) in the ratio of 2 parts angiotensin to one part RSA. The conjugation was accomplished using 1-ethyl-3-(3-dimethyl-amino propyl) carbodiimide hydrochloride in a ten-fold excess over the amount of angiotensin. The angiotensin-RSA mixture was purified by overnight dialysis against water and mixed with an equal volume of Freund's Complete Adjuvant for injection. Rabbits of the Dutch Belt, New Zealand Albino, and Chinchilla strains were injected with the immunogen in either the footpads or intramuscularly. The injections were repeated at weekly intervals until a high-titre antibody was obtained.

Antibody was harvested by bleeding the rabbits from an ear vein. After the blood was allowed to clot at room temperature, the serum was removed and inactivated by heating at 60^{o}C for 30 minutes. The serum was then treated with Dowex AG50W-X4 ion exchange resin to remove any endogenous angiotensin. Serial dilutions of the antiserum in 100 microliters of 0.01 M PO_4 buffer, pH 7.6, were incubated with radioiodinated angiotensin and the binding capacity of the antiserum dilutions was established. A serum dilution yielding 35-45% binding of labeled antigen was the dilution used for the radioimmunoassay.

Labeling of angiotensin

Radioactive antigen was prepared in two steps. The first step consisted of labeling valine^{5}- asparagine^{1}- angiotensin II with I^{125}. The second step involved the purification of the labeled material.

In the first step of the labeling procedure, 5 mcg of angiotensin was allowed to react with Chloramine T, 4.0 millicurie I^{125}, and crystallized bovine serum albumen. Sodium metabisulfite was added to halt the reaction and the mixture was quickly added to a chromatograph column containing Dowex 1-X8 anion exchange resin. Fifteen to twenty 0.5 ml aliquots were collected from the column for purification.

The purification of angiotensin-I^{125} was accomplished by descending ion exchange paper chromatography. The chromatographic step proceeded for two hours in a pyridine, acetic acid, water, and ethanol system. After overnight drying, peaks of radioactivity from the chromatograms were determined using a radiochromatogram scanner. Two peaks of high radioactivity were found on each chromatogram. The peak nearest the origin represented the free I^{125} and the peak immediately behind the solvent front represented the angiotensin-I^{125}. This area of the strip behind the solvent front was eluted with 0.01 M PO_4 buffer, pH 7.6, and filtered on a millipore system. The resulting purified angiotensin-I^{125} was stored frozen until use in the assay.

Assay protocol

Previous studies using this radioimmunoassay indicated that non-specific binding of the antigen by serum proteins made it difficult to extrapolate points from a standard curve performed in buffer to points obtained from plasma samples. This binding of the antigen by serum proteins varies from animal to animal. For this reason, a standard curve was prepared in resin-treated plasma for each animal from which samples for assay were obtained. The standard curve was performed in plasma which had been pretreated with Dowex AGW-50X4 resin to remove any angiotensin present. The plasma from the 50 ml blood sample obtained from each animal prior to hypotension was divided into two parts; one to serve as the pre-hemorrhage sample and the portion resin-treated for use in the preparation of the standard curve.

Incubation mixtures for the assay were prepared in chilled ($4^{o}C$) cellulose acetate tubes which had previously been rinsed with deionized water. Each tube contained 0.250 ml plasma (either resin-treated, or sample plasma), 0.100 ml of diluted antiserum, 0.100 ml of labeled angiotensin (about 3000 cpm), and 0.01 M PO_4 buffer, pH 7.6 to bring the total volume of each tube to 0.470 ml. The standard curve was established by addition of 0.10 ng to 30 ng of valine^{5}-asparagine^{1}- angiotensin II in 0.020 ml of buffer to a

series of tubes which contained resin-treated plasma. A co-precipitate tube containing treated plasma and buffer served to indicate the extent of the non-specific protein binding of angiotensin-I^{125} by the plasma. A second tube containing labeled angiotensin and buffer was used to determine background radioactivity. The entire assay was performed in triplicate and average values obtained from each set of three tubes were used in the calculations.

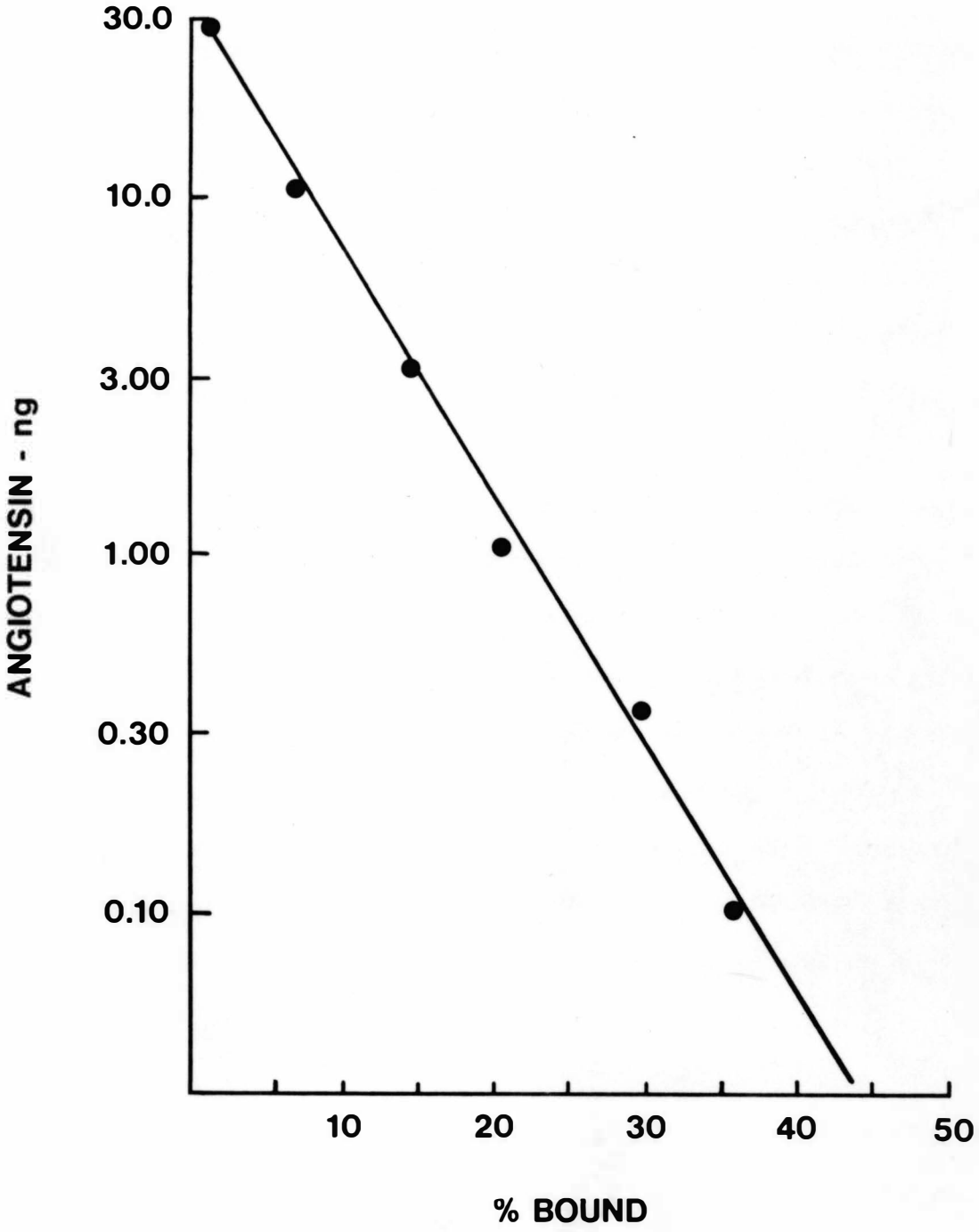
The assay mixtures were incubated for 18 hours at 4°C. After incubation, the free labeled angiotensin was separated from the antibody bound angiotensin by adsorbtion of the free material to dextran-coated charcoal. The charcoal was prepared by mixing a 5% charcoal suspension with a 0.5% Dextran 10 solution. Approximately 250 mg of the coated-charcoal suspension was added to each tube. The tubes were then vortex mixed and centrifuged at 1000 X g for fifteen minutes at 4°C. The supernatants were aspirated and discarded. The remaining pellets were counted on a Model 8742 Nuclear Chicago Scintillation Counter.

The per cent of labeled angiotensin which was bound by the antibody for each set of tubes was calculated by subtracting the counts of each tube from the counts of the co-precipitate tube and dividing this difference by the total counts added. A typical standard curve is presented in Figure 2.

FIGURE 2

STANDARD CURVE USED FOR DETERMINATION

OF SAMPLE ANGIOTENSIN LEVELS


30.0
10.0
3.00
1.00
0.30
0.10
ANGIOTENSIN - ng
10
20
30
40
50
% BOUND

Experimental Procedures

Dogs were anesthetized and surgically prepared by nephrectomy or sham-operation for nephrectomy. One hour after the completion of the surgical procedures, the animals were subjected to a two hour hypotensive period by allowing them to bleed freely into a stabilization reservoir against a constant pressure of 45 mm Hg. The volume of blood mobilized into the reservoir (bleeding volume) indicated changes in vascular capacity. A radioimmunoassay for angiotensin II was performed on blood samples taken during hypotension.

Four series of experiments were performed. The first series consisted of sham-operated and nephrectomized animals with intact autonomic nervous systems. This series of animals was designated as controls. The second series consisted of sham-operated and nephrectomized animals pretreated with spinal anesthesia prior to hypotension. The third series consisted of hexamethonium pretreated sham-operated and nephrectomized animals. A fourth series consisted of nephrectomized animals to which angiotensin infusions were given during hypotension.

Bleeding volumes, as well as angiotensin levels, were determined for each series. Mean differences were compared statistically using Student's *t* test. Significant differences were indicated by *t* values which were less than the 5% probability value.

RESULTS

Control Animals

Bleeding volume

The average blood loss of 12 sham-operated and 8 nephrectomized animals during the two hour hypotensive period is presented in Figure 3. It is apparent that little difference in blood loss between the two groups occurred during the first 30 minutes of hypotension. After this time, the sham-operated animals lost progressively more blood than the nephrectomized animals.

As seen in Table 1, the difference between the two groups reached a maximum of 14.1 ml/kg after 120 minutes of hypotension. The maximum blood loss of the sham-operated animals (50.1 ml/kg) occurred after 90 minutes of hypotension and the maximum blood loss of the nephrectomized animals (39.6 ml/kg) occurred at 60 minutes. The renal contribution to blood loss in these animals is apparent in the progressively increasing difference between the two groups after 30 minutes of hypotension. This difference was found to be significant at 60, 90, and 120 minutes after the onset of hypotension.

FIGURE 3

EFFECT OF NEPHRECTOMY ON BLOOD LOSS IN CONTROL ANIMALS

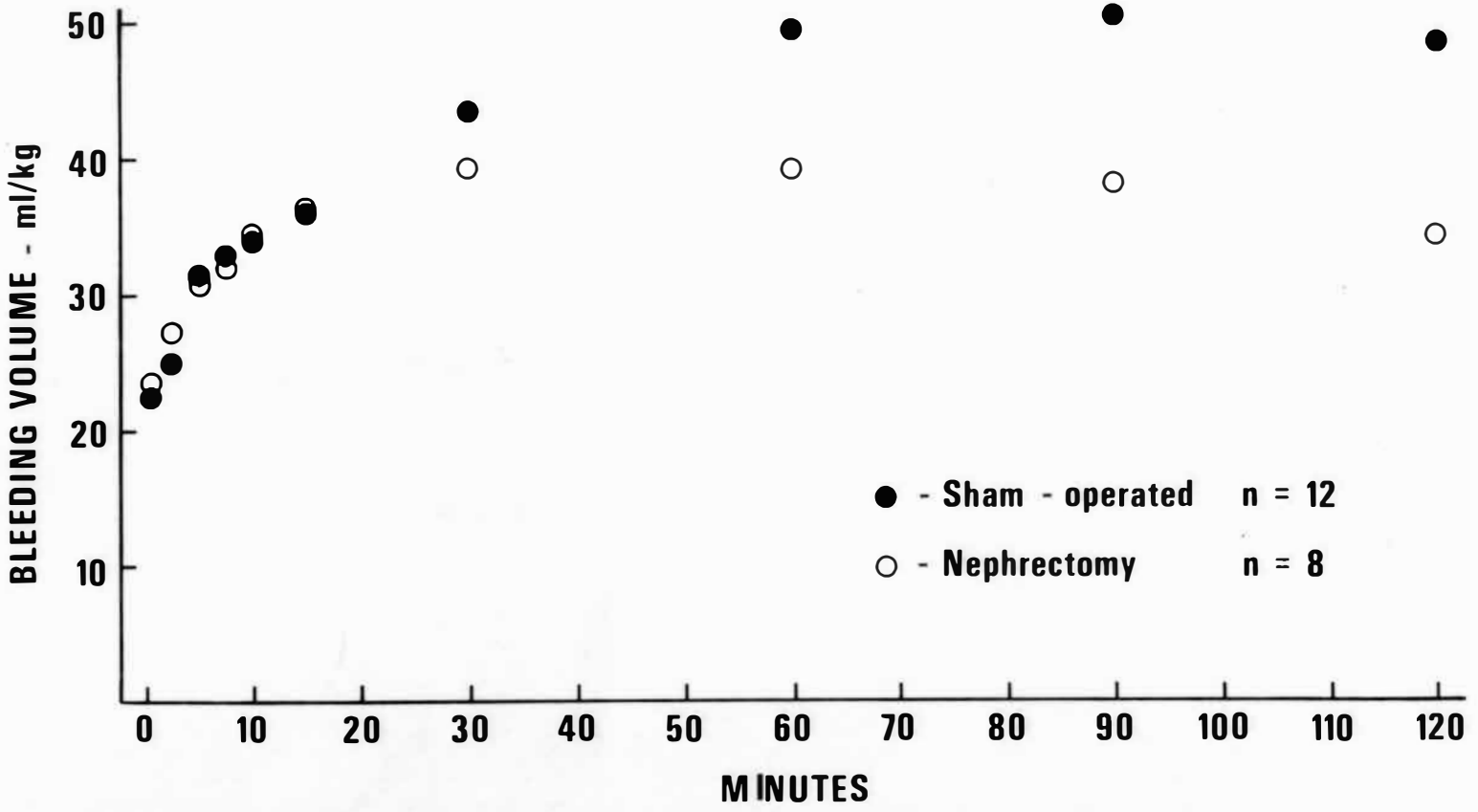

BLEEDING VOLUME - ml/kg
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
110
120
MINUTES
● - Sham - operated n = 12
○ - Nephrectomy n = 8

TABLE 1
BLEEDING VOLUMES OF SHAM-OPERATED AND NEPHRECTOMIZED CONTROL ANIMALS AND ARTERIAL ANGIOTENSIN LEVELS OF THE SHAM-OPERATED ANIMALS

Time (mins)	Bleeding Volumes (ml/kg ± S.E.)		Difference (sham-nephrectomy)	Angiotensin (ng/ml ± S.E.)
	Sham-operated n=12	Nephrectomized n=8		
0				1.2 ± 0.3
1	22.6 ± 1.3	23.7 ± 1.5	-1.1	1.8 ± 0.5
2	25.1 ± 2.6	27.3 ± 1.5	-2.2	2.6 ± 0.6
5	31.1 ± 1.4	30.8 ± 2.4	0.3	2.6 ± 0.9
7	32.6 ± 1.4	32.1 ± 2.7	0.5	3.0 ± 1.1
10	33.2 ± 1.6	34.2 ± 3.0	-1.0	2.9 ± 1.5
15	35.2 ± 2.1	35.7 ± 2.7	-0.5	2.2 ± 0.7
30	43.5 ± 2.3	39.3 ± 2.8	4.2	3.5 ± 0.7**
60	49.9 ± 2.0	39.6 ± 3.1	10.3*	8.1 ± 1.4**
90	50.1 ± 1.7	38.5 ± 3.4	11.6*	9.6 ± 1.4**
120	48.7 ± 2.2	34.6 ± 2.7	14.1*	9.6 ± 1.5**

* Difference is significant p less than 0.05

** Difference from 0 time is significant p less than o.o5

Angiotensin levels

The arterial angiotensin levels determined by radioimmunoassay for the 12 sham-operated dogs are presented in Table 1. No significant increase from the pre-hemorrhage level of 1.2 ng/ml was observed until 30 minutes. The maximum value of 9.6 ng/ml was found in both the 90 and 120 minute samples.

The average angiotensin levels determined for 5 nephrectomized animals during hypotension are presented in Table 2. It is obvious that little change from the pre-hemorrhage value of 0.6 ng/ml occurred during the hypotensive period. The pre-hemorrhage value of 0.6 ng/ml represents the level of angiotensin in arterial plasma one hour after nephrectomy.

Animals Pretreated with Spinal Anesthesia

Bleeding volume

The average blood loss for 6 sham-operated and 6 nephrectomized animals pretreated with spinal anesthesia is presented in Figure 4. It is readily apparent that spinal anesthesia eliminated any renal component of blood loss. As indicated in Table 3, no statistically significant difference in blood loss between the two groups existed at any time.

TABLE 2
ARTERIAL ANGIOTENSIN LEVELS OF NEPHRECTOMIZED CONTROL ANIMALS DURING HEMORRHAGE

	Time (mins)										
	0	1	2	5	7	10	15	30	60	90	120
Angiotensin (ng/ml ± S.E.) n=5	0.6 ± 0.2	0.6 ± 0.2	0.4 ± 0.2	0.6 ± 0.1	0.3 ± 0.2	0.7 ± 0.4	0.7 ± 0.2	0.5 ± 0.2	0.7 ± 0.3	0.8 ± 0.4	0.6 ± 0.2

FIGURE 4

EFFECT OF NEPHRECTOMY ON BLOOD LOSS

IN ANIMALS PRETREATED WITH SPINAL ANESTHESIA

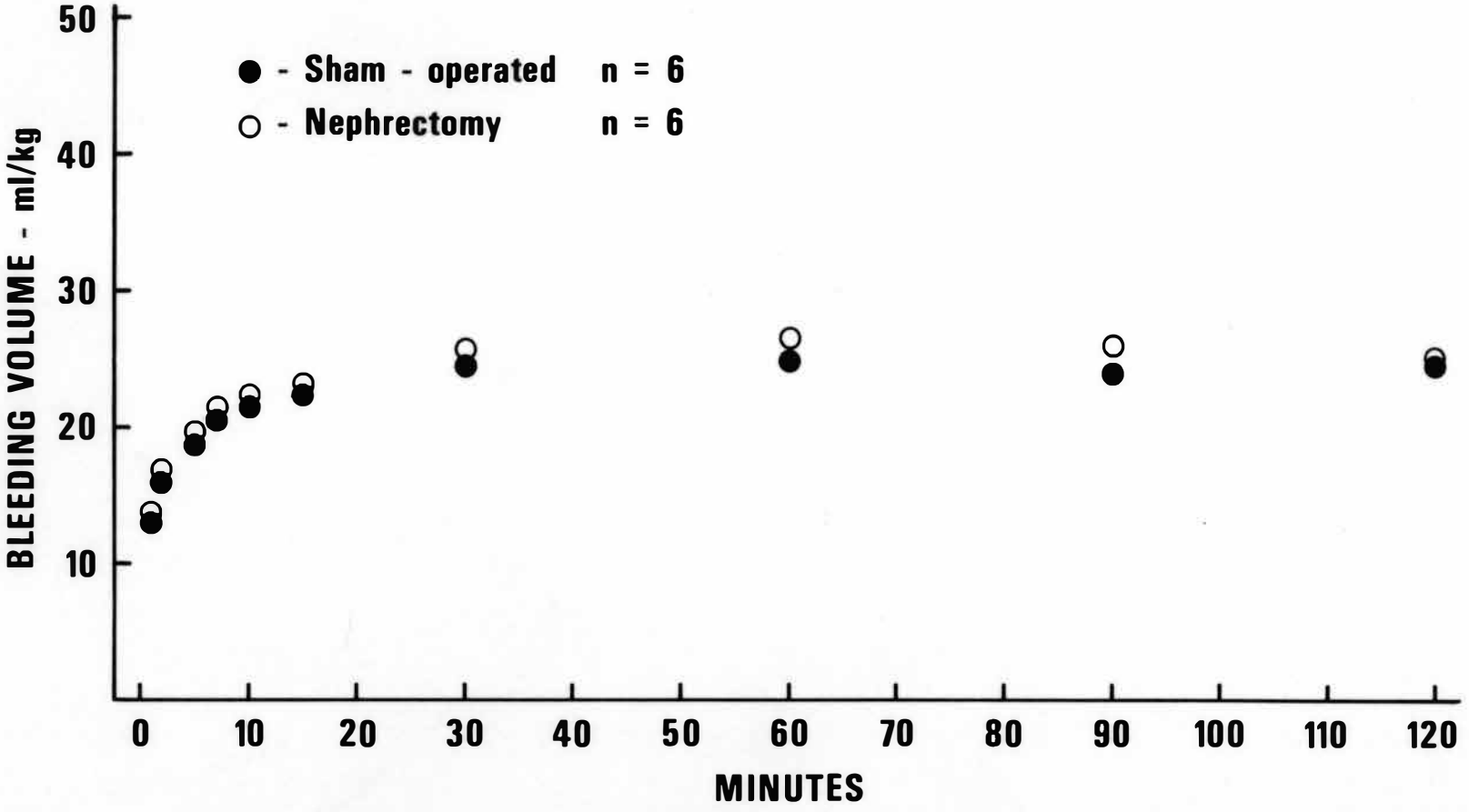

● - Sham - operated n = 6
○ - Nephrectomy n = 6
BLEEDING VOLUME - ml/kg
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
110
120
MINUTES

TABLE 3

BLEEDING VOLUMES OF SHAM-OPERATED AND NEPHRECTOMIZED ANIMALS PRETREATED WITH SPINAL ANESTHESIA AND ARTERIAL ANGIOTENSIN LEVELS OF THE SHAM-OPERATED ANIMALS

Time (mins)	Bleeding Volumes (ml/kg ± S.E.)		Difference* (sham-nephrectomy)	Angiotensin (ng/ml ± S.E.)
	Sham-operated n=6	Nephrectomized n=6		
0				1.1 ± 0.1
1	13.7 ± 1.4	13.1 ± 2.4	0.6	1.7 ± 0.5
2	15.5 ± 1.4	16.9 ± 2.9	-1.4	2.1 ± 0.4**
5	18.9 ± 1.6	19.7 ± 3.4	-0.8	4.0 ± 1.0**
7	20.4 ± 1.4	21.3 ± 3.1	-0.9	1.9 ± 0.6
10	21.8 ± 1.5	22.3 ± 2.9	-0.5	2.7 ± 0.8
15	22.4 ± 1.1	23.1 ± 3.0	-1.3	2.3 ± 0.6
30	25.4 ± 1.5	24.2 ± 2.7	1.2	2.5 ± 0.7
60	25.0 ± 1.9	26.3 ± 3.6	-1.3	4.7 ± 1.2**
90	24.0 ± 1.8	26.0 ± 3.9	-2.0	4.7 ± 1.1**
120	24.2 ± 1.5	24.6 ± 3,9	-0.4	4.2 ± 0.8**

* No significant difference at any time p less than 0.05.

** Difference from 0 time is significant p less than 0.05.

FIGURE 5

EFFECT OF NEPHRECTOMY ON BLOOD

LOSS IN ANIMALS PRETREATED WITH HEXAMETHONIUM

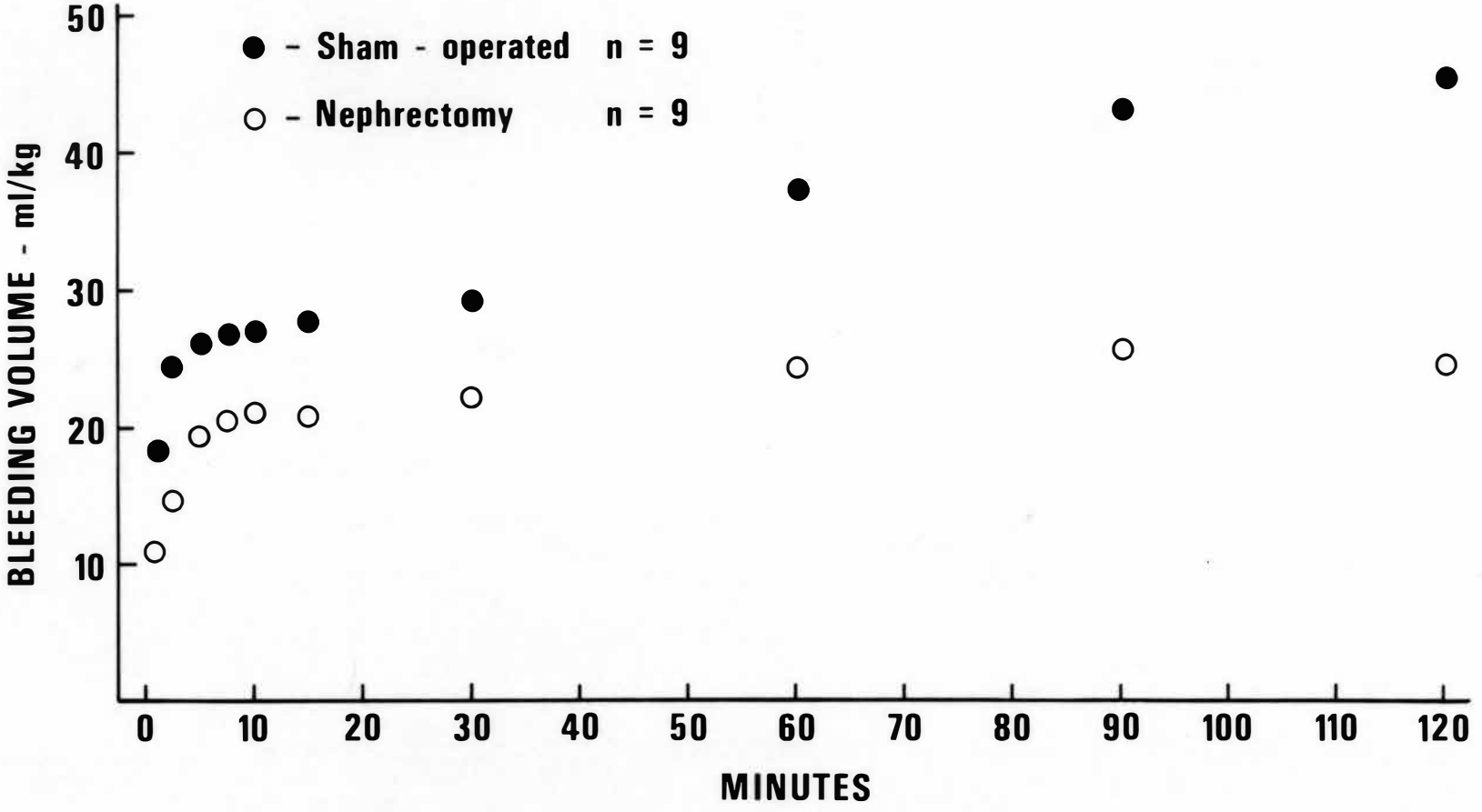

● - Sham - operated n = 9
○ - Nephrectomy n = 9
BLEEDING VOLUME - ml/kg
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
110
120
MINUTES

TABLE 4

BLEEDING VOLUMES OF SHAM-OPERATED AND NEPHRECTOMIZED ANIMALS PRETREATED WITH HEXAMETHONIUM AND ARTERIAL ANGIOTENSIN LEVELS OF THE SHAM-OPERATED ANIMALS

Time (mins)	Bleeding Volumes (ml/kg ± S.E.)		Difference (sham-nephrectomy)	Angiotensin (ng/ml ± S.E.)
	Sham-operated n=9	Nephrectomized n=9		
0				0.3 ± 0.1
1	18.2 ± 1.8	11.0 ± 1.0	7.2*	0.5 ± 0.2
2	22.5 ± 2.3	14.3 ± 1.1	8.2*	0.7 ± 0.2
5	26.6 ± 2.5	19.4 ± 1.2	7.2*	1.1 ± 0.8
7	26.9 ± 2.4	20.2 ± 1.1	6.7*	0.3 ± 0.2
10	26.8 ± 2.1	21.0 ± 1.2	5.9*	0.7 ± 0.3
15	27.2 ± 2.3	20.5 ± 0.9	6.7*	2.1 ± 0.6**
30	29.3 ± 2.7	22.0 ± 1.3	7.3*	1.8 ± 0.7
60	37.8 ± 3.2	24.6 ± 2.6	13.2*	1.5 ± 0.7
90	43.0 ± 3.2	25.2 ± 2.7	17.8*	1.6 ± 0.5**
120	45.9 ± 3.5	24.1 ± 3.1	22.8*	1.6 ± 0.6

* Difference is significant p less than 0.05.

** Difference from 0 time is significant p less than 0.05.

Angiotensin levels

Table 3 shows that the pre-hemorrhage angiotensin level of 1.1 ng/ml found in the spinal anesthetized sham-operated animals differed little from the 1.2 ng/ml level found in the control sham-operated animals. The angiotensin levels in the spinal anesthetized sham-operated animals increased significantly at 2 and 5, as well as 60, 90, and 120 minutes after the onset of hypotension. The 4.7 ng/ml maximum value was found at 60 and 90 minutes.

Animals Pretreated with Hexamethonium

Bleeding volume

Figure 5 illustrates the average blood loss of 9 sham-operated and 9 nephrectomized animals pretreated with the ganglion blocking agent, hexamethonium. At all of the selected times during hypotension the sham-operated animals had a greater bleeding volume than the nephrectomized animals. Table 4 shows that the blood loss of the sham-operated animals was statistically greater than the blood loss of the nephrectomized animals as early as one minute after the onset of hypotension. The greater blood loss of the sham-operated animals continued to increase throughout the 120 minute hypotensive period. At 120 minutes the blood loss of the sham-operated animals was 22.8 ml/kg greater than that of the nephrectomized animals.

TABLE 5
EFFECT OF PHENTOLAMINE ON VASCULAR CAPACITY OF SHAM-OPERATED AND NEPHRECTOMIZED ANIMALS PRETREATED WITH HEXAMETHONIUM

	Bleeding volumes (ml/kg ± S.E.)		Difference
	Sham-operated n=5	Nephrectomized n=5	(sham-nephrectomy)
120 minutes after the onset of hypotension	47.5 ± 4.0	28.3 ± 4.0	19.2
10 minutes after phentolamine (2 mg/kg I.V.)	39.2 ± 3.3	23.0 ± 2.9	16.2
Net reinfusion	8.3 ± 1.2	5.3 ± 1.6	3.0

Angiotensin levels

Table 4 shows that the angiotensin levels of tha ganglion blocked sham-operated animals failed to increase greatly during hypotension. The only levels statistically different from the pre-hemorrhage value of 0.3 ng/ml were the 2.1 ng/ml level found at 15 minutes and the 1.6 ng/ml level found at 90 minutes. After 120 minutes of hypotension, only 1.6 ng/ml of angiotensin was found.

To determine the extent of adrenergic vasoconstriction present in the hexamethonium treated animals, the alpha adrenergic blocking agent, phentolamine, was administered to 5 sham-operated and 5 nephrectomized dogs. The phentolamine (2 mg/kg) was given intravenously shortly after the end of the two hour hypotensive period. The total amount of blood translocated from the reservoir to the animal during a 10 minute period after the administration of phentolamine is presented in Table 5. The average net reinfusion of the sham-operated animals was 8.3 ml/kg. The nephrectomized animals showed an average net reinfusion of 5.3 ml/kg. The difference in reinfusion between the two groups was not found to be statistically significant.

Angiotensin Infusion

Bleeding volume

Table 6 shows the results of graded angiotensin infusions on blood loss in nephrectomized hemorrhaged animals. The increased bleeding volume as a result of infusion was determined by comparison of the bleeding volume at the tenth minute of infusion with the bleeding volume of control nephrectomized animals at the same time after the onset of hypotension.

It is apparent that the 0.01 mcg/kg/min angiotensin infusion had little effect on the bleeding volume. The 0.10 mcg/kg/min infusion caused an increased bleeding volume of 5.4 ml/kg. The 1.0 mcg/kg/min infusion resulted in an 8.6 ml/kg increase in bleeding volume

Angiotensin levels

Table 6 shows that as a result of the 0.01 mcg/kg/min angiotensin infusion there was an increase of arterial angiotensin from 0.7 ng/ml to 2.2 ng/ml. After the infusion of 0.10 mcg/kg/min the angiotensin levels in the plasma rose to 37.8 ng/ml. The 10 minute infusion of 1.0 mcg/kg/min of angiotensin caused the arterial plasma level to increase to 89.3 ng/ml. Thirty minutes after the termination of infusions, 2.7 ng/ml of angiotensin was detected in the plasma.

TABLE 6
EFFECT OF GRADED ANGIOTENSIN INFUSIONS ON
BLOOD LOSS IN NEPHRECTOMIZED ANIMALS*

	Infusion Rate		0.01mcg/ kg/min	0.10mcg/ kg/min	1.0mcg/ kg/min	
Time (mins)	0	60	70	80	90	120
Bleeding volume (ml/kg ± S.E.)		37.9 ± 4.1	38.8 ± 4.2	44.6 ± 2.1	47.1 ± 2.2	39.4 ± 2.3
Angiotensin levels (ng/ml ± S.E.)	0.7 ± 0.1		2.2 ± 1.2	37.8 ± 13.3	89.3 ± 16.4	2.7 ± 0.8
Blood loss difference from nephrectomized control animals (ml/kg)		1.7	0.3	5.4	8.6	4.8

* n=3

DISCUSSION

The method used in this study involved the production of hypotension by allowing an animal to mobilize a volume of blood into a reservoir against a constant pressure of 45 mm Hg. The pressure equilibrium between the animal and stabilization reservoir was maintained by allowing the free flow of blood between the animal and the reservoir. The changes in the volume of blood within the reservoir indicated changes in the vascular capacity of the animal.

It is usually assumed that about 80% of the circulating blood volume is contained in the venous segments of the circulation. For this and other reasons, the veins are referred to as capacity vessels. Therefore, the observed changes in vascular capacity reflect changes in the venous volume.

Venous volume can be altered either actively or passively. In either case the net result is an alteration in venous capacity which would be manifest by a change in the volume of blood mobilized into the stabilization reservoir.

An active process of decreasing venous capacity would involve venoconstriction. Venoconstriction would cause a transient increase in central venous volume and increased venous return to the heart, which in turn would cause an increased cardiac output. Since the animal is in pressure equilibrium with the stabilization reservoir, an increased

amount of blood would be mobilized into the reservoir.

A passive alteration of vascular capacity would occur as a result of arteriolar constriction, which would cause a decreased capillary and post-capillary pressure. This would result in a decreased pressure against the venous walls and allow shortening of the elastic elements of the wall causing a transient increase in central venous volume. This in turn would increase cardiac output and increase the volume of blood mobilized into the reservoir.

DuCharme and Beck (1971) indicated that the renal pressor component of blood loss might cause an active venoconstriction. They found that in the perfused hindlimb of a hemorrhaged dog, changes in perfusion pressure did not correlate positively with loss of blood from the animal to the stabilization reservoir. An increase in perfusion pressure during constant flow would primarily indicate arteriolar constriction. Failure of the perfusion pressure to increase at a time when blood continued to accumulate in the reservoir would suggest that the blood loss was due to active venoconstriction.

Schwinghamer et al. (1970) have presented data indicating that an active increase in venous resistance occurs in the perfused forelimb of the dog in response to hemorrhage. This increase was observed in cutaneous veins, but not veins draining muscle. A similiar observation was made by Bond et al. (1970). Dow and Fry (1967) also presented data

showing an active venoconstriction in response to hemorrhage in the dog. These investigators measured venous wall tension by means of an extraluminal force transducer attached to the inferior vena cava. The progressive increase in venous wall tension without changes in central venous pressure during hemorrhage was abolished by phenoxybenzamine. This would suggest that the venoconstriction was adrenergic in nature.

Based on these preceding reports, it can be inferred that in the present study, at least a portion of the observed blood loss was due to active constriction of the capacity vessels.

Hemorrhagic hypotension provides an adequate stimulus for the release of renin and consequent formation of angiotensin. This fact is well documented by the reports of Paladini and Scornik (1963), McKenzie et al. (1966) and Regoli and Vane (1966). All of these authors indicated that large amounts of angiotensin appeared in the circulation as a result of hemorrhage.

The exact nature of the stimulus provided by hemorrhagic hypotension for the release of renin is difficult to determine. Hemorrhage results in activation of the sympathetic nervous system, a decrease in renal perfusion pressure, and a decreased sodium load at the level of the macula densa. Therefore, hemorrhagic hypotension could stimulate renin release by any of these three postulated mechanisms.

Whatever the mechanism it is evident from Table 1 that The reduction of arterial pressure to 45 mm Hg is a sufficient stimulus to cause the release of renin and consequent formation of angiotensin. Table 1 shows that there is a large renal contribution to blood loss. This is indicated by the large bleeding volume difference between the sham-operated and nephrectomized dogs at 60, 90, and 120 minutes after the onset of hypotension.

It is also shown in Table 1 that this renal contribution to bleeding volume is associated with high levels of angiotensin in the arterial plasma. This correlation would suggest that angiotensin is the renal pressor material responsible for the renal contribution to blood loss. This would support the postulate of Sapirstein et al. (1941) that the renin-angiotensin system is the renal homeostatic mechanism involved in the maintainance of vascular tone during hemorrhagic hypotension.

If angiotension was the renal pressor substance causing the greater blood loss in sham-operated dogs it follows that in any situation where there is a renal component to blood loss there would be a concomitant increase in angiotensin. It also follows that in situations where there is no renal component to blood loss, there would be no increase in angiotensin. Also, exogenous administration of angiotensin would cause an increased bleeding volume in nephrectomized animals that is comparable to the blood loss

of sham-operated animals. This exogenous administration of angiotensin would also result in plasma levels of angiotensin similiar to those found in the sham-operated animals.

It is quite evident from Table 6 that angiotensin was able to exert a negative capacity effect when infused into nephrectomized animals during hemorrhage. The nature of this negative capacity effect, whether active or passive, cannot be determined from the methods used in this study. The data in Table 6, however, raise some question as to the ability of the observed levels of angiotensin found in the sham-operated animals to cause the increased blood loss of this group. The maximum bleeding volume difference between sham-operated and nephrectomized animals of 14.1 ml/kg is associated with an angiotensin level of 9.6 ng/ml in the sham-operated animals. In contrast, a 1.0 mcg/kg/min angiotensin infusion into nephrectomized animals which caused an additional blood loss of 8.6 ml/kg was associated with an angiotensin level of 89.3 ng/ml. The additional blood loss as a result of infusion was about 60% of the maximum bleeding difference between the sham-operated and nephrectomized control animals. This additional blood loss was associated with an angiotensin level almost nine times greater than that found in the sham-operated animals at the time of maximum bleeding volume difference. Thus, it appears that the observed angiotensin levels in the sham-operated animals may not be responsible for the large bleeding volume difference.

This conclusion must be made with some reservations, however. There is a general acceptance that exogenous administration of a hormone must be in quantities several times greater than the observed endogenous levels to produce the same degree of effect. This is usually due to the impurity of the exogenous agent or to minute chemical differences between the endogenous and exogenous hormones.

The fact that both the endogenous and exogenous angiotensins will bind with the same antibody would suggest that their chemical composition is very similiar, if not the same. Also, the conclusion that the angiotensin levels found in the sham-operated animals may not be great enough to cause the increased bleeding difference is based on the amount of angiotensin found in the plasma and not the quantity of angiotensin administered.

Figure 4 illustrates that pretreatment with spinal anesthesia eliminated the renal component of bleeding volume found in the control sham-operated dogs. If angiotensin was responsible for the increased bleeding volume of the sham-operated control animals then no change in angiotensin levels would be expected in the sham-operated spinal anesthetized animals.

There was, however, a significant increase in angiotensin levels in the sham-operated dogs. The observed elevated levels late in hemorrhage occur at the same time as the elevated levels in the control animals. It is interest-

ing to note that although the pre-hemorrhage levels are very similiar to those found in the control animals, the maximum value of 4.7 ng/ml found in the spinal anesthetized animals is only about 50% of the 9.6 ng/ml angiotensin level found in the control animals. These results would indicate that the angiotensin levels found in the spinal anesthetized animals are not sufficient to cause a decrease in vascular capacity.

It is conceivable that the angiotensin levels of the sham-operated spinal anesthetized dogs exert no negative capacity effect due to the reduction of sympathetic tone. As indicated by Zimmerman (1962), intact sympathetic pathways are necessary for the total pressor effect of angiotensin.

The reduction, but not abolition, of angiotensin formation as a result of spinal anesthesia would suggest that some neurogenic pathway is necessary for the maximal release of renin in response to hemorrhage. It is also possible that the elevated angiotensin levels late in hemorrhage reflect a return of neurogenic function due to diminution of the spinal anesthesia. However, in several dogs that were given additional spinal anesthesia late in hemorrhage, no large reinfusion of blood occurred suggesting that the spinal cord was still anesthetized.

Table 4 also shows that no large increase in plasma angiotensin occurred in the sham-operated animals pretreated with hexamethonium, at any time during the hypotensive

period. The 0.3 ng/ml angiotensin pre-hemorrhage level found in the sham-operated animals of this series is less than that found in the other sham-operated animals (Table 1 and Table 3). This reduction in angiotensin would suggest that either a ganglionated neurogenic pathway is involved in the release of renin or hexamethonium possesses other properties which prevent the release of renin and/or formation of angiotensin.

Since in this study the release of renin was determined by the ultimate formation of angiotensin II, it is conceivable that hexamethonium did not block the release of renin but inhibited one of the enzymatic steps involved in the formation of angiotensin II. Scornik and Paladini (1963) and other investigators have reported that ganglioplegic agents prevent the increase in plasma renin activity usually associated with hemorrhage and other renin-releasing situations. This would indicate that the inhibiting action of ganglion blocking agents is on renin secretion and not inhibition of the conversion of angiotensinogen to angiotensin I.

It was hypothesized that the renal contribution to bleeding volume in the hexamethonium pretreated animals was adrenergic in nature. To test this hypothesis, the alpha adrenergic blocking agent, phentolamine was given to sham-operated and nephrectomized hexamethonium pretreated animals at the end of the hypotensive period. If the renal

contribution to the observed greater bleeding volume of the sham-operated animals was of an adrenergic origin, then the sham-operated animals should have exhibited a greater reinfusion of blood than the nephrectomized animals after the administration of phentolamine. Also, the reinfusion in the sham-operated animals should have been comparable to the bleeding volume difference between the two groups at the end of the hypotensive period.

As shown in Table 5, no large reinfusion was found in either group as a result of the administration of phentolamine. The 8.3 ml/kg average net reinfusion found in the sham-operated animals was not found to be significantly different than the 5.3 ml/kg average net reinfusion of the nephrectomized animals. It is also apparent from Table 5 that 10 minutes after the administration of phentolamine, there still existed a 16.2 ml/kg bleeding volume difference between the two groups. This indicates that there existed a non-angiotensin, non-adrenergic renal pressor material present in the circulation of the sham-operated animals.

It is shown by Figure 5 that this non-adrenergic, non-angiotensin renal pressor substance exerted a negative capacity effect in the sham-operated animals as early as one minute after the onset of hypotension. A renal pressor system showing a rapid response to decreased blood pressure could conceivably function to maintain normal blood pressure. If this was the case, then the sham-operated hexa-

methonium pretreated animals would be expected to have a higher pre-hemorrhage blood pressure than the nephrectomized animals. Such a difference was not observed, however. Apparently this renal pressor system functions only in response to acute reduction of the blood pressure.

The fact that this non-angiotensin renal pressor system is responsible for the renal component of blood loss in the hexamethonium pretreated animals would suggest that it may contribute to the blood loss of the sham-operated control animals. The data obtained from the angiotensin infusions would seem to suggest that although angiotensin is capable of exerting a negative capacity effect, the levels found in the control sham-operated animals are not great enough to account for the difference in blood loss between sham-operated and nephrectomized animals. This would then indicate that some other vasoactive material released by the kidney in response to hemorrhage must lead to the observed reduction in vascular capacity. This additional renal pressor agent appears to be under neurogenic control involving a non-ganglionated pathway.

The overall conclusions of these observations are a) the renin-angiotensin system exerts little control over the reduction of vascular capacity during hemorrhagic hypotension and b) that there is a renal pressor system that does not involve angiotensin.

SUMMARY

It has been well illustrated by many investigators that the kidney is responsible for a portion of the blood pressure homeostasis in response to hemorrhage. The fact that hemorrhage results in the liberation of renin from the kidney in response to hemorrhage has also been well established. The purpose of the present investigation was to determine if the renin-angiotensin system was the renal homeostatic mechanism of blood pressure maintainance activated by hemorrhage.

Pentobarbital-anesthetized dogs were bled to and maintained at a fixed hypotensive level of 45 mm Hg for a two hour period. Controlled hypotension was achieved by means of an arterial stabilization reservoir. Changes in vascular capacity were determined by changes in the volume of blood within the reservoir. Arterial plasma samples taken periodically throughout the hypotensive period were analyzed for angiotensin II content by means of radioimmunoassay.

The blood loss of dogs sham-operated for nephrectomy was significantly greater than that of nephrectomized dogs after 90 minutes of hypotension. The difference between the two groups after 120 minutes of hypotension was 14.1 ml/kg. The angiotensin content in arterial plasma of the sham-operated dogs increased from a pre-hemorrhage value of 1.2 ng/ml to 3.5 and 9.6 ng/ml after 30 and 90 minutes

respectively of hypotension. Sham-operated and nephrectomized dogs subjected to spinal anesthesia prior to hypotension exhibited no difference in blood loss. However, the angiotensin levels of the sham-operated dogs increased from a pre-hemorrhage value of 1.1 ng/ml to 4.7 ng/ml at 90 minutes after the onset of hypotension. Sham-operated dogs pretreated with the ganglion blocking agent, hexamethonium, lost considerably more blood than their nephrectomized counterparts (22.8 ml/kg at 120 minutes), even though the arterial angiotensin levels differed little from the pre-hemorrhage value of 0.3 ng/ml throughout the hypotensive period. Intravenous infusion of angiotensin into nephrectomized dogs at doses which increased blood loss to a level comparable to sham-operated control animals resulted in an arterial plasma level of angiotensin several times greater than the maximum level found in the control dogs.

It was concluded that, in addition to the renin-angiotensin system, some other renal pressor system is activated during hemorrhagic hypotension.

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