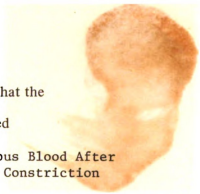
VASOACTIVITY OF RENAL VENOUS BLOOD AFTER UNILATERAL RENAL ARTERY CONSTRICTION

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
MOTILAL B. PAMNANI
1975



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Vasoactivity of Renal Venous Blood After Unilateral Renal Artery Constriction

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Physiology

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Date December 16, 1974

O-7639

ABSTRACT

VASOACTIVITY OF RENAL VENOUS BLOOD AFTER UNILATERAL RENAL ARTERY CONSTRICTION

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Data from several laboratories indicate that unilateral renal artery constriction is associated with hypertension and release of vasoconstrictor and vasodilator agents from the kidney into the circulation. Thus it has been hypothesized that the abnormal hemodynamic state of renovascular hypertension may represent a state of vascular constriction attributable to an absolute or relative increase in circulating vasoconstrictor agents and/or to an absolute or relative decrease in circulating vasodilator agents. The main purposes of the present investigation were two fold: 1. to bioassay renal venous blood following acute unilateral renal artery constriction for the presence of vasoactive substances and, 2. to identify vasoactive substances thus found. We pump perfused the isolated denervated gracilis muscle of each of 27 pentobarbital anesthetized dogs at constant flow with blood from his vena cava (VC), from each of his renal veins (RRV and LRV) and from the abdominal aorta (FA) sequentially before and during the 45 minutes after partial constriction of his left renal artery ("experimental animals"). Twenty-seven "control dogs" underwent sham constriction. Of these 54 dogs, 7 experimental and 7 control dogs were

immunized against renin before the constriction (Group B) and 8 experimental and 8 control dogs received indomethacin before the constriction (Group C). Group A was composed of the remaining 12 experimental and 12 control dogs. Venous and arterial blood hematocrits, PO_2 and PCO_2, Na^+, K^+, Ca^{++} and Mg^{++} concentrations did not change during the procedure. Following constriction of the renal artery mean arterial pressure rose, and plasma renin concentration (PRC) of LRV and VC blood increased in experimental animals of groups A and C only, as did gracilis perfusion pressure during perfusion with LRV blood ($P < 0.001$). Similar increases in gracilis resistance were evoked by VC and FA blood in dogs of group A. As there was no change in the hematocrit and therefore probably viscosity of the blood, the rise in gracilis resistance may be attributable to increased vasoconstriction. Interestingly, no vasoconstriction was evoked by RRV blood ($P > 0.2$) in experimental animals of group A. In group C similar levels of gracilis vasoconstriction were evoked by LRV, RRV, VC and FA blood. Gracilis perfusion pressures in experimental dogs of group B were not significantly different from those in control dogs. This study therefore shows that following acute unilateral renal artery constriction, a vasoconstrictor substance, probably angiotensin II, is generated systemically by renin added to venous blood by the constricted kidney. Besides the renin-angiotensin system no other vasoconstrictor seems to play a role in this response. Our data further suggest that the elevated vasoconstrictor activity of systemic

blood is apparently "titrated" within the untouched kidney by increased release of prostaglandins probably PGE_2 by this kidney. Thus the opposite untouched kidney may attenuate Goldblatt hypertension not only by its excretory function but also by release of vasodilator substances.

VASOACTIVITY OF RENAL VENOUS BLOOD AFTER UNILATERAL RENAL ARTERY CONSTRICTION

By

Motilal B. Pamnani

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology

1975

Dedication

To my parents and to my
wife Renu and my son Anil.

ACKNOWLEDGMENTS

The author wishes to express his sincere and deep gratitude to his major advisor, Dr. H. W. Overbeck, for his guidance, advice and assistance throughout the course of this study and during the preparation of this thesis.

Thanks and gratitude are extended to Dr. J. B. Scott, co-chairman of the author's guidance committee for his direction, advice and encouragement. Appreciation and thanks are extended to the members of the guidance committee, Drs. C. C. Chou, M. D. Bailie, J. B. Hook and G. D. Reigle for their competent guidance and expert professional expertise in review of this dissertation and especially to Dr. M. D. Bailie for taking extra time to estimate plasma renin concentration (PRC) in his laboratory.

A special word of thanks is due to Dr. F. J. Haddy for his constant encouragement, support and guidance.

The author also acknowledges the skillful technical assistance of Mr. Donald L. Anderson, the chief technician, Mrs. Josephine Jhonston, specialist, Mr. Booker Swindal, chief surgical technician, and Mrs. Lynn Dietrick and Nancy Turner for their expert typing of this manuscript.

Last, but not the least, thanks are extended to Ms. Amylou Davis for assistance in overcoming administrative details.

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LITERATURE SURVEY

I. Introduction

The mechanism of hypertension following renal artery constriction remains obscure. Data from other investigators suggests that partial constriction of the renal artery may stimulate the kidney to release not only vasoconstrictor but also vasodilator agents. It has also been reported that the opposite untouched kidney may also respond by release of vasoactive agents into the blood. These vasoactive agents released by the kidneys may contribute to the abnormal vasoactivity present in Goldblatt hypertension. In this survey, the literature regarding various vasoactive agents released by the kidneys during acute and chronic stages of one and two kidney renal hypertension is reviewed.

II. Hypertensive Function of the Kidney

A. Experimental Renal Hypertension

The association of renal disease and hypertension was recognized by Richard Bright in 1836 but it was not until 1934 when the renal etiology of hypertension became an important concept. In 1934 Goldblatt and co-workers for the first time demonstrated that diastolic hypertension could be produced in experimental animals by constriction of the renal artery.

1. Methods of production of experimental renal hypertension

Various procedures have been used to produce renal hypertension in experimental animals.

a. Clamping of renal artery

The Goldblatt experiment was to partially constrict one renal artery in dogs by means of a silver clamp, after which hypertension developed in 24 to 48 hours. The hypertension progressed slowly, reached maximum in one to two weeks and usually lasted 4 to 6 weeks. Then pressure slowly returned to normal. Hypertension could be made permanent if the contralateral kidney was removed or if both renal arteries were constricted. Braun-Menendez et al. (1946) using the Goldblatt technique also produced hypertension in the monkey, rabbit, goat and rat. In the rat in contrast to most other species constriction of one renal artery with the opposite kidney untouched leads to the development of chronic renovascular hypertension.

b. Compression of renal parenchyma

1) Cellophane wrapping

Page (1939) produced persistent hypertension in dogs, cats, and rabbits by enveloping the kidneys in cellophane. Within three to five days of wrapping, an inflammatory reaction to the cellophane occurred and during the next two or three weeks a constricting hull formed around the parenchyma of the kidney. Although the hypertension due to wrapping one kidney was less marked and was not persistent, hypertension occurred whether one or both kidneys were placed in cellophane.

2) Figure of 8 ligature

Grollman (1944) using figure of 8 ligature method produced experimental renal hypertension in mouse, rat, rabbit and dog. This method essentially consists in passing a cotton thread or a tape over the pole and the body of the kidney and drawing it tight so as to compress renal parenchyma.

c. Radiation of the kidney

Hartman et al. (1926) demonstrated that irradiation of the kidneys with Roent'gen rays produced renal lesions and a moderate rise of blood pressure in dogs.

d. Constriction of renal vein

Pederson (1927) produced chronic hypertension in the rabbit by constricting the renal vein with aluminum wire and placing the kidney in a loose pouch of a fixed animal membrane to prevent the development of collateral circulation.

e. Renal arteriovenous anastomosis

Major et al. (1939) produced hypertension and renal insufficiency in dogs by anastomosing the renal artery and vein of one kidney and removing the other kidney.

Lasher and Glenn (1939) reported their findings from a series of 12 dogs in which they had surgically created unilateral renal arteriovenous fistulae. The authors did not statistically analyze their data, but there was no apparent rise in blood pressure until a contralateral renal a-v fistula was created. Soon after this second procedure the blood pressure rose only slightly and the dogs died of uremia.

f. Ligature of both ureters

Hartwich (1930) produced renal hypertension by ligation of both ureters in dogs. Enger and his colleagues (1938) grafted the kidney which had had ureteral ligation into a normal dog. The recipient dog developed hypertension. This suggested to the authors that hypertension due to ligation of ureters was produced by passage of substances from the grafted kidney into the blood rather than interference with the excretory function of the kidney.

g. Coarctation of abdominal aorta

Yet another method that has been used to produce experimental renal hypertension is coarctation of the abdominal aorta. Goldblatt et al. (1939) demonstrated that constriction of abdominal aorta just above the origin of both main renal arteries in dogs invariably resulted in elevation of carotid artery pressures. The hypertension developed with about the same time course as after constriction of the main renal arteries. Constriction of the abdominal aorta just below the origin of both main renal arteries had no significant effect on the carotid pressures. The investigators, therefore, concluded that hypertension following the constriction of the abdominal aorta just above the origin of both main renal arteries was of renal origin. Rytand (1938) has shown that in rats partial occlusion of the aorta between the origin of the two renal arteries was followed by left ventricular hypertrophy only when there was living renal tissue beyond the constriction. As other causes of left ventricular hypertrophy were absent, the authors concluded that the rats were hypertensive. On the other hand, when renal tissue

distal to the constriction was excised, hypertrophy (hypertension) did not occur. These authors, therefore, concluded that the hypertension due to coarctation of the aorta was of renal origin.

All these methods for producing experimental renal hypertension in animals have in common the fact that they involve interference with the hemodynamics of the kidney or obvious damage to the renal parenchyma. Of all these experimental procedures, constriction of renal arteries, wrapping of the kidney (causing perinephritis) or coarctation of aorta above the renal artery(s) give more consistent results and have been extensively used to produce renal hypertension in experimental animals.

2. Evidence for the release of vasoconstrictor(s) from the kidney

The mechanism underlying the development of hypertension due to manipulation of the kidney is still being debated. The concept that the constricted kidney releases a vasopressor substance was first advanced by Goldblatt et al. (1937). However, it was not until 1938 that Houssay and Taquini detected vasopressor substances released by the affected kidney. Work of several other investigators also indicates that the manipulated kidney releases vasoconstrictor(s) which is responsible for the peripheral vasoconstriction and elevation of the systemic arterial pressure. According to the renin hypothesis development of renal hypertension is brought by liberation of renin into the blood by the manipulated kidney, with the subsequent formation of the potent pressor substance angiotensin II. A number of physiological, biochemical and pharmacological procedures have been used to investigate vasoconstrictor(s) released by the manipulated kidney.

a. Physiological procedures

1) Return of the blood pressure to normal on release of the clamp on the renal artery or on excision of the clamped kidney

Goldblatt et al. (1937) showed that hypertension due to renal artery constriction disappeared if the constricting clamp on the renal artery was removed. The blood pressure returned to normal in hours or days. These findings of Goldblatt et al. were confirmed by the work of Blacklock and Levy (1937) and Braun-Menendez et al. (1946). Braun-Menendez et al. (1946) further showed that excision of the clamped kidney in the early stages of the development of renal hypertension brings about a prompt decline of blood pressure to normal. Reversibility of acute renal hypertension by removal of the manipulated kidney has been confirmed by several investigators. The authors, therefore, concluded that, at least during the early stage of the development of hypertension, the manipulated kidney releases a vasoconstrictor substance(s) responsible for the hypertension. However, the possibility that the hypertension was due to the derainged excretory function, neural stimulation or failure of the manipulated kidney to release an antihypertensive substance was not ruled out in these early experiments.

2) Additional evidence indicating role of renin-angiotensin system in experimental renal hypertension

The fact that intravenous infusion of renin and/or angiotensin can cause hypertension has been cited as one piece of evidence incriminating the renin system in the mechanism of renal hypertension. Blacket et al. (1950) have shown that the pressor effect of small doses of renin can

be maintained in unanesthetized rabbits for up to 3 weeks without tachyphylaxis. When the infusion was stopped, pressure fell to normal. McCubbin et al. (1965) infused subpressor amounts of angiotensin into dogs and observed that after 1 to 3 days arterial pressure began to rise and increased progressively thereafter until severe sustained hypertension developed. Tachyphylaxis to this infusion did not develop and the raised pressure persisted for the duration of the infusion, more than a month in some cases. If the infusion was stopped after only a few days, arterial pressure returned to the control value within 30 to 60 minutes. After termination of infusions lasting 3 to 4 weeks, pressure did not return entirely to control levels within the following 2 days. Using chronically implanted aortic electromagnetic flow probes, the authors found that the hypertension was due entirely to a rise in total peripheral resistance.

Although these experiments demonstrated that infusion of angiotensin may cause sustained rises in arterial pressure, they did not prove that the hypertension in renal hypertension is attributable to angiotensin. Masson et al. (1966) showed that the subcutaneous administration of saline extracts from endocrine kidneys (clipped kidneys), but not of the contralateral kidneys, to uninephrectomized rats produced renal hypertension with the same degree of severity as that elicited by unilateral clipping of the renal artery. This effect was also obtained by injecting crude renin extracts from rats and hogs. The injected animals showed the same changes as those seen in rats made hypertensive by unilateral clipping of the renal artery, i.e., renal and

vascular lesions and decrease in renin content of the remaining kidney. These investigators (1966) also showed that administration of renin prevented the remission of renal hypertension produced by removal of the clipped kidney.

b. Levels of renin and/or angiotensin in blood in renal hypertension

Several investigators have attempted to measure plasma renin and/or angiotensin levels using various methods, attempting to demonstrate that the rise of blood pressure or increase in vasoconstrictor activity following renal artery clamping is due to activation of the renin-angiotensin system.

1) Direct bioassay technique

Direct bioassay was one of the first methods used for the determination of renin-angiotensin levels in blood. Houssay and Taquini (1938) drew blood from the renal vein of a dog with two kidney Goldblatt hypertension of a few days duration. The vasoconstrictor effect of the plasma was tested in the isolated perfused hind limb of the toad. Plasma from the clipped kidney produced marked vasoconstriction whereas plasma from the normal kidney did not show elevated vasoconstrictor activity. Pritchard et al. (1964) produced acute renal hypertension in 8 dogs. In 5 dogs both renal arteries were constricted and in 3 dogs the right kidney was removed and a clamp was applied to the left renal artery. One ml of arterial plasma from these hypertensive animals was injected into the circulation of normal rats. Pressor activity was readily demonstrated in the first 7 days after clamping. Govaerts and Verniory (1950) produced acute one kidney Goldblatt hypertension in 6 dogs by

removing the right kidney and constricting the left renal artery. The venacaval blood downstream to the renal veins was sampled when the hypertension had existed for 3 to 100 days. Plasma was assayed for vasoconstrictor activity. Utilizing hind limb perfusion in bilaterally nephrectomized dogs, Govaerts and Verniory could not demonstrate vasoconstrictor activity when they assayed blood from dogs which had been hypertensive for more than 35 days. On the other hand, blood from dogs having hypertension from 3 to 31 days readily evoked vasoconstrictor activity.

Haddy et al. (1965) produced unilateral constriction in the renal artery in anesthetized dogs. The renal venous outflow from the constricted kidney was perfused through isolated vascular bed of the forelimb of the animal to determine whether a vasoconstrictor agent is released from the kidney into renal venous blood during a 10 minute period of renal artery constriction. The brachial arterial perfusion pressure rose above control value indicating release of vasoconstrictor substance by the constricted kidney. As the systemic arterial pressure did not change, the authors concluded that constrictor substance was released in quantities insufficient to raise the general blood pressure.

Koletsky et al. (1963) produced renal hypertension in rats by bilateral constriction of the renal arteries, external compression by figure of eight ligature or by ligation of a branch of each main renal artery. Blood from the hypertensive rats was tested for the presence of vasoconstrictor material by injecting 1 cc into the circulation of normal rats. In contrast to injection of blood from control normotensive rats,

the injections of blood from hypertensive rats produced significant pressor response in the recipients. Positive responses, however, were obtained only during the first two weeks of renal hypertension. After the second week assays were uniformly negative. This difference in vasopressor response prompted the authors to conclude that during the acute stage, i.e., up to the first two weeks or so of onset of renal hypertension, a vasopressor substance is present in the circulation of the hypertensive animal. In contrast to the acute stage, no vasoactive material could be demonstrated in the blood after the second week of hypertension. These data of several investigators are thus consistent with the view that constriction of the renal artery brings about release of vasoconstrictor substance(s), presumably the renin-angiotensin system. The results of these procedures also indicate that the mechanism of acute experimental renal hypertension may differ from that of the chronic stages. Nevertheless, it was possible that in the chronic stages of renal hypertension renin levels were too low to be detected by this rather crude bioassay technique.

2) Biochemical methods of generation of angiotensin in blood

To increase the sensitivity of the bioassay technique Leloir et al. (1940) developed biochemical techniques to increase formation and recovery of angiotensin. They incubated renin-containing blood with renin substrate in the absence of angiotensinases. The angiotensin thus generated could then be estimated either by bioassay or by radioimmunoassay technique.

a) Bioassay method for determination of generated angiotensin

The amount of angiotensin thus formed was then tested for its vasoconstrictor action on a perfused isolated limb or organ. Scornik and Paladini (1961) using similar but improved techniques of generating angiotensin which yielded 60 to 65% recovery of angiotensin, bioanalyzed the blood of 17 dogs during the first week of one kidney Goldblatt hypertension. This blood yielded an average value of 21 rat units of angiotensin/L. In 8 dogs with hypertension from 4½ to 40 months duration, assay of blood yielded an average of 4.7 rat units of angiotensin/L, equivalent to values in their normal control dogs.

Peart et al. (1961) produced experimental one kidney Goldblatt hypertension in rabbits by applying a silver clip to the left renal artery and removing the right kidney. Renin levels in renal vein blood samples were estimated by bioassay. The blood was incubated with renin substrate in the absence of angiotensinases and then bioassayed for its pressor activity in anesthetized rats. These investigators were unable to demonstrate renin release into the renal vein in any of the rabbits which they studied from 3 to 259 days after the onset of renal hypertension. Thus these authors could not demonstrate the presence of vasoconstrictor substance even during the acute stage of hypertension perhaps because by the time (3rd day) the authors started measuring renin, the renin levels had already returned to normal. It has been reported by Koletsky et al. (1971) that in this one kidney type of hypertension, constriction of the sole remaining kidney is followed by elevation of plasma renin level. However, increased plasma renin level is not

maintained and it decreases close to normal as early as one day. Ebihara et al. (1968) determined pressor activity and renin content of renal venous blood daily for 10 days following the constriction of one renal artery of 10 dogs, with the other kidney intact. They correlated these variables with the observed rise in arterial blood pressure. Their results showed that there was a gradual rise in blood pressure which appeared on the day following constriction of the renal artery, reached its maximum on the third day and declined to the preoperative level by the 10th day. In these animals the pressor activity of renal venous effluent of the constricted kidney was demonstrably elevated in 2 hours following constriction and attained maximal pressor activity within 24 hours and then gradually declined to its preoperative level by the 10th day. In two dogs the authors collected venous effluent from both renal veins. The pressor activity of the blood from the opposite untouched kidney remained at its preoperative level throughout the post-operative period. The pressor substance in the renal effluent of the constricted kidney was found to be heat stable, ultrafiltrable, and destroyed by proteolytic enzymes. Its molecular size was found to be of the same order of magnitude as angiotensin. The authors therefore concluded that the pressor substance released in the effluent of a kidney with renal artery constriction was probably angiotensin formed by the action of renin on its substrate. Harris and Ayers (1972) studied renin activity during acute and chronic renal hypertension produced by an externally controlled renal arterial snare in conscious uninephrectomized dogs (one kidney Goldblatt model). Plasma renin activity was measured

by the rat bioassay method and results were expressed as nanograms of angiotensin II generated per 100 ml of plasma per hour incubation time. With tightening of the snare, the plasma renin activity rose immediately, began to drop on the first day after constriction, and was essentially normal by the second day.

It appears that most, but not all, results obtained by these measures of plasma renin activity (PRA) are more or less in agreement with those obtained by direct bioassay of blood from renal hypertensive animals; acute renal hypertension of both one and two kidney type appears to be renin dependent, at least in part. Measurement of PRA by this technique (bioassay of the generated angiotensin) has greater sensitivity than the direct methods but suffers from many of the same disadvantages; namely, its nonspecificity and lack of absolute units of expression. The possible effects of other activators and inhibitors are ignored. Moreover, these methods require large quantities of blood.

b) Radioimmunoassay method of determination of angiotensin generated

A valuable step forward in this respect has been made by Habber et al. (1965) and Boyd et al. (1967) with their development of a radioimmunoassay technique for measuring angiotensin. Employing radioimmunoassay techniques for the estimation of plasma renin activity Cowley and Guyton (1972) have studied the quantitative relationships between renal perfusion pressure, renin secretion, arterial renin activity and systemic arterial blood pressure in dogs in which the cardiovascular control loops of the central nervous system were eliminated by spinal cord destruction and decapitation. They produced stepwise decreases in renal perfusion

pressures, at four different levels (100, 85-80, 65-70 and 55 to 50 mm Hg). Blood samples for renin analysis were withdrawn after each step decrease in renal perfusion pressure had been maintained constant for 20 minutes. Renin activity was measured by radioimmunoassay of angiotensin I. Each decrease of 15 mm Hg in renal perfusion pressure elevated the net secretion of renin nearly 20 ng min^{-1} g^{-1} kidney and the arterial renin activity nearly 8 ng ml^{-1} $hour^{-1}$. These increments in renin secretion and arterial renin activity decreased when renal perfusion pressure was below 50 mm Hg. Correlation of renal perfusion pressure and systemic blood pressure response showed that the arterial blood pressure continued to rise until renal pressure was lowered below 50-55 mm Hg. These experiments positively indicate that the kidney responds to decreased perfusion pressure by rapidly releasing renin.

Gutmann et al. (1973) studied the rate of renin release and its effect on systemic blood pressure in trained unanesthetized dogs with an externally adjustable cuff on the renal artery. Their results show that in the basal state renal vein renin activity measured by radioimmunoassay technique was 0.9 ± 1.1 ng/ml^{-1}-hr^{-1} (2 SD) in 3 control normal dogs. The renal vein renin activity rose in the first minute after constriction of the renal artery and continued to rise during the first 4 minutes. This effect was seen in uninephrectomized dogs as well as in normal dogs. The rate of rise was greater in the dog in which the renal arterial pressure had been reduced to a lower level compared to other animals. By the 5th minute the authors found that systemic pressure was definitely elevated. Systemic renin activity also had

doubled from 1.0 to 2.0 ng/ml/hour in 5 minutes. Renal venous and systemic renin activity continued to rise for the 90 minute period of constriction although renal artery pressure was maintained at a predetermined level of 50 mm Hg by increasing the pressure in the cuff as systemic pressure rose. In contrast when renal arterial pressure was regulated at a higher level (75 mm Hg), renal venous renin activity reached a maximum value by 5 minutes and remained relatively constant for the ensuing hour. In general, at higher perfusion pressures, renal venous renin activity plateaued soon after constriction. A rapid decline in peripheral and renal venous renin activity, as well as in arteriovenous renin difference after the release of the cuff, was demonstrated. Thus the results of these experiments indicate that secretion of renin is increased instantaneously in two kidney as well as in uninephrectomized dogs by reduction of renal artery pressure by as little as 10-15 mm Hg below systemic pressure. This reduction produced no changes in renal blood flow as measured by ^{85}Kr--washout-technique. These observations agree with those reported by Skinner and McCubbin (1964) in the anesthetized dog, also offering no evidence that renal ischemia is responsible for renin secretion. Thus use of this very sensitive radioimmunoassay method for the estimation of plasma renin activity, definitely shows that when the kidney perfusion pressure is lowered to a critical level the kidney immediately responds by increased release of renin in both one kidney and two kidney models. Though this radioimmunoassay method has the great advantages of being highly specific and sensitive, the various preparatory chemical manipulations may effect the assay

system. Furthermore, this assay system cannot assess the role of other vasoactive agents, both vasoconstrictors and vasodilators, that may be released by the kidney at the time its perfusion pressure is lowered.

c. Return of blood pressure to normal by the use of inhibitors or blockers of the renin-angiotensin system components

For many years it has been realized that the availability of effective blocking agents for renin or for angiotensin II might help greatly to assess the degree of involvement of the renin-angiotensin system in experimental renal hypertension. During the past few years, an increasing number of blocking agents for the renin-angiotensin system have been developed. These agents act at one of the three sites: (1) they block the action of renin in plasma, (2) they prevent converting enzyme from forming angiotensin II from angiotensin I, or (3) they block the receptor sites for angiotensin II.

1) Renin antibodies

Renin antibodies have been used by several investigators to block the action of renin in plasma in investigations of the role of the renin-angiotensin system in experimental hypertension. Walkerlin (1958) produded renin antibodies by injecting hog renal extracts containing renin into dogs with chronic renal hypertension due to bilateral renal artery constriction. As the anti-renin titers increased in these animals, the blood pressure gradually fell to normotensive levels. As the anti-renin injections were stopped, the blood pressure rose presumably as the anti-renin titer decreased. These investigators obtained a good correlation between the blood anti-renin titer and the

anti-hypertensive effect. Control tissue extracts produced no anti-hypertensive effect. Therefore, the anti-hypertensive action associated with the production of anti-renin was not a foreign protein effect or nonspecific toxic action. Deodhar et al. (1964) immunized rats, rabbits and dogs with acetylated rat, rabbit and dog renin, respectively, resulting in each case in the development of anti-renin to the homologous, untreated as well as to the acetylated renin. In order to test the efficiency of this anti-renin in vivo, a chronic renal hypertensive dog was immunized with acetylated dog renin. This animal had hypertension of more than 6 years duration, produced by constriction of both main renal arteries. Subcutaneous acetylated dog renin was given repeatedly resulting in progressive rise in anti-renin titer of the serum. The blood pressure fell progressively, until it reached a normotensive level in about 10 weeks. After immunization was discontinued, the blood pressure began to rise, gradually reached the previous hypertensive level in about 10 weeks, at which point the anti-renin titer was undetectable. These experiments with anti-renin antibodies suggest that renin is involved in the development and maintenance of elevated blood pressure during not only the acute but also in the chronic stages of renal hypertension of both the one kidney and the two kidney models. However, these early studies have been repeatedly criticized, because the renin preparations used were crude and could have contained other agents.

2) Converting enzyme inhibitor

The converting enzyme acts on angiotensin I and splits off a dipeptide histdyl-leucine to form active vasopressor agent angiotensin II.

Green et al. (1972) showed that a group of peptides from Bothrops jararaca venom inhibits the converting enzyme and thus block the conversion of angiotensin I to angiotensin II. Engel et al. (1972) have synthesized two polypeptides, one a pentapeptide and another a nonapeptide both of which are converting enzyme inhibitors. Of the two peptides, nonapeptide is the longer acting blocking agent of the converting enzyme. Many investigators have used these peptides to assess the role of the renin angiotensin system in renal hypertension.

Krieger et al. (1971) using pentapeptide as converting enzyme blocking agent have shown a reduction in arterial blood pressure of rats with two kidney Goldblatt hypertension (7-19 days duration). But this pentapeptide had no effect on blood pressure of rats with one kidney Goldblatt hypertension both acute and chronic. These results would indicate that two kidney Goldblatt hypertension is renin-angiotensin dependent whereas elevated arterial blood pressure in one kidney Goldblatt, both acute and chronic hypertension is maintained by some other mechanism. However, Miller et al. (1972) have reported that acute constriction of the renal artery and controlled reduction of renal artery pressure is followed by a prompt increase in systemic renin activity and a concomitant rise in blood pressure in conscious unilaterally nephrectomized dogs. However, the elevation of blood pressure induced by renal artery constriction could be prevented by prior treatment with the nonapeptide converting enzyme inhibitor.

The results of Miller et al. are therefore not in agreement with those of Krieger et al. regarding the role of renin angiotensin system

in the acute stage of one kidney Goldblatt hypertension. Results of Miller et al. indicate that renin-angiotensin is involved in the development of hypertension in the acute stage of one kidney Goldblatt hypertension.

3) Angiotensin II antibodies

The role of angiotensin in the pathogenesis of RV hypertension has also been investigated by use of conjugates of angiotensin capable of producing angiotensin antibodies which are biologically effective.

Christlieb et al. (1969) produced experimental renal hypertension in rats by clipping one renal artery or by clipping one renal artery with contralateral nephrectomy. One hundred fifty-three to 223 days after the onset of hypertension, twenty rats with both types of hypertension received immunizing injections of angiotensin chemically linked to a carrier protein. Eleven of these rats developed significant antibody titers. Ten of these rats showed a significant blood pressure reduction. None of the 9 renal hypertensive rats without demonstrable antibodies, and none of the 5 renal hypertensive rats with sham immunization, showed a fall in blood pressure. The renal hypertensive rats were all refractory to exogenously injected angiotensin after immunization. These results, therefore, suggest that angiotensin plays an important role in both one kidney and two kidney types of hypertension. MacDonald et al. (1970) also studied the role of angiotensin in renal hypertension in rabbits by this immunological approach. These investigators produced one kidney Goldblatt hypertension in rabbits. These animals were immunized against valyl 5-angiotensin II amide absorbed

onto carbon. Despite immunization three rabbits developed hypertension. When hypertension was established, in vivo studies showed complete absence of a pressor response to intravenous injection of up to 800-1600 units of rabbit renin and 0.8 μg angiotensin II amide, doses which caused marked elevation of blood pressure in normal and hypertensive non-immune rabbits. On the basis of these results the authors concluded that it is extremely difficult to postulate any major role for circulating renin or angiotensin in either the initiation or maintenance of one-kidney renovascular hypertension in rabbits. Thus the results of McDonald et al. were apparently not in agreement with those of Christlieb and his co-workers. Eide (1972) also has studied renovascular hypertension in rats immunized against angiotensin II. In 29 highly immunized and 33 control rats, one renal artery was partially constricted and the other kidney left intact. Both groups developed hypertension. Intravenous doses of large amounts of renin and angiotensin had no effect on the blood pressure of the immunized rats. These data provided evidence against angiotensin II playing a role in the genesis of 2-kidney renovascular hypertension in rats. Thus several studies have shown that antibodies to angiotensin II are not successful in interfering with the development of even 2-kidney renovascular hypertension, suggesting that angiotensin is not involved in this type of hypertension. Recently, however, it has been reported by Walker et al. (1972) that adequate neutralization of angiotensin II by presence of circulating antibodies does not exclude a role for angiotensin II in the pathogenesis of renal hypertension. These authors have shown that the level of free (unbound)

angiotensin II is adequate for angiotensin II to participate in physiological processes despite high titers of antibodies for the octapeptide. These investigators also speculate that angiotensin II may have more affinity for the vascular receptor sites than it has for the antibodies.

4) Angiotensin II analogues

The use of antibodies against renin and angiotensin have thus failed to completely clarify the role of the renin-angiotensin system in the pathogenesis of renal hypertension. The most extensive attempts to block the renin angiotensin, and thereby, to drop arterial blood pressure in experimental hypertension have been made with synthetic angiotensin II analogues which act as specific antagonists. Bumpus et al. (1973) prepared an angiotensin II analog by substituting the amino acid alanine at position 8 of the angiotensin molecule. Khairallah et al. (1970) showed that this 8 substituted angiotensin II analogue (Ala^8) inhibited the action of angiotensin on guinea pig ileum. Turker et al. (1972) have shown that the inhibition of angiotensin II by its 8-alanine analog is specific and competitive on the rabbit aorta. Not only was the response to angiotensin II inhibited in the presence of the 8-alanine analog, but the aortic strip contracted by angiotensin II was relaxed by this competitive inhibitor. Pals and co-workers (1971) have reported that infusions of 1-sar-8 Ala-angiotensin II analogue reduce arterial blood pressure in conscious rats in the acute phase (less than two weeks) of unilateral renal hypertension (produced by constriction of aorta between the origins of two renal arteries) but are ineffective during the chronic phase of such hypertension (2-5 weeks). About the same time, Brunner et al. (1971) studied one-kidney and 2 kidney Goldblatt

hypertension of 6 weeks duration in rats. They used the same analogue of angiotensin II as did Pals and his associates. The infusion of this analogue in their study reduced arterial blood pressure in the two kidney but not in one kidney renal hypertensive rats. Bing and Neilsen (1973) have observed that angiotensin II analogue reduced arterial pressure in chronic two kidney Goldblatt hypertensive rats. More recently, Bumpus et al. (1973) using 8-Ile-angiotensin II analogue have found that this specific competitive antagonist reduced arterial blood pressure in rats with acute (4-6 weeks duration) one-kidney or two-kidney Goldblatt hypertension, but in chronic one-kidney Goldblatt hypertension of more than 30 weeks duration 8-Ile-angiotensin II analogue failed to reduce arterial blood pressure. Similarly the infusion of 1-sar-8 Ile-angiotensin II by these authors in conscious dogs with acute one kidney Goldblatt type renal hypertension of 6 days duration, resulted in a fall of arterial blood pressure. In contrast, when 1-sar-8 Ile-angiotensin II was given to conscious dogs with chronic one kidney type perinephritic hypertension or to dogs with one kidney type Goldblatt hypertension of 10 to 15 days duration, arterial blood pressure failed to fall. Thus the results of various investigators seem to be consistent in that animals with acute and chronic two kidney Goldblatt type hypertension as well as animals with acute one kidney Goldblatt hypertension respond to competitive antagonist with a fall in arterial pressure indicating participation of angiotensin II as the cause of elevated blood pressure in this type of renal hypertension. On the other hand, more work is needed to clarify the role of the renin-angiotensin system

in chronic one kidney type hypertension (Goldblatt and perinephritic) which does not respond to the angiotensin II analogue blocking action.

Recently, Gravas et al. (1973) have re-investigated this problem. They have reported decreases in arterial blood pressure in response to infusion of 1-sar-8-Ala angiotensin II in chronic one kidney Goldblatt hypertensive (4 weeks duration) rats, only when these animals were sodium depleted with concurrent increase in plasma renin activity. The authors, therefore, concluded that the chronic one kidney type hypertension is primarily dependent on the body fluid volume rather than on the renin-angiotensin system.

Summarizing the results of these several investigators there is much evidence incriminating the renin-angiotensin system in the development of two kidney Goldblatt hypertension. For example: 1) removal of clipped kidney during the early stages of two kidney Goldblatt hypertension rapidly returns blood pressure to normal levels, arguing for the participation of a rapidly acting vasopressor agent, 2) anti-renin antibodies or angiotensin II analogues may prevent or alleviate acute and chronic two kidney Goldblatt hypertension. These agents have also been found effective during acute stages of one kidney type Goldblatt and perinephritic hypertension thus indicating the involvement of the renin-angiotensin system in this type of hypertension also. Most of the present evidence on the other hand does not seem to implicate the renin-angiotensin system in the maintenance of elevated blood pressure in the chronic stage of one kidney Goldblatt and perinephritic hypertension. Haddy (1974) has recently reviewed the role of renin-angiotensin in this

type of renal hypertension. Haddy concludes that the renin-angiotensin system need not be considered in the maintenance of the chronic stages of one kidney Goldblatt and perinephritic hypertension on the basis of the following evidence: 1) juxtaglomerular granulation and renin content of the sole remaining kidney in this type of hypertension are normal, 2) renin secretion, renin and angiotensin levels in blood are normal, 3) elevated blood pressure is unaffected by immunization against angiotensin II or administration of angiotensin II analogues. Bianchi et al. (1970) have sequentially measured renin activity, cardiac output and fluid volume following renal artery constriction in dogs with one kidney Goldblatt hypertension. They observed that following renal artery constriction there was a temporary increase in plasma renin activity which was followed by a period of increased cardiac output and fluid volume expansion. At the end of their experimental period, the blood volume, extracellular fluid volume and cardiac output were close to normal. The peripheral resistance first increased markedly with increased plasma renin concentration and then decreased towards normal. A secondary increase in peripheral resistance was observed by the authors subsequent to increase in cardiac output. This time the peripheral resistance remained elevated even though cardiac output later returned toward normal. Ledingham and Cohen (1964) also observed an early increase in cardiac output and extracellular fluid volume in one kidney Goldblatt rats. These changes resemble Bianchi's results in dogs. A subsequent study by Ledingham et al. (1967) showed an early increase in cardiac output without a return to normal values.

Further evidence of fluid volume expansion in chronic one kidney Goldblatt hypertension has been obtained from measurements of fluid volume and sodium balance studies. Swales et al. (1972) found a positive sodium balance of 2 mEq in rats with one kidney Goldblatt hypertension of two weeks duration, whereas their two kidney Goldblatt hypertensive rats showed a negative sodium balance during the same time. Conway (1968) observed a positive sodium balance of 123 mEq in dogs with one kidney Goldblatt hypertension of 15 days duration. The data, in total, therefore support the concept that the elevated arterial pressure at the onset of one kidney Goldblatt hypertension is supported by renin-angiotensin mediated vasoconstriction but later an expanded extracellular fluid volume and total body fluid volume, and not increased plasma renin activity, are responsible for the hypertension.

3. Identification of other vasoactive agents

The role of the renin-angiotensin system in Goldblatt hypertension is strongly indicated by the work of the several investigators cited above. However, these experiments do not rule out the possibility that other vasoactive agents of renal origin may play a role in the development of experimental renal hypertension. This possibility has been investigated by Grollman and Krishinamurty (1971) who have recently postulated that the pressor agent produced by the kidney following renal artery constriction is neither angiotensin nor renin, but a hitherto undescribed pressor agent which they named "nephrotensin". These investigators constricted both renal arteries of normotensive mongrel dogs,

and collected blood from the femoral vein at 8-12 hour intervals during the first post-operative day. They assayed this blood for its pressor activity. Two to four days post-operatively, when the blood pressure of their experimental animals had reached levels of 180 to 200 mm Hg the plasma exhibited a pressor activity equivalent to that of at least 5 μg of synthetic angiotensin II equivalents per milliliter. In order to establish the identity of the pressor agent the authors investigated its chemical properties, and its action on blood pressure, isolated organs, and various smooth muscle preparations. The pressor response to the injection of this pressor agent in the anesthetized ganglion blocked rat resembled that of angiotensin II, but unlike the latter, it was not dialyzable through a cellophane, visking or collodian membrane, nor was it absorbed by Dowex 50W-X2 at pH 5.5, Fuller's earth or activated magnesium silicate. The authors also showed that the rat's ascending colon did not respond to this pressor agent, even though it readily contracted on the addition of angiotensin I or II to the bath fluid, angiotensin I being only about one-tenth as active as angiotensin II in inducing a contractile response of the ascending colon. The addition of α-amylase which converts angiotensin I to II rendered the contractile response to the two compounds equal. When the authors added α-amylase to their plasma they observed no change in the contraction of the ascending colon, also suggesting that the pressor agent was not angiotensin I. The authors further showed that this described agent differed from renin in many respects. The pressor agent is precipitated by $(NH_4)_2SO_4$ at a concentration of 40-70% in contrast to renin which is precipitated at

lower concentrations. Renin is eluted from DEAE at a pH of 3.0; whereas, their pressor agent was eluted at pH 1.0. The pressor agent is stable at 80°C for 10 minutes in contrast to renin which is destroyed under the same conditions. The pressor agent, when injected repeatedly into anesthetized rats, did not evoke the phenomenon of tachyphylaxis as did renin. The authors found that the pressor activity of this agent, unlike renin, was not influenced by prior nephrectomy. These results, therefore, suggested to the investigators that this renal pressor substance is not renin, angiotensin I or angiotensin II. Schweikert et al. (1972), on the other hand, believe that the renal pressor substance described by Grollman and Krishinamurty is related to angiotensin I. In order to characterize this factor, these investigators carried out simultaneous bioassay and radioimmunoassay for angiotensin I on renal vein plasma and plasma extracts from patients with renovascular hypertension. The renal vein plasma of the stenotic kidney exhibited pressor activity when it was injected into suitably prepared rats. There was a positive correlation of pressor activity with immunoassayable angiotensin I level. To further test their hypothesis that the renal pressor substance may be angiotensin I in some form, these investigators incubated plasma containing renal pressor activity with specific angiotensin I antiserum. In all specimens the original pressor activity was completely neutralized after incubation. Similar results were obtained when normal plasma to which synthetic angiotensin I had been added was assayed. Since the anti serum does not cross react significantly with any other known material, these data suggested to the authors that the renal pressor

substance is immunologically similar to angiotensin I. The authors also incubated plasma containing the renal pressor substances with converting enzyme. The renal pressor substance was altered by converting enzyme to a substance which retained the pressor activity but lacked the immunoreactivity of angiotensin I; presumably angiotensin I had been converted to angiotensin II. Furthermore these investigators found that the pressor material in plasma was relatively non-dialyzable and was not well absorbed on Dowex 50W-X2 ion exchange resin; angiotensin I also behaved similarly when added to plasma. The authors, therefore, concluded that the "renal pressor substance" of Grollman is in fact angiotensin I bound to globulin. The experiments of Schweikert do not explain why addition of α-amylase which converts angiotensin I to angiotensin II failed to activate the renal pressor agent isolated by Grollman and Krishinamurty. Moreover, these experiments of Grollman et al. and Schweikert et al. were carried out in two different species under entirely different experimental conditions. Thus the possibility that renal artery constriction initiates renal release of pressor agents other than those of the renin-angiotensin system has not been completely ruled out. Shorr et al. (1951) described a vasopressor material of renal origin which he called vasoexcitator material (VEM). He postulated the presence of oppositely acting factors, vasoexcitator material (VEM) and vasodepressor material (VDM) which originate in specific organs under specific metabolic conditions. Thus the VDM which has been identified as ferritin was found to originate chiefly in the liver. The kidney was believed to be the sole source of VEM. According to these investigators

the application of a Goldblatt clamp to constrict the renal artery results in the derangement of kidney metabolism with the consequent release of VEM by the constricted kidney and the elevation of the blood pressure. The authors assayed VEM in the peripheral blood using meso-appendix as the assay organ. VEM, normally undetectable in the blood, promptly appeared after application of the clamp and persisted during the acute stage of hypertension. At about the time the blood pressure attained chronic hypertensive levels, the VEM activity waned and eventually disappeared. On fractionation, blood samples showed both VEM and VDM activity. These authors, therefore, concluded that in the acute stage of renal hypertension a vasopressor material of renal origin in the form of VEM appears in the blood. Later VDM of hepatic origin also appears, initially in low concentration, thereafter, in progressively increasing amounts until, with transition to the chronic stage, VEM and VDM concentrations reach physiologically equivalent ratio as measured by the rat mesoappendix test. These authors did not explain why the blood pressure did not return to normal when equilibrium between VEM and VDM was reached. The chemical nature of VEM has as yet not been determined. This postulate of Shorr needs further work before it finds any acceptance.

In view of the known ability of the kidney to synthesize and secrete several vasoactive agents, the possibility exists that besides renin the kidney elaborates some other vasopressor substance when renal hemodynamics are altered. Most research work to date has centered around the renin-angiotensin system and its role in renal hypertension. The role of other renal vasopressor agents in renal hypertension, if any, has not been fully investigated.

B. Renin-angiotensin System

The role of the renin-angiotensin system in renal hypertension has been extensively studied. It took several years of intensive investigation to understand various biophysiological aspects of this system.

1. Discovery of renin

In the latter part of the nineteenth century, the ideas of Brown-Sequard on internal secretion of chemical substances into the blood-stream came to have considerable influence on thought and work in the area of Physiology. Tigerstedt and Bergman (1898) decided to investigate the possible endocrine function of the kidney. They found that crude saline extracts of rabbit kidney injected into anesthetized rabbits produced a pressor response. They named this pressor substance "renin". Tigerstedt and Bergman's initial observation was disputed for many years. Pearce (1909) and Hartwich (1932) were unable to confirm it, perhaps because these authors used alcohol as extractant, resulting in denaturation of renin. On the other hand, Vincent and Sheen (1903) were also able to demonstrate the pressor effect of renal extracts. The production of sustained hypertension in dogs by renal artery constriction by Goldblatt et al. (1934) rekindled interest in the role of renin in the physiological control of blood pressure in health and disease.

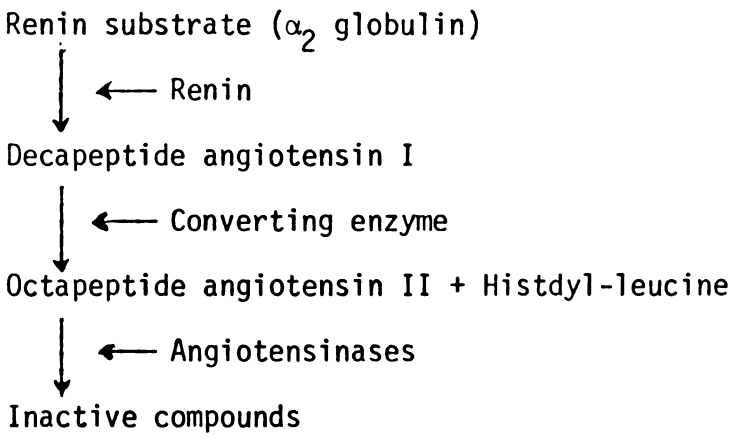
2. Chemistry of renin, renin substrate, converting enzyme, angiotensin I and angiotensin II

Though renin has as yet not been isolated in pure form, the chemical characterization of renin revealed that it is protein in nature and

behaves like a proteolytic enzyme. The first clue that renin is an enzyme and by itself a non-pressor substance came from observations of Kohlstaedt et al. (1938). They showed that purified renin produced marked pressor activity when tested in the intact animal, but in the dog tail preparation perfused with Ringer solution only weak vasoconstriction, finally disappearing was evoked. It was only when plasma was added to the perfusing fluid that full activity was restored. Thus the authors concluded that renin acts as an enzyme on some substrate in plasma to form a pressor substance they called "angiotonin". Braun-Menendez et al. (1940) simultaneously came to the same conclusion and named the pressor substance "hypertensin". Subsequently, the two groups adopted a compromise name "angiotensin". Hessel (1938) defined a unit of renin as that amount of enzyme required to raise arterial blood pressure by 30 mm Hg when injected intravenously into a 10 kg dog. Green and Bumpus (1954) showed that the plasma factor on which renin acts is a non pressor α_2 globulin (angiotensinogen or hypertensinogen), formed in the liver. Knowledge of the structure of angiotensin was hampered by the presence in renin and renin substrate preparations of angiotensinases. This latter term is a generic one for enzymes capable of destroying angiotensin. Peart (1956) isolated angiotensin from ox serum and found it to be a decapeptide. Helmer (1955) working with an isolated aortic strip, had observed a factor in plasma that enhanced the activity of angiotensin and suggested that it might be the angiotensin converting enzyme. Earlier, Skeggs et al. (1954) had discovered that there are two forms of angiotensin that could be separated by

counter current distribution. One of these, the decapeptide, angiotensin I, is the initial product of the action of renin on renin substrate. The second, angiotensin II, an octapeptide is formed by the removal of histdyl-leucine from angiotensin I by a peptidase in plasma called converting enzyme. Sequence analysis studies showed that the structure of angiotensin II (horse) to be asp-arg-val-tyr-ileu-his-pro-phe. The possibility that angiotensin II is the pressor substance and not angiotensin I was tested by Skeggs et al. (1956) in the isolated rat kidney perfused with physiological salt solution. It was found that angiotensin II was vasoconstrictor, while angiotensin I, in the absence of plasma, was not. Helmer (1957) using purified angiotensin I and II and converting enzyme, obtained similar results. On the other hand, Gross and Turrian (1960) showed that angiotensin I stimulates the smooth muscle in preparations of isolated guinea pig ileum. Bumpus et al. (1960) proposed an explanation for these contradictions when they discovered that homogenates of heart, liver, aorta and ileum contained an enzyme that is able to convert angiotensin I to II. They suggested that the enzyme was either intracellular or was adsorbed on the surfaces of tissues and, therefore, could convert angiotensin I in the absence of plasma. Ng and Vane (1967) have since then shown that almost all the angiotensin I in the circulating blood was converted to angiotensin II in one passage through the lungs. These observations have been confirmed by Biron et al. (1968). These data of Ng, Vane and Biron indicate the presence of high concentrations of converting enzyme in pulmonary tissues. Bakhle (1968) has isolated and partially purified converting enzyme from

the lungs. The lung enzyme resembled plasma converting enzyme in that both were inhibited by ethylenediamine tetracetic acid (EDTA), and both required chloride ions. Thus to summarize the steps involved in the formation of angiotensin II:



To summarize further, renin released by the manipulated kidney in renal hypertension is by itself a non-pressor substance, but through its action on renin substrate produces angiotensin I which in turn is acted upon by converting enzyme to form the potent pressor substance angiotensin II.

3. Anatomical location of renal renin and its level under different experimental conditions

On a purely morphological basis Goormaghtigh (1939) concluded that the granulated juxtaglomerular (JG) cells elaborate renin. Bing et al. (1958) found renin only in the cortical areas of the kidney containing glomeruli. Renin content decreased with increasing distance from the kidney surface. Cook and Pickering (1959) showed that nearly all the renin is in the glomerular fraction, the tubular fraction being devoid of it. Furthermore, glomeruli with attached fragments of

juxtaglomerular complex contained more renin than those without such fragments. Clear evidence that renin is present in juxtaglomerular apparatus came from two independent laboratories. Cook and Pickering (1962) and Cook (1967) bisected a few hundred magnetically isolated glomeruli so that one-half always included the vascular pole region of each glomerulus. They found virtually all the renin in these halves. Microdissection work of Faarup (1967, 1968) showed that, of the renin in the whole juxtaglomerular apparatus, 90% or more was contained in the afferent arteriole close to the glomerulus where many JG cells were clustered. Up to 5% was found in macula densa cells, 3% in Goormaghtigh cells and small amounts further down the afferent arteriole. Hartford et al. (1964) using fluorescent antibody techniques did not find any renin in the macula densa. These experiments thus clearly demonstrate that JG cells contain renin; however, the presence of renin in macula densa cells is still uncertain.

The amount of renin extractable from the kidney and the juxtaglomerular index show a close relationship. Juxtaglomerular index is a semiquantitative method of determination of the renin content of the kidney first described by Hartfort and Hartfort (1953). In this method, all the glomeruli of the entire cortical area of one section of the kidney are counted along with granular cells. The granulations are estimated and recorded on 1+ to 4+ scale, and these totals multiplied by 1, 2, 3, and 4 respectively. The weighted totals are then expressed in terms of 100 glomeruli as an index of granulation. Though this method of measuring renin content is useful it must be remembered that such

methods are highly personal and could be misleading; whereas, determination of renin content of the kidney by extraction procedures is more precise. A great many observations indicate that the amount of renin extractable from the kidney varies under different experimental conditions. These changes, therefore, can provide useful information about the activity of the renin angiotensin system. Tobian et al. (1959) showed that if one renal artery is clamped and the other kidney left untouched, the renin content of the ischemic kidney increases in a few days, reaches a maximum amounting to 3 to 4 times the normal value within a week, and then remains elevated for periods up to one year; in the opposite untouched kidney the renin content decreases. In contrast, in one kidney Goldblatt hypertension, Regoli et al. (1962) showed that the renal renin content of the sole constricted kidney does not change. Furthermore, if a contralateral, unmanipulated kidney is removed several weeks after a clip has been placed on the artery of the other kidney, the increased renin concentrations in the constricted kidney slowly falls to normal values. Tobian et al. (1959) also showed that JG granulation is decreased by DOCA plus salt or overloading with salt. Hartford et al. (1959) found increased JG granulation accompanying a sodium deficient diet. Although determination of the renin content of the kidney either by juxtaglomerular index or by extraction procedures does not necessarily permit any conclusion as to the amount of renin released from the kidney, a direct correlation between renin content of kidneys and its concentration in plasma has been indicated by studies with grafted kidneys. Omae et al. (1961) have shown that kidneys from DCA treated rats which contain

little or no renin do not cause any pressor response when they are grafted onto nephrectomized recipient rats. These authors also showed that in rats with 2 kidney Goldblatt hypertension, the clipped kidney used as donor elicits a much greater pressor response than does the untouched contralateral one. Hatt et al. (1966) by their electron microscopic studies also have shown that high renin content of the kidney is related to signs of increased secretory activity; whereas, morphological evidence of diminished secretory activity is found in the presence of reduced renin content. In contrast to these long term experiments, acute experimental procedures; such as, unilateral constriction of renal artery or hemorrhage do not cause immediate changes in renal renin content. Zeigler and Gross (1964) showed that acute hemorrhage in rats caused release of renin by the kidneys, indicated by a 4 fold increase in the plasma concentration of a renin-like substance in a few minutes. This effect was not demonstrable if the kidneys were removed 30 minutes before bleeding. In contrast to this increase in circulating renin, acute hemorrhage did not affect the renin content of the kidney per se. Similarly, Gross et al. (1965) showed that repeated transfusions in rats resulted in marked decrease in normal concentration of renin-like activity in blood. The content of the kidney was not affected by over-transfusion. These experiments, therefore, indicate that significant variations in the rate of renin release can take place without affecting the renin content of the kidneys.

In general, variations in renin content of the kidney following experimental procedures occur slowly, over the course of several days or even weeks.

4. Measurement of renin and angiotensin levels in blood

The measurement of plasma renin levels remains a fundamental problem in the study of the renin-angiotensin system. To elucidate the role of the renin-angiotensin system in physiological and pathological states there is a need for a precise, specific and accurate assay. Renin was first assayed by direct bioassay methods, the determination of the pressor effect of an intravenous injection of renin-containing solution into an animal. Haas et al. (1966) believe that if enough renin is available the ideal animal for the assay is the dog, as dogs respond to the intravenous injection of renin from all species and bioassay can be carried out on unanesthetized animals. Nairn et al. (1959) used a similar direct method for the assay of renin using trained unanesthetized rabbits. Pritchard et al. (1964) in their experiments used rats to bioassay the renin content of blood.

The direct bioassay method for renin estimation is essentially a crude nonspecific method which does not take into account the presence of various other vasoactive substances in the biological fluid that is being bioassayed. It also does not take into account cross reactions between the blood of two different animals or animals belonging to different species. Besides these disadvantages the direct bioassay method is a relatively insensitive technique for detecting renin in blood. Leloir et al. (1940), therefore, devised a method for the quantitative detection of renin based on its enzymic action. This assay procedure, since improved by various other investigators, is based on the principle that renin may be readily quantitated in terms of amounts of angiotensin

to which it gives rise under controlled conditions. The various factors which influence the formation of angiotensin are controlled. These include the time of incubation, the pH, the concentration of renin substrate and the elimination of angiotensinases. In general, plasma is incubated at 37°C for a varying period of time, under strictly controlled pH (usually either 5.5 or 7.4). The time of incubation must be long to produce sufficient angiotensin for bioassay detection. Plasma angiotensinases may be inhibited by partial acidification (Helmer and Judson (1963); Gunnels et al. (1967); Fasciolo et al. (1964); Boucher et al. (1964); Lever et al. (1964); Brown et al. (1964); Pikens et al. (1965)). Khairallah et al. (1963) have shown that angiotensinase A is Ca^{++} dependent. Therefore, it may be inhibited by chelating agents such as EDTA and BAL, as in the procedure of Skinner (1967), Gould et al. (1966), and Ryan et al. (1968). Diisopropylfluorophosphate inhibits the plasma endopeptidase angiotensinase B (Khairallah and Page, 1967) and is used as an additional inhibitor in the method of Pickens et al. (1965). Boucher et al. (1964) in their method used Dowex 50W resin to bind the angiotensin that is formed and thus protect it from degradation. The end product of the reaction of renin on its substrate is the decapeptide angiotensin I which in vivo is rapidly converted to an active octapeptide angiotensin II by converting enzyme. Skeggs et al. (1956) have shown that converting enzyme is Cl dependent, inhibited by EDTA and is labile at low pH. Thus the peptide formed under incubation conditions where convering enzyme is inhibited is angiotensin I. The angiotensin formed is then measured by a bioassay method. The preparations used for bioassay

include the pentobarbitone anesthetized, bilaterally vagotomized ganglion blocked rat (Peart, 1955). This preparation responds to nanogram amounts of angiotensin, a sensitivity 50-200 times greater than that obtained by direct injection techniques. The disadvantage of this method is that renin concentration in plasma is not measured; what is actually measured is the capacity of a very crude system to generate angiotensin. Thus the values obtained represent only the effective "renin activity". Unknown inhibitors and activators may be present or foreign proteins may alter reaction rates. Also, the extraction procedures may alter plasma renin concentration. These disadvantages of the bioassay method have been overcome by the development of a radioimmunoassay for angiotensin I. Radioimmunoassay has several advantages. It is rapid, simple, highly reproducible, specific and many determinations can be made simultaneously. Thus incubation time and plasma sample volumes have been greatly reduced. Haber et al. (1965), by means of a radioimmunoassay employing gel filtration, was able to assay nano-gram amounts of angiotensin. The technique is as follows: Antibodies to angiotensin produced in rabbits by injection of an angiotensin-poly-L-lysine polymer, are allowed to react with isotope-labeled angiotensin. Upon addition of unlabeled angiotensin to this mixture, some of the labeled angiotensin is displaced from the complex. There is a quantitative relationship between the amount of the unlabeled angiotensin added, and labeled angiotensin released from the complex. Radioactive ^{125}I is absorbed onto charcoal dextran. The amount of radioactive tracer displaced from the antibody is a measure of AI formed from added plasma.

Boyd et al. (1967) developed a similar radioimmunoassay for determining plasma levels of angiotensin II. The authors showed that this assay method can detect 30 pg/ml amounts of angiotensin and is not influenced by angiotensin I.

In these assay procedures when endogenous renin substrate in the plasma is used to generate angiotensin, the value obtained is called the plasma renin activity (PRA). When endogenous substrate is removed and exogenous substrate made from pig, ox or sheep plasma is added, the value obtained is referred to as the plasma renin concentration (PRC).

C. Control of Renin Secretion

There are a number of major known stimuli which influence the release of renin by the kidneys and thus may play a role in renal hypertension. The precise receptors of these stimuli in the kidney are not known but certainly the afferent arteriolar wall and macula densa stand out as the most likely sites.

1. Theories of receptor sites in the kidney for renin release

a. Baroreceptor theory

After the early experiments of Goldblatt and others on the production of renal hypertension it was assumed that the factor stimulating renin release was renal ischemia. However, Huidobro and Braun-Menendez (1942) demonstrated that the arterial pressor activity in the dog was not increased by breathing 7-8% O_2, carbon monoxide or 2-5% CO_2, nor by the production of histotoxic anoxia by cyanide. The lack of effect of low arterial PO_2 on acute renin release in dogs has also been confirmed

by Skinner and McCubbin (1964). Dirvy (1951) demonstrated that no detectable renin release occurred during perfusion of a dog kidney with venous blood at normal perfusion pressure. Spath et al. (1971) have shown that reduction of the oxygen tension did not significantly influence the renin activity of renal venous plasma in dogs either at normal flow or at low flow, thus indicating absence of acute local effect of hypoxemia on renin secretion. Thus these experiments demonstrate that renin release may not be attributable to renal ischemia. Kohlstaedt and Page (1940) demonstrated that in perfused dog kidneys a decrease in pulse pressure without a change in mean pressure was an adequate stimulus for renin release. Their experiments, however, were complicated by changes in urine flow and blood flow. This problem was carefully studied by Skinner et al. (1963). They showed that small reductions in renal perfusion pressure within physiologic ranges, with renal blood flow held constant, caused the kidney to release renin. In six of seven normal anesthetized dogs, the renal venous plasma showed increased pressor activity within 5 minutes after reduction of mean arterial perfusion pressure to 80 to 95 mm Hg from a control level of 100-120 mm Hg. Total renal blood flow and O_2 saturation of renal venous blood was unaffected. Reduction in the pulse pressure alone did not increase the pressor activity of renal venous blood. Further constriction decreased the mean arterial perfusion pressure without changing mean blood flow and pressor activity of venous blood rose further. Therefore, the authors concluded that release of renin from the kidney involves a baroreceptor that is sensitive to changes in mean arterial perfusion pressure. These findings have been confirmed by the work of Gutmann et al. (1973).

These investigators lowered the renal arterial pressure (mean), but not blood flow, to a controlled level by inflating a cuff around the renal artery in trained unanesthetized dogs. In these experiments, renin activity in renal venous and aortic blood was measured by a radioimmunoassay method.

Advocates of the baroreceptor theory propose that the granular cells in the media of the afferent arterioles are sensitive to stretch. Increased mean pressure stretches the granular cells and inhibits renin release; whereas, decreased pressure reduces granular cell distention and enhances renin release.

b. Macula densa theory

The anatomy of the juxtaglomerular apparatus has stimulated speculation concerning the function of the macula densa. Goormaghtigh (1945) suggested a functional relationship between the macula densa and the juxtaglomerular cells. This hypothesis received its support from experimental evidence provided by Vander and Miller (1964). These authors prevented the usual increase in renin secretion which follows aortic constriction above renal arteries by administration of diuretics. They suggested that the diuretics increased the sodium load at the macula densa and, thereby, blocked the increase in renin release; accordingly, they proposed that increased renin release is stimulated by a decrease in sodium load at the macula densa. In contrast to the concept of Vander and Miller, Thurau et al. (1967) supported the idea that it is the increased sodium concentration to which the macula densa responds by releasing renin. In their experiments, retrograde injection of hypertonic sodium solution into the distal tubule was associated with a decrease in

the diameter of the proximal tubule or with proximal tubule collapse; this finding was interpreted to reflect a reduction in glomerular filtration rate. From these observations, Thurau et al. proposed the following sequence of changes: increased renin release secondary to increased sodium concentration in the tubule fluid perfusing the macula densa, local angiotensin II formation, afferent arteriolar constriction and decreased glomerular filtration rate with resultant proximal tubule collapse. Support for the idea that increased sodium concentration rather than decreased sodium load leads to renin release has also been provided by Meyer et al. (1968). These authors gave furosemide to rabbits and observed an increase in plasma renin activity during reinfusion of ureteral urine into the femoral veins to prevent volume depletion. Since available data (Laragh et al., 1966) indicates that furosemide increases early distal tubule sodium, Meyer et al. believe that increase in sodium concentration at the macula densa is responsible for renin release. On the other hand, Gross et al. (1965) have shown that plasma sodium depletion increases renin secretion, indicating a reciprocal relationship between sodium load and renin release, a finding in support of the Vander and Miller hypothesis. Nash et al. (1968) have produced evidence that the stimulus for renin release is neither decreased sodium load nor increased tubular fluid sodium concentration but sodium flux across the macula densa into the interstitium surrounding the contiguous juxtaglomerular cells.

Thus many lines of evidence implicate the macula densa as a sensor for renin release. But it remains unclear specifically what the macula densa senses.

2. Other factors affecting renal renin secretion

Besides renal perfusion pressure and sodium, renin secretion by the kidney is affected by a number of other factors.

a. The role of K ion on renal renin release

Vander (1970) studied the direct effects of potassium ion on renin secretion. He carried out experiments on mongrel dogs maintained on a standard laboratory diet. Intrarenal-arterial infusion of KCl in anesthetized normal or acutely salt depleted dogs produced inhibiton of renin release and natriuresis in every case. Arterial blood pressure and renal hemodynamics were not changed. The natriuresis was limited only to the infused kidney; kaliuresis was of much greater magnitude in the infused kidney than in the contralateral kidney. Therefore, these investigators concluded that increased plasma potassium acts directly on the kidney to inhibit renin release and sodium reabsorption. Similar findings have also been reported by Maebashi et al. (1970) in normal human volunteers and in patients with reno-vascular hypertension. When these investigators gave KCl orally to patients with reno-vascular hypertension, renin activity decreased markedly as compared with control values. In normal volunteers, severe restriction of salt intake to 30 mEq/day was accompanied by a significant increase in plasma renin activity. However, when potassium chloride was added to this low salt diet, plasma renin activity reduced markedly. In another series of experiments, these investigators administered the diuretic hydrochlorothiazide to normal human volunteers and this resulted in an elevation of

the plasma renin on the 5th day of diuretic administration increasing further by the 10th day. Some of these subjects were given potassium supplements from the 6th to the 10th day of diuretic administration. This supplement resulted in a decrease in plasma renin activity. The remaining volunteers received potassium chloride concomitantly with hydrochlorothiazide from the beginning. Under these conditions plasma renin activity failed to increase. These findings show that potassium loading suppresses renin release by the kidney. Several investigators have shown that potassium administration stimulates aldosterone secretion (Funder et al., 1969; Eisenstein, 1967), and Conn (1964) has suggested that an increase in aldosterone secretion suppresses renin release through its effect on plasma volume. Thus Maebashi et al. concluded that the stimulatory effect of potassium upon the adrenal cortex increases aldosterone secretion which in turn suppresses renin release by the kidney. Experiments of Brunner et al. (1970) showed that potassium administration reduced plasma renin activity in 18 of 28 studies of both normal and hypertensive subjects. Suppression of renin activity occurred despite sodium diuresis. These subjects also showed an increase in aldosterone secretion. The failure of suppression of plasma renin in 10 studies was due to smaller amounts of potassium administered to these subjects. Potassium administration always produced an initial natriuresis. The amounts of sodium lost and increase in aldosterone secretion were not different whether or not plasma renin activity was suppressed. Therefore, the authors concluded that the suppression of plasma renin activity induced by potassium administration occurred independently of

associated changes in either aldosterone secretion or sodium balance. Sealy et al. (1970) in their experiments on rats have confirmed the findings of Brunner and his co-workers. Both these groups of investigators speculate that potassium might modify renin secretion 1) by acting directly on the juxtaglomerular cells, or 2) by inducing intrarenal changes in sodium transport which could modify the amount or the concentration of the sodium ions presented to the macula densa.

b. Negative feedback control of renin release by angiotensin II

The possibility that circulating levels of angiotensin II might control renin secretion by a negative feedback mechanism, such as that which exists in other endocrine systems, has been investigated by Vander and Geelhoed (1965). This hypothesis was tested by evaluating the effect of intravenously infused angiotensin II upon renin secretion. As the arterial blood pressure increased due to pressor activity of the infused angiotensin the authors tightened a clamp on the aorta above the renal arteries to maintain mean renal arterial blood pressure at a previous control level (110-140 mm Hg). In every dog they studied, angiotensin produced a decrease of renal venous renin concentration. In the second series of their experiments, the clamp on the aorta was further tightened to reduce renal arterial pressure to 80-90 mm Hg. By this means, the effects of angiotensin on renin secretion were measured at a constant reduced renal arterial pressure. Here again in every dog, angiotensin reduced renin secretion despite the continued stimulus of the low renal blood pressure. The authors, therefore, concluded that intravenous infusion of angiotensin II inhibited renin secretion in dogs

under conditions of basal or elevated renin secretion. This inhibition was independent of changes in mean renal arterial blood pressure. Therefore, these authors proposed that circulating angiotensin has a negative feedback effect on renin secretion. Genest et al. (1965) working with humans were able to reduce the elevated plasma renin secretion induced by salt depletion by infusing suppressor quantities of angiotensin II. Blair West et al. (1971) produced sodium depletion in conscious sheep by withdrawing parotid saliva for 24 to 48 hours. Angiotensin II was infused intravenously or into the renal artery during a 24 hour control period and throughout the sodium depletion period. This infusion of angiotensin II into the renal artery blocked the expected rise in plasma renin concentration (PRC) that normally occurs during sodium depletion. This effect was produced independently of changes in renal arterial pressure, renal sodium excretion or plasma potassium level, suggesting that the inhibition of renin release was due to a direct intrarenal action of angiotensin II. The findings of these groups, therefore, further support the hypothesis that renin secretion may be regulated partly by the circulating levels of angiotensin II through a short loop negative feedback mechanism.

c. Hormones affecting renin release

The possibility exists that renin release may be altered by circulating hormones other than angiotensin II. This possibility has been investigated by a number of investigators.

1) Role of vasopressin and oxytocin in renin release

Bunag et al. (1967) tested the effect of vasopressin and oxytocin on the release of renin induced by lowered renal perfusion pressure.

Vasopressin was infused into the renal artery in two dogs at a rate of 2 to 4 mU/Kg/min. Renin release was inhibited in both dogs. Vasopressin at this dose level also produced a small rise of mean arterial pressure (average +15 mm Hg) and a fall in renal blood flow (average -45 ml/min). Oxytocin infused at the same dose as vasopressin had no significant effect on renin release. When the rate of infusion of oxytocin was 10 times greater than that used for vasopressin, oxytocin also inhibited renin release. The degree of inhibition, however, was less than that produced by vasopressin. Oxytocin at either concentration had no effect on systemic blood pressure or renal blood flow. Vander (1968) has confirmed the findings of Bunag et al. (1967) that renin release can be inhibited by vasopressin. Vander obtained inhibition of renin release with 10 mU/min infusion of vasopressin compared to 2 to 4 mU/Kg/min used by Bunag et al. Both these groups of investigators have postulated that inhibition of renin release by vasopressin may be due to its natriuretic effect as explained by the Vander Miller Hypothesis (1964). According to this hypothesis, decreased tubular reabsorption of sodium--i.e., increased urinary sodium excretion accounts for the inhibition of renin release (see above). These investigators did not rule out the possibility that vasopressin acts directly on JG cells. Goodwin et al. (1970) studied the physiological role of vasopressin in the control of aldosterone secretion, plasma renin activity and sodium metabolism in man. Their data show that in the absence of overhydration, vasopressin has no demonstrable effects upon plasma renin activity, aldosterone excretion rate or sodium balance. Even during overhydration producing suppressed vasopressin secretion the changes that occurred in plasma

renin activity and aldosterone secretion were not significant. The work of these investigators, therefore, suggests that in normal man, changes in circulating levels of vasopressin do not play a physiological role in the control of sodium excretion or renin release.

2) Role of aldosterone in renin release

The effect of adrenal steroids on renin secretion have been studied by a number of investigators. Sokabe et al. (1963) reported that chronic adrenal insufficiency in rats results in an increase in renin release and renal renin content or JG gradulation. Conversely, chronic administration of exogenous aldosterone or DCA plus salt reduced renin release and renal renin content or JG granulation. Conn et al. (1964) showed that in humans, primary aldosteronism is associated with markedly reduced plasma renin, plasma angiotensin and JG granulation. Conversely Addison's disease in humans is associated with an increase in plasma renin level. A detailed analysis of the mechanism by which mineralocorticoids reduce plasma renin activity was made by Robb et al. (1969). These workers gave DOCA to normal conscious dogs for 2 weeks and studied the response to several potent renin releasing stimuli. Their data suggested that DOCA depressed plasma renin activity either by expansion of the extracellular fluid volume or by an increase in total body sodium, and not by a direct inhibitory effect on the JG apparatus.

Thus, it is apparent, from the work of several investigators, that in addition to changes in renal perfusion pressure, it is also necessary to consider the effect of circulating level of angiotensin II, vasopressin, K^+, extracellular fluid volume and total body sodium on the release of renin by the kidney.

3. Role of sympathetic nervous system in release of renal renin

Barajas (1964) has shown that the juxtaglomerular apparatus receives a rich supply of non-myelinated and presumably sympathetic nerves. Collins (1936) removed all the nerve tissue surrounding the renal artery, vein and ureter. These structures were then scrubbed with gauze to clean them more thoroughly and then painted with 5% phenol water. The kidney was then shelled out of the perirenal fat and peritonium, leaving the renal artery, vein and the ureter as the only connection between kidney and the animal. In 4 dogs the renal arteries were constricted after denervation, producing an increase in arterial blood pressure. These experiments suggest that renal nerves are not necessary to the hypertension resulting from renal artery constriction. Page (1935) has reported similar findings. In their experiments, about six weeks after performing unilateral nephrectomy, the remaining kidney was denervated and a clamp applied to the renal artery. Their results showed that unilateral nephrectomy by itself did not cause a significant rise in the level of blood pressure, but constriction of the renal artery did so shortly after recovery from anesthesia. A few days after operation mean arterial pressure in some cases measured 220 to 290 mm Hg. Similar responses occurred in two control animals in which the renal nerves were left intact. These studies also indicate that the mechanism of renal hypertension does not necessarily involve a nervous reflex originating in the constricted kidney. Taquini et al. (1964), on the other hand, reported that denervation of the rat kidney decreased the renin content in that kidney, compared with the opposite untouched kidney.

Vander (1965) showed that intravenous infusion of either epinephrine (5-6 μg/min) or norepinephrine (12-16 μg/min) with mean renal arterial pressure maintained constant by suprarenal aortic constriction produced enhanced renin secretion. Similar results were obtained by stimulation of the renal nerves. These maneuvers also caused a decrease in glomerular filtration rate (GFR), renal plasma flow (RPF) and sodium excretion. Simultaneous induction of osmotic diuresis and catecholamine infusion or renal nerve stimulation prevented the increase in renin secretion, but did not alter changes in GFR or RPF. Therefore, the authors suggested that the increased renin secretion induced by catecholamine infusion or renal nerve stimulation might be 1) the indirect result of a decrease in filtered sodium produced by these procedures or 2) due to direct effects of catecholamines and renal nerves on the renin secreting cells.

Passo et al. (1971) have studied effects of stimulation of the pressor region of the medulla oblongata on renin secretion in dogs. Chronically implanted electrodes in the dorsal medulla oblongata near the obex were electrically stimulated while changes in plasma renin activity and blood pressure were monitored. Stimulation in 16 dogs significantly increased the plasma renin activity. In almost all instances, these increases were associated with marked increases in blood pressure. Stimulation of the medulla 4 hours after renal denervation was not associated with any significant increase in plasma renin activity. The response was also absent when the medulla oblongata was stimulated two weeks after renal denervation. These results provide further evidence

that sympathetic stimulation can increase renin secretion. The same authors, Passo et al. (1971) have further investigated the effect of α and β adrenergic blocking agents on the increase in renin secretion produced by stimulation of the medulla oblongata in dogs. Stimulation of the medulla oblongata in those animals given phenoxybenzamine resulted in no attenuation of the rise in plasma renin. On the other hand, β blockade abolished the rise in plasma renin concentration induced by central stimulation. Allison et al. (1970) studied the effect of isoproterenol and adrenergic blocking agents on plasma renin activity (PRA). The investigators infused isoproterenol, a beta stimulating agent, into the renal artery of uninephrectomized dogs. This catecholamine caused a significant increase in plasma renin activity. This increase was not seen in the presence of systemic beta blockade. In contrast, systemic alpha blockade with phenoxybenzamine did not prevent the rise in PRA evoked by isoproteronol infusion; if anything, the authors noticed potentiation of the PRA response. Thus these experiments indicate that beta rather than alpha receptors are involved in the sympathetic nervous system control of renin secretion.

To separate the various factors controlling the release of renin, Davis et al. (1972) prepared a non-filtering kidney model thereby eliminating the effect of the macula densa on renin secretion. The role of the renal nerves in renin release was also excluded by denervating the kidney. The influence of circulating catecholamines was eliminated by adrenalectomy. These authors prepared the non-filtering kidney model by clamping the left renal artery for 2 hours duration and by ligating and

sectioning the left ureter. On the third post-operative day they removed the right kidney and on the fourth day the experiment was performed. Blood flow to the non-filtering kidney was measured by electromagnetic flowmeter. The rate of renin secretion was calculated from measurements of the renal venous-aortic difference in plasma renin activity and the rate of renal plasma flow. Dogs prepared in this manner were subjected to acute hemorrhage (20 ml/Kg). A significant increase in renin secretion occurred. In another series of experiments, the aorta was constricted above the renal arteries. This reduced renal perfusion pressure by 20 mm Hg but renal blood flow was unchanged (autoregulation). Here again there was significant increase in renin secretion. These data suggested to the authors that increased renin secretion occurs in association with renal autoregulation. Release of the aortic constriction was accompanied by reduction in renin secretion to the control level. These findings, therefore, provide strong support for the concept of an intrarenal vascular receptor.

To identify the vascular receptor, papaverine was infused intra-arterially into a denervated, non-filtering kidney at a rate to achieve maximum arteriolar dilation while minimally decreasing systemic arterial pressure. During papaverine infusion, the dog was hemorrhaged 20 ml/Kg blood body weight. The expected increase in renin secretion was completely blocked by the papaverine infusion. These data, therefore, suggest an afferent arteriolar locus for the vascular receptor since papaverine is known to dilate the renal afferent arterioles and prevent autoregulation. To determine the role of the renal nerves in renin

secretion, the response of renin secretion to hemorrhage was also studied during intrarenal arterial infusion of papaverine into the innervated, non-filtering kidney. Papaverine produced an increase in renal blood flow from 105 to 143 ml/min but failed to block the renin response to hemorrhage in the innervated kidney. As the macula densa was non-functioning and arterioles were (maximally) dilated by papaverine, the data imply that this increase in renin was mediated through the renal nerves. In the same preparation, the renal nerves were directly stimulated. Here, again, an increase in renin secretion occurred in spite of the infusion of papaverine and absence of the macula densa, again suggesting that renal nerves exert a direct effect on the JG cells. The response to intra-renal infusion of epinephrine and norepinephrine was also tested in the same preparation. Epinephrine infusion at a level comparable to that occurring in hemorrhage increased renin release but this effect was completely blocked by papaverine. The level of epinephrine used was approximately 11 μg/liter. Since papaverine blocked the action of epinephrine the authors concluded that epinephrine may increase renin release by its influence on renal arteriolar smooth muscle tone. In the same preparation however, norepinephrine, on the other hand, produced a striking increase in renin release and papaverine failed to block the response. These findings suggested to the authors that norepinephrine acts directly on the JG cells.

Available evidence thus provides support for (1) the existence of both a renal vascular receptor and a macula densa receptor, (2) a role of renal sympathetic nerves, and (3) a role of various humoral agents including sodium and potassium ions, angiotensin II and catecholamines.

The intermediate link between vascular receptors macula densa and JG cells remains unknown. According to Davis (1971) the juxtaposition of the macula densa to the JG cells suggests the possibility of a local hormone that is released by the macula densa and diffuses into the JG cells with the subsequent release of renin.

III. Antihypertensive Function of the Kidney

There is evidence to suggest that the regulation of the systemic blood pressure by the kidney is, in part, an expression of a renal antihypertensive function. According to this hypothesis, states of animal and human hypertension may be the result of a deficiency of renal depressor system(s), rather than solely an increase in renal pressor mechanism.

A. Evidence in Support of Antihypertensive Function of the Kidney

The evidence for the existence of such a renal antihypertensive function is derived from a variety of investigations.

1. Removal of untouched kidney in two kidney Goldblatt hypertension

Beginning with the pioneering studies of Fasciolo et al. (1938) and Goldblatt et al. (1934) it has been appreciated that the normal kidney exerts a protective role in two kidney Goldblatt hypertension. Removal of such a kidney leads to a much more marked elevation in blood pressure, suggesting an antihypertensive role for the intact kidney.

2. Renoprival hypertension

Braun-Menendez and Von Euler (1947) and Grollman et al. (1949) demonstrated that bilateral nephrectomy in the dog is followed by development of hypertension (renoprival hypertension). Floyer (1955) also demonstrated development of renoprival hypertension following bilateral nephrectomy in rats. However, the results of these experiments were believed to be a relatively non-specific effect of the associated state of uremia. To investigate the possibility that the development of renoprival hypertension is not due to absence of excretory renal function, Grollman et al. (1949) showed that implantation of ureters into the inferior vena cava, in which the same degree of uremia was achieved as with removal of both kidneys, was not associated with the development of hypertension. These results were interpreted by the authors as consistent with the view that the kidney elaborates a substance(s) necessary for maintaining the normotensive state. Leonard and Heisler (1951), on the other hand, have noted that hypertension did not develop when bilaterally nephrectomized dogs were fed a diet lacking in protein and low in sodium and when significant weight gain during this period was prevented by dialysis. These authors, therefore, concluded that the hypertension following bilateral nephrectomy is not due to absence of any renal antihypertensive agent but may be due to expansion of the body fluid volume. Grollman et al. (1951) have studied this problem in great detail. These investigators maintained bilaterally nephrectomized dogs for a prolonged period by intermittent peritoneal lavage; such animals had relatively normal volume, cardiac output, and

venous pressure but still developed hypertension. Muirhead et al. (1953) also confirmed that hypertension occurs in nephrectomized dogs maintained alive by dialysis. The work of Grollman and Muirhead strongly indicates that renoprival hypertension is neither entirely dependent on exogenous sodium load nor apparently on expansion of the extracellular fluid volume.

Utilizing two separate approaches Kloff and Page (1954) and Muirhead (1953) demonstrated a recession of renoprival hypertension when the circulation of the hypertensive animal was connected to intact renal tissue. Kloff and Page perfused blood from the nephrectomized hypertensive animal through the intact kidneys of a normal animal, a procedure which promptly lowered the arterial pressure. Muirhead and colleagues demonstrated a prompt and sustained lowering of renoprival hypertension following placement of a renal homotransplant in the neck of the hypertensive recipient. As the transplanted kidney deteriorated one week later, the hypertensive state recurred.

These experiments, therefore, strongly support the hypothesis that the normal mammalian kidney has an anti-hypertensive function, and that this function is not attributable solely to renal excretory function.

3. Kidney extracts demonstrating anti-hypertensive function

As early as 1940 Grollman et al. prepared kidney extracts which, when given by mouth or by vein, lowered arterial pressure in hypertensive rats and even in some hypertensive patients. Hamilton and Grollman (1958) purified the active principle and found it to be water soluble and probably a peptide. Sokabe (1962) showed that this anti-hypertensive

principle is present in the cortex and not in the medulla of the kidney. Page et al. (1940) also prepared whole kidney extracts that lowered the arterial pressure of hypertensive dogs and patients. In contrast with Grollman's material, the active principle was not dialyzable. When injected into dogs with renal hypertension, these extracts caused a gradual fall in pressure to normal levels. In patients with essential hypertension, benign or malignant, there was a significant reduction in arterial pressure. Muirhead et al. (1960) prepared medullary extracts from the dog kidney that protected other dogs against renoprival hypertension when administered either locally or intravenously. These medullary extracts also caused a significant fall in pressure in dogs and rabbits with Goldblatt hypertension. Muirhead et al. (1964, 1965) showed that, as with extracts of whole kidney, administration of extracts of renal medulla also required several days for the arterial pressure to begin to recede and then to reach its lowest level. On cessation of treatment, the return of blood pressure to hypertensive levels was also gradual. Muirhead et al. (1969), in an attempt to further test the antihypertensive action of the renal medulla, conducted a transplantation experiment using a highly inbred strain of Wistar rats. Siblings of these rats were shown to be histocompatible to skin. One sibling was made hypertensive by constricting the left renal artery and removing the right kidney; the other siblings were used as donors of renal tissue. There were two hypertensive groups; one group was injected subcutaneously with fragmented renal medulla, the other with fragmented renal cortex. Fourteen days later these transplants were removed surgically. The control

arterial pressure averaged 115 and 119 mm Hg, and the hypertensive pressure, 169 and 170 mm Hg, respectively. While the renal medullary transplants were in place, the pressure dropped an average of 25 mm Hg. In contrast, while the renal cortical transplants were present, the pressure rose by an average of 12 mm Hg. A short time following removal of medullary transplants, the pressure returned to pre-transplant hypertensive levels. Removal of the cortical transplant caused no significant change in pressure. The early drop in pressure resulted from non-specific depressor agent(s) injected with the tissue but the sustained results suggest that the renal medullary tissue may have an endocrine like anti-hypertensive action. Muirhead et al. (1966) extracted anti-hypertensive neutral lipid (ANRL) from renal medullary extracts. These lipids prevented renoprival hypertension at an oral dose of 3 μg/kg/day and caused partial remission of renal hypertension. In doses up to 500 μg/kg, this material had no acute vasodepressor activity in normal dogs.

In 1963, Lee et al. found that some medullary extracts from rabbit kidneys contained substance(s) that, in contrast with Muirhead's substance, caused an acute vasodepressor effect in anesthetized, vagotomized, pentolinium treated normal rats. It also differed from the substances of Page and Grollman in being an acid lipid, poorly soluble in water but dialyzable. Moreover, in contrast to Grollman's substance, it was derived from the medulla and not from the cortex. In 1965, Lee et al. isolated from these extracts three biologically active acidic lipids related to the prostaglandins.

Osvaldo and Latta (1966) have shown that medullary tissue contains interstitial cells with osmiophilic droplets while the cortical tissue had no osmiophilic interstitial cells. These medullary interstitial cells are probably the source of the medullary antihypertensive factor(s).

Identification of prostaglandins in the renal medulla which were potent vasodilators provided the first direct evidence that the kidney contains compounds that could mediate an antihypertensive function.

B. Prostaglandins

The first convincing evidence of the existence of this class of biologically active compounds was provided by Maurice Goldblatt (1935) and Von Euler (1935) who independently studied and described the smooth muscle stimulating and blood pressure lowering effects of human seminal plasma. The name prostaglandin was given by Von Euler. Bergstrom and Sjovall (1960) succeeded in isolating prostaglandin E_1 (PGE_1) and prostaglandin F_1 (PGF_1) from sheep seminal vesicular glands.

Horton (1969) discovered that unsaturated analogues are present in various other tissues, including lung, thymus, kidney, brain and iris. In addition, prostaglandins are released from certain other tissues on chemical and electrical stimulation. These include such a diverse group as fat pads, adrenals, ovaries, stomach, intestine, lung, nerves and spleen.

1. Chemistry of prostaglandins

All the prostaglandins isolated from natural sources, except for some metabolites formed by β-oxidation, contain 20 carbon atoms and may be considered as derivatives of a hypothetical acid for which the trivial

name "prostanoic acid" was proposed by Bergstrom et al. (1963). Four series of natural prostaglandins have so far been described, designated by letters, E, F, A, and B corresponding to the differences in the five member ring. All the primary prostaglandins are hydroxylated in the 15 position and contain a 13, 14-trans double bond. The degree of unsaturation of the side chains is indicated by the subscript numeral after the letter, thus prostaglandins E_1, F_1, A_1, and B_1 have only the trans double bond, prostaglandins E_2, F_2, A_2 and B_2 have in addition, a cis double bond in the 5, 6 position while prostaglandins E_3, F_3 and B_3 have an additional cis double bond in the 17, 18 position. The three vasodepressor prostaglandin-like material isolated by Lee et al. (1965) were further identified by the authors (1967) as PGE_2, $PGF_2\alpha$ and PGA_2. The natural occurrence of PGA_2 was questioned since dehydration of PGE_2 consequent to extraction and purification of PGE_2 may have accounted for the formation of PGA_2. Independently and concurrently, Daniels et al. (1967) identified PGE_2 as the principal vasodepressor lipid of the rabbit renal medulla.

2. Localization of renal prostaglandins synthesis and degradation

Lee et al. (1965) first isolated renal prostaglandins from renal medullary extracts, indicating to the authors that prostaglandins are synthesized in the renal medulla. Further work in this field has confirmed this conclusion. Hamberg (1969) demonstrated the presence within the renal medulla of the prostaglandin-synthesizing enzyme system (prostaglandin synthetase). This investigator added to medullary homogenates tritiated arachnidonic acid, the precursor of PGE_2 and $PGF_2\alpha$.

After incubation of homogenates with tritiated arachnidonic acid, the bulk of the radioactivity recovered (22-47%) was definitely identified as PGE_2, while a lower yield (5-7%) of $PGF_2\alpha$ was obtained. Only traces of a labeled compound, tentatively identified as PGA_2 were isolated from these homogenates. Crowshaw and Szlyk (1970) failed to show the presence of appreciable quantities of prostaglandins in the renal cortex. Absence of prostaglandins in the renal cortex may be due either to the absence of prostaglandin synthetase in renal cortex or degradation of prostaglandins to inactive metabolites after synthesis. However, these workers did not detect the major metabolites of PGE_2 and $PGF_2\alpha$ in extracts of pooled rabbit renal cortices and, therefore, concluded that the prostaglandin synthetases are mainly located in renal medulla. On the other hand, Larsson and Änggård (1973) using a microsomal fraction obtained from rabbit renal medulla and cortex, reported that the capacity of the cortex to biosynthesize a tritrium-labeled PGE-like material from added arachnidonic acid was 10% of that of the renal medulla and papilla. However, formation of biologically active PGE_2 from endogenous precursors by the cortex was much smaller, perhaps 1-2%, than that formed by either the medullary or the papillary enzyme preparations. Thus, the results of these investigations indicate that the cortex is able to synthesize small quantities of prostaglandins. The presence of prostaglandin synthetase in the cortex may have important functional implications in terms of regulation of the renal circulation.

Änggård et al. (1972) have studied in detail the subcellular localization of prostaglandin synthetase in the papilla of the renal

medulla. These investigators demonstrated that prostaglandin synthetase was associated mainly with microsomes. In contrast to the localization of synthetase, prostaglandins themselves were located mainly in the supernatant fraction. These observations suggest that prostaglandins are synthesized by microsomal enzymes and then are released from the endoplasmic reticulum into the cytoplasm or into the extracellular fluid of the inner medulla. Only traces of prostaglandins were found associated with microsomes, mitochondria, and lipid droplets suggesting that there are no sites of storage within the renal medulla. These conclusions have been confirmed by Crowshaw (1973) who demonstrated that the prostaglandins synthesized from 1-C^{14} arachnidonic acid by rabbit renal medullary slices were all located in the incubation medium. None were present in the tissue slices even though the slices had incorporated large quantities of arachnidonic acid into tissue phospholipids and neutral lipids. Thus prostaglandins do not seem to be stored. This interpretation is further confirmed by tissue levels of prostaglandins which vary acutely according to experimental conditions. Crowshaw (1971) in his experiments on renal medullary homogenates has estimated that concentration of PGE_2 may be as high as 24 µg/g of tissue. In contrast, Änggård et al. (1972) in their experiment quickly froze the renal medullary tissue; the concentration of PGE_2 in this quickly frozen tissue was found to be only 0.005 µg/g. Thus the reported concentration of prostaglandins in a tissue are more an index of biosynthetic activity of the tissue rather than endogenous content of the prostaglandins. Larsson and Änggård (1973) have also studied metabolism of prostaglandins in

three microscopically defined regions of rabbit kidney, the cortex, the outer medulla and the inner medulla. The results showed that the metabolism of prostaglandins by 15-hydroxy prostaglandin dehydrogenase (PGDH) occurred mainly in the cortex with rates 10 times those seen in the inner medulla. Results of these investigators seem to be in good agreement with the histochemical studies by Nissen and Andersen (1968) on the distribution of PGDH in the rat kidney. These authors found the highest PGDH activity in the distal ascending loop of Henle. Appreciable quantities were also found in the tunica media of the cortical arterioles. These results thus demonstrate a clear-cut dissociation between the sites of biosynthesis and metabolism of prostaglandins in the kidney. The absence of the major metabolizing enzyme, 15-hydroxy prostaglandin dehydrogenase, from the medulla of the kidney, where synthesizing enzymes have been shown to be most active, has intriguing implications concerning the duration and site of activity of prostaglandins. After their release from the renal medulla in response to appropriate stimuli prostaglandins cannot be degraded appreciably locally because of the virtual absence of degrading enzymes in the renal medulla; thus, prostaglandins find their way into circulation and may produce remote effects.

Ferreira and Vane (1967) have shown that two major renal prostaglandins, PGE_2 and PGF_{2}^{α}, are stable in blood. Prostaglandins were incubated with dog or cat blood for 1-2 minutes before being assayed. None of these prostaglandins lost activity on incubation with blood. In contrast, these investigators found that these same prostaglandins are almost completely inactivated on passage across the lung. More than

95 percent of the infused prostaglandin was removed in one circulation through the pulmonary vascular bed of the cat. In two other species (rabbit and dog) the disappearance of E_1 in pulmonary circulation was found to be 90 percent. Thus in all three species, the lungs removed almost all of the infused prostaglandins. These investigators also found 70 percent of the infused prostaglandins disappeared in the portal circulation and 50 percent were removed as they circulated through the hindquarter of the animal. Therefore the authors concluded that, though prostaglandins E_1, E_2 and $F_2\alpha$ are stable in blood, they are rapidly removed from the circulation by lungs, liver, and, to lesser extent, by the hindquarters. Removal of prostaglandins A_1 and A_2 by the lung was not tested by these authors, as their assay organ (rat stomach strip) was insensitive to these particular prostaglandins. McGiff et al. (1969), using the renal and mesenteric vasculature as assay organs, have studied selective passage of prostaglandins A_1 and A_2 across the lung. The physiological activity of prostaglandin A_1 and A_2 was the same either by intravenous or by intra-aortic infusion indicating that PGA is not degraded on its passage through the lung. Thus, unlike PGE_2 and $PGF_2\alpha$, PGA_2 could function as a circulating hormone. Whether PGA_2, in fact, is present in sufficient concentration in blood to produce effects as a circulating hormone is still under question. Crowshaw (1973), using rabbit and dog renomedullary slices and homogenates could not demonstrate biosynthesis of PGA_2 after addition of either labelled arachidonic acid, the natural substrate for prostaglandin synthetase, or PGE_2, the immediate precursor of PGA_2. These observations support the work of Hamberg

(1969) previously mentioned. Itskovitz et al. (1973) measured PGE_2, $PGF_2\alpha$ and PGA_2 simultaneously by radioimmunoassay and bioassay procedures in the effluent of the isolated blood perfused canine kidney. Though concentrations of PGE_2 and $F_2\alpha$ were found elevated in the effluent due to exclusion of the lungs from the perfusion system, PGA_2 remained depressed throughout the experiment. Therefore, the authors came to the conclusion that PGA_2, if present in the kidney, is present in small amounts.

Jaffee et al. (1973) measured prostaglandins A, E, and F in human plasma by radioimmunoassay techniques. Twenty-six normal individuals had mean plasma concentration of PGA, PGE and PGF of 960 $\pm$ 126 pg/ml, 340 $\pm$ 34 pg/ml, and 128 $\pm$ 17 pg/ml, respectively. These results indicate high levels of PGA in the peripheral circulating blood. Zusman et al. (1973) confirmed the work of Jaffee et al. (1973). However, the values of PGA, PGE and PGF in human volunteers on ad lib. sodium intake were found to be 1.60 $\pm$ 0.06 ng/ml, 0.24 $\pm$ 0.02 ng/ml and 0.38 $\pm$ 0.03 ng/ml respectively, values much higher than those reported by Jaffee and his co-workers.

Thus the studies of Hamberg (1969), Crowshaw (1973) and Itskovitz (1973), who found either very low biosynthesizing capacity of PGA_2 by renal medulla as well as low levels of PGA_2 in the renal venous effluent cannot explain the high levels of PGA_2 in human systemic plasma reported by Zusman (1973) and Jaffee (1973). However, differences in the results may have been due to species differences. Whether prostaglandin A may be present in the circulation in sufficient quantities to produce its effects as a circulating hormone still remains an open question.

3. Biological actions of renal prostaglandins

The biological actions of prostaglandins A, E and F are often quite dissimilar and can, in fact, sometimes be opposite. Thus PGE's and PGA's are vasodepressor in dogs, cats and rabbits while PGF's are vasopressor. The PGE's and PGF's also have powerful effects on the smooth muscle of the digestive, urinary, respiratory, and reproductive systems. PGA's have little or no effect on nonvascular smooth muscle. Von Euler (1935) reported the first observations of the hemodynamic effects of prostaglandins. He noted that prostaglandins (probably mostly PGE) markedly lowered the blood pressure of rabbits, cats and dogs and caused vasodilation of hind limb vessels of the frog. Atropine had no effect on this response. Subsequent work has confirmed these observations. Carlson (1965) showed that intravascular infusion of PGE_1 in dogs lowered blood pressure and increased heart rate. The effect was, however, dependent upon the mode of administration. Infusion of PGE_1 high in the aorta had a more pronounced effect than infusion low in the aorta or intravenously. The increase in the heart rate could be blocked by a ganglion blocking agent or by pretreatment with reserpine. Blocking of β receptors did not change the blood pressure response to injected PGE_1 but markedly reduced the effect on the heart. Similar results were recorded in other species. Thus the increase in heart rate is believed to be entirely reflex, probably secondary to a baroreceptor-mediated increase in sympathetic activity occasioned by the fall in systemic blood pressure. Horton and Main (1963) showed that the effects of PGE_2 on the blood pressure are the same as E_1. Weeks et al. (1969)

have studied the relative depressor activity of PGE_1, PGE_2, PGA_1, and PGA_2 in anesthetized, vagotomized rats, treated with pentolinium, in similarly prepared dogs and in unanesthetized dogs under basal conditions. The vasodepressor activity of two PGA forms was approximately two and one-half times greater than that of the corresponding PGE's in the dogs but, in rats, PGA's have only one-third the activity of PGE's. In contrast, Lonigro et al. (1973) have reported that PGE_2 is a more potent vasodilator than PGA_2 in renal vascular beds in dogs. These authors demonstrated that the renal vasoconstriction and antidiuresis produced by renal nerve stimulation was reversed by PGE_2 and PGA_2 whereas $PGF_2\alpha$ had no effect. PGE_2 (100 ng/ml) inhibited the renal actions of adrenergic stimuli and angiotensin II to the same degree as PGA_2, at a dose one-fifth that of PGA_2. Thus, although both PGE's and PGA's are vasodepressor, their relative potency is different in different species and in different vascular beds of the same animal.

Smith et al. (1967) have studied the vasodilator action of PGE_1 on hindlimb blood flow in anesthetized dogs. Comparison of dose response curves obtained following intra-arterial injection revealed the following vasodilator molar potencies: PGE_1 > acetylcholine = isoproteronol > histamine. The vasodilator action of PGE_1 was not abolished by atropine, propranolol, methysergide or pyribenzamine. These experiments, therefore, demonstrate that PGE is a potent peripheral vasodilator acting directly on vascular smooth muscle without apparent involvement of adrenergic, cholinergic or histaminergic components. Strong and Bohr (1967) have also confirmed the relaxant effect of E_1 and A_1 on small

blood vessels by in vitro studies. On the other hand, prostaglandin F's are vasoconstrictors, at least in some species; for example, rat and dog. Horton and Main (1965) and DuCharme and Weeks (1967) showed that $PGF_2\alpha$ lowers blood pressure in cat and rabbit but elevates blood pressure in rat, dog and spinal chick. DuCharme et al. (1968) have studied the effect of $PGF_2\alpha$ on the peripheral segmental vascular resistance by the method of Haddy et al. (1961). This study revealed little effect by $PGF_2\alpha$ on perfusion pressure in the limb of the dog, but a considerable increase in small vein pressure. This effect is abolished by denervation and restored by electrical stimulation of the lumbar sympathetic trunk. In the whole animal $PGF_2\alpha$ decreased venous capacitance. This effect was clearly demonstrated in a preparation in which cardiac output was maintained constant by pumping blood from the right atrium to the pulmonary artery. $PGF_2\alpha$ caused an increase in atrial pressure with little or no effect on systemic pressure. Hexamethonium, phenoxybenzamine, or denervation abolished the effect of $PGF_2\alpha$ on the cutaneous vein of the perfused paw, but neither of these drugs prevented the $PGF_2\alpha$ reduction of venous capacitance in the whole animal. The reason for this discrepancy has not been studied. Emerson et al. (1971) have studied the cardiovascular effects of continuous intraarterial infusion of prostaglandin $F_2\alpha$ in the anesthetized dog. In these experiments, prostaglandin $F_2\alpha$ caused only a transient increase in venous return which returned to control level after the first minute of infusion. However, the total peripheral resistance and mean systemic arterial blood pressure were elevated throughout the infusion, when

cardiac inflow was adjusted continually to match venous return. In contrast, the increase in these variables was not maintained when cardiac inflow was held constant. These investigators also reported elevated heart rate and increased myocardial contractile force during the infusion. The increased total peripheral resistance noted in these animals would suggest direct vasoconstrictor action on vascular beds. This work, therefore, is in agreement with the work of Nakano and McCurdy (1968) who have reported that intraarterial injection of prostaglandins $F_2\alpha$ increases resistance and decreases flow in vasculature supplied by the femoral, brachial, and renal arteries. These experiments, therefore, indicate that, although there are species differences, $PGF_2\alpha$ in the dog constrict both arteries and veins.

Taken together, these data indicate that the renal prostaglandins are potent vasoactive agents. Release of these prostaglandins by the kidney could play an important role in maintenance of blood pressure both in health and in disease.

4. Control of prostaglandin synthesis and release

It has been shown by several investigators that the kidney can be induced to release prostaglandins by several different stimuli.

There is suggestive evidence that there may be an inverse relationship between extractable content of renomedullary prostaglandins and renal perfusion pressure. Thus Hickler et al. (1966) noted that isolated rabbit kidneys subjected to a high perfusion pressure lost much of their extractable activity. Strong et al. (1966) extracted prostaglandins from a kidney removed from a 62 year old woman with renovascular

hypertension. The kidney had two renal arteries, of each of which the main branch was completely thrombosed. Separate extractions revealed that the prostaglandin content of the ischemic part was at least 50 times more than that of the nonischemic portion. Edwards et al. (1969) measured the PGE_2 content of renal venous blood from eight patients who had hypertension associated with unilateral renal artery stenosis. Higher PGE_2 concentrations, ranging from 40 to 234 ng/ml, were found in blood from the involved kidney. No PGE_2 was measurable from the venous blood of the contralateral kidney. In a sixth patient with "severe bilateral renal dysfunction", measurable PGE_2 levels were present in both renal veins. In two patients, who had previously had surgical repair of renal artery stenosis, no prostaglandin was detected in renal venous blood. The work of Edwards et al. indicates that prostaglandins in the renal venous blood of the stenosed kidneys are elevated in human renal hypertension, but it does not explain the mechanism of this elevation.

The relationship of the release of prostaglandins from the renal medulla and constriction of the renal arteries, has been investigated by Sweet et al. (1972). These authors studied the effect of acute reduction of renal perfusion pressure on vascular responses to adrenergic stimuli, using the isolated hindpaw of the dog perfused at constant flow with renal venous blood as the assay organ. Adrenergic responses were studied before, and after 15 minutes of reduced renal pressure. During the period of reduced renal pressure responses to nerve stimulation were decreased, while the responses to intraarterial norepinephrine were

unchanged. In the second series of experiments, the investigators passed the renal venous blood through the lung of the dog in an attempt to determine if the depressant factor is cleared from the lung like many of the prostaglandins. In contrast to experiments in which no lung tissue was involved, the vasoconstrictor response to neurologically released and exogenous administered norepinephrine were both increased. These experiments, therefore, suggest that when the renal perfusion pressure is reduced acutely, the kidney elaborates a humoral material whose actions resemble, in part, the effects of prostaglandins E and A on the cutaneous vascular bed, i.e., the vasoconstrictor response to sympathetic nerve stimulation is depressed. When the renal venous blood was passed through the lungs, not only was the blockade of adrenergic responses reversed, but vasoconstrictor responses to both types of adrenergic stimuli was enhanced above control. Thus, it may be concluded that during acute reduction in renal pressure the kidney can elaborate a humoral factor, probably prostaglandins. This conclusion has been further confirmed by a detailed study of this problem by McGiff et al. (1970). They used a bioassay method, i.e., the blood superfused organ technique developed by Vane (1969), to identify prostaglandins released into renal venous effluent. By use of three banks of organs, arranged in parallel for superfusion, simultaneous assay of blood from each renal vein and aorta permitted determination of the generation or disappearance, or both, of blood-borne substances of renal origin having activity on the assay organ. Each organ bank consisted of three tissues continuously superfused in series by renal venous blood: rat stomach

strip, rat colon, and chick rectum. This combination of assay organs has the specificity and sensitivity required for detection of prostaglandins. PGE_2 and $PGF_2\alpha$ when injected into the extracorporeal circuit of this assay system, produced contraction of all three tissues. Of the blood-borne vasoactive substances (angiotensin, catecholamines, kinins, histamine) which are likely to be present in renal venous effluent, only prostaglandins would elicit contraction of all three tissues. By this technique PGA's cannot be detected since they do not have effects on non-vascular smooth muscle. Renal venous blood samples at the same time are extracted and bioassayed for prostaglandins. These are then compared to PGE_2 and $PGF_2\alpha$ standards for their effects on assay organs. Extracts of the renal medulla are extracted and purified and chemically characterized for prostaglandin activity. During unilateral renal artery constriction in anesthetized dogs, in 13 of 14 experiments, the authors demonstrated by bioassay prostaglandin-like substances appearing in the venous blood of the constricted kidney. In 11 of 13 experiments, these substances also appeared in the venous blood from the contralateral kidney. The assay organs superfused with aortic arterial blood demonstrated elevated angiotensin levels (by increased contraction of rat colon) during renal artery constriction in all experiments. In the one experiment the authors did not demonstrate the prostaglandin-like activity in renal venous blood from the constricted artery, the animal was spontaneously hypertensive. Prostaglandin-like substances were detected only in renal venous blood. Infusion of exogenous angiotensin II into the renal artery produced a

prostaglandin-like response on bioassay of renal venous effluent. Therefore, the authors concluded that angiotensin II may be responsible for the release of prostaglandin-like substances into renal venous effluent. This conclusion was further confirmed by the fact that increases in plasma renin level (greater than tenfold of control) occurred at the time prostaglandins appeared in the renal venous blood of the contralateral kidney. Extracts of renal medulla were prepared and purified and appeared to contain a mixture of E and F prostaglandins. By thin layer chromatography they were further identified as PGE_2 and $PGF_2\alpha$ and PGA's. In three experiments renal venous blood, obtained during renal artery constriction at the time that prostaglandin-like substances were detected by assay organs, was extracted and purified and then assayed by the superfusion technique. The purified material exhibited PGE_2 and $PGF_2\alpha$ activity. From these observations the authors concluded that following renal artery constriction, the kidney released renin and prostaglandins (predominantly PGE) into the venous blood as an initial response to constriction. Increased release of renin from the constricted kidney accelerated formation of angiotensin II which, the authors hypothesized, stimulated release of prostaglandins from the constricted and also from the contralateral intact kidney.

To test this hypothesis these authors (1970) also studied the effect of infusion of angiotensin II into the renal arteries of anesthetized dogs. A bioassay technique using superfused blood-bathed organs was used to detect release of vasoactive substances into the renal venous blood. In all experiments, infusion of angiotensin II into the renal

artery released prostaglandin-like substances into the renal venous blood. Infusion of angiotensin II initially reduced renal blood flow. Within 2 to 5 minutes after initial reduction in renal blood flow, release of prostaglandins occurred simultaneously with recovery of renal blood flow. On chromatographic purification of the renal venous blood obtained during infusion of angiotensin II, the authors found mainly PGE_2 and did not find evidence for the presence of $PGF_2\alpha$. The authors, therefore, concluded that released PGE_2 might function as a regulator of renal blood flow and may also have an antihypertensive function in the peripheral circulation when angiotensin II levels are increased.

Aikin and Vane (1973) confirmed the findings of McGiff et al. (1970) and again showed that infusion of angiotensin II into the renal artery of anesthetized dogs, caused a dose dependent decrease in renal artery blood flow and release of prostaglandin E_2-like substance into renal venous blood detected by superfusion techniques. Administration of indomethacin or meclofenamate, which block the synthesis of prostaglandins by inhibiting the prostaglandin synthetase enzyme (Vane, 1971), prior to infusion of angiotensin II reduced or abolished prostaglandin release from the kidney provoked by the angiotensin II infusions.

To summarize, these experiments strongly suggest that when the hemodynamics of the kidney are altered by constriction of the renal artery, the manipulated kidney responds by an increased release of vasoconstrictor substances (renin-angiotensin). These may then stimulate both the manipulated and the opposite untouched kidney to synthesize and release elevated amounts of vasodepressor prostaglandins. McGiff et al.

(1972) have further shown that norepinephrine shares with angiotensin II the ability to release prostaglandins from the kidney. When noradrenaline was infused continuously into the right renal artery, its initial vasoconstrictor and antidiuretic effects were diminished with concurrent appearance of prostaglandins of the E series in the renal venous effluent. In contrast, during renal nerve stimulation, early rapid recovery of renal blood flow and urine flow did not occur and the concentration of PGE-like substances in the renal venous blood did not increase. Although the authors concluded that noradrenaline stimulates the release of prostaglandins, they did not explain why direct renal nerve stimulation failed to stimulate prostaglandin release from the kidney. In contrast to the findings of McGiff et al. (1972), Dunham and Zimmerman (1970) showed that renal vascular constriction elicited by renal nerve stimulation or by infusion of norepinephrine is followed by release of prostaglandin E_2 in the renal venous blood.

Another factor that stimulates PGE_2 release from the kidney is bradykinin. McGiff et al. (1972) have studied the effects of the vasodilator peptides bradykinin and eledoisin on "PGE" and "PGF" concentrations in renal venous blood. Bradykinin infusion into the renal artery increased mean renal blood flow initially without affecting aortic blood pressure. After 2 minutes the concentration of a PGE compound in renal venous blood increased several fold. In contrast, eledoisin infused in equidilator doses did not increase the concentration of PGE in renal venous blood. The concentration of PGF was not affected by either polypeptide. These results suggest that bradykinin may have a specific stimulatory effect on PGE release, brought about even in the presence of

increased renal blood flow. It is thus evident from these data that several distinct types of stimuli; such as, elevated renal perfusion pressure, renal nerve stimulation, and vasoactive agents, both vasoconstrictors and vasodilators, can stimulate the kidney to release increased amounts of renal prostaglandins.

5. Antihypertensive function of renal prostaglandins

Several experiments have now indicated that these renomedullary prostaglandins may have an important antihypertensive action. Hickler et al. (1964) demonstrated that prolonged administration of large doses of purified renal medullary extracts injected once daily into the peritoneum of rats with renovascular hypertension produced a moderate reduction of arterial pressure. The more severely hypertensive rats required relatively large doses for the achievement of a similar reduction in blood pressure. Muirhead et al. (1967) injected an average dose of 2.1 mg/kg/day of pure PGE_2 subcutaneously into renovascular hypertensive rats. In one group of rats, arterial pressure dropped significantly and remained depressed as long as PGE_2 was injected. Pretreatment pressure was 154 $\pm$ 5 mm Hg; treatment pressure was 124 $\pm$ 3 mm Hg. An average of 4 days was required for the blood pressure to reach the lowest level. When treatment was stopped blood pressure returned to hypertensive level in an average of 3.8 days. A second group of rats did not respond to PGE_2 treatment. The hypertensive pressures in this group (166 $\pm$ 2.5 mm Hg) were significantly higher than those in the first group. At autopsy fibrinoid necrosis of the renal vessels was noted in this group of rats. The authors, therefore, suggested that PGE_2 has an

anti-hypertensive effect in rats with benign but not malignant hypertension. Lee (1974) has proposed an intrarenal action of renal prostaglandins to explain their role as antihypertensive agents. According to this author, elevation of arterial blood pressure is associated with a corresponding rise in cortical arteriolar constriction so that blood flow to the renal cortex remains unchanged in the face of a rise in arteriolar pressure (autoregulation). However, as such, autoregulation does not exist for the kidney medulla, increases in blood pressure being accompanied by corresponding increments in medullary blood flow. This increase in medullary blood flow stimulates release of PGA_2, PGE_2 or both by the interstitial cells of renal medulla. Evidence for this release has been provided by Ishi and Tobian (1969). These authors observed that in two kidney Goldblatt hypertension in rats there was depletion of the osmophilic granules of the interstitial cells in the constricted as well as in the untouched kidney, which was interpreted as release of prostaglandins from both the kidneys. According to Lee there is intrarenal circulation of these released prostaglandins from the medulla to the cortex producing prostaglandin induced cortical vasodilation. The resultant increase in cortical blood flow leads to a decrease in arterial blood pressure by lowering plasma volume by diuresis and natriuresis.

Lee (1974) also has proposed an alternate hypothesis based on systemic actions of prostaglandins. According to this hypothesis hypertension produces redistribution of blood from the cortex to the medulla resulting in increased release of renal prostaglandins PGA_2, PGE_2 or both.

The released prostaglandins enter the general circulation producing systemic vasodilation and antihypertensive effects. According to this hypothesis, only PGA_2 could exert an antihypertensive function, since PGA_2, unlike PGF_2, is not destroyed during passage through the lung.

C. Kallikrein-kinin System

Though considerable attention has been focused in recent years on the antihypertensive actions of renal prostaglandins, the possibility that the kidney exerts its antihypertensive action through additional vasodepressor systems of renal origin cannot be ruled out. One such system that has been investigated is the kallikrein-kinin system.

1. Discovery of kinin

Frey and his co-workers (1950) demonstrated that urine from experimental animals has hypotensive activity. This hypotensive activity was ascribed to a substance called kallikrein. Werle et al. (1937) demonstrated that kallikrein is an enzyme which acts on a plasma α_2 globulin called kininogen or kallidinogen to form an active hypotensive polypeptide called kallidin. Rocha e Silva (1949) also described a hypotensive polypeptide formed as a result of action of snake venom or trypsin on plasma α_2 globulin. The authors called this hypotensive polypeptide bradykinin. Since the discovery of kallidin and bradykinin numerous additional kinins have been found. Schachter (1968) has suggested the generic term "kinin" to apply to the expanding number of polypeptides having properties similar to those of kallidin and bradykinin.

2. Chemistry and physiology of kinins

Kinins are thus derived from alpha-2-globulin precursors in plasma called kininogens, kallidinogens or prokinins.

Elliott et al. (1960) have shown that bradykinin is a polypeptide with a molecular weight of 1060, and is composed of 9 amino acids. Both the N-terminal and the carboxyl or C-terminal are arginine. Kallidin differs from bradykinin in that a tenth amino acid, lysine, is found in the N-terminal position as shown by Pierce (1968). Elliott et al. (1963) isolated another biologically active kinin from ox blood which consists of 11 amino acids and is called methonyllysl-bradykinin. The structure of several other kinins has been characterized in recent years. Erdos (1966) has shown that though there are several proteolytic enzymes, including kallikrein, trypsin and plasmin, that can cleave kinins from kinogens; physiologically kallikreins are the most important of these kinins. Kallikrein originally was thought to represent a single enzyme but Webster (1968) has shown that there are several distinct kallikreins derived from different tissues of the body. These kallikreins have been found in the urine, kidney, intestines, pancreas, salivary glands and plasma. Nustad (1970) showed that both the kidney and urinary kallikreins have similar molecular weights, pH optimums and are inhibited by the same inhibitors. Carretero and Oza (1973) have demonstrated that rabbit antisera specific for urinary kallikreins, cross reacted with renal tissue kallikreins suggesting that urinary kallikreins have an antigenicity which is similar to that of kidney kallikreins. Webster and Pierce (1963) showed that urinary kallikrein differed from plasma kallikrein in several respects. These data, therefore, suggest that the source of urinary kallikreins is the kallikreins secreted by the kidney. Nustad (1970) has demonstrated that the rat kidney kallikrein is mainly located in the cortex.

Webster and Gilmore (1964) and Barroclough and Mills (1965) have shown that kinins are potent vasodilator agents and when infused into the renal artery are natriuretic. Stein et al. (1972) have demonstrated that kinins produce natriuresis by inhibiting sodium reabsorption in the distal tubule; thus suggesting that the natriuretic effect is specific and not secondary to vasodilation. Marin-Grez et al. (1972) have found in dogs that blood kinins and urinary kallikreins are increased immediately after acute salt loading. However, blood kinins do not increase in nephrectomized, sodium loaded dogs, indicating that such increases depend on the presence of the kidneys. These authors also noted that kallikrein excretion was greater in the urine of sodium-loaded dogs than in those that were water-loaded, suggesting that the presence of sodium increases kallikrein excretion.

3. Possible role of kinins as anti-hypertensive agents

The role of the kallikrein-kinin system in hypertension has not been delineated. Elliot and Nazzum (1934) reported that the amount of kallikrein in urine was reduced in patients with essential hypertension. Frey et al. (1950) confirmed this findings. Similarly, Marin-Grez and Carretero (1972) and Margolius et al. (1972) have shown that the urinary excretion of kallikreins is reduced in experimental renal hypertension. On the other hand, Margolius et al. (1971) have reported increased excretion of urinary kallikreins in spontaneously hypertensive rats and deoxycorticosterone salt hypertensive rats. Thus it is evident that urinary kallikrein excretion is abnormal in many types of hypertension, but the abnormality may not be the same in all types of hypertension.

The exact mechanism of such differences is still not clear; it may be linked to urinary sodium excretion in these different types of hypertensions. Adetuyili and Mills (1972) found a good correlation between urinary sodium ($U_{Na}.V$) and kallikrein excretion in patients with essential hypertension.

The mechanism by which kinins could exert antihypertensive effects is still not clear. It has been proposed that renal kallikreins could participate in regulation of blood pressure by releasing kinins which, in turn, produce a direct peripheral vasodilation; the increase in blood pressure might be caused by a decrease of kallikrein released by the kidney. However, Carretero and Oza (1973) have pointed out that a direct role of renal kallikrein in the regulation of peripheral resistance is unlikely, as considerable amounts of kallikrein inhibitors are normally present in the blood. Therefore, these authors have proposed that kallikrein exerts an antihypertensive effect by regulating sodium excretion by the kidney. Another mechanism by which renal kinins may produce their antihypertensive effect is through secondary release of renal prostaglandins, as demonstrated by McGiff et al. (1972) (see above).

Thus the work of several investigators shows that renal kinins are potent vasodilators and natriuretic agents. The exact role of this system in regulation of blood pressure in both health and disease remains unclear. More work is also required to determine the mechanism of regulation of secretion of the renal kallikrein-kinin system. The significance of the decrease in urinary kallikrein excretion observed in renal and essential hypertensives remains to be investigated.

D. Renin Inhibitor

Another antihypertensive agent of renal origin which has aroused interest is renin inhibitor.

1. Discovery of renin inhibitor

As early as 1898 Tigerstedt and Bergman reported that the nephrectomized animal gave a greater and more sustained pressor response to an injection of renin than did a normal animal. Page and Helmer (1940) reported that this increased pressor response in dogs without kidneys could be reduced by the transfusion of normal dogs' blood into the nephrectomized animal. This observation was confirmed by Gross et al. (1962) by cross circulation experiments between nephrectomized and normal rats. These authors, therefore, concluded that a humoral factor was involved in the sensitization of the nephrectomized animal to injected renin. Blaquier (1965) and Bing (1964) showed that renin added to plasma from nephrectomized animals produced more angiotensin and at a more rapid rate than did the same amount of renin added to the plasma from normal animals. Blaquier (1965), by studying the kinetics of angiotensin generation in plasma of normal and nephrectomized animals, showed that the increased rate of angiotensin generation was due to increased renin substrate in plasma from the nephrectomized animals. However, Smeby (1967) found the amount of renin substrate to be constant 24 hours after bilateral nephrectomy in dogs, but the rate of angiotensin formation by added renin continued to increase. This observation separated the increase in renin substrate which occurred 24 hours after bilateral nephrectomy from the increase in the rate of angiotensin generated which occurred soon after bilateral nephrectomy.

This observation has been confirmed in humans (Devaux et al., 1968). These observations clearly suggest that mechanisms other than elevated levels of renin substrate are involved in the sensitization of the bilaterally nephrectomized animal to injected renin. As a corollary to these experiments Sen et al. (1967) in their in vitro studies showed that the addition of normal dog plasma reduced the rate of angiotensin production in plasma from nephrectomized dogs. Similarly, when these investigators added plasma from normal dogs to a partially purified system of renin reacting with partially purified renin substrate, the rate of angiotensin generated was reduced. These experiments suggest the presence of agent(s) of renal origin in normal plasma which inhibit the generation of angiotensin. Sen et al. (1967) isolated a phospholipid from the kidney which inhibited the reaction of renin with renin substrate in vitro. However, Smeby et al. (1967) showed that this phospholipid was not a renin inhibitor but rather the precursor of a renin inhibitor which on incubation with enzyme phospholipase A is converted to lysophospholipid, the renin inhibitor. To distinguish between these two substances, it has been proposed that the isolated phospholipid be termed "renin preinhibitor" and the lysophospholipid "renin inhibitor".

2. Antihypertensive effect of renin inhibitor

To evaluate the role of renin inhibitor in the maintenance of blood pressure in health and disease in vivo studies were undertaken by a number of investigators.

Boucher et al. (1967) injected phospholipid (renin preinhibitor) intramuscularly daily into normotensive rats. After 4 days the plasma

renin activity was reduced significantly, but the blood pressure was unchanged. In contrast, when they injected the preinhibitor into rats with acute two kidney Goldblatt hypertension, both the plasma renin activity and blood pressure were reduced. Similarly, on injection of renin preinhibitors into rats with chronic two kidney Goldblatt hypertension (more than 48 weeks duration) both plasma renin activity and blood pressure were again reduced. These data again demonstrate that renin-angiotensin system participates in acute and chronic two kidney Goldblatt hypertension and suggests a possible antihypertensive role of renin preinhibitor.

There is, as yet, no evidence available to show under what experimental conditions the release of renin-preinhibitor by the kidney is increased. Recently, an interesting observation has been made by Gryglewski and Vane (1972). These authors have shown that synthesis of renal prostaglandins from phospholipids by the action of phospholipase gives rise to the byproduct lysophopholipid, the renin inhibitor. Thus, it is conceivable that the release of renal prostaglandins and renal renin inhibitor are linked to one another and the release of both antihypertensive agents occurs under the same experimental conditions. However, further work is needed before any antihypertensive role can be attributed to renal renin inhibitor or preinhibitor.

PURPOSE OF THE INVESTIGATION

The data from several laboratories indicates that unilateral renal artery constriction is associated with hypertension and release of vasoconstrictor and vasodilator agents into the circulation. Thus it has been suggested that the abnormal hemodynamic state of renovascular hypertension may represent a state of vascular constriction attributable to an absolute or relative increase in vasoconstrictor agents and/or to an absolute or relative decrease in vasodilator agents released into the circulation. These hypotheses are capable of being tested by bioassay procedures. Most such assays have been performed indirectly using processed blood from renal hypertensive animals injected into recipient animals or by using radioimmunoassay techniques. The few direct assay studies reported involving perfusion of vascular beds, suggest that the vasoconstrictor activity of renal venous blood from the constricted kidney increases. This increased vasoconstrictor activity is associated with increased release of renin by the constricted kidney. However, most of these studies represented attempts to demonstrate specific agents such as renin and angiotensin and the participation of other vasoconstrictor agents has not been ruled out.

Experiments using superfused organ systems have provided evidence that vasodepressor prostaglandins are also released into the venous blood of both kidneys following unilateral renal artery constriction. Again these studies were attempts to demonstrate specific agents and the

possibility exists that the kidneys may release some additional vasodilator agent(s); for example, kinins or renin inhibitor. The main purposes of the present investigation were twofold: 1) to investigate the possibility that vasoactive agents other than renin-angiotensin and vasodilator prostaglandins are released into the circulation following unilateral renal artery constriction, and 2) to assess the hemodynamic effects on a vascular bed of released vasoactive agents. Thus, we directly bioassayed renal venous blood and systemic blood using the gracilis muscle as test organ. Using pharmacologic techniques, we also attempted to identify the vasoactive agents responsible for the hemodynamic changes we observed.

MATERIALS AND METHODS

Healthy male mongrel dogs with blood hematocrit between 40-50% and weighing between 18-28 kg were divided into three groups.

I. Bioassay of Renal Venous and Systemic Bloods after Unilateral Renal Artery Constriction (Group A)

Twenty-four animals randomly distributed into 12 control and 12 experimental animals were studied. The dogs were anesthetized with sodium pentobarbital, 35 mg/kg. Additional doses of pentobarbital were administered during surgery if necessary but no supplementary doses were given during the period of data collection. The animals were mechanically ventilated with a Harvard constant-volume respirator (Harvard Apparatus Co., Model #607, Dover, Mass.) via an intratracheal tube. Respiratory rate was set at twenty per minute and tidal volume was adjusted so that systemic arterial blood pH was between 7.39 and 7.41. The pH was determined on pH M 71 Mark II Acid Base Analyser Radiometer (Copenhagen). The right gracilis muscle of the dog was isolated and all the blood vessels, with the exception of the main gracilis artery and vein, were cut between ligatures or cauterized. Heavy occlusive cord-ligatures were placed at each end of the muscle to eliminate colateral flow. The muscle was denervated by cutting the gracilis nerve. A mid-line abdominal incision was then made and the left renal artery was freed

from surrounding fascia and carefully placed in an externally adjustable renal artery clamp (Figure 1). The clamp was not tightened and was fixed to a stand so as not to cause any displacement or distortion of the artery. The left femoral vein and its three branches were then exposed. Three polyethylene catheters (P 240 Clay Adams, Intramedic Cat. No. 7451 I.D. 0.066" O.D. 0.095") were passed through the branches of the femoral vein and placed one in each renal vein and inferior vena cava (Figure 2). The tips of the renal catheters lay deep in the renal vein near the kidney. The tip of the inferior vena cava catheter was placed 3-4 inches upstream to the openings of the renal veins. A fourth catheter of the same size and length was passed through the left femoral artery into the abdominal aorta. All four catheters were then connected to a glass manifold arrangement, allowing blood from individual vessels to be pump perfused by a Sigmamotor pump (Model T8) at a constant flow into the isolated vascular bed of the right gracilis muscle. As the gracilis muscle was perfused with blood from one vessel at a time, the blood in the remaining three canulae was diverted into a glass reservoir to prevent stagnation in the catheters. Blood from the reservoir was continuously returned to the animal via the external jugular vein by means of a second Sigmamotor pump. The abdominal incision was then carefully closed without disturbing the clamp on the left renal artery. The gracilis muscle preparation was maintained moist with 37°C saline. The temperature of the preparation was maintained at 37°C by using a heat lamp.

Figure 1. Externally adjustable clamp used for constriction or sham constriction of left renal artery (LRA).

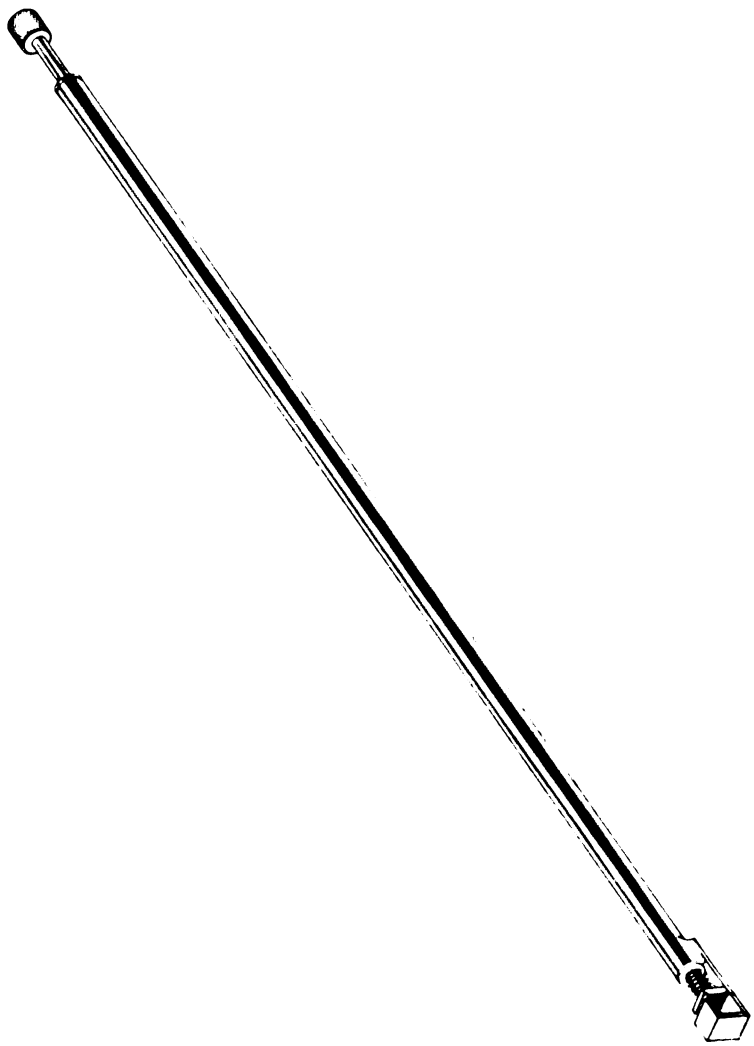


Figure 1

Figure 2. Schematic drawing of the preparation for constant flow perfusion of the isolated denervated right gracilis vascular bed with blood from the left renal vein (LRV), right renal vein (RRV), vena cava (VC) and femoral artery (FA) before and after constriction (sham constriction) of the left renal artery (LRA).

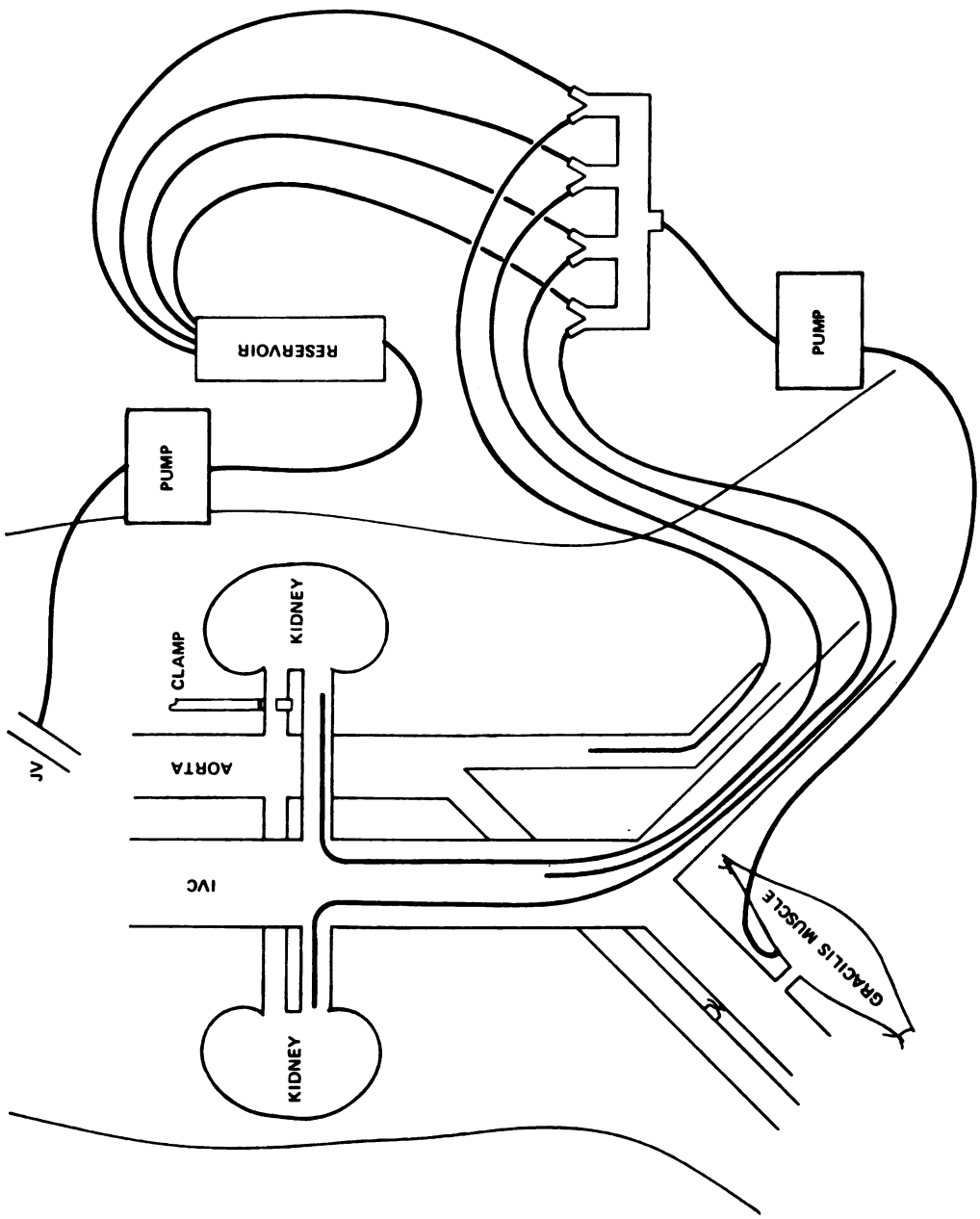

RESERVOIR
PUMP
PUMP
KIDNEY
CLAMP
JV
AORTA
IVC
KIDNEY
GRACILIS MUSCLE

Figure 2

Following the surgical procedure 30 minutes were allowed to establish a steady state. At the end of the 30 minute period heparin sodium 5 mg/kg (Wolins Pharmacol. Corp., Framingdale, New York) was injected intravenously for systemic anticoagulation. The artery of the gracilis muscle was cannulated and the gracilis muscle perfusion commenced initially with blood drawn from the arterial cannula. Perfusion pressure was monitored through a 20 gauge hypodermic needle inserted into the rubber tubing downstream from the pump. Pressures were similarly monitored upstream to the pump. Aortic blood pressure was continuously monitored through a side tube connected to the femoral artery cannula. All pressures were monitored by low volume displacement pressure transducers (Statham Laboratories, Model P23 G b, Hato Ray, Puerto Rico) which served as inputs into a direct writing oscillograph (Sanborn Co., Boston, Mass.). A few minutes after beginning the perfusion of the gracilis muscle with arterial blood, when steady state perfusion pressure was recorded, the perfusion pump was stopped to test the isolation of the vascular bed of the gracilis muscle. Fall of the perfusion pressure to 0-10 mm Hg was accepted as an indication that the vascular bed of the gracilis was isolated. The pump flow was then set at a rate that produced a perfusion pressure approximately equal to aortic pressure. Thereafter, pump flow was not changed during the experiment. Pump flow was measured by calibrated cylinder and stopwatch through a T tube interposed between the pump and the gracilis artery cannula. The gracilis muscle venous return flowed freely into the femoral vein. The gracilis muscle was then serially perfused with blood from the left renal vein (LRV), vena cava (VC), right renal vein (RRV) and in some dogs with blood

from the femoral artery (FA). Steady state perfusion pressures were recorded. Duration of each perfusion was as long as necessary to establish steady state perfusion pressure or 10 minutes, whichever occured first. These perfusions were repeated in the same order until two consecutive readings of perfusion pressure during perfusion with blood from the same vessel were within ± 10 mm Hg of each other, indicating steady state conditions. Pressure drop across the outflow cannula was subtracted from each perfusion pressure reading to obtain net perfusion pressure readings. To calculate baseline gracilis vascular resistance during perfusion with blood from each source, these net perfusion pressure readings were averaged. These baseline resistance values were plotted on graphs. After these control perfusion pressures had been recorded and while the muscle was being perfused with LRV blood, the left renal artery was constricted in the 12 experimental dogs. This was done in each dog by turning the knob of the externally adjustable clamp until the artery was completely occluded, then quickly opening the clamp to produce a 2 mm gap. The control group of animals underwent the same procedures except that the clamp on the left renal artery was not tightened but only handled (sham constriction). Following constriction or sham constriction the gracilis muscle was then perfused sequentially with blood from the LRV, VC and RRV, and, in some dogs, with femoral arterial blood, recording steady state perfusion pressures during the 45 minute period after constriction (or sham constriction). At least 2 perfusion pressure readings were recorded during perfusion with blood from each vessel. In the case of gracilis perfusion with blood from each

vessel in each dog, changes in net perfusion pressure, compared to baseline perfusion pressure during the control period, were plotted against time. By extrapolation from these plots we derived net gracilis perfusion pressures at standardized time intervals following constriction (or sham constriction). Extrapolation was necessary to derive these pressures at standardized time intervals, because the muscle could be perfused by blood from only one vessel at a time and the duration of perfusion was dependent on the time required to achieve a steady state perfusion pressure. These extrapolated net perfusion pressures were used to calculate net percent change in perfusion pressure compared to the respective baseline readings. For perfusion with left renal vein blood, percent changes in the net perfusion pressures were calculated for 5 minute intervals up to 45 minutes post left renal artery manipulation. Similarly, net percent changes in perfusion pressure were also calculated for perfusion with vena caval blood for 5 minute intervals from 20 until 45 minutes, perfusion with right renal vein blood for 5 minute intervals from 25 until 45 minutes, and perfusion with femoral artery blood for 5 minute intervals from 35 until 45 minutes post renal artery constriction (or sham constriction). At each interval, percent changes in net perfusion pressure while the gracilis muscle was perfused with blood from each vessel were compared to values during perfusion with blood from each other vessel by the Student's t test. Similarly, percent changes in net perfusion pressure in dogs of the experimental group were compared to values in the control group by Student's t test. Towards the end of

perfusion experiment 0.1 ml sodium nitroprusside solution dissolved in saline in the concentration of 5 mg per 100 ml was injected into rubber tubing upstream to the pump to test whether the gracilis muscle vessels were still capable of responding to vasoactive agents. At the end of all experiments, the perfused gracilis muscle was excised and weighed to the nearest 0.5 gm. Perfusion flow rate in ml/min/100 gm of gracilis muscle was calculated. Preliminary examination of data indicated differences between gracilis resistance responses to perfusion with LRV and RRV blood that were maximal about 25 minutes post renal artery constriction. Therefore, in some dogs, samples of LRV, RRV, VC and FA blood (each sample less than 2 ml) were collected at that point and pH, PO_2, PCO_2, serum concentrations of Na^+, K^+, Mg^{++}, Ca^{++} and hematocrit estimated. We also obtained blood samples from ten additional dogs, 5 experimental and 5 control, not otherwise included in Group A but subjected to an identical protocol. No hemodynamic measurements were made in these dogs, but 25 minutes after constriction (or sham constriction) of the left renal artery, samples of LRV, RRV, VC and FA blood (each sample less than 2 ml) were collected and pH, PO_2, PCO_2, serum concentrations of Na^+, K^+, Mg^{++}, and Ca^{++} and hematocrit estimated. Additionally at this time, in these dogs, 10 ml of blood from each vessel were collected in EDTA tubes for determination of plasma renin concentrations (PRC). pH and PCO_2 determinations were made using potentiometric methods (Radiometer-Copenhagen, blood micro system, acid base analyser, Copenhagen, Denmark). PO_2 was determined by Radiometer Copenhagen; serum Na^+ and K^+ concentrations were measured by flame

photometry (Beckman Instruments, Inc., Model 105, Fullerton, California): Mg^{++} and Ca^{++} concentrations were measured on a Perkin-Elmer Atomic Absorption Spectrometer (Model 290, Perkin-Elmer Corp., Norwalk, Conn.). PRC was estimated by Dr. Micheal D. Bailie, Departments of Human Development and Physiology, Michigan State University, using the radioimmunoassay technique of Haber et al. (1969) slightly modified in that an excess of exogenous renin substrate was added to the incubation medium.

II. Bioassay of Renal Venous and Systemic Blood after Unilateral Renal Artery Constriction Following Immunization Against Renin (Group B)

In the second series of experiments in 7 control and 7 experimental dogs the same experimental protocol was followed as in group A except that the animals were immunized against renin during the control perfusion period. Following baseline perfusion of the gracilis muscle with blood from LRV, RRV, VC and FA, both the experimental and the control animals were immunized against renin by bolus intravenous injection of 0.5 ml of homologous antirenin serum (supplied courtesy of Dr. Sibley Hoobler) containing 75 units of antirenin. Following immunization, the gracilis muscle was again perfused in a sequential manner with blood from the LRV, RRV, VC and FA to determine baseline perfusion pressures before constriction (sham constriction) of the left renal artery. Towards the end of the experiment, the animals were challenged with 2 units of lyophilized dog renin (supplied courtesy of Dr. E. Hass, Ph.D., L.D. Beumont Memorial Research Laboratories) in 1 cc saline

injected intravenously. In all these dogs, blood samples were collected for estimation of hematocrit, pH, PO_2, PCO_2, and serum electrolytes 25 minutes post left renal artery manipulation. As in group A, gracilis vascular response to injection of sodium nitroprusside solution was tested in each animal.

III. Bioassay of Renal Venous and Systemic Blood after Unilateral Renal Artery Constriction Following Indomethacin Infusion (Group C)

In another group of 8 experimental and 8 control dogs the same protocol as described for group A was followed with the addition of an attempt to block the synthesis of prostaglandins. After the gracilis muscle was perfused with LRV, RRV, VC and FA blood to obtain baseline resistance values, indomethacin dissolved in buffered saline in the concentration of 0.9 mg per ml was infused intravenously at the rate of 5.7 ml per min. Both control and experimental animals received a total dose of 5 mg of indomethacin per kilogram body weight, requiring 15-20 minutes to complete the infusion. During and after the infusion the gracilis muscle was perfused sequentially with blood from LRV, RRV, VC and FA. Net perfusion pressures during perfusion with blood from each vessel during and after indomethacin infusion were averaged and taken as baseline perfusion pressures. Perfusion pressures were then recorded during the 45 minutes after constriction (or sham constriction) of the left renal artery. Percent changes in perfusion pressure at intervals following manipulation of the left renal artery were calculated as before.

Blood samples were collected before and 25 minutes following manipulation of the renal artery for estimation of hematocrit. Also, at these times in 10 dogs, 5 control and 5 experimental, blood samples (5 ml) were collected in EDTA tubes for estimation of plasma renin concentration by the method mentioned above.

As in group A, gracilis vascular response to injection of sodium nitroprusside was tested in each animal.

IV. Statistical Methods

A. Student's t test for paired replicates was used within the control or experimental animals of each group: 1) To compare mean arterial pressures after renal artery constriction (or sham constriction) with control pressures; 2) to compare mean arterial pressures after administration of antirenin (Group B) or indomethacin (Group C) with control pressures; 3) to compare the percent changes in gracilis perfusion pressures when perfused with the various bloods; and 4) To compare pH, PO_2, PCO_2, serum Na^+, K^+, Ca^{++}, Mg^{++} and plasma renin concentrations of the various bloods.

B. The unpaired Student's t test was used among control and experimental animals of each group: 1) To compare percent changes in gracilis perfusion pressures when perfused with each blood; and 2) To compare pH, PO_2, PCO_2, serum Na^+, K^+, Ca^{++}, Mg^{++} and plasma renin concentrations in blood samples.

C. The unpaired Student's t test was used to compare gracilis perfusion pressures in animals that received indomethacin infusion with those that did not.

D. Slopes of the curves in Figure 3 for experimental animals were determined by regression analysis and were compared by Student's t test.

RESULTS

I. Group A Bioassay Studies

General data on the 12 control and 12 experimental dogs of Group A are presented in Table 1. To summarize, there were no significant differences in body weights, hematocrits, mean arterial blood pressures, weights of the gracilis muscles perfused and gracilis muscle perfusion flow rates between the two groups. Table 2 shows the baseline net perfusion pressures during control perfusions of the gracilis muscle with blood from left renal vein, right renal vein and vena cava. These values did not differ in the two groups of animals. In Table 3 are presented percent changes in gracilis net perfusion pressures during perfusion of the muscle with blood from LRV, RRV and VC following sham constriction or constriction of the left renal artery (LRA) in control and experimental animals respectively. The data show that in the control animals following sham constriction there is a slow steady increase in the perfusion pressure (1-7 percent) with the passage of time. This increase in perfusion pressure was similar whether the muscle was perfused with LRV, RRV or VC blood (Figure 3). This steady increase in perfusion pressure may have been due in part to gradual recovery from anesthesia and in part to slight bleeding from the surgery. In the experimental animals, as compared to control animals, perfusion with either LRV or VC blood after tightening the clamp on the left renal artery evoked significant

Table 1. Group means ± SEM of body weights, hematocrits, control mean arterial pressures, weights of gracilis muscles perfused and perfusion flow rates of group A.

	Control Group (N = 12)	Experimental Group (N = 12)	P Value for Comparison of Control Group with Experimental Group
Body wt. (kg)	24.0 ± 0.7	24.4 ± 4.0	> 0.5
Hematocrit (%)	43.3 ± 0.5	44.0 ± 0.8	> 0.9
Control mean arterial pressure (mm Hg)	139.2 ± 3.2	130.5 ± 4.0	> 0.1
Wt. of gracilis muscle perfused (gm)	84.6 ± 2.5	92.2 ± 3.9	> 0.2
Perfusion flow rate (ml/min 100 gm^{-1})	13.4 ± 0.4	13.6 ± 0.4	> 0.5

Table 2. Group means ± SEM of baseline net perfusion pressures during control perfusions of gracilis muscle with blood from left renal vein (LRV), right renal vein (RRV) and vena cava (VC) in experimental and control animals of group A.

	Baseline Net Perfusion Pressures mm Hg		P Value for Comparison of Control Group with Experimental Group
Vessel	Control Group (N = 12)	Experimental Group (N = 12)	
LRV	129.1 ± 3.1	125.3 ± 4.1	> 0.4
RRV	128.5 ± 3.6	126.2 ± 5.0	> 0.9
VC	117.5 ± 3.2	118.8 ± 5.6	> 0.9

Table 3. Group means ± SEM of percent increase in net perfusion pressures during perfusion with LRV, RRV and VC blood following sham constriction or constriction of the left renal artery (LRA) in control (C) and experimental (E) animals of group A, respectively. (N = 12)

Time#	LRV¢		RRV¢		VC¢	
	C	E	C	E	C	E
5 min	0.6±0.6	14.1±2.2***				
10 min	1.6±0.7	19.5±2.6***				
15 min	2.5±0.6	21.6±2.5***				
20 min	3.3±0.7	23.1±2.4***			1.9±1.4	20.5±2.3***
25 min	4.3±0.7	24.7±2.4***	2.8±0.9	5.8±3.5	2.7±1.5	22.4±2.4***
30 min	5.0±0.9	26.3±2.5***	3.9±1.1	7.0±3.5	3.4±1.7	23.6±2.7***
35 min	5.8±1.0	27.7±2.6***	4.9±1.2	8.7±3.5	3.8±1.9	25.1±3.0***
40 min	6.4±1.2	29.1±2.8***	6.0±1.4	10.3±3.5	4.1±2.1	26.5±3.3***
45 min	7.3±1.4	30.9±3.1***	6.8±1.6	12.3±3.4	4.5±2.2	28.1±3.8***

#Time in minutes following sham constriction or constriction of the LRA in control and experimental animals, respectively.

¢Percent increase in net perfusion pressures with LRV, RRV and VC blood, respectively.

***$P < 0.001$ for comparison of control group with experimental group.

Figure 3. Effect on gracilis perfusion pressure as the muscle is perfused with LRV, RRV and VC blood following constriction (sham constriction) of the left renal artery (LRA). The ordinate represents the net percent change in perfusion pressure compared to the respective baseline readings. The abcissa represents time in minutes after the renal artery is constricted or sham constricted in the experimental and control animals, respectively. Solid symbols represent the experimental animals and open symbols represent the control animals.

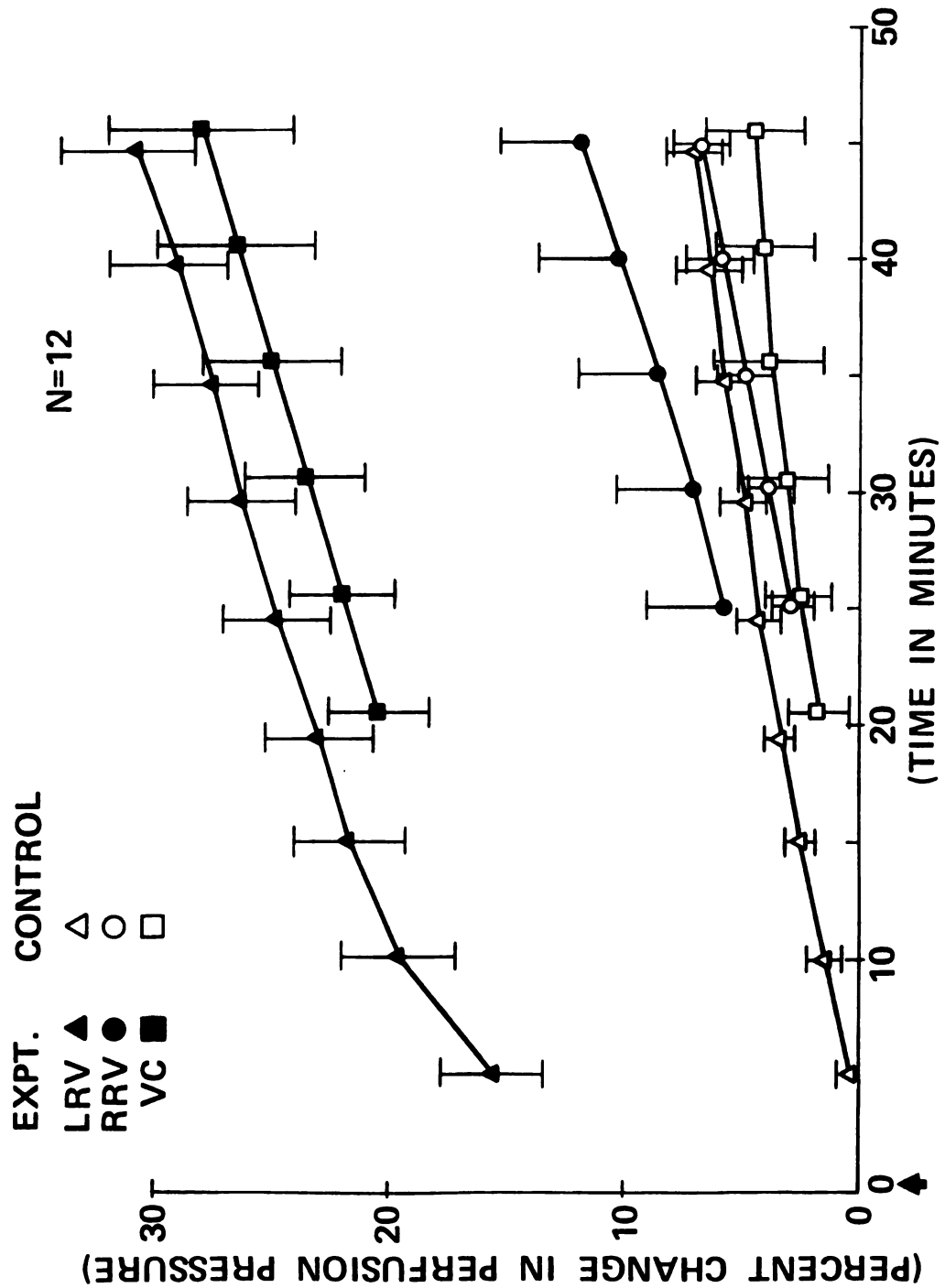

EXPT.
LRV
RRV
VC
CONTROL
N=12
(PERCENT CHANGE IN PERFUSION PRESSURE)
30
20
10
0
0
10
20
30
40
50
(TIME IN MINUTES)

Figure 3

and similar increases ($P < 0.001$) in gracilis perfusion pressure (Figure 3). In contrast, no similar increases ($P > 0.1$) in gracilis perfusion pressure were evoked by RRV blood. Despite this significant difference in mean values when gracilis was perfused by RRV blood in experimental animals, the slope of the RRV curve (Figure 3) did not differ from slopes of the LRV and VC curves over the period 25 to 45 minutes post renal artery constriction. These data, therefore, suggest that a significant increment in gracilis resistance evoked by LRV and VC blood occurred during the first 25 minutes after renal artery constriction. This increment was not produced by perfusing with RRV blood. In Table 4 are presented percent changes in gracilis net perfusion pressures, 35, 40 and 45 minutes post left renal artery constriction in 8 experimental animals in which the gracilis muscle was perfused with femoral arterial (FA) blood in addition to LRV, RRV, and VC blood. The data again show that perfusing the muscle with blood from LRV, VC or FA blood evoked similar increases in perfusion pressure which were significantly greater ($P < 0.02$) than perfusion pressures evoked by perfusion of the muscle with RRV blood (Figure 4). In Table 5 are presented group means of blood hematocrit, pH, PCO_2, PO_2, and serum electrolytes of LRV, RRV, VC and FA blood of experimental and control animals taken 25 minutes after LRA constriction or sham constriction respectively. The data show that pH, PO_2 and PCO_2 measured in LRV blood post constriction were similar to values in RRV blood. Blood from the VC, compared to LRV and RRV blood, had significantly lower pH and PO_2 ($P < 0.001$) and significantly higher PCO_2 ($P < 0.001$). The pH, PO_2 and PCO_2 of LRV, RRV, VC and FA blood from

Table 4. Group means ± SEM of percent increase in net perfusion pressure during perfusion of the gracilis with blood from LRV, RRV, VC and FA 35 to 45 minutes following constriction of the left renal artery (LRA) in 8 experimental animals of group A. (N = 8)

	Time in minutes following constriction of LRA		
	35 min	40 min	45 min
Percent increase in perfusion pressure with LRV blood	25.9 ± 3.3**	27.5 ± 3.6**	29.3 ± 4.0**
Percent increase in perfusion pressure with RRV blood	10.0 ± 4.1	11.7 ± 4.5	13.5 ± 4.8
Percent increase in perfusion pressure with VC blood	24.1 ± 3.8**	25.8 ± 4.3**	27.8 ± 4.9**
Percent increase in perfusion pressure with FA blood	25.0 ± 4.7**	24.8 ± 4.9**	25.1 ± 5.1*

*P < 0.02 comparison of RRV blood with other bloods

**P < 0.01 comparison of RRV blood with other bloods

Figure 4. Effect on gracilis perfusion pressure as the muscle is perfused with blood from LRV, RRV, VC and FA blood following constriction of the left renal artery (LRA) in experimental animals of group A. The ordinate represents the net percent change in perfusion pressure compared to the respective baseline readings. The abcissa represents time in minutes following constriction of LRA.

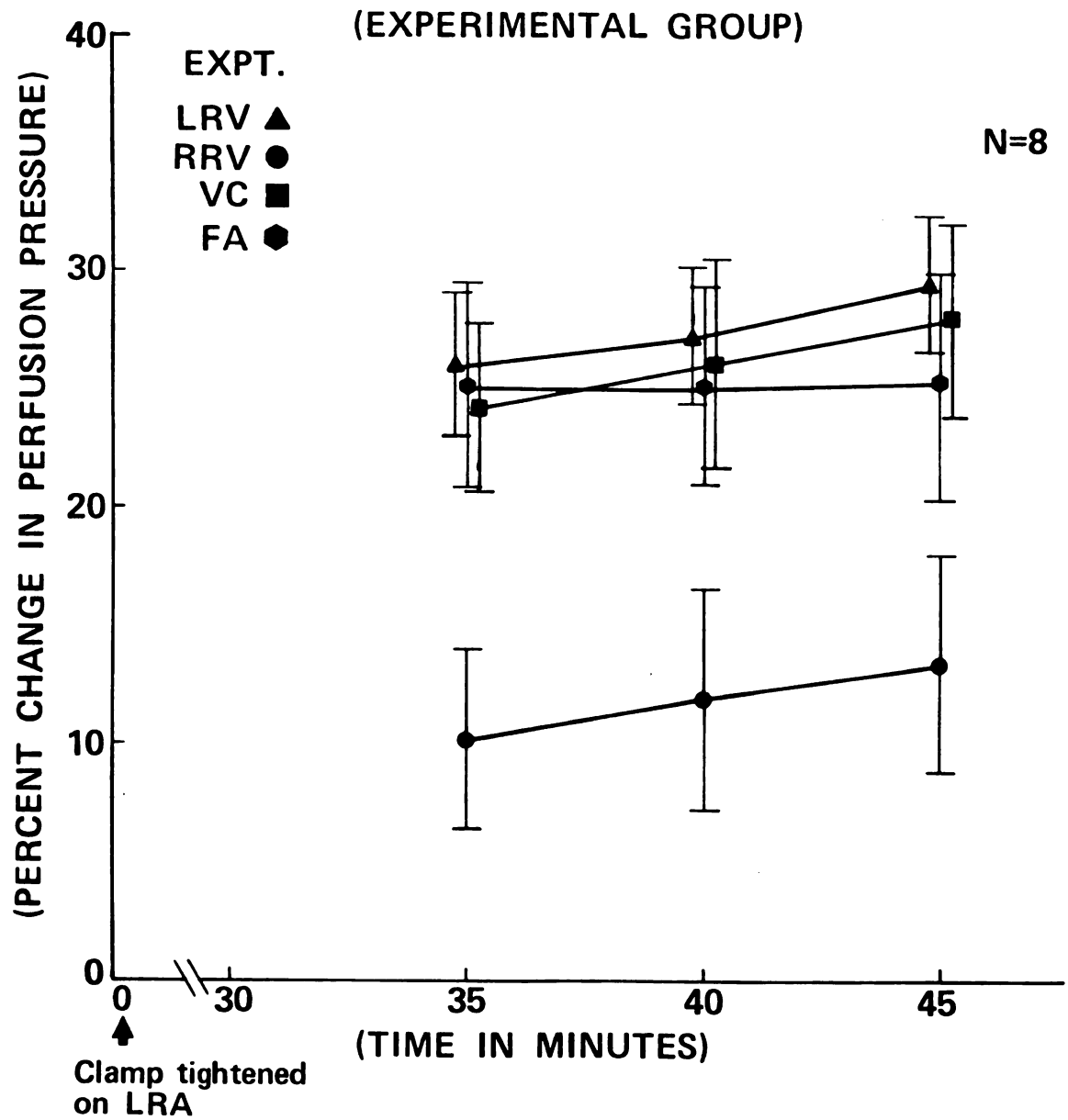

(EXPERIMENTAL GROUP)
EXPT.
LRV ▲
RRV ●
VC ■
FA ⬢
N=8
(PERCENT CHANGE IN PERFUSION PRESSURE)
40
30
20
10
0
0
30
35
40
45
(TIME IN MINUTES)
Clamp tightened on LRA

Figure 4

Table 5. Group means ± SEM of blood hematocrit, pH, blood gas tensions and serum electrolytes of blood samples removed from LRV, RRV, VC and FA 25 minutes after constriction or sham constriction of the left renal artery in experimental and control animals of group A, respectively.

	N	Experimental Group				Control Group			
		LRV	RRV	VC	FA	LRV	RRV	VC	FA
Blood hematocrit, vol. percent	10	43.7±1.0	43.9±0.9	43.8±1.1	44.1±0.9	42.7±0.5	43.1±0.4	43.1±0.4	42.4±0.4
Blood pH and gas tensions	9								
pH		7.40±0.01	7.40±0.01	7.37±0.01	7.42±0.01	7.39±0.01	7.39±0.01	7.37±0.01	7.40±0.01
PO_2 (mm Hg)		60.0±5.1	56.7±6.0	41.6±4.1	87.7±8.1	55.8±3.0	56.0±3.7	41.1±3.0	76.0±1.7
PCO_2 (mm Hg)		27.0±2.5	26.9±3.7	32.6±2.5	22.6±1.6	24.9±0.9	25.6±1.2	33.6±1.4	23.6±1.1
Serum electrolytes	7								
Na^+(mEq/L)		142.1±1.6	143.1±1.5	142.1±1.9	142.7±1.6	144.6±1.3	144.6±1.2	145.0±1.5	144.9±1.4
K^+(mEq/L)		4.0±0.2	3.9±0.2	4.1±0.1	4.0±0.2	4.2±0.1	4.1±0.1	4.2±0.2	4.1±0.2
Ca^{++} (mEq/L)		5.0±0.1	4.9±0.1	4.9±0.1	4.9±0.1	4.8±0.1	4.9±0.1	4.9±0.1	4.9±0.1
Mg^{++} (mEq/L)		1.93±0.03	1.97±0.04	1.94±0.03	1.95±0.02	1.98±0.04	1.96±0.03	1.99±0.06	1.98±0.05

experimental animals did not differ from values in blood from control animals. (The low PO_2 probably was related to small amount of lung atelectasis due to continuous positive pressure mechanical ventilation; the low PCO_2 was probably attributable to the hyperventilation which was carried out to maintain the pH.) The hematocrit and serum electrolytes of samples drawn from LRV, RRV, VC or FA in experimental and control animals also did not differ. Table 6 presents mean aortic blood pressures before and after constriction (or sham constriction) of the left renal artery. Following left renal artery constriction in the experimental group there was a significant increase ($P < 0.05$) in the mean arterial pressure as indicated by paired t test. No increase occurred in the control group. Injection of sodium nitroprusside into the perfusion system towards the end of the experiment lowered the perfusion pressure by 30 to 50 mm Hg in each dog, indicating that the gracilis muscle was capable of responding to vasoactive agents.

II. Group B Bioassay Studies

Control and experimental animals were subjected to the same protocol as group A animals, following immunization against renin during the control period. The general data of group B animals are presented in Table 7. Again the body weights, blood hematocrits, mean arterial blood pressures, weights of the gracilis muscles perfused and perfusion flow rates in control animals did not differ from values in experimental animals. Table 8 indicates that bolus intravenous injection of homologus antirenin had no effect on mean arterial pressure ($P > 0.5$) during the

Table 6. Group means ± SEM of mean aortic blood pressure (mm Hg) before (control time) and after (experimental time) the sham constriction or constriction of the left renal artery in control and experimental animals, respectively. N = 12

	Mean arterial pressure (mm Hg) during control time	Mean arterial pressure (mm Hg) during experimental time (minutes after (sham) constriction)								
		5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min
Control group	139.2	138.8	138.8	138.9	138.8	138.8	138.9	138.9	138.1	138.1
Δ Control group		-0.4±0.4	-0.4±0.4	-0.3±0.5	-0.4±0.7	-0.4±0.7	-0.3±0.8	-0.3±0.8	-1.1±1.1	-1.1±1.1
Experimental group	130.5	133.1	137.6	138.2	138.4	138.2	137.4	138.4	137.4	138.5
Δ Experimental group		2.6±1.8	7.1±2.9*	7.7±3.1*	7.9±3.5*	7.7±3.3*	6.9±3.6	7.9±3.5*	6.9±3.9	8.0±4.2

*Significantly different from blood pressure during control time ($P < 0.05$)

Table 7. Group means ± SEM of body weights, hematocrit, control mean arterial pressure, weight of gracilis muscle perfused and perfusion flow rate of group B animals.

	Control Group N = 7	Experimental Group N = 7	P Value for Comparison of Control Group with Experimental Group
Body weight (kg)	24.4 ± 0.7	24.5 ± 0.4	> 0.9
Hematocrit (%)	41.9 ± 0.8	43.5 ± 0.7	> 0.2
Control mean arterial pressure (mm Hg)	135.1 ± 6.7	142.7 ± 4.6	> 0.4
Weight of gracilis muscle perfused (gm)	82.0 ± 5.0	83.3 ± 3.3	> 0.9
Perfusion flow rate (ml/min 100 gm^{-1})	12.8 ± 1.0	13.1 ± 0.5	> 0.9

Table 8. Group means ± SEM of control mean arterial pressure and mean arterial pressure following antirenin injection in group B animals as well as separately in control and experimental animals.

Group	N	Control mean arterial pressure	Mean arterial pressure following immunization against renin			
			10 min	P value for comparison of control BP with BP after immunization in the same group	20 min	P value for comparison of control BP with BP after immunization in the same group
Group B animals Δ in Group B	14	138.7	138.9 0.2±1.0	> 0.5	138.9 0.2±1.0	> 0.5
Control animals Δ in control animals	7	133.6	135.1 1.5±1.6	> 0.2	135.1 1.5±1.6	> 0.2
Experimental animals Δ in experimental animals	7	143.9	142.7 -1.3±1.2	> 0.2	142.7 -1.3±1.4	> 0.2

20 minutes following injection. In Table 9 are presented percent changes in gracilis net perfusion pressures following constriction (or sham constriction) of the left renal artery in animals of group B. The data show that, following immunization against renin, there was a slow steady increase in gracilis perfusion pressure in the control group with time as in group A controls. In contrast to group A, however, constriction of the left renal artery in experimental animals did not increase gracilis perfusion pressure over that of controls when perfused with LRV, RRV, VC or FA blood (Figures 5 and 6). In Table 10 are presented blood hematocrits, pHs, blood gas tensions and serum electrolytes of LRV, RRV, VC and FA blood removed 25 minutes post constriction (or sham constriction) of the left renal artery. Again, as in group A, the pH, PO_2 and PCO_2, blood hematocrit and serum electrolytes of LRV, RRV, VC and FA blood from the experimental animals did not differ ($P > 0.2$) from values in the control group. Table 11 presents the mean aortic blood pressures before and after constriction (or sham constriction) of the left renal artery following immunization against renin. In contrast to group A, constriction of the left renal artery failed to produce a rise in blood pressure in these immunized experimental animals. As in group A, no increase in mean arterial pressure was again observed in the control animals.

As in group A, sodium nitroprusside injection decreased gracilis resistance in each animal.

Table 9. Group means ± SEM of percent increase in net perfusion pressure during perfusion of the gracilis muscle with blood from LRV, RRV, VC and FA following sham constriction or constriction of the left renal artery (LRA) in control (C) and experimental (E) animals, respectively, of group B after these animals had been immunized against renin. N = 7

Time[#]	LRV[¢]		RRV[¢]		VC[¢]		FA[¢]	
	C	E	C	E	C	E	C	E
5 min	2.0±0.6	2.1±0.9						
10 min	2.2±0.6	3.2±0.9						
15 min	2.6±0.7	3.9±1.0						
20 min	2.8±0.8	4.9±1.2			3.4±2.1	3.6±1.5		
25 min	2.9±1.0	5.7±1.3	3.4±0.8	4.2±1.2	3.9±2.2	4.4±1.6		
30 min	3.3±1.2	6.6±1.5	5.1±1.4	5.0±1.1	4.0±2.5	5.1±1.8		
35 min	3.6±1.4	7.6±1.7	5.2±1.3	5.3±1.3	4.6±2.5	5.9±2.1	6.2±1.6	5.4±2.1
40 min	4.0±1.3	8.2±2.0	5.4±1.3	5.9±1.3	4.9±3.2	6.8±2.2	7.0±1.6	6.0±2.0
45 min	5.0±1.1	8.8±2.1	6.3±1.1	6.5±1.5	5.1±3.4	7.4±2.5	7.8±1.4	6.2±2.0

[#]Time in minutes following sham constriction or constriction of the LRA in control and experimental animals, respectively.

[¢]Percent increase in perfusion pressure with LRV, RRV, VC and FA blood, respectively.

Figure 5. Effect on gracilis perfusion pressure as the muscle is perfused with LRV, RRV, VC and FA blood after sham constriction of the left renal artery (LRA) following immunization against renin in control animals of group B. The ordinate represents the net percent change in perfusion pressure compared to the respective baseline readings. The abcissa represents time in minutes following sham constriction of LRA.

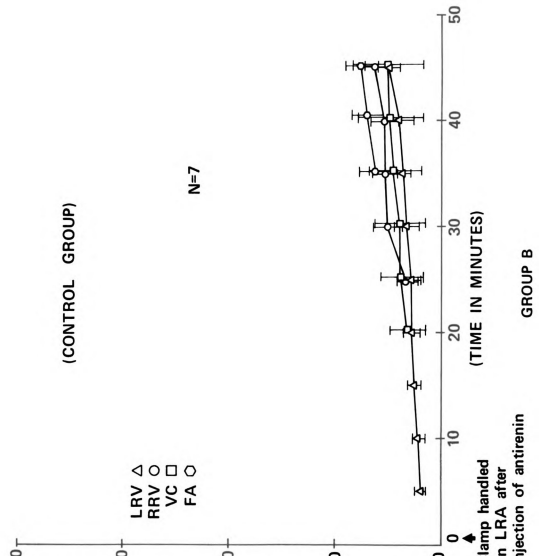

(CONTROL GROUP)
N=7
LRV △
RRV ○
VC □
FA ○
(PERCENT CHANGE IN PERFUSION PRESSURE)
40
30
20
10
0
0
10
20
30
40
50
(TIME IN MINUTES)
Clamp handled on LRA after injection of antirenin
GROUP B

Figure 5

Figure 6. Effect on gracilis perfusion pressure as the muscle is perfused with LRV, RRV, VC and FA blood after constriction of the left renal artery (LRA) following immunization against renin. The ordinate represents percent change in perfusion pressure compared to the respective baseline readings. The abcissa represents time in minutes following constriction of LRA.

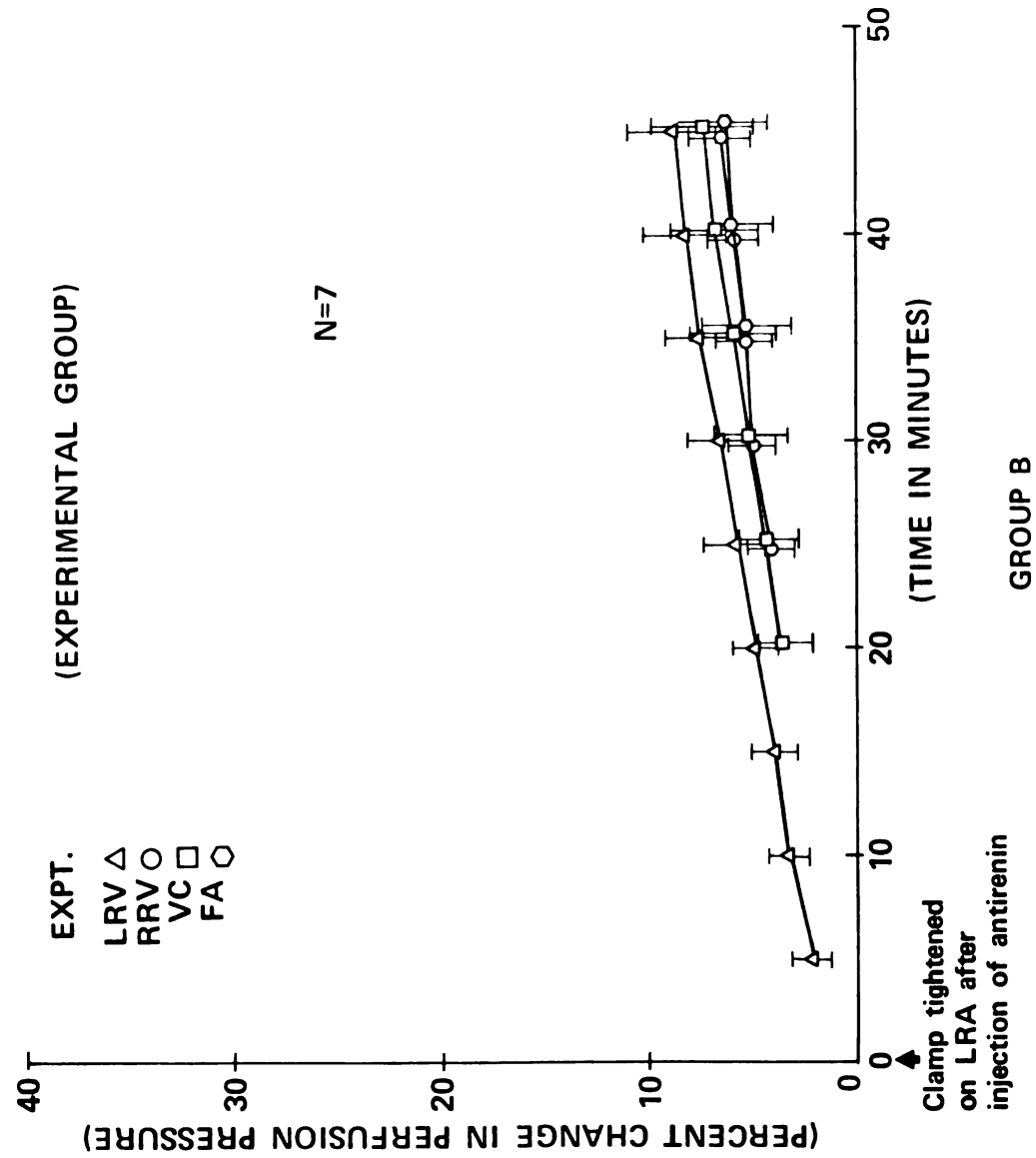

(PERCENT CHANGE IN PERFUSION PRESSURE)
40
30
20
10
0
0
10
20
30
40
50
(TIME IN MINUTES)
Clamp tightened on LRA after injection of antirenin
EXPT.
LRV △
RRV ○
VC □
FA ⬡
(EXPERIMENTAL GROUP)
N=7
GROUP B

Figure 6

Table 10. Group means ± SEM of blood hematocrit percent, pH, blood gas tensions and serum electrolytes of blood samples removed from LRV, RRV, VC and FA 25 minutes after sham constriction or constriction of the left renal artery in control and experimental animals of group B, respectively.

	N	Control Animals				Experimental Animals			
		LRV	RRV	VC	FA	LRV	RRV	VC	FA
Blood hematocrit (%)	7	42.5±1.2	42.7±1.2	42.4±0.9	42.5±1.2	42.9±0.6	43.1±0.7	42.3±0.8	43.1±0.9
Blood pH and gas tensions	7								
pH		7.39±0.01	7.39±0.01	7.36±0.01	7.4±0.01	7.39±0.01	7.39±0.01	7.36±0.01	7.41±0.01
PO_2 (mm Hg)		59.6 ±2.7	56.1 ±2.7	45.4 ±1.8	66.7±2.1	60.3 ±1.7	57.8 ±1.6	45.0 ±1.1	70.3 ±1.7
PCO_2 (mm Hg)		22.6 ±0.8	22.0 ±0.6	30.1 ±0.8	20.4±0.5	22.4 ±1.2	21.1 ±0.7	31.9 ±1.2	20.4 ±0.9
Serum electrolytes	7								
Na^+ (mEq/L)		142.4±1.5	141.7±1.4	141.3±1.2	143.4±1.3	143.3±1.4	142.7±0.6	142.4±1.4	142.6±1.7
K^+ (mEq/L)		4.0±0.1	3.9±0.1	4.0±0.1	4.0±0.1	3.8±0.1	4.0±0.1	3.9±0.1	4.0±0.1
Ca^{++} (mEq/L)		4.9±0.2	5.1±0.1	5.0±0.2	5.1±0.1	5.1±0.1	5.1±0.1	5.1±0.1	5.3±0.1
Mg^{++} (mEq/L)		2.05±0.05	2.07±0.06	2.03±0.04	2.07±0.06	2.11±0.04	2.09±0.04	2.08±0.07	2.07±0.07

Table 11. Group means ± SEM of mean aortic pressure (mm Hg) before (control time) and after (experimental time) sham constriction or constriction of the left renal artery following immunization against renin in the control and the experimental animals of group B, respectively. N = 7

Group	Control mean arterial pressure#	Mean arterial pressure (mm Hg) during experimental time (minutes following (sham) constriction of LRA)								
		5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min
Control group	135.1	134.7	134.3	133.9	133.9	133.9	134.0	134.0	135.7	134.6
Δ in control group		-0.4±1.2	-0.8±1.2	-1.2±1.2	-1.2±1.2	-1.2±1.2	-1.1±1.2	-1.1±1.2	0.6±1.2	-0.5±1.4
Experimental group	142.7	143.4	142.9	143.0	142.7	143.1	142.6	142.9	142.3	143.0
Δ in experimental group		0.7±0.7	0.2±0.9	0.3±0.5	0.0±0.4	0.4±0.8	-0.1±0.9	0.2±0.9	-0.4±1.2	0.3±1.1

#Mean arterial pressure (mm Hg) during control time after immunization against renin.

III. Group C Bioassay Studies

Eight control and eight experimental animals received intravenous infusions of indomethacin during the control period. The general data of this group are presented in Table 12. Again body weights, hematocrits, control mean arterial pressures, weights of gracilis muscles perfused and perfusion flow rates in control and experimental groups did not differ. By twenty minutes after beginning the intravenous infusion of indomethacin, mean arterial pressure was significantly elevated ($P < 0.01$) in both control and experimental animals (Table 13). The gracilis resistance was also elevated ($P < 0.01$) in these animals compared to animals that did not receive indomethacin. In Table 14 are presented percent changes in gracilis net perfusion pressures following constriction (or sham constriction) of the left renal artery in group C animals. In the experimental animals, as compared to the controls gracilis perfusion with LRV, RRV, VC or FA blood after tightening the clamp on the left renal artery evoked similar significant increases ($P < 0.01$) in perfusion pressures (Figure 7). Again, blood hematocrit estimated before and 25 minutes after the manipulation of the left renal artery did not differ in the two groups (control animals Hct. before 42.4 ± 0.5 after 42.2 ± 0.5, experimental animals Hct. before 42.4 ± 0.4 after 41.5 ± 0.5).

IV. Radioimmunoassay of Plasma Renin Concentration

In Table 15 are presented measured plasma renin concentrations (PRC) in LRV, RRV, VC and FA blood, collected from some animals of group C (and group A) 25 minutes post manipulation of the left renal artery. Blood from the left renal vein and vena cava in the experimental animals

Table 12. Group means ± SEM of body weights, hematocrits, control mean arterial pressures, weights of gracilis muscles perfused and perfusion flow rates of group C animals.

	Control Group (N = 8)	Experimental Group (N = 8)	P Value for Comparison of Control Group with Experimental Group
Body Weight (kg)	23.9 ± 0.8	24.5 ± 0.6	> 0.9
Hematocrit (%)	43.9 ± 0.5	43.8 ± 0.5	> 0.9
Control mean arterial pressure (mm Hg)	135.6 ± 3.6	138.8 ± 2.6	> 0.5
Weight of the gracilis muscle perfused (gm)	80.9 ± 3.4	90.8 ± 3.0	> 0.05
Perfusion flow rate (ml/min 100 gm^{-1})	13.8 ± 0.3	12.3 ± 0.6	> 0.9

Table 13. Data showing effect of intravenous infusion of indomethacin on arotic blood pressure in the control and the experimental animals of group C.

	Control mean aortic pressure	Mean aortic pressure (mm Hg) following the beginning of I.V. infusion of indomethacin			
		10 min	20 min	30 min	40 min
Control animals	135.6	137.5	140.6	141.2	144.0
Δ in control animals		1.9±1.4	5.0±1.6*	5.6±1.5**	8.4±2.0**
Experimental animals	138.8	141.2	143.8	147.2	147.5
Δ in control animals		2.4±1.2	5.0±1.2**	8.4±1.2***	8.7±1.2***

* $P < 0.02$
** $P < 0.01$
*** $P < 0.001$

for comparison of mean aortic pressure before and after indomethacin infusion in each group.

Table 14. Group means + SEM of percent increase in gracilis net perfusion pressure during perfusion with blood from LRV, RRV, VC and FA following sham constriction or constriction of the left renal artery (LRA) in conrrol (C) and experimental (E) animals of group C, respectively, following intravenous infusion of indomethacin. N = 8

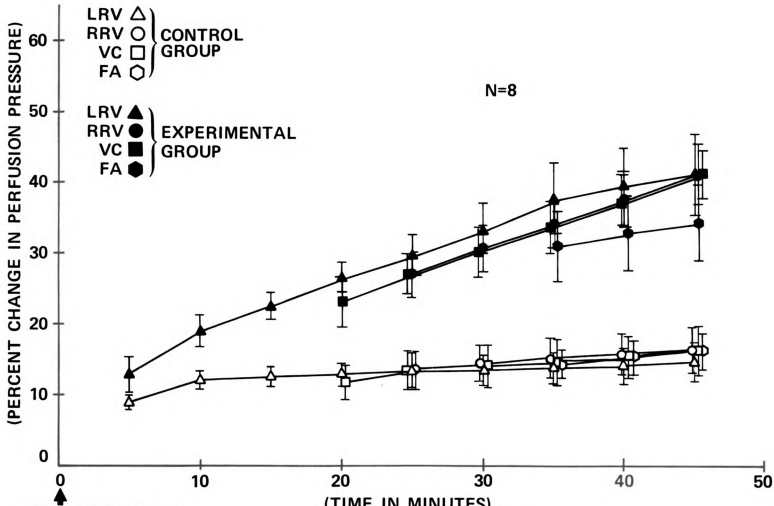
Time[#]	LRV[¢]		RRV[¢]		VC[¢]		FA[¢]	
	C	E	C	E	C	E	C	E
5 min	8.8±0.8	12.8±3.2						
10 min	12.1±1.3	19.0±2.2*						
15 min	12.5±1.4	22.5±2.0**						
20 min	12.9±1.6	26.3±2.4**			10.8±2.0	23.0±3.4*		
25 min	13.2±1.8	29.6±2.4**	13.7±2.5	27.1±3.2*	11.6±2.2	26.7±3.4**		
30 min	13.5±1.8	33.2±3.9**	14.6±2.7	30.7±3.2**	12.8±2.4	30.3±3.6**		
35 min	13.9±2.3	37.6±5.1**	15.3±3.0	34.2±3.3**	14.0±2.7	33.8±3.7**	14.6±1.8	31.0±5.0*
40 min	14.2±2.5	39.6±5.6**	15.9±3.2	37.8±3.6**	14.9±3.0	37.2±3.8**	15.8±2.0	32.7±5.0*
45 min	15.0±2.7	41.2±5.7**	16.7±3.3	41.2±3.9**	15.9±3.3	40.4±4.1**	16.8±2.2	34.1±5.1*

[#]Time in minutes following sham constriction or constriction of the LRA in control and experimental animals, respectively.

[¢]Percent increase in net perfusion pressure with LRV, RRV, VC and FA blood, respectively.

* P < 0.05
** P < 0.01
} for comparison of blood from experimental group with appropriate control blood.

Figure 7. Effect on gracilis perfusion pressure as the muscle is perfused with LRV, RRV, VC and FA blood after constriction (sham constriction) of the left renal artery following indomethacin infusion. The ordinate represents percent change in perfusion pressure compared to the respective baseline readings. The abcissa represents time in minutes following constriction or sham constriction of LRA in the experimental and control animals, respectively. Solid symbols represent the experimental animals and open symbols represent the control animals.



GROUP C

Figure 7

Table 15. Plasma renin concentration (PRC) estimated in LRV, RRV, VC and FA blood 25 minutes post sham constriction or constriction of the left renal artery in some control and some experimental animals, respectively, of both group A (N = 8) and C (N = 10).

Group	PRC (ng/ml/hr)			
	LRV blood	RRV blood	VC blood	FA blood
Control group	29.0± 7.6	33.4±10.2	15.7± 4.2	20.1± 5.6
Experimental group	118.0±21.6**	50.5±12.6	42.5±11.5*	44.7±11.3

**P < 0.01 comparing control group blood to the respective blood from the experimental group.

*P < 0.05 comparing control group blood to the respective blood from the experimental group.

Table 16. Group means ± SEM of mean aortic pressure (mm Hg) before (control time) and after (experimental time) the sham constriction or constriction of the left renal artery following indomethacin infusion in the control and the experimental animals of group C, respectively. N = 8

Group	Control mean aortic pressure+	Mean aortic pressure (mm Hg) during experimental time (minutes following (sham) constriction of LRA)								
		5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min
Control group	144.5	144.1	144.9	145.2	144.9	143.4	143.1	143.8	143.0	143.0
Δ in control group		-0.4±0.9	0.4±0.7	0.7±0.9	0.4±1.0	-1.1±1.0	-1.4±1.2	-0.7±1.9	-1.5±1.5	-1.5±1.8
Experimental group	147.5	153.4	154.6	155.2	157.0	158.8	159.0	159.4	159.0	159.6
Δ in experimental group		5.9±1.6**	7.1±1.7**	7.7±1.6**	9.5±1.6***	11.3±1.9***	11.5 ±1.7***	11.9±1.9***	11.5±2.2**	12.1±1.8***

+ Mean aortic pressure (mm Hg) during control time after indomethacin infusion.

** Significantly different from blood pressure during control time ($P < 0.01$).

*** Significantly different from blood pressure during control time ($P < 0.001$).

had significantly higher plasma renin concentrations than that from the left renal vein and vena cava respectively in control animals. However, there was no significant difference in PRC of RRV and FA blood from experimental animals compared to the respective blood from the control animals. In control animals PRC in right and left renal vein bloods compared by paired t test was significantly higher ($P < 0.05$) than that in the vena cava blood. Similarly in experimental animals PRC in right and left renal vein bloods was significantly higher ($P < 0.05$) compared to vena cava blood. Table 16 presents mean aortic blood pressure before and after constriction (or sham constriction) of the left renal artery following indomethacin infusion. Following left renal artery constriction in the experimental group there was a significant increase ($P < 0.001$) in the mean arterial pressure. Elevation in blood pressure occurred within 5 minutes of renal artery constriction and remained elevated throughout the experiment. Again no increase occurred in the control group. As in group A sodium nitroprusside injection decreased gracilis resistance in each animal.

DISCUSSION

These studies demonstrate that following acute unilateral renal artery constriction in dogs there is a significant increase in the systemic arterial pressure as previous investigators have reported. In the present experiments blood pressure was elevated within 10 minutes of renal artery constriction. However, by the end of 25 minutes after constriction the blood pressure returned to its preconstriction level. This rise in blood pressure was associated with a significant increase in plasma renin concentration (PRC) in venous blood from the constricted kidney and vena caval blood, indicating an increased release of renin by the constricted kidney. After acute unilateral renal artery constriction the systemic blood increased gracilis muscle vascular resistance. As there was no change in the hematocrit and therefore probably the viscosity of this blood, the rise in gracilis resistance may be attributed to vasoconstriction. This is the first report indicating that the vasoconstrictor activity of systemic blood becomes elevated as early as 20 min after unilateral renal artery constriction. Thus, systemic vasoconstriction probably accounts, at least in part, for the rapid rise observed in systemic blood pressure. Increase in the vasoconstrictor activity of the systemic blood suggests that renin released by the constricted kidney generated angiotensin II which circulated in the systemic blood producing generalized vasoconstriction, thus elevating the blood

pressure. That the circulating vasoconstrictor agent is related to renin release is confirmed by the effect of immunization against renin. Such immunization prevented the increase in mean arterial pressure after renal artery constriction. Furthermore, the systemic blood in these immunized animals did not elicit gracilis vasoconstriction. These data support our conclusion that the early elevation of arterial pressure observed in experimental animals following renal artery constriction is due to the renin-angiotensin system and argue against participation by other vasoconstrictors released by the kidney. However, our data do not allow us to speculate about mechanisms involved in the chronic stages of Goldblatt hypertension.

These results are in agreement with those of several other investigators (Ebihara and Grollman, 1968; Bianchi et al., 1972; Gutmann et al., 1973) who have associated the development of hypertension following acute unilateral renal artery constriction with an increase in plasma renin activity (PRA). Gutmann et al. (1973) found that PRA in the renal venous blood of a constricted kiendy was elevated within one minute of renal artery constriction in conscious dogs. In their experiments blood pressure was elevated within 5 minutes of renal artery constriction and remained elevated for at least 1-7 hours. The failure of the blood pressure in our experiments to remain elevated may have been due in part to intervention of the baroreceptor depressor mechanism. Cowley et al. (1972) have reported that only a slight rise in the systemic arterial pressure is observed following infusion of angiotensin II in the presence of an intact nervous system in anesthetized animals.

Our data from animals immunized against renin appear to exclude the possibility that "nephrotensin" or some precursor of nephrotensin is released by the constricted kidney, as suggested by Grollman and Krishinamurty (1971). However, our data cannot rule out the possibility that "nephrotensin" or some vasoconstrictor agent in addition to angiotensin II is formed systemically as a result of the enzymatic action of renin. This possibility could be best examined by the use of specific angiotensin II blocking agents. In this regard Bumpus et al. (1973) and Bing and Neilsen (1973) showed that injection of angiotensin II analogues completely reversed the hypertension induced by unilateral renal artery constriction. These data, therefore, strongly support our conclusion that angiotensin is the only vasopressor agent formed by the release of renin due to renal artery constriction.

In the animals that received indomethacin, the systemic arterial pressure was elevated within 5 minutes of the renal artery constriction and remained elevated throughout the experiment. The more sustained elevation of blood pressure in these animals cannot be attributed to an independent vasoconstrictor effect of indomethacin (if any) as the control animals also received indomethacin but showed no elevation of mean arterial pressure following sham constriction of the renal artery. Because indomethacin inhibits prostaglandin synthesis, these findings suggest that the more sustained hypertension in these animals was probably due to absence of prostaglandin release. These data, therefore, strongly suggest that in the early stages of two kidney Goldblatt hypertension elevation of blood pressure is attenuated not only by baroreceptor depressor mechanism but also by the release of prostaglandins.

In the present experiments, venous blood from the constricted kidney increased gracilis vascular resistance within 5 minutes after constriction. The systemic blood of these animals elicited similar increases in gracilis resistance. As there was no change in the hematocrit and therefore probably in the viscosity of renal venous and systemic blood, the rise in gracilis resistance may be attributed to vasoconstriction. This vasoconstriction cannot be attributed to changes in serum concentrations of Na^{+}, K^{+}, Ca^{++} and Mg^{++}, nor to changes in blood gas tension or pH. Furthermore, renin immunization blocked this vasoconstrictor activity, indicating that the vasoconstriction is due to increase in the activity of the renin-angiotensin system.

As unilateral constriction of renal artery stimulates that kidney to release renin with subsequent formation of angiotensin II we might expect higher levels of vasoconstrictor in the venous blood from the constricted kidney compared to systemic blood. However, several investigators have shown that renin itself is not a vasoactive agent (Kohlstaedt et al.,1938; Braun-Menendez et al.,1940). A number of investigators (Ng et al., 1967; Bakhle, 1968 and Oparil et al., 1970) have further shown that conversion of angiotensin I to angiotensin II mainly takes place in the lung. Most investigators now agree that kidney tissue also contains converting enzyme and has the capacity to convert angiotensin I to angiotensin II. This capacity, however, is very small, only 6-7% of angiotensin I being converted to angiotensin II within the kidney. Bailie et al. (1971) have further shown that newly synthesized angiotensin II within the kidney is not in equilibrium with renal venous plasma but

instead enters the renal lymphatics. Thus generation of active vasoconstrictor agent (angiotensin II) in the systemic circulation probably explains the similar increase in the vasoconstrictor activity of venous blood from the constricted kidney and systemic blood in the present experiments. However, it is also possible that venous blood from the constricted kidney contained vasodilator substance which reduced net vasoconstrictor activity of renal blood to the level of systemic blood.

In striking contrast to the significant increase in gracilis vasoconstriction evoked by venous blood from the constricted kidney and also by systemic blood, no vasoconstriction occurred when the gracilis was perfused by venous blood from the opposite untouched kidney. This difference in response to venous blood from the opposite untouched kidney was probably not due to a difference in blood viscosity as the hematocrits were similar. Similarly, concentrations of Na^{+}, K^{+}, Ca^{++} and Mg^{++}, pH, PO_2 and PCO_2 of the venous blood from the constricted kidney did not differ from those in venous blood from the opposite kidney. The difference in response to venous blood from the untouched kidney also cannot be explained by differences in PRC, which if anything are in the wrong direction. Thus the decreased vasoconstrictor activity of venous blood from the untouched kidney means that this kidney inactivated the circulating angiotensin II or secreted vasodilator substance of its own, or both. To investigate the role of prostaglandins in this response, animals were infused with indomethacin. Indomethacin, by its inhibitory action on the enzyme prostaglandin synthetase, blocks the synthesis of prostaglandins (Herbacznska-Cedra and Vane, 1972). Moreover, prostaglandins are not stored in any appreciable amounts (Crowshaw, 1971).

Thus blocking their synthesis is equivalent to completely inhibiting their secretion. In contrast to animals not receiving indomethacin, constriction of the renal artery following indomethacin evoked increases in the vasoconstrictor activity of venous blood from the untouched kidney. These increases were similar to those in venous blood from the constricted kidney, vena caval blood, and arterial blood. Thus the difference in the vasoactivity of venous blood from the untouched kidney disappeared after indomethacin infusion. It is, therefore, reasonable to conclude that partial constriction of the renal artery in some way stimulates the opposite kidney to release vasodilator prostaglandin, probably PGE_2, into its venous blood. Further, these data strongly argue against the possibility that the untouched kidney releases vasodilator substances other than prostaglandins, because the only known function of indomethacin on vasoactive agents is to inhibit prostaglandin synthesis. In this regard this is the first report providing such evidence that no vasodilator substances, other than prostaglandins, are released by the untouched kidney following unilateral renal artery constriction. Regarding inactivation of circulating vasoconstriction agent, previous reports suggest that renal tissue does extract and excrete angiotensin II (Oparil and Bailie, 1973 and Oparil and Haber, 1974) but the present data strongly suggest that such a function occurs no more in the untouched than in the constricted kidney.

Our results in regard to release of prostaglandins by the opposite untouched kidney are in agreement with those of McGiff et al. (1970). However, these investigators demonstrated release of prostaglandins not only from the opposite kidney but also from the constricted kidney.

In contrast, our data argue against release of hemodynamically significant amounts of prostaglandin from the constricted kidney, at least in the very early stages of Goldblatt hypertension. Following renal artery constriction there was a similar increase in the vasoconstrictor activity of venous blood from the constricted kidney and systemic blood. If significant amounts of prostaglandin were present in blood from the constricted kidney we would have expected attenuated vasoconstrictor activity. However, it is possible, but unlikely, that release of vasodilator prostaglandins by the constricted kidney exactly counteracted the vasoconstrictor effect of denovosynthesized angiotensin II within that kidney.

If significant amounts of prostaglandins were being released from the constricted kidney, the vasoconstrictor activity of its venous blood should be enhanced following indomethacin. However, the vasoconstrictor activity of venous blood from the constricted kidney and the systemic blood, were again similar after indomethacin, strongly arguing against the possibility of significant release of prostaglandins by the constricted kidney (and also against significant synthesis of angiotensin II within that kidney). These results are, therefore, not entirely in agreement with those of McGiff et al. (1970). We suggest two possible explanations for this difference in results: 1) A bank of organs consisting of rat stomach, rat colon and chick rectum were used by McGiff et al. to detect prostaglandins. Prostaglandins produce contraction of all three tissues. However, angiotensin II also contracts rat colon and rat stomach, raising the possibility of difficulties in differentiation between prostaglandins and angiotensin II, if both agents are present in the superperfusate. 2) Our bioassay system registered only net change in vasoactivity.

Therefore, it is possible that in our experiments gracilis vasoconstriction by angiotensin II might have overshadowed the vasodilation produced by small amounts of prostaglandins released by the constricted kidney. The opposite kidney, on the other hand, apparently releases much greater amounts of prostaglandins.

In their subsequent study, McGiff et al. (1970) showed that the stimulus for the release of renal prostaglandins following renal artery constriction is the elevated levels of angiotensin II. Our findings in animals immunized against renin seem to agree with the findings of these investigators. In these immunized animals, constriction of the renal artery evoked neither the vasoconstrictor response to systemic blood nor the vasodilator response to blood from the untouched kidney. These results indicate that constriction of the renal artery and, therefore, decrease in renal perfusion pressure per se does not stimulate release of prostaglandins, and suggest that in this experimental setting prostaglandins are probably released in response to elevated circulating levels of angiotensin II. However, the present data do not rule out the possibility that increased systemic arterial pressure may stimulate the release of prostaglandins.

In these experiments, immunization against renin by itself produced no significant change in the mean arterial pressure indicating that the renin-angiotensin system probably does not play a significant role in the maintenance of arterial pressure, at least in anesthetized dogs. This finding is in agreement with the work of other investigators (Samuels et al., 1973; Pals et al., 1971; and Johnson and Davis et al.,

1973) who have shown that in normotensive dogs on a normal salt intake, angiotensin II is not required for maintenance of the blood pressure. On the other hand, following indomethacin infusion in our experiments, there was a significant increase in gracilis vasoconstriction and elevation of the mean arterial pressure, suggesting that prostaglandins, by altering peripheral resistance, may play an important role in maintenance of normal blood pressure. However, it is possible that indomethacin itself has vasoconstrictor properties.

SUMMARY AND CONCLUSIONS

To summarize, our data show that following unilateral renal artery constriction there is an increase in the arterial pressure within 10 minutes. This increase in the arterial pressure is associated with release of renin by the constricted kidney. Renin stimulates the synthesis of angiotensin II in systemic blood. The elevated levels of angiotensin II increase peripheral resistance, as shown by the increase in vasoconstrictor activity of systemic blood within 20 minutes. It is unlikely that any other vasoconstrictor system plays a role in this response. Our data further show that the elevated vasoconstrictor activity of systemic blood is apparently "titrated" within the untouched kidney by an increased release of prostaglandins, probably PGE_2, by this kidney. There is no evidence for release of vasodilator substances other than prostaglandins. These prostaglandins reduce the duration of the hypertension. Thus, in addition to the antihypertensive role of its excretory function, the opposite untouched kidney in early stages of two kidney Goldblatt hypertension importantly attenuates the hypertension by release of vasodilator prostaglandins.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adetuyibi, A., and Mills, I. H.: Relation between urinary kallikrein and renal function, hypertension, and excretion of sodium and water in man. Lancet, 2:203, 1972.

Aikin, J. W., and Vane, J. R.: Intra-renal prostaglandin release attenuates the renal vasoconstrictor activity of angiotensin. J. Pharmacol. Exp. Ther. 184:678, 1973.

Allison, D. J., Clayton, P. A., Passo, S. S., and Assaykeen, T. A.: The effect of isoproterenol and adrenergic blocking agents on plasma renin activity and renal function in anesthetized dogs. Fed. Proc. 29:782, 1970.

Anggärd, E., Bohman, S. O., Griffin, J. E., III, Larsson, C., and Maunsbach, A. B.: Subcellular localization of the prostaglandin system in rabbit-renal papilla. Acta. Physiol. Scand. 84:231, 1972.

Bailie, M. D., Rector, F. C., Jr., Seldin, D. W.: Angiotensin II in arterial and renal venous plasma and renal lymph in the dog. J. Clinc. Invest., 50:119, 1971.

Bakhle, V. S.: Conversion of angiotensin I to angiotensin II by cell free extracts of dog lung. Nature (Lond.) 220:219, 1968.

Barajas, L.: The innervation of juxtaglomerular apparatus. An electron microscopic study of the innervation of the glomerulus arterioles. Lab. Invest. 13:916, 1964.

Barroclough, M. A., and Mills, I. H.: Effect of bradykinin on renal function. Clin. Science, 28:69, 1965.

Bergostrom, S., Rhyage, R., Samuelsson, B., and Sjovall, J.: The structure of prostaglandin E, F_1 α and F_1 β. J. Biol. Chem. 238:3555, 1963.

Bergostrom, S., and Sjovall, J.: The isolation of prostaglandin E from sheep prostate gland. Acta Chem. Scand. 14:1701, 1960.

Bianchi, G., Baldoli, E., Lucca, R., and Barbin, P.: Pathogenesis of arterial hypertension after the constriction of the renal artery leaving the opposite kidney intact both in the anesthetized and in the conscious dog. Clin. Sci., 42:651, 1972.

Bianchi, G., Tilde Tenconi, L., and Lucca, R.: Effect in conscious dog of constriction of renal artery to sole remaining kidney on hemodynamics, sodium balance, body fluid volumes, plasma renin concentration and pressor responsiveness to angiotensin. Clin. Sci. 38:741, 1970.

Bing, J., and Wieberg, B.: Localization of renin in the kidney. Acta. Path. et Microbiol. Scandivan. 44:138, 1958.

Bing, J.: Mechanism of increased sensibility to renin in nephrectomized animals. Acta. Path. Microbiol. Scand. 60:311, 1964.

Bing, J., and Nielsen, K.: Role of renin angiotensin system in normo- and hypertension. Acta, Patho. Microbiol. Scand. 78:6, 1973.

Biron, P., Meyer, P., Panisset, J. C.: Removal of angiotensins from systemic circulation. Can. J. Physiol. Pharmacol. 46:175, 1968.

Blacket, R. B., Depoorter, A., Pickering, J. B., Sellers, A. L., and Wilson, G. M.: Hypertension produced in rabbit by long continued infusion of renin. Clin. Sci. 9:205, 1950.

Blacklock, A., and Levy, S. E.: Studies on the etiology of renal hypertension, Ann. Surg. 106:826, 1937.

Blair-West, Jr., Coghlan, J. P., Denton, D. A., Funder, J. W., Scoggins, B. A., and Wright, R. D.: Inhibition of renin secretion by systemic and intrarenal angiotensin infusion. Am. J. Physiol. 220: 1309, 1971.

Blaquire, P.: Kinetic Studies on renin-angiotensinogen reaction after nephrectomy. Am. J. Physiol. 208:1083, 1965.

Boucher, R., Menard, J., and Genest, J.: A micro-method of measurement of renin in the plasma and kidney of rats. Can. J. Physiol. Pharmacol. 45:881, 1967.

Boucher, R., Veyrat, R., de Champlain, J., and Genest, J.: New procedure for measurement of human plasma angiotensin and renin activity levels. Canad. M. A. J. 90: 194, 1964.

Boyd, G. W., Landon, J. and Peart, W. S.: Radioimmunoassay for determination of plasma levels of angiotensin II in man. Lancet, 2:1002, 1967.

Braun-Menendez E., Fasciolo, J. C., Leloir, L. F., Munoz, J. M., and Taquini, A. C.: Renal hypertension. Translated by Dexter, L. Springfield, Ill.: Charles C. Thomas, 1946.

Braun-Menendez E., Fasciolo, J. C., Leloir, L. F., and Munoz, J. M.: The substance causing renal hypertension. J. Physiol. 98:283, 1940.

Braun-Menendez E., Van Euler, U. S.: Hypertension after bilateral nephrectomy in the rat. Nature (London) 160:905, 1947.

Bright, R.: Cases and observations illustrative of renal disease accompanied with albuminous urine. Guy"s Hospital Rep., 1:388, 1836.

Brown, J. J., Davies, D. L., Lever, A. F., Robertson, J. I. S., and Tree, M.: The estimation of plasma renin concentration in the dog. Biochem. J. 93:3C, 1964.

Brunner, H. R., Baer, L., Sealey, J. E., Ledingham, G. G. and Laragh, J. H.: The influence of potassium administration and of potassium deprivation on plasma renin in normal and hypertensive subjects. J. Clin. Invest. 49:2128, 1970.

Brunner, H. R., Krishman, J. D., Sealey, J. E., and Laragh, J. H.: Hypertension of renal origin. Evidence for two different mechanisms. Science 174:1344, 1971.

Bumpus, F. M., Sen, S., Smeby, R. R., Sweet, C., Ferrario, C. M., and Khosla, M. C.: Use of angiotensin II antagonists in experimental hypertension. Cir. Res. 32(Suppl. I): I-150, 1973.

Bumpus, F. M., Smeby, R. R., and Page, I. H.: Angiotensin, and renal pressor hormone. Hypertension 9:762, 1960.

Bunag, R. D., Page, I. H., and McCubbin, J. W.: Inhibition of renin release by vasopressin and angiotensin. Cardiovas. Res. 1:67, 1967.

Carlson, L. A., and Oro, L.: Effect of prostaglandin E_1 on blood pressure and heart rate in dog. Acta. Physiol. Scand. 67:89, 1965.

Carretero, O. A. and Oza, N. B.: Urinary kallikrein, sodium metabolism and hypertension. Int. Congress Series, 302:290, 1973.

Christlieb, A. R., Biber, T. V. L., and Hickler, R. B.: Studies on the role of angiotensin in experimental renovascular hypertension: Immunological approach. J. Clin. Invest. 48:1506, 1969.

Collins, D. A.: Hypertension from constriction of the arteries of denervated kidneys. Am. J. Physiol. 17:334, 1936.

Conn, J. W.: Plasma renin activity in primary aldosteronism: Importance in differential diagnosis and in research of essential hypertension. J. A. M. A. 190:222, 1964.

Cook, W. F.: The detection of renin in juxtaglomerular cells. J. Physiol. 174:73, 1967.

Cook, W. F., and Pickering, G. W.: The location of renin in the rabbit kidney. J. Physiol. 149:526, 1959.

Cook, W. F., and Pickering, G. W.: The location of renin in the kidney. Biochem. Pharmacol. 9:165, 1962.

Conway, J.: Changes in sodium balance and hemodynamics during development of experimental renal hypertension in dogs. Cir. Res. 22: 763, 1968.

Cowley, A. W. Jr., and Guyton, A. C.: Quantification of intermediate steps in renin-angiotensin vasconstrictor feedback loop in the dog. Cir. Res. 30:557, 1972.

Crowshaw, K.: The incorporation of (1-14C) arachidonic acid into the lipids of rabbit renal slices and conversion to prostaglandins E_2 and F_2, Prostaglandins, 3:607, 1973.

Crowshaw, K.: Prostaglandin biosynthesis from endogenous precursors in rabbit kidney. Nature [New Biol.] 231:240, 1971.

Crowshaw, K., Szlyk, J. Z.: Distribution of prostaglandins in rabbit kidney. Biochem. J. 116:421, 1970.

Daniels, E. G., Hinman, J. B., Leach, B. E., and Muirhead, E. E.: Identification of prostaglandin E_2 as the principle vasodepressor lipid of rabbit renal medulla. Nature, 215:1298, 1967.

Davis, J. O., Blane, E. H., Witty, R. T., Johnson, J. A., Shade, R. E., and Braverman, B.: The control of renin release in non-filtering kidney: Control of renin secretion. Advances in Experimental Medicine and Biology. Volume 17, ed. T. A. Assaykeen, pp. 117, 1972.

Davis, J. O.: What signals the kidney to release renin. Cir. Res. 28: 301, 1971.

Deodhar, S. D., Hass, E., and Goldblatt, H.: Production of antirenin to homologous renin and its effect on experimental renal hypertension, J. Exper. Med. 119:425, 1964.

DeVaux, C., Alexandre, J. M., Menard, J., Meyer, P. and Milliez, P.: Mise en evidence d'un inhibiteur naturel d'origine rénale du systeme rénine-angiotensine chez l'homme. C. R. Acad. Sc. [D] Paris. 266:2029, 1968.

Dirvy, A.: The mechanism of hypertension action of the kidney. Arch. Internat. Physiol. 59:211, 1951.

Ducharme, D. W., and Weeks, J. R.: Prostaglandin $F_2\alpha$, a unique pressor substance. Fed. Proc. 26:1-681, 1967.

Ducharme, D. W., Weeks, J. R., and Montgomery, R. G.: Studies on the mechanism of the hypertensive effect of protaglandin $F_2\alpha$. J. Pharmacol. Exp. Therap. 160:1, 1968.

Dunham, E. W., and Zimmerman, B. G.: Release of protaglandin-like material from dog kidney during nerve stimulation. Am. J. Physiol. 219:1279, 1970.

Ebihara, A., and Grollman, A.: Pressor activity of renal venous effluent following constriction of renal artery in dogs. Am. J. Physio., 214:1, 1968.

Edwards, W. G., Jr., Strong, G. C., and Hunt, J. G.: A vasodepressor lipid resembling prostaglandin E_2 (PGE_2) in the renal venous blood of hypertensive patients. J. Lab. and Clin. Med. 74:389, 1969.

Eide, I.: Renovascular hypertension in rats immunized with angiotensin II. Cir. Res. 30:149, 1972.

Eisenstein, A. B., editor: The adrenal cortex. Boston Little, Brown and Co. 1967.

Elliot, A. H., and Nazum, F. R.: Urinary excretion of a depressor substance (kallikrein of Frey and Kraut) in arterial hypertension. Endocrinology 18:462, 1934.

Elliot, D. F., Lewis, G. P., and Horton, E. W.: Structure of bradykinin. Biochem. J. 76:16, 1960.

Elliot, D. F., Lewis, G. P., and Smyth, D. G.: New kinins from ox blood Biochem. J. 87:21, 1963.

Emerson, T. E., Jr., Jelks, G. W., Daughterty, R. M., Jr., and Hodgman, R. E.: Effects of prostaglandin E_1 and $F_2\alpha$ on venous return and other parameters in the dog. Am. J. Physiol. 220:243, 1971.

Engel, S. L., Schaeffer, J. R., Gold, B. I., and Rubin, B.: Inhibition of pressor effects of angiotensin I and augmentation of depressor effects of bradykinin by synthetic peptides. Proc. Soc. Exp. Biol. Med. 140:240, 1972.

Enger, R., Linder, F., and Sarre, H.: Die Wirkung quantitative abgestufter Drosselung der Nierendurchblutung auf den Blutdruck. Z. ges. exp. Med. 104:18, 1938.

Erdös, E. G.: Hypertensive peptides, kallidin and eledoisin. Advances in Pharmacol. 4:1, 1966.

Euler-von, N. S.: A depressor substance in the vesicular gland. J. Physiol. (Lond.) 84:21, 1935.

Farrup, P.: Renin location in the different parts of the juxtaglomerular apparatus in the cat kidney. Acta. Path. Microbiol. Scand. 71: 509, 1967.

Farrup, P.: Renin location in the different parts of the juxtaglomerular apparatus in the cat kidney. Acta. Path. Microbiol. Scand. 72: 109, 1968.

Fasciolo, J. C., Houssay, B. A., and Taquini, A. C.: The blood pressure raising secretory of the ischemic kidney. J. Physiol. 94:281, 1938.

Fasciolo, J. C., DeVito, E., Romero, J. C., and Cucchi, J. N.: The renin content of the blood of humans and dogs under several conditions. Canad. M. A. J. 90:206, 1964.

Ferriera, S. H., and Vane, J. R.: Prostaglandins: Their disappearance from and release into the circulation. Nature (Lond.) 216:868, 1967.

Floyer, M. A.: Further studies on the mechanism of experimental hypertension in the rat. Clin. Sci. 14:163, 1955.

Frey, E., Kraut, H., and Werle, E.: Kallikrein-kinin system und Seine Inhibitoren, Ferdinand Enke Verlag. Stuttgart, 1968:290, 1950.

Funder, J. W., Blair—West, Jr., Coghlan, J. P., Denton, D. A., Scoggins, B. A., Wright, R. D.: Effect of plasma $[K^+]$ on the secretion of aldosterone. Endocrinology 85:381, 1969.

Genest, J., de Champlain, J., Veyrat, R., Boucher, R., Tremblay, G. Y., Strong, C. G., Koiw, E., and Marcus-Aurele, J.: Role of renin angiotensin system in various physiological and pathological states. Hypertension 13:97, 1965.

Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W.: Studies on experimental hypertension. The production of persistent elevation of systolic blood pressure by means of renal ischemia. J. Exper. Med., 59:347, 1934.

Goldblatt, H., Kahn, J. R., and Hanzal, R. F.: Studies on experimental hypertension IX. The effect on blood pressure of the constriction of the abdominal aorta, above and below the site of the origin of both main renal arteries. J. Exp. Med. 69:649, 1939.

Goldblatt, H.: Studies on experimental hypertension. The pathogenesis of experimental hypertension due to renal ischemia. Ann. Int. Med. 11:69, 1937.

Goldblatt, M. W.: Properties of human seminal plasma. J. Physiol. Lond. 84:208, 1935.

Goodwin, F. J., Ledingham, J. G., Laragh, J. H.: The effects of prolonged administration of vasopressin and oxytocin on renin, aldosterone and sodium balance in normal man. Clin. Sci. 39:641, 1970.

Goormaghtigh, N.: Existence of an endocrine gland in the media of the renal arterioles. Proc. Soc. Exp. Biol. & Med. 42:688, 1939.

Goormaghtigh, N.: Facts in favour of endocrine function of renal arterioles. J. Path. and Bact. 57:392, 1945.

Gould, A. B., Skeggs, L. T., and Kahn, J. R.: Measurement of renin and substrate concentration in human serum. Lab. Invest. 15:1802, 1966.

Govaerts, P., and Verniory, A.: The vasoconstrictor properties of the vena cava blood of the dogs with acute and chronic renal hypertension. Bull. Acad. Roy. Med. Belg. 15:375, 1950.

Gravas, H., Brunner, H. R., Vaughan, E. D., Jr., and Laragh, G. H.: Angiotensin-sodium interaction in blood pressure maintenance of renal hypertensive and normotensive rats. Science, 180:1369, 1973.

Green, L. J., Camargo, A. C. M., Krieger, E. M., Stewart, J. M., and Ferreira, S. H.: Inhibition of the conversion of angiotensin I to II and potentiation of bradykinin by small peptides present in Bothrops jararaca venom. Cir. Res. 30 (Suppl. II):II-62, 1972.

Green, A. A., and Bumpus, F. M.: The purification of hog renin substrate. Canad. Med. Ass. J. 90:185, 1954.

Grollman, A., and Krishinamurty, V. S. R.: A new pressor agent of renal origin, its differentiation from renin and angiotensin. Am. J. Physiol. 221:1499, 1971.

Grollman, A., Muirhead, E. E., and Vanatta, J.: Role of the kidney in pathogenesis of hypertension as determined by a study of the effects of bilateral nephrectomy and other experimental procedures on the blood pressure of the dog. Am. J. Physiol. 157:21, 1949.

Grollman, A., Turner, L. B., Leuitch, M., and Hill, D.: Hemodynamics of bilaterally nephrectomized dog subjected to intermittent peritoneal lavage. Am. J. Physiol. 165:167, 1951.

Grollman, A., Williams, J. R., and Harrison, T. R.: The reduction of elevated blood pressure by administration of renal extracts. J. A. M. A. 115:1169, 1940.

Grollman, A.: A simplified procedure for inducing Chronic renal hypertension in the mammal. Proc. Soc. Exper. Biol. Med. 57:102, 1944.

Gross, F., Brunner, H., and Zeigler, M.: Renin-angiotensin system, aldosterone and sodium balance. Recent. Prog. Hormone Res. 21: 119, 1965.

Gross, F., and Turrain, H.: Pharmacology of hypertension and synthetic analogues, in polypeptides which affect smooth muscle and blood vessels. ed. by M. Schachter, Pergamon Press, Oxford, 1960.

Gross, F., Buschor, O., and Zengin, H.: Reduction of increased sensitivity to renin by cross circulation. Am. J. Physiol. 202: 1095, 1962.

Gryglewski, R., and Vane, J. R.: Release of prostaglandins and rabbit aorta contracting substance (RCS) from rabbit spleen and its antagonism by anti-inflammatory drugs. Br. J. Pharmacol. 45:37, 1972.

Gunnels, J. C., Grim, C. E., Robinson, R. R., and Wildermann, N. M.: Plasma renin activity in healthy subjects and patients with hypertension. Arch. Int. Med. 119:332, 1967.

Gutmann, F. D., Tagawa, H., Haber, E., and Barger, A. C.: Renal arterial pressure, renin secretion, and blood pressure control in trained dogs. Am. J. Physiol. 224:66, 1973.

Haas, E., Goldblatt, H., Gipson, E. C., and Lewis, L.: Extraction, purification and assay of human renin free of angiotensinases. Cir. Res. 19:739, 1966.

Haber, E., Page, L. B., Jacoby, G. H.: Synthesis of antigenic branch-chain copolymers of angiotensin and poly-L-lysine. Biochemistry 4:693, 1965.

Haddy, F. J.: Cat ion and cardiovascular system. 1974.

Haddy, F. J., Molnar, J. I., and Cambell, R. W.: Effects of denervation and vasoactive agents on vascular pressures and weight of dog forelimb. Am. J. Physiol. 201:631, 1961.

Haddy, F. J., Scott, J. B. and Molnar, J. I.: Mechanism of volume replacement and vascular constriction following hemorrhage. Am. J. Physiol. 208:169, 1965.

Hamberg, M.: Biosynthesis of prostaglandin in renal medulla of rabbit. Federation European. Biochem. Soc. Letters 5:127, 1969.

Hamilton, J. G., and Grollman, A.: The preparation of renal extracts effective in reducing blood pressure in experimental hypertension. J. Biol. Chem. 233:528, 1958.

Harris, R. C., and Ayers, C. R.: Renal hemodynamics and plasma renin activity after renal artery constriction in conscious dogs. Cir. Res. 31:520, 1972.

Hartfort, P. M., and Hartfort, W. S.: Studies on renal juxtaglomerular cells. i. Variations produced by sodium chloride and deoxy-corticosterone acetate. J. Exp. Med. 97:415, 1953.

Hartfort, P. M., Newmark, L. N., and Pitcock, J. A.: Relationship of renal juxtaglomerular cells to sodium intake, adrenal cortex and hypertension in Hypertension ed. by J. Moyer. W. B. Saunders Co. Philadelphia, 1959.

Hartfort, P. M., Sutherland, L. E., and Hartfort, W. S.: Juxtaglomerular cells as the source of renin: Further studies with the fluorscent antibody technique and the effect of passive transfer of anti-renin. Canad. M. A. J. 90: 163, 1964.

Hartman, F. W., Bolliger, A., and Doub, H. P.: Experimental nephritis produced by irradiation. Amer. J. Med. Sci. 172:487, 1926.

Hartwich, A.: Der Blutdruck bei expermenteller uramie und partieller Nierenauscheidung. Z. ges. exp. Med. 69:462, 1932.

Hartwich, A., and Hessel, G.: Der Einfluss der Blutdruck Zuglerauschaltung auf den Adrenat ingehalt des Blutes. Z. ges. exp. Med., 53:612, 1930.

Hatt, P. Y., Berjal, G., and Bonvalet, J. P.: Structure arterielles et arteriolaires au cours d L'hypertension experimentale du rat. Etude au microscope electronique, in L'hypertension Arterielle, ed. by P. Milliez and P. Teherdakoff, L'Expansion Scientifique Francaise, Paris, 1966.

Helmer, O. M.: A factor in plasma that enhances contraction produced by angiotonin on rat aortic strip. Fed. Proc. 14:225, 1955.

Helmer, O. M.: Differentiation between two forms of angiotonin by means of spirally cut strips of rabbit aorta. Am. J. Physiol. 188:571, 1957.

Helmer, O. M., and Judson, W. E.: The quantitative determination of renin in the plasma of patients with arterial hypertension. Circulation 27:1050, 1963.

Herbaczynska-Cedro, K., and Vane, J. R.: International Conference on prostaglandins, Advances in the BioSciences 9. Vieweg: Pergamon p. 45, 1972.

Hessel, G.: Uber das renin Ein Experimenteller Beitrag Zur Pathogenese des renalen Hochdrucks. Arch. exp. Path. Pharmak. 190:180, 1938.

Hickler, R. B., Birbari, A. E., Kamm, D. E., and Thorn, G. W.: In "L'Hypertension Arterielle" (P Milliez and P. Tcherdakoff, eds.) pp. 182. L'Expansion Scientifique Francaise, Paris, 1966.

Hickler, R. B., Lauler, D. P., Saravis, C. A., Vagnucci, A. I., Steiner, G., and Thorn, G. W.: Vasodepressor lipid from the renal medulla. Canad. Med. Assoc. J. 90:280, 1964.

Hickler, R. B., Leach, B. E., Brown, G. B., Daniel, E. G., and Hamilton, J. W.: Antihypertensive effect of prostaglandin E_2 (PGE_2) in renovascular hypertension. J. Lab. & Clin. Med. 70:986, 1967.

Horton, E. W.: Hypothesis on physiological roles of prostaglandins. Physiol. Rev. 49:122, 1969.

Horton, E. W., and Main, I. H. M.: Comparison of the biological activities of four prostaglandins. Brit. J. Pharmacol. Chemotherap. 21:182, 1963.

Horton, E. W., and Main, I. H. M.: A comparison of the action of prostaglandins $F_2\alpha$ and E_1 on smooth muscle. Brit. J. Pharmacol. Chemotherap. 24:470, 1965.

Houssay, B. A., and Taquini, A. C.: Especificidad de la accion vasoconstrictora de la sangre venosa del rinon isquemiado. Rev. Soc. Biol. 14:86, 1938.

Huidobro, F., and Braun-Menendez, E.: The secretion of renin by the intact kidney. Am. J. Physiol. 13:47, 1942.

Ishi, M., and Tobian, L.: Interstitial cell granules in renal papilla and the solute composition of renal tissue in rats with Goldblatt hypertension. J. Lab. Clin. Med. 74:47, 1969.

Itskovitz, H. D., Stemper, J., Pacholczyk, D., and McGiff, J. C.: Renal protaglandins: Determination of intrarenal distribution of blood flow. Clin. Sci. 45:(Suppl I):321, 1973.

Jaffee, B. M., Beherman, H. R., and Parker, C. W.: Radioimmunoassay measurement of prostaglandin E, A, and F in human plasma. J. Clin. Invest. 52:398, 1973.

Jhonson, J. A., and Davis, J. O.: Angiotensin II: Important role in the maintenance of the arterial blood pressure. Science 179:906, 1973.

Judson, W. E., and Helmer, O. M.: Diagnostic and prognostic value of renin activity in renal venous plasma in renovascular hypertension. Hypertension, 13:79, 1965.

Khairallah, P. A., and Page, I. H.: Plasma angiotensinases. Biochem. Med. 1:1, 1967.

Khairallah, P. A., Bumpus, F. M., Page, I. H., and Smeby, R. R.: Angiotensinase with a high degree of specificity in plasma and red cells. Science (N.Y.) 140:672, 1963.

Khairallah, P. A.: Mechanism of receptor interaction of angiotensin II analogues. J. Med. Chem. 13:148 (181), 1970.

Kloff, J. W., and Page, I. H.: Blood pressure reducing function of the kidney: reduction of renoprival hypertension by kidney perfusion. Am. J. Physiol. 178:75, 1954.

Kohlstaedt, K. G., Helmer, O. M., and Page, I. H.: Activation of renin by blood colloids. Proc. Soc. exp. Biol. (N.Y.) 39:214, 1938.

Kohlstaedt, K. G., and Page, I. H.: Liberation of renin by perfusion of kidneys following reduction in pulse pressure. J. Exper. Med. 72:201, 1940.

Koletsky, S., and Pritchard, W. H.: Vasopressor material in experimental renal hypertension. Cir. Res. 13:552, 1963.

Koletsky, S., Paulicko, K. M., and Rivera-Velez, J. M.: Renin-angiotensin activity in hypertensive rats with single ischemic kidney. Lab. Invest. 24:41, 1971.

Krieger, E. M., Salgado, H. C., Assan, C. J., Green, L. L. J., and Ferreira, S. H.: Potential screening test for detection of over-activity of renin-angiotensin system. Lancet, 1:269, 1971.

Laragh, J. H., Cannon, P. J., Stason, W. B., and Heinemann, H. D.: Physiologic and clinical observations on furosemide and ethacrynic acid. Ann. N. Y. Acad. Sci. 139:453, 1966.

Larsson, C., and Anggård, E.: Regional differences in the formation and metabolism of prostaglandins in the rabbit kidney. Eur. J. Pharmacol. 21:30, 1973.

Lasher, E. P., Jr., and Glenn, F.: Effects on kidney and blood pressure of artificial communication between renal artery and vein. Arch. Surgery. 38:886, 1939.

Ledingham, J. M., and Cohen, R. D.: Changes in extracellular fluid volume and cardiac output during the development of experimental renal hypertension. Canad. M. A. J. 90:292, 1964.

Ledingham, J. M., and Pelling, D.: Cardiac output and peripheral resistance in experimental renal hypertension in rats. Cir. Res. 21(Suppl. II):II-187, 1967.

Lee, J. B.: Renomedullary vasodepressor substance, Medullin: Isolation, chemical characterization and physiological properties. Cir. Res. 17:57, 1965.

Lee, J. B.: Cardiovascular-renal effects of protaglandins. Arch. Intern. Med. 133:56, 1974.

Lee, J. B., Hickler, R. B., Saravis, C. A., and Thorne, G. W.: Sustained depressor effect of renal medullary extract in the normotensive rat. Cir. Res. 13:359, 1963.

Lee, J. B.: The identification of prostaglandin E_2, F_2 and A_2 from rabbit kidney medulla. Biochem. J. 105:1251, 1967.

Leloir, L. F., Munoz, J. M., Braun-Menendez, E., and Fasciolo, J. C.: Dosaje de la renina. Rev. Soc. Argent. Biol. 16:635, 1940.

Leonards, J. R., and Heisler, C. R.: Maintenance of life in bilaterally nephrectomized dogs and its relation to maligant hypertension. Am. J. Physiol. 167:553, 1951.

Lever, A. F., and Robertson, J. I. S.: Renin in plasma of normal and hypertensive rabbits. J. Physiol. 170:212, 1964.

Lonigro, A. J., Terrangno, N. A., Malik, K. U., and McGiff, J. C.: Differential inhibition by prostaglandins of the renal actions of pressor stimuli. Prostaglandin 3:595, 1973.

McCubbin, J. W., DeMoura, R. S., Page, I. H., and Olmsted, F.: Arterial hypertension elicited by subpressor amounts of angiotensin. Science, 149:1394, 1965.

McDonald, G. J., Louis, W. J., Renzini, V., Boyd, G. W., and Peart, W. S.: Renal clip hypertension in rabbits immunized against angiotensin II, Cir. Res. 27:197, 1970.

McGiff, J. C., Crowshaw, K., Terragno, N. A., Lonigro, A. J.: Release of a prostaglandin-like substance into renal venous blood in response to angiotensin II, Cir. Res. 26(Suppl. I):I-121, 1970.

McGiff, J. C., Crowshaw, K., Terragno, N. A., Lonigro, A. J., Strand, J. C., Williams, M. A., Lee, J. B., and Ng, K. K. F.: Prostaglandin-like substance appearing in canine renal venous blood during renal ischemia. Cir. Res. 27:765, 1970.

McGiff, J. C., Crowshaw, K., Terragno, N. A., Malik, K. U., and Lonigro, A. J.: Differential effect of noradrenaline and renal nerve stimulation on vascular resistance in the dog kidney and the release of prostaglandin E-like substance. Clin. Sci. 42:223, 1972.

McGiff, J. C., Terragno, N. A., Stand, J. C., Lee, J. B., Lonigro, A. J., and Ng, K. K. F.: Selective passage of prostaglandins across the lung. Nature, 223:742, 1969.

Major, R. H., Weber, C. H., and Rumold, M. H.: Blood guanidine: Further observations. Arch. Intern. Med. 64:988, 1939.

Margolius, H. S., Geller, R. G., DeJong, W., Pisano, J. J. and Sjoerdsma, A.: Urinary kallikrein excretion in hypertension. Cir. Res. 31(Suppl. II):II-125, 1972.

Margolius, H. S., Geller, R., Pisano, J. J., and Sjoerdsma, A.: Altered urinary kallikrein excretion in human hypertension. Lancet, 2:1063, 1971.

Marin-Grez, M., and Carretero, O. A.: Urinary kallikrein excretion in rats with acute hypertension. Proc. of IX Internation. Congress of Cardiology:22, 1972.

Maebashi, M., Yoshinaga, K., and Muira, K.: Suppressed plasma renin activity in hypertensive patients--its evaluation for the diagnosis of primary aldosteronism. Tohoku. J. Exp. Med. 99:393, 1970.

Marin-Grez, M., Cottone, P., and Carretero, O. A.: Evidence for an involvement of kinins in regulation of sodium excretion. Am. J. Physiol. 223:794, 1972.

Masson, G. M. C., Aoki, K., and Deodhar, Sh.D.: Course of hypertension during prolonged treatment with heterogenous renin. Experimentia, 22:531, 1966.

Meyer, P., Menard, J., Papanicolaov, N., Alexandre, J. M., DeVaux, C., and Milliez, P.: Mechanism of renin release following furosemide diuresis in the rabbit. Am. J. Physiol. 215:908, 1968.

Miller, E. D., Samuels, A. I., Haber, E., and Barger, A. C.: Inhibition of angiotensin conversion in experimental renovascular hypertension. Science. 177:1108, 1972.

Muirhead, E. E., Brown, G. B., Germain, G. S., Brooks, B., and Leach, B. E.: The renal medulla as an anti-hypertensive organ. J. Lab. & Clin. Med. 74:992, 1969.

Muirhead, E. E., Daniels, E. G., and Hinman, J. W.: Renomedullary antihypertensive principle in renal hypertension. J. Lab. & Clin. Med. 67:778, 1966.

Muirhead, E. E., Leach, B. E., Brown, G. B., Daniels, E. G., and Hinman, J. W.: Antihypertensive effect of prostaglandin E_2 (PGE_2) in renovascular hypertension. J. Lab. & Clin. Med. 70:986, 1967.

Muirhead, E. E., Booth, E., and Brooks, B.: Kosnski, M., Daniels, E. G., and Hinman, J. W.: Renomedullary anti-hypertensive principle in renal hypertension. J. Lab. & Clin. Med. 64:989, 1964.

Muirhead, E. E., Daniels, E. G., Booth, E., Freyburger, W. A., and Hinman, J. W.: Ronomedullary vasodepression and anti-hypertensive function. Arch. Path. 80:43, 1965.

Muirhead, E. E., Jones, F., and Stirman, J. A.: Antihypertensive property in renoprival hypertension of extracts from renal medulla. J. Lab. & Clin. Med. 56:167, 1960.

Muirhead, E. E., Stirman, J. A., Jones, F., Lesch, W., Burns, M., and Fogelman, M. J.: Cardiovascular lesions following bilateral nephrectomy of dog. Role of hypertension and other factors on pathogenesis. Arch. Int. Med. 91:250, 1953.

Ng, K. K. F., and Vane, J. R.: Conversion of angiotensin I to angiotensin II. Nature (Lond.) 216:762, 1967.

Nakano, J., and McCurdy, J. R.: Hemodynamic effects of prostaglandins E_1, A_1, and $F_2\alpha$ in dogs. Proc. Soc. Expt. Biol. Med. 128:39, 1968.

Narin, R. C., Raser, K. B., and Chadwick, C. S.: The histological localization of renin with fluorescent antibody. Brit. J. Exper. Path. 40:155, 1959.

Nash, F. D., Rostorfer, H. H., Bailie, M. D., Wathen, R. L., and Schneider, E. G.: Renin release: relation to renal sodium load and dissociation from hemodynamic changes. Cir. Res. 22:473, 1968.

Nissen, H. M., and Andersen, : On the localization of a prostaglandin dehydrogenase activity in the kidney. Histochemie. 14:189, 1968.

Nustad, K.: Localization of kininogenase in the rat kidney. Brit. J. Pharmacol. 39:87, 1970.

Nustad, K.: The relationship between kidney and urinary kininogenase. Brit. J. Pharmacol. 39:73, 1970.

Omae, T., Masson, G. M. C., and Page, I. H.: Release of pressor substance from renal grafts originating from rats with renal hypertension. Circ. Res. 9:441, 1961.

Oparil, S., Sandars, C. A., and Haber, E.: In-vivo and in-vitro conversion of angiotensin-I to angiotensin-II in dog blood. Cir. Res. 26:591, 1970.

Oparil, S., Bailie, M. D.: Mechanism of renal handling of angiotensin II in the dog. Circ. Res. 33:500, 1973.

Oparil, S., and Haber, E.: The renin angiotensin system. New England J. Med. 291:389, 1974.

Osvaldo, L., and Latta, H.: Interstitial cells of renal medulla. J. Ultrastructure Res. 15:589, 1966.

Page, I. H.: Relationship of extrinsic renal nerves to origin of experimental hypertension. Am. J. Physiol. 112:116, 1935.

Page, I. H.: The production of persistent hypertension by cellophane nephritis. J. A. M. A., 113:2046, 1939.

Page, I. H., and Helmer, O. M.: Angiotonin activator renin and antiotensin inhibitor and mechanism of angiotonin tachyphylaxis in normal, hypertensive and nephrectomized animals. J. Exper. Med. 71:495, 1940.

Page, I. H., Helmer, O. M., Kohlstaedt, K. G., Fouts, P. G., Kemp, G. F., and Corcoran, A. C.: Substances in kidneys and muscle eliciting prolonged reduction of blood pressure in human and experimental hypertension. Proc. Soc. Exper. Biol. & Med. 43:722, 1940.

Pals, D. T., Masucci, F. D., Denning, G. S., Jr., Sipos, F., and Fesseler, D. C.: Role of the pressor action of angiotensin II in experimental hypertension. Cir. Res. 29:673, 1971.

Passo, S. S., Assaykeen, T. A., and Otsuka, K.: Effect of stimulation of the medulla oblongata on renin secretion in dogs. Neuroendocrinology 7:1, 1971.

Pearce, R. M.: An experimental study of the influence of kidney extracts and of the serum of animals with renal lesions up on the blood pressure. J. Exp. Med. 11:430, 1909.

Peart, W. S., Robertson, J. I. S., and Grahame-Smith, D. G.: Examination of the relationship of the renin release to hypertension produced in the rabbit by renal artery constriction. Cir. Res. 9:1171, 1961.

Peart, W. S.: A new method of large scale preparation of hypertensin, with a note on its assay. Biochem. J. 59:300, 1955.

Peart, W. S.: The isolation of hypertensin. Biochem. J., 62:520, 1956.

Penderson, A. H.: A method of producing experimental chronic hypertension in rabbits. Arch. Path. 3:912, 1927.

Pickens, P. T., Dustan, H. P., Bumpus, F. M., and Page, I. H.: Measurement of plasma renin activity in hypertension. Hypertension. 13:90, 1965.

Pierce, J. V.: Physiological Society Symposium. Vasoactive peptides. Fed. Proc. 27:52, 1968.

Pritchard, W. H., Ormond, A. P., Jr., and Koletsky, S.: Differences in levels of circulating materials in dogs with acute and chronic renal hypertension. Proc. Exp. Biol. Med. 117:127, 1964.

Regoli, D., Brunner, H., Peters, G., and Gross, F.: Changes in renin content in kidneys of renal hypertensive rats. Proc. Soc. Exper. Biol. & Med. 109:142, 1962.

Robb, C. A., Davis, J. O., Jhonston, C. I., Hartfort, P. M.: Effects of deoxycorticosterone on plasma renin activity in conscious dogs. Am. J. Physiol. 216:884, 1969.

Rocha e Silva, M., Beraldo, W. T., and Rosenfield, G.: Bradykinin hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin. Am. J. Physiol. 156:261, 1949.

Ryan, J. W., McKenzie, J. K., and Lee, M. R.: A rapid simple method for the assay of renin in rabbit plasma. Biochem. J. 108:679, 1968.

Rytand, D. A.: The renal factor in arterial hypertension with coarctation of aorta. J. Clin. Invest. 17:391, 1938.

Samuels, A. I., Miller, E. D., Fray, C. S., Haber, E., and Barger, A. C.: Regulation of pressure by the renin-angiotensin system (Abstr.) Fed. Proc. 32:380, 1973.

Schachter, M.: Physiological Society Symposium: Vasoactive peptides. Fed. Proc. 27:49, 1968.

Schaechtelin, G., Regoli, D., and Gross, F.: Bioassay of circulating renin-like pressor material by isovolemic cross circulation. Am. J. Physiol. 205:303, 1963.

Schweikert, R. J., Carey, R. M., Liddle, G. W., and Island, D. P.: Evidence that the renal pressor substance of Grollman is related to angiotensin I. Cir. Res. 31(Suppl. II):II-132, 1972.

Scornik, O. A., and Paladini, A. C.: Angiotensin blood levels in dogs with experimental renal hypertension. Am. J. Physiol. 201:526, 1961.

Sealey, J. E., Clark, I., Bull, M. B., and Laragh, J. H.: Potassium balance and the control of renin secretion. J. Clin. Invest. 49:2119, 1970.

Sen, S., Smeby, R. R., and Bumpus, F. M.: Isolation of a phospholipid renin inhibitor from kidney. Biochemistry 6:1572, 1967.

Shorr, E., Zewifach, B. W., and Black, M. M.: Histochemical studies with triphenyl tetrazolium of the kidney and adrenal cortex in experimental and human essential hypertension. Trans. Assn. Am. Phys. 64:445, 1951.

Skeggs, L. T., Kahn, J. R., and Shumway, N. P.: The preparation and function of the hypertensin converting enzyme. J. Exper. Med. 103:295, 1956.

Skeggs, L. T., Marsh, W. H., Kahn, J. R., and Shumway, N. P.: The existence of two forms of hypertensin. J. Exp. Med. 99:275, 1954.

Skeggs, L. T., Marsh, W. H., Kahn, J. R., and Shumway, N. P.: Amino acid composition and electrophoretic properties of hypertensin I. J. Exp. Med. 102:435, 1955.

Skinner, S. L., McCubbin, J. W., and Page, I. H.: Renal baroreceptor control of renin secretion. Science 141:814, 1963.

Skinner, S. L., and McCubbin, J. W.: Control of renin secretion. Cir. Res. 15:64, 1964.

Skinner, S.: Improved assay methods for renin "concentration" and "activity" in human plasma. Cir. Res. 20:391, 1967.

Smeby, R. R., Sen, S., and Bumpus, F. M.: Naturally occurring renin inhibitor. Cir. Res. 20(Suppl. II):II-129, 1967.

Smith, E. R., McMorrow, J. V., Jr., Covino, B. G., and Lee, J. B.: Mechanism of the hypotensive and vasodilator action of prostaglandin E_1. Clin. Res. 15:222, 1967.

Sokabe, H., and Grollman, A.: Localization of blood pressure regulating and erythropoietic function in rat kidney. Am. J. Physiol. 203: 991, 1962.

Sokabe, H., Mikasa, A., Yasuda, H. and Masson, G. M. C.: Adrenal cortex and renal pressor function. Cir. Res. 12:94, 1963.

Spath, J. R., Daugherty, R. M., Jr., Scott, J. B., and Haddy, F. J.: Effect of local renal hypoxia on renin activity in renal venous plasma. Proc. Soc. Exper. Biol. and Med. 137:484, 1971.

Stein, J. H., Congbalay, R. C., Karsh, D. L., Osgood, R. W., and Ferris, T. F.: The effect of bradykinin on proximal tubular sodium reabsorption in the dog.: Evidence for functional nephron heterogeneity. J. Clin. Invest. 51:1709, 1972.

Strong, C. G., and Bohr, D. F.: Effects of prostaglandins E_1, E_2, A_1 and $F_1\alpha$ on isolated vascular smooth muscle. Am. J. Physiol. 213:725, 1967.

Strong, C. G., Boucher, R., Nowacznski, W., and Genest, J.: Renal vasodepressor lipid. Mayo Clinic Proc. 41:433, 1966.

Sweet, C. S., Kadowitz, P. J., Forker, E. L., and Brody, M. J.: Depression of adrenergic transmission by a factor in renal venous blood. New evidence for an antihypertensive function of the kidney. Arch. Intern. Pharmacodyn. 198:229, 1972.

Swales, J. D., Thurston, H., Quiroz, F. P., and Medina, A.: Sodium balance during the development of experimental hypertension. J. Lab. & Clin. Med. 80:539, 1972.

Taquini, A. C., Blaguier, P., and Taguini, A. C., Jr.: On the production and role of renin. Canad. Med. Assoc. J. 90:210, 1964.

Thurau, K., Schnermann, J., Nagel, W., Hoster, M., and Whol, M.: Composition of tubular fluid in the macula densa segment as a factor regulating the function of the juxtaglomerular apparatus. Cir. Res. 21(Suppl.II):II-79, 1967.

Tigerstedt, R., and Bergman, P. G.: Niere und kreislauf. Skand. Arch. Physiol. 8:223, 1898.

Turker, R. K., Hall, M. M., Yamamoto, M., Sweet, C. S., and Bumpus, F. M.: New, long lasting competitive inhibitor of angiotensin. Science. 177:1203, 1972.

Tobian, L., Janeck, J., and Tombaulian, A.: Correlation between granulation of juxtaglomerular cells and extractable renin in rats with experimental hypertension. Proc. Soc. Exper. Biol. & Med. 100:94, 1959.

Vander, A. J.: Effect of catechol amines and the renal nerves on renin secretion in anesthetized dogs. Am. J. Physiol. 209:659, 1965.

Vander, A. J.: Direct effects of potassium on renin secretion and renal function. Am. J. Physiol. 219:455, 1970.

Vander, A. J., and Geelhoed, G. W.: Inhibition of renin secretion by angiotensin II. Proc. Soc. Exp. Biol. Med. 120:399, 1965.

Vander, A. J., Luciano, J. R.: Neural and humoral control of renin release in salt depletion. Cir. Res. 20, 21(Suppl. II):69, 1967.

Vander, A. J.: Inhibition of renin release in dogs by vasopressin and vasotocin. Cir. Res. 23:605, 1968.

Vander, A. J., and Miller, R.: Control of renin secretion in anesthetized dog. Am. J. Physiol. 207:537, 1964.

Vane, J. R.: Release and fate of vasoactive hormones in circulation. Brit. J. Pharmacol. 35:209, 1969.

Vane, J. R.: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature [New Biol.] 231:232, 1971.

Vincent, S., and Sheen, W.: The effect of intravenous injections of extracts of animal tissues. J. Physiol. (Lond.) 29:242, 1903.

Walker, W. G., Ruiz-Maza, F., and Horvath, J. S.: Demonstration of free (unbound) angiotensin II in immunized rabbits. Proc. 5th int. Cong. Nephrol. p. 115, 1972.

Walkerlin, G. E.: Antibodies to renin as proof of the pathogenesis of sustained renal hypertension. Circulation. 17:653, 1958.

Webster, M. E.: Physiology Society Symposium: Vasoactive peptides. Fed. Proc. 27:84, 1968.

Webster, M. E., and Gilmore, J. P.: Influence of kallidin-10 on renal function. Am. J. Physiol. 206:714, 1964.

Wester, M. E., and Pierce, J.V.: The nature of the kallidins released from human plasma by kallikreins and other enzymes. An. N. Y. Acad. Sci. 104:91, 1963.

Weeks, J. R., Sekhar, N. C., and Ducharme, D. W.: Relative activity of prostaglandin E_1, A_1, E_2 and A_2 on lipolysis, platelet aggregation, smooth muscle and the cariovascular system. J. Pharmacol. 21:103, 1969.

Werle, E.: Uber die Wirkung des kallikrein auf den isolierten darn und über eine neuse darmkontrabierende substanz. Biochem. Ztschr. 289:217, 1937.

Zeigler, M., and Gross, F.: Effect of blood volume on renin-like activity in blood. Proc. Soc. Exper. Biol. & Med. 116:774, 1964.

Zusman, R. M., Spector, D., Caldwell, B. V., Speroff, L., Schneider, G., and Murlow, P. J.: The effect of chronic sodium loading and sodium restriction on plasma prostaglandin A, E and F concentration in normal humans. J. Clin. Invest. 52:1093, 1973.