University of Nebraska Medical Center
BREAKTHROUGHS FOR LIFE

University of Nebraska Medical Center
DigitalCommons@UNMC

MD Theses

Special Collections

1966

Regulation of aldosterone secretion

Bruce Allan Sebek
University of Nebraska Medical Center

This manuscript is historical in nature and may not reflect current medical research and practice. Search PubMed for current research.

Follow this and additional works at: https://digitalcommons.unmc.edu/mdtheses

Recommended Citation

Sebek, Bruce Allan, "Regulation of aldosterone secretion" (1966). *MD Theses*. 2869.
https://digitalcommons.unmc.edu/mdtheses/2869

This Thesis is brought to you for free and open access by the Special Collections at DigitalCommons@UNMC. It has been accepted for inclusion in MD Theses by an authorized administrator of DigitalCommons@UNMC. For more information, please contact digitalcommons@unmc.edu.

THE REGULATION OF ALDOSTERONE SECRETION

Bruce Allan Sebek

Submitted in Partial Fulfillment for the Degree of

Doctor of Medicine

College of Medicine, University of Nebraska

February 1, 1966

Omaha, Nebraska

TABLE OF CONTENTS

THE REGULATION OF ALDOSTERONE SECRETION

Long before aldosterone was isolated and identified, it was observed that a potent sodium-retaining hormone is present in the amorphous fraction of extracts of the adrenal cortex. Furthermore, the concept of receptors concerned with the control of the volume of the intravascular space originated before aldosterone was isolated and characterized. In his works on the pathogenesis of edema in 1948 and 1952, Peters (1, 2) set forth the view that fluid and electrolytes escape from the intravascular space with a subsequent decrease in total circulating blood volume. He further proposed that in certain clinical states in which there is an increase in total vascular volume, such as congestive heart failure, a decrease in "effective circulating blood volume" existed. Peters suggested the decrease in "effective circulating blood volume or some ramification thereof stimulated receptors which he termed volume meters or "volumeters." He further reasoned that these receptors might control the peripheral blood level of the yet unidentified sodium-retaining hormone of the adrenal cortex.

In 1950, Deming and Luetscher (3) reported the presence of increased sodium-retaining activity in the urine of patients with congestive heart failure. Additional reports followed which demonstrated that increased sodium-retaining activity is present in the urine from patients with cardiac failure (4), cirrhosis (5), and nephrosis (6). Following the isolation and crystallization of

aldosterone by Simpson and associates in 1953 (7) and its structural identification (8), it was shown that the excretion of aldosterone in urine from patients (9) and from dogs (10), accumulating edema or ascites, is increased. Furthermore, a rough inverse correlation of aldosterone output with sodium excretion in urine was described by Luetscher and Johnson (11). Other reports appeared (12, 13, 14, 15, 16, 17, 18) which described an inverse relationship between expansion of body fluid volume and the rate of aldosterone secretion. Thus with the isolation and characterization of aldosterone and with the development of methods for analysis of this mineralocorticoid in adrenal vein blood and urine, attention was focused on the mechanisms regulating the rate of aldosterone secretion.

The studies that have followed concerning the control of aldosterone secretion have been designed to identify the various components of a receptor-effector system. Two general hypotheses have emerged to explain the regulation of aldosterone secretion. According to the earlier of the two views, there are peripheral nervous receptors possibly in the upper arterial tree or cardiac atria. These receptors then form the origin of an afferent nervous limb with integration of their stimuli in the central nervous system. A neurohormone, pineal hormone, and ACTH have all been proposed as the efferent limb for such a regulatory system. Proponents of the second hypothesis have maintained that a peripheral receptor

effects the release of a hormone from an extracranial organ and that this hormone leads (directly or indirectly) to increased aldosterone secretion.

The evidence for these two hypotheses will be presented. First, the evidence for the afferent side of the aldosterone regulatory system according to the first hypothesis will be presented. Various data concerning peripheral nervous receptors, an afferent nervous limb, and a central integrating mechanism will be reviewed. Secondly, the immediate stimulus to aldosterone production and possible mediators of an efferent limb of a regulatory system will be considered. The role of ACTH, plasma electrolytes, pineal extracts, and other tissue extracts will be examined. Finally, the voluminous works implicating the renin-angiotensin system as a major component of the control of aldosterone secretion will be presented. This will involve consideration of a receptor-effector system according to the second hypothesis in which the central nervous system plays no essential role.

The original concept of "volume receptors," as proposed by Peters (1, 2), has been supported by many investigators and several extensions of the Peter's hypothesis have been made. In the early reports of Epstein, Post, and McDowell (19, 20), it was suggested that "volume receptors" might be located in the central arterial tree. This hypothesis was based on the observation that a tendency toward inadequate filling of the systemic arterial tree is a factor common to those circulatory states in which the kidneys retain abnormal amounts of sodium.

One of the first attempts (16) to examine local regions of the arterial system was concerned with the moderator reflex, of which the baroreceptors in the carotid sinus and the aortic arch constitute the afferent limb and sympathetic fibers the efferent limb. Barger et al (21) set forth the thesis that a fall in arterial blood pressure secondary to cardiac failure may lead to decreased stimulus of the carotid sinus and aortic arch nerves and a reflex increase in sympathetic activity, resulting in decreased Na excretion. Evidence for increased sympathetic activity was provided by a marked increase in sodium and water excretion following the renal intra-arterial injection of Dibenzyline into a dog with experimental tricuspid insufficiency while little significant effect was observed in a normal dog. Barger and his coworkers (22) also carried out experiments in which they clamped the common carotid arteries reducing the carotid sinus pressure. Normal arterial pressure was maintained in the left kidney by means

of an aortic clamp placed immediately superior to the left renal artery. Sodium excretion, GFR, and RPF were observed to be decreased in the left kidney. This evidence was presented in support of a reflex which increases renal sympathetic activity and results in sodium retention.

Subsequent experiments by Carpenter et al (23), in which these workers transplanted the left kidney and adrenal to the neck, removed the right kidney and adrenal, and constricted the thoracic inferior vena cava, demonstrated marked sodium retention, ascites formation, and increased urinary excretion of aldosterone. Similar transplant studies in dogs with secondary hyperaldosteronism demonstrated that denervation of the adrenal did not prevent the development of sodium retention and increased urinary aldosterone. They concluded that neither intact renal and adrenal nerves nor increased renal venous pressure are essential to the hypersecretion of aldosterone and virtually complete sodium retention following thoracic inferior vena cava constriction.

Bartter and associates (24, 25) have proposed that receptors located at the thyrocarotid arterial junction initiate impulses which lead to hypersecretion of aldosterone after thoracic caval constriction and after common carotid arterial constriction in the dog. They have reported that the interruption of afferent nervous pathways arising at the thyrocarotid arterial junction prevented an increase in aldosterone secretion rate following chronic constriction

of the thoracic inferior vena cava and that denervation of the thyrocarotid artieral junction in two dogs with ascites secondary to thoracic inferior vena cava constriction resulted in natriuresis. Seven dogs were subjected to constriction of the common carotid artery low in the neck (25). The constriction was sufficient to markedly reduce the pulse pressure in the lingual artery. The data suggested that aldosterone secretion increased slightly but not significantly following constriction. Two of the dogs also demonstrated a concomitant increase of corticosterone suggesting increased release of ACTH.

The role of arterial baroreceptors in the central arterial tree in the control of aldosterone secretion was further investigated by Carpenter and colleagues (26). Extensive denervation was performed in the dog and studies were conducted after each of three stages of denervation. The first stage consisted of sectioning of the carotid sinus nerves and stripping of the adventitia of the entire cervical carotid system including the thyrocarotid junction. The arteries were then painted with five per cent phenol and subsequently washed with ethanol. Two to three weeks following this procedure, the thoracic inferior vena cava was chronically constricted. This extensive denervation of the carotid baroreceptor areas did not prevent the hypersecretion of aldosterone and the virtually complete sodium retention which followed caval constriction. These results were also in agreement with the work of Davis et al (27).

Combined denervation of the cervical carotid arterial system (stage 1) and the aortic arch with additional left cervical vagotomy (stage 2) was carried out in five dogs. Again there was no effect on the observed sodium retention and hypersecretion of aldosterone in these dogs with chronic thoracic inferior vena caval constriction. This demonstration that aortic arch baroreceptors were not essential to almost complete sodium retention in the dog with secondary hyperaldosteronism was consistent with the findings of Bartter and Gann (24) and of Davis and associates (27) that bilateral vagotomy with destruction of afferent fibers from the aortic arch baroreceptors failed to prevent the significant rises which occur in aldosterone secretion due to chronic thoracic inferior vena caval constriction in the dog.

Additional denervation (stage 3) consisted of right vagotomy and bilateral splanchnic nerve section. As was found previously, sodium retention remained almost complete and ascites formation contined unabated in these extensively denervated dogs with chronic thoracic inferior vena caval constriction.

To support the findings of the above denervation experiments, Carpenter and co-workers (26) conducted experiments in which the common carotid arteries were constricted proximally causing a marked reduction in pulse pressure and a moderate reduction in mean arterial pressure in the area of the carotid sinus and thyrocarotid junction. These dogs were intact. A small increase in

aldosterone production was observed in only 2 of 7 animals. These data are in contrast to those of Bartter et al (25) presented previously.

Biglieri and Ganong (28) have also provided evidence that the increase in aldosterone secretion which follows carotid constriction results from ACTH release. They observed an elevation in the rate of aldosterone secretion after proximal common carotid constriction in the normal dog, but found that aldosterone production failed to increase after similar carotid arterial constriction in the hypophysectomized dog.

The right atrium has been considered as a possible location for receptors controlling aldosterone secretion. Farrell (29) proposed that the increased rate of aldosterone secretion in dogs with chronic thoracic caval constriction is the result of a reflex mediated by right atrial stretch receptors. In support of this view, Anderson, McCally, and Farrell (30) reproted that stretching the right atrial wall by means of external sutures was associated with an abnormally low rate of aldosterone secretion in contrast to left atrial stretching which was without effect.

However, Davis (31) has pointed out that the consistent finding of hypersecretion of aldosterone in the presence of both a reduced right atrial pressure during acute thoracic inferior vena caval constriction in dogs and a high right atrial pressure in experimental heart failure due to pulmonic stenosis is irreconcilable with the right atrial stretch hypothesis.

Mills, Bartter, and Casper (32) have maintained that right atrial receptors and vagal pathways are concerned only with inhibition of aldosterone secretion. They reported that aldosterone secretion failed to fall significantly following release of a constricting caval ligature if the vagi were cut.

Gann and Travis (33, 34) proposed that carotid constriction, by increasing atrial pulse pressure, may increase vagal inhibition of aldosterone and thus obscure the response to carotid constriction. Secretion of aldosterone increased in response to carotid constriction in four vagectomized dogs, however.

Davis et al (27) provided further evidence that neither the vagus nerve nor right atrial receptors play an essential role in the control of aldosterone secretion. He concluded that if the vagus nerve constitutes the afferent neural pathway, aldosterone should decline following vagotomy of normal dogs and of dogs with hyperaldosteronism secondary to caval constriction; neither occurred. In dogs with caval constriction, a decrease in aldosterone production failed to occur after acute vagotomy and in chronic studies of vagotomized dogs (27, 35).

Considerable attention has also been directed toward the possible role of various areas of the central nervous system in the regulation of aldosterone secretion.

In an early attempt to evaluate the role of the diencephalon in the control of aldosterone secretion, Rauschkolb and Farrell (36) reported that aldosterone secretion decreased after decerebration

and removal of brain tissue rostral to the midcollicular level but remained unchanged after decortication. In these early experiments, no mention was made as to the disposition of the anterior pituitary which is presently recognized to play a role in the control of aldosterone secretion. Damage to or accidental removal of the adenohypophysis by decerebration but not by decortication might account for the observed low rate of aldosterone secretion following decerebration. This idea is supported by the fact that cortisol secretion dropped following decerebration but failed to occur after decortication.

Farrell and co-workers (37, 38) conducted additional experiments using cats which were fed either regular diets or salt deprived for two weeks, which is a potent stimulus to aldosterone secretion. Both groups had similar cortisol levels. Lesions were produced in the rostral dorsal midbrain by high frequency coagulation. The lesions involved mainly the central region around the cerebral aqueduct leaving the pineal gland intact. A significant reduction in aldosterone secretion irrespective of the dietary intake of sodium resulted. The levels of cortisol did not vary significantly in the experimental or control group. On the other hand, lesions in the ventral tegmentum of the midbrain had no effect on aldosterone secretion.

It is of interest to note that the pretectal region is included in the area which is coagulated in the rostral dorsal midbrain lesions. Krieger and Krieger (39) reported the response of seven

patients with specific pretectal lesions to salt restriction in the diet. Four of these patients showed no elevation of urinary aldosterone levels following sodium restriction, whereas all six control subjects with other focal central nervous system disease showed the expected increase. Basal levels of aldosterone excretion in these patients with pretectal disease varied widely within the normal range. These investigators thus concluded that the defect in pretectal disease is one affecting mechanisms of expected homeostatic response to sodium depletion and not that controlling basal secretion.

Palkovits et al (40) has presented further evidence to support the work of Farrell and his group. Electrocoagulation of the subcommissural organ was performed in the rat. This area is also ablated in Farrell's experiments in which he coagulates the rostral dorsal midbrain. After 21 days of normal diet, the control and experimental animals are sacrificed and their adrenals removed. The adrenals were quartered and incubated. The in vitro production of aldosterone in the experimental animals was 2.58 ug/hour/100 mg adrenal tissue as compared to only 0.97 ug/hour for the controls. Aqueous extracts of the subcommissural organ was added to the incubation medium and found to have no effect. In all cases the pineal gland was intact. The authors suggested that the basal production of aldosterone is dependent on the integrity of the subcommissural organ or at least of the epithalamus.

Additional data concerning the role of the subcommissural organ in the control of aldosterone secretion in the rat has been obtained by VanDerWal and associates (41). The subcommissural organ was destroyed by electrocoagulation and four days later one half of the animals in the control and experimental group were given experimental nephrosis as a stimulus to increase aldosterone production. Subsequent sacrifice, quartering of the adrenals, and incubation revealed a significant decrease in the aldosterone production rate of the rats with the subcommissural organ lesions as compared to the controls. These investigators likewise concluded that the subcommissural organ may be involved in the regulation of basal aldosterone secretion.

Farrell (29) called attention to the pineal body as a possible locus for the production and/or release of an aldosterone stimulating hormone which he referred to as adrenoglomerulotropin.

In a preliminary report, Farrell, Koletsky, and Lapham (42) described a decrease in aldosterone secretion after pinealectomy in dogs. Davis (18) attempted to confirm Farrell's finding of decreased aldosterone secretion after pinealectomy but was unable to do so. The pineal gland was removed from several normal dogs and later the animals were subjected to chronic thoracic inferior vena cava constriction. Marked sodium retention, ascites development, increase of adrenal vein aldosterone, and increase of urinary aldosterone occurred despite pinealectomy. The pineal region was

examined histologically and no evidence of the pineal gland was found. In two of the dogs, the subcommissural organ was also destroyed.

In a subsequent report, Farrell (43) observed normal levels of aldosterone in pinealectomized dogs and an expected rise in aldosterone secretion in response to sodium depletion. That pinealectomy is without effect on aldosterone secretion has been confirmed in sheep with isolated adrenal glands by Coghlan, et al (44) and in rats by VanDerWal and associates (41) who incubated the adrenal glands at intervals from 2 to 28 days following pinealectomy.

Farrell (29) also reported that extracts of the pineal gland selectively stimulated aldosterone secretion. Farrell and McIsaac (45) presented evidence that the active agent in extracts of pineal tissue has colorimetric, chromatographic, and fluorometric characteristics identical with 1-methyl-6-methoxy-1,2,3,4-tetrahydro-2-carboline. These workers observed an increase in aldosterone secretion in response to carboline infusions in the midcollicular decerebrate dog. In subsequent experiments in normal dogs, Taylor and collaborators (46) reported the effect of carboline infusions upon aldosterone secretion was inconsistent.

Mulrow and co-workers (47) infused from 4 to 10 ug per hour of 1-methy-6-methoxy-1,2,3,4-tetrahydro-2-carboline into hypophysectomized and nephrectomized dogs. These

dosages represented larger amounts than those claimed to be stimulatory by Farrell (45). No dosage had a stimulatory effect on aldosterone or 17 hydroxycorticoids. Also no differences were observed between the infusion of carboline in 10 per cent ethanol in saline or 0.1 per cent ascorbic acid in saline.

Blair-West and colleagues (48), utilizing adrenal glands isolated and transplanted to the neck, infused carboline into the arterial supply of the gland in conscious, trained sheep. They reported quantities of 0.025 to 8.0 micrograms per hour did not increase either aldosterone or 17 hydroxycorticosteroids significantly.

Davis (32) has noted that 1-methyl-6-methoxy-1,2,3,4-tetrahydro-2-carboline has never been demonstrated in peripheral plasma and thus is of questionable physiological importance.

Further studies have been conducted which tend to lend weight to the faction which claims no stimulatory role for the midbrain regions in aldosterone secretion (29,49). The effects of midbrain transection have been studied in an effort to resolve the question whether nervous afferents, said to be involved in the control of aldosterone secretion enter the lower brain stem to form ascending pathways. If these pathways do in fact exist and terminate in higher centers in the midbrain or diencephalon and there perform a stimulatory role, then transection of the midbrain should impede appropriate stimuli to aldosterone production.

Newman, et al (37) were the first to report that neither the rate of aldosterone nor cortisol secretion was altered following transection of the midbrain reticular formation in the cats.

In a more recent experiment, Davis and his group (50) performed complete midbrain transections in normal dogs and observed that this manipulation failed to influence the rates of secretion of aldosterone, corticosterone, and Porter-Silber chromogens. Subsequent bleeding of these dogs, a potent, known stimulus to aldosterone secretion in the intact as well as hypophysectomized animal, resulted in a striking increase in aldosterone secretion while corticosterone output was unaltered. Also, in dogs with existing hyperaldosteronism secondary to thoracic inferior vena cava constriction, the high rates of aldosterone secretion persisted after complete midbrain transection. This data was taken to indicate, therefore, that neither the response in aldosterone secretion to acute blood loss or to chronic thoracic caval constriction is dependent upon the upper brain stem or pineal region. The results also implied that the upper brain stem and pineal region are not the source for an aldosterone stimulating hormone released in response to acute hemorrhage (32) and in dogs with hyperaldosteronism secondary to thoracic caval constriction (51).

Additional experiments were designed to exclude the lower brain stem as a source of an aldosterone stimulating factor in response to acute hemorrhage (32). Dogs were initially hypophysectomized, establishing low control values for aldosterone

secretion. Decapitation which followed after approximately two hours was without effect on the previously observed control levels of aldosterone. The animal was then subjected to acute blood loss after which a two hundred per cent increase in aldosterone secretion was observed while no change in adrenal blood flow took place. Up to one hour following decapitation, aldosterone levels were noted to be comparable to those following hypophysectomy in the absence of hemorrhage. It was thus concluded that there was nothing to suggest that an aldosterone stimulating factor could be secreted from the brain.

The work of the Blair-West laboratories (48) confirm the above findings of Davis in the sheep. Following hypophysectomy, pinealectomy, and decerebration in sodium replete sheep with transplanted, cervical adrenal glands, these animals were subjected to thoracic inferior vena cava constriction to which they responded with a significant increase in aldosterone secretion.

Recent evidence has been accumulating, however, favoring the possible existence of an inhibitory regulatory system in the central nervous system. One of the main problems in the detection and definition of inhibitory systems has been that the control levels of aldosterone secretion have been too low in most assay systems used. Most, if not all, evidence then for the existence of inhibitory systems is of an indirect nature.

In 1960, Coghlan et al (44) performed a series of experiments in conscious, trained sheep with transplanted cervical adrenals

and who were made sodium depleted by a permanent parotid fistula. Among these, they observed that in sodium depleted animals demonstrating increased aldosterone secretion, midcollicular section with anterior brain removal did not lower this level, but resulted in failure of the expected fall in aldosterone secretion following sodium repletion.

Further data has been provided by Barbour and associates (52), who have shown that removal of the brain rostral to the midcollicular level in hypophysectomized, nephrectomized dogs causes some increase in the secretion of aldosterone.

A larger series of experiments were conducted by the Blair-West group (48) in an attempt to strengthen the concept of an inhibitory role for the central nervous system. Experiments were conducted on sheep who had a single, transplanted, cervical adrenal gland and who were made sodium deficient by means of an unilateral parotid fistula.

One group of these animals was bilaterally nephrectomized prior to decerebration. Systemic infusions of 280 milliequivalents of sodium chloride were administered subsequent to decerebration in order to make the sheep replete with respect to sodium chloride. In spite of restoration to sodium balance, the aldosterone secretion rate was still eight times the basal level three hours later. These findings were in accord with previous data (44, 53).

Other experiments were conducted in sodium depleted sheep (48) which underwent right nephrectomy one to six weeks before the

experiment. The experiment then began with the sheep undergoing left nephrectomy. The aldosterone secretion rate slowly began to decline following the second nephrectomy whereas the cortisol and corticosterone levels were high secondary to elevated ACTH resulting from the surgical trauma. The next step consisted of hypophysectomy and midcollicular decerebration after which cortisol and corticosterone levels fell to basal secretory rates observed in animals deprived of ACTH. Aldosterone secretion rates continued to gradually fall but were still significantly increased eight fold over basal rates eleven hours after complete nephrectomy and seven hours after midcollicular decerebration. In all cases, the plasma ionic composition was the same at the end of the experiment as at the beginning, eliminating the electrolytes as possible stimulants to aldosterone secretion.

Finally in an attempt to further minimize the effects of elevated levels of ACTH on aldosterone secretion which result as a consequence of surgery, sodium deficient sheep were hypophysectomized two to four weeks previous to experimentation. These animals were maintained on appropriate replacement therapy until two to three days before commencement of the experiment. Further manipulations included bilateral nephrectomy and midcollicular decerebration one hour later. Even though cortisol levels fell to below basal levels for normal conscious animals, the aldosterone secretory rate was six to ten times basal observations after 24

hours had elapsed following nephrectomy. Again it was possible to exclude the changing effect of sodium and potassium.

In their report, Bartter and co-workers (54) compared basic adrenal steroid secretion in hypophysectomized, nephrectomized dogs to that in dogs who had been subjected to additional removal of tissue rostral to the superior colliculi. This decortication not only significantly increased the rate of aldosterone secretion but also the cortisol and corticosterone secretion rates. It was noted that following decerebration the aldosterone levels observed were comparable to those seen in normal or hypophysectomized animals following hemorrhage.

It seems apparent then that the brain tissue between the intercollicular and pulvinar levels is required for the fall of secretion of aldosterone which follows repletion of sodium in sheep which have been deficient. Indeed, sodium deficiency may not even be necessary to demonstrate this effect. Additional data from dogs also supports a possible inhibitory function for certain areas of the central nervous system.

Since it is well known that ACTH exerts a profound influence on adrenocortical function, the possible role of ACTH was one of the first factors to be studied in the regulation of aldosterone secretion.

The first evidence to suggest a role for the adenohypophysis in the control of aldosterone secretion was the finding of a fall in aldosterone secretion following hypophysectomy. This result was reported by Singer and Stack-Dunne (55) in rats in 1955 and by Rauschkolb, Farrell, and Koletsky (56) in dogs in 1956.

Other data favoring this hypothesis soon emerged. In studies by Ganong and co-workers (57) on the relationship of the hypothalamus to aldosterone secretion, the only hypothalamic lesions which influenced aldosterone production significantly were those involving the median eminence. Their dogs with chronic median eminence lesions showed a lower rate of aldosterone secretion than normal dogs after the surgical trauma of adrenolumbar cannulation.

Similar results were obtained by Davis et al (58). In chronic studies of dogs with experimental secondary hyperaldosteronism, the only hypothalamic lesions which were associated with decreased urinary excretion of aldosterone were those involving the median eminence. Moderate to marked adrenocortical atrophy was present in the animals with median eminence lesions. These findings (57, 58) were consistent with a decrease in ACTH secretion which follows median eminence lesions.

Most early observers (56, 57, 59) were reporting approximately an 80 to 90 per cent decline in aldosterone secretion following acute and subacute hypophysectomy. In all of the early experiments the animals were subjected to the stress of an operative procedure in order to cannulate the adrenolumbar vein for collection of adrenal vein blood. In regard to this point, Davis (60) noted that stressful stimuli, provoked by anesthesia, operative procedure, and cannulation, stimulate aldosterone secretion. It was his conclusion that the observed 80 to 90 per cent fall in aldosterone production following hypophysectomy occurred from this elevated level secondary to stress.

In a subsequent report, Davis and colleagues (61) studied the effect of hypophysectomy on steroid secretion by means of a chronic indwelling catheter in trained, conscious dogs with hyperaldosteronism secondary to thoracic inferior vena cava constriction. Again, an 80 to 90 per cent fall in aldosterone secretion occurred following hypophysectomy.

Abundant evidence has also been presented that hypophysectomized human subjects exhibit a somewhat lower than normal rate of urinary aldosterone excretion (62, 63, 64).

Further evidence also was supplied by Haynes and Berthat (65), who demonstrated that ACTH exerts an effect on the biosynthesis of aldosterone in vitro in beef adrenal slices. The work on Pincus (66) was in accord with this finding.

Thus with the establishment of the fact that ACTH is the sole pituitary factor responsible at least in part for aldosterone production in the adrenal gland, studies were focused on determining the role ACTH plays in the overall control of aldosterone secretion.

Davis (60) suggested a dual role for ACTH in the production of aldosterone in various conditions of experimental secondary hyperaldosteronism. He was of the opinion that ACTH plays both an initiative and supportive role in the production of aldosterone.

Following operative procedures (60) and after acute blood loss (67), the striking elevation in the rates of secretion of corticosterone and cortisol suggests the presence of a high plasma level of ACTH. In both experimental situations, the pattern of steroid response was very similar to the increase in aldosterone, corticosterone, and cortisol secretion which occur after the intravenous injection of ACTH. Following laparotomy, no mechanism other than elevated ACTH release has been described to explain the augmented aldosterone output; therefore, in this situation the role of ACTH appears to be an initiative one as well as one of prime importance in maintenance. After acute blood loss, however, renin as well as ACTH is released (68) so that increased angiotensin II is also present to augment aldosterone secretion. (The role of the renin-angiotensin system will be discussed in detail elsewhere in this paper. It will only be noted here for those unfamiliar with this subject, that a great body of evidence exists showing that angiotensin II has a direct effect on the adrenal gland causing an

increased output of aldosterone.) Nevertheless, the greater response to acute blood loss in the intact animal (67,69,70), than in the hypophysectomized dog (67,69,70) makes it clear that ACTH contributes substantially to the increase in steroid production following acute hemorrhage. It was concluded that after acute blood loss, ACTH probably supports steroidogenesis by favoring the action of angiotensin II, which appears to be the primary mechanism leading to increased aldosterone secretion.

The observation by Liddle and associates (71) that the daily administration of ACTH in human subjects results in a diminution in the response in aldosterone output in urine after 3-5 days of hormone injection is consistent with the concept that ACTH is supportive rather than initiative in the regulation of aldosterone secretion.

In experimental secondary hyperaldosteronism due to thoracic inferior vena caval constriction, ACTH appears to be of importance (59,60,61). In this situation, ACTH appears to play a supportive role rather than an initiative one. It has been shown by Davis et al (59) that the 80 to 90 per cent drop in aldosterone secretion which follows hypophysectomy in dogs with hyperaldosteronism secondary to thoracic inferior vena caval constriction was blocked by the intravenous administration of ACTH during the post-hypophysectomy period. This experiment indicated that ACTH has an important function in the maintenance of the elevated

level of aldosterone. On the other hand, Davis and co-workers (61) noted that in conscious dogs with chronic indwelling adrenal catheters and thoracic caval constriction only a low plasma level of ACTH is needed for near maximal aldosterone production. Very high rates of aldosterone secretion were observed in the presence of a low basal output of corticosterone and of cortisol, a finding which reflects a low plasma level of ACTH. Again they concluded that the role of ACTH was merely supportive, whereas angiotensin II was primarily responsible for the elevated aldosterone levels.

In a recent study, Mulrow and Ganong (72) attempted to delineate a supportive role for ACTH in secondary hyperaldosteronism in dogs. As has been previously stated (59) a marked drop has been observed in aldosterone secretion following hypophysectomy. In their study, Mulrow and Ganong (72) observed that acute angiotensin II infusions in the nephrectomized, hypophysectomized dog did not raise the level of aldosterone secretion to that observed in secondary hyperaldosteronism. The addition of ACTH (2-50 mU), an amount which alone has no effect on aldosterone production but stimulates cortisol and corticosterone, to constant infusions of angiotensin II in nephrectomized, hypophysectomized dogs resulted in marked stimulation of 17 hydroxycorticoids and corticosterone secretion but no change in aldosterone secretion. The amount of angiotensin II given was insufficient to stimulate production of the 17 hydroxycorticoids or corticosterone. This data then seems

to be in disagreement with that presented above which favors a supportive role for ACTH in secondary hyperaldosteronism.

Blair-West and colleagues (48) have contributed significantly to the information on the part played by ACTH in aldosterone secretory control. These investigators conducted a series of experiments in conscious, trained sheep with transplanted cervical adrenal glands by which they were able to conduct intra-arterial infusions on this isolated gland in vivo. Sodium replete sheep were infused with β and δ_1 ACTH at rates calculated to give adrenal arterial plasma concentrations of ACTH of approximately 0.5-10 mU per 100 milliliters. The expected relationship between ACTH infusion and cortisol and corticosterone secretion rate was observed but a feature of importance shown was that no significant effect on aldosterone secretion occurred until the cortisol secretion rate exceeded one thousand micrograms per hour and the corticosterone rate was in excess of 45 micrograms per hour. In subsequent experiments, these workers observed that in sodium depleted sheep with high aldosterone levels aldosterone secretion rates often remained elevated after nephrectomy and also in a large majority of the determinations the cortisol and corticosterone levels were high. The majority of cortisol levels were between 380-400 micrograms per hour, whereas the corticosterone levels ranged between 16-20 micrograms per hour. It thus appeared that increased levels of ACTH must be present to account for the

elevated levels of cortisol and corticosterone. Even so, the levels of cortisol and corticosterone were not elevated to the levels at which one begins to observe a rise in aldosterone secretion rate. The authors then suggested that possibly the adrenal glomerulosa might be more sensitive to ACTH in the sodium depleted state. To test this hypothesis, they gave from 280-450 milliequivalents of sodium chloride systemically at between 48 and 52 hours. A large fall of aldosterone secretion took place without an observed drop in the levels of cortisol or corticosterone. Thus the aldosterone secretion rate decreased in the sodium depleted, nephrectomized animal which was made sodium replete, although the index of ACTH release remained high. It appeared that the sodium chloride infusion changed the sensitivity of the adrenal gland to ACTH, but a local effect of sodium chloride on the adrenal gland could not be excluded. Subsequent experiments were performed in conscious, intact sheep with transplanted cervical adrenals. Adrenal arterial infusions of β_1 ACTH was conducted on these sodium deficient animals. Large rises of aldosterone appeared in adrenal vein blood in response to infusion rates of only 0-60 mU per hour, which had no effect in sodium replete sheep. It would appear that the body stores of sodium play an important part in the aldosterone secretory response of the zona glomerulosa to ACTH.

Since the early work of Haynes, other data has appeared in which ACTH has been shown to have a direct effect on the biosynthesis of aldosterone.

Early 1962 data, published by Kaplan and Bartter (73), showed a direct effect of ACTH upon aldosterone biosynthesis by beef adrenal slices. In their initial work, 8 U per gram tissue of ACTH stimulated the biogenesis of all three steroids, i.e., hydrocortisone, corticosterone, and aldosterone. A quantitatively greater response was observed in the case of hydrocortisone, however.

In more recent works, Kaplan (74) has found a dose of ACTH which will stimulate the synthesis of hydrocortisone and corticosterone but will not increase the production of aldosterone in vitro. This dose of ACTH was 1 U per gram of adrenal tissue. He also found that at a level of 2 U per gram of adrenal tissue, aldosterone biogenesis was significantly stimulated at an incubation time of 30 minutes. Examination of the biosynthetic rate at one hundred and eight minutes revealed the aldosterone production rate to be identical with the control value. At both incubation times the effect of ACTH upon hydrocortisone synthesis was pronounced.

Muller (75) has provided additional data on the effects of ACTH on aldosterone biogenesis in vitro. Using rat adrenal quarters, he demonstrated that 5 IU units of ACTH was responsible for at least a 150 per cent increase in aldosterone and concomitant

450 per cent increase in corticosterone values. These levels represented minimal values above the mean controls. To study the effect of sodium deficiency on adrenal response to ACTH, he used adrenals from rats which had been sodium deficient for two weeks prior to sacrifice. An increased level of aldosterone biogenesis was observed in both the control vessels and those vessels with ACTH added to them. In both cases, the levels were significant when compared with adrenals derived from sodium replete animals. The response was also significantly greater in the ACTH stimulated quarters derived from sodium depleted rats when compared to the control. Corticosterone levels in both cases were unaffected; in fact, a small decrease was noted in its synthesis. These results agree with the in vivo findings of Blair-West et al (48) that the response of the adrenal tissue to ACTH, as manifest by increased aldosterone biosynthesis, seems to be greater in animals which are sodium deficient. Since aldosterone is also intimately involved in the metabolism of potassium, the response of adrenal tissue to ACTH under the influence of varying amounts of potassium in the incubation medium was studied. It was found that in the presence of a potassium concentration of 3.6 milliequivalents the base line aldosterone production was 40 per cent lower than the levels in the standard potassium concentration of 5.9 milliequivalents. Maximal ACTH stimulation gave comparable aldosterone levels in both concentrations of potassium ion.

Although the evidence available unequivocally demonstrates that ACTH can exert an independent and pronounced effect on aldosterone secretion, this type of analysis of the functional role of ACTH in the control of aldosterone secretion is a descriptive one. Even in a descriptive analysis, we are not yet able to state with any assurance to what extent ACTH participates in the minute to minute and day to day control of aldosterone secretion.

Only until knowledge of the specific biochemical pathways by which ACTH exerts its effect are known, will one be able to ascertain the exact role of ACTH on the overall picture of aldosterone secretion regulation. The precise action of ACTH in the biosynthesis of the adrenal mineralo- and glucocorticoids is unknown. It will only be briefly mentioned here that Haynes and Berthat (65,76) proposed a theory concerning the mechanism of ACTH action. Their theory states that initially ACTH stimulates the formation of cyclic adenosine-3'5'-monophosphate (cyclic AMP), which in turn activates the enzyme phosphorylase. Active phosphorylase then mediates the conversion of glycogen to glucose-1-phosphate, which is converted to glucose-6-phosphate by the isomerase, phosphoglucomutase. Glucose-6-phosphate may then be metabolized aerobically via the hexose monophosphate shunt with the resultant production of reduced nicotinamide-adenine dinucleotide phosphate (NADPH). It is well known that $NADPH_2$ is vital for hydroxylation reactions at the 11, 17, 18 and 21 positions in steroidogenesis (66).

Although their theory has remained popular over the years and the increased metabolism of cyclic AMP and an increase in phosphorylase following ACTH stimulation appears to be well founded, sequential steps from this point are not solidly established (77).

The relationship between the effects of ACTH and of angiotensin II in steroidogenesis is also poorly defined. That doses of ACTH exist which stimulate the production of corticoids other than aldosterone is well known (48, 74, 75). That higher levels stimulate production of all the adrenal steroids is also a well established fact (48, 74, 75). In a like manner, doses of angiotensin II are known which stimulate increased aldosterone production only, while higher doses stimulate increased production of all the adrenocorticoids (48,74,75,78,79).

Kaplan (74), noting that ACTH exerts its effect through cyclic 3'5' AMP, incubated adrenal slices with maximal stimulatory doses of cyclic 3'5' AMP, ACTH, and the combination of the two agents. A vigorous response was observed in all cases with a marked increase in all corticosteroids. As expected, the response to stimulation with ACTH and cyclic 3'5' AMP together was no greater than the response to each of these agents alone. Adrenal slices were then incubated with maximal stimulatory doses of angiotensin II, angiotensin II plus ACTH, and angiotensin II plus cyclic 3'5' AMP. Significant elevations of all the corticosteroids were again observed. The combination of angiotensin II with either ACTH or cyclic 3'5' AMP had an effect significantly greater than that obtained with

angiotensin II alone. The finding of an additive effect of both ACTH and cyclic 3'5' AMP with that of angiotensin II suggests that these stimulatory substances may be producing their effects via different mechanisms.

Finally as a matter of completeness, it should be pointed out that the ACTH control mechanism, thus far mentioned, has been only an efferent one. Besides the well known feed-back mechanism which exists to control ACTH, it is well established that peripheral stimuli may initiate nervous impulses which are integrated in higher centers in the central nervous system. These modified stimuli are relayed to the hypothalamus, where a corticotropin releasing factor may be released to stimulate ACTH production and release (80,81). To consider these mechanisms would be to describe the control of ACTH release which is not germane to the present subject.

Since aldosterone occupies a position of importance in the metabolism of sodium and potassium, much attention has also been devoted to dietary and plasma levels of these electrolytes and their relationship to the secretion of aldosterone.

The importance of the sodium ion on the control of aldosterone secretion was first reported by Luetscher and Axelrad (82) in 1954. Since this initial report, it has been repeatedly demonstrated that a low sodium intake augments the rate of aldosterone secretion in several mammals including man, and that sodium loading decreases aldosterone (83,84,85). The hypersecretion of aldosterone has been observed in hypophysectomized, sodium depleted animals (83,48). Although ACTH is unnecessary for sodium depletion to increase aldosterone production, in the presence of the adenohypophysis ACTH may function to support steroidogenesis at a higher level than in the hypophysectomized animal during chronic sodium depletion. The role of ACTH in the control of aldosterone secretion during sodium depletion has previously been thoroughly reviewed and will not be considered any further at this point. The primary mechanism by which sodium depletion stimulates aldosterone secretion appears to be mediated via the renin-angiotensin system (83,84,85,86,87,88, 89,90) which will be considered later.

In contrast to the unequivocal evidence for the influence of alterations in sodium intake on aldosterone secretion, there has been considerable divergence of opinion on the role of dietary

potassium in the control of aldosterone secretion. Several workers (91,92,93,94,95,96) have reported that potassium loading augments the rate of aldosterone excretion, whereas potassium depletion results in decreased urinary values for aldosterone. On the other hand, Hernando and associates (97) reported that four of five human subjects failed to show an increase in urinary aldosterone output in a situation in which potassium loading was superimposed on a low sodium regimen. Bartter (17) stated he observed an increase in urinary aldosterone output in response to potassium loading in the presence of a normal intake of sodium. Rosnagle and co-workers (98) were unable to detect an increase in aldosterone secretion in intact dogs during potassium loading.

Bartter et al (99) stated that a fall in aldosterone excretion with potassium depletion may occur even when restriction of sodium intake prevents significant sodium retention, and that the rise in aldosterone excretion with the administration of potassium could not be prevented by immediate replacement of sodium excreted in response to the potassium load. He further concluded that changes in body potassium influenced aldosterone secretion by a mechanism not dependent upon alterations in intravascular volume.

In a more recent study, Gann and colleagues (100) examined the effects of changes in body potassium in normal human subjects. Dietary potassium was varied, while sodium was kept constant and losses which occurred in the urine were immediately replaced.

Mannitol infusions were used to vary potassium levels. Their studies confirmed the previous reports which show that the administration of potassium increases and a depletion of potassium decreases the excretion of aldosterone. They further concluded that the changes in excretion of aldosterone which follow potassium loading or depletion are independent of changes in sodium balance or intravascular volume.

It naturally followed that when sodium and potassium were implicated in the control of aldosterone release, attention was also focused on the plasma and serum concentrations of these electrolytes.

In considering the mechanism of action of potassium, Laragh and co-workers (101) proposed that the level of plasma potassium is the primary determinant of the rate of aldosterone secretion during changes of potassium intake. Both normal human subjects and patients with benign essential hypertension were depleted of sodium by dietary restriction and diuretics. They gradually shut off sodium excretion without an increase in aldosterone. A concomitant hypokalemia was produced by the diuretics. Upon repletion of the potassium level, a gradual rise in aldosterone excretion was observed. These investigators (91) also reported a similar response in dogs. Increases in plasma potassium in normal dogs was accompanied by elevated aldosterone levels. Laragh et al (102) were unable to conclude whether or not a fall in plasma sodium concentration per se stimulates aldosterone output in man. They noted that a reduced plasma level is not predictably associated with elevated aldosterone excretory rates.

Bartter et al (12) were in agreement that hyponatremia per se is not a stimulus to the secretion of aldosterone in man. They deliberately induced hyponatremia by giving water to subjects receiving Pitressin tannate in oil. Aldosterone excretion never rose even when hyponatremia was accompanied by potassium loading such that the serum sodium/potassium ratio was sharply reduced.

Moran and associates (103) reported that elevation of the peripheral plasma level of potassium by intravenous infusions of potassium chloride in intact dogs augmented the aldosterone secretory rate.

In a more recent study, Gann and collaborators (100) have shown in normal human subjects that hyperkalemia is accompanied by an elevated level of urinary aldosterone and that hypokalemia causes a reduction in aldosterone excretion. Plasma potassium levels were regulated by dietary potassium and diuretics. Plasma sodium levels were kept constant and the plasma volume did not vary significantly.

Using a more direct approach to study the effect of the potassium ion, Urquhart et al (104) conducted perfusion experiments in the isolated adrenal glands of hypophysectomized, nephrectomized dogs. They were able to show that increased plasma levels of potassium alone stimulated aldosterone by an apparent direct action on the adrenal cortex. Plasma sodium levels were decreased by infusions of five per cent glucose or mannitol. An increased secretory rate was also observed in response to this hyponatremia. In both cases the corticosterone secretion rate was not significantly affected.

Similar studies to these were conducted by the Australian workers, Blair-West, Denton, Goding, and Wright (48). These workers perfused the isolated, transplanted cervical adrenal glands of intact, conscious, sodium replete sheep. They were also able to demonstrate a direct effect on aldosterone secretion by variations in sodium and potassium concentration perfusing the adrenal gland. It was concluded that the only significant effect in aldosterone stimulation could be obtained by a concurrent decrease of sodium and increase of potassium. The largest increase in aldosterone production was observed when the plasma sodium concentration was reduced by 19 milliequivalents per liter and the potassium concentration was increased by 1.9 milliequivalents per liter. Increased aldosterone secretion occurred independent of changes in cortisol and corticosterone.

These investigators were also interested in determining whether a change in dietary sodium exerts its effect by alteration of the plasma concentration and thence exerting a local, direct effect on the adrenal glomerulosa. Sodium deficient animals, secreting high levels of aldosterone, underwent adrenal perfusion with an increased concentration of sodium. Little or no reduction of the high aldosterone secretory rate occurred. However, when systemic infusions of hypertonic sodium were given, correcting the body sodium deficit, a large decrease in aldosterone production occurred. They concluded that the systemically infused sodium acted elsewhere

in the body than on the adrenal and that aldosterone control in this case involved at least a two component system.

Additional in vitro experiments are available which tend to support a local stimulatory role for the potassium ion. Kaplan (74) demonstrated that the presence of potassium in the incubation medium in the concentration of ten milliequivalents per liter caused beef adrenal slices to synthesize a significantly greater amount of aldosterone and corticosterone but not hydrocortisone. Lowering the sodium concentration in the medium to 107 milliequivalents per liter had no effect upon the synthesis of any of the adrenocortical steroids.

Muller (75) obtained similar in vitro results using rat adrenal quarters. An aldosterone stimulating effect was observed with increased potassium in the incubation medium. The potassium levels were increased from 3.6 to 8.4 milliequivalents per liter with a maximal stimulation of more than 200 per cent increase above the mean control value at 8.4 milliequivalents per liter of potassium concentration. Corticosterone production was not significantly affected by these changes in potassium concentration.

It should be noted that although an increased potassium concentration has been shown to be a stimulant of aldosterone synthesis in vitro, the effective concentrations are not at physiologic levels. However, this is true in general of in vitro steroid experiments (74).

In 1958, Yankopoulos, Davis, Kliman, and Peterson (51) presented direct evidence that a humoral agent is present in peripheral blood which provides a stimulus to aldosterone secretion. Cross circulation of blood from dogs with thoracic inferior vena caval constriction and secondary hyperaldosteronism through normal isolated adrenals consistently resulted in the hypersection of aldosterone by the isolated adrenal glands. The adrenals were isolated by the technique of Hilton and associates (105). Control observations were made by circulation of blood from the carotid artery of the normal recipient through the isolated adrenals and by return of blood to the external jugular vein of the recipient. During cross circulation, peripheral blood from the donor dog with chronic secondary hyperaldosteronism was circulated from the donor's femoral artery through the isolated adrenals and returned to the femoral vein of the donor. Aldosterone secretion increased in every experiment; the average increase was 129 per cent which was highly significant. As a control experiment, blood from normal dogs was circulated through the isolated adrenals of normal dogs. In this case, no consistent changes in aldosterone secretion occurred and the averages of the control and the experimental values were the same. It was also noted that throughout the perfusion experiments the concentration of sodium and potassium in the plasma perfusing the isolated adrenals did not vary significantly throughout the control, cross circulation, and recovery periods. These findings did clearly

indicate that a circulating humour in peripheral blood acts on the adrenal cortex to increase the aldosterone secretory rate. It was suggested that this hormone be designated the aldosterone stimulating hormone (ASH). Concurrent independent cross circulation studies were conducted in conscious, trained sheep by Denton, Goding, and Wright (94). Their results were essentially the same as those in the dog indicating the presence of an aldosterone stimulating hormone. It was clear from these observations (51,94) and from subsequent reports (60,69,83,106,107,108) that this aldosterone stimulating factor in peripheral blood is not ACTH. As previously presented, it was also reported that neither the upper brain stem or pineal region (32,51) nor the lower brain stem (32) was the site of production of this particular aldosterone stimulating hormone.

Prior to the demonstration of the existence of an aldosterone stimulating hormone in peripheral blood (51,94) the suggestion of a close functional relationship between the adrenal cortex and the kidney had been made by several workers. In 1949, Dunihue (109) found that adrenalectomized cats demonstrated a hypergranularity and hypertrophy if the juxtaglomerular apparatus of the kidney and that this hypergranularity could be prevented or reversed by the administration of desoxycorticosterone acetate in the adrenalectomized animals Hypergranularity of the juxtaglomerular cells has also been observed in Addison's Disease (110). In 1951, Deane and Masson (111) reported that the injection of partially purified solutions of renin

produced widening of the zonaglomerulosa of the adrenal cortex in rats. Additional data which supported an inerrelation between the juxtaglomerular cells and the adrenal gland was supplied by Hartroft and Hartroft (112,113). They demonstrated that sodium deficiency in adult rats did indeed produce hypergranulation of the juxtaglomerular cells. High salt feeding on the other hand produced a kidney with juxtaglomerular apparati which were degranulated. Degranulation was further intensified if the high sodium diet was supplemented with desoxycorticosterone acetate injections (112). Conducting further experiments, these investigators studied the changes in the zonaglomerulosa of the adrenal glands of rats on low sodium diets and on high sodium diets with desoxycorticosterone supplements. The rats on low sodium diets revealed a significantly hypertrophic zonaglomerulosa whereas those on high sodium diets with desoxycorticosterone supplements had an atrophic zonaglomerulosa. These findings were in accord with the earlier work of Deane et al (114). Hartroft and Hartroft (113) made further observations showing that a highly significant degree of correlation existed in these animals between juxtaglomerular cell granularity and the adrenal status. Those rats on low sodium diets with hyperplasia of the zonaglomerulosa demonstrated a great degree of granularity of the juxtaglomerular cells. On the other hand, those animals receiving high sodium diets and desoxycorticosterone acetate presented with atrophy of the zonaglomerulosa and markedly decreased granularity of the juxtaglomerular

apparatus. A similar correlation between the status of the zona-glomerulosa and the granularity of the juxtaglomerular apparatus was shown in patients with varying degrees of hyponatremia (115). Hartroft and Edelman (116) also observed that hypophysectomy in rats neither alters the juxtaglomerular cells nor prevents their stimulation (hypergranularity) by sodium deficiency and degranulation (inhibition) by sodium loading and desoxycorticosterone acetate. Kuhn, Hartroft, and Pitcock (117) reported on experiments in which rats were injected twice daily with extracts of dog kidneys that contained high concentrations of renin, whereas control rats were injected with similar extracts but from normal dog kidneys with a very low content of renin. During the first few days the zona-glomerulosa underwent hypertrophy concomitant with a progressive degranulation of the juxtaglomerular cells. Both effects were seen to reverse when antibody formation to the foreign protein took place. This degranulation of the juxtaglomerular apparatus was interpreted as suppression of activity, which is what would be expected with an exogenous source of endogenous secretory product. Pressor activity of renal extracts (renin) from these same rats was correlated with the degree of granulation of the juxtaglomerular cells. As shown previously (118,119) increasing pressor activity was accompanied by increasing granularity of the juxtaglomerular cells.

With this mass of incriminating evidence available, it thus remained for Davis and his colleagues (60,69,83,106,120,121,122,

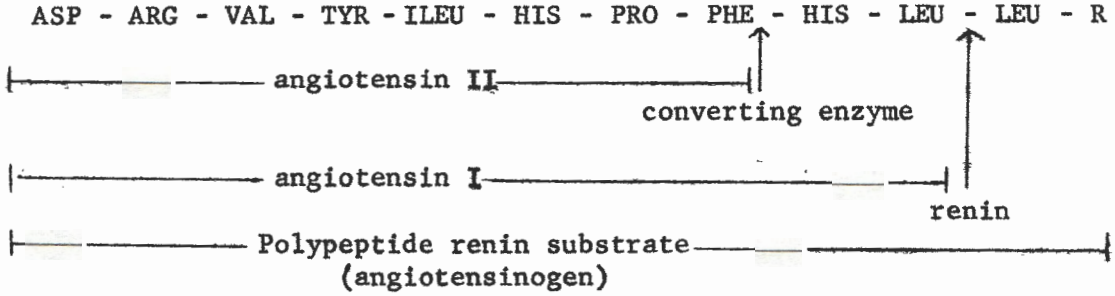
123) to provide more direct evidence that the kidney was the source of the aldosterone stimulating hormone found in the peripheral circulation and that this hormone was in fact the enzyme, renin. Classic endocrine techniques were used in uncovering of these facts. Control observations were made in hypophysectomized, nephrectomized dogs. Hypophysectomy was also done in these experiments to eliminate a rise in aldosterone secretion which could occur in response to elevated ACTH levels as a result of operative procedure to cannulate the adrenal vein. The stimulus of acute blood loss failed to elicit a rise in either aldosterone or corticosterone secretion in the hypophysectomized, nephrectomized dog. In the presence of kidneys in hypophysectomized dogs, however, a 100 per cent rise in aldosterone production occurred in response to acute hmorrhage. Saline extracts were then made from the dog kidneys and infused into hypophysectomized, nephrectomized dogs. A significant increase in aldosterone secretion was observed in all cases. It was further noted that in the hypophysectomized, nephrectomized animals, the basal rate of aldosterone secretion was a mean value of 4.5 mug per minute, whereas the mean basal secretory rate for aldosterone in dogs which had undergone hypophysectomy only was 9.0 mug per minutes. This then represented a 50 per cent reduction in the mean basal rate of aldosterone secretion in those hypophysectomized dogs having had bilateral nephrectomies. It was further shown, that in cases of experimental secondary hyperaldosteronism which included cardiac

failure, thoracic inferior vena caval constriction, and sodium deprivation, a significant and striking decrease in aldosterone secretion occurred upon bilateral nephrectomy. In all cases the dogs were hypophysectomized. A significant rise in aldosterone secretion was again observed when these animals were infused with saline extracts of kidneys. Lastly, fractionation studies of kidney extracts showed that the only fractions with aldosterone stimulating hormone activity were the renin fractions. The independent work of Ganong and Mulrow (107) showed essentially the same series of findings in similar experiments.

At essentially the same time in 1960, Genest and associates (124,125) and Laragh and colleagues (78) reported that the intravenous infusion of synthetic angiotensin II increased aldosterone secretion in man. Other confirmatory reports followed from various independent groups describing increased aldosterone secretion in response to partially purified renin (126) or synthetic angiotensin II (126,127).

As a point of completeness and in order that the role of the renin-angiotensin in the regulation of aldosterone secretion may be better understood, the components in the system and their interactions will be briefly discussed. Haas, Lamfrom, and Goldblatt (128,129) have partially purified renin from kidney extracts. It is a proteolytic enzyme which is thermolabile and has a short biologic lifespan. Renin is both the initial and rate limiting substance of the system. It was discovered that renin acts on a

protein in the α_2 globulin fraction of plasma to split off a decapeptide, angiotensin I (129, 130). This renin substrate is synthesized in the liver. Three major and two minor forms of renin substrate have been isolated and purified from hog plasma (131,132). Analysis shows that all are glycoproteins with molecular weights of about 57,000 with a similar amino-acid composition. These substrates apparently differ only in their carbohydrate moiety. Differences in sialic acid, neutral hexose, and glucosamine are observed in the various forms of renin substrate (134). Nevertheless, all yield the same decapeptide (angiotensinI) when hydrolyzed by renin. Angiotensin I in turn is converted into an octapeptide (angiotensin II) by the splitting off of a histadyl leucine residue by the enzyme known as the converting enzyme. This enzyme is present in plasma and is chloride activated (135). The structure of angiotensin II was determined and found to be composed of the following amino acids from the N terminal amino-acid: aspartic acid, arginine, valine, tyrosine, isoleucine, histidine, proline, phenylalanine (136,137,138). The N terminal amino-acid of angiotensin II, aspartic acid, is the N terminal amino-acid of renin-substrate (angiotensinogen) (134). The components and reactions of the renin-angiotensin system may be summarized as follows:



That the end product of the renin-angiotensin system, angiotensin II, is a direct stimulant to the zonaglomerulosa of the adrenal cortex, causing secretion of increased amounts of aldosterone, has been shown in many acute, subacute, and chronic experiments.

Ziegler and Gross (139) demonstrated that severe acute hemorrhage in the rat accompanied by a steep fall in blood pressure increased the plasma concentration of renin-like pressor substance to about four times the normal value within a few minutes. This effect is not demonstrable if the kidneys are removed 30 minutes prior to bleeding. In the dog a similar increase in either circulating angiotensin II (140,86) or renin (141) is obtained after severe bleeding. Coupled with the earlier evidence that hemorrhage stimulates aldosterone secretion in the dog and this effect is blocked if the pituitary and kidneys, but not the kidneys alone, are removed (70,108,142,143), this data indicates that the renin-angiotensin system is one humoral factor operative in the increased secretion of aldosterone in response to hemorrhage. It is yet not clear, however, whether the reduction of blood volume or the steep fall in blood pressure is responsible for the release of renin. Of interest in this respect is the observation that retransfusion of the amount of blood removed during hemorrhage leads to a rapid return to normal of the elevated plasma renin concentration (141) and a reduction of

the elevated aldosterone secretion rate to normal (70) in the dog. Also of note is the work of Paladini and Scornik (144) who showed that acute hypotension produced in the dog by ganglionic blocking agents was not followed by an increase in blood angiotensin levels.

That the renin-angiotensin system is responsible for the elevated aldosterone levels observed in sodium depletion and the reduced aldosterone levels observed in sodium loading has been well-established. Gross and associates (90) conducted acute sodium depletion experiments in rats. The rats were depleted by giving intraperitoneal injections of one hundred milliliters of five per cent glucose and withdrawing it after one hour. Cross circulation studies demonstrated that within three hours of the initial administration of glucose solution, an increased concentration of renin like substance was detected equal in magnitude to that observed in acute hemorrhage. A prolonged sodium load, either dietary alone or enhanced by the simultaneous administration of mineralocorticoids, was shown to lead to the disappearance of renin from the blood and kidneys in rats within a few weeks (145). Brown et al (89) demonstrated that normal human subjects on a sodium diet of 420-560 milliequivalents daily showed a fall in plasma renin in all cases. When the diet was restored to normal, a rise in plasma renin to normal levels was again observed. Veyrat and colleagues (87) further demonstrated a parallel relationship between arterial renin activity and urinary

aldosterone valves in normal human subjects. Both values were low on high sodium diets. In contrast to chronic loading experiments, chronic sodium depletion has been shown to significantly increase the concentration of circulating renin and the content of renal renin in rats. No concomitant changes in blood pressure or hematocrit are observed (90,146). Brown et al (89,147) studied the effect of sodium depletion in normal human subjects as sodium depletion persisted. Sodium repletion led to a fall in renin values to a level observed in the normal individual. Veyrat and associates (87) went further and again demonstrated a parallel relationship between arterial renin activity and urinary aldosterone levels. Both values were seen to be significantly elevated in sodium depleted human subjects. It has been stated that small decreases in plasma sodium levels may lead to increased levels of aldosterone secretion (although not statistically significant) by a direct action on the zonaglomerulosa (48) whereas other investigators (12,48,102) are of the opinion that hyponatremia per se is not a stimulus to increased aldosterone secretion, but that the renin angiotensin system is the regulatory mechanism important in the response of aldosterone production to dietary sodium. This is borne out by the preceeding statements. Furthermore, in an attempt to determine by what mode sodium deficiency stimulates the renin-angiotensin system, Scornik and Paladini (86) measured the blood angiotensin levels, plasma sodium, and the

apparent volume distribution of Na^{24} in normal dogs made sodium deficient. They found that with an eighteen per cent depletion of the total exchangeable Na^{24}, the angiotensin blood levels increased significantly. The dogs were nephrectomized and 24 hours later no pressor activity was found. Moreover a significant correlation was found between angiotensin blood levels and the sodium space but not between angiotensin levels and the sodium plasma concentration. Conn and associates (148) feel that changes in the plasma volume secondary to variations in dietary sodium play an important role in the control of renin release and ultimately the rate of aldosterone production. They have also pointed out that when plasma volume becomes overexpanded a second mechanism also participates in the regulation of sodium (149). This mechanism involves the partial inhibition of proximal tubular reabsorption of sodium due to the action of an unidentified hormone (salt-losing hormone).

Conn and co-workers (148,150) have also shown that posture significantly affects renin secretion. It was shown in normal human subjects that there is a significant increase in renin secretion and renin activity in plasma on assuming the upright posture. That this is not a mere function of exercise was observed when these subjects exercised in the recumbent position and then were tilted to the upright position, passively. A significant increase in plasma renin activity was observed in

the upright position. Differences in dietary sodium does not obscure this phenomenon. The upright posture stimulates renin release even when subjects have been sodium loaded and as expected, renin release is intensified in sodium depleted subjects who assume an erect position.

Gross, Ziegler, and Brunner (90) observed rats that had been subjected to bilateral adrenalectomy and were not given replacement therapy. In about five to seven days, a marked increase in circulating renin and renal renin content were noted. However, these animals also demonstrated hemoconcentration, lowered blood pressure, negative sodium balance and hyponatremia. Human subjects with Addison's Disease have also been shown to exhibit extremely high levels of plasma renin when they are left untreated (84,89). When given appropriate replacement therapy, plasma renin values are seen to return to normal or near normal levels in patients with Addison's Disease (84,89). In contrast to this data, Barbour (151) stated he was unable to detect any angiotensin in peripheral arterial blood of patients with Addison's Disease. Conn and colleagues (152,153) have shown that the converse situation also exists in man in the case of primary aldosteronism, in which apparently autonomous adrenal cortical tissue (tumor) is secreting aldosterone at an unabated rate. They demonstrated complete supression of the renin angiotensin system in patients with primary aldosteronism. Renin activity could not be defected in the

supine or upright position. No renin activity was observed after severe restriction of sodium for as long as 16 days. Following removal of the aldosterone secreting tumors, these patients had measurable plasma renin activity in one to nine days. Furthermore, they were able to respond to sodium deprivation with increased renin levels. Aldosteronopenia persisted in these patients, however, indicating the adrenal cortex was now unresponsive. Barbour (151) has also indicated that he was unable to detect any angiotensin activity in the peripheral blood of human subjects with primary aldosteronism.

The production of experimental hypertension has also led to many pertinent observations concerning the role of the renin-angiotensin system in the control of aldosterone secretion (90, 154). The placement of a clip on one renal artery, while leaving the opposite kidney untouched, results in a characteristic distribution pattern for renin. Renin is observed to increase in the kidney with reduced blood flow due to the clip, whereas the opposite untouched kidney has no detectable renin in a short period. Circulating renin levels are seen to increase parallel with the increase in renin content of the clipped kidney. A parallel increase in aldosterone secretion is also seen with the increase in circulating renin. This response has been observed in the dog, cat, rat, and rabbit. It should be noted in the rat that the presence of a contralateral untouched kidney is necessary for the

elevation of renin content of the clipped kidney and a rise in circulating renin. Hence, the untouched kidney is also necessary for a rise in aldosterone secretion.

A similar situation is also seen in human subjects with hypertensive disease due to unilateral narrowing lesions of the renal artery such as atherosclerotic plaques or fibrous dysplasia. Many of these patients have a secondary aldosteronism and increased renin activity may be demonstrated in the venous blood from the kidney supplied by the stenotic artery (155) or in the peripheral blood (152,153).

That infusions of renin and angiotensin II are capable of stimulating increased aldosterone secretion in many species, including man, has been shown both indirectly and directly by many investigators. As previously cited, Genest and associates (124, 125) and Laragh and colleagues (78) reported that brief intravenous infusions of synthetic angiotensin II into human subjects four periods of eight to 24 hours resulted in increased secretion (78) and excretion (124,125) of aldosterone. Some of the earlier studies involved the infusions of dog renin into hypophysectomized, nephrectomized dogs (127,157). The amount of renin given was large enough to cause an increase in blood pressure but also a striking increase in the secretion of aldosterone. Corticosterone and Porter-Silber chromogen secretion also increased but the increment was slight. In subsequent experiments, Mulrow et al (158) used

smaller doses of more pure hog and human renin. They demonstrated a significant increase in aldosterone secretion and blood pressure in hypophysectomized, nephrectomized dogs. Increases in corticosterone and Porter-Silber chromogens were observed only with the larger doses of renin. The first infusions of synthetic angiotensin II gave results similar to those observed with renin infusions (126,127). A rise in blood pressure, as well as in aldosterone secretion, was observed. There also was some stimulation of corticosterone and 17 hydroxycorticoid production. In a later study (158), the infusion of angiotensin II into hypophysectomized, nephrectomized dogs was shown to increase blood pressure and aldosterone secretion, but have no effect on corticosterone or 17 hydroxycorticoid production. Urquhart and co-workers (156,159), noting that experiments up until this time had been acute, attempted to demonstrate a more physiologic basis for the control of aldosterone by angiotensin II. They infused normal conscious dogs for periods as long as 11 days with doses of synthetic angiotensin too low to increase blood pressure, but definite increments in urinary aldosterone excretion occurred over this period. There was no evidence for tachyphylaxis as regards the response in aldosterone secretion. The results of continuous intravenous infusion of synthetic angiotensin II in normal human subjects was reported by Laragh et al (160,161). Nonpressor doses were infused for periods up to ten days with a resultant continuous augmentation of the aldosterone secretory rate. No significant increase in

hydrocortisone secretion was observed. Thus the angiotensin II effect was both persistent and selective with nonpressor doses. Finally, the direct action of angiotensin II upon the adrenal cortex in the stimulation of the production of aldosterone has been shown. The selective stimulation of aldosterone biogenesis by the infusion of synthetic angiotensin II (valine 5 angiotensin) into the arterial supply of the isolated adrenal glands was demonstrated in the sheep (48) and in the dog (162). Unlike the dog, sheep, and man, considerable disagreement exists as to the importance of angiotensin II in the stimulation of the adrenal cortex and aldosterone production in the rat. Marx et al (163) injected angiotensin II amide in oil subcutaneously into rats for periods up to four weeks. A sustained rise in blood pressure resulted and on sacrifice the width, volume, and lipid content of the zonaglomerulosa was significantly increased. The quartered rat adrenals were also incubated alone and with ACTH. When compared to normal controls, the aldosterone values for the adrenals from the injected rats were significantly greater. Singer and coworkers (164) also reported that the infusion of val-5-angiotensin II into the jugular vein of hypophysectomized rats with ligated renal vessels generally resulted in an increase in aldosterone production as measured by the secretory rate into adrenal vein blood. Marieb and Mulrow (165) presented contradictory evidence. Acute infusions of angiotensin II and semipure rat renin failed to stimulate aldosterone secretion in intact rats.

Angiotensin II infusions into hypophysectomized, nephrectomized animals did not increase the aldosterone levels to those observed in intact rats. Aldosterone secretion was also not stimulated by the continuous infusion of angiotensin II in seven to twelve days. Moreover, during sodium deficiency nephrectomy failed to lower the high aldosterone secretion rates of these rats. Other investigators (166,167) have reported essentially the same findings.

Having firmly established that angiotensin II is a direct stimulant of the adrenal cortex for the secretion of aldosterone in dog, man, sheep and possibly the rat, brief consideration will be given to the juxtaglomerular apparatus and intrarenal location of renin, in order that the stimuli for renin release may be reviewed and the overall picture of the renin-angiotensin aldosterone axis may be presented.

The juxtaglomerular apparatus may be considered to include the following units: juxtaglomerular cells, lacis or polkissen cells, and the macula densa (168,169). The juxtaglomerular cells are specific granulated cells having an epithelial like appearance and are located in the wall of the afferent arteriole just before it enters the glomerulus (170,171). The juxtaglomerular cells are in such a position as to be in contact with the arteriolar endothelium on one side and the smooth muscle cells of the media on the other side (170). The granules observed in the cytoplasm of the juxtaglomerular cells are thought to represent renin or its precursor (168), since in the acute phase of sodium depletion a significant degree of degranulation is seen to occur before the hypergranulation of chronic sodium deficiency appears (172). Hypergranularity, hyperplasia, and hypertrophy of the juxtaglomerular cells which occur during experiments in which renin levels are chronically elevated are consistent with the idea that these granules represent synthetic and secretory activity (168). As already

previously stated, it was also observed that the granules in the juxtaglomerular cells increased and decreased in parallel with the renin content of the kidney in several different experimental situations (118,119).

The "lacis" or polkissen cells are located in the angle formed by the entry of the afferent arteriole into and the exit of the efferent arteriole from the glomerulus. (168). The polkissen cells are thought to be continuous with the mesangial cells of the glomerular tuft and it has been suggested that these cells may be transformed into juxtaglomerular cells in conditions stimulating hyperactivity (173).

The macula densa represents a specialized portion of the distal tubule which is adjacent to the afferent arteriole. It consists of those cells of the distal tubule which abut directly upon the juxtaglomerular cells, that are in that side of the arteriolar wall (168). Electron microscope studies have shown that there is no basement membrane separating the cells of the macula densa and the juxtaglomerular cells. Furthermore, these same investigators observed apparent intercellar channels between these two elements, which appeared to be continuous with the endoplasmic reticulum of both cells (171). This finding may be of functional significance.

Cook and Pickering (174,175,176) in an attempt to locate the site of renin production and storage within the kidney, infused a

suspension of magnetic iron oxide particles into the renal artery. These particles lodged in the glomerular capillaries and, by means of an electromagnet, the magnetic glomeruli were separated from the non-glomerular tissue of the fragmented cortex. By microdissection the glomeruli were separated into approximately equal parts, which consisted of a vascular pole half, including the afferent arteriole and the opposite distal half. Extracts of these halves were injected into pentolinium treated rats. Almost all the pressor material was contained in the half which included the afferent arteriole. It was concluded that renin is stored in cells located in the region of the vascular pole of the glomeruli.

As had been previously implied by earlier studies in which variations in granularity were assumed to be indicative of variations in renin content in the juxtaglomerular cells, Hartroft, et al (168, 177,178) demonstrated that antibodies to partially purified renin were localized to the juxtaglomerular cells. Frozen dried or cold acetone fixed sections of dog and rabbit kidney were incubated with fluorescein-labeled antiserum to partially purified hog renin which had been obtained from dog serum. Since antihog renin also neutralizes dog and rabbit renin (179), antibodies to renin in these two species are effective, while antibodies to other hog protein are less likely to interfere. Very specific staining of juxtaglomerular cells in the rabbit and dog was observed. No staining of the macula densa was noted.

In contrast to the concept that renin is located in the juxtaglomerular cells, Bing and Kazimierczak (180,181,182,183, 184,185,186) have demonstrated that the majority of renin is located within the macula densa. These investigators studied the kidneys of cats and rabbits by means of a freezing microtome, selective staining of glomeruli, and microdissection. Renin was assayed by means of its pressor action in rats. These investigators studied the various components of the kidney cortex by a method of elimination. They showed that when the distal tubule was removed from preparations containing glomeruli, vasa afferentia, and the full complement of tubular cells surrounding the cells, then the remaining preparation was found to contain from less than ten per cent to 40 or 50 per cent of the renin activity. They thus concluded that the major portion of renin is located in the macula densa containing part of the distal tubules, which contain from 50 or 60 per cent to 90 per cent. They also showed that the subcapsular region of the kidneys of newborn pigs, which contains only immature nephrons with tubular tissue and no afferent vessels or glomeruli, has abundant amounts of renin (185). This confirms an earlier report by Kaplan and Friedman (187). Hartroft et al (178) claim that juxtaglomerular cells are demonstrable by both Bowie stain and fluorescent antibody technique in the developing metanephros of the pig embryo and even in the less mature glomeruli of the outer cortex. Bing and Kazimierczak (186) have proposed

that renin formed in the macula densa may pass to the afferent arteriole where it is stored and released into the blood stream.

Two major theories have evolved as to the regulation of the release of renin from the kidney. One is the concept of a "stretch" receptor in the juxtaglomerular cells of the afferent glomerular arteriole whereas the other involves the idea of a "sodium load chemoreceptor" in the cells of the macula densa.

In 1960, Tobian (188, 189) proposed that the juxtaglomerular cells may act as "stretch" receptors due to their anatomic location within the walls of the afferent arterioles. He based this conclusion on earlier experiments (119) in which it was observed that in kidneys, which had been subjected to reduced arterial flow by the placement of a clip on the main renal artery in rats, both an increase in granularity of the juxtaglomerular cells and an increase in the amount of renin in that kidney occurred which was significant. It was also noted that when systemic hypertension resulted from stenosis of the renal artery, a marked decrease in granularity of the juxtaglomerular cells and in the renin content of the contralateral kidney resulted. Tobian et al (191) conducted further experiments in which isolated kidneys were perfused at various pressures and then a juxtaglomerular cell count was made and taken to be indicative of renin production and release. One rat provided blood via a pump to the renal artery of normal isolated kidneys which were perfused under pressures normal for the healthy rat or which were considered hypertensive. Whenever an isolated kidney

was perfused, the contralateral kidney was also counted as a control. These investigators found that after 3 hours of perfusion at normotensive pressures the juxtaglomerular cell mean granularity did not differ from the mean values of the control. On the other hand, after three hours of perfusion at levels between 85 to 110 mm. Hg. higher than normotensive levels a distinct decrease of 50 per cent in the mean juxtaglomerular cell granularity was observed. This was thought to represent a decrease in renin synthesis and release. Tobian (188, 189, 190) thus suggested that the afferent signal for renin release might be provided by a decreased stretch of the afferent arterioles, since it was observed that in cases of chronic reduction of renal blood flow, both the granularity of the juxtaglomerular cells and the renal renin content were increased. Conversely, it was thought that increased perfusion pressures with a resultant increase in stretching of the afferent arteriole resulted in the inhibition of renin release. By this mechanism of renin release, a negative feedback system is thought to exist. The afferent signal thus appears to be a decrease in renal arterial pressure and in renal blood. This in turn results in a decreased "stretch" of the afferent arteriole with resultant release of renin. Consequent to the release of renin, angiotensin II stimulates the zonaglomerulosa to secrete increased amounts of aldosterone. Aldosterone in turn acts on the distal tubules of the nephrons to promote retention of sodium. (The effects of

angiotensin on the renal tubule will not be considered here for the sake of simplicity.) Sodium retention is then accompanied by a secondary retention of water with a resultant expansion of the circulating blood volume. According to this concept, the blood pressure and blood flow is thus restored to optimal levels. A subsequent increase in the amount of "stretch" in the afferent arteriole takes place suppressing the release of renin.

Following its inception, other data began to appear which supported the "stretch" concept and the idea that renal perfusion pressure and renal blood flow were important determinants in the release of renin. Davis et al (106) and Ganong and Mulrow (192) demonstrated that constriction of the aorta above the renal arterial origin produced a fairly prompt rise in the aldosterone secretory rate in hypophysectomized dogs. Lever and Peart (193) constricted the renal artery in dogs lowering the mean pressure and found a prompt increase in the renin concentration of renal lymph. In recent experiments, Skinner, McCubbin, and Page (194,195,196) have studied the effects of renal perfusion pressure on renin release in dogs by use of balloon inserted into the aorta above the origin of the renal arteries. They demonstrated that progressive reduction of the pulse pressure and pulsatile flow without reduction in mean pressure or flow caused no increase of pressor material in renal vein blood. With further reduction in pulse pressure and pulsatile flow, the mean pressure began to drop

without change of mean flow. At this time a significant increase in pressor material appeared in renal vein blood. This pressor material was found to be identical with angiotensin II. It was noted that a reduction in mean renal perfusion pressure of as little as five to twenty mm Hg. is followed almost immediately by an increase in renin like activity in renal venous blood and a consequent rise in renal perfusion pressure is followed by a rapid reduction of the renin concentration to normal. Scornik and Paladini (86,140,144) have been unable to detect an increase in angiotensin like activity in the blood of dogs after a reduction in systemic or renal perfusion pressure up to 80 mm Hg. However, when they infused noradrenaline in conjunction with aortic constriction above the origin of the renal arteries, a marked increase in angiotensin like material was observed in the blood.

On the other hand, Vander and Miller (197) have suggested that the lowering of mean renal perfusion pressure does not increase renin release per se, but does so through its influence on some renal function. In their experiments on dogs in which they had reduced the mean renal perfusion pressure by clamping of the renal artery and observed a rise in renin secretion, they were able to abolish the rise in renin levels with osmotic diuretics, as well as other diuretics, without changing the renal perfusion pressure or glomerular filtration rate. Gross et al (90) have

noticed that in their experiments with rats, in which water restriction or overtransfusion was performed, a negative correlation exists between intravascular volume and renin release. Furthermore, the parallelism between glucose 6 phosphate dehydrogenase activity in the macula densa and the renin content in the juxtaglomerular cells indicates the functional states of these two cell types are closely related (90,198).

Vander and Miller (197) have recently suggested that the macula densa may act as a receptor area where variations in flow or composition of the tubular fluid are responsible for renin release. They postulated that the macula densa might function as a "chemoreceptor" detecting variation in tubular sodium load. Because of their proximity and interconnection, the macula densa might then act via the juxtaglomerular cells causing the appropriate increase or decrease of renin secretion.

The macula densa is located at the beginning of the distal tubule and thus receives urine hypotonic compared to plasma from the end of the ascending lib of Henle's loop. If the filtered sodium load is lowered either by a decrease in glomerular filtration rate (e.g. reduced renal perfusion pressure) or a diminished plasma sodium (e.g. sodium depletion), the sodium load at the macula densa will be low as well, since the reabsorption of sodium in the proximal tubule is a constant percentage of the filtered sodium load. Therefore variations in the sodium load

of the proximal tubule are passed to the macula densa. If then for some reason the filtered sodium load is decreased, the macula densa responds via the juxtaglomerular cells with an increased release of renin. The subsequent increase in angiotensin II operates to restore equilibrium in two ways. By constriction of the efferent arteriole, the glomerular filtration pressure is raised with a concomitant rise in the total filtered sodium load. Secondly, the adrenal cortex is stimulated to secrete increased amounts of aldosterone which then promotes distal tubular reabsorption of sodium. In this manner, then, sodium is conserved with restoration of the appropriate sodium load at the macula densa (an increase) which in turn now inhibits the release of renin.

It is then not unreasonable to assume that diuretics may inhibit the increase in renin secretion following a reduction in renal perfusion pressure by decreasing proximal tubular reabsorption. In spite of a diminished filtered load, a normal sodium load at the macula densa would result (197).

In support of the sodium load chemoreceptor function of the macula densa, Gross and associates (90) presented a series of experiments in rats, all of which manifested increased rates of renin secretion which correlated with a decreased tubular sodium load. They enumerated several experiments in which a decreased filtered sodium load, decreased sodium load at the macula densa,

decreased urinary sodium, and increased renin secretion were observed. Among these were severe acute hemorrhage, increased ureteral pressure, dehydration (thirst), acute sodium depletion, adrenal insufficiency, chronic sodium depletion, and reduced renal perfusion pressure. It was also shown that in rats which were sodium loaded and given desoxycorticosterone acetate supplements, the renin secretory rate was significantly depressed. In this case, the filtered sodium load, sodium load at the macula densa, and urinary sodium level were all elevated.

SUMMARY

The evidence concerned with mechanisms controlling aldosterone secretion has been reviewed. Two main hypotheses have been examined.

Various clinical and experimental data has been presented in support of various regions of the central arterial tree and cardiac atria functioning as peripheral nervous receptors. The evidence implied these receptors form the origin of an afferent limb with integration of their stimuli in the central nervous system. Further, experiments have been cited which favor the pineal gland and an extracted substance, 1-methyl-6-methoxy-1,2,3,4-tetrahydro-2-carboline, as the efferent, stimulatory limb of this system. Evidence to the contrary, namely, that neither extensive denervation of the central arterial system and cardiac atria nor decerebration, pinealectomy, and decapitation failed to block the mechanisms leading to increased aldosterone secretion was presented. Extensive data was also reviewed which demonstrated that 1-methyl-6-methoxy-1,2,3,4-tetrahydro-2-carboline had no direct effect on aldosterone secretion. These latter results support the alternative view that an extracranial system provides the immediate stimulus to aldosterone production. Current findings do appear to define a possible inhibitory role for certain regions of the central nervous system.

That ACTH stimulates the biogenesis of aldosterone has been well demonstrated both in vitro and in vivo in several species. Evidence has been presented which expresses a difference of opinion as to whether ACTH is important in the basal production of aldosterone. Recent data indicates those levels of ACTH which stimulate increased aldosterone synthesis are such that a large rise in glucocorticoids precede and accompany this elevation. Data has been cited which indicates ACTH stimulates aldosterone via pathways which are independent of other stimulatory substances.

Discussion has been made concerning the role of dietary sodium and potassium in the control of aldosterone secretion. Experiments have indicated that sodium depletion most probably exerts its stimulatory effect via the renin-angiotensin system. Evidence has been cited which implies that potassium loading increases aldosterone secretion through the hyperkalemia which results. More direct experiments have been discussed which demonstrate that a local increase of the potassium ion in the arterial supply of the adrenal gland stimulates aldosterone secretion. Similar experiments which have attempted to provide evidence that hyponatremia is a direct stimulus to aldosterone production have been inconclusive.

The renin-angiotensin system has been defined. Data has been presented which shows that the end product of this system,

angiotensin II is a direct stimulant to aldosterone biogenesis. This stimulatory effect appears to be independent of other stimulatory factors. The renal site of renin production has been considered. Contradictory data favoring the juxtaglomerular cells on one hand and the macula densa on the other as this site has been presented. Evidence as to the nature of the immediate stimulus for the release of renin has been cited. Current views appear to have followed two main lines of thought. Those investigators favoring the juxtaglomerular cells as the site of renin production have stated that renin release is mediated by the amount of "stretch" of the juxtaglomerular cells which occurs secondary to changes in mean perfusion pressure and/or mean blood flow. On the other hand, those favoring the macula densa as the site of renin production have stated that renin is secreted in response to changes in the tubular sodium load at this site.

BIBLIOGRAPHY

1. Peters, J.P.: The Role of Sodium in the Production of Edema. New Eng. J. Med., 239:353, 1948.

2. Peters, J.P.: The Problem of Cardiac Edema. Amer. J. Med., 12:66, 1952.

3. Deming, G.B. and Luetscher, J.A., Jr.: Bioassay of Desoxycorticosterone-Like Material in Urine. Proc. Soc. Exp. Biol. Med., 73:171, 1950.

4. Singer, Bertha and Wener, Joseph: Excretion of Sodium Retaining Substances in Patients With Congestive Heart Failure. Amer. Heart. J., 45:795, 1953.

5. Chart, J.J. and Shipley, E.S.: The Mechanism of Sodium Retention in Cirrhosis of the Liver. J. Clin. Invest., 32:560, 1953.

6. McCall, M.F. and Singer, Bertha: Studies in Nephrosis: Chemical Corticoids, Salt Retaining Factor, and Effect of ACTH. J. Clin. Endocr., 13:1157, 1953.

7. Simpson, S.A., Tait, J.F., Wettstein, A., Neher, R., Euw, J.V. and Reichstein, T.: Isolierung Eines Neuen Kristallisierten Hormons Aus Nebennieren Mit Besonders Hoher Wirksamkeit Auf Den Mineralstoffwechsel. Experientia, 9:333, 1953.

8. Simpson, S.A., Tait, J.F., Wettstein, A., Neher, R., Euw, J.V. Schindler, O., and Reichstein, T.: Konstitution Des Aldosterons, Des Neuen Mineralocorticoids. Experientia, 10:132, 1954.

9. Luetscher, J.A., Jr., Neher, R., and Wettstein, A.: Isolation of Crystalline Aldosterone From the Urine of a Nephrotic Patient. Experientia, 10:456, 1954.

10. Davis, J.O., Goodkind, M.J., Pechet, M.M. and Ball, W.C., Jr.: Increased Excretion of Aldosterone in Urine From Dogs With Right Sided Congestive Heart Failure and From Dogs With Thoracic Inferior Vena Cava Constriction. Amer. J. Physiol., 187:45, 1956.

11 Luetscher, J.A., Jr. and Johns B.B.: Observations on the Sodium-Retaining Corticoid (Aldosterone) in the Urine of Children and Adults in Relation to Sodium Balance and Edema. J. Clin. Invest., 33:1441, 1954.

23. Carpenter, C.C.J., Davis, J.O., Holman, J.E., Ayers, C.R., and Bahn, R.C.: Studies on the Response of the Transplanted Kidney and the Transplanted Adrenal Gland to Thoracic Inferior Vena Caval Constriction. J. Clin. Invest., 40:196, 1961.

24. Bartter, F.C. and Gann, D.S.: On the Hemodynamic Regulation of the Secretion of Aldosterone. Circulation, 21:1016, 1960.

25. Bartter, F.C., Mills, I.H., and Gann, D.S.: Increase in Aldosterone Secretion by Carotid Artery Constriction in the Dog and Its Prevention by Thyrocarotid Arterial Junction Denervation. J. Clin. Invest., 39:1330, 1960.

26. Carpenter, C.C.J., Davis, J.O., and Ayers, C.R.: Concerning the Role of Arterial Baroreceptors in the Control of Aldosterone Secretion. J. Clin. Invest., 40:1160, 1961.

27. Davis, J.O., Yankopoulos, N.A., and Holman, J.: Chronic Effects of Carotid Sinus Denervation, Cervical Vagotomy and Aortic Depressor Nerve Section on Aldosterone and Sodium Excretion. Amer. J. Physiol., 197:207, 1959.

28. Biglieri, E.G., and Ganong, W.F.: Effect of Hypophysectomy on the Adrenocortical Response to Bilateral Carotid Constriction. Physiologist, 3:21, 1960.

29. Farrell, Gordon: Regulation of Aldosterone Secretion. Physiol. Rev., 38:709, 1958.

30. Anderson, C.H., McCally, M., and Farrell, G.L.: The Effects of Atrial Stretch on Aldosterone Secretion. Endocrinology, 64:202, 1959.

31. Davis, J.O.: A Critical Evaluation of the Role of Receptors in the Control of Aldosterone Secretion and Sodium Excretion. Progr. Cardiov. Dis., 4:27, 1961.

32. Mills, I.H., Casper, A. and Bartter, F.C.: On the Role of the Vagus in the Control of Aldosterone Secretion. Science, 128:1140, 1958.

33. Gann, D.S. and Travis, R.H.: Mechanism of Hemodynamic Control of Secretion of Aldosterone in the Dog. Amer. J. Physiol., 207:1095, 1964.

34. Gann, D.S., Gould, K.L., Morley, J.E., and Mumma, J.V.: Effects of Vagotomy and of Carotid Constriction on Corticosteroid Secretion in the Dog. Proc. Soc. Exp. Biol. Med., 115:944, 1964.

35. Carpenter, C.C.J., Davis, J.O., and Ayers, C.R.: Arterial Baroreceptors and Aldosterone Secretion. Clin. Res., 8:364, 1960.

36. Rauschkolf, E.W. and Farrell, G.L.: Evidence for Diencephalic Regulation of Aldosterone Secretion. Endocrinology 59:526, 1956.

37. Newman, A.E., Redgate, E.S., and Farrell, G.L.: The Effects of Diencephalic-Mesencephalic Lesions on Aldosterone and Hydrocortisone Secretion. Endocrinology 63:723, 1958.

38. Taylor, A.N. and Farrell, G.L.: Effects of Brain Stem Lesions on Aldosterone and Cortisol Secretion. Endocrinology, 70:556, 1962.

39. Krieger, D.T., and Krieger, H.P.: Aldosterone Excretion in Pretectal Disease. J. Clin. Endocr., 24:1055, 1964.

40. Palkovits, M., Monos, E., and Fachet, J.: The Effect of Subcommissural Organ Lesions on Aldosterone Production in the Rat. Acta Endocr., 48:169, 1965.

41. VanDerWal, B., Moll, J. and DeWied, D.: The Effect of Pinealectomy and of Lesions in the Subcommissural Body on the Rate of Aldosterone Secretion by Rat Adrenal Glands in Vitro. Progr. Brain Res., 10:635, 1965.

42. Farrell, G.L., Koletsky, S., and Lapham, L.W.: Decreased Aldosterone Secretion Following Pinealectomy. Fed. Proc., 18:44, 1959.

43. Farrell, G.L.: Adrenoglomerulotropin. Circulation, 21:1009, 1960.

44. Coghlan, J.P., Denton, D.A., Goding, J.R., and Wright, R.D.: The Control of Aldosterone Secretion. Postgrad. Med., 36:76, 1960.

45. Farrell, G.L. and McIsaac, W.M.: Adrenoglomerulotropin. Arch. Biochem., 94:543, 1961.

46. Taylor, A.N., McIsaac, W.M. and Farrell, G.L.: Steroidogenic Properties of 1-Methyl-6-Methoxy Tetrahydrocarboline. Fed. Proc., 21:186, 1962.

47. Mulrow, P.J., Ganong, W.F., and Boryczka, Angela: Further Evidence for a Role of the Renin-Angiotensin System in Regulation of Aldosterone Secretion. Proc. Soc. Exp. Biol. Med., 112:7, 1963.

48. Blair-West, J.R., Coghlan, J.P., Denton, D.A., Goding, J.R., Wintour, M., and Wright, R.D.: The Control of Aldosterone Secretion. Recent Progr. Hormone Res., 19:311, 1963.

49. Bartter, F.C., Mills, I.H., Biglieri, E.G., and Delea, C.: Studies on the Control and Physiologic Action of Aldosterone. Recent Progr. Hormone Res., 15:311, 1959.

50. Davis, J.O., Anderson, E., Carpenter, C.C.J., Ayers, C.R., Haymaker, W., and Spence, W.T.: Aldosterone and Corticosterone Secretion Following Midbrain Transection. Amer. J. Physiol., 200:437, 1961.

51. Yankopoulos, N.A., Davis, J.O., Kliman, Bernard, and Peterson, R.E.: Evidence That a Humoral Agent Stimulates the Adrenal Cortex to Secrete Aldosterone in Experimental Secondary Hyperaldosteronism. J. Clin. Invest., 38:1278, 1959.

52. Barbour, B.H., Testerman, D., Casper, A., and Bartter, F.C.: On the Role of the Central Nervous System in Control of Adrenal Cortical Steroid Production. Clin. Res., 11:213, 1963.

53. Blair-West, J.R., Coghlan, J.P., Denton, D.A., Goding, J.R., Munro, J.A., and Wright, R.D.: The Effect of Neuraxial Ablations Upon the Secretion of Electrolyte Active Steroid. J. Physiol., 153:52, 1960.

54. Bartter, F.C., Barbour, B.H., Carr, A.A., Delea, C.S., and Slater, J.D.H.: Control of Adrenocortical Steroid Secretion Dependent Upon and Independent of Pituitary and Kidneys. Canad. Med. Ass. J., 90:240, 1964.

55. Singer, Bertha, and Stack-Dunne, M.P.: The Secretion of Aldosterone and Corticosterone by the Rat Adrenal. J. Endocr., 12:130, 1955.

56. Rauschkolb, E.W., Farrell, G.L. and Koletsky, S.: Aldosterone Secretion After Hypophysectomy. Amer. J. Physiol., 184:55, 1956.

57. Ganong, W.F., Lieberman, A.H., Daily, W.J.R., Yuen, V.S., Mulrow, P.J., Luetscher, J.A.,Jr., and Bailey, R.E.: Aldosterone Secretion in Dogs with Hypothalamic Lesions. Endocr., 65:18, 1959.

58. Davis, J.O., Bahn, R.C., and Ball, W.C.,Jr.: Subacute and Chronic Effects of Hypothalamic Lesions on Aldosterone and Sodium Excretion. Amer. J. Physiol., 197:387, 1959.

59. Davis, J.O., Bahn, R.C., Yankopoulos, N.A., Kliman, B. and Peterson, R.E.: Acute Effects of Hypophysectomy and Diencephalic Lesions on Aldosterone Secretion. Amer. J. Physiol., 197:380, 1959.

60. Davis, J.O.: Mechanisms Regulating the Secretion and Metabolism of Aldosterone in Experimental Secondary Hyperaldosteronism. Recent Progr. Hormone Res., 17: 293, 1961.

61. Davis, J.O., Carpenter, C.C.J., Ayers, C.R., and Bahn, R.C.: Relation of Anterior Pituitary Function to Aldosterone and Corticosterone Secretion in Conscious Dogs. Amer. J. Physiol., 199:212, 1960.

62. Dingman, J.F., Gaitan, E., Staub, M.C., Arimura, A. and Peterson, R.E.: Hypoaldosterone in Panhypopituitarism. J. Clin. Invest., 39:981, 1960.

63. Kliman, B., and Peterson, R.E.: Double Isotope Derivative Assay of Aldosterone in Biological Extracts. J. Biol. Chem., 235:1639, 1960.

64. Ross, E.J., Van't Hoff, W., Crabbe, J., and Thorn, G.W.: Aldosterone Excretion in Hypopituitarism and After Hypophysectomy in Man. Amer. J. Med., 28:229, 1960.

65. Haynes, R.C.,Jr., and Berthat, L.: Studies on the Mechanism of Action of the Adrenocorticotropic Hormone. J. Biol. Chem., 225:115, 1957.

66. Pincus, G.: The Biosynthesis of Adrenal Cortical Steroids. Bull. N.Y. Acad. Med., 33:587, 1957.

67. Carpenter, C.C.J., Davis, J.O., Holman, J.E., Ayers, C.R. and Bahn, R.C.: Studies on the Response of the Transplanted Kidney and the Transplanted Adrenal Gland to Thoracic Inferior Vena Caval Constriction. J. Clin. Invest., 40:196, 1961.

68. Dexter, L., Frank, H.A., Haynes, F.W., and Alschule, M.D.: Traumatic Shock. VI. The Effect of Hemorrhagic Shock on the Concentration of Renin and Hypertensinogen in the Plasma in Unanesthetized Dogs. J. Clin. Invest., 22:847, 1943.

69. Davis, J.O., Carpenter, C.C.J., Ayers, C.R., Holman, J.E., and Bahn, R.C.: Evidence for Secretion of an Aldosterone-Stimulating Hormone by the Kidney. J. Clin. Invest., 40:684, 1961.

70. Mulrow, P.J., and Ganong, W.F.: The Effect of Hemorrhage Upon Aldosterone Secretion in Normal and Hypophysectomized Dogs. J. Clin. Invest., 40:579, 1961.

71. Liddle, G.W., Duncan, L.E.,Jr., and Bartter, F.C.: Dual Mechanism Regulating Adrenocortical Function in Man. Amer. J. Med., 21:380, 1956.

72. Mulrow, P.J., and Ganong, W.F.: Effect of Simultaneous Infusions of ACTH and Angiotensin II Upon Aldosterone Secretion of Hypophysectomized Nephrectomized Dogs. Proc. Soc. Exp. Biol. Med., 118:795, 1965.

73. Kaplan, N.M. and Bartter, F.C.: The Effect of ACTH, Renin, Angiotensin II and Various Precursors on Biosynthesis of Aldosterone by Adrenal Slices. J. Clin. Invest., 41:715, 1962.

74. Kaplan, N.M.: The Effect of ACTH and Angiotensin II Upon Adrenal Steroid Synthesis. In: Metcoff, Jack. Proceedings, Fourteenth Annual Conference on the Kidney. Angiotensin Systems and Experimental Renal Diseases. Boston; Little, Brown, and Company, 1963. p. 97.

75. Muller, Jurg.: Aldosterone Stimulation in Vitro. I. Evaluation of Assay Procedure and Determination of Aldosterone Stimulative Activity in a Human Urine Extract. Acta Endocr., 48:283, 1965.

76. Haynes, R.C.,Jr., Sutherland, E.W., and Rall, T.W.: Role of Cyclic Adenylic Acid in Hormone Action. Recent Progr. Hormone Res., 16:121, 1960.

77. Hilf, Russell: The Mechanism of Action of ACTH. New Eng. J. Med., 273:798, 1965.

78. Laragh, J.H., Angers, M., Kelley, W.G. and Lieberman, S.: Hypotensive Agents and Pressor Substances. J.A.M.A., 174:234, 1960.

79. Biron, P., Koiw, E., Nowaczynski, W., Brouillet, J., and Genest, J.: The Effects of Intravenous Infusions of Valine 5 Angiotensin II and Other Pressor Agents on Urinary Electrolytes and Corticosteroids, Including Aldosterone. J. Clin. Invest., 40:338, 1961.

80. Reichlin, Seymour: Neuroendocrinology. New. Eng. J. Med., 269:1182-1191, 1246-1250, 1296-1303, 1963.

81. Tepperman, Jay: Metabolic and Endocrine Physiology. An Introductory Text. Chicago Year Book Medical Publishers, Inc., 1962, p. 120.

82. Luetscher, J.A.,Jr., and Axelrad, B.J.: Increased Aldosterone Output During Sodium Deprivation in Normal Man. Proc. Soc. Exp. Biol. Med., 87:650, 1954.

83. Davis, J.O., Ayers, C.R., and Carpenter, C.C.J.: Renal Origin of an Aldosterone-Stimulating Hormone in Dogs With Thoracic Caval Constriction and in Sodium Depleted Dogs. J. Clin. Invest., 40:1466, 1961.

84. Peart, W.S.: The Functions for Renin and Angiotensin. Recent Progr. Hormone Res., 21:73, 1965.

85. Davis, J.O.: The Angiotensin System and Control of Aldosterone. In: Metcoff, Jack. Proceedings, Fourteenth Annual Conference on the Kidney. Angiotensin Systems and Experimental Renal Diseases. Boston; Little, Brown and Company, 1963. p.78.

86. Scornik, O.A., and Paladini, A.C.: Significance of Blood Angiotensin Levels in Different Experimental Conditions. Canad. Med. Ass. J., 90:269, 1964.

87. Veyrat, R., deChamplain, J., Boucher, R., and Genest, J.: Measurement of Human Arterial Renin Activity in Some Physiological and Pathological States. Canad. Med. Ass. J., 90:215, 1964.

88. 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. Med. Ass. J., 90:206, 1964.

89 Brown, J.J., Davies, D.L., Lever, A.F., and Robertson, J.I.S.: Variations in Plasma Renin Concentration in Several Physiological and Pathological States. Canad. Med. Ass. J., 90:201, 1964.

90. Gross, F., Brunner, H. and Ziegler, M.: Renin-Angiotensin System, Aldosterone, and Sodium Balance. Recent Progr. Hormone Res., 21:119, 1965.

91. Laragh, J.H., and Stoerk, H.C.: On the Mechanism of Secretion of the Sodium Retaining Hormone (Aldosterone) Within the Body. J. Clin. Invest., 34:913, 1955.

92. Liddle, G.W., Bartter, F.C., Duncan, L.E.,Jr., Barber, J.K. and Delea, C.: Mechanisms Regulating Aldosterone Production in Man. J. Clin. Invest., 34:949, 1955.

93. Luetscher, J.A.,Jr., and Curtis, R.H.: Observations on the Regulation of Sodium and Potassium Balance. Ann. Intern. Med., 43:658, 1955.

94. Denton, D.A., Goding, J.R., and Wright, R.D.: Control of Adrenal Secretion of Electrolyte-Active Steroids. Brit. Med. J., 2:447, 1959.

95. Johnson, B.B., Lieberman, A.H., and Mulrow, P.T.: Aldosterone Excretion in Normal Subjects Depleted of Sodium and Potassium. J. Clin. Invest., 36:757, 1957.

96. Nowaczynski, W., Koiw, E. and Genest, J.: Chemical Method for the Determination of Urinary Aldosterone. Canad. J. Biochem. Physiol., 35:425, 1957.

97. Hernando, L., Crabbe, J., Ross, E.J., Reddy, W.J., Renald, A.E., Nelson, D.H. and Thorn, G.W.: Clinical Experience with a Physico-Chemical Method for Estimation of Aldosterone in Urine. Metabolism, 6:518, 1957.

98. Rosnagle, R.S., and Farrell, G.L.: Alterations in Electrolyte Intake and Adrenal Steroid Secretion. Amer. J. Physiol., 187:7, 1956.

99. Bartter, F.C., Mills, I.H., Biglieri, E.G., and Delea, C.S.: Studies on the Control and Physiologic Action of Aldosterone. Recent Progr. Hormone Res., 15:311, 1959.

100. Gann, D.S., Delea, C.S., Gill, J.R.,Jr., Thomas, J.P. and Bartter, F.C.: Control of Aldosterone Secretion by Changes of Body Potassium in Normal Man. Amer. J. Physiol., 207:104, 1965.

101. Laragh, J.H., Vlick, S.U., Januszewicz, V., Kelley, W.G. and Lieberman, S.: Electrolyte Metabolism and Aldosterone Secretion in Benign and Malignant Hypertension. Ann. Intern. Med., 53:259, 1960.

102. Laragh, J.H., Cannon, P.J., Ames, R.P., Sicinski, A.M. and Borkowski, A.J.: Aldosteronism in Man: Mechanism Controlling Secretion of the Hormone; Role of Angiotensin. In: Metcoff, Jack. Proceedings, Fourteenth Annual Conference on the Kidney. Angiotensin Systems and Experimental Renal Diseases. Boston; Little, Brown and Company, 1963. p. 108.

103. Moran, W.H.,Jr., Rosenberg, J.C. and Zimmerman, B.: The Regulation of Aldosterone Output: Significance of Potassium Ion. Sug. Forum 9:120, 1959.

104. Urguhart, J., Davis, J.O., and Higgins, J.J.,Jr.: Stimulation of Aldosterone Secretion by an Increase in Plasma Potassium Concentration. Fed. Proc., 21:186, 1962.

105. Hilton, J.G., Weaver, D.C., Muelheims, G., Glaviano, V.V. and Wegria, R.: Perfusion of the Isolated Adrenals in Situ. Amer. J. Physio., 192:525, 1958.

106. Davis, J.O., Hartroft, P.M., Titus, E.O., Carpenter, C.C.J., Ayers, C.R., and Spiegel, H.E.: The Role of the Renin-Angiotensin System in the Control of Aldosterone Secretion. J. Clin. Invest., 41:378, 1962.

107. Ganong, W.F., and Mulrow, P.J.: Evidence of Secretion of an Aldosterone Stimulating Substance by the Kidney. Nature, 190:1115, 1961.

108. Ganong, W.F., and Mulrow, P.J.: Role of the Kidney in Adreno-Cortical Response to Hemorrhage in Hypophysectomized Dogs. Endocrionology, 70: 182, 1962.

109. Dunihue, F.W.: The Effect of Adrenal Insufficiency and of Desoxycorticosterone Acetate on the Juxtaglomerular Apparatus. Anat. Rec., 103:442, 1949.

110. McManus, J.F.A.: Medical Diseases of the Kidney. Philadelphia, Pa., Lea and Febiger, 1950. p. 38.

111. Deane, H.W., and Masson, G.M.C.: Adrenal Cortical Changes in Rats With Various Types of Experimental Hypertension. J. Clin. Endocr., 11:193, 1951.

112. Hartroft, P.M., and Hartroft, W.S.: Studies on Renal Juxtaglomerular Cells. I. Variations Produced by Sodium Chloride and Desoxycorticosterone Acetate. J. Exp. Med., 97:415, 1953.

113. Hartroft, P.M. and Hartroft, W.S.: Studies on Renal Juxtaglomerular Cells. II. Correlation of Degree of Granulation of Juxtaglomerular Cells With the Width of the Zona-glomerulosa of the Adrenal Cortex. J. Exp. Med., 102: 205, 1955.

114. Deane, H.W., Shaw, J.H., and Greep, R.C.: The Effect of Altered Sodium or Potassium Intake on Width and Cytochemistry of the Zonaglomerulosa of the Rat's Adrenal Cortex. Endocrinology, 43:133, 1948.

115. Pitcock, J.A., and Hartroft, P.M.: The Juxtaglomerular Cells in Man and Their Relationship to the Level of Plasma Sodium and to the Zonaglomerulosa of the Adrenal Cortex. Amer. J. Path., 34:863, 1958.

116. Hartroft, P.M., and Edelman, Robert: Renal Juxtaglomerular Cells in Sodium Deficiency. In: Moyer and Fuchs, Edema. Philadelphia, Pa., Saunders and Company, 1960, p. 63.

117. Kuhn, C., Hartroft, P.M., and Pitcock, J.A.: Effects of Renal Extracts on Juxtaglomerular Cells, Renin Content, and Zonaglomerulosa. Fed. Proc., 20:404, 1961.

118. Pitcock, J.A., Hartroft, P.M., and Newmark, L.N.: Increased Renal Pressor Activity (Renin) in Sodium Deficient Rats and Correlation With Juxtaglomerular Cell Granulation. Proc. Soc. Exp. Biol. Med., 100:868, 1959.

119. Tobian, L., Janecek, J., and Tomboulian, A.: Correlation Between Granulation of Juxtaglomerular Cells and Extractable Renin in Rats With Experimental Hypertension. Proc. Soc. Exp. Biol. Med., 100:94, 1959.

120. Mulrow, P.J. and Ganong, W.F.: Discussion of J.O. Davis. Mechanisms Regulating the Secretion and Metabolism of Aldosterone in Experimental Secondary Hyperaldosteronism. Recent Progr. Hormone Res., 17:293, 1961.

121. Davis, J.O.: Adrenocortical and Renal Hormonal Function in Experimental Cardiac Failure. Circulation, 25:1002, 1962.

122. Davis, J.O.: The Control of Aldosterone Secretion. Physiologist, 5:65, 1962.

123. Davis, J.O.: Discussion of Gordon Farrell. The Physiological Factors Which Influence the Secretion of Aldosterone. Recent Progr. Hormone Res., 15:275, 1959.

124. Genest, J.E., Koiw, E., Nowaczynski, W., and Sandor, T.: Study of Urinary Adrenocortical Hormones in Human Arterial Hypertension. Absts. 1st. Intern. Congr. Endocr. Copenhagen, Denmark. p. 173, 1960.

125. Genest, J.E., Biron, P., Koiw, E., Nowaczynski, W., Chretien, M., and Boucher, R.: Adrenocortical Hormones in Human Hypertension and Their Relation to Angiotensin. Circ. Res., 9:775, 1961.

126. Carpenter, C.C.J., Davis, J.O., and Ayers, C.R.: Relation of Renin, Angiotensin II, and Experimental Hypertension to Aldosterone Secretion. J. Clin. Invest., 40:2026, 1961.

127. Mulrow, P.J., and Ganong, W.F.: Stimulation of Aldosterone Secretion by Angiotensin II. Yale J. Biol. Med., 33: 386, 1961.

128. Haas, E., Lamfrom, H., and Goldblatt, H.: Biochemical and Physio-Chemical Studies on Renin. Arch. Biochem., 44:63, 1953.

129. Haas, Erwin, and Goldblatt, Harry: Studies on Renin. Biochem. Z., 338:164, 1963.

130. Page, I.H. and Helmer, O.M.: A Crystalline Pressor Substance (Angiotensin) Resulting From the Reaction Between Renin and Renin Activator. J. Exp. Med., 71:29, 1940.

131. Skeggs, L.T.,Jr., Lentz, K.E., Hochstrasser, Harry and Kahn, J.R.: The Purification and Partial Characterization of Several Forms of Hog Renin Substrate. J. Exp. Med., 118:73, 1963.

132. Lentz, K.E., Skeggs, L.T.,Jr., Hochstrasser, Harry, and Kahn, J.R.: Countercurrent Distributions of Renin Substrate and Serum Proteins in Polyethylene Glycol Salt Systems. Biochem. Biophys. Acta., 69:263, 1963.

133. Davis, J.O., Urquhart, J. and Higgins, J.T.: Renin, Angiotensin and Aldosterone in Experimental Secondary Hyperaldosteronism. Canad. Med. Ass. J., 90:245, 1964.

134. Skeggs, L.T.,Jr., Lentz, K.E., Hochstrasser, Harry and Kahn, J.R.: The Chemistry of Renin Substrate. Canad. Med. Ass. J., 90:185, 1964.

135. Skeggs, L.T., Kahn, J.R., and Shumway, N.P.: The Preparation and Function of Hypertension Converting Enzyme. J. Exp. Med., 103:295, 1956.

136. 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.

137. Elliot, D.F., and Peart, W.S.: The Amino Acid Sequence in a Hypertensin. Biochem. J., 65:246, 1957.

138. Smeby, R.R.: Angiotensin, a Hormone of the Kidney. In: Metcoff, Jack. Proceedings, Fourteenth Annual Conference on the Kidney. Angiotensin Systems and Experimental Renal Diseases. Boston; Little, Brown, and Company, 1963. p. 29.

139. Ziegler, M., and Gross, F.: Effect of Blood Volume Changes on Renin-Like Activity in Blood. Proc. Soc. Exp. Biol. Med., 116:774, 1964.

140. Scornik, O.A., and Paladini, A.C.: Angiotensin Blood Levels in Hemorrhagic Hypotension and Other Related Conditions. Amer. J. Physiol., 206:553, 1964.

141. Brown, J.J., Davies, D.L., Lever, A.F. and Robertson, J.I.S.: Unpublished Data. Second International Congress of Endocrinology, London, 1964. Cited by: Gross, F., Brunner, H., and Ziegler, M.: Renin-Angiotensin System Aldosterone, and Sodium Balance. Recent Progr. Hormone Res., 21:119, 1965.

142. Mulrow, P.J. and Ganong, W.F.: The Mechanism of Aldosterone Stimulation Following Hemorrhage in the Hypophysectomized Dog. J. Clin. Invest., 40:1065, 1961.

Mulrow, P.J., Ganong, W.F.: Role of the Kidney and the Renin-Angiotensin System in the Response of Aldosterone Secretion to Hemorrhage. Circulation, 25:1002, 1962.

144. Paladini, A.C. and Scornik, O.A.: Blood Levels of Angiotensin in Different Experimental Conditions. In: Williams, P.C. Memoirs of the Society for Endocrinology, Hormones and the Kidney. London; Academic Press, 1963. p. 265.

145. Gross, F., Loystalot, P. and Meier, R.: Production of Experimental Hypertension by Aldosterone. Acta Endocr., 26:417, 1957.

146. Hartroft, P.M. and Eisenstein, A.B.: Alterations in the Adrenal Cortex of the Rat Induced by Sodium Deficiency: Correlation of Histologic Changes With Steroid Hormone Secretion., Endocrinology, 60:641, 1957.

147. Brown, J.J., Davies, D.L., Lever, A.F., and Robertson, J.I.S.: Influence of Sodium Loading and Sodium Depletion on Plasma Renin in Man. Lancet II: 273, 1963.

148. Conn, J.W., Rovner, D.R. and Cohen, E.L.: Normal and Altered Function of the Renin-Angiotensin-Aldosterone System in Man. Ann. Intern. Med., 63:266, 1965.

149. Rovner, D.R., Conn, J.W., Knopf, R.F., Cohen, E.L., and Hsueh, M. T-Y.: The Nature of Renal Escape From the Sodium-Retaining Effect of Aldosterone in Primary Aldosteronism and in Normal Subjects. J. Clin. Endocr., 25:33, 1965.

150. Cohen, E.L., Rovner, D.R., Conn, J.W., and Blough, W.M.,Jr.: Effects of Position, Exercise, and Sodium Intake on Plasma Renin Activity in Normal People. Clin. Res., 12:362, 1964.

151. Barbour, B.H.: Discussion of W.S. Peart: The Function for Renin and Angiotensin. Recent Progr. Hormone Res., 21: 73, 1965.

152. Conn, J.W., Cohen, E.L., and Rovner, D.R.: Suppression of Plasma Renin Activity in Primary Aldosteronism. Distinguishing Primary From Secondary Aldosteronism in Hypertensive Disease. J.A.M.A., 190:213, 1964.

153. 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.

154. Gross, F. and Lichtlen, P.: Experimental Renal Hypertension: Renin Content of Kidneys in Intact and Adrenalectomized Rats Given Cortexone. Amer. J. Physiol., 195:543, 1958.

155. Helmer, O.M.: Renin Activity in Blood From Patients With Hypertension. Canad. Med. Ass. J., 90:221, 1964.

156. Urquhart, John, Davis, J.O. and Higgins, J.T.,Jr.: Effects of Prolonged Infusion of Angiotensin II in Normal Dogs. Amer. J. Physiol., 205:1241, 1963.

157. Mulrow, P.J., Ganong, W.F., Cera, G. and Kuljian, A.: Nature of Aldosterone Stimulating Factor in Dog Kidneys. J. Clin. Invest., 41:505, 1961.

158. Mulrow, P.J., Ganong, W.F., and Boryczka, Angela: Further Evidence for a Role of the Renin-Angiotensin System in Regulation of Aldosterone Secretion. Proc. Soc. Exp. Biol. Med., 112:7, 1963.

159. Urguhart, John, Davis, J.O. and Higgins, J.J.,Jr.: Effects of Prolonged Infusion of Angiotensin II in Unrestrained Dogs. Physiologist 5:224, 1962.

160. Laragh, J.H., Cannon, P.J., Ames, R.P., Sicinski, A.M., Bentzel, C.J. and Meltzer, J.I.: Angiotensin II and Renal Sodium Transport: Natriuresis and Diuresis in Patients With Cirrhosis and Ascites. J. Clin. Invest., 41:1375, 1962.

161. Laragh, J.H.: Interrelationships Between Angiotensin, Norepinephrine, Epinephrine, Aldosterone Secretion and Electrolyte Metabolism in Man. Circulation, 25:203, 1962.

162. Ganong, W.F., Mulrow, P.J., Boryczka, A., and Cera, G.: Evidence for a Direct Effect of Angiotensin II on the Adrenal Cortex of the Dog. Proc. Soc, Exp. Biol. Med., 109:381, 1962.

163. Marx, A.J., Deane, H.W., Mowles, T.F. and Sheppard, Herbert: Chronic Administration of Angiotensin in Rats: Changes in Blood Pressure, Renal and Adrenal Histophysiology and Aldosterone Production. Endocrinology, 73:329, 1963.

164. Singer, Bertha, Losito, Carine, and Salmon, Sheila: Some Studies on the Effect of Angiotensin II on Adrenocortical Hormone Secretion in Hypophysectomized Rats With Renal Pedicle Ligation. Endocrinology, 74:325, 1964.

165. Marieb, N.J. and Mulrow, P.J.: Role of the Renin Angiotensin System in the Regulation of Aldosterone Secretion in the Rat. Endocrinology, 76:657, 1965.

166. Eilers, E.A. and Peterson, R.A.: Proceedings 46th Meeting Endocrine Society, San Francisco, 1964. Abstract No. 80 p. 62. Cited by: Gross, F., Brunner, H., and Ziegler, M.: Renin Angiotensin System, Aldosterone, and Sodium Balance. Recent Progr. Hormone Res., 21:119, 1965.

167. Mulrow, P.J.: Second International Congress of Endocrinology. London, 1964. Cited by: Gross, F., Brunner, H. and Ziegler, M.: Renin Angiotensin System, Aldosterone, and Sodium Balance. Recent Progr. Hormone Res., 21: 119, 1965.

168. Hartroft, P.M.: Juxtaglomerular Cells. Circ. Res., 12:525, 1963.

169. Hartroft, P.M.: Histologic and Functional Aspects of Juxtaglomerular Cells. In: Metcoff, Jack. Proceedings, Fourteenth Annual Conference on the Kidney. Angiotensin Systems and Experimental Renal Diseases. Boston; Little, Brown and Company, 1963. p. 27.

170. Ruyter, J.N.C.: Uber Einen Merkwurdigen Abschnitt der Vasa Afferentia in der Mauseniere. Ztschr. f. Zellforch u. Mikr. Anat. 2:242, 1925. Cited by: Hartroft, P.M.: Juxtaglomerular Cells. Circ. Res., 12:525, 1963.

171. Hartroft, P.M. and Newmark, L.N.: Electron Microscopy of Renal Juxtaglomerular Cells. Anat. Rec., 139:185, 1961.

172. Miller, G. and Hartroft, P.M.: Renal Juxtaglomerular Cells in Acute Dietary Sodium Deficiency. Fed. Proc., 20:404, 1961.

173. Latta, Harrison, and Maunsback, A.B.: The Juxtaglomerular Apparatus as Studied Electron Microscopically. J. Ultrastruct. Res., 6:547, 1962.

174. Cook, W.F., and Pickering, G.W.: The Location of Renin in the Rabbit Kidney. J. Physiol., 149:526, 1959.

175. Cook, W.F.: The Location of Renin Within the Kidney. J. Physiol., 152:27, 1960.

176. Cook, W.F.: Renin and the Juxtaglomerular Apparatus. In: Williams, P.C. Memoirs of the Society for Endocrinology. Hormones and the Kidney. London; Academic Press, 1963, p.247

177. Edelman, Robert and Hartroft, P.M.: Localization of Renin in Juxtaglomerular Cells of Rabbit and Dog Through Use of the Fluorescent Antibody Technique. Circ. Res., 9: 1069, 1961.

178. Hartroft, P.M., Sutherland, L.E. and Hartroft, W.S.: Juxtaglomerular Cells as the Source of Renin: Further Studies With the Fluorescent Antibody Technique and the Effect of Passive Transfer of Antirenin. Canad. Med. Ass. J., 90: 163, 1964.

179. Lamfrom, H., Haas, E., and Goldblatt, H.: Studies on Antirenin. Amer. J. Physiol., 177:55, 1954.

180. Bing, J., and Wiberg, B.: Localization of Renin in the Kidney I. Acta Path. Microbiol. Scand., 44:138, 1958.

181. Bing, J. and Kazimierczak, J.: Localization of Renin in the Kidney II. Acta Path. Microbiol. Scand., 47:105, 1959.

182. Bing, J. and Kazimierczak, J.: Localization of Renin in the Kidney III. Renin Content of Different Parts of the Periglomerular Circumference. Acta Path. Microbiol. Scand., 50:1, 1960.

183. Bing, J. and Kazimierczak, J: Localization of Renin in the Kidney IV. Renin Content of Different Parts of the Juxtaglomerular Apparatus. Acta Path. Microbiol. Scand., 54: 80, 1962.

184. Bing, J. and Kazimierczak, J.: Further Studies on the Localization of Renin in the Kidney. Ann. Histochem., 6:537, 1961.

185. Bing, J. and Kazimierczak, J.: Renin in Nephrogenic Renal Tissue Devoid of Bothe Granular and Nogranular Epithelioid Juxtaglomerular Cells. Acta Path. Microbiol. Scand, 54:80, 1964.

186. Bing, J. and Kazimierczak, J.: Location of Renin. In: Williams, P.C. Memoirs of the Society for Endocrinology. Hormones and the Kidney. London; Academic Press, 1963, p. 255.

187. Kaplan, A. and Friedman, M.: Site of Renin Formation in the Kidney III. J. Exp. Med., 76:307, 1942.

188. Tobian, L.: Interrelationships of Electrolytes, Juxtaglomerular Cells, and Hypertension. Physiol. Rev., 40:280, 1960.

189. Tobian, L.: Relationship of Juxtaglomerular Apparatus to Renin and Angiotensin. Circulation, 25:189, 1962.

190. Tobian, Louis: The Juxtaglomerular Cells and Experimental Hypertension. In: Metcoff, Jack. Proceedings, Fourteenth Annual Conference on the Kidney. Angiotensin Systems and Experimental Renal Disease. Boston; Little, Brown, and Company, 1963. p. 17.

191. Tobian, L., Tomboulian, A., and Janecek, J.: The Effect of High Perfusion Pressures on the Granulation of Juxtaglomerular Cells in an Isolated Kidney. J. Clin. Invest., 38:605, 1959.

192. Ganong, W.F. and Mulrow, P.J.: The Effect of Aortic Constriction on Aldosterone Secretion in Hypophysectomized Dogs. J. Clin. Invest., 41:1503, 1962.

193. Lever, A.F. and Peart, W.S.: Renin and Angiotensin Like Activity in Renal Lymph. J. Physiol., 160:548, 1962.

194. Skinner, S.L., McCubbin, J.W., and Page, I.H.: Renal Baroceptor Control of Renin Secretion. Science, 141:814, 1963.

195. Skinner, S.L., McCubbin, J.W. and Page, I.H.: Angiotensin in Blood and Lymph Following Reduction in Renal Arterial Perfusion Pressure in Dogs. Circ. Res., 13:336, 1963.

196. Skinner, S.L., McCubbin, J.W. and Page, I.H.: Control of Renin Secretion. Circ. Res., 15:64, 1964.

197. Vander, A.J. and Miller, R.: Control of Renin Secretion in the Anesthetized Dog. Amer. J. Physiol., 207:537, 1964.

198. Fisher, E.R.: Correlation of Juxtaglomerular Granulation, Pressor Activity and Enzymes of Macula Densa in Experimental Hypertension. Lab. Invest., 10:707, 1961.