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# THE ROLE OF THE ADRENAL CORTEX AND THE KIDNEY IN THE PATHOGENESIS OF CARDIAC EDEMA*

It is a distinct privilege to present this lecture in honor of Dr. John Peters. As a fine doctor, a very productive scientist and a most distinguished citizen, his achievements were truly remarkable. The breadth and the depth of his many interests and contributions in medicine are striking. One of his areas of interest was the pathogenesis of edema. His first paper on this subject appeared in 1929; from then until the end of his highly productive career, he published many provocative papers on edema. His analysis of the mechanisms regulating the excretion of salt and water led him to postulate the existence of a "volume receptor."[1,2] As you may know, this hypothesis stimulated an intensive search for such a receptor and for the intimate mechanisms involved. We, in our laboratory, are one of the many groups who has sought a receptor concerned with the regulation of aldosterone secretion and sodium excretion. At this time, I would like to recognize that the data which I describe represent the combined efforts of a number of doctors whose names have appeared on our definitive publications.

In 1950, Deming and Luetscher[3] reported the presence of increased sodium-retaining activity in urine from patients with congestive heart failure. This finding and the classic papers of Dr. Peters on the concept of a "volume receptor" marked the beginning of a new era in our knowledge of the relation of the adrenal cortex and the kidney to sodium retention. Also, this finding by Deming and Luetscher provided the first suggestive evidence for a high blood level of aldosterone in heart failure, and thus raised the question of the importance of aldosterone in the pathogenesis of cardiac edema. In the series of experiments which I will describe today, the role of aldosterone has been studied in experimental right heart failure. Three major aspects of the problem are considered. First, what is the relation of the high blood level of aldosterone to sodium retention in heart failure? Second, what are the mechanisms

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leading to hyperaldosteronemia? Is there a decrease in the rate of metabolism of aldosterone as well as an increased rate of secretion of the hormone? Third, what stimulates the adrenal cortex to secrete large amounts of aldosterone in cardiac failure?

To study these problems we have used two chronic experimental preparations in dogs. In the first, ascites is produced by constriction of the inferior vena cava in the thorax; marked sodium retention ensues and large quantities of fluid accumulate in the peritoneal cavity.[4] Although these animals do not have a failing heart, the mechanisms of hyperaldosteronism and of salt and water retention appear to be essentially the

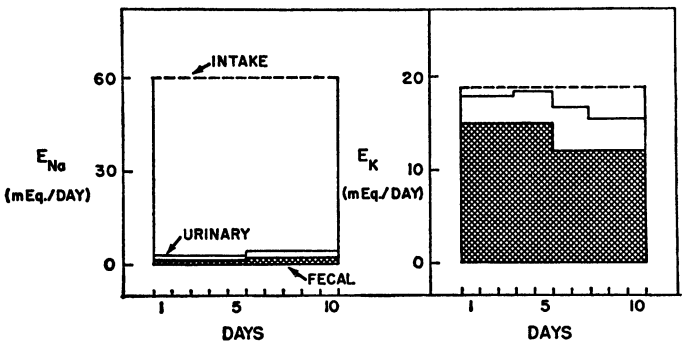


FIG. 1. Typical pattern of sodium (Na) and potassium (K) excretion in a dog with thoracic caval constriction and ascites. Renal sodium retention is almost complete and the low sodium and high potassium pattern of fecal electrolyte excretion is characteristic of experimental secondary hyperaldosteronism.[4]

same as in experimental heart failure. The second preparation is the dog with right-sided congestive heart failure secondary to progressive pulmonic stenosis.[5] This experimental model resembles right heart failure in man very closely, especially the syndrome which results from congenital pulmonic stenosis.

To provide you with background information and to show you our first evidence for the importance of the adrenal cortex in experimental ascites formation, the typical pattern of electrolyte excretion in a dog with thoracic caval constriction is presented in Figure 1.[4] On an intake of sodium of 60 mEq./day, sodium retention is almost complete; both fecal and urinary sodium excretion are reduced. These changes are associated with a marked elevation in fecal potassium output and a reduction in urinary potassium excretion. The normal rate of fecal potassium excretion in the dog is 1-2 mEq./day. This low sodium and high potassium

pattern of fecal electrolyte excretion suggests that a high plasma level of aldosterone is present. It has been demonstrated[6] that a low sodium and a high potassium concentration appears in saliva and in sweat in response to large doses of aldosterone.

#### RELATION OF ALDOSTERONE TO SODIUM RETENTION

Let us consider the first question, "Does aldosterone result in the retention of sodium in heart failure?" If so, then bilateral adrenalectomy of an animal with ascites should lead to a natriuresis in the absence of

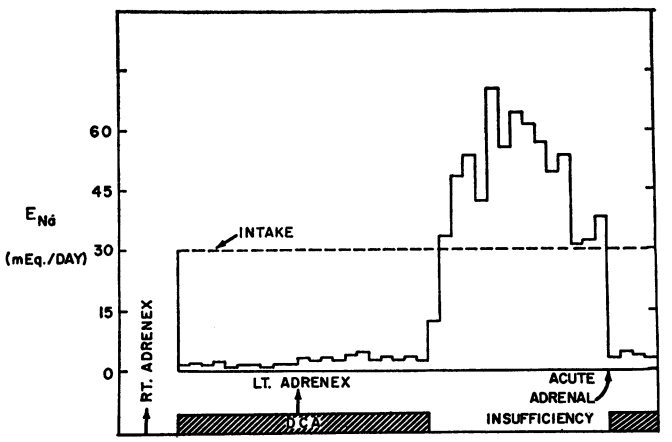


FIG. 2. Effects of discontinuation of desoxycorticosterone acetate (DCA) (3 mg./day) therapy in an adrenalectomized dog with thoracic inferior vena cava constriction and ascites. $E_{Na}$ is the abbreviation for sodium excretion. Caval constriction was performed before right adrenalectomy. Sodium retention and ascites formation continued after both right and left adrenalectomy until DCA therapy was discontinued; a striking diuresis ensued after DCA was stopped.[7]

hormone therapy, and this is what happens (Fig. 2).[7] After removal of the adrenal glands and discontinuation of desoxycorticosterone acetate (DCA) therapy in a dog with ascites produced by constriction of the thoracic inferior vena cava, a striking natriuresis ensued and lasted for 12 days until all ascites disappeared. Upon administration of DCA again, sodium retention recurred and ascites reaccumulated.

Similar observations were made in adrenalectomized dogs with experimental cardiac failure and, in addition, several different types of hormone therapy were given (Fig. 3).[8] In the absence of hormone therapy, sodium balance was negative. Also, a natriuresis occurred during injection of

either cortisone acetate or hydrocortisone acetate in a dose of 25 mg./day. In the presence of 25 mg./day of cortisone acetate to maintain cardiovascular function, DCA was administered in doses ranging from 0.5 to 25.0 mg./day. On the low dose of 0.5 mg./day of DCA, sodium excretion was variable; in some animals there was slight retention of sodium

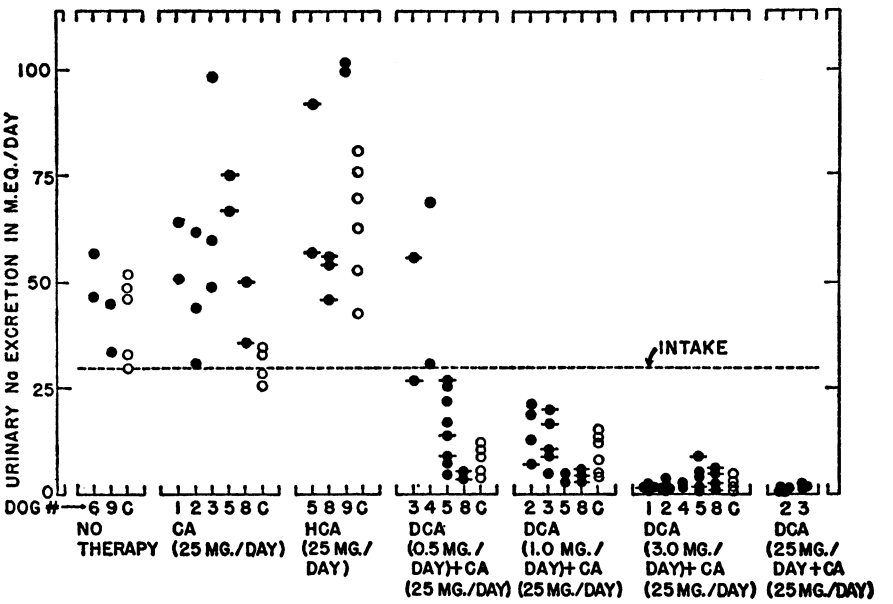


FIG. 3. Effects of adrenocortical hormones and of no hormone therapy on renal sodium excretion in a group of adrenalectomized dogs with cardiac failure and in one adrenalectomized dog (C) with thoracic caval constriction which was studied concurrently. The solid symbols with a horizontal bar indicate animals receiving digoxin. Some of the animals were given digoxin to sustain cardiovascular function but in no instance was enough digoxin given to effect cardiac compensation.[8] (CA = cortisone acetate; HCA = hydrocortisone acetate.)

while in others sodium balance was present or sodium balance was negative. As the dose of DCA was increased there was a progressive drop in sodium excretion until with 25 mg./day of DCA sodium retention was virtually complete. Since the effects of DCA and of aldosterone on the renal excretion of sodium are qualitatively identical[9] it was concluded that a high blood level of aldosterone is an important causative factor in the sodium retention of experimental heart failure.

If aldosterone or DCA were alone sufficient to effect chronic sodium retention, then administration of large amounts of either of these steroids

to normal humans or animals should produce sustained sodium retention. This does not occur; instead, both normal and adrenalectomized animals, including man, retain sodium for only a few days in the presence of large amounts of a sodium-retaining hormone and then escape from the sodium-retaining effects of the hormone. The typical response to administration of 25 mg./day of DCA to a normal dog is presented in Figure 4. Sodium

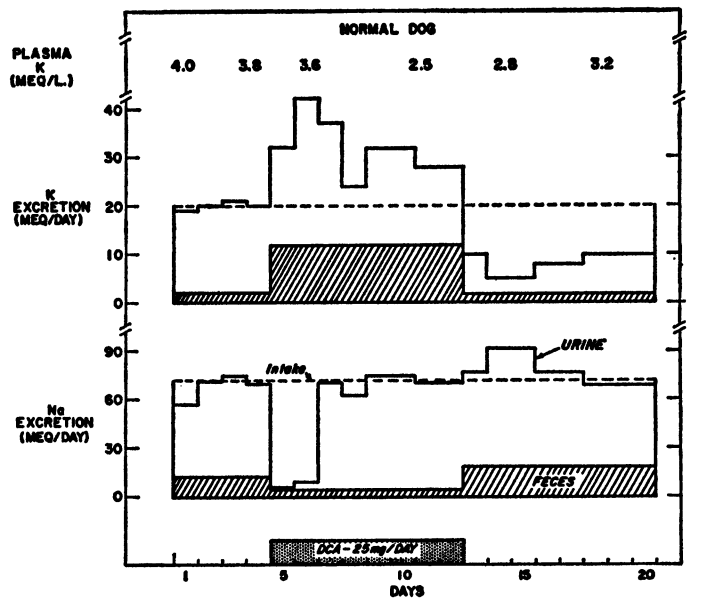


FIG. 4. Effects of 25 mg./day of DCA on sodium (Na) and potassium (K) excretion and plasma potassium concentration in a normal dog.

retention occurred for only two days while a negative potassium balance persisted for several days and hypokalemia resulted; essentially the same response occurs in the simple adrenalectomized dog.

In contrast, adrenalectomized dogs with a constricting ligature on the thoracic inferior vena cava show almost complete sodium retention and form large quantities of ascites for weeks to months during injection of 25 mg./day of DCA.[7] It appears, therefore, that the presence of the constricting caval ligature sensitizes the animal to the sodium-retaining action of DCA.

To examine the nature of this sensitizing factor, four possible mechanisms have been considered.[10] First, is the additional factor elevated renal venous pressure? Second, is the mechanism mediated by the renal nerves?

Third, does a reduction in glomerular filtration rate (GFR) or renal blood flow (RBF) change the response of the renal tubules to DCA or to aldosterone? Finally, is the factor a hormone which is essential for sodium retention in the presence of an excess of aldosterone or DCA?

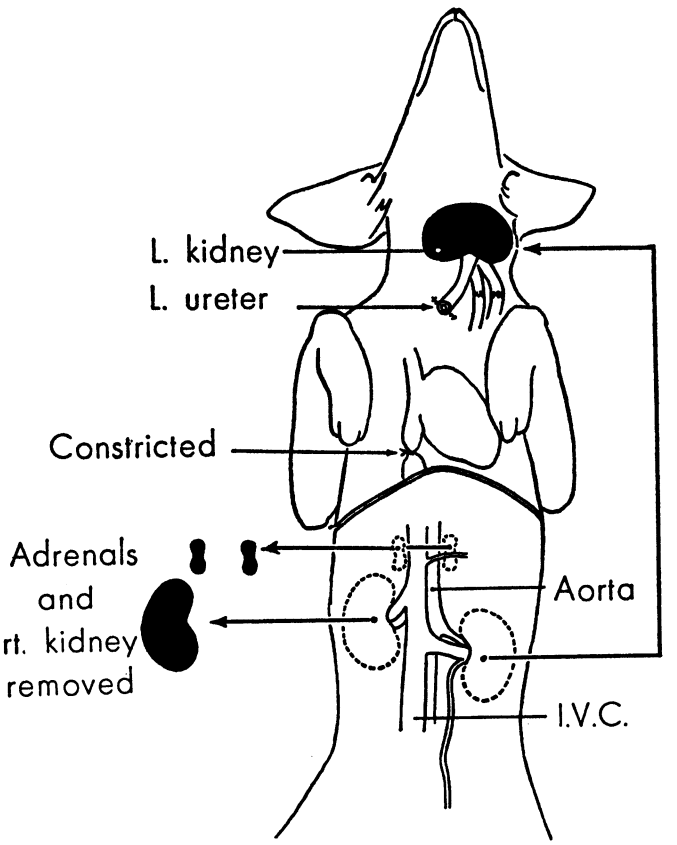


FIG. 5. Diagram of the experimental design used to examine the nature of the extra-adrenal factor essential for chronic renal sodium retention in the presence of increased sodium-retaining hormone. The left kidney was transplanted to the neck, the right kidney and both adrenal glands were removed and the inferior vena cava was constricted in the thorax.[10]

An experiment was designed to test these four possibilities.[10] The left kidney was transplanted to the neck, the right kidney was extirpated, both adrenal glands were removed and the thoracic inferior vena cava was constricted (Fig. 5). By transplantation of the kidney to the neck, it was removed from the area subjected to a high venous pressure and, of

course, the kidney was denervated. The response to a large dose of DCA was studied before and after removal of the caval ligature in a series of these animals. The results of a typical experiment are presented in Figure 6. In the presence of caval constriction, the administration of 25 mg./day of DCA was associated with almost complete sodium retention

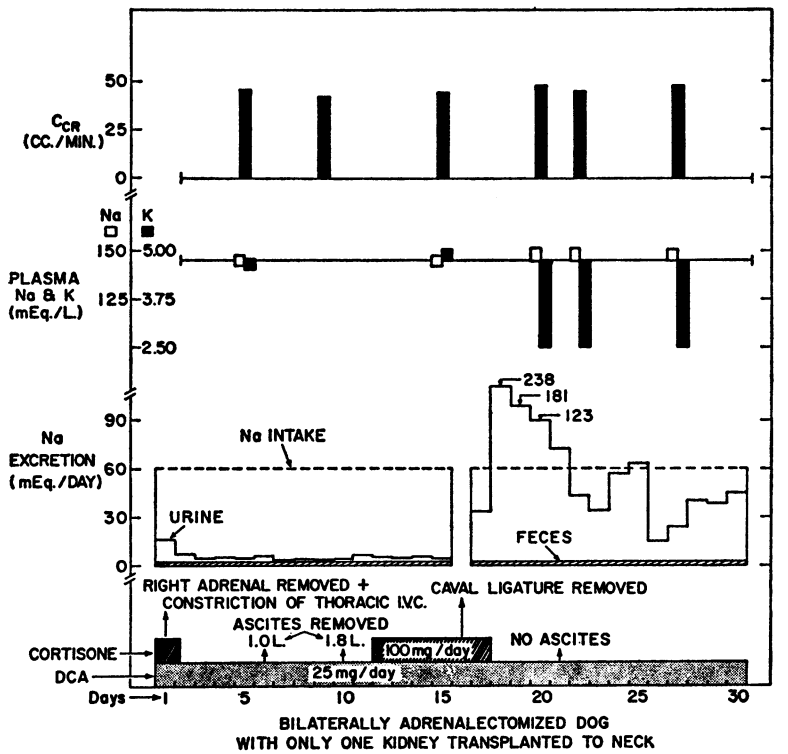


FIG. 6. Sodium excretion, plasma electrolyte concentrations and creatinine clearance during caval constriction and ascites formation and after removal of the caval ligature in a bilaterally adrenalectomized dog with only one kidney which was transplanted to the neck.[10]

and large quantities of ascites formed. To maintain the animals in better health and to prepare them for the surgery of removal of the ligature, 100 mg./day of cortisone acetate were given in addition to the DCA from days 12-17 (Fig. 6); the marked sodium retention continued. Following removal of the caval ligature, a large loss of sodium occurred, all ascites disappeared and sodium retention ceased. Similar findings were obtained in two animals in which aldosterone was given after completion of the

studies with DCA. The data demonstrate that the caval ligature altered the response of the transplanted neck kidney to DCA and to aldosterone so that sodium retention occurred. Since the venous pressure in the neck kidney was not elevated and the renal nerves were absent, the first two possibilities of venous hypertension and a nervous mechanism were eliminated.

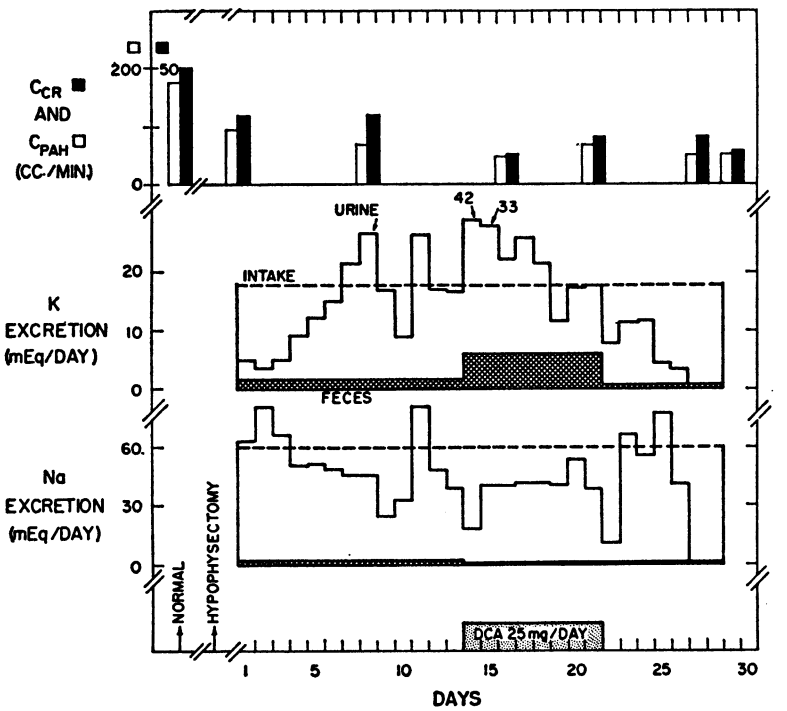


FIG. 7. Response to a large dose of DCA in a hypophysectomized dog with very low rates of glomerular filtration and renal plasma flow.[10]

Extensive studies[10] of GFR and RPF in the transplanted cervical kidney of these animals failed to demonstrate that renal hemodynamic function was lower during the presence of the caval ligature and the associated sodium retention than following removal of the ligature and in the absence of sodium retention. Furthermore, following removal of the caval ligature from one animal, a progressive drop in both GFR and RPF occurred over a period of several weeks but sodium retention failed to occur despite continued administration of 25 mg./day of DCA. Further study[10] of the effects of a large dose of DCA in dogs with very low renal hemodynamic

function secondary to hypophysectomy revealed that again an "escape" from the sodium-retaining action of DCA occurred (Fig. 7). It is concluded, therefore, than an extra-adrenal factor in addition to DCA or aldosterone is essential for chronic renal sodium retention. The present experiments provide evidence for postulating that this extra-adrenal factor is either 1) some as yet undefined renal functional change, or 2) a humoral agent. Additional experimental work is necessary to distinguish between these two alternatives.

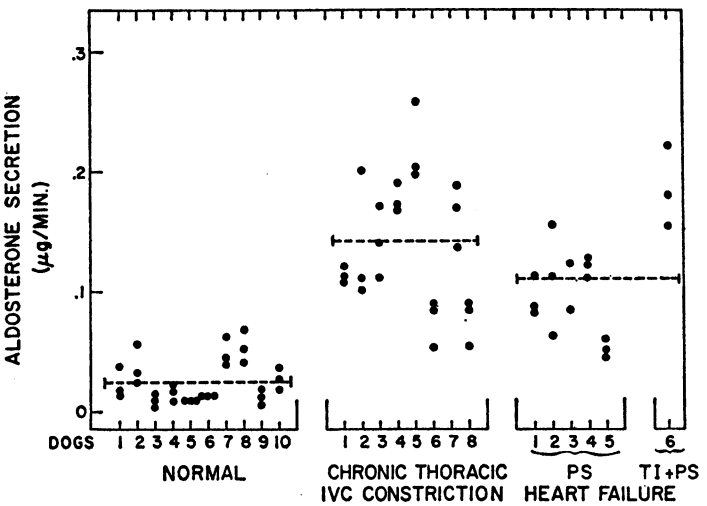


FIG. 8. Aldosterone secretion by the right adrenal gland in a group of normal dogs, in dogs with thoracic caval constriction, and in dogs with right heart failure. The measurements were made in anesthetized dogs subjected to the stress of laparotomy.[18]

#### MECHANISMS OF HYPERALDOSTERONEMIA

The second major problem is concerned with the mechanisms leading to the high blood level of aldosterone in heart failure. Evidence for hypersecretion of aldosterone in dogs with thoracic caval constriction and in dogs with experimental heart failure was obtained by cannulation of the right adrenolumbar vein and collection of adrenal venous blood by the method of Hume and Nelson.[11] The concentrations of aldosterone and corticosterone in adrenal vein plasma were measured by the double isotope derivative assay of Kliman and Peterson.[18] The high rates of aldosterone secretion by the right adrenal gland in 8 dogs with thoracic caval constriction and in 6 dogs with experimental right heart failure are compared with aldosterone production in 10 normal dogs (Fig. 8).[18] These findings

led to the conclusion that increased secretion of aldosterone occurs in experimental right heart failure.

The other mechanism which contributes to the high blood level of aldosterone is that of a decreased rate of metabolism of the hormone.[14] Since the liver is the principal site of metabolism of adrenal cortical steroids and the liver is congested in right-sided cardiac failure, the possibility was considerd that aldosterone is inactivated at a subnormal rate. To study this mechanism, $H^3$-d-aldosterone was injected intravenously into normal dogs and into dogs with chronic hepatic congestion and the rate of disappearance of the hormone from peripheral plasma was studied.[14] In the majority of the animals with a congested liver, there was a marked prolongation of the biological half-life of $H^3$-d-aldosterone, and the fractional turnover rate of tritiated aldosterone was reduced. Following hepatectomy, the disappearance curve of tritiated aldosterone was almost flat, a finding which reflects the important role of the liver in the metabolism of aldosterone. These results indicate that a decreased rate of metabolism of aldosterone may contribute to the high blood level of aldosterone in congestive heart failure. However, the more consistent occurrence and the marked increase in secretion of aldosterone in dogs with caval constriction and in experimental heart failure indicate that hypersecretion is the primary mechanism leading to hyperaldosteronemia.

From the combined data on aldosterone secretion and the turnover rate of aldosterone, the peripheral plasma level of the hormone was calculated for normal dogs and for dogs with secondary hyperaldosteronism produced by thoracic caval constriction.[14] A value of .002 μg./100 ml. of plasma was obtained for normal dogs, whereas .090 μg./100 ml. of plasma was estimated for dogs with experimental secondary hyperaldosteronism. These values indicate that a 45-fold increase in the peripheral plasma level of aldosterone is present in dogs with thoracic inferior vena cava constriction.

## HUMORAL MECHANISM LEADING TO HYPERSECRETION OF ALDOSTERONE IN HEART FAILURE

### A. *Evidence for an aldosterone-stimulating hormone*

The third major question is the intriguing one of "What stimulates the adrenal cortex to secrete large amounts of aldosterone in experimental heart failure?" Available evidence clearly demonstrates that the immediate stimulus is humoral. Indirect evidence for a humoral mechanism was obtained by stimulation of the isolated, denervated adrenal to secrete in-

creased amounts of aldosterone.[15] The left adrenal and kidney were transplanted to the neck and one week later the cervical kidney and the abdominal adrenal gland were removed (Fig. 9). Subsequent constriction of the thoracic inferior vena cava resulted in marked sodium retention and ascites formation and in the low sodium and high potassium pattern of fecal electrolyte excretion which is typical of hypersecretion of aldo-

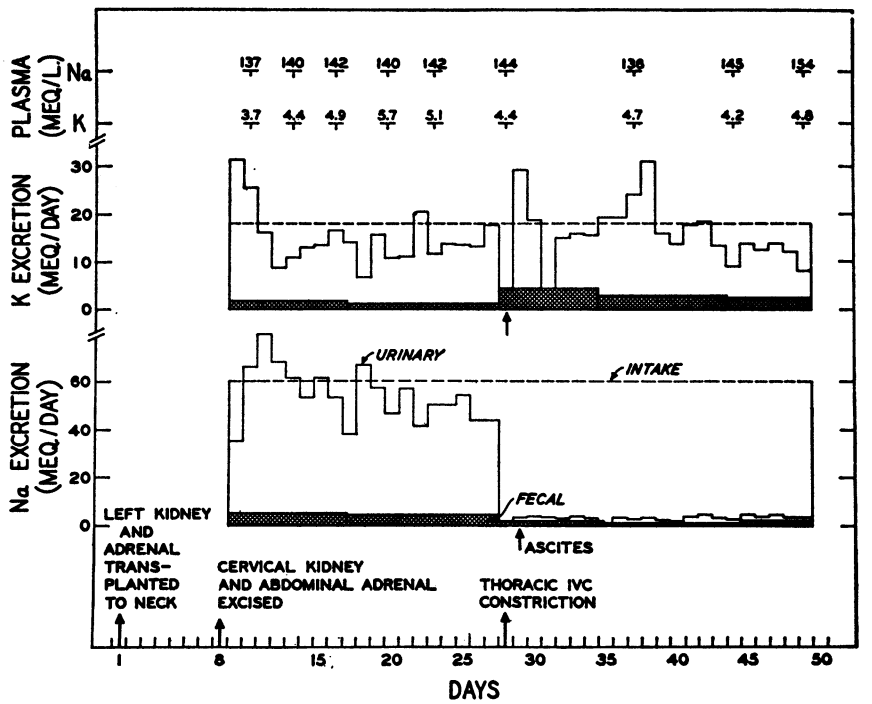


FIG. 9. Effects of constriction of the thoracic inferior vena cava (IVC) on sodium and potassium excretion and on plasma sodium and potassium concentrations in a dog with one transplanted adrenal in the neck and one abdominal kidney *in situ*.[15]

sterone. The external jugular vein draining blood from the transplanted adrenal gland was cannulated and blood collected; aldosterone secretion by this denervated adrenal was elevated. The stimuli of acute blood loss[15] and of chronic sodium depletion[16] have also been demonstrated to invoke hypersecretion of aldosterone by the denervated adrenal. The data clearly imply that the immediate stimulus to aldosterone production is humoral.

Direct evidence for a humoral mechanism was provided by cross circulation of blood from dogs with hyperaldosteronism secondary to

thoracic caval constriction through normal isolated adrenals (Fig. 10);[17] hypersecretion of aldosterone by the isolated adrenals occurred consistently during cross circulation. The adrenals were isolated by the technique of Hilton and associates.[18] Control observations were made during circulation of blood from the carotid artery of the normal recipient, through the

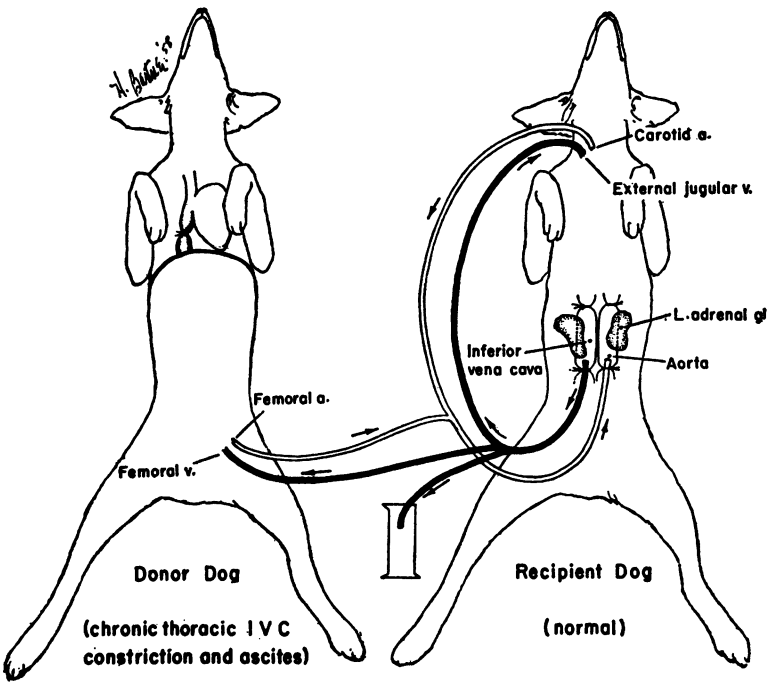


FIG. 10. Diagram of scheme for cross circulation of blood from a chronic donor dog with hyperaldosteronism on the left through the isolated adrenals of a normal recipient on the right.[17]

isolated adrenals, and to the external jugular vein of the recipient. During cross circulation, peripheral blood from the chronic donor dog with secondary hyperaldosteronism was circulated from the donor's femoral artery through the isolated adrenals and returned to the femoral vein of the donor. Observations were made during a recovery period with the same experimental conditions as during the control period. Aldosterone secretion increased during cross circulation in every experiment and returned to the control level in all experiments with recovery observations (Fig. 11); the average increase was 129 per cent, which was highly

significant. It should be pointed out that aldosterone cannot be detected in peripheral plasma by the technique used for analysis of the hormone (earlier data were calculated values); consequently, the increase in aldosterone in adrenal vein blood from the recipient's adrenal glands represents an actual increase in the rate of hormone secretion. Since the blood from

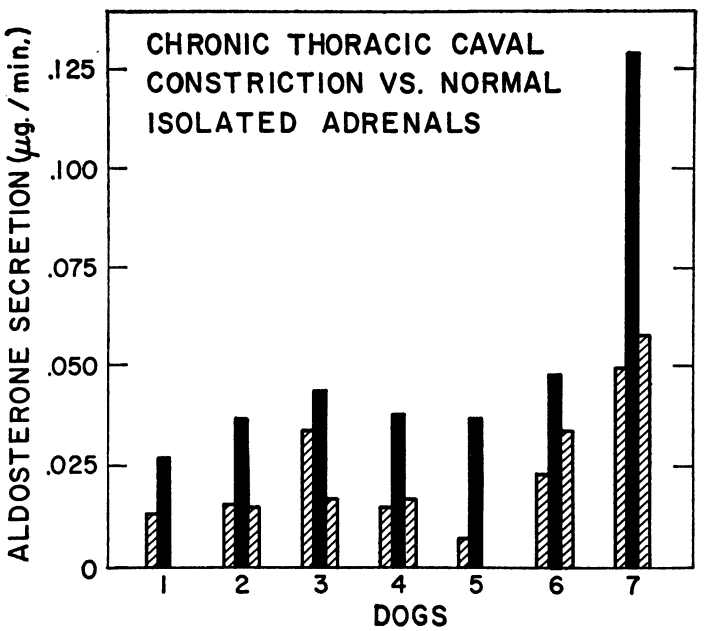


FIG. 11. The effects of cross circulation of blood from dogs with chronic thoracic caval constriction through normal isolated adrenals. The bars with the diagonal lines represent control and recovery periods whereas the solid bars indicate aldosterone secretion during cross circulation.[17]

donor dogs perfused isolated adrenal glands, the data show a direct effect of a humoral agent in donor blood. As a control experiment, blood from normal dogs was circulated through the isolated adrenals of normal animals; no consistent changes in aldosterone secretion occurred and the averages of the control and experimental values were the same. Also, the concentrations of sodium and potassium in plasma perfusing the isolated adrenals were essentially unchanged during the control, cross circulation, and recovery periods. These results demonstrate the presence of an aldosterone-stimulating agent in peripheral blood of dogs with experimental secondary hyperaldosteronism. Denton, Goding and Wright[19] performed similar cross circulation experiments in conscious sheep in which

chronic sodium depletion was used to stimulate aldosterone secretion and they obtained essentially the same results. It has been proposed[17] that this humoral agent is a hormone and that it be designated, the *aldosterone-stimulating hormone,* (ASH).

B. *Role of adrenocorticotrophic hormone (ACTH)*

Another hormone, in addition to ASH, is important in the production of aldosterone and this is the well-known adenohypophysial hormone,

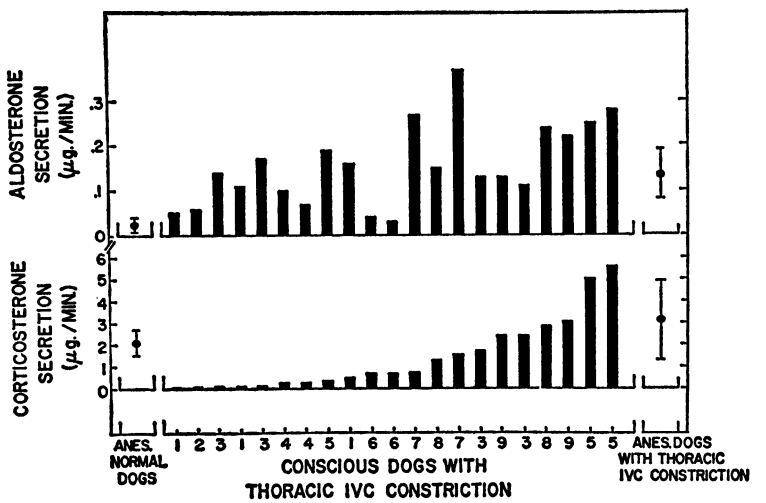


FIG. 12. Simultaneous rates of secretion of aldosterone and corticosterone in 9 conscious dogs with thoracic inferior vena cava (IVC) constriction showing very high secretion rates for aldosterone in the presence of low corticosterone outputs. The different values for the same animals were obtained on different days and are plotted in order of progressively increasing rates of corticosterone output. For comparison, aldosterone and corticosterone secretion is presented (average value and standard deviation) for 10 anesthetized (anes.) and stressed normal dogs, on the left, and 16 anesthetized, stressed dogs with thoracic IVC constriction, on the right.[20]

ACTH. In conscious dogs with a high rate of aldosterone secretion secondary to thoracic caval constriction, hypophysectomy resulted in an 80-90 per cent fall in aldosterone secretion.[20] The striking acute fall in aldosterone secretion which follows hypophysectomy was completely blocked by an infusion of ACTH during the posthypophysectomy period.[21] These findings clearly indicate that ACTH plays an important role in the production of aldosterone. Other observations,[20] however, demonstrate that maximal or near maximal rates of aldosterone secretion were present in conscious, unstressed dogs with thoracic caval constriction in which cortico-

sterone secretion was very low (Fig. 12); also, Porter-Silber chromogen secretion was low. These results indicate that a low circulating level of ACTH is sufficient for very high rates of aldosterone secretion, but the low basal output of ACTH is obviously quite important as demonstrated by the striking effect of hypophysectomy. It is suggested that ACTH provides support for a high rate of aldosterone production while ASH plays a more direct role in promoting hypersecretion of aldosterone.

C. *Relation of plasma electrolyte changes to aldosterone secretion*

It seems clear that the levels of plasma sodium or plasma potassium are not primary determinants of the rate of secretion of aldosterone in heart failure. It has been demonstrated (Refs. 16, 22 and personal observations) that either 1) a drop in plasma sodium, or 2) an increase in plasma potassium will augment the rate of aldosterone output by isolated adrenals. However, the concentrations of both plasma sodium and potassium are usually normal in uncomplicated heart failure, a finding which excludes a primary steroidogenic role of either hyponatremia or hyperkalemia in this diseased state.

D. *Renal origin of the aldosterone-stimulating hormone*

The finding of an ASH in peripheral blood of animals with hyperaldosteronism secondary to thoracic caval constriction[17] or secondary to chronic sodium depletion[19] raises the question of the locus of secretion of this hormone. To determine the source of ASH, the stimulus of acute hemorrhage was first used to promote aldosterone secretion.[23] Our approach was a relatively simple one, namely, to remove an organ or region of the body and to bleed the animal. A subsequent increase in aldosterone secretion was interpreted as evidence that the extirpated organ did not secrete ASH. On the other hand, removal of a region of the body and failure of aldosterone secretion to increase following stimulation by bleeding was considered indicative of removal of the locus of secretion of ASH.

The anterior pituitary was examined first as a possible source of ASH because of the striking drop in aldosterone secretion which follows hypophysectomy. As pointed out above, ACTH is important in the production of aldosterone but the possibility remained that ASH might be another anterior pituitary hormone. To examine this hypothesis, hypophysectomized dogs were bled to determine if aldosterone secretion would increase. An average increase of 100 per cent in aldostrone output ($P<.02$) (left section of Fig. 13) was observed for a group of eight hypophysectomized dogs.[23] Additional evidence that ASH is not secreted

by the anterior pituitary was obtained from studies of conscious hypophysectomized dogs with thoracic caval constriction in which aldosterone secretion was elevated five to sixfold.[20] This evidence that ASH is secreted by an extra-pituitary organ suggested that various regions of the body could be systematically removed, one at a time, along with the anterior pituitary and the response in aldosterone secretion to acute blood loss determined. The anterior pituitary was removed as well as the specific organ ablated because in animals stressed by laparotomy large

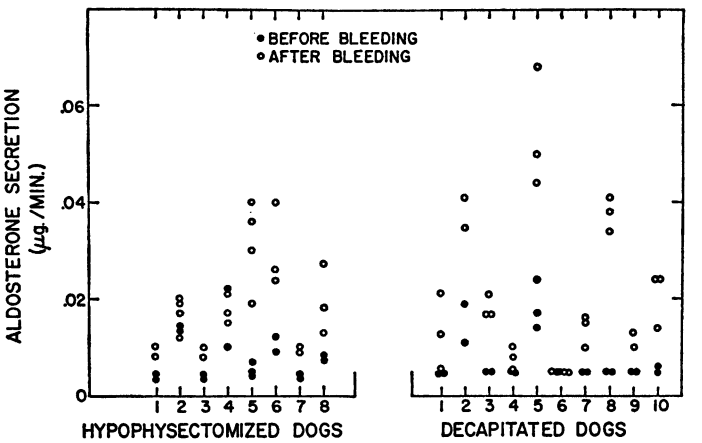


FIG. 13. Comparison of the response in aldosterone secretion to acute bleeding in 8 simple hypophysectomized dogs and in 10 decapitated animals.[28]

amounts of ACTH are released and a high circulating level of ACTH might obscure the response in aldosterone secretion to a change in ASH.

The head was next examined as a possible source of ASH. Dogs were decapitated and bled to determine if bleeding would stimulate aldosterone secretion in the absence of the brain and the anterior pituitary. An average increase of 200 per cent in aldosterone secretion ($P<.01$) (right section of Fig. 13) was observed for a group of ten decapitated dogs.[28] As a control experiment, studies were conducted in another group of dogs in which the head was removed but the animals were not bled. One hour following decapitation, which was the usual time the response to acute hemorrhage was observed in the previous series of animals, additional measurements were made; aldosterone secretion remained at the low control level. This control experiment indicates that the increase in aldosterone secretion observed following acute bleeding in decapitated

animals was a positive response to acute hemorrhage rather than the result of loss of an inhibitory factor or the result of a change secondary to the trauma of decapitation. It was concluded that ASH is secreted by an extracranial organ.

The liver and the kidneys were considered next as possible loci for secretion of ASH. The liver was excluded by bleeding hepatectomized-hypophysectomized dogs and observing an increase in aldosterone secretion.[23] The first indication that the aldosterone-stimulating hormone is secreted

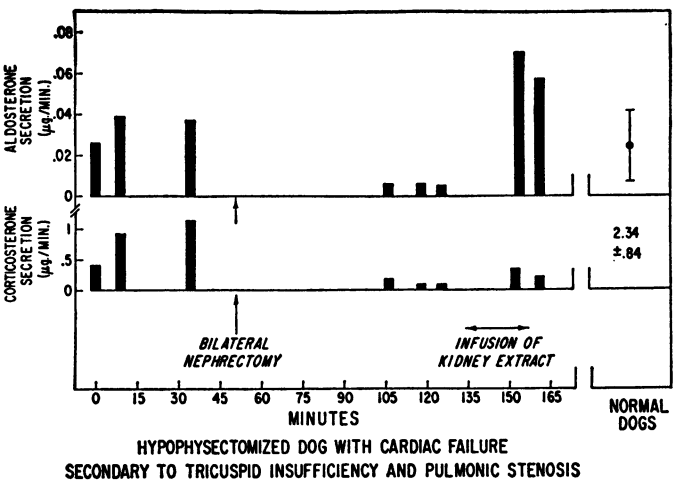


FIG. 14. The effects of acute nephrectomy and subsequent infusion of a saline extract of the animal's two kidneys on aldosterone and corticosterone secretion.[25]

by the kidney was the failure to obtain an increase in aldosterone secretion after bleeding of nephrectomized-hypophysectomized dogs.[23] Also, acute bilateral nephrectomy of hypophysectomized dogs produced a 50 per cent reduction in the rate of aldosterone secretion and the postnephrectomy level of aldosterone secretion was almost nil. Finally, injection of saline extracts of each animal's two kidneys produced a striking rise in aldosterone production. Similar studies in nephrectomized-hypophysectomized dogs were carried out by Ganong and Mulrow[24] with essentially the same results.

These initial studies were extended to include observations in dogs with hyperaldosteronism secondary to chronic congestive heart failure,[25] chronic thoracic caval constriction,[26] and chronic sodium depletion.[26] Although these animals were hypophysectomized two or three days prior to the acute study, many of them showed hypersecretion of aldosterone in the absence of the anterior pituitary. Following acute bilateral

nephrectomy, aldosterone secretion decreased 80-90 per cent and the final absolute level of aldosterone in adrenal vein plasma was almost undetectable (see Fig. 14). A similar marked drop in corticosterone secretion followed nephrectomy. Return of ASH to these animals by injection of saline extracts of their kidneys effected a marked increment in aldosterone secretion and a slight increase in corticosterone output. The evidence that an aldosterone-stimulating factor is secreted by the kidney in a variety of

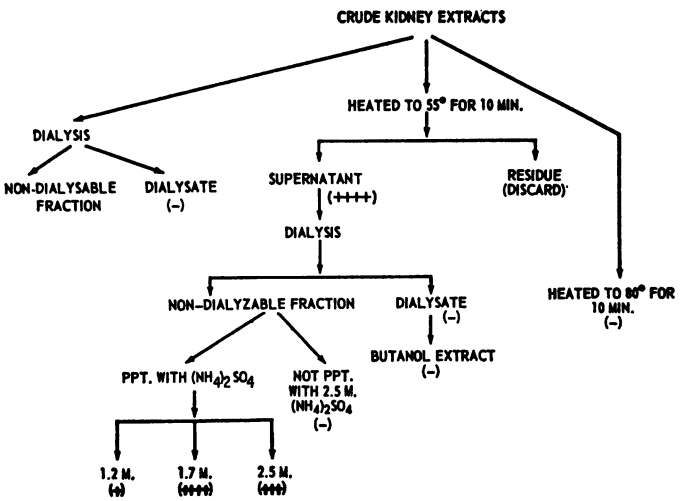


FIG. 15. Scheme for fractionation of crude kidney extracts for aldosterone-stimulating and pressor activity.[27]

experimental situations suggests the existence of an ASH common to these conditions.

### *E. Evidence for the identity of ASH and renin*

What is the chemical nature of the aldosterone-stimulating hormone secreted by the kidney? To answer this question crude kidney extracts were fractionated according to the scheme in Figure 15.[27] The extracts were heated to 55° C. for 10 minutes; the supernatant showed marked aldosterone-stimulating and pressor activity. The supernatant was dialyzed to obtain evidence on the protein nature of the active agent. Both the dialysate and butanol extracts of the dialysate were inactive. The non-dialyzable protein fraction was, however, highly active. Further fractionation of this material with ammonium sulfate demonstrated that the 1.7 and 2.5 M. ammonium sulfate fractions were highly active for increasing

aldosterone secretion and blood pressure; these fractions are known to precipitate renin selectively. Protein not precipitated with 2.5 M. ammonium sulfate was inactive. Heating to 80° C. for 10 minutes, which is known to denature renin,[28] destroyed all aldosterone-stimulating and pressor activity, suggesting again that ASH is renin. Finally, assay of the dialysate from non-heated kidney extracts revealed no activity. Collectively, these fractionation studies provide strong support for the identity of ASH and renin.

According to the classical view, renin is an enzyme which is secreted by the kidney and which acts upon renin substrate, hypertensinogen, to produce hypertensin I or angiotensin I, a decapeptide. Hypertensinogen is an $\alpha$-2 globulin; it has been suggested[29] that hypertensinogen is secreted by the liver. Angiotensin I is transformed by a plasma "converting enzyme" to angiotensin II, an octapeptide and the active pressor agent.

These considerations raise the question "Will synthetic angiotensin II stimulate aldosterone secretion?" Genest and associates[30] demonstrated an increase in urinary aldosterone excretion in man during infusion of synthetic angiotensin II and Laragh and coworkers[31] found that synthetic angiotensin II augments aldosterone secretion in humans. The steroidogenic action of angiotensin II has been confirmed by several workers[16, 32-34] and, more recently, evidence has been provided for a direct action of angiotensin II on the adrenal cortex.[33, 35]

Since there is evidence that ASH is renin, the renin content of the kidneys was examined in experimental secondary hyperaldosteronism. Renin was extracted and assayed by a slight modification of the method of Haas and Goldblatt.[36] The renin content of the kidneys was elevated sixfold above normal in dogs with thoracic caval constriction, and in two dogs with experimental cardiac failure the renin content of the kidney was higher than in any of the normal animals.[37] These data on increased renin content of the kidney do not necessarily reflect renin secretion but, in view of the large body of other evidence in support of the importance of the renin-angiotensin system in secondary hyperaldosteronism,[35] increased renin secretion appears to be a reasonable interpretation. This result and interpretation are consistent with the finding of Merrill, Morrison, and Brannon in 1946[37] that the renin content of renal vein blood was elevated in patients with heart failure.

More recently, the rate of renin release into lymph has been studied in a group of dogs with hyperaldosteronism secondary to thoracic caval constriction and in normal animals (Ref. 38 and personal observations). Thoracic duct lymph was collected and was prepared for assay by the technique of Helmer.[39] Lymph rather than renal vein blood was studied

because the relatively slower flow of lymph in comparison with renal vein blood might provide a greater concentration of renin in lymph than in blood. The substance in lymph which was assayed was presumably angiotensin II. Normal animals were found to have a large excess of renin substrate in lymph so that differences in the amount of angiotensin II formed in the two groups of dogs reflect differences in the amount of renin released.

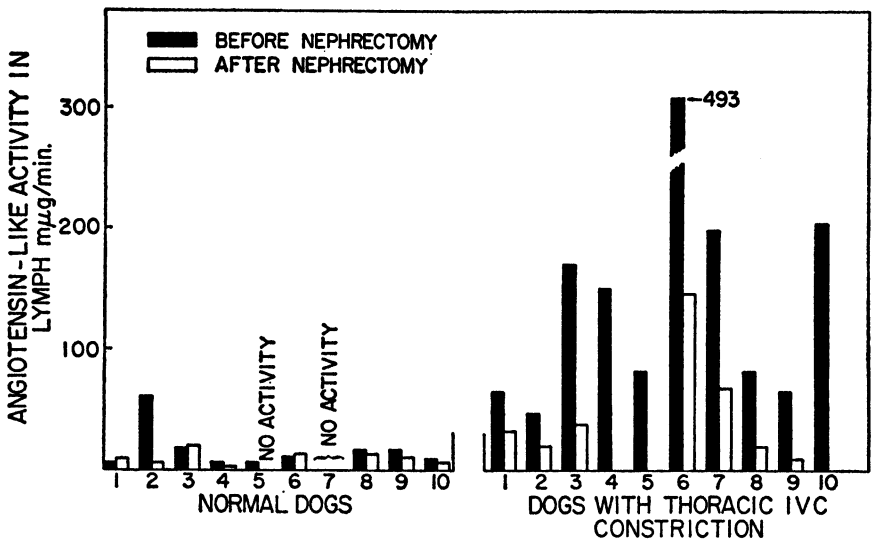


FIG. 16. Angiotensin-like activity (blood pressure assay) in thoracic duct lymph from normal dogs and dogs with secondary hyperaldosteronism produced by thoracic caval constriction.[88]

A double assay was used to estimate the quantity of angiotensin-like material in prepared lymph. First, the blood pressure response in the pentolinium-treated, vagotomized rat was used and, second, the steroidogenic response by the isolated adrenals of a hypophysectomized-nephrectomized dog was studied. It should be emphasized that the results obtained from use of a steroid assay for the angiotensin II in lymph provide strong additional evidence for the specificity of the Helmer technique.

There was a striking increase in angiotensin-like activity in prepared thoracic duct lymph from dogs with caval constriction compared with normal dog lymph (Fig. 16). These data were obtained by the blood pressure assay; for quantitation a dose response curve was obtained in each assay rat with synthetic angiotensin II. An increase in angiotensin-

like activity from an average value of 15 mμg/min. for normal lymph to 156 mμg/min. for lymph from dogs with caval constriction was found ($P<.01$). Following nephrectomy, angiotensin-like activity decreased in all animals in which an appreciable amount of activity was present initially.

The steroid response to infusion of a 30-minute collection of lymph from a normal dog and from a dog with thoracic caval constriction is

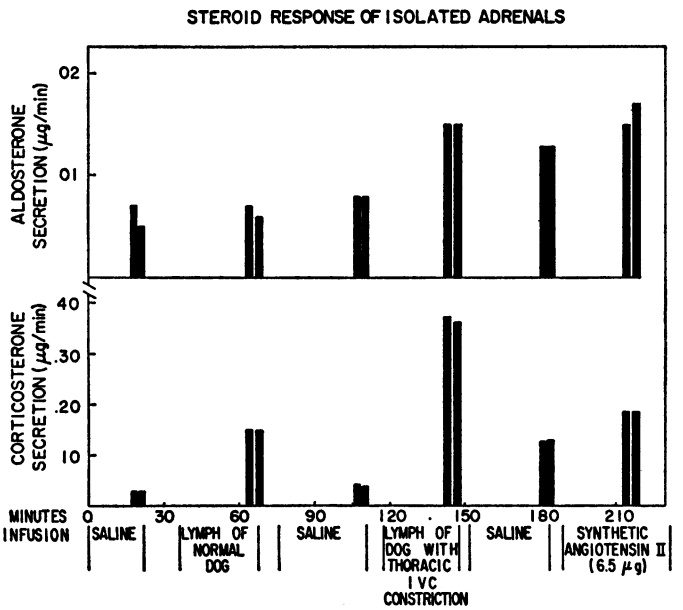


FIG. 17. A typical response in aldosterone and corticosterone secretion by isolated adrenals to prepared thoracic duct lymph from a 30-minute collection from a normal dog and from a dog with thoracic caval constriction. Synthetic angiotensin II was injected after the lymph in the same dose estimated by the pressor response to be present in the lymph from the dog with caval constriction.

presented in Figure 17. During infusion of normal dog lymph, there was no effect on aldosterone secretion and only a slight increase in corticosterone output. In contrast, the lymph from a dog with caval constriction and hyperaldosteronism produced a definite increase in both aldosterone and corticosterone secretion. Finally, infusion of the same dose (6.5 μg.) of synthetic angiotensin II as estimated by the pressor assay to be present in the 30-minute collection of lymph from the dog with caval constriction increased both aldosterone and corticosterone production. For the entire study, the response in aldosterone secretion

from isolated adrenals to prepared lymph from 7 normal dogs and from 6 dogs with thoracic caval constriction was determined. The lymph from the dogs with caval constriction produced an 80 per cent increase in aldosterone secretion ($P<.01$), whereas no aldosterone-stimulating activity was detected in normal dog lymph. These results provide evidence for release of increased amounts of renin by the kidney into lymph in dogs with thoracic caval constriction.

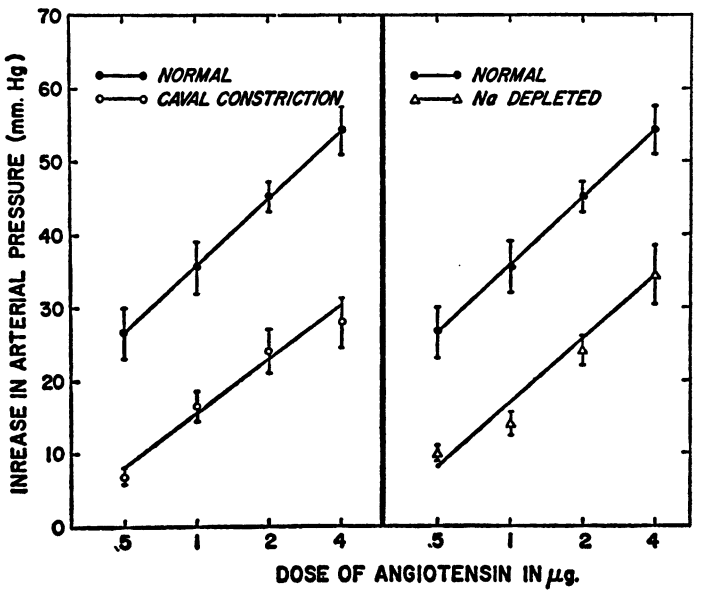


FIG. 18. Response in mean arterial pressure to synthetic angiotensin II in conscious dogs.[87]

The evidence from 1) fractionation studies, 2) the demonstration that angiotensin II has a direct action on the adrenal cortex, and 3) the observations on the renin content of kidneys and of renal vein blood and the rate of renin release into lymph indicates that ASH is renin.

*F. Pressor response to angiotensin II in secondary hyperaldosteronism*

If dogs with experimental secondary hyperaldosteronism have a high circulating level of angiotensin II as the data strongly indicate, the question arises as to explanation for the absence of hypertension. It seemed quite possible that these animals with secondary hyperaldosteronism might be less sensitive to angiotensin II. To test this hypothesis, a single intra-

venous injection of angiotensin II was given to conscious dogs and the blood pressure response measured.[27] Four dose levels (½, 1, 2 and 4μg.) of synthetic angiotensin II were studied twice in each of 5 normal dogs and 5 dogs with caval constriction (Fig. 18). The expected log-dose response occurred for both groups, but more important was the marked reduction in the response of the dogs with caval constriction. Similarly, dogs with hyperaldosteronism secondary to chronic sodium depletion were less reponsive to angiotensin II than were normal animals.

*G. Intrarenal locus of secretion of renin*

What specific cells in the kidney secrete ASH or renin in experimental secondary hyperaldosteronism? Examination of the juxtaglomerular cells from dogs with thoracic caval constriction revealed the presence of hypergranulation and, occasionally, of hyperplasia (Fig. 19).[27] These findings together with all other data on the association of renin with juxtaglomerular cell changes are consistent with the view that renin is secreted by the juxtaglomerular cells in experimental secondary hyperaldosteronism. A logical site or location for the so-called "volume receptor" and possibly the one referred to by Dr. Peters is the renal afferent arteriole since the juxtaglomerular cells are located in the media of these arterioles.

*H. Afferent signal to the kidney for release of renin*

This leaves us with one additional question, "What is the stimulus to the juxtaglomerular cells to release renin?" From earlier studies on the effects of acute hemorrhage, it was reasoned that a drop in blood pressure and blood flow through the kidney might provide the stimulus for the release of renin and hypersecretion of aldosterone. A test of this hypothesis was provided by constricting the aorta immediately above the renal arteries because this manuever decreased the renal perfusion pressure and renal blood flow. The results of a typical experiment are presented in Figure 20.[27] Suprarenal aortic constriction produced a drop in arterial pressure below the kidney; renal blood flow (not presented in Fig. 20) was also measured and found to be reduced. Both aldosterone and corticosterone secretion increased; the increase in corticosterone output, although definite, was to a level less than ten per cent of the level observed in normal dogs stressed by laparotomy and was probably of no physiologic significance. In contrast, aldosterone output increased to a level greater than the rate of aldosterone production in normal dogs subjected to the stress of laparotomy; consequently, the level of aldosterone secretion achieved was relatively high and of physiologic importance. Similar

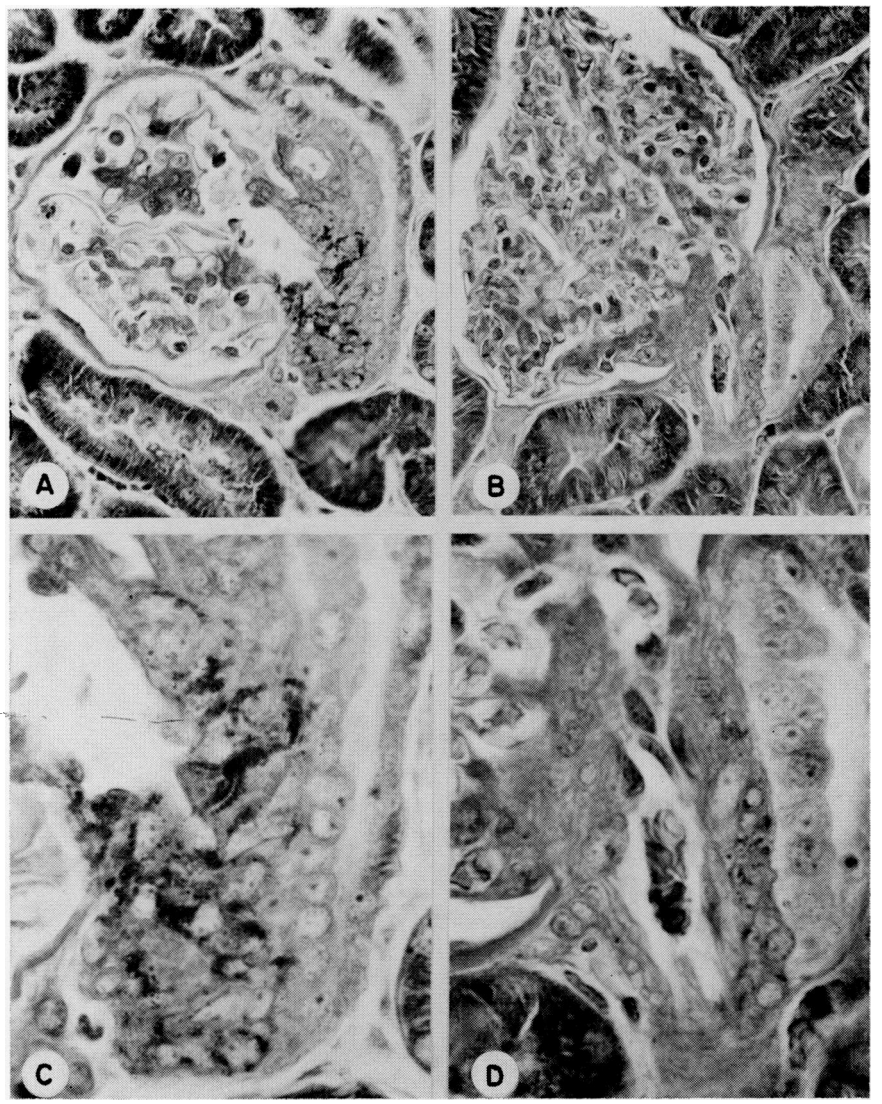


FIG. 19. Paraffin sections of kidneys stained by the Bowie technique and photographed with a Wratten G filter. A. Kidney from a dog with caval constriction. A glomerulus occupies most of the field with the afferent arteriole and macula densa at the lower right. Even at this low power (X 450), granules in juxtaglomerular cells appear prominent. This field is seen at higher magnification (X 1100) in Figure C. Note hyperplasia and hypergranulation of juxtaglomerular cells. B. Kidney from a normal dog for comparison with Figure A. At this magnification (X 450), juxtaglomerular cell granules are barely discernible in the wall of the arteriole at the lower right. This portion of the field is shown at higher magnification (X 1100) in Figure D. Granules are small and sparse, as is normal for juxtaglomerular cells in this species.[27]

results were obtained in fifteen hypophysectomized dogs subjected to aortic constriction[37] and the response was almost identical for both aldosterone and corticosterone secretion to that observed following acute hemorrhage in hypophysectomized dogs.[38] Also, in both low[5] and high[40] output experimental heart failure, the arterial pressure and blood flow through the kidney are decreased. Although the precise signal to the kidney is unknown, the

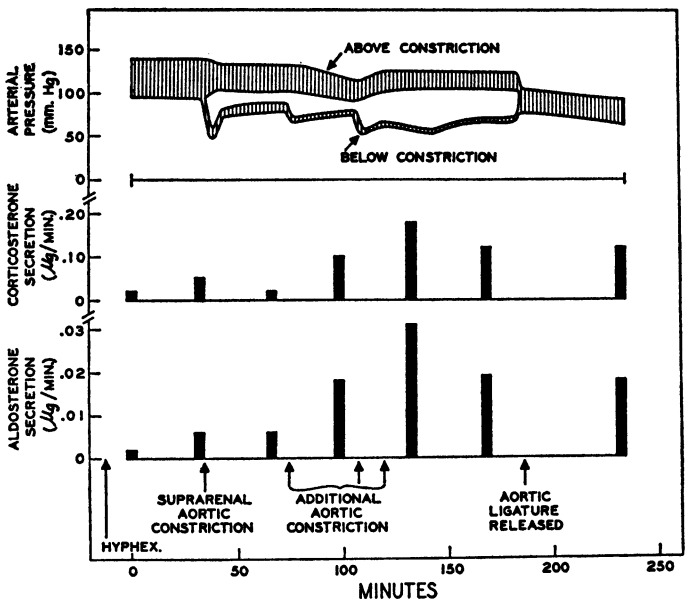


FIG. 20. Effects of acute constriction of the aorta immediately above the renal arteries in a hypophysectomized dog.[37]

signal is provided in several experimental situations by decreased pressure and flow of blood through the organ.

Tobian[41] has proposed that a decrease in stretch of the walls of the renal afferent arterioles provides the afferent signal for release of renin by the juxtaglomerular cells. This is a reasonable working hypothesis but there are certain findings left unexplained.[35] Also, it is possible that there is a dynamic component to the signal. The precise nature of the afferent signal to the kidney remains to be determined by future research.

## SUMMARY

Our current view of the mechanisms leading to hypersecretion of aldosterone, sodium retention and ascites formation in experimental right

heart failure is summarized in Figure 21. In either low or high output experimental heart failure, systemic venous pressure is elevated, arterial pressure is decreased and the flow of blood through the kidney is reduced. These changes are associated with the secretion of an aldosterone-stimulating hormone by the kidney and this hormone appears to be renin. There is a substantial body of evidence to show that increased secretion

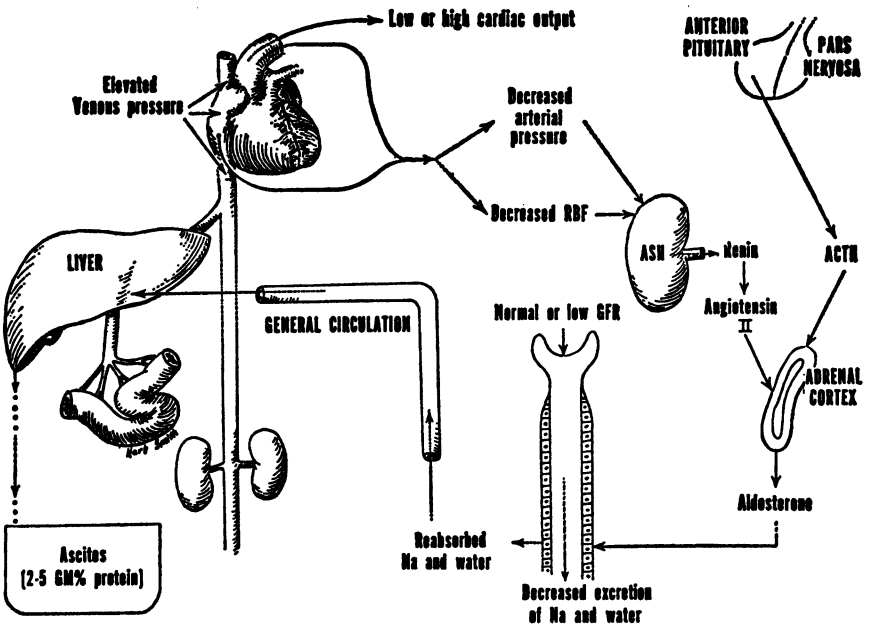


FIG. 21. Diagram to depict the mechanisms leading to hypersecretion of aldosterone, sodium retention and ascites formation in experimental heart failure.

of renin occurs in experimental right heart failure and results in an increased plasma level of angiotensin II. This octapeptide, in the presence of a basal rate of ACTH output by the anterior pituitary, acts directly on the adrenal cortex to promote increased aldosterone secretion. Aldosterone, in association with an extra-adrenal factor, acts on the renal tubule cells to promote sodium transport and, thereby, sodium retention by the kidney. Acute reductions in GFR also result in retention of sodium by the kidney. Sodium retention is accompanied by retention of water and by expansion of the circulating blood volume. Reabsorbed fluid and electrolytes are extravasated to form edema by localization of fluid in the regions of the body where a high hydrostatic pressure favors transudation. Large quan-

tities of ascites are formed by transudation of fluid from the hepatic lymphatics.

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