Adrenocortical and Renal Hormonal Function in Experimental Cardiac Failure

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In 1950, Deming and Luetscher reported that urine from patients with congestive heart failure showed an increase in sodium-retaining activity. This finding was the first suggestive evidence that a high blood level of aldosterone is present in heart failure and this result raised the question of the pathogenic importance of aldosterone in cardiac failure. In the present series of experiments, the role of aldosterone has been studied in experimental cardiac failure. Three important aspects of the problem are considered. First, does aldosterone promote the retention of salt and water and, if so, under what circumstances? Secondly, is hyperaldosteronemia the result of both an increase in the rate of aldosterone production and a decreased rate of metabolism of the hormone? Thirdly, 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. The first of these is the dog with ascites produced by constricting the inferior vena cava in the thorax. These animals show marked sodium retention and form large quantities of ascitic fluid. Although this preparation does not contain a failing heart, the mechanisms leading to hyperaldosteronism and the changes in salt and water metabolism appear to be essentially the same as in experimental right heart failure. The second preparation is the dog with right-sided congestive heart failure secondary to progressive pulmonic stenosis. This experimental model resembles right heart failure in man very closely.

Let us consider first the question “Does aldosterone result in the retention of sodium in heart failure?” If so, then bilateral adrenalectomy of a dog with experimental ascites should lead to a diuresis in the absence of hormone therapy. The results of an experiment of this type in a dog with thoracic caval constriction are presented in figure 1. After removal of each of the adrenal glands separately and discontinuation of desoxycorticosterone acetate (DCA), a striking natriuresis occurred. All ascites disappeared after 12 days and, then, signs of adrenal insufficiency became evident. 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. 2). In the absence of hormone therapy, net loss of sodium occurred. Also, a natriuresis was observed during administration of either cortisol acetate or hydrocortisone acetate in a dose of 25 mg./day. In the presence of cortisol acetate (25 mg./day) to maintain cardiovascular function, DCA was given in doses ranging from 0.5 to 25 mg./day. With 0.5 mg./day of DCA, slight retention of sodium occurred; as the dose of DCA was increased progressively, sodium retention became more marked until with 25 mg./day of DCA, sodium retention was virtually complete. Since the effects of DCA and aldosterone on the renal excretion of sodium are qualitatively identical, it was concluded that a high blood level of aldosterone is an important causative factor in the sodium retention of experimental heart failure.

In clinical or experimental states with edema, there appears to be a factor in addition to aldosterone that is essential for chronic
sodium retention. The evidence for this factor is that adrenalectomized dogs fail to show chronic sodium retention on a large dose of DCA, whereas adrenalectomized dogs with thoracic caval constriction or with heart failure given the same large dose of DCA retain salt and water. Some functional change secondary to the presence of caval constriction or cardiac failure promotes the renal retention of sodium in the presence of sufficient sodium-retaining hormone. It is possible that the absence of this factor accounts for the failure of a normal animal to retain sodium in the presence of a large amount of DCA or aldosterone.

Figure 1

Effects of discontinuation of DCA (3 mg./day) therapy in an adrenalectomized dog with thoracic inferior vena caval 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. Data from Davis, J. O., Howell, D. S., and Southworth, J. L.: Circulation Research 1:260, 1953.

The second question is concerned with the mechanisms leading to a high blood level of aldosterone. Evidence for hypersecretion of aldosterone in dogs with thoracic caval constriction and in dogs with experimental heart failure was obtained by direct cannulation of the right adrenolumbar vein and collection of adrenal venous blood by the method of Hume and Nelson. The concentrations of aldosterone and corticosterone in adrenal vein plasma were measured by the double isotope derivative assay of Kliman and Peterson. The high rate of aldosterone secretion by the right adrenal gland in eight dogs with thoracic caval constriction and in six dogs with experimental right heart failure is compared with aldosterone production in 10 normal dogs (fig. 3). These findings led to the conclusion that increased production of aldosterone occurs in experimental heart failure.

What about the other alternative mechanism for hyperaldosteronemia, namely, a decreased rate of metabolism of aldosterone? Since the liver is the principal site for metabolism of adrenocortical steroids and the liver is congested in right-sided heart failure, the possibility was considered that aldosterone is...
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 dog (C) with thoracic caval constriction, which was studied concurrently. The solid symbols with a horizontal bar indicate that the animal was 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. CA and HCA are abbreviations for cortisol acetate and hydrocortisone acetate. Data from Davis, J. O., Howell, D. S., and Hyatt, R. E.: Am. J. Physiol. 183:263, 1955. (Reproduced with permission of the publisher.)

The third question is the intriguing one of the mechanisms leading to hypersecretion of aldosterone in heart failure. Evidence that the immediate stimulus is hormonal was obtained by cross circulation of blood from a donor dog with thoracic caval constriction and secondary hyperaldosteronism through hepatectomy, the peripheral blood level of aldosterone was calculated for normal dogs and for dogs with secondary hyperaldosteronism produced by thoracic caval constriction. A value of .002 μg.m./100 ml. of blood was obtained for normal dogs, whereas .090 μg.m./100 ml. of blood was estimated for dogs with experimental secondary hyperaldosteronism. Consequently, the calculations indicate a 45-fold increase in the blood level of aldosterone in dogs with thoracic caval constriction.

From the combined data on aldosterone secretion and the turnover rates of the hormone, the peripheral blood level of aldosterone was markedly reduced. Following hepatectomy, the disappearance curves of tritiated aldosterone were almost flat, a finding that reflects the important role of the liver in the metabolism of aldosterone. These results indicate that a decreased rate of metabolism of aldosterone may also contribute to the high blood level of aldosterone in congestive heart failure.
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. Data from Davis, J. O.: Rec. Prog. Hormone Research 17:293, 1961.13 (Reproduced with permission of the publisher.)

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. From Yankopoulos, N. A., Davis, J. O., Kliman, B., and Peterson, R. E.: J. Clin. Invest. 38:1278, 1959.10 (Reproduced with permission of the publisher.)
The results of a typical experiment are presented in figure 5. A striking increase in aldosterone secretion occurred during cross circulation and aldosterone production returned to the control level during the recovery period. The concentration of aldosterone in adrenal vein plasma increased while adrenal blood flow was essentially unchanged. The results of all seven experiments of this type demonstrated an increase in aldosterone secretion in every instance and a return to the control level of aldosterone production in the experiments in which recovery periods were obtained (fig. 6). The average increase in aldosterone secretion for the group was 129 per cent ($p<.01$). It should be pointed out that aldosterone cannot be detected in peripheral plasma by the technic used for analysis of the hormone (earlier data were calculated values); consequently, the increase in aldosterone in adrenal vein blood of the recipient's adrenal glands represents an actual increase in the rate of secretion. Since the blood from donor dogs perfused isolated adrenal glands, the data show a direct effect of a humoral agent in donor blood.

To provide a control on the effects of cross circulation per se, or of any event associated with this experimental design, blood from normal dogs was circulated through normal isolated adrenal glands. There were no consistent changes in the secretory rate of aldosterone, and the mean control and mean experimental values were the same. Measurements of plasma electrolytes during cross circulation of blood showed no consistent changes. These observations demonstrate, therefore, the existence of an aldosterone-stimulating agent in the blood of dogs with hyperaldosteronism secondary to thoracic caval constriction, a factor perhaps comparable to such an agent in human hyperaldosteronism. We have suggested the name aldosterone-stimulating hormone, ASH, for the humor. Concurrent independent observations by Denton, Goding, and Wright have provided similar evidence for

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*Figure 5*

ASH from cross circulation studies in conscious sheep.

Where is the aldosterone-stimulating hormone secreted? In experimental heart failure and in dogs with hyperaldosteronism secondary to thoracic caval constriction, ASH is secreted by the kidney. Evidence for the renal origin of ASH was obtained by studying the acute response to bilateral nephrectomy in hypophysectomized dogs with heart failure or caval constriction. In these studies it was necessary to remove the anterior pituitary before nephrectomy because ACTH exerts an important influence on aldosterone production, and changes in ACTH might obscure alterations in aldosterone production secondary to loss of ASH. The results of a typical response to bilateral nephrectomy and reinjection of the hormone, ASH, are presented in figure 7. During the control period, hypersecretion of aldosterone was present although a marked reduction in aldosterone secretion had resulted from hypophysectomy 2 days previously. Following nephrectomy, both aldosterone and corticosterone secretion fell markedly; injection of a saline extract of the animal's two kidneys produced a striking increase in aldosterone secretion and a slight rise in corticosterone output.

The effects of bilateral nephrectomy in 10
hypophysectomized dogs with thoracic caval constriction and ascites are presented in figure 8. Aldosterone secretion fell in every instance; the average decrease was from .033 to .006 μg/m. min., an 81 per cent fall. A similar drop in corticosterone secretion occurred.

What, then, is the chemical nature of ASH? To determine this, crude kidney extracts were fractionated for aldosterone-stimulating and pressor activity. The fractionation scheme used and the results are summarized in figure 9. Crude kidney extracts were heated to 55° for 10 minutes; the supernatant from these heated extracts showed marked aldosterone-stimulating and pressor activity (as indicated by +++++). The supernatant was dialyzed overnight to determine if the active agent is protein. Both the dialysate and butanol extracts of the dialysate failed to produce (see minus sign in figure 9) a steroidogenic and a pressor response. In contrast, the nondialyzable protein fraction was highly active. Further fractionation of the protein demonstrated that most of the aldosterone-stimulating activity was in the 1.7 and 2.5 M. ammonium sulfate fractions, which are known to precipitate renin. Also, both fractions increased blood pressure markedly. Heat to 80° C. for 10 minutes, which denatures renin, destroyed all aldosterone-stimulating and pressor activity, a finding that also suggests that ASH is renin. Finally, assay of the dialysate from unheated crude kidney extracts showed no aldosterone-stimulating activity. Collectively, these results provide strong suggestive evidence that the renal aldosterone-stimulating factor is renin.

Before we proceed further, let us review briefly the classical concept of the renin-angiotensin system. The kidney secretes the enzyme renin, which acts upon hypertensigen, an alpha-2 globulin in the blood, to produce angiotensin I, a decapptide. Angiotensin I is converted by a plasma "converting enzyme" to angiotensin II, which is an octapeptide and the active pressor agent.
Will angiotensin II stimulate aldosterone secretion? Genest and co-workers and Laragh and associates have demonstrated an increase in aldosterone secretion during administration of synthetic angiotensin II to man. Carpenter et al. and Mulrow and Ganong have observed an increase in aldosterone secretion during injection of synthetic angiotensin II in nephrectomized-hypophysectomized dogs.

Since available evidence indicates that ASH is renin, the renin content of the kidneys was examined in dogs with secondary hyperaldosteronism (fig. 10). Renin was extracted and assayed by a slight modification of the method of Haas and Goldblatt. The renin content of the kidneys was elevated 6 to 7 fold in dogs with hyperaldosteronism secondary to thoracic caval constriction. In two dogs with cardiac failure, the renin content of the kidneys was higher than the renin present in kidneys from normal animals. Although these data on renin content do not necessarily re-
Figure 9


Figure 10

flect renin secretion, the most reasonable interpretation of the finding is that the rate of renin release by the kidney is augmented in experimental secondary hyperaldosteronism. This result and the interpretation are consistent with the finding of Merrill, Morrison, and Brannon in 1946 that secretion of renin by the kidney is increased in patients with heart failure.

If dogs with secondary hyperaldosteronism have a high level of angiotensin II, as the data strongly suggest, the question arises as to the explanation for the absence of hypertension in these animals. We reasoned that the blood pressure response to angiotensin II might be less in these animals. To test this hypothesis, a single intravenous injection of synthetic angiotensin II was given to conscious dogs and the blood pressure response was measured. The results are presented in the left section of figure 11. Synthetic angiotensin II (valine-5-angiotensin II from Ciba) was injected on two occasions into each of five normal dogs and into five dogs with thoracic caval constriction and secondary hyperaldosteronism. A log-dose response was obtained in both groups, and the blood pressure response was markedly reduced in the dogs with caval constriction. A similar reduction in the response to synthetic angiotensin II was observed in another group of five dogs with hyperaldosteronism secondary to chronic sodium depletion (right section of figure 11).

Let us now consider what specific cells in the kidney secrete ASH or renin. Piteock, Hartroft, and Newmark and Tobian, Janecek, and Tomboulian have provided evidence that renin is secreted by the juxta-
glomerular cells. A direct correlation was found between the amount of renin by bioassay and the degree of granulation of the juxtaglomerular cells in sodium deficient and control rats by Piteock et al., while Tobian and associates demonstrated a similar correlation in hypertensive rats and in rats fed an excess of dietary salt. Hartroft examined the juxtaglomerular cells from the kidneys of a group of dogs with thoracic caval constriction and compared the results from a control group of normal dogs. There was a marked increase in the granularity of the juxtaglomerular cells in the dogs with caval constriction and, in the animals with the most marked juxtaglomerular cell granularity, the juxtaglomerular cells were hyperplastic. Also, in a recent report, Edelman and Hartroft provided more specific evidence by the fluorescent-antibody technic that the juxtaglomerular cells secrete renin. This finding and the observation of Hartroft of increased granularity and hyperplasia of juxtaglomerular cell in dogs with secondary hyperaldosteronism produced by thoracic caval constriction are consistent with the view that increased secretion of renin by the juxtaglomerular cells occurs in experimental secondary hyperaldosteronism. A logical site or location, then, for the *receptor,* the so-called "volume receptor" would be the renal afferent arterioles, since the juxtaglomerular cells are located in the media of the afferent arterioles of the kidney.

This leaves us with the additional question, "What is the stimulus to the juxtaglomerular
cells to release renin? From earlier studies on the effects of acute hemorrhage on aldosterone secretion, we reasoned that a drop in blood pressure and flow through the kidney might provide the stimulus for release of renin and, thereby, for increased aldosterone secretion. A test of this hypothesis would be to determine the effects of constriction of the aorta immediately above the renal arteries with a resultant decrease in renal arterial pressure and renal blood flow, which should augment aldosterone secretion. A typical experiment and result are presented in figure 12. As in earlier studies, hypophysectomy was performed before control observations were made. Suprarenal aortic constriction decreased aortic pressure below the constriction. Measurements of paraaminohippurate clearance (not shown in figure 12) demonstrated a decrease in renal blood flow. Both aldosterone and corticosterone secretion increased; the elevation in corticosterone output, although definite, was to a level less than 10 per cent of the "stressed" level in the normal dog and was probably of no physiologic significance. In contrast, aldosterone secretion reached a level greater than the rate of aldosterone production observed in normal dogs subjected to the stress of laparotomy; consequently, the level of aldosterone secretion achieved was relatively high and of physiologic importance. Similar results were obtained in 15 hypophysectomized dogs subjected to aortic constriction and the response was almost identical for both aldosterone and corticosterone production to that observed following acute hemorrhage of hypophysectomized dogs. We agree, therefore, with Tobian that a decrease in stretch of the walls of the renal afferent arterioles constitutes a likely explanation for the stimulus to hypersecretion of aldosterone by the renin-angiotensin system.

Our current view of the sequence of events from the primary myocardial defect in experimental heart failure to the changes in adrenocortical function is presented in figure 13. As a result of myocardial failure, a decrease in cardiac output and an elevation in venous pressure occur. Renal arterial pressure and renal blood flow are consistently decreased in experimental heart failure, the consistent occurrence of decreased arterial pressure is a reflection of the severity of cardiac failure secondary to progressive pulmonic stenosis. It is suggested that some functional alteration in renal hemodynamic function occurs secondary to decreased pressure and flow through the kidney in experimental heart failure. As suggested by Tobian, a decrease in stretch of the renal arterioles provides a reasonable explanation for release of renin by the juxtaglomerular cells. Increased circulating renin leads to increased angiotensin II, which augments aldosterone secretion by a direction action on the zona glomerulosa of the adrenal cortex.

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