

## The renin-angiotensin-aldosterone system in congestive failure in conscious dogs.

L Watkins Jr, ... , F W Smith, A C Barger

*J Clin Invest.* 1976;**57**(6):1606-1617. <https://doi.org/10.1172/JCI108431>.

### Research Article

The role of the renin-angiotensin-aldosterone system in the development of congestive failure has been assessed in the conscious dog by use of the nonapeptide converting enzyme inhibitor. Constriction of the pulmonary artery or thoracic inferior vena cava was maintained for 2 wk while daily measurements were made of plasma renin activity, plasma aldosterone, plasma volume, hematocrit, serum sodium and potassium concentrations, sodium and water balance, body weight, and arterial, caval, and atrial pressures. The initial response to constriction was a reduction in blood pressure, a rise in plasma renin activity, plasma aldosterone, and water intake, and nearly complete sodium retention. In the days after moderate constriction plasma volume and body weight increased (with development of ascites and edema); blood pressure, sodium excretion, plasma renin activity, and plasma aldosterone returned to normal. In animals in which blood pressure was not restored, plasma renin activity and plasma aldosterone remained elevated throughout the period of constriction. Single injections of converting enzyme inhibitor reduced blood pressure when plasma renin activity was elevated. Chronic infusion of the inhibitor in dogs with thoracic inferior vena caval constriction prevented the restoration of blood pressure and suppressed the rise in plasma aldosterone; sodium retention and volume expansion were less than in control experiments. Thus the renin-angiotensin-aldosterone system plays an essential role in the maintenance of blood pressure during [...]

**Find the latest version:**

<https://jci.me/108431/pdf>



# The Renin-Angiotensin-Aldosterone System in Congestive Failure in Conscious Dogs

LEVI WATKINS, JR., JAMES A. BURTON, EDGAR HABER, JAMES R. CANT, FRANKLIN W. SMITH, and A. CLIFFORD BARGER with the technical assistance of SARA E. MCNEIL and STEPHEN M. SHERRILL

*From the Department of Physiology and Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02115*

**ABSTRACT** The role of the renin-angiotensin-aldosterone system in the development of congestive failure has been assessed in the conscious dog by use of the nonapeptide converting enzyme inhibitor. Constriction of the pulmonary artery or thoracic inferior vena cava was maintained for 2 wk while daily measurements were made of plasma renin activity, plasma aldosterone, plasma volume, hematocrit, serum sodium and potassium concentrations, sodium and water balance, body weight, and arterial, caval, and atrial pressures. The initial response to constriction was a reduction in blood pressure, a rise in plasma renin activity, plasma aldosterone, and water intake, and nearly complete sodium retention. In the days after moderate constriction plasma volume and body weight increased (with development of ascites and edema); blood pressure, sodium excretion, plasma renin activity, and plasma aldosterone returned to normal. In animals in which blood pressure was not restored, plasma renin activity and plasma aldosterone remained elevated throughout the period of constriction. Single injections of converting enzyme inhibitor reduced blood pressure when plasma renin activity was elevated. Chronic infusion of the inhibitor in dogs with thoracic inferior vena caval constriction prevented the restoration of blood pressure and suppressed the rise in plasma aldosterone; sodium retention and volume expansion were less than in control experiments. Thus the renin-angiotensin-aldosterone system plays an essential role in the maintenance of blood pressure during the genesis of congestive

failure. Initially, the restoration of blood pressure is dependent upon circulating angiotensin II; in the later stages, blood pressure is dependent upon the increase in plasma volume.

## INTRODUCTION

The increased renal venous renin activity first observed in patients with congestive heart failure by Merrill et al. (1) 30 years ago, suggested that renin may play a role in the maintenance of blood pressure in cardiac failure. Several years before aldosterone was identified, Deming and Luetscher (2) also reported that patients with congestive failure excreted increased amounts of a salt-retaining steroid, now known to be aldosterone. However, the plasma levels of renin and aldosterone in patients and experimental animals with congestive heart failure have subsequently been reported as high, low, or normal (3-9). As a result of this variability, the role of the renin-angiotensin-aldosterone (RAA)<sup>1</sup> system in the syndrome has not been clearly defined.

The recent availability of inhibitors of the renin-angiotensin system, as well as an appropriate animal model, has provided means for a more precise analysis of the problem. Congestive failure has been induced in the conscious trained dog by inflation of a previously implanted constricting cuff around the thoracic inferior vena cava (TIVC) or the pulmonary artery. The response of the RAA system was monitored from the time of constriction to the development of frank congestive failure, thus enabling us to study the sequence of events

This work was presented in preliminary form at the 59th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., 1975.

Received for publication 20 November 1975 and in revised form 6 February 1976.

<sup>1</sup>Abbreviations used in this paper: BP, blood pressure; CEI, converting enzyme inhibitor; PA, plasma aldosterone; PRA, plasma renin activity; PV, plasma volume; RAA, renin-angiotensin-aldosterone; TIVC, thoracic inferior vena cava.

in the progressive course of the disease uncomplicated by anesthesia and surgery. Pulmonary artery stenosis in the dog produces a right-sided congestive heart failure similar to that seen in man, with cardiac enlargement, elevated right ventricular end diastolic pressure, low cardiac output, inability to exercise, and retention of sodium and water with resultant ascites and edema. TIVC constriction, although not a model of cardiac failure, simulates the hemodynamic consequences of impaired cardiac performance (10, 11).

By the use of the nonapeptide converting enzyme inhibitor (CEI) (12–14) which blocks the conversion of angiotensin I to the physiologically active pressor agent angiotensin II, we have been able to assess quantitatively the role of the RAA system in the regulation of systemic pressure during the development of congestive failure. We have also examined the role of the renin-angiotensin system in the regulation of plasma aldosterone levels, sodium excretion, and water balance. Finally, the nonapeptide has enabled us to show that the ability of the animals to compensate for the circulatory impairment imposed by constriction is markedly impaired if the generation of angiotensin II and the rise in plasma aldosterone is suppressed.

## METHODS

Mongrel dogs (20–30 kg) were kept in metabolic cages in a room of constant temperature and fed a fixed diet containing approximately 75 meq of sodium and of potassium. Free access to water was allowed and 24-h intake and urinary output measured. The animals were trained to lie quietly on a padded table in an air-conditioned room. After training the animals were prepared surgically for the experiments.

### Surgical procedures

For surgical procedures the animals were anesthetized with intravenous pentobarbital sodium (30 mg/kg). Under sterile conditions, with the animal on artificial ventilation, thoracotomy was performed through the left 4th intercostal space. The pericardial sac was opened widely exposing the pulmonary artery and the TIVC. A 2.0 × 1.5-cm inflatable silastic rubber cuff was placed around the main pulmonary artery and a 2 × 2-cm cuff was placed around the TIVC. A polyvinyl chloride catheter (0.04 inch ID, 0.07 inch OD) was placed in the right atrium through the atrial appendage; in several dogs a catheter was also implanted in the left atrium. The abdominal aorta and vena cava were approached retroperitoneally through a left flank incision and were catheterized with polyvinyl chloride catheters (0.025 inch ID, 0.056 inch OD) using the techniques of Herd and Barger (15). The intravascular catheters and the catheters attached to the inflatable cuffs were exteriorized through subcutaneous tunnels over the left flank and protected by a cotton jacket. The animals were allowed to recover for 2 wk before experiments were started.

### Experimental protocol

*Thoracic inferior vena caval constriction.* For 3 days before caval constriction the following control measure-

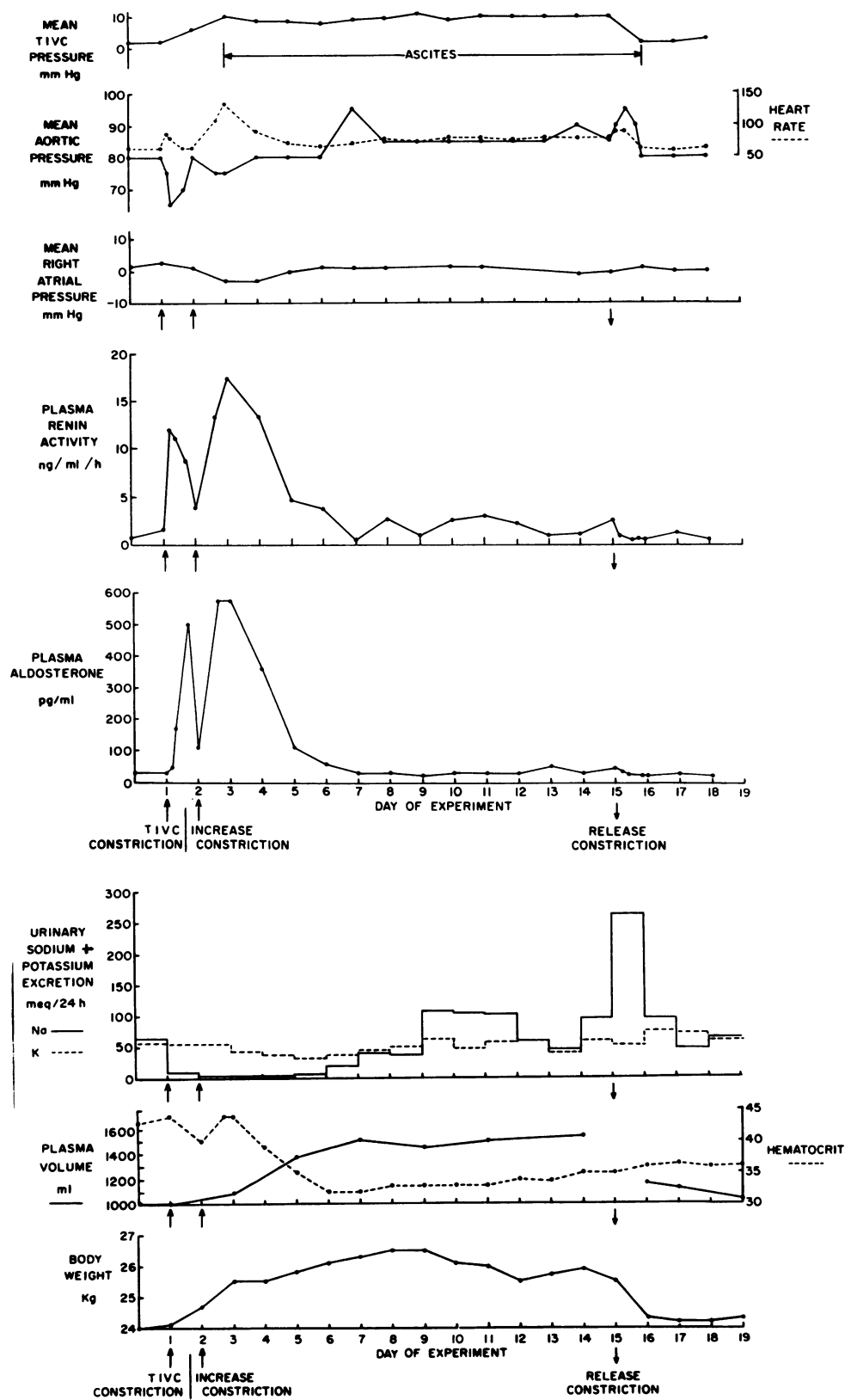
ments were made between 8 and 10 a.m.: inferior vena caval, atrial, and aortic pressures; plasma renin activity (PRA) (16) and plasma aldosterone (PA) (17); plasma volume (PV); and hematocrit; 24-h urinary sodium and potassium excretion, serum sodium and potassium concentrations, water balance (intake minus urinary output), and body weight were also measured. The caval constriction was done in two stages on the first 2 days to produce a 10-mm Hg rise in abdominal caval pressure. Preliminary experiments indicated that some animals did not tolerate a more rapid or more severe constriction. The initial constriction, raising the inferior vena caval pressure by 5 mm Hg, was done over a period of 5–10 min by inflating the caval cuff with saline; after the desired constriction was obtained the catheter was clamped. Blood samples for PRA and PA were taken at 15 min before and at 15, 30, and 45 min after constriction while pressures were continuously recorded. The animal was then returned to the cage. 5 h later all pressures were again measured and blood samples taken for PRA and PA. If elevated caval pressure had not been maintained, minor adjustments in the inflation of the cuff were made.

24 h later the cuff was further inflated to raise the caval pressure an additional 5 mm Hg. Samples for PRA and PA were taken before and 15 min after inflation. 5 h later the measurements were repeated. After 48 h, all measurements listed previously were done daily. At the end of 2 wk, or after a steady state of salt balance had been achieved, the constriction was released. The measurements were continued for 3 days after release.

*Acute converting enzyme blockade during thoracic inferior vena caval constriction.* Thoracic caval constriction was performed in two stages as described above. On days 3, 5, 7, and 10, while pressures were measured in two dogs, 5 mg of the CEI (< Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) was given intravenously as a single bolus. In previous experiments in this laboratory, no rise in plasma bradykinin was detected with such a dose, or during constant infusion as below (18). Blood samples for PRA were taken before and 15 min after injections.

*Chronic converting enzyme blockade during thoracic inferior vena caval constriction.* Before inflation of the caval cuff, a 5–10-mg bolus of the inhibitor was given intravenously followed by a constant infusion for 3 days. The infusion pump, secured to the shoulder harness of the dog, infused the inhibitor through the caval catheter. The completeness of blockade was tested 2–4 times daily by the injection of 1 µg of angiotensin I. In the normal animal this injection caused a 30–35-mm Hg rise in arterial pressure; during adequate blockade no change in pressure was produced. In the first experiment with chronic infusion of the CEI, 5 mg/h were administered. On the 2nd day, a small rise in arterial pressure was observed after the injection of 1 µg of angiotensin I. Therefore, the dose of the inhibitor was raised to 10 mg/h for the remainder of the infusion. In the following two experiments with chronic converting enzyme blockade, a dose of 10 mg/h was used throughout the 3-day infusion period. This dose produced effective blockade in experiments 1 and 2 as evidenced by the lack of a pressor response to injected angiotensin I. In the 3rd experiment, 10 mg/h did not completely block the pressor response to angiotensin I in the latter part of the experiment; on several occasions a 5–10-mm Hg rise in blood pressure (BP) was produced. However, because of the limited supply of the CEI the dose was not raised.

*Pulmonary artery constriction.* The experimental protocol for the pulmonary artery constriction was similar to



**TABLE I**  
*Summary of Data from Experiments on Congestive Failure Induced by Thoracic Inferior Vena Caval Constriction*

|                                     | Aortic pressure         | TIVC pressure                | Right atrial pressure        | PRA                         | PA                        | PV                           | Sodium excretion            | Water intake                 | Weight                      |
|-------------------------------------|-------------------------|------------------------------|------------------------------|-----------------------------|---------------------------|------------------------------|-----------------------------|------------------------------|-----------------------------|
|                                     | mm Hg                   | mm Hg                        | mm Hg                        | ng/ml/h                     | pg/ml                     | ml                           | meq/24 h                    | ml/24 h                      | kg                          |
| Average control value (3 days)      | 84±3                    | 3.3±1.2                      | 2.9±0.9                      | 0.5±0.1                     | 21±4                      | 1,551±180                    | 71±5                        | 715±102                      | 26±1.2                      |
| Max or min value after constriction | 75±3<br><i>P</i> < 0.01 | 13.6±1.7<br><i>P</i> < 0.001 | -3.4±0.8<br><i>P</i> < 0.001 | 14.5±2.2<br><i>P</i> < 0.01 | 389±68<br><i>P</i> < 0.01 | 2,131±245<br><i>P</i> < 0.02 | 1.0±0.1<br><i>P</i> < 0.001 | 1,854±254<br><i>P</i> < 0.01 | 29.5±0.9<br><i>P</i> < 0.01 |
| Value before release                | 85±3<br><i>P</i> > 0.1  | 11.8±0.8<br><i>P</i> < 0.001 | 2.1±0.9<br><i>P</i> > 0.1    | 1.4±0.4<br><i>P</i> > 0.1   | 23±5<br><i>P</i> > 0.1    | 1,889±180<br><i>P</i> < 0.01 | 70±14<br><i>P</i> > 0.1     | 802±79<br><i>P</i> < 0.02    | 29.0±0.9<br><i>P</i> < 0.02 |

*n* = 5.

Figures are mean±SEM. *P* = B vs. A; C vs. A. Maximum value refers to peak level of TIVC pressure, PRA, PA, PV, water intake, and weight. Minimum value refers to nadir of aortic pressure, right atrial pressure, and sodium excretion.

that for TIVC except that the end point pressures were different. The pulmonary artery cuff was inflated in one or two stages to induce either an immediate 5-mm Hg drop in systemic arterial pressure, a 1–2-mm Hg rise in right atrial pressure, or a 3–4-mm Hg drop in left atrial pressure. The systolic murmur of pulmonic stenosis was clearly audible under these conditions.

### Hemodynamic measurements

The aortic, inferior, vena caval, and atrial pressures were monitored through the corresponding catheters connected to pressure transducers. The outputs were recorded simultaneously on a polygraph with the instantaneous aortic and mean pressures recorded on separate channels. Only mean pressures were recorded from the inferior vena cava and atria. The spinous processes of the thoracic vertebrae were used as a zero reference for all pressures. Heart rate was determined from the BP record, and did not vary more than ±5 beats/min during a single control experimental session; BP varied ±3 mm Hg. Control BP in the dogs in this series was 84±3 mm Hg.

### Laboratory measurements

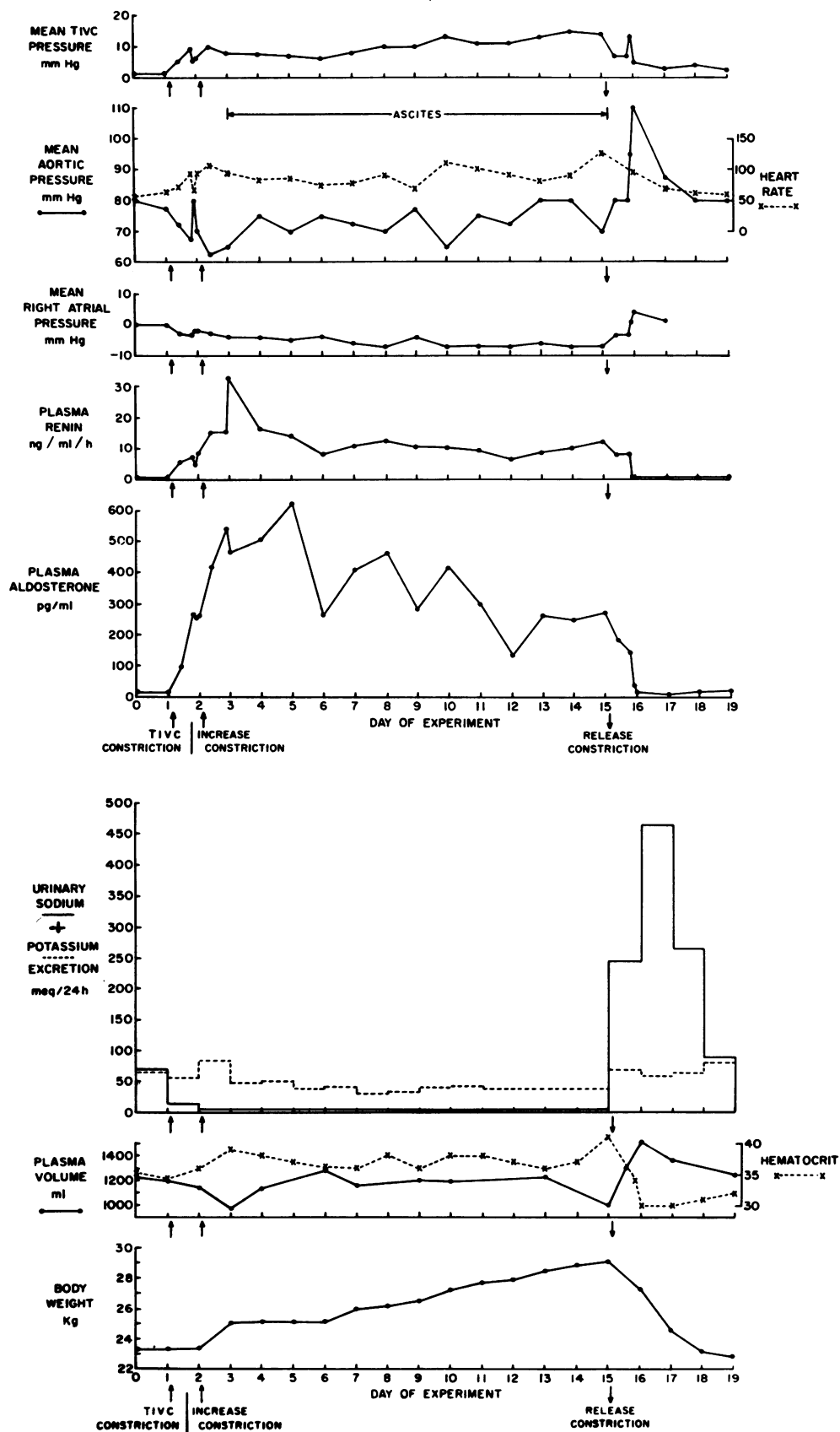
PRA was determined in duplicate by the radioimmunoassay of Haber et al. (16) and expressed as ng/ml per h of angiotensin I generated at pH 7.4. PA was determined in duplicate by the radioimmunoassay of Poulsen et al. (17) and expressed as picograms per milliliter. For both determinations a 3-ml sample of aortic blood was collected in an iced tube containing Na<sub>2</sub>EDTA and immediately centrifuged in the cold; the plasma was separated and frozen within 15 min of drawing the blood. PV was determined by dye dilution technique using Evans blue dye with blood samples drawn at 10, 15, and occasionally 30 min after injection. Serum and urinary sodium and potassium were determined with a flame photometer.

## RESULTS

*Thoracic inferior vena caval constriction.* The data for five experiments in four dogs are summarized in Table I; the *P* values after constriction indicate that all

the changes were statistically significant. To demonstrate the sequence of events that occur in the genesis of congestive failure induced by caval constriction, a representative example (Fig. 1) will be described in detail. With cuff inflation (indicated by the upright arrows), abdominal caval pressure rose to 6 mm Hg, and then to 10 mm Hg with the second constriction; it remained at this level until release. Arterial pressure fell from 80 to 65 mm Hg but was restored at 24 h; right atrial pressure also fell. Within 15 min of the first constriction, PRA rose from 1.5 to 12.8 ng/ml per h and PA rose more slowly, going from a control value of 33 to a peak level of 500 pg/ml in the first 24 h. With the second constriction, arterial pressure again fell and right atrial pressure was reduced to -3 mm Hg. Arterial pressure was restored at day 4, and thereafter remained at or slightly above control level. Restoration of right atrial pressure was slower than that of arterial pressure and was not back to control until day 6. PRA reached a peak value of 17.6 ng/ml per h on the 3rd day and gradually fell to normal thereafter. PA reached a peak of 530 pg/ml on the 3rd day and then also fell to control levels. Urinary sodium excretion was reduced from 65 to < 5 meq/day for 5 days; urinary potassium excretion remained relatively constant. Serum sodium and potassium concentrations (not shown in figure) did not change significantly. Water intake (not shown in figure) more than doubled in the first 24 h after constriction, while urine volume was relatively unchanged. PV rose from 1,000 to 1,550 ml by day 7, while body weight increased from 24 to 26.5 kg; the increase in PV was 20% of the fluid gain. Hematocrit fell from 48 to 32%. Heart rate rose from a control value of 55 to a peak level of 135 by day 3 and then fell to control levels. Ascites, determined by paracentesis, was noted on day

FIGURE 1 A representative example of the response of the conscious dog to thoracic inferior vena caval constriction and the development of moderate congestive failure.



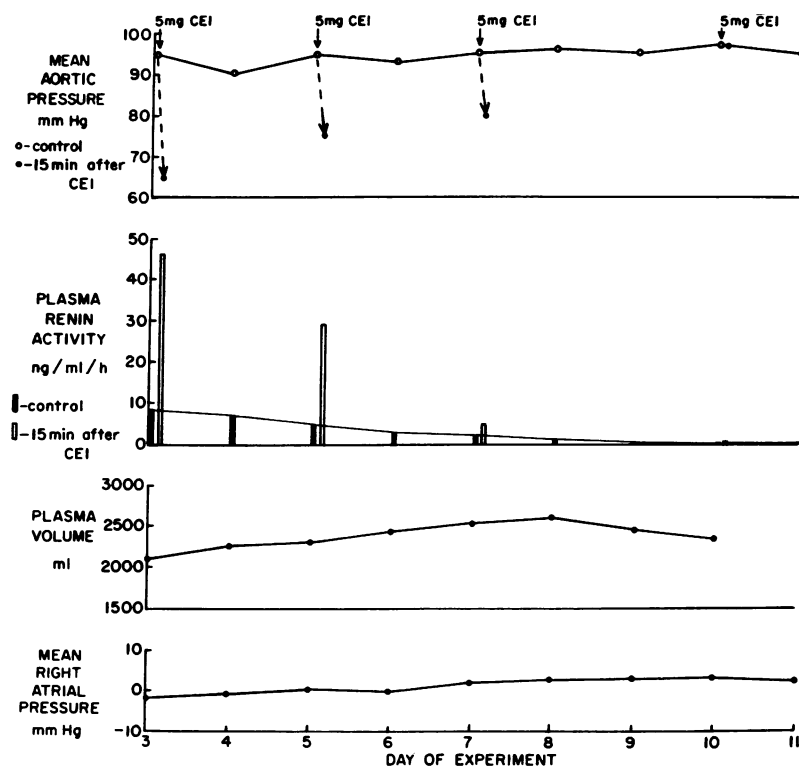


FIGURE 3 Hypotension (broken line and arrow) induced in a dog with congestive failure (TIVC constriction) by injection of 5 mg of the nonapeptide CEI on days 3, 5, and 7. The rise in PRA observed after the injection of the inhibitor is indicated by the open bars. On the 10th day, when PRA was normal, the CEI did not lower BP.

3. With the expansion of the PV, arterial and right atrial pressure returned to control value and sodium excretion gradually rose. At the same time, PRA and PA decreased to normal. Release of the constriction (inverted arrow) was accompanied by a fall in caval pressure and an initial rise in arterial and right atrial pressures during reabsorption of ascites and edema. The animal excreted 265 meq of sodium in the first 24 h after release and PV fell by 400 ml, while body weight decreased approximately 1.5 kg.

In one animal (Fig. 2) the circulatory impairment was so severe that arterial and right atrial pressures were not restored. This enabled us to examine the response of the RAA system in an animal unable to compensate adequately. In contrast to the response of the dog illustrated in Fig. 1, PRA and PA remained elevated throughout the 2-wk period of caval constriction; PRA and PA were 12 and 14 times control value, respectively, when the constriction was released. Although sodium retention was nearly complete during the entire experiment, and body weight increased 6 kg, PV did not in-

crease. However, after deflation of the cuff, PV rose 50% during the reabsorption of the ascites and edema, and BP increased from 80 to 110 mm Hg.

*Acute converting enzyme blockade during thoracic inferior vena caval constriction.* The previous data indicate that PRA (and by inference angiotensin II) is elevated early in the development of congestive failure. To determine the physiologic significance of the elevated angiotensin II in the maintenance of arterial pressure, single injections of the CEI were given on sequential days in two of the chronic dogs. In the normal animal, intravenous injection of 5 mg of the nonapeptide causes no change in arterial pressure, but blocks the usual pressor response to angiotensin I; the response to angiotensin II is unaltered. Fig. 3 illustrates the effects of acute converting enzyme blockade during TIVC constriction. The experiment was similar to that shown in Fig. 1, except that on experimental days 3, 5, 7, and 10 a single injection of 5 mg of the nonapeptide was given intravenously. Only days 3 through 10 are graphed in the figure. On day 3, when arterial pressure was 95 mm Hg

FIGURE 2 Persistent elevation of PRA and PA in severe congestive failure induced by thoracic inferior vena caval constriction.

**TABLE II**  
*Comparison of Effects of Thoracic Inferior Vena Caval Constriction on Sodium Excretion, Water Intake, and Plasma Volume in Dogs with and without Chronic Blockade of Converting Enzyme*

| Dog           | Duration of constriction | Urinary Na excretion during TIVC constriction | Dietary Na intake during same period | Net Na retained during same period | Increase in PV |
|---------------|--------------------------|---|--------------------------------------|------------------------------------|----------------|
|               |                          | meq   | meq                                  | meq                                | ml             |
| <b>Fran</b>   |                          |   |                                      |                                    |                |
| Control TIVCC | 3 days                   | 36  | 225                                  | 189                                | 439            |
| CE blockade*  | 3 days                   | 92  | 225                                  | 133                                | 350            |
| <b>Blue</b>   |                          |   |                                      |                                    |                |
| Control TIVCC | 3 days                   | 17  | 225                                  | 208                                | 316            |
| CE blockade   | 3 days                   | 81  | 225                                  | 144                                | 166            |
| <b>Yancy</b>  |                          |   |                                      |                                    |                |
| Control TIVCC | 5 days                   | 28  | 375                                  | 347                                | 421            |
| CE blockade   | 5 days                   | 146   | 375                                  | 229                                | 100            |

\* CE, converting enzyme.

and the PRA was 9 ng/ml per h, 5 mg of the nonapeptide caused a 30-mm Hg drop in arterial pressure as indicated by the dashed line and arrow; PRA rose to 46 ng/ml per h after the injection. As PV and right atrial pressure increased in the following days, control PRA (indicated by the solid bars) fell progressively. On day 5, PRA was 4.6 and on day 7, 2.2 ng/ml per h. Since PRA was falling, the effect of the nonapeptide on arterial pressure was progressively less. Arterial pressure fell by 20 mm Hg on day 5 and by 15 mm Hg on day 7. On day 10, when PRA was again normal, arterial pressure did not fall with the injection of the inhibitor. Similar results were observed in the second animal.

*Chronic converting enzyme blockade during thoracic inferior vena caval constriction.* The previous experiments demonstrate the elevated levels of PRA and angio-

otensin II are important for the maintenance of arterial pressure during the development of congestive failure. To determine whether an animal can restore and maintain blood pressure when subjected to the circulatory impairment of caval constriction in the absence of generation of angiotensin II, a constant infusion of the CEI was given to three dogs. Fig. 4 illustrates the effect of chronic blockade by the CEI for 3 days during TIVC constriction. The animal with chronic inhibitor infusion tolerated only the initial constriction which increased inferior vena caval pressure by only 5 mm Hg. Arterial pressure fell from 80 to 65 mm Hg. Unlike the animal with an intact RAA system, arterial pressure was not restored and at day 3 arterial pressure was still 60 mm Hg. Although PRA was markedly elevated, PA did not change significantly during the first 48 h but rose

**TABLE III**  
*Summary of Data from Experiments on Congestive Heart Failure Induced by Pulmonary Artery Constriction*

|                                     | Aortic pressure         | TIVC pressure              | Right atrial pressure     | Left atrial pressure      | PRA                        | PA                       | PV                           | Sodium excretion        | Water intake                 | Weight                      |
|-------------------------------------|-------------------------|----------------------------|---------------------------|---------------------------|----------------------------|--------------------------|------------------------------|-------------------------|------------------------------|-----------------------------|
|                                     | mm Hg                   | mm Hg                      | mm Hg                     | mm Hg                     | ng/ml/h                    | pg/ml                    | ml                           | meq/24 h                | ml/24 h                      | kg                          |
| Average control value (3 days)      | 78±2                    | 0.3±0.3                    | 3±1.2                     | 3±0.5                     | 0.5±0.1                    | 14±2                     | 1,650±149                    | 63±0                    | 666±95                       | 27±0.3                      |
| Max or min value after constriction | 69±2<br><i>P</i> < 0.05 | 8.5±0.3<br><i>P</i> < 0.05 | 9±0.5<br><i>P</i> < 0.01  | -1±1<br><i>P</i> < 0.02   | 2.1±0.3<br><i>P</i> < 0.05 | 70±14<br><i>P</i> < 0.05 | 2,450±160<br><i>P</i> < 0.01 | 4±1<br><i>P</i> < 0.01  | 1,368±197<br><i>P</i> < 0.05 | 29.3±0.3<br><i>P</i> < 0.01 |
| Value before release                | 81±1<br><i>P</i> > 0.1  | 8.5±0.3<br><i>P</i> < 0.05 | 8.6±0.9<br><i>P</i> > 0.1 | 2.8±1.2<br><i>P</i> > 0.1 | 0.6±0.2<br><i>P</i> > 0.1  | 14±2<br><i>P</i> > 0.1   | 2,383±191<br><i>P</i> < 0.01 | 75±10<br><i>P</i> > 0.1 | 893±214<br><i>P</i> > 0.1    | 29±0.8<br><i>P</i> < 0.01   |

*n* = 4.

Figures are mean±SEM. *P* = B vs. A; C vs. A.



slightly near the end of the infusion when PRA exceeded 12 ng/ml per h, suggesting that blockade may have been incomplete at this time. In the normal dog after caval constriction, urinary sodium excretion fell to levels of <5 meq/day, with 90% of dietary sodium retained; in the chronically blocked animal, urinary sodium excretion was reduced to an average of 25 meq/day, with only 60% of intake retained (Table II). Consequently, despite more severe and persistent hypotension during blockade, 30% less dietary sodium was retained in the blocked animals during the CEI infusion. On the other hand, it should be noted that two-thirds of the sodium could be retained without a rise in plasma angiotensin II and aldosterone. As shown in Table II, the increase in PV was also less in the blocked animal. Urinary potassium concentrations did not change significantly. During the period of infusion (3–5 days) no ascites was detected by abdominal tap and pitting edema was not present. Thus, when the generation of angiotensin II was suppressed by the CEI, dogs with moderate TIVC constriction were unable to restore arterial pressure during the 3–5-day infusion period. During chronic infusion in control experiments, no significant changes were noted in the measured parameters.

**Pulmonary artery constriction.** Four experiments were performed in three dogs and the data are summarized in Table III. Under the conditions of these experiments, right-sided congestive heart failure developed over the course of several days, with the presence of ascites and edema. While right atrial pressure rose, left atrial and arterial pressures were significantly reduced. Although the increases in PRA and PA were much smaller than those observed with caval constriction, the changes in salt and water balance, and PV were comparable in the two series. By the 8th to 10 day after pulmonary artery constriction, aortic pressure was restored to normal, as was left atrial pressure, PRA, PA, and sodium excretion.

In one animal pulmonary stenosis severe enough to prevent restoration of BP was induced. In this experiment aortic pressure fell to 60 mm Hg and remained at least 10 mm Hg below control for the entire period of stenosis (14 days). PRA rose to a peak value of 11.1 ng/ml per h by day 4 and was still 7.6 ng/ml per h before the release of the constriction. PA rose to a peak of 290 pg/ml by day 4 and was still 50 pg/ml at the end of the constriction. After the constriction, sodium retention was virtually complete for the entire period; water intake increased with a marked increase in body weight (4.1 kg). However, PV rose only from 1,000 to 1,175 ml, an observation similar to that noted in severe caval constriction. With the release of the stenosis the large amount of ascitic fluid and edema was reabsorbed with a marked rise in PV (from 1,175 to 1,680 ml) within 48 h of release, an observation similar to

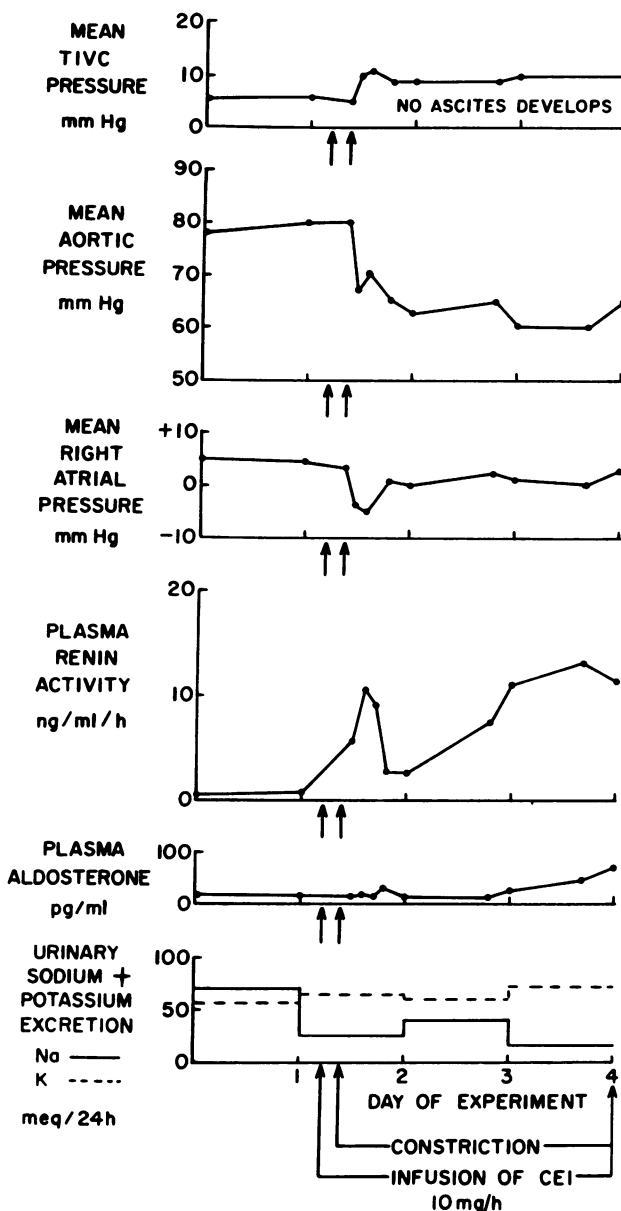


FIGURE 4 Response of the conscious dog to thoracic inferior vena caval constriction during the constant infusion of the nonapeptide CEI (10 mg/h). The animal was able to tolerate only a single constriction of the cava and was unable to restore BP to normal.

that noted in severe caval constriction. Aortic pressure rose above control while caval and right atrial pressures fell. The dog excreted 916 meq of sodium in the first 72 h while body weight decreased from 28.2 to 22.6 kg.

**Animal behavior.** During the first few days after TIVC constriction the animal became sluggish and fatigued more easily while walking, indicating some decrease in exercise tolerance. Later, with increase in PV

and restoration of BP, behavior appeared normal. After pulmonary artery constriction not only was there a decrease in exercise tolerance, but also frequent episodes of syncope during exertion; again this was not present when circulatory compensation was adequate. The animals with chronic CEI blockade during TIVC constriction functioned remarkably well despite more persistent and severe hypotension (50–60 mm Hg). No clinical evidence of cerebral, renal, or gastrointestinal damage was noted in these animals with persistent hypotension during converting enzyme blockade.

## DISCUSSION

To understand the role of the RAA system in the development of congestive failure, the natural history of the syndrome must be studied from the onset of the cardiovascular insult through the period of reflex compensation and subsequent attainment of a new steady state. In man, because of the long life history of the disease, it has not been feasible to perform such a study. As a result, the sequential changes in the RAA system in congestive heart failure in man are not well defined. Previous attempts in the experimental animal to define a role of the RAA system have often been episodic or have been complicated by anesthesia, surgery, and traumatic experimental procedures. In our laboratory, congestive heart failure was previously induced by the surgical production of tricuspid insufficiency followed by pulmonary artery stenosis. The animals were first studied several weeks later after recovery from surgery. At this time many of the important compensatory changes had already taken place. To circumvent these problems in the current study, the trained animals were chronically catheterized and inflatable cuffs were placed on the pulmonary artery and TIVC. No measurements were made until the animals were fully recovered.

The sequential data obtained in these trained conscious dogs provide evidence for an essential role in the RAA system in the genesis of congestive failure and demonstrate that chronic blockade of the system markedly impairs the ability of the animal to compensate. We have defined compensation as a restoration of arterial pressure with normal PRA and PA levels. The circulatory impairment caused by the imposition of TIVC or pulmonary artery constriction initiates a series of compensatory responses, among which is a rise in PRA and, by inference, angiotensin II. This elevation in angiotensin II is important for the restoration of arterial pressure in the early stages of failure, indicated by the marked fall in pressure when the CEI is injected during this period. When the converting enzyme is blocked chronically by a constant infusion of the inhibitor, more severe hypotension follows constriction and hypotension persists throughout the period of infu-

sion, which is further evidence for the importance of the elevation of angiotensin II. Johnson and Davis (19) have reported similar data in congestive failure using the [Sar<sup>1</sup>, Ala<sup>8</sup>] angiotensin II analog. The drop in arterial pressure, induced by two agents which block by different mechanisms, strongly suggests that it is angiotensin II which is responsible for the maintenance of arterial pressure in congestive failure when PRA is elevated.

The rise in PA after TIVC constriction was closely correlated with the elevation of PRA. Although such correlation does not prove a causal relationship, the absence of any rise in PA during chronic converting enzyme blockade and constriction strongly suggests that under these conditions angiotensin II is responsible for the increased PA levels. With the constant infusion of 10 mg/h of the CEI, PA did not rise until PRA exceeded the range of 10–12 ng/ml per h. During TIVC or pulmonary artery constriction, sodium and water retention occurred during the period of elevation of PRA and PA. With compensation PRA and PA fell, sodium excretion returned to normal, and a new steady state was reached. However, when compensation was inadequate, the PRA and PA levels remained elevated and sodium retention persisted throughout the duration of the constriction. The constant infusion of the CEI, which suppresses the generation of angiotensin II, and a rise in PA made it possible to determine the quantitative significance of the elevated PA and angiotensin II in the retention of sodium. Animals with chronic blockade during TIVC constriction retain less sodium than the unblocked animals despite a greater degree of hypotension. It is of interest that sodium retention was as complete when PRA was elevated 2–3-fold as it was when PRA was 10–20 times normal, suggesting that only small increases in PRA and PA are necessary for nearly complete sodium retention, and that the higher values for PRA are more important for the maintenance of arterial BP.

Water retention in congestive failure resulted primarily from increased water intake as urine output was not significantly altered after TIVC constriction. Increased water intake was apparent soon after constriction and before any change in sodium balance, suggesting that the renin-angiotensin system may be an important mechanism in the stimulation of thirst during the development of congestive failure. Fitzsimons (20), Fernandez (21), and Simpson and Routtenberg (22) have provided evidence that the angiotensin II may act as a dipsogenic factor important in the maintenance of body fluid balance.

As a result of the sodium and water retention, intravascular and extravascular fluid volume expand. The increased PV is apparently an essential compensa-

tory response in congestive failure. As a consequence of the increased PV, ventricular end-diastolic volume is restored or elevated, augmenting ventricular performance. When expansion of the PV was sufficient to restore arterial pressure, and PRA was again normal, injection of the CEI had no effect on arterial pressure, indicating that angiotensin II no longer played a significant role in the maintenance of arterial pressure. With further expansion of the extravascular fluid volume, the ascites and edema characteristic of right-sided congestive failure developed.

In the animals that were unable to compensate, PV was not significantly increased, although ascites and edema were more severe than in the compensated animal. Similar data were reported by Ross et al. (23) who found that dogs with severe congestive failure (tricuspid insufficiency and pulmonic stenosis) had normal or reduced total blood volume. These observations on PV in the compensated vs. the uncompensated animal may help to explain the variable results reported in patients with congestive heart failure.

Release of the TIVC constriction was invariably associated with a marked but transient rise in arterial pressure; right atrial pressure increased while inferior vena caval pressure decreased. Arterial and atrial pressures stabilized at control values within 48–72 h. The effects on PRA and PA depended upon the levels before release. If PRA and PA levels were elevated, they fell promptly upon release; if they were at control levels no significant change was noted. In the animals that were able to compensate, PV fell to control levels within 72 h; in animals with severe failure PV initially rose during the mobilization of ascites and edema and then gradually fell to control. Diuresis and natriuresis invariably followed the release of the constriction, the magnitude depending upon the total amount of sodium and water retained during the period of constriction. The same general pattern followed pulmonary artery release except that right atrial pressure fell while left atrial pressure increased with release.

The mechanisms controlling renin release in congestive failure have not been well defined. By comparing the changes in PRA after TIVC constriction with that observed after pulmonary artery constriction, some important clues have emerged, particularly as regards the relative role of high and low pressure receptors. With TIVC constriction BP fell and PRA increased markedly, suggesting the rise in PRA may be related to a fall in arterial pressure through activation of the renal baroreceptor mechanism as proposed by Witty et al. (24). When a comparable drop in arterial pressure was induced by pulmonary artery constriction the rise in PRA was much less. Since the fall in arterial pressure was similar in both models (a 10–15-mm Hg decrease) some

other stimulus in addition to renal artery hypotension may have been responsible for the greater release of renin after TIVC constriction, or for the lesser release after pulmonary artery constriction. From experiments such as that illustrated in Fig. 3, it is apparent that high levels of PRA may be sustained in the absence of arterial hypotension.

Although the role of vagal cardiac afferents in the regulation of renin secretion is not yet clear, a growing body of evidence suggests that cardiopulmonary pressures (atrial, pulmonary venous, or ventricular end-diastolic) may modify the release of renin. Reports indicate that high cardiopulmonary pressures may suppress renin release, while low pressures or blockade of vagal afferents may stimulate the release of renin (25–28). In the present experiments with TIVC constriction, right and left atrial pressures were reduced, whereas with pulmonary artery constriction right atrial pressure increased while left atrial pressure was decreased. In the TIVC experiments PRA returned to control level simultaneously with the restoration of atrial pressures; arterial pressures were usually back to normal several days earlier, probably as a result of the high levels of circulating angiotensin II. After pulmonary artery constriction, PRA returned to normal when left atrial pressure was back to control level. Thus, the high PRA noted with TIVC constriction may result from the combined effects of renal artery hypotension and reflex neurogenic stimuli resulting from decreased cardiopulmonary pressures. In contrast, with pulmonary artery constriction, the fall in the left atrial pressure may be counterbalanced by the rise in right atrial pressure. Consequently, the major stimulus to renin release with pulmonary artery constriction may be the renal artery hypotension. In support of such a view is the quantitatively similar rise in PRA observed when renal perfusion pressure was reduced to comparable levels by either pulmonary artery or renal artery constriction (29).

In summary, the compensatory responses to mild TIVC or pulmonary artery constriction may be divided into an early and late stage. In the early stage of TIVCC, arterial and atrial pressures fell; after pulmonary artery constriction, right atrial pressure rose while left atrial pressure fell. PRA, angiotensin II generation, and PA increased. The elevation of systemic angiotensin II was essential for the maintenance of arterial pressure; intrarenally, the increase in angiotensin II and in aldosterone may be important in the retention of sodium and water. As a consequence of the salt and water retention, intravascular and extravascular fluid volumes expanded. In the later stage, if compensation was adequate, arterial and atrial pressures returned toward normal and PRA and PA fell to control levels. Sodium excretion was restored and the animal with venous congestion, ascites,

and edema reached a new steady state. As a result of the expanded PV, ventricular performance was restored or augmented and the elevated levels of angiotensin II were no longer essential for the maintenance of arterial pressure. When circulatory impairment was severe or compensation was inadequate, PRA and PA remained elevated during the entire period of constriction. A single injection of the CEI caused a fall in arterial pressure when PRA was elevated, but produced no change when PRA was normal. Chronic infusion of the CEI, which suppressed the generation of angiotensin II and in turn a rise in aldosterone, markedly impaired the ability of the animal to compensate. After constriction with CEI, severe hypotension persisted and the ability to conserve sodium and water was diminished. By examination of the sequential changes in the RAA system during the initiation and maintenance of congestive failure, the variability in PRA and PA levels reported in the literature may now be understood. Early in the development of the failure they may be elevated; during the later or compensated stage they may be normal. The particular PRA or PA level observed in congestive failure depends, then, upon the state of compensation.

We conclude that the RAA system plays an essential homeostatic role in the development of congestive failure and is important for both restoration of arterial pressure and the expansion of intravascular volume. Secondly, changes in cardiopulmonary pressures may be important in the regulation of renin secretion. Finally, the nonapeptide converting enzyme inhibitor has proven to be a useful pharmacologic tool to define the role of the RAA system in the development of congestive failure.

#### ACKNOWLEDGMENTS

We thank Dr. J. S. Parsons of the National Institute for Medical Research, London, for the use of the Mill-Hill miniature infusion pump. We also thank Merck Sharp & Dohme for the thrombolytin and Mrs. Birthe Creutz for secretarial assistance.

This research was supported by U. S. Public Health Service grants 5 RO1 HL02493 and 5 TO1 GM01541.

#### REFERENCES

1. Merrill, A. J., J. L. Morrison, and E. S. Brannon. 1946. Concentration of renin in renal venous blood in patients with chronic heart failure. *Am. J. Med.* 1: 468-472.
2. Deming, Q. B., and J. A. Luetscher, Jr. 1950. Bioassay of desoxycorticosterone-like material in urine. *Proc. Soc. Exp. Biol. Med.* 73: 171-175.
3. Brown, J. J., D. L. Davies, V. W. Johnson, A. F. Lever, and J. I. S. Robertson. 1970. Renin relationships in congestive cardiac failure, treated and untreated. *Am. Heart J.* 80: 329-342.
4. Ayers, C. R., R. E. Bowden, and J. P. Schrank. 1972. Mechanisms of sodium retention in congestive heart failure. *Adv. Exp. Med. Biol.* 17: 227-243.
5. Belleau, L., H. Mion, S. Simard, P. Granger, E. Bertranou, W. Nowaczynski, R. Boucher, and J. Genest. 1970. Studies on the mechanism of experimental con-

- gestive heart failure in dogs. *Can. J. Physiol. Pharmacol.* 48: 450-456.
6. Sanders, L. L., and J. C. Melby. 1964. Aldosterone and the edema of congestive heart failure. *Arch. Intern. Med.* 113: 331-341.
7. Davis, J. O., M. M. Pechet, W. C. Ball, Jr., and M. J. Goodkind. 1957. Increased aldosterone secretion in dogs with right-sided congestive heart failure and in dogs with thoracic inferior vena cava constriction. *J. Clin. Invest.* 36: 689-694.
8. Genest, J., P. Granger, J. de Champlain, and R. Boucher. 1968. Endocrine factors in congestive heart failure. *Am. J. Cardiol.* 22: 35-42.
9. Davis, J. O. 1962. Adrenocortical and renal hormonal function in experimental cardiac failure. *Circulation.* 25: 1002-1014.
10. Davis, J. O. 1965. The physiology of congestive heart failure. *Handb. Physiol.* Section 2. Circulation. 3: 2071-2122.
11. Barger, A. C. 1956. The pathogenesis of sodium retention in congestive heart failure. *Metab. Clin. Exp.* 5: 480-489.
12. Ferreira, S. H. 1965. A bradykinin-potentiating factor (BPF) present in the venom of *Bothrops jararaca*. *Brit. J. Pharmacol. Chemother.* 24: 163-169.
13. Bakhle, Y. S. 1971. Inhibition of angiotensin I converting enzyme by venom peptides. *Brit. J. Pharmacol.* 43: 252-254.
14. Ondetti, M. A., N. J. Williams, E. F. Sabo, J. Plušec, E. R. Weaver, and O. Kocy. 1971. Angiotensin-converting enzyme inhibitors from the venom of *Bothrops jararaca*. Isolation, elucidation of structure, and synthesis. *Biochemistry.* 10: 4033-4039.
15. Herd, J. A., and A. C. Barger. 1964. Simplified technique for chronic catheterization of blood vessels. *J. Appl. Physiol.* 19: 791-792.
16. Haber, E., T. Koerner, L. B. Page, B. Kliman, and A. Purnode. 1969. Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J. Clin. Endocrinol. Metab.* 29: 1349-1355.
17. Poulsen, K., J. Sancho, and E. Haber. 1974. A simplified radioimmunoassay for plasma aldosterone employing an antibody of unique specification. *Clin. Immunol. Immunopathol.* 2: 373-380.
18. Miller, E. D., Jr., A. I. Samuels, E. Haber, and A. C. Barger. 1975. Inhibition of angiotensin conversion and prevention of renal hypertension. *Am. J. Physiol.* 228: 448-453.
19. Johnson, J. A., and J. O. Davis. 1973. Angiotensin II: Important role in the maintenance of arterial blood pressure. *Science (Wash. D. C.).* 179: 906-907.
20. Fitzsimons, J. T. 1969. The role of a renal thirst factor in congestive heart failure in dogs. *Trans. Assoc. Am. Physiol. (Lond.).* 201: 349-368.
21. Fernandez, L. A. 1972. Effects of renin and angiotensin on water intake. *Medicine (B. Aires).* 32: (Suppl. I) 63-67.
22. Simpson, J. B., and A. Routtenberg. 1973. Subfornical organ: site of drinking elicitation by angiotensin II. *Science (Wash. D. C.).* 181: 1172-1175.
23. Ross, J. F., A. C. Barger, S. C. Finch, R. S. Ross, H. L. Price, and E. J. Freireich. 1953. The blood volume before and following experimentally produced congestive heart failure in dogs. *Trans. Assoc. Am. Physicians Phila.* 66: 278-293.

24. Witty, R. T., J. O. Davis, R. E. Shade, J. A. Johnson and R. L. Prewitt. 1972. Mechanisms regulating renin release in dogs with thoracic caval constriction. *Circ. Res.* **31**: 339-347.
25. Brennan, L. A., Jr., R. L. Malvin, K. E. Jochim, and D. E. Roberts. 1971. Influence of right and left atrial receptors on plasma concentrations of ADH and renin. *Am. J. Physiol.* **221**: 273-278.
26. Mancia, G., J. C. Romero, and J. T. Shepherd. 1975. Continuous inhibition of renin release in dogs by vagally innervated receptors in the cardiopulmonary region. *Circ. Res.* **36**: 529-535.
27. Kurz, K. D., J. A. Hasbargen, and J. E. Lehr. 1975. Effects of increased left atrial transmural pressure on renin secretion. *Fed. Proc.* **34**: 367. (Abstr.)
28. Epstein, M., D. S. Pins, J. Sancho, and E. Haber. 1975. Suppression of plasma renin and plasma aldosterone during water immersion in normal man. *J. Clin. Endocrinol. Metab.* **41**: 618-625.
29. Tagawa, H., F. D. Gutmann, E. Haber, E. D. Miller, Jr., A. I. Samuels, and A. C. Barger. 1974. Reversible renovascular hypertension and renal arterial pressure. *Proc. Soc. Exp. Biol. Med.* **146**: 975-982.