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EXTRACTION AND BIOASSAY OF RENIN FROM KIDNEYS OF  
SODIUM-DEPLETED AND SODIUM-LOADED RATS

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The major stimulus for renin release in renal ischemic hypertension is well known. The juxtaglomerular granules release the enzyme renin which interacts with the plasma alpha - 2 globulin (renin substrate) resulting in the decapeptide Angiotensin I. A plasma converting enzyme which is halide dependent then completes the reaction yielding the octapeptide Angiotensin II. The latter is the potent vasopressor agent.

Our experiments in rats were designed to determine concentrations of renin within the kidney tissue after increasing levels of sodium chloride were added to a diet deficient only for sodium chloride. A second objective in the three groups of rats on the variable sodium diet was to compare renal tissue levels of renin after Angiotensin II administration for 14 or 21 days.

METHOD AND MATERIALS

Forty-eight Sprague-Dawley rats (250 gm males) were divided into two major divisions; one-half of each division (12 animals) received Angiotensin II subcutaneously for 14 days. The other half (12 animals) of each division served as experimental controls (no Angiotensin II) for the 14 days. In the second division another group of 12 rats received Angiotensin II for 21 days while the remaining 12 served as experimental controls.

Each division of 24 animals was further subdivided into three groups based on the amount of sodium administered by dietary means. Group I rats (low sodium) received a diet of 0.5 to 0.7 mEq Na<sup>+</sup> per 24 hours. Dietary consumption was approximately 25 grams of food per day for these 16 rats. Group II rats (normal sodium intake) received 1.0 to 1.2 mEq Na<sup>+</sup> per 24

hours. In Group III each of 16 rats was fed a diet containing 1.8 to 2.0 mEq of Na<sup>+</sup> per 24 hours (highsodium).

Injected rats sacrificed at 14 days were given 0.1 mg of angiotensin per kilogram daily. Angiotensin injected rats sacrificed at 21 days were given 0.1 mg/kg for the first 14 days and 0.4 mg/kg for the last week.

Appropriate numbers of rats were sacrificed from each group making up both subdivisions 14 and 21 days after the start. Kidneys from each animal were pooled within the same sub group for renin extraction and bioassay.

The method employed for renin extraction by our group has been described previously by our group<sup>1</sup>. After sufficient samples of renin were obtained, a rat bioassay technique was employed. Sprague-Dawley rats weighing 350 grams were used. The femoral artery was cannulated and mean arterial pressure was monitored on a standard pressure transducer-amplifier recorder system. Renin extracts in solution (0.2 ml) was injected into the femoral vein for assay. Angiotensin II\* (2 to 20 nanograms) were injected between assay procedures for standardization.

## RESULTS

After 14 days renin tissue concentration was highest in Group I - low sodium rats - 52 renin units (Fig. 1). Rats on normal sodium and high sodium diets had 35 and 33 renin units respectively.

Figure 2 is a bargraph depicting results after 21 days in three groups of rats. Again three levels of sodium intake were employed. An inverse dose response relationship of dietary sodium to renin concentration is seen.

In Figure 3 Angiotensin II was injected subcutaneously into four rats

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\* Angiotensin II was generously supplied by CIBA, Summit, New Jersey.

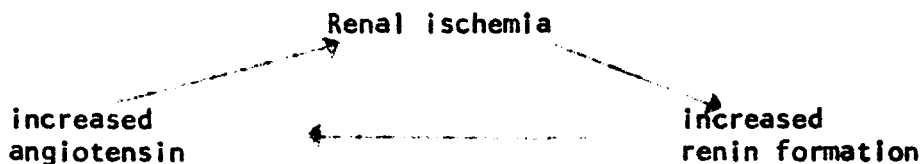
in each group for 21 days. The angiotensin injected rats had levels of tissue renin which exceeded the corresponding experimental controls in all three instances.

In Figure 4 angiotensin was injected into 12 rats for 14 days. Four rats from the angiotensin injected group were placed into each of the groups on the variable sodium diet. Tissue renin concentrations were variable in the angiotensin injected rats. Highest renin concentrations were in the kidneys of the low sodium rats; they included both control and angiotensin injected animals. Animals on high sodium and injected with angiotensin also had increased concentrations of renin.

#### DISCUSSION

Angiotensin, considered to be a product of renal ischemia, appears to promote additional renal ischemia. It has been shown by investigators to cause a decrease in renal blood flow<sup>2,3,4</sup>. McQueen and his co-workers<sup>5</sup> found that angiotensin caused a greater decrease in renal plasma flow than epinephrine.

Katz and his co-workers have suggested that angiotensin might cause a change in renal circulation similar to that of renal artery constriction and might be involved in a feed back mechanism as follows:



Tobian et al.<sup>7,8</sup> have shown that the JG (juxtaglomerular index) in the rat varies inversely with kidney perfusion pressure. If angiotensin causes

sufficient constriction in vessels proximal to the afferent arterioles, it might lower the pressure in these vessels. This preferential lowering in the renal arterial arcade might occur even while raising the pressure in the systemic circulation. Thus the increase in JG index and renin concentration in the kidney may be explained by this means. Our data in Figure 3 supports the above concept since renin was found in higher concentrations in the three groups of rats injected with angiotensin for 21 days.

Hartroft and Hartroft<sup>9</sup> showed that a high sodium diet caused a decrease in the JG index. This decrease accounts for the lower renin levels found in both of our rat groups receiving a high sodium diet for 14 and 21 days respectively.

Katz and co-workers<sup>10</sup> found that giving aldosterone together with angiotensin did not result in the increased JG index found with angiotensin administration alone. Our data does not support this concept. The low sodium, angiotensin injected rats did not have a lowered concentration of renin. It would appear that this new concept needs further study.

#### SUMMARY

1. Rats placed on a low sodium diet for 14 or 21 days had higher concentrations of renin than the controls. A third group - high sodium animals had even lower tissue renin levels.
2. The addition of angiotensin II for 21 days to the three groups of rats on a variable sodium intake resulted in higher concentrations of tissue renin.
3. Angiotensin probably has a preferential effect in the kidney producing further renal ischemia.

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## LEGENDS

- Figure 1. Increased renin concentration in the kidneys of rats on variable salt intake for 14 days.
- Figure 2. Illustrates the inverse relationship between renin concentration in kidney tissue and amount of salt in the diet after 21 days.
- Figure 3. Reveals increased concentration of renin in rats administered angiotensin for 21 days as compared with their controls in each diet group.
- Figure 4. Shows some variability in the renin concentration in rats administered angiotensin for 14 days compared to their controls.

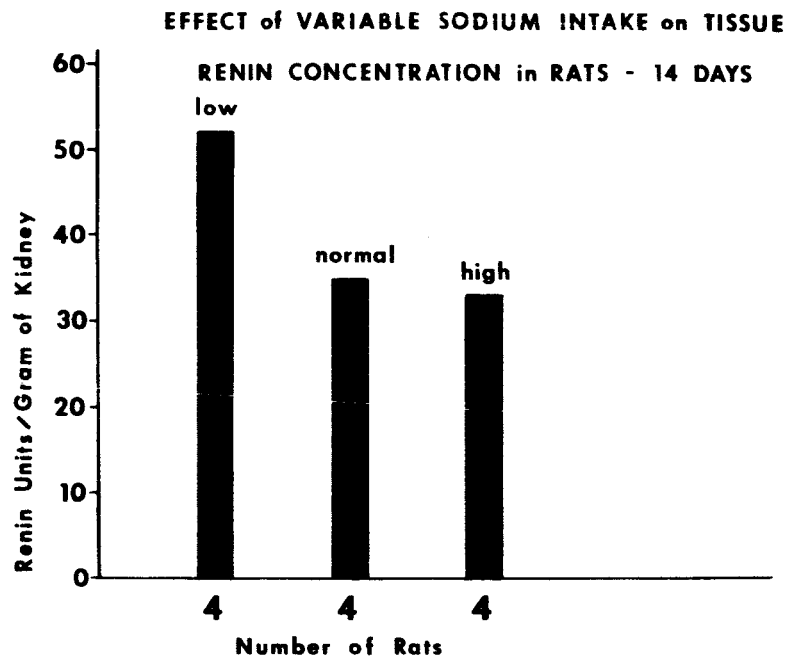


Figure 1

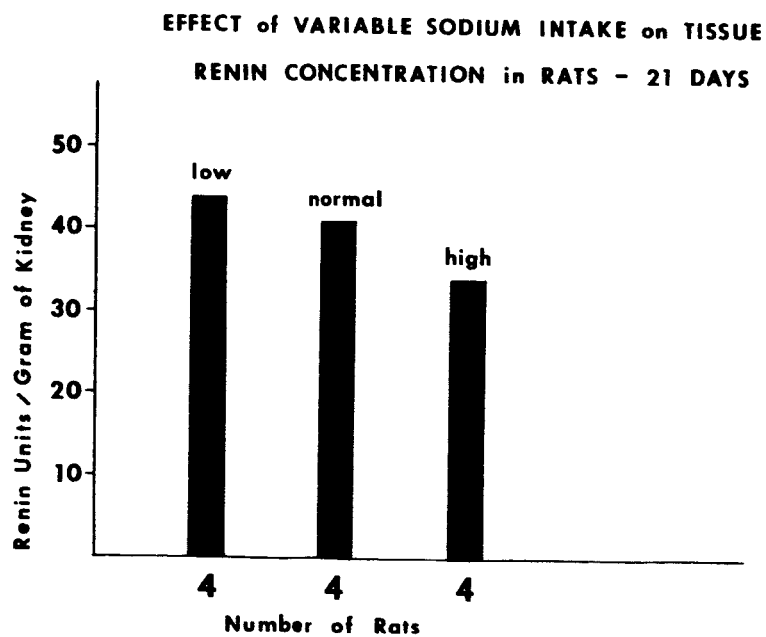


Figure 2



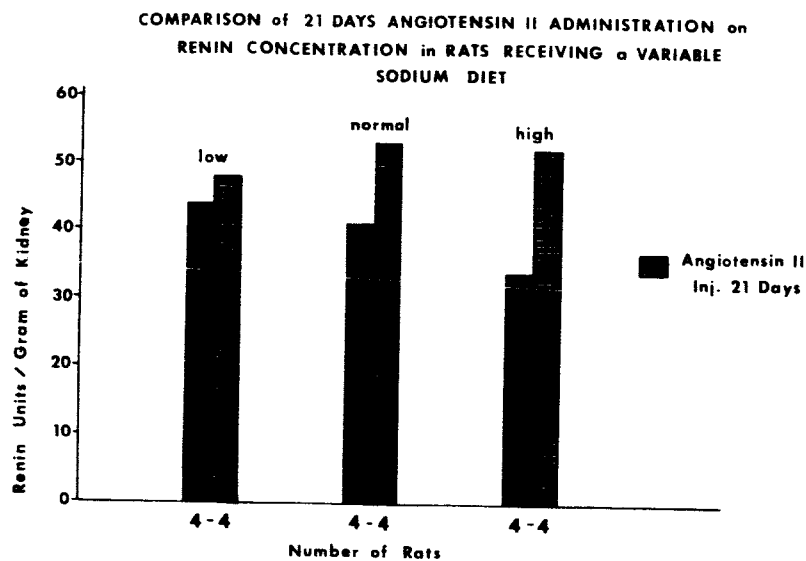


Figure 3

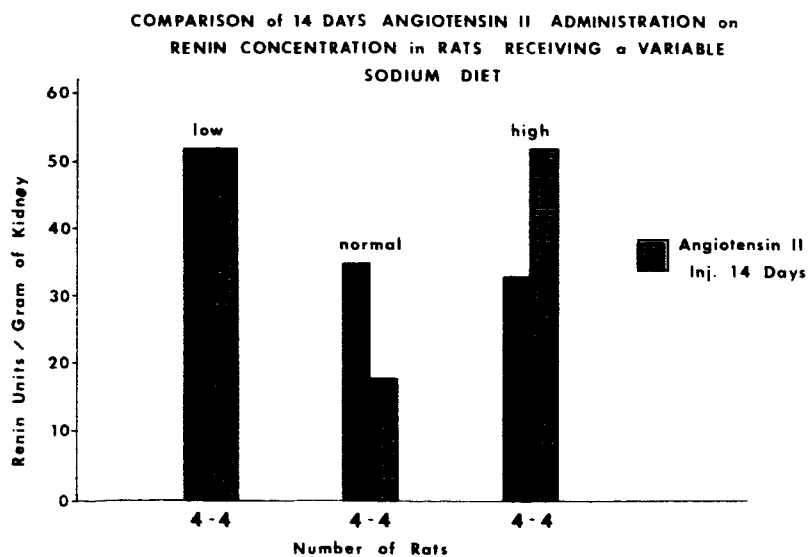


Figure 4