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From these various data it can be seen that changes in plasma renin seem closely connected with changes in sodium balance and aldosterone secretion. If the levels of plasma renin are, in fact, paralleled by changes in plasma angiotensin, regarded as the final stimulus, then the renin-angiotensin system must be seriously considered as a controller of aldosterone secretion. The relation of these findings to the mechanism of hypertension still, however, requires further study.

We are grateful to Prof. W. S. Peart for encouragement and help.

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The Renin Content of the Blood of Humans and Dogs Under Several Conditions

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SKEGGS, Kahn and Shumway,[1] Scornik and Paladini,[2] and Boucher, Biron and Genest[3] have developed methods for the estimation of angiotensin in blood and found minute amounts of this peptide in the blood of normal human beings and dogs.

The angiotensin contained in the circulating blood could be secreted as such by the kidney and formed by the action of renin upon angiotensinogen, within the cells of the kidney that contain the renin. The other possibility is that kidneys secrete renin into the circulating blood, where angiotensin is formed.

In a previous investigation[4] it was shown that the angiotensin content of the renal vein of normal unanesthetized dogs is similar to that of the arterial blood. Calculations showed that, if angiotensin was secreted by the kidney, its concentration in the renal vein should be two to three times that of the arterial blood.

Consequently, the conclusion was reached that the kidneys normally secrete renin, thus deserving the name of renal hormone. As a result, renin should be a normal component of the blood, and the physiological actions of the renin-angiotensin system should be mediated through changes in the renin output by the kidney.

To investigate this matter it is necessary to devise a specific method for renin, sensitive enough to detect renin in normal blood. In the past, several methods have been described for the estimation of the renin content of the blood. Most of them were not sensitive enough to detect renin in normal blood; others were not specific, so that the results obtained were open to dispute.

METHOD

The method developed by us combines that of Leloir *et al.*[5] for the estimation of renin with that of Scornik and Paladini[2] for the estimation of angiotensin. In short, it is as follows: Blood is collected in a cooled centrifuge tube with heparin as an anticoagulant. After centrifuging in the cold, 10 ml. of plasma* is acidified to pH 3.8, kept at 25° C. during 30 min. and then brought to pH 5.1. By means of this treatment the activity of the angiotensinase is kept low enough so that no destruction of the angiotensin added to the acidified plasma is observed. The renin and the angiotensinogen are not affected.

The original sample is divided into two parts. Half of the volume is immediately precipitated with four volumes of alcohol. This is the blank for the angiotensin content. The other half is incubated for two hours at 37° C. and then precipitated in the same way as the first one.

The angiotensin contained in the supernatant is purified and concentrated according to the method of Scornik and Paladini.[2] This includes ether extraction, n-butanol extraction of the watery phase saturated with sodium chloride, extraction of butanol by 0.01 hydrochloric acid, and adsorption with an Aminex column 50 W X 2 equilibrated to pH 7.5. The eluates are assayed on the blood pressure of the anesthetized rat.

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*Since the renin content of human plasma is lower than that of dogs, double the volume of plasma (20 ml.) is used for determination of the former.

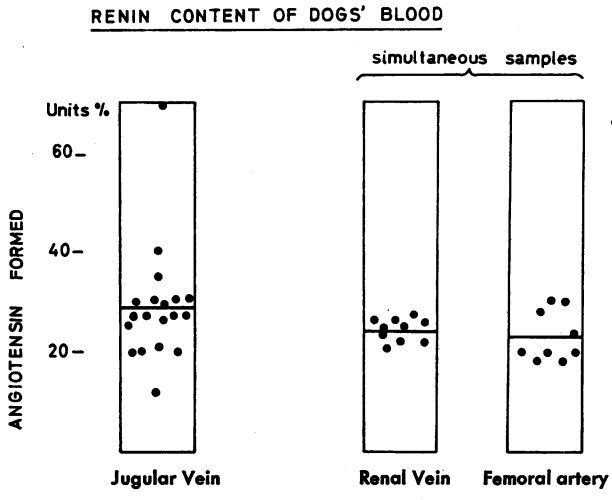

Fig. 1.—Comparison of the renin content of arterial, jugular and renal vein blood in normal unanesthetized dogs.

The claims for the specificity of this method rest on the following observations: Renin activity is found in normal dogs but disappears after nephrectomy. No pressor substance is recovered when angiotensinase is present in the plasma. The pressor substance in the eluate is inactivated by renal peptidases or by trypsin. Scornik and Paladini have shown, furthermore, that only angiotensin is recovered through their method of purification, so that other pressor substances can be excluded.

The renin activity is expressed in units (rat units) of angiotensin formed by 100 ml. of plasma under the conditions described here.*

*Although we cannot state the proper correspondence of this unit with the Goldblatt renin unit, a rough approximation is that our unit is around 1/10,000 that of the Goldblatt unit.

Our rat angiotensin unit corresponds to 6 ng. of the synthetic valine-5 angiotensin II amide (CIBA).

RESULTS

Renin Content of the Blood of Normal Dogs

In 20 normal unanesthetized dogs the renin content of the jugular vein blood was estimated. The values found ranged from 5 to 40 units per 100 ml. of plasma, averaging 29.5 units.

The renin content of the renal venous blood was estimated in nine dogs. A polyethylene catheter was introduced into the left renal vein; several days after the operation, blood was drawn from the renal vein in the unanesthetized undisturbed dog and processed as described above.

Blood samples from the femoral artery were also processed and their renin content was estimated. Fig. 1 shows the results obtained.

No significant difference was found between the renin content of the renal and that of the arterial plasma. This is to be expected, since calculations show that, for renin, with a half-life in circulation of some 15 minutes, the concentration in the renal vein should exceed arterial levels by about only 15%.

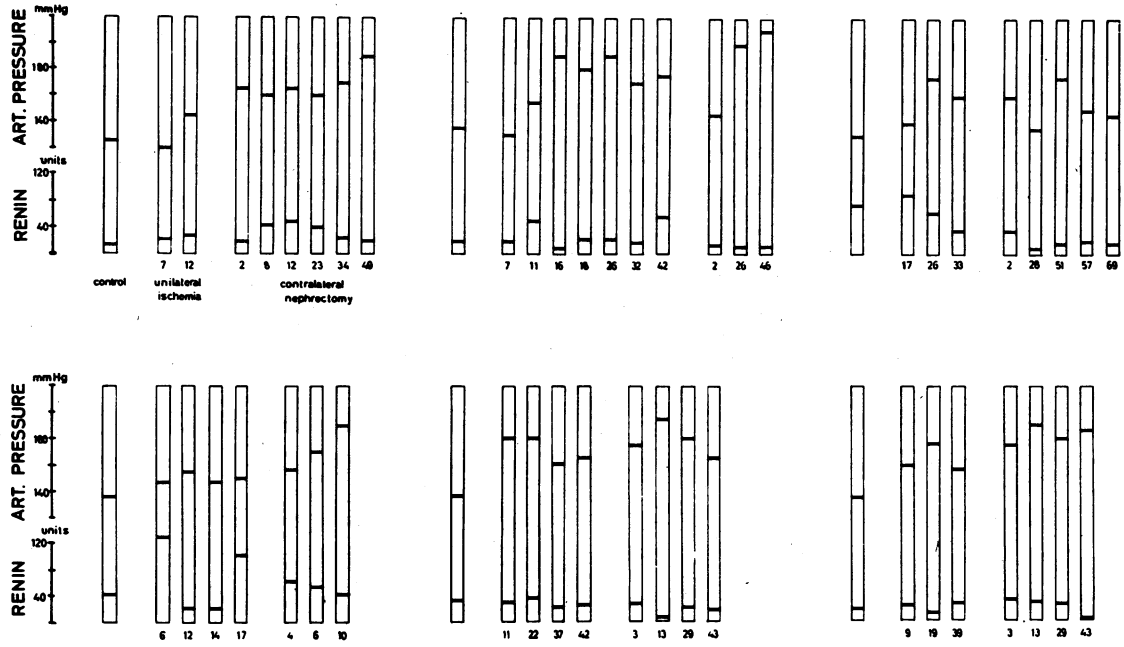

Fig. 2.—Arterial pressure and renin content in six dogs. The left renal artery was constricted by a silver clamp, and after a variable period of time the right kidney was extirpated. The numbers at the foot of the columns represent days after renal ischemia (first set) or after right nephrectomy (second set).

Effect of a Non-Ischemic Kidney on the Renin Content of the Blood

After unilateral ischemia, the arterial pressure rises in the dog and shows a tendency to return to normal afterwards. Contralateral nephrectomy is followed by a steep increase in the arterial pressure, as if some protective mechanism had been withdrawn.[6] One possibility is that the non-ischemic

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kidney is able to control the release of renin by the ischemic one.

To test this hypothesis, the left renal artery was compressed in six mongrel dogs by a small silver clamp. The arterial pressure was measured at frequent intervals by femoral puncture, and the renin content of the jugular vein blood was estimated. After a variable period of time, when the pressure had dropped to the previous levels, or near them, the right kidney was extirpated.

Results are represented in Fig. 2. It can be seen that after unilateral clamping of the renal artery the blood pressure rises, but the rise shows a tendency to disappear later on. This rise is not accompanied by an elevation of the renin content of the blood, although in some cases a small increase was found. After contralateral nephrectomy the blood pressure went up again, but the renin content of the blood did not show any significant change. We can conclude that the hypertension due to renal ischemia in the dog (benign type) is not caused by an increase in blood renin and that the protective action of the non-ischemic kidney is not mediated through interference with the renin secretion of the ischemic kidney.

Renin Content of the Blood in Human Secondary Hyperaldosteronism

The experiments of Genest *et al.*,[7] Laragh *et al.*,[8] Mulrow and Ganong,[9] and Davies *et al.*[10] strongly suggest that the renin-angiotensin system is involved in the mechanism of aldosterone secretion. The renin content of the kidneys of dogs which underwent ligation of the supradiaphragmatic cava vein has been found to be increased.[10] However, under certain conditions it is possible that the kidney content does not reflect the renin content of the blood, so we decided to investigate the problem further. We contacted Dr. Genest and it was agreed to carry out the experiments in collaboration. A group of patients with secondary hyperaldosteronism were to be studied. Renin estimations would be done in Mendoza and the aldosterone determinations in Dr. Genest's laboratory in Montreal. The renin determinations only will be reported, since we do not yet have the results of aldosterone determinations.

Edematous Patients

The renin content of the venous blood of nine cardiac patients with congestive failure was estimated (Fig. 3). In five the renin content was found to be within the normal range (less than five units per 100 ml. plasma) but in four, high renin values were found. All four of these patients had severe edema. Of the five patients with normal values, two were under treatment with diuretics and digitalis and the edema was receding; two had only slight maleolar edema and the fifth was ingesting several grams of sodium bicarbonate daily.

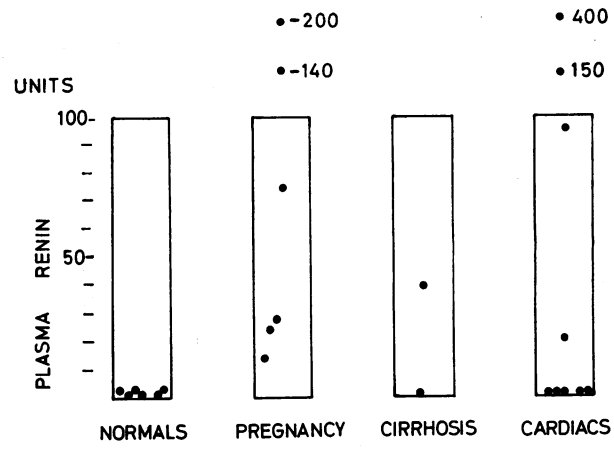

Fig. 3.—Renin content in various conditions in humans. Renin values are expressed in units per 100 ml. of plasma.

Only two cirrhotic patients with ascites were studied (Fig. 3). A high renin content was found in one and normal values in the other. The patient with the normal renin level was under treatment and the ascites was receding.

Pregnancy

Rinsler and Rigby[11] and Kumar, Feltham and Gornall[12] showed that, in the last three months of pregnancy, aldosterone excretion increases considerably. We investigated the renin content of the venous blood of five normal women in the last three months of pregnancy. The results (Fig. 3) show clearly that in every case the renin content was well above the normal values.

Human Hypertension

Two hypertensive patients with unilateral renal vascular disease were studied. In one the renin content was normal, i.e. less than 3 renin units per 100 ml. In the other patient the renin content was very high: 140 and 200 units per 100 ml. plasma on two different determinations (Fig. 4).

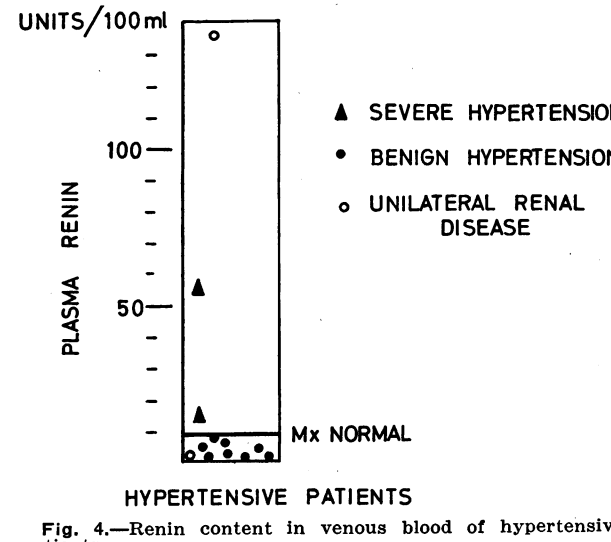

Fig. 4.—Renin content in venous blood of hypertensive patients.

In two patients with severe hypertension values of 14 and 55 units were found.

In six patients suffering from benign hypertension the renin content of the blood was found to be normal (Fig. 4).

Low Sodium Diet

Five normal individuals, three males and two females, were studied.

Urine was collected and renin was determined the day before the beginning of the experiment. The subjects were then submitted to a diet of rice and fruit for four days. No limitation to the amount of food or water ingested was imposed. The sodium content of the urine was measured by a flame photometer.

Renin was again determined on the fourth day of the diet. Urinary sodium dropped below 20 mEq. in 24 hours in every case. Fig. 5 shows that there is a clear increase in the renin content of the blood after four days of low sodium diet.

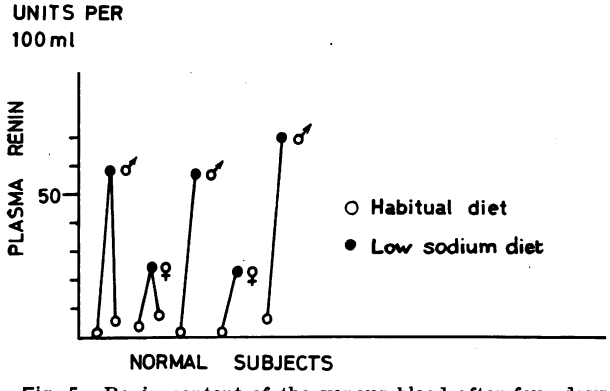

Fig. 5.—Renin content of the venous blood after four days of a diet of rice and fruit.

DISCUSSION

We found an increase in the renin content of the blood in certain conditions in which greater amounts of aldosterone than normal are excreted. These results support the idea that renin is the mediator for the aldosterone secretion.

Renin was found to be a normal component of the blood of men and dogs, confirming the previous results of Fasciolo and Taquini[13] obtained by a much less specific method.

The physiological actions of the renin-angiotensin system appear to be mediated through changes in the rate of renin secretion by the kidney, so that renin fully deserves the name of renal hormone.

With regard to the mechanisms which increase the renin output by the kidney, little can be said. Renal perfusing pressure does not appear to be the stimulus, since blood renin increases markedly after a few days of sodium deprivation but not in hypertension due to renal ischemia.

Since renin increases in normal human pregnancy and in sodium deprivation in man, conditions associated with a normal blood pressure, but not in benign hypertension, either experimental or clinical, the conclusion can be drawn that renin is not the cause of the rise of arterial pressure.

SUMMARY

A specific method for renin estimation in blood has been developed, sensitive enough to detect renin in the blood of normal dogs and human beings.

In dogs in which transient hypertension had been produced by unilateral clamping of one renal artery, blood renin did not show significant increases.

After extirpation of the contralateral, non-ischemic kidney, no rise in the blood renin was observed.

Renin was not increased in the blood of patients with benign hypertension.

Important increases in blood renin were found in four cardiac patients, in one cirrhotic and in two patients with malignant hypertension.

In five women in their last three months of pregnancy a great increase of blood renin was observed.

Renin showed an increase five to ten times above the previous levels in normal humans submitted to a low salt diet for four days.

It is concluded that renin is a hormone of the kidney and that changes in aldosterone production by the adrenal cortex appear to be mediated through changes in the amount of renin secreted by the kidneys.

Neither experimental renal hypertension in dogs nor benign hypertension in humans seems to be caused by an increase in the renin content of the blood.

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