A Sensitive Method for Assay of Plasma Renin Activity


ABSTRACT

Renin activity is separated from rabbit plasma by globulin precipitation, dialysis, and DEAE cellulose column chromatography with a Tris chloride buffer system and acid elution, followed by ultrafiltration. The plasma renin extract contains no renin substrate and no angiotensinase activity can be detected after incubation with angiotensin for 200 hr. Incubation with a known concentration of rabbit renin substrate, similarly free of renin activity and angiotensinase, at pH 6.0 in 0.1 M phosphate buffer forms angiotensin-like activity at a constant rate for up to 200 hr. Recovery and reproducibility are satisfactory. Evidence is presented that the material in the plasma extract behaves similarly to renin and that the incubation product is indistinguishable from angiotensin.

ADDITIONAL KEY WORDS kidney enzyme renin substrate DEAE cellulose chromatography plasma and bacterial peptidase surface adsorption controlled conditions conscious rabbits

In 1938, Pickering and Prinzmetal1 described the first method for assaying renin by finding the dose of the unknown that would give the same rise of blood pressure on intravenous injection in the conscious rabbit as a given dose of a standard. Their standard was an alcohol-dried rabbit-kidney powder which was stable for several years. Other workers used the rise of blood pressure in a test animal, but did not compare this with the rise produced by a standard renin preparation, thus introducing a large source of error because of variation in sensitivity between animals. This type of assay had many disadvantages: it was not very precise; it was slow; and it was incapable of assaying small quantities.

A great increase in sensitivity came when renin activity was determined by the quantity of angiotensin, produced in a standard in vitro incubation with plasma or more purified plasma substrate, and assayed in a test animal.

Kohlstaedt and Page2 assayed the angiotensin by its vasoconstrictor activity in the perfused rabbit ear. A more reproducible and more specific method was that of Leloir et al.,3 who incubated plasma at pH 4.2 at 37°C for 20 min to destroy most of the angiotensinase activity and much of the angiotensinogen (renin substrate) present. They then brought the pH to 7.5 and incubated the plasma with a known large excess of renin substrate in the presence of an antibacterial agent. The angiotensin-like activity was separated from protein, concentrated by ethanol extraction and evaporation, and assayed by the rise of blood pressure produced in an unanesthetized dog.

The standard methods in use today all measure renin activity by means of angiotensin formed. Various devices are used to prevent angiotensinase action, and the angiotensin is assayed in rather more sensitive preparations than were available in earlier years. However, they require blood samples varying from 20 to 100 ml.

Such a large amount of blood cannot be taken from smaller animals, such as the rabbit, without raising the plasma renin activity.

From the Department of the Regius Professor of Medicine, Radcliffe Infirmary, Oxford, Great Britain. Dr. McKenzie is a Nuffield Commonwealth Fellow. Accepted for publication March 2, 1966.
ASSAY OF PLASMA RENIN ACTIVITY during the course of sampling. We describe here a more sensitive method, which will assay renin in samples as small as 2 ml of plasma. The outstanding new features in this method, are strict asepsis and the use of cadmium ions to inactivate angiotensinase during the preparation of the renin substrate.4

**Methods**

**PREPARATION OF PLASMA EXTRACT FREE FROM ANGIOTENSINASE AND SUBSTRATE**

**Sampling Procedure**

Four-to six-milliliter blood samples were taken slowly (3 to 6 min) from the central ear artery of the conscious rabbit in all experiments not involving hemorrhage. Saline (0.9%) was injected simultaneously into the marginal ear vein in similar volume. Such samples may be taken at hourly intervals for at least 6 hr without raising plasma renin activity. Indeed, there was no more than a 50% rise in plasma renin activity in the fourth 5-ml sample taken at 15-min intervals without saline replacement.

**Sterile Technique**

Samples were taken aseptically. All containers were sterilized by dry heat (170°C for 5 hr) or boiling, and all solutions were sterilized by autoclave (115°C for 20 min). In addition, chlorhexidine gluconate 0.01% was added to all solutions. Bacteriological techniques were used during transfer of solutions and unsealed containers were stoppered with sterile cotton wool plugs. Aliquots of solutions were inoculated into broth from time to time to check the sterile technique.

**Other Precautions**

To reduce losses by adsorption, all containers were of polypropylene, polystyrene or siliconized glass. All the procedures up to incubation were carried out at 4°C as tests showed that there were losses of up to 20% of renin activity when column chromatography was done at room temperature.

**SEPARATION OF RENIN ACTIVITY FROM PLASMA (FIG. 1)**

The blood samples were centrifuged at 2,000 rpm. Samples of plasma were mixed with an equal volume of saturated ammonium sulfate solution. After standing at 4°C for at least half an hour, the precipitate was firmly sedimented by high speed centrifugation at 18,000 rpm (20,000 g) for 40 min. The supernatant was discarded and the precipitate dissolved in 20 to 30 ml of 0.01 M Tris (Tris hydroxymethylaminomethane) chloride buffer at pH 7.4.

Sulfate ion, which interferes with chromatography, was removed by dialysis against distilled water and then 0.01 M Tris chloride buffer pH 7.4 [the starting buffer for diethylaminoethyl (DEAE) cellulose chromatography]. When sulfate-free, the dialysis sac contents were centrifuged at 2,200 rpm to remove the euglobulin precipitate, which formed during dialysis. As the precipitate may adsorb small amounts of renin it was extracted twice with 20 ml of 0.01 M Tris chloride buffer pH 7.4 to minimize losses.

DEAE cellulose, Whatman grade 50, was prepared in the chloride phase and equilibrated with 0.01 M Tris chloride pH 7.4, autoclaved and packed aseptically in 8 X 2 cm columns.4 The supernatant and washings from dialysis were run on in 0.01 M Tris chloride pH 7.4, autoclaved and packed aseptically in 8 × 2 cm columns.4 The supernatant and washings from dialysis were run on in 0.01 M Tris chloride pH 7.4, autoclaved and packed aseptically in 8 × 2 cm columns.4

This fraction was then concentrated to 5 to 10 ml by ultrafiltration for 24 hr using 700 mm Hg negative pressure around 8/32 Visking dialysis membrane sterilized by boiling. The ultrafiltrate was then discarded and replaced with 0.1 M phosphate buffer pH 6.0. Equilibration was allowed for 24 hr, the negative pressure being adjusted to give a final volume of 1 to 2 ml at pH 6.0.

The residue and several washings of the membrane with 0.1 M phosphate buffer pH 6.0 were centrifuged to remove the fine precipitate usual-

---

**RENIN RECOVERY**

**PLASMA 2-3 ml ALIQUOTS (NH₄)SO₄ PRECIPITATION (50% SATURATION) GLOBULIN PRECIPITATE REDISSOLVED & DIALYZED TO EQUILIBRATE DEAE-CELLULOSE RENIN ELUTED IN 100 ML 0.02M CHLORIDE pH 2.6 (ANGIOTENSINASE REMOVED) CONCENTRATED BY ULTRAFILTRATION INCUBATED 1 WITH SUBSTRATE 2 ALONE 3 WITH ANGIOTENSIN**

**FIGURE 1**

Flow sheet of renin recovery from plasma.
ly present. The 2.5 to 4.5 ml of water-clear supernatant was used for incubation.

**PREPARATION OF RENIN SUBSTRATE (FIG. 2)**

This is fully described in a previous paper. In summary, the plasma of rabbits whose kidneys had been removed 48 hr previously was precipitated with ammonium sulfate at a final concentration of 3.0 M. The precipitate, after high speed centrifugation, was dissolved in 0.01 M Tris chloride buffer pH 7.4 and dialyzed against distilled water followed by the same buffer until sulfate-free. The dialysis sac contents were centrifuged and the supernatant run onto 20 X 2 cm sterile DEAE cellulose columns equilibrated with 0.01 M Tris chloride buffer pH 7.4.

The columns were washed with a solution containing 0.01 M Tris chloride pH 7.4 and 0.03 M sodium chloride. This removed much unwanted protein, mainly gamma globulins. The substrate-containing fraction was then eluted with a solution containing 0.01 M Tris chloride pH 7.4 and 0.08 M sodium chloride, and any trace of angiotensinase activity in this was removed by incubation with 0.001 M cadmium chloride for 2 hr at 37°C. After precipitating most of the cadmium by adding phosphate buffer to a final concentration of 0.001 M at pH 7.4 the centrifuged precipitate was discarded. The substrate activity in the supernatant may be concentrated by ultrafiltration if necessary.

The final substrate solution produced 1.3 to 2.5 μg of angiotensin per ml when incubated with excess of renin and usually had a specific activity of about 0.6 μg of available angiotensin per mg of protein. It had no angiotensinase activity and produced no pressor or depressor activity when incubated alone for 120 to 200 hr.

**INCUBATION**

If no angiotensinase is present, a mixture of renin with a large excess of renin substrate under standard conditions will produce angiotensin at a constant rate and the amount of angiotensin in the mixture will increase in linear fashion with time. In our incubations, substrate concentration, temperature, pH, ionic strength, concentration of antiseptic and time were controlled.

**Substrate Concentration**

Substrate sufficient to produce at least 600 ng of angiotensin was used in the reaction mixture. Provided that the reaction was allowed to produce a maximum of 300 to 350 ng, the rate of angiotensin formation remains constant. This relatively large consumption of substrate activity could alter the rate of angiotensin formation. In this event, the incubation tube with the largest amount of angiotensin formed should show a lower rate of formation of the peptide than tubes with smaller amounts of angiotensin. However, in 30 randomly chosen experiments where more than 200 ng of angiotensin was present in the final tube, the rate of angiotensin formation in this tube was, on average, 1.5% greater than the mean of the other tubes. We have found with normal levels of renin activity in the rabbit, that angiotensin is formed at a rate of about 1 ng/hr from a 3-ml plasma sample. Thus incubation times of 48, 72 and 96 hr produce enough angiotensin for accurate assay.

**Temperature**

Although reaction velocity increases with temperature, losses of renin occur above 37°C; 37°C was therefore selected as a suitable temperature.

**Hydrogen ion concentration**

For rabbit renin acting on rabbit renin substrate, several experiments have shown that the pH optimum was close to 6.0 in 0.1 M phosphate with a fairly sharp decline on either side of this value. This applied to renin derived from kidney and also renin activity from plasma. A typical experiment is shown in figure 3.

Ionic strength of the buffer was not critical as no difference has been found between rates of reaction in phosphate buffer varying between 0.025 and 0.25 M. Similarly, when chlorhexidine...
ASSAY OF PLASMA RENIN ACTIVITY

pH optimum for rabbit renin and substrate. Rate of angiotensin formation by rabbit renal renin from rabbit renin substrate in 0.1 M phosphate buffer at various pH's (renin 0.006 unit and substrate 1 µg activity in 1 ml expressed in terms of angiotensin formed on complete conversion, in each estimation).

Gluconate concentration was increased from 0.01 to 0.1 %, reaction rate was not affected.

INCUBATION PROCEDURE

Five incubation tubes were set up for each plasma extract. The first three contained in 1 ml an aliquot of plasma extract and substrate capable of producing 600 ng or more of angiotensin. The fourth tube contained a similar aliquot of plasma extract incubated alone in 0.1 M phosphate buffer pH 6.0; this was used to detect any pressor or depressor activity in the extract. The last tube contained, in addition to the aliquot of extract and phosphate buffer, 100 ng of synthetic ValB-angiotensin II-asp-β-amide (Hypertensin, Ciba) to test for angiotensinase activity in the extract (table 1).

The first three tubes were then incubated for three different times, so that the shortest time was sufficient to generate an accurately assayable amount of angiotensin. The reaction was stopped by freezing the mixture rapidly and assay was performed with the mixture thawed and the incubation tube in ice water. This was not important for very slow rates of angiotensin formation. The rate of angiotensin formation per hour for each tube was determined and a mean rate calculated.

The fourth and fifth tubes were removed at the same time as the third tube. The fourth tube very rarely showed more than 5 ng/ml of pressor activity while the fifth tube showed loss of added angiotensin only on rare occasions, and these results were discarded. A typical example of the results of these control tubes is seen in figure 4.

EXPRESSION OF RENIN ACTIVITY

In this laboratory the renin unit employed has been in terms of a known weight (100 mg) of an ethanol-dried rabbit kidney preparation similar to, but not identical with, that used by Pickering and Prinzmetal. This unit quantity has been found to generate angiotensin at the rate of 1.8 µg/hr in phosphate buffer 0.1 M pH 8.0 at 37°C when acting on a large excess of rabbit renin substrate. Thus, when for each sample, the average rate of angiotensin formation of the aliquot of extract has been found, the result may be expressed in terms of this standard.

TABLE 1

Incubation Tubes for a Plasma Renin Activity Estimation

<table>
<thead>
<tr>
<th>Tube</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma extract</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Substrate</td>
<td>2 µg angiotensin/ml</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>pH 6.0, 0.1 M</td>
<td>0.4</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin</td>
<td>1 µg/ml</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Circulation Research, Vol. XIX, August 1966
be converted to renin units per L by the following expression:

\[
\frac{R}{1800} \times \frac{V_e}{V_A \cdot V_p} \times 1000 \text{ units/L}
\]

where \( R \) is average rate of angiotensin formation in nanograms per hour, \( V_e \) the total volume of the extract, \( V_A \) the extract aliquot volume and \( V_p \) the original plasma sample volume, all in milliliters (the incubation mixture volume was always 1 ml).

**ANGIOTENSIN ASSAY**

The preparation used was essentially that of Peart\(^5\) using the response of the rat's arterial pressure as an index with synthetic angiotensin II (Val\(_5\)-Angiotensin II-asp-\(\beta\)-amide, Hypertensin, Ciba, Batch 4208) as standard. Rats weighing 150 to 500 g were anesthetized with pentobarbitone sodium, 60 mg/kg, intraperitoneally and given pentolinium tartrate in polyvinylpyrollidone (Ansolysen retard, May & Baker) 2.5 mg/kg, and atropine sulfate, 1.5 mg/kg, subcutaneously. The blood pressure was measured by a direct ink-writing mercury manometer\(^*\) attached to a carotid artery cannula. Doses of pressor agents were given via an external jugular vein cannula, the total injection with saline washing varying from 0.20 to 0.25 ml.

Sensitivity varied greatly between rats and in the same rats at different times. In general, sensitivity was lower with deeper anesthesia, especially when first set up, and tended to increase after the first hour. A satisfactory rat gave an initial response of at least 15 mm Hg rise to a dose of 5 ng of angiotensin.

The standard solution of angiotensin, sterilized by microfiltration, was stored in sterile siliconized ampules at 4°C at a concentration of 10 \(\mu\)g/ml. A working standard was made each day by dilution of a fresh ampule to 100 ng/ml in well siliconized glass, and discarded at the end of the day. Assay was by the bracket method with alternate doses of unknown and standard. The result was determined from the mean of a dose of standard which gave a definitely smaller response and a dose which gave a definitely larger response than a given volume of the unknown, on two or three occasions in each case. For a typical assay see figure 5.

**Results**

**RECOVERY OF RENIN FROM PLASMA**

Recovery has been tested by addition of known amounts of rabbit kidney renin extract to plasma from rabbits whose kidneys had been removed 48 hr previously. Recovery in 20 experiments over the range mainly found in rabbits (1 to 10 units/L) is shown in table 2. Mean recovery was 48%, with a range of 38.5 to 60% and a standard deviation of 6.2. There was no detectable difference between recoveries at low and high renin concentrations in this range. Losses occur during DEAE cellulose chromatography (25 to 40%), with

---

some loss during ammonium-sulfate precipitation and dialysis (10 to 15%), while losses with ultrafiltration are small (5% or less).

Reproducibility of Plasma Renin Estimations

The results of 20 duplicate estimations of plasma renin activity over a wide range of activity showed a mean difference between determinations of 6.4% with a range of 0 to 18% and a standard deviation of 5.6 (see table 3).

EVIDENCE THAT THE PLASMA ACTIVITY WAS RENIN

The plasma activity assayed was similar to kidney renin extract in the following respects. It was precipitated by 50% saturation with ammonium sulfate, adsorbed and eluted similarly from DEAE cellulose, was stable at pH 2.5 at 4°C, would not pass through a dialysis membrane and was destroyed by boiling. It behaved like an enzyme in forming a product at a rate dependent on substrate concentration, temperature and pH and proportional to time of incubation. The plasma extract activity was not consumed in the reaction. The pH optima of kidney and plasma extracts were similar at 6.0 for rabbit renin substrate.

Rabbits nephrectomized 48 hr previously rarely have detectable plasma activity, whereas activity has always been detectable when a kidney is present.

The blood pressure-raising material produced by incubation of the plasma extract with the rabbit renin substrate, behaved like angiotensin in giving a similar pressor response in the rat, being unaffected by boiling and being destroyed by extracts of red cells, liver and kidney, but not by 0.1 M sodium thioglycollate. It was also similar to angiotensin in adsorption and elution from Dowex 50 W-X2 resin.

Discussion

A method for estimating renin activity should combine high specificity, high sensitivity, high recovery and reliability of recovery. If possible it should also be simple and not unduly time consuming. None of the presently published methods is ideal.

The use of an incubation and measurement of rate of angiotensin formation is common to all procedures, but there is some divergence of opinion on what the attributes of the method should be. Many authors 8-9 use methods which cause as little alteration as possible to the plasma sample in the belief that it normally contains an excess of substrate and that if angiotensinase is controlled renin is the chief factor determining the amount of angiotensin produced in unit time. Factors such as substrate concentration, 'inhibitor' or 'activator' concentration, and even angiotensinase concentration are theoretically allowed to act in their normal fashion by carrying out the incubation using the original plasma. However, conditions do have to be altered in that all plasmas have to be treated to inactivate angiotensinase by dialysis, treatment with chelating agents or other enzyme poisons. In addition pH is always lowered, at least for the incubation, usually to between pH 5.0 and 6.0. Thus, conditions are not the same as obtain in vivo.

The other type of method, a more direct descendant of that of Leloir et al., does not attempt to reproduce in vivo conditions, but

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Renin activity Estimate 1</th>
<th>units/L Estimate 2</th>
<th>Difference %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4</td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>9.1</td>
<td>11.1</td>
<td>18.0</td>
</tr>
<tr>
<td>3</td>
<td>1.3</td>
<td>1.1</td>
<td>15.0</td>
</tr>
<tr>
<td>4</td>
<td>14.9</td>
<td>15.6</td>
<td>4.5</td>
</tr>
<tr>
<td>5</td>
<td>0.8</td>
<td>0.8</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>2.7</td>
<td>3.1</td>
<td>12.0</td>
</tr>
<tr>
<td>7</td>
<td>13.1</td>
<td>13.9</td>
<td>8.0</td>
</tr>
<tr>
<td>8</td>
<td>2.5</td>
<td>2.9</td>
<td>14.0</td>
</tr>
<tr>
<td>9</td>
<td>1.4</td>
<td>1.4</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>2.5</td>
<td>2.5</td>
<td>0.0</td>
</tr>
<tr>
<td>11</td>
<td>9.6</td>
<td>10.4</td>
<td>8.0</td>
</tr>
<tr>
<td>12</td>
<td>7.4</td>
<td>8.1</td>
<td>9.0</td>
</tr>
<tr>
<td>13</td>
<td>13.0</td>
<td>13.5</td>
<td>4.0</td>
</tr>
<tr>
<td>14</td>
<td>2.0</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>15</td>
<td>6.4</td>
<td>6.6</td>
<td>3.0</td>
</tr>
<tr>
<td>16</td>
<td>8.5</td>
<td>8.9</td>
<td>4.5</td>
</tr>
<tr>
<td>17</td>
<td>3.5</td>
<td>3.7</td>
<td>5.0</td>
</tr>
<tr>
<td>18</td>
<td>5.7</td>
<td>5.9</td>
<td>3.0</td>
</tr>
<tr>
<td>19</td>
<td>6.4</td>
<td>7.0</td>
<td>9.0</td>
</tr>
<tr>
<td>20</td>
<td>1.3</td>
<td>1.5</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Mean, 6.4%; so, 5.6

Circulation Research, Vol. XIX, August 1966
extracts the renin as completely as possible in a form free from angiotensinase and renin substrate. The conditions of incubation, with addition of a known excess of angiotensinase-free, renin-free substrate, though highly artificial, can then be accurately controlled to make the renin activity the only known variable.

Recent evidence suggests that a given quantity of kidney renin forms angiotensin at different rates when incubated with different plasmas all free from angiotensinase and all containing an excess of substrate. The rate of angiotensin formation, in conditions of substrate excess, has also been found to be greater for a given renin concentration in nephrectomized than in normal animals. This evidence suggests the possibility of the presence of renin inhibitors in normal plasma. Comparisons of renin activity obtained by using both types of method may well give valuable information on such factors.

At least four variations of the first type of method are in use. The method of Helmer and Judson inactivates angiotensinase which is dependent on calcium ion, by dialysis of the plasma sample against buffer or distilled water at pH 5.5. After a 1-hr incubation at 37°C the angiotensin-like activity is assayed by the rabbit aortic strip or nephrectomized pithed cat. Angiotensinase activity is evident even after a 1-hr incubation. The first order reaction equation of Plentl and Page is used to take account of the variable plasma substrate concentration but does not allow for angiotensinase activity. Experiments for recovery and reliability of recovery of renin are not quoted and subnormal levels of renin activity cannot be assayed.

In the procedure of Boucher et al., angiotensinase is partially inhibited by chelation of calcium with the ammonium salt of ethylenediamine tetracetic acid (EDTA), while angiotensin formed during a 3-hr incubation is protected from destruction by adsorption on Dowex 50 W-X2 resin in the incubation mixture. The angiotensin is eluted with diethylamine from the resin into acetic acid and concentrated to dryness. After several extractions with ethanol it is assayed in the nephrectomized rat. Their results fail to find renin activity in some normal subjects, and although reproducibility of renin recovery at one high level was good, results at lower renin levels are not quoted. Results of recovery of various amounts of added angiotensin, as quoted in their renin method, show a mean of 84%, but also suggest that recovery of small amounts is less than recovery of large amounts. Substrate concentration is not taken into account.

De Vito and Fasciolo use either acidification or EDTA to inhibit angiotensinase activity. One portion of a sample is estimated for angiotensin directly and another incubated for 2 hr at pH 5.1. The angiotensin-like activity is purified and concentrated by the method of Scornik and Paladini. They quote recoveries of added angiotensin of the order of 50 to 60%. However, no recoveries of added renin are given and in some normal human plasma samples renin does not seem to be assayable (angiotensin formation range <0.2 to 0.5 ng/ml).

The most recent procedure of Pickens et al. is able to use a 4-hr incubation at pH 5.5 without detectable angiotensinase activity by dialyzing the plasma against EDTA and then distilled water and treating it with diisopropyl fluorophosphate (DFP). Substrate concentration in the plasma is determined and is always shown to be in large excess and not to fall by more than 16% during the incubation of the plasma for 4 hr. (This fall was produced by the highest renin activity they have yet measured.) Recovery of renin as measured by angiotensin-like activity assayed in the ganglion-blocked rat is 85% with variability of SD ± 7. They do not quote a large amount of evidence for specificity. The method still requires 10 ml of plasma and if smaller volumes were used low renin activities might not be assayable. (The lowest normal renin activity from a 10-ml plasma sample seems to be only about twice the sensitivity limit of the method.) If angiotensinase inhibition were sufficient to allow incubations for longer than...
4 hr then falling substrate concentration and bacterial activity could become important.

The methods of Brown et al. and that we describe are examples of the second type. They have the merit of three estimates of the rate of angiotensin production which improves reliability.

The method of Brown et al. controls incubation conditions rigidly, renin-like activity being made, as far as possible, the only variable. Renin is extracted from plasma by dialysis, chromatographic separation on DEAE cellulose and acidification to pH 2.5 at high salt concentration to remove substrate and angiotensinase. The plasma renin extract is incubated at pH 5.7 with a known excess of prepared ox renin substrate which is almost free of angiotensinase but produces a small amount of pressor activity on incubation alone, a property which may produce a significant error at low levels of renin activity. Such low values have not been observed under their conditions. The substrate preparation also has rather low activity of 0.005 μg of angiotensin available per mg of protein as compared to our preparation with 0.6 μg/mg of protein. This means considerable dilution of the renin activity in the final incubation mixture to achieve a sufficient substrate excess, and a greater possibility of nonspecific pressor activity developing. Finally, the substrate does contain angiotensinase since only 80% of angiotensin added to it and incubated for 24 hr is recovered. They have not found any angiotensinase activity in the plasma renin extract, though a small amount of substrate activity in the renin extract has occasionally been found.

Our method for extracting plasma renin-like activity is very similar to that of Brown et al., and this is reflected in the recoveries of added rabbit renin of 48% (sd ± 6.2) compared with theirs of 40% (sd ± 6.7). However it has the advantages that angiotensinase cannot be detected in the substrate preparation on incubation for 200 hr with angiotensin; the substrate has a higher activity per milligram of protein and does not form significant pressor or depressor material on prolonged incubation. In addition, in our method the plasma renin extract is checked to ensure freedom from other vasoactive substances and from angiotensinase activity over the period of the incubation.

By maintaining asepsis, it is thus possible to incubate a plasma extract for 100 to 200 hr and obtain three estimates of rate of angiotensin production. In this way the renin activity in small plasma samples of 2 to 3 ml is readily assayed with satisfactory accuracy even in rabbit plasmas with renin activities below the normal range.

The method described in this paper combines satisfactory specificity, recovery and reproducibility with high sensitivity. Its complexity, unfortunately, would make its routine use difficult.

References


A Sensitive Method for Assay of Plasma Renin Activity
MICHAEL R. LEE, WILLIAM F. COOK and JOHN K. MCKENZIE

Circ Res. 1966;19:260-268
doi: 10.1161/01.RES.19.2.260

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1966 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/19/2/260

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/