The Quantitative Determination of Renin in the Plasma of Patients with Arterial Hypertension

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With the technical assistance of R. M. Sanders

IN PREVIOUS papers we have described the presence of vasoconstrictor and vasopressor activity in dialyzed renal and peripheral venous plasma of patients with arterial hypertension. Plasma from such patients caused a contraction of the rabbit aorta strip and elevated the blood pressure of nephrectomized cats. Evidence has also been presented that an enzyme with the characteristics of "renin" is responsible for the vasoactivity of the plasma and that it reacts with renin-substrate to form a product that has the properties of angiotensin.

In this paper a method for the quantitative determination of renin in human plasma is described. In addition, further data are presented showing that the renin-angiotensin system is responsible for activity being assayed. The method is based on measuring the amount of the reaction product, angiotensin II, produced by the incubation of dialyzed plasma under standardized conditions. Renin-substrate (angiotensinogen) is also measured because the amount of angiotensin formed by renin depends upon the amount of substrate present in the plasma. Converting enzyme is present in excess in the plasma; so it is not a limiting factor.

All the components for the formation of angiotensin II are present in plasma: renin, renin-substrate, and converting enzyme. These are all proteins, and therefore do not pass through the dialyzing membranes. Consequently, by dialysis it is possible to remove most vasoactive agents not related to this system. Unfortunately, the dialyzed plasma also contains angiotensinase.

Methods

Preparation of the Blood for Assay

Renal venous blood obtained by catheterization and peripheral blood obtained by arterial or venous puncture were collected with heparin as an anticoagulant and centrifuged immediately. During transportation to the laboratory the vessel in which the blood was collected was immersed in an ice bath. If the plasma was not dialyzed at once, it was frozen. Dialyzing sacs were prepared from cellophane tubing, three-fourths inch in diameter. If care was taken to squeeze all of the air out of the sacs before dialysis, no increase in volume of the plasma occurred during this procedure. Most of the plasmas in the work presented in this paper were adjusted to pH 5.5 before transfer to dialyzing sacs, then dialyzed against cold running tap water (13 C.) for 18 to 20 hours. Dialysis has also been done in a cold room at 4 C. against 4 liters of 0.01 M phosphate buffer at pH 5.5 and 7.4. When aliquots of the same plasma were treated in these three different ways, no significant difference in the renin content was found.

After dialysis, the plasma was transferred to a centrifuge tube and adjusted again to pH 5.5 and centrifuged. The insoluble proteins were discarded and the supernatant fluid was made isotonic by the addition of 0.26 ml. of saturated sodium chloride solution for each 10 ml. of plasma. The resultant product was stored in a frozen state.

Assay Methods

Two preparations were employed for the measurement of angiotensin—a spirally cut strip of rabbit aorta and a 2-day nephrectomized, pithed cat. The former is quite useful for plasmas of low renin content and for screening plasmas for the presence of renin. The latter is used for the assay of renin-substrate as well as for plasmas of high renin content.

In order to obtain a high degree of sensitivity, the aorta has to be handled with care. Rabbits

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weighing 5 to 7 lb. are killed by a blow on the back of the neck and bled by severing the neck vessels. The descending thoracic aorta is removed and placed in a Petri dish containing Krebs bicarbonate solution at room temperature. Excessive fat and connective tissue are trimmed off with iris scissors. The whole length of the aorta is then cut along a close spiral into strips approximately 4 to 5 mm. in width. For the assay, strips 45 to 50 mm. in length (unstretched) are used. With the aid of stainless-steel hooks, they are mounted in a muscle chamber of 20-ml. capacity. The bath is maintained at 37.5 C. The isotonic lever is adjusted to give a nine-fold amplification and is counterweighted to exert 4-Gm. tension on the strip. Throughout these procedures, undue stretching of the muscle is avoided.

The Krebs solution used in the muscle chamber has been modified by doubling the potassium chloride concentration and by the addition of 10 mg. per liter of di-sodium salt of ethylenediaminetetraacetic acid (EDTA). The latter chemical without interfering with electrolyte balance of the Krebs solution prevents the tetany that occurs only rarely in some sensitive muscle preparations.

Angiotensin Standard

The angiotensin was prepared from hog renin and hog renin-substrate. It had been standardized against a pure sample of Skegg’s natural angiotensin II, 1 µg. of which is equivalent to 2.38 Goldblatt units (G.U.), (one Goldblatt unit being equivalent to 0.42 µg.). The unit used in our laboratory for many years was worked out in cats and is one sixth that of Goldblatt’s dog unit. Therefore, our angiotensin unit (A.U.) is equivalent to 0.07 µg. of angiotensin II. Synthetic 5-valyl aspartyl amide angiotensin II was not available at the start of this work. When it became available, it was found that its constrictor activity on the aortic strip was only one fourth that of natural angiotensin although the synthetic angiotensin II caused the same elevation in mean pressure as the natural angiotensin II when injected intravenously in the pithed cat. The sensitivity of our test preparations is such that 0.05 unit, equivalent to 0.0035 µg. of angiotensin, can be readily assayed. In sensitive preparations, as little as 0.01 A.U. or 0.0007 µg. can be detected.

Experimental

Before the quantitative method for determination of “renin” in human plasma is described, further evidence will be presented that the enzymatic activity we are measuring is “renin.” Plasma dialyzed and treated in the manner described contains at least four factors: renin, renin-substrate, converting enzyme, and angiotensinase. Any treatment that modifies any of these components will alter the amount of angiotensin formed. Since the amount of angiotensin produced is the index of renin activity, care must be taken in planning and interpreting experiments designed to compare the characteristics of “renin” released into the blood stream with “renin” extracted from kidney tissue. In the following sections, the characteristics of the “renin” in human plasma are described.

Optimal pH for Activity of “Renin” in Human Plasma

In the preparation of plasma for assay, “angiotensinase” was not removed because we wished to modify the plasma as little as possible. The presence of this enzyme could modify the optimal pH for the formation of angiotensin. In order to determine the optimal pH, “active” dialyzed plasma from a patient with arterial hypertension was incubated at 37 C. for 1 hour at pH 4.4 to 7.4 in increments of 1.0 pH and assayed on the aortic strip. The results of this experiment are shown in figure 1. The vasoactivity was greatest at pH 5.4 with a value equivalent to 0.135 A.U./ml.; at pH 7.4 only 0.045 A.U./ml. was found.

![Graph](image)

Figure 1

Constrictor activity of dialyzed human plasma incubated for 1 hour at 37 C. at pH units shown in abscissa. Assay on aortic strip.
Previously it has been shown with unincubated plasma that the constrictor activity as assayed on the aortic strip paralleled the pressor activity tested by intravenous injection in the nephrectomized-pithed cat preparation. To determine whether the incubation product behaved in the same way a similar experiment was done with an "active" plasma from a patient with hypertension. The reaction product was assayed by intravenous injection in a 2-day-postnephrectomized cat. Three milliliters of plasma were mixed with an equal volume of 0.9 per cent sodium chloride solution and the pH of the mixture was adjusted with hydrochloric acid or sodium hydroxide from pH 4.5 to 8.5 in increments of 1.0 pH. In this experiment, the samples were incubated for 2 hours at 37°C and at the end of the incubation all samples were adjusted to pH 5.5 and boiled 10 minutes. The coagulated protein was removed by centrifugation and 1.0 ml. of the clear supernatant fluid, equivalent to 0.5 ml. of original plasma, was used for the assay. The pressor activity of the incubation mixtures is illustrated in the kymograph record in figure 2. The greatest response was at pH 5.5, the pressor activity of 1 ml. of original plasma being equivalent to 0.50 A.U. or 0.035 μg. of angiotensin II. At pH 7.4 the response was only equivalent to 0.08 A.U. or 0.0056 μg. of angiotensin II.

A similar experiment was done to determine the pH dependence of an angiotensinase-free renin, prepared from human kidneys, when incubated with "inactive" human plasma. Three-milliliter aliquots of such a plasma were mixed with an equal volume of 0.9 per cent sodium solution containing 0.006 G.U./ml. of angiotensinase-free renin and the pH of the mixtures was adjusted from pH 4.5 to 8.5 as in the previous experiment. The samples were incubated for 2 hours at 37°C. The reaction was stopped by boiling as described above. The pressor activity of the incubated mixtures assayed in a nephrectomized, pithed cat are shown in figure 3. The greatest response was at pH 5.5. These experiments show that the "renin-like" enzyme in plasma from patients with arterial hypertension has the same pH dependence as renin prepared from human kidneys.

**Optimal pH of Angiotensinase in Human Plasma**

In order to determine the relationship of these findings to angiotensinase, the angiotensinase activity of a nonhemolyzed, dialyzed plasma was determined in the same pH range as used previously to find the optimal pH for angiotensin formation. For these experiments an inactive human plasma was chosen which, when incubated for 1 hour at pH 5.5, did not form pressor substances. To 2-ml. aliquots of this plasma an equal volume of angiotensin in 0.9 per cent sodium chloride

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**Figure 2**

Pressor activity of dialyzed human plasma incubated for 1 hour at 37°C at pH units shown. Mean blood pressure tracing of pithed cat. Ordinate scale, mm. Hg. Time marker, 1 minute. At markers 2 to 6, 1.0 ml. of incubation mixtures equivalent to 0.5 ml. of plasma; at 7, 1.0 ml. of saline control; at 1, 8, 9, angiotensin standard.

**Figure 3**

Pressor activity of "inactive" dialyzed human plasma incubated with angiotensinase-free human renin prepared from kidney tissue. Mean blood pressure tracing of pithed cat. Ordinate scale, mm. Hg. Time marker, one minute. At markers 4 to 8, 0.8 ml. of incubation mixture equivalent to 0.4 ml. of plasma; at 3, 0.8 ml. of plasma control; at 1, 2, 9, angiotensin standard.
solution, containing 2.0 angiotensin units, was added and the mixture incubated for 1 hour at 37 C. At the end of an hour, after being adjusted to pH 5.5, the samples were boiled to stop the reaction. After centrifuging, the supernatant fluid was assayed in a cat. The results are shown in figure 4. The greatest angiotensinase activity was at pH 7.5. These results are of the same order as found by Munoz et al.\(^9\) for the angiotensinase in ox blood.

**Optimal pH Activity of "Renin" in an Angiotensinase-Free System**

The optimal pH of renin on ox globulin, according to Munoz and colleagues,\(^9\) is between 7.5 and 8.5. Obviously, this is quite different from the values reported in this paper for dialyzed human plasma. In our laboratory, as in many other laboratories, we routinely have used pH 7.4 for renin assays. In most instances the absence of angiotensinase in both the renin and renin-substrate has been determined by incubating these preparations with angiotensin at pH 7.4. Whereas plasma angiotensinase is active between pH 7 and 8, in kidney tissue an angiotensinase with an optimal activity at pH 4.0 has been demonstrated as well as one with the same optimal activity as that in plasma.\(^10\) To determine the true pH dependence of renin activity, it is necessary to ascertain the amount of angiotensinase present in both the renin and renin-substrate at the pH levels at which the incubations are conducted.

Because of loss of renin substrate in human plasma when angiotensinase was destroyed, hog renin and hog renin-substrate were used as a model.

The angiotensinase content of both the standard hog renin and hog renin-substrate preparations was assayed at pH 4.5 to 8.5 in increments of 1.0 pH unit. The assay was done, as described earlier, for the angiotensinase in dialyzed human plasma. For controls, angiotensin was mixed with the renin or renin-substrate, boiled immediately, then incubated at pH 5.5 for the same length of time as the mixtures being assayed. As shown in figure 5 the renin was free from angiotensinase. Only small amounts of angiotensinase were present in the renin-substrate at the higher pH values.

![Figure 4](image-url)

**Figure 4**

Ordinate scale, mean blood pressure in a pithed cat. Two A.U. of angiotensin incubated 1 hour at 37 C, at pH values shown with 2 ml. of dialyzed human plasma. Plasma control with boiled plasma, 31 mm. Dose, 0.6 ml. equivalent to 0.3 ml. of plasma.

![Figure 5](image-url)

**Figure 5**

Left ordinate, angiotensin units formed by incubating hog renin with hog renin-substrate (white dots with solid line). Right ordinate, mean blood pressure of pithed cat. Angiotensinase content of hog renin (white dots with broken line). Angiotensin control with boiled renin, 41 mm. Angiotensinase content of hog renin-substrate (black dots with solid line). Angiotensin control with boiled renin-substrate, 38 mm. Reaction time 1 hour at 37 C. Abscissa, pH range of incubations.
To determine the pH dependence of renin in this practically angiotensinase-free system, 0.05 G.U. of renin in 2 ml. of 0.9 per cent sodium chloride solution was added to 3 ml. of 2 per cent renin-substrate and incubated for 1 hour at 37 C. at pH 4.5 to 8.5 in increments of 1.0 pH unit. The reaction was stopped by boiling at pH 5.5, then centrifuged to remove the coagulated protein. The supernatant fluid was assayed in a cat. As can be seen in figure 3, the amount of angiotensin found at pH 5.5 and 7.5 is the same (1.33 and 1.35 A.U. respectively). The data presented in this figure are an average of three separate series of incubations.

Effect of Acid Treatment to Destroy Angiotensinase on Activity of Plasma

To determine whether the angiotensinase content of the plasma could be lowered without loss of activity, an "active" plasma was adjusted to pH 3.9 with phosphoric acid and incubated at 37 C. for 20 minutes. The plasma was from the same patients on whom the data in figure 2 were obtained. After an equal volume of saline was added to this plasma, aliquots were adjusted to pH 5.5, 6.5, and 7.4 and incubated for 2 hours at 37 C. The reaction was stopped by boiling as described earlier. The differential between the amount of angiotensin formed at the different pH levels was decreased compared to that of untreated plasma, indicating a decreased angiotensinase content at the higher pH levels. One milliliter of plasma at pH 5.5 caused a rise in mean pressure equivalent to 0.56 A.U./ml., at pH 6.5 to 0.46 A.U., and at pH 7.4 to 0.42 A.U. The untreated plasma, after incubation for 2 hours at pH 5.5, had an activity equivalent to 0.68 A.U./ml. Because of this loss of activity at pH 5.5, further efforts were not made to destroy angiotensinase in plasma that was to be assayed quantitatively. The angiotensinase content of a small series of inactive and "active" plasmas was determined. No difference was found.

pH Dependency of "Renin" in Plasma

The data presented in the preceding sections indicate that optimal formation of angiotensin in plasma at pH 5.4 to 5.5 could be accounted for by a balance between optimal conditions for renin activity and minimal conditions for angiotensinase action. Angiotensinase-free human renin incubated with dialyzed human plasma has the same pH dependency.

Alkal Stability of "Renin" in Human Plasma

Dr. Erwin Haas has found that human renin can be treated at pH 9.8 for 1 hour at 0 C. without loss of activity. He suggested that we try the effect of such treatment in "active" plasma from patients with hypertension. If no loss of activity occurred as the result of this treatment, more evidence would be obtained for the presence of "renin" in plasma. If renin-substrate were not destroyed by this treatment, these conclusions would be valid. The original plasma, which had a renin-substrate concentration of 10.2 A.U./ml., when incubated for 1 hour at pH 5.5 at 37 C. formed 0.43 A.U./ml. The plasma, after treatment at pH 9.8 for 1 hour, on incubation at pH 5.5 for 1 hour showed the same activity as the original plasma. No change in renin-substrate occurred. The alkal stability of "renin" in human plasma is the same as that of human renin. A comparison of the acid stability of the renin-like enzyme in plasma to human renin was not feasible because of the instability of renin-substrate of plasma at pH concentrations necessary to denature renin.

Reaction of Human Renin with Rat Plasma

Shipley and Helmer have shown human kidney extracts containing 60 times as much renin activity as a rat kidney extract caused only an insignificant rise in blood pressure when given intravenously to a pithed rat compared to the rise produced by rat renin. Furthermore, when human renin was incubated with rat plasma in vitro, only a very small quantity of angiotensin was formed. The following experiment was done to determine whether the enzyme in human plasma with the characteristics of renin reacted in the same way as renin prepared from human kidneys. The substrates were dialyzed plasmas from a nephrectomized cat and rat and from

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a patient with chronic glomerulonephritis. The cat plasma was included because earlier we had demonstrated that unincubated plasmas from patients with arterial hypertension would cause an elevation in pressure when injected intravenously in a pithed-nephrectomized cat. All of these samples when incubated for 1 hour at 37 C. at pH 5.5 caused no constriction of the aortic strip. Then 0.25 ml. of an “active” plasma from a patient with hypertension was added to 0.75 ml. of each of the substrates. For a control, 0.25 ml. of the “active” plasma was diluted with 0.75 ml. of 0.9 per cent sodium chloride solution. All samples were adjusted to pH 5.5 and incubated for 1 hour at 37 C. At the end of the incubation, the samples were frozen rapidly. The constrictor activity was tested in the aortic strip using 0.8 ml. of the sample. The contraction of the strip to the mixtures was as follows:

Angiotensin II (0.09 A.U.) 27 mm.
Control “active” human plasma 15 mm.
“Active” human plasma + rat plasma 14 mm.
“Active” human plasma + cat plasma 24 mm.
“Active” human plasma + “inactive” plasma 25 mm.

The specificity of angiotensinase-free human renin prepared from kidneys was tested on inactive plasma from the same species. The renin substrate concentration of the rat plasma was 18 A.U./ml., that of the cat and human plasma was 6.4 A.U./ml. To 1 volume of the plasmas at pH 5.5 an equal volume of saline containing approximately 0.006 G.U./ml. of plasma was added and the mixtures were incubated for 1 hour at 37 C. The reaction was stopped by boiling. The amount of angiotensin was as follows:

Human renin + rat plasma 0.27 A.U./ml.
Human renin + cat plasma 2.0 A.U./ml.
Human renin + human plasma 2.0 A.U./ml.

These experiments show that renin in “active” human plasma has the same specificity as that of renin prepared from human kidneys.

Temperature Coefficient

Early in the work it was found that incubation of “active” plasma at pH 5.5 at 4 C. for 24 hours resulted in insignificant vaso-activity compared to incubation at 37 C., a characteristic of human renin. However, this difference was not quantitated.

To calculate the velocity constant of the renin in “active” plasma, samples of renal venous plasma were incubated at pH 5.5 at 5 C. for 24 hours and at 37 C. for 2 hours.

For comparison, an “inactive” plasma was incubated with a dilute solution of human renin at 5 and 37 C. under the same conditions as above. The results were as follows:

“Active” plasma at 5 C. for 24 hours at pH 5.5
Angiotensin formed = 0.05 A.U./ml.
Renin-substrate = 9.6 A.U./ml.
K = 0.000332 \times 10^{-2}

“Active” plasma at 37 C. for 2 hours at pH 5.5
Angiotensin formed = 0.68 A.U./ml.
Renin-substrate = 8.0 A.U./ml.
K = 0.074 \times 10^{-2}

The velocity of the reaction was 220 times faster at 37 C. than at 5 C.

Results for “inactive” plasma plus human renin were as follows:

At 5 C. for 24 hours at pH 5.5
Angiotensin formed = 0.05 A.U./ml.
Renin-substrate = 7.3 A.U./ml.
K = 0.000487 \times 10^{-2}

At 37 C. for 1 hour at pH 5.5
Angiotensin formed = 0.375 A.U./ml.
Renin-substrate = 6.4 A.U./ml.
K = 0.1014 \times 10^{-2}

The velocity of the reaction was 210 times faster at 37 C. than at 5 C.

The data show that the renin-like enzyme in plasma has the same temperature coefficient as human renin prepared from kidney tissue.

Effect of Incubation of “Active” Plasma on Renin-Substrate

Further evidence for the renin nature of the enzyme in plasma of patients with arterial hypertension could be obtained if after incubation at 37 C. there would be a decrease in renin-substrate. Leloir and his colleagues used such a method for the indirect determination of small amounts of renin in plasma.

To study the phenomenon, a sample of peripheral blood plasma, which was vasoinactive after incubation at 1 hour at 37 C., and a plasma from a patient with malignant hypertension, which liberated 0.3 A.U./ml. of angio-
tensin under the same conditions, were selected.

An aliquot of plasma was incubated with an excess of human renin to determine the renin-substrate content. Another aliquot was diluted with 1 volume of 0.9 per cent sodium chloride solution, adjusted to pH 5.5 and incubated 4 hours at 37 C. To adsorb the angiotensin formed, the incubate was shaken with 10 mg. of acid-washed animal charcoal per ml. of original plasma at 0 C. for 10 minutes and centrifuged to remove the charcoal. The supernatant fluid was then incubated with an excess of human renin to determine the amount of renin-substrate remaining after these procedures.

Incubation of the ‘active’ plasma resulted in a decrease of renin-substrate from 8.4 A.U./ml. to 6.6 A.U./ml.; whereas, inactive plasma treated in the same way showed no reduction in renin-substrate.

Nature of the Heat Stable Vasoactive Factor Liberated by the Incubation of Human Plasma

In earlier papers, we have shown that the physical and pharmacologic properties of the vasoactive polypeptide in every way tested were identical to angiotensin. The rate of inactivation of this polypeptide by kidney and red-cell angiotensinase, as well as by trypsin and chymotrypsin, was the same as that for purified angiotensin. The dependence on a chloride-activated enzyme (converting enzyme) for constrictor activity on the aortic strip identifies the polypeptide with angiotensin.

Summary of Evidence for the Presence of Renin in Human Plasma

The renin-like enzyme in human plasma is of renal origin, that is, the concentration of this substance is higher in renal venous blood than in peripheral blood.

It has the following characteristics which are the same as those in human renin: the same specificity to rat, cat, and human plasma, the same temperature coefficient, the same alkali stability, the same pH dependency, and splits renin-substrate.

The demonstration of the converting enzyme and the presence of angiotensin I and II are further proof that the renin-angiotensin system is responsible for the vasoactivity of human plasma from patients with hypertension.

In addition, the physical and pharmacologic properties of the polypeptide formed by incubation of human plasma in every way tested are identical to angiotensin.

Quantitative Determination of Renin Content of Plasma

With the great variation in the renin-substrate concentration in plasma of subjects with arterial hypertension, any attempt at quantitation of renin in the plasma must take this factor into consideration. The first order reaction constant in the form used by Plentl and Page for the assay of renin,

$$K = \frac{2-\log_{10} 100}{t} \left( \frac{M-X}{M} \right) \times 2.303$$

appeared suitable. In this equation ‘M’ is the amount of angiotensin formed by incubation with an excess of renin, or the renin-substrate concentration, and ‘X’ is the amount of angiotensin formed by the renin present in the incubation mixture in time ‘t’ expressed in minutes. Since the expression $M-X \over M$ is a measure of substrate utilization, any standard of angiotensin activity can be used, units or micrograms of angiotensin.

The validity of the use of the reaction constant in the presence of the angiotensinase in dialyzed human plasma was tested. An ‘inactive’ plasma was diluted with an equal volume of saline containing 0.1, 0.2, and 0.4 ml. of a dilute solution of angiotensinase-free human renin. The mixtures were incubated for 1 and 2 hours at pH 5.5 and 37 C. The renin-substrate was determined by incubation with an excess of human renin. The reaction was stopped by heating to 100 C. The values obtained were as follows:

$$K = \frac{2-\log_{10} 100(5.85 - 2.0)}{60} \times 2.303 = 0.7 \times 10^{-2}$$
RENIN IN ARTERIAL HYPERTENSION

<table>
<thead>
<tr>
<th>Renin</th>
<th>1 Hour incubation A.U./ml</th>
<th>K × 10²</th>
<th>2 Hour incubation A.U./ml</th>
<th>K × 10²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 ml.</td>
<td>0.60</td>
<td>0.16</td>
<td>0.92</td>
<td>0.15</td>
</tr>
<tr>
<td>0.2 ml.</td>
<td>1.14</td>
<td>0.32</td>
<td>1.71</td>
<td>0.30</td>
</tr>
<tr>
<td>0.4 ml.</td>
<td>2.00</td>
<td>0.62</td>
<td>2.80</td>
<td>0.58</td>
</tr>
<tr>
<td>Renin-substrate 6.4</td>
<td></td>
<td></td>
<td></td>
<td>5.6</td>
</tr>
</tbody>
</table>

When an "active" plasma was incubated without the addition of renin for 0.5, 1, and 2 hours at pH 5.5 at 37 C., the values obtained were:

<table>
<thead>
<tr>
<th>Hours</th>
<th>A.U./ml.</th>
<th>Renin-substrate</th>
<th>K × 10²</th>
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</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.50</td>
<td>7.73</td>
<td>0.22</td>
</tr>
<tr>
<td>1.0</td>
<td>0.62</td>
<td>6.13</td>
<td>0.18</td>
</tr>
<tr>
<td>2.0</td>
<td>0.80</td>
<td>5.33</td>
<td>0.20</td>
</tr>
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</table>

Most of the renin-substrate concentrations found in human plasma fall in the range of 5 to 15 A.U./ml., so three plasmas in this range were incubated with 0.001 G.U. of angiotensinase-free human renin per ml. of dialyzed plasma at pH 5.5 for 1 hour at 37 C. as before. This amount of renin was chosen because the majority of the renin concentrations found in human plasma fall below this value. The following values were obtained:

<table>
<thead>
<tr>
<th>Renin-substrate</th>
<th>A.U./ml.</th>
<th>K × 10²</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>0.33</td>
<td>0.109</td>
</tr>
<tr>
<td>9.3</td>
<td>0.52</td>
<td>0.096</td>
</tr>
<tr>
<td>14.4</td>
<td>0.78</td>
<td>0.093</td>
</tr>
</tbody>
</table>

Mean = 0.099

Since some plasmas from renal veins of the involved kidneys in renal vascular occlusive disease have higher renin contents, a similar experiment was done with a higher concentration of renin (0.003 G.U./ml. of plasma). Only one renal vein plasma had a renin value higher than 0.003 G.U./ml. The renin-substrate concentration was varied from 5.6 to 17.8 A.U./ml. (eight different concentrations). The mean of the K × 10² (first order) was 0.306; the standard deviation was 0.047; and the standard error of the mean was 0.016.

These data show that it is possible to express the renin activity of dialyzed human plasma in terms of the first-order reaction constant in the presence of the angiotensinase present when the incubation is conducted at pH 5.5. Plentl and Page suggested a renin unit could be defined as: 1 R.U. = K × 10².

Even though the activity of the renin in human plasma could be expressed in terms of the reaction constant, it was thought that it would be worth while to correlate reaction constant with Goldblatt units, since in many studies in the literature this has been the unit of activity of renin. For these studies, angiotensinase-free renin was prepared from human kidneys and standardized against hog renin by use of hog substrate. Human plasma that was vasoinactive when incubated for 1 hour at pH 5.5 at 37 C. was used as a source of renin-substrate. When incubated under the same conditions with an excess of human renin, it was capable of releasing 7.2 A.U./ml. of plasma. For the renin-substrate assay, 1 ml. of plasma was diluted with 3 ml. of saline containing 0.2 G.U. of renin.

The reaction mixtures were made up of 2 ml. of plasma and 2 ml. of 0.9 per cent sodium chloride solution containing the renin. Concentrations of renin per ml. of plasma ranged from 0.0005 to 0.02 Goldblatt units. The samples were incubated for 1 hour at 37 C. at pH 5.5. The reaction was stopped as described earlier and assayed as before in the cat preparation.

Angiotensinase-free hog renin and renin-substrate were incubated under the same conditions for comparison with the human material. In this experiment, 9 ml. of 2 per cent renin-substrate in a total incubation mixture of 10 ml. were used. The range of renin concentration was the same as for the human plasma incubates. On incubation for 1 hour at 37 C. at pH 5.5 with an excess of renin, 2.8 A.U. of angiotensin were formed in 1 ml. of reaction mixture (fig. 5).

The data are presented in figure 6, human in black dots, hog in white dots. There was a close correlation between renin concentration and reaction constant. The reaction constant of human renin incubated with human plasma and hog renin incubated with hog substrate paralleled each other under these conditions.

With this information, then, it was possible
Reaction constant for incubation of human renin with human plasma (black dots) and hog renin with hog renin-substrate (white dots). Incubation 1 hour at 37°C. at pH 5.5.

**Figure 6**

To express renin in Goldblatt units per liter of dialyzed plasma, as well as in terms of the reaction constant. For instance, renal venous plasma from the involved kidney of a patient with renal vascular occlusive disease when incubated for 1 hour produced 2.0 A.U. of angiotensin per ml. of plasma. When incubated with excess of human renin, 5.85 A.U. of angiotensin was formed per ml. of plasma. Substituting these values in the formula and referring to figure 6, $0.7 \times 10^{-2}$ is equivalent to 0.007 G.U. per ml. or 7.0 G.U. per liter.

Brachial artery plasma from this same patient obtained 5 days before the renal vein plasma, when incubated for 1 hour, produced 0.90 A.U. of angiotensin per ml. of plasma. When incubated with excess of renin, 10.5 A.U. of angiotensin was formed per ml. When calculated the same way, $K = 0.15 \times 10^{-2}$ or 1.5 G.U. per liter of plasma.

No renin could be detected in brachial artery plasma 1 hour after the removal of the involved kidney. Blood pressure before operation was 200/130, after operation 140/80. The renin concentrations in the plasmas of this patient are the highest found to date.

Useful information on the release of renin by the kidney can be obtained without determining the renin-substrate concentration of the plasma. Routinely, dialyzed plasmas are screened for activity on the aortic strip after incubation at pH 5.5 for 1 hour at 37°C.

By the techniques described in this paper and by assaying the angiotensin formed on the aortic strip, it is possible to quantitate the renin concentration in the peripheral blood of healthy normotensive subjects. The "M" value or renin-substrate of the dialyzed plasma of 14 such subjects, ranging in age from 19 to 46 years, averaged 6.07 A.U./ml., with a range of 4.8 to 7.6. The "X" value, or angiotensin formed by incubation of plasma, averaged 0.023 A.U./ml., with a range of 0.005 to 0.04; the renin content averaged 0.07 G.U./L., with a range of 0.03 to 0.12.

Since this paper is concerned chiefly with the description of the methods used, only a brief summary of the findings in arterial hypertension will be presented.

The highest concentration of constrictor activity was found in patients with renal vascular occlusive disease. High values were also found in malignant hypertension.

The values for both renin and renin-substrate varied greatly from patient to patient and in the same patient during the course of therapy and change in clinical state. An example is shown in table 1. This patient was treated with a combination of a diuretic, reserpine, and hydralazine.

On admission to the hospital, the renin concentration of peripheral plasma was 0.47 G.U./L. With the reduction in pressure the renin concentration dropped to a normal value of 0.07 G.U./L. All of the values for renin-substrate were higher than normal in the peripheral plasma (8.0 to 16.0 A.U./ml).

Even greater variation was found in a patient with malignant hypertensive cardiovascular disease. In this patient 14 assays were done on peripheral venous plasma over a
period of 4½ months. The renin values averaged 0.35 G.U./L., with a range from 0.12 to 0.82 G.U./L.; the renin-substrate from 8.4 to 57 A.U./ml., of plasma. The high value of 57 A.U./ml. of renin-substrate occurred when the blood urea nitrogen was 154 mg. per cent—a week before the patient died in uremia. The renin-substrate and blood urea nitrogen concentration did not necessarily parallel each other. Soon after the patient was admitted to the hospital with a blood urea nitrogen of 138 mg. per cent, the renin-substrate was equivalent to 10.2 A.U./ml. Later when the blood urea nitrogen had dropped to 23 mg. per cent, the renin-substrate concentrations of 10.4 and 11.2 A.U./ml. were found.

In two patients diagnosed as pre-eclampsia with blood urea nitrogen concentrations of 17 and 11 mg. per cent, the renin-substrate values were 24 and 18 A.U./ml., respectively.

In contrast to these data a patient with a blood pressure of 220/160 and a blood urea nitrogen of 20 mg. per cent had renin and renin-substrate values in the normal range.

Discussion

In our earlier work the constrictor and pressor activity of dialyzed plasma of patients with arterial hypertension was expressed in terms of angiotensin. In our current work, vasoactivity is expressed in renin units. The amount of angiotensin formed depends upon the concentration of renin and renin-substrate available for it to act upon. Both these factors have been taken into account in the development of the method.

The great variation observed in the concentration of renin-substrate in the plasma of patients with arterial hypertension may be of real physiologic importance, since the adrenal glands and the liver are both concerned with the formation of this factor. In the plasma of many of these patients elevated values for renin-substrate have been found. The highest levels occurred in the presence of uremia but the renin-substrate and blood urea nitrogen concentrations did not parallel each other. Much more work will have to be done before the meaning of these findings can be interpreted.

With a quantitative method for the determination of renin in small amounts of plasma, it will be possible to evaluate the role of renin in different states of arterial hypertension as well as in other pathologic conditions.

Summary

A method was described for the quantitative determination of renin in small amounts of dialyzed human plasma, utilizing the first order reaction constant.

A wide variation was found in the renin-substrate (angiotensineogen) content of plasma of patients with arterial hypertension. Many of these patients had high concentrations of this factor.
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William Heberden 1710-1801

It may be noted that among his colleagues and friends were John and William Hunter, Fothergill, Jenner, Sir George Baker, Withering, Piteairn and Robert Gooch, men who in point of time followed Heberden, and bridged the road to the development of the great group headed by Richard Bright.—Prefatory Essay, by LeRoy Crummer. WILLIAM HEBERDEN. An Introduction to the Study of Physic. New York, Paul B. Hoeber, Inc., 1929, p. 17.
The Quantitative Determination of Renin in the Plasma of Patients with Arterial Hypertension

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