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A Rapid Simple Method for the Assay of Renin in Rabbit Plasma

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1. EDTA (10mM), 2,3-dimercaptopropan-1-ol (10mM) and chlorhexidine gluconate (0·005%, w/v) cause complete inactivation of plasma enzymes that degrade angiotensin I, but have no effect on the reaction of renin with its substrate. The reagents were termed the selective inhibitors. 2. Thus it is possible to measure renin in plasma by its ability to catalyse the release of angiotensin I. 3. Sterile plasma, treated with the selective inhibitors, is incubated with renin substrate (500–1000ng. of angiotensin content/ml.) at pH 6 at 42° for 6hr. 4. Under these conditions the reaction obeys first-order kinetics. Renin activity is calculated in terms of the percentage release of the angiotensin content/hr. 5. As described, the assay is sufficiently sensitive to measure renin in the plasma of all normal rabbits. By extending the length of the incubation, much lower activities can be measured.

When Tigerstedt & Bergman (1898) discovered renin in extracts of rabbit kidney, they found a similar substance in the plasma of the renal venous effluent. Subsequent studies have shown that kidney renin is an enzyme that reacts with a plasma protein, renin substrate, to release the vasopressor decapeptide, angiotensin I (Braun-Menéndez & Fasciolo, 1939; Page & Helmer, 1940; Skeggs, Marsh, Kahn & Shumway, 1954; Lentz, Skeggs, Woods, Kahn & Shumway, 1956; Skeggs, Lentz, Kahn, Shumway & Woods, 1956; Peart, 1956; Elliott & Peart, 1957; Skeggs, Lentz, Hochstrasser & Kahn, 1963). It is likely that plasma renin behaves similarly, and several methods have been proposed for measuring plasma renin activity by determining the rate of release of angiotensin. Angiotensin, in turn, is measured by bioassay, most commonly by using the blood-pressure response of the pentolinium-treated anaesthetized rat (Peart, 1955).

Measurement of renin activity by this approach requires very careful control of incubation conditions. This has proved difficult. In addition to controlling pH, temperature and substrate concentration, it is necessary to eliminate the effect of plasma enzymes that degrade angiotensin I and modify its bioassay response. Also, it is necessary to eliminate the effect of vasoactive substances other than angiotensin I that may be formed in plasma. Converting enzyme removes the *C*-terminal dipeptide of angiotensin I, forming the more active octapeptide, angiotensin II (Lentz *et al.* 1956; Gross & Turrian, 1960). Other plasma peptidase enzymes, commonly called angiotensinase enzymes, degrade angiotensin to inactive peptides and free amino acids (Khairallah, Bumpus, Page & Smeby, 1963; Regoli, Riniker & Brunner, 1963; Nagatsu, Gillespie, Folk & Glenner, 1965). Plasma also contains a precursor of kallikrein (Eisen, 1963). Active plasma kallikrein releases bradykinin, a polypeptide causing an initial lowering of rat blood pressure (Croxatto & Belmar, 1962).

A vast number of methods have been proposed for the assay of plasma renin activity, but none of these methods is at once rapid, simple, quantitative and sensitive. Two methods (Lever, Robertson & Tree, 1964; Lee, Cook & McKenzie, 1966) have proved reproducible and sufficiently sensitive to measure renin-like activity in the plasmas of all normal rabbits. However, these methods require preliminary physicochemical separation of renin from endogenous renin substrate and angiotensinase enzymes. Recovery of renin is far from complete (38–50%). This partially purified renin is then incubated with renin and angiotensinase enzymes. These methods are tedious, time-consuming and expensive. Though the incubation systems are free of angiotensinase enzymes, it has not been shown that converting enzyme has been removed or inactivated. Thus it is possible that these methods do not measure renin as such, but detect coupled reactions of renin and converting enzyme.

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In this study it was found that the addition of EDTA (disodium salt), BAL* and chlorhexidine gluconate to rabbit plasma causes the complete inhibition of converting enzyme and angiotensinase enzymes, but leaves the reaction of renin with its substrate unmodified. The further addition of SBTI prevents the action of plasma kallikrein. Thus it has become possible to measure renin in plasma by its ability to catalyse the release of angiotensin I.

MATERIALS AND METHODS

Sterile technique. Methods recommended by Cook & Lee (1965) and Lee *et al.* (1966) were used to avoid bacterial contamination. Glass containers were sterilized by heating at 170° for 5hr. Polystyrene containers were sterilized by γ-irradiation. Aqueous solutions were either heated at 115° at 15lb./in.^{2} for 20min. or passed through a Millipore bacteriological filter. Aqueous reagents contained the antibacterial reagent chlorhexidine gluconate [1,6-di-(4-chlorophenyldiguanido)hexane gluconate, Hibitane; Imperial Chemical Industries Ltd., Pharmaceutical Division, Wilmslow, Cheshire] at 0·01% (w/v).

Glass adsorption. All glassware was coated with silicone (Siliclad; Clay–Adams, New York, N.Y., U.S.A.) to prevent loss of angiotensin by glass adsorption. Clean glassware was immersed in an aqueous solution of Siliclad (1%, v/v) for 10sec., rinsed thoroughly with water and then dried in an oven at 100° for 10min.

Blood sampling. Small blood samples (10ml. or less) were taken under xylocaine local anaesthesia by puncture of the central ear artery. Blood was collected into polypropylene syringes containing sufficient heparin to give a final concentration of 20–30 units/ml. Plasma was collected after centrifugation of blood at 1690*g* at 4° for 10min.

Reagents. The following reagents were used: 0·1M-sodium phosphate buffer, pH6·0; EDTA–phosphate solution, 40mM-EDTA (disodium salt) in 0·1M-sodium phosphate buffer, pH6·0; SBTI–phosphate solution, 1mg. of SBTI (3× crystallized; Sigma Chemical Co., St Louis, Mo., U.S.A.)/ml. of 0·1M-sodium phosphate buffer, pH6·0; BAL (Dimercaprol; Boots Pure Drug Co. Ltd., Nottingham), 0·4M solution in arachis oil. All solutions containing 0·1M-sodium phosphate buffer, pH6·0, also contained chlorhexidine gluconate (0·01%). The SBTI–phosphate solution was sterilized by filtration.

Renin substrate. The renin substrate (preparation 1) used in most experiments was prepared as described in the accompanying paper (Ryan & McKenzie, 1968), but in some (see Fig. 1) the partially purified preparation recommended by Cook & Lee (1965) was used. This was designated preparation 2. Preparation 2 had a specific activity 10–12 times that of plasma and no angiotensinase activity was detected during incubation for up to 200hr. at 37°. Renin substrate was measured as described in the accompanying paper (Ryan & McKenzie, 1968). Its concentration is expressed in terms of ng. of angiotensin content.

Preparation of renin. The renin solution (A) used in most experiments was prepared by a modification (Ryan & McKenzie, 1968) of the method of Skeggs, Lentz & Kahn (1967). Renin solution B was used in some experiments. Ethanol-dried kidney powder (Pickering & Prinzmetal, 1938) was extracted at room temperature with 5ml. of water/g. of powder. Chlorhexidine gluconate was added to 0·01%. After being stirred for 1hr. the mixture was centrifuged. The supernatant was adjusted to pH3·0–3·5 with acetic acid, left at room temperature for 30min. and then neutralized with 5N-NaOH. After 2hr. at 4° the suspension was centrifuged. The clear faint-yellow supernatant was tested for renin activity and residual angiotensinase activity. If, as was usual, this solution was not free of angiotensinase activity, it was again acidified and then neutralized. The second acidification usually caused a substantial loss of renin activity (more than 30%). About one-third of these preparations contained no angiotensinase activity detectable during incubation for 24hr. Angiotensinase activity was measured by incubating samples of the renin preparation at 37° with [1-asparagine-5-valine]-angiotensin II (100 or 200ng./ml.) in 0·05M-sodium phosphate buffer, pH6·0.

Assay of angiotensin. Angiotensin was assayed by its effect on the mean arterial blood pressure of the pentolinium-treated anaesthetized rat (Peart, 1955; Peart *et al.* 1966). [5-Isoleucine]-angiotensin I (200ng./ml.) or [1-asparagine-5-valine]-angiotensin II (100ng./ml.) was used as the external reference standard. Since the angiotensin released by renin under the conditions of these experiments is probably angiotensin I, an angiotensin I reference solution is to be preferred. Unfortunately, both [5-isoleucine]- and [5-valine]-angiotensin I are in short supply. The [5-isoleucine]-angiotensin I used in these experiments was a kind gift from Dr Leonard T. Skeggs, jun., Cleveland Veterans Administration Hospital, Cleveland, Ohio, U.S.A., and [1-asparagine-5-valine]-angiotensin II (Hypertensin) was obtained from Ciba, Basle, Switzerland.

Purification of angiotensin. A 50ml. sample of plasma was mixed with 25ml. of the SBTI–phosphate solution, 25ml. of the EDTA–phosphate solution and 2·5ml. of BAL. This mixture was incubated for 22hr. at 37°. The reaction was stopped by adding 50ml. of 10% (w/v) trichloroacetic acid. The mixture was chilled at 4° for 1hr. and then centrifuged. The supernatant was saved and the precipitate was washed with 20ml. of 5% (w/v) trichloroacetic acid. After centrifugation, the wash supernatant was added to the first. The combined supernatants were extracted four times with equal volumes of diethyl ether. Residual ether was removed under reduced pressure. The solution was adjusted to pH7·0 with 2N-NaOH and then concentrated in a rotary evaporator to about 1ml. The resulting yellow viscous fluid was extracted twice with 4ml. portions of butan-1-ol previously equilibrated with 0·05M-sodium phosphate buffer, pH7·0. The butan-1-ol extracts were combined and applied as the upper phase of the first two tubes of a countercurrent train. The countercurrent-distribution system developed was modified after that recommended by Skeggs *et al.* (1954). Butan-1-ol was 2vol. with respect to the lower phase, 0·05M-sodium phosphate buffer, pH7·0. In this system the distribution coefficient of [5-isoleucine]-angiotensin I was 1·7–2·2 and the distribution coefficients of [5-isoleucine]- and [5-valine]-angiotensin II were 0·17–0·21 and 0·10–0·13 respectively.

Inactivation studies. The angiotensin formed by plasma renin was incubated with trypsin (Tryptar; Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex), chymo-

* Abbreviations: BAL, 2,3-dimercaptopropan-1-ol; SBTI, soya-bean trypsin inhibitor.

trypsin (Chymar; Armour Pharmaceutical Co. Ltd.), or carboxypeptidase A treated with di-isopropyl phosphorofluoridate (Carboxypeptidase-A DFP; Sigma Chemical Co.). Angiotensin and enzyme (1:1, by wt.) were incubated in 0·01 M-tris–HCl buffer, pH 7·5, at 37° for 1 hr. and 2 hr.

Inhibition of plasma kallikrein activity. By avoiding exposure to glass surfaces and by adding SBTI to concentrations above 100 μg./ml., release of bradykinin was prevented. This is a necessary step, since angiotensin must be measured by a bioassay preparation that responds to bradykinin.

RESULTS

Reaction of plasma renin with renin substrate

Inhibition of plasma angiotensinase activity. The effects of EDTA, BAL and chlorhexidine gluconate on the activity of rabbit plasma angiotensinase enzymes are shown in Table 1. No matter whether plasma was in a dilution of 1:4 or 1:15, the combination of EDTA, BAL and chlorhexidine gluconate prevented the degradation of angiotensin for at least 48 hr. at 37° at pH 6·0 or 7·4.

Effect of EDTA, BAL and SBTI on the reaction of renin with renin substrate. Lee *et al.* (1966) have shown that chlorhexidine gluconate in concentrations up to 0·1% (w/v) does not modify the reaction of renin with renin substrate. Fig. 1 shows that SBTI, EDTA and BAL have no effect on the rate of release of angiotensin by partially purified renin (preparation B) and substrate (preparation 2). All incubation mixtures contained chlorhexidine gluconate (0·01%). Similar results were obtained with renin and substrate preparations of much lower purity. The reagents EDTA, BAL, SBTI and chlorhexidine gluconate have been designated the selective inhibitors.

Effect of pH, *temperature and substrate concentration.* The rate of release of angiotensin by renal or plasma renin was greatest near pH 6·0 (Fig. 2). Renin and substrate were stable at 50° for at least 8 hr. As shown in Fig. 3, the rate of angiotensin formation was 1·4-fold greater at 42° than at 37°. The velocity of the reaction of substrate with plasma renin obeyed first-order kinetics to substrate concentrations of at least 1000 ng. of angiotensin content/ml. at pH 6·0 (Fig. 4). Similar results were obtained with renal renin (Ryan & McKenzie, 1968). As yet, it has not been possible to use substantially higher concentrations of substrate and the K_m has not been established.

Characterization of the incubation product. The

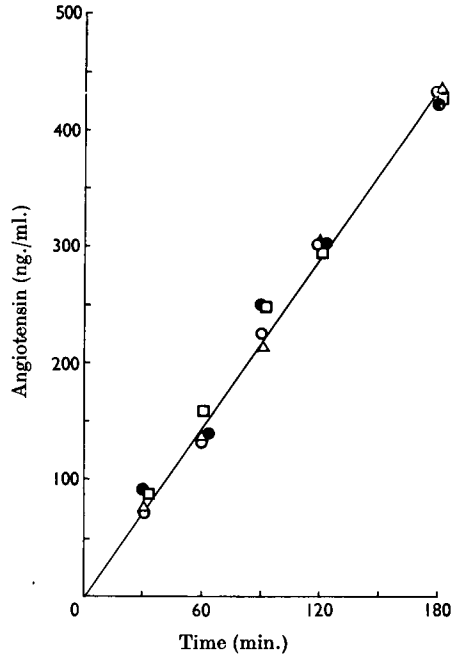

Fig. 1. Effect of EDTA, BAL and SBTI on the reaction of renin and renin substrate. Each reaction mixture contained renin (preparation B) and renin substrate (preparation 2), each free of angiotensinase activity, in 0·1 M-sodium phosphate buffer, pH 6·0, with chlorhexidine gluconate (0·01%, w/v). The initial substrate concentration was 1200 ng./ml. □, Control; △, with EDTA (10 mM); ●, with SBTI (250 μg./ml.); ○, with SBTI (250 μg./ml.), EDTA (10 mM) and BAL (10 mM).

Table 1. *Inhibition of plasma angiotensinase enzymes*

Incubation mixtures contained plasma diluted 15-fold with 0·1 M-sodium phosphate buffer, pH 6·0. [1-Asparagine-5-valine]-angiotensin II was added to a concentration of 500 ng./ml. These mixtures were incubated at 37°.

Inhibitor	Inactivation of angiotensin (ng./min.)	Inhibition (%)
None	44·0	0
Chlorhexidine gluconate (0·01%)	16·7	62
Chlorhexidine gluconate (0·01%) + EDTA (10 mM)	6·3	86
Chlorhexidine gluconate (0·01%) + BAL (10 mM)	3·3	95
Chlorhexidine gluconate (0·01%) + EDTA (10 mM) + BAL (10 mM)	No loss in 48 hr.	100

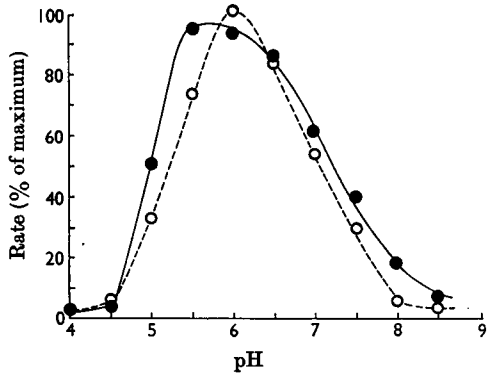

Fig. 2. Effect of pH on the rate of release of angiotensin. NaH_2PO_4 and Na_2HPO_4 were used to prepare the buffer solutions, each of which was used in a final concentration of 0·05 M with respect to phosphate. All reaction mixtures contained SBTI (250 μg./ml.), EDTA (10 mM), BAL (10 mM) and chlorhexidine gluconate (0·005%). These mixtures were incubated at 37°. The maximum rate of release of angiotensin by renal renin (○, mean of two experiments) was 100 ng./hr., and plasma renin (●, mean of three experiments) released angiotensin at a maximum rate of 25 ng./hr.

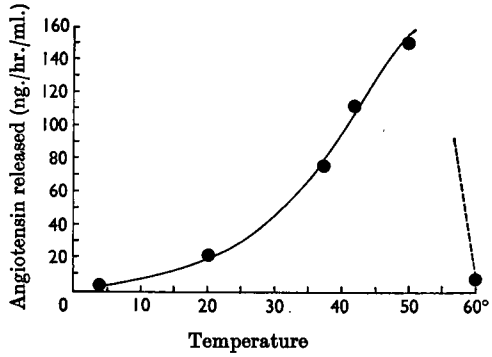

Fig. 3. Effect of temperature on the rate of release of angiotensin. Each reaction mixture contained plasma and renin substrate (preparation 1) in 0·05 M-sodium phosphate buffer, pH 6·0. Inhibitors were added as described in Fig. 2.

product formed on incubation of substrate with renin of rabbit plasma was not distinguished from angiotensin I. The product caused a sharp transient rise of the mean arterial pressure of the pentolinium-treated anaesthetized rat. It was stable at 100° for 20 min. and was soluble in water, dilute salt solutions, 5% trichloroacetic acid, 0·5 M-phosphoric acid, 70% ethanol, water-saturated butan-1-ol and water-saturated butan-2-ol. It was not soluble in diethyl ether. Its biological activity was lost on incubation with trypsin, chymotrypsin and di-

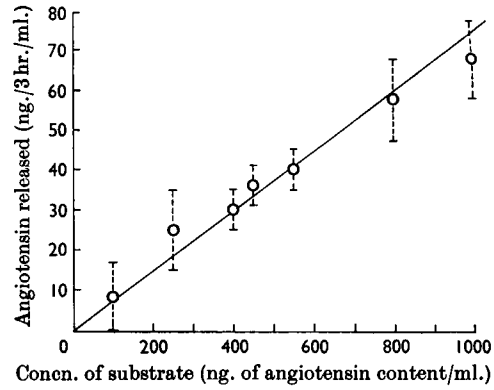

Fig. 4. Effect of substrate concentration on the initial velocity of angiotensin release by plasma renin. Plasma, treated with the selective inhibitors as indicated in Fig. 2, was incubated at pH 6·0 with various concentrations of renin substrate (preparation 1) at 42° for 3 hr. Conversion of substrate into angiotensin did not exceed 10%.

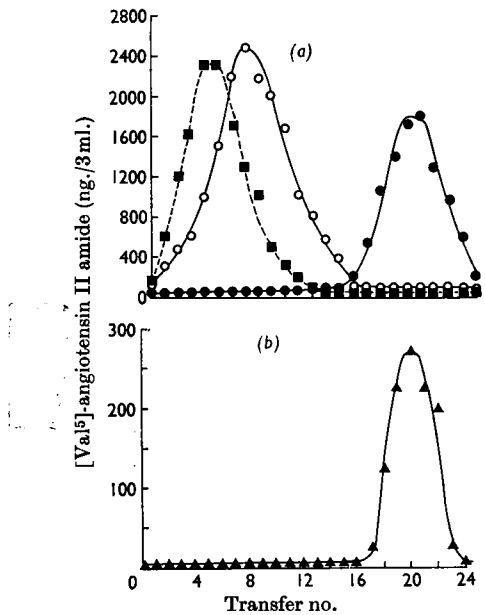

Fig. 5. Countercurrent distributions between butan-1-ol (2 vol.) and 0·05 M-sodium phosphate buffer, pH 7·0 (1 vol.), of (*a*) [5-isoleucine]-angiotensin I (●), [5-isoleucine]-angiotensin II (○) and [5-valine]-angiotensin II (■) and (*b*) the reaction product (▲).

isopropyl phosphorofluoridate - treated carboxy - peptidase A. Unlike vasopressin, the product was not inactivated by thioglycollate or BAL.

In the countercurrent-distribution system used, the product moved like [5-isoleucine]-angiotensin I.

Table 2. *Preparation of plasma for renin assay*

Plasma (2ml.) was mixed with 1ml. of the SBTI–phosphate solution, 1ml. of the EDTA–phosphate solution and 0·1ml. of BAL in arachis oil. The mixture was shaken vigorously to suspend the arachis oil. This mixture (plasma plus inhibitors) was used as indicated.

		Rate		Substrate		Angiotensinase	
Tube no.	Zero 1	2	3	4	5	6	7
	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)
Plasma + inhibitors	0·5	0·5	0·5	0·2	0·2	0·5	0·5
Renin substrate*	1·0	1·0	1·0	0·4	0·4	—	—
[Ile^5]-angiotensin I (1 μg./ml.)	—	—	—	—	—	0·2	0·2
Renal renin	—	—	—	0·4	0·4	—	—
Phosphate buffer†	—	—	—	—	—	0·8	0·8
Time of incubation at 42°	0	6hr.		6 × $t_{\frac{1}{2}}$‡		0	6hr.
Stop reaction	Snap freezing	Snap freezing		8·0ml. of phosphate buffer† added. Heating in boiling-water bath for 5min.		Snap freezing	

* Renin substrate, preparation 1.

† 0·1M-Sodium phosphate buffer, pH 6·0, with chlorhexidine gluconate (0·01%, w/v).

‡ These tubes are incubated six times as long as required for the renin preparation used to release half the angiotensin content (Ryan & McKenzie, 1968).

[5-Valine]-angiotensin I was not available for comparison. Distributions are shown in Fig. 5. These data suggest strongly that the incubation product is angiotensin I. It could not be either known form of angiotensin II. The distribution curve of the reaction product was narrower than expected. The cause of this was not determined.

Assay of plasma renin

As shown above, the selective inhibitors did not modify the reaction of renin with substrate, but prevented the actions of kallikrein, converting enzyme and angiotensinase enzymes. The reaction product, probably angiotensin I, was fully preserved during incubation. Thus the rate of accumulation of this product should be a direct reflection of plasma renin activity.

Incubation conditions allowing a zero-order reaction were not found, and it was necessary to estimate renin activity by comparing the rate of accumulation of angiotensin with the total angiotensin content of substrate.

A protocol for the assay of plasma renin activity is shown in Table 2. Incubations were at 42°. The initial substrate concentrations in reaction tubes 1–5 varied in the range 500–1000ng. of angiotensin content/ml. Angiotensin content of substrate was determined after incubating substrate with a standard renin solution for a period six or more times as long as the time required for that renin solution to release half the angiotensin content. The principles of this substrate assay are presented in the following paper (Ryan & McKenzie, 1968).

The following assay routine was used. After it had been shown that the unincubated reaction mixture (1) did not contain a vasoactive substance, the angiotensin contents of the incubated rate tubes (2 and 3) were compared directly with the angiotensin contents of the substrate tubes (4 and 5). By comparing the angiotensin concentrations of mixtures 6 and 7, the absence of angiotensinase activity was shown. As demonstrated below, it was not necessary to determine angiotensin concentrations by assay with a standard reference solution of angiotensin.

Renin activity was calculated by using the integrated form of the first-order rate equation:

$$k[\mathrm{E}]t = \ln\left(\frac{[\mathrm{S}]}{[\mathrm{S}-\mathrm{X}]}\right) \qquad (1)$$

where [E] is renin activity, k is a proportionality constant, [S] is the initial substrate concentration and [S − X] is the substrate concentration at time t.

A unit of renin activity was defined as that net enzyme activity causing the release of 1% of the angiotensin content of substrate in 1hr. at 42° at pH 6·0 when the initial substrate concentration is within the first-order range. From this definition, k is 0·01 renin unit^{-1}hr.$^{-1}$.

Angiotensin released by plasma renin and the angiotensin content of substrate were compared by finding some volume of the rate-tube contents (2 and 3) causing the same blood-pressure rise as some

volume of the substrate-tube contents (4 and 5). This equality was expressed:

$$V_R[A]_R = V_S[A]_S \quad (2)$$

where V_R is the volume of the rate-tube contents causing the same blood-pressure rise as a determined volume of the substrate tube contents, V_S. $[A]_R$ and $[A]_S$ represent the angiotensin concentrations of the rate and substrate tubes.

In practice, it was always necessary to dilute the substrate-tube contents before assay, thus:

$$[A]_S D_S = [S] \quad (3)$$

where [S] is the true initial substrate concentration and D_S is the reciprocal of the dilution of [S] before assay. In the protocol shown in Table 2, the substrate-tube contents were diluted 15-fold before assay.

Eqns. (2) and (3) can be combined to give:

$$[S] = \frac{V_R[A]_R D_S}{V_S} \quad (4)$$

It was assumed that the rate of release of angiotensin was equal to the rate of utilization of substrate, thus:

$$[S-X] = \frac{V_R[A]_R D_S}{V_S} - [A]_R \quad (5)$$

or

$$[S-X] = \frac{[A]_R}{V_S}(V_R D_S - V_S) \quad (6)$$

The integrated form of the first-order rate equation can be expressed:

$$[E] = \frac{2{\cdot}303}{kt}\log\left(\frac{V_R D_S}{V_R D_S - V_S}\right) \quad (7)$$

From eqn. (7) it is clear that plasma renin activity can be calculated without knowing the absolute amount of angiotensin released by plasma renin or the angiotensin content of substrate, provided that the initial substrate concentration is known to be within the first-order range (1000ng. of angiotensin content or less/ml.). This approach makes it unnecessary to assay against a standard reference compound and thus eliminates part of the assay error.

When renin activity is less than 10units/ml. there is very little error in calculating renin activity by a rearrangement of the first-order (since [E] is constant) rate equation:

$$[E] = \frac{1}{k}\left(\frac{\text{rate}}{[S]}\right) \quad (8)$$

If rate is considered to be $[A]_R/t$, the calculation of renin activity by using eqn. (8) can be done in terms of the volume of the rate-tube contents causing the same blood-pressure response as a determined volume of the substrate tube contents:

$$[E] = \frac{1}{k}\left(\frac{[A]_R/t}{V_R[A]_R D_S/V_S}\right) = \frac{1}{k}\left(\frac{V_S}{V_R D_S t}\right) \quad (9)$$

As k is 0·01:

$$[E] = 100\left(\frac{V_S}{V_R D_S t}\right) \quad (10)$$

Replication studies. By following the protocol of Table 2, the renin activity of every plasma sample was measured in replicate. The range of plasma renin activities tested was 50–600% of the normal average value. The rate replicates were compared directly with each other and with the substrate assay replicates by bracket assay. By this approach replicates were in perfect agreement. It must, however, be emphasized that the bracket assay incorporates an error of ±10–15% and may approach ±30% when used to measure low renin activities (less than 1 unit). A more precise replication study would require a better means of assay of angiotensin.

Renin activity of plasmas of normal rabbits. The plasmas of 50 normal rabbits were assayed for renin activity. Blood was taken from mature rabbits of either sex maintained on a normal diet. Samples were collected at 9–11a.m. on any given day and should not reflect diurnal variation. Values were in the range 1·3–5·3units/ml.; the arithmetic mean was 2·7 (±0·3 S.D.) units/ml.

Sensitivity. With the usual rat-blood-pressure assay, it is difficult to measure angiotensin concentrations of less than 20ng./ml. By following the protocol shown in Table 2, with an initial substrate concentration near 1000ng. of angiotensin content/ml., the renin activities of all normal rabbits were measurable. It was occasionally necessary to extend the incubation time to 18hr. to measure renin activities of the order of 0·3–0·7unit/ml.

Plasma renin recovery. As shown above, the addition of the selective inhibitors to plasma prevented the actions of converting enzyme and angiotensinase enzymes while leaving the reaction of renin with its substrate unmodified. Since plasma was assayed without prior extraction, dialysis or fractionation, this was taken to indicate that 'recovery' of renin activity was complete. High concentrations of heparin, the anticoagulant used in this study, can decrease the rate of angiotensin formation (Sealey, Gerten, Ledingham & Laragh, 1967; Ryan & McKenzie, 1968) and could thus lead to an underestimation of renin activity. This problem can be avoided by strict control of the amount of heparin used to collect blood samples or by using EDTA as the anticoagulant.

DISCUSSION

Renin assay methods proposed since 1963 have been reviewed by Lee *et al.* (1966), Gould, Skeggs & Kahn (1966) and Skinner (1967). Gould *et al.* (1966) were the first to study in detail the kind of angiotensin formed by a plasma renin reaction mixture. Under their incubation conditions the ultimate vasopressor product formed is angiotensin II. Thus their method does not measure renin activity as such, but reflects coupled reactions of renin and converting enzyme. By using the method presented here, these coupled reactions are avoided. By the selective inactivation of converting enzyme, renin can be measured by the rate of formation of angiotensin I.

Our method for the assay of renin has the disadvantage that the reaction kinetics are first-order, and renin substrate concentration must be determined in each assay. Since substrate concentration must be measured in terms of angiotensin content, a standard renin solution free of angiotensinase enzyme activity is required. Though preparation of such renin solutions has in the past been difficult, the new technique developed by Skeggs *et al.* (1967) is simple and efficient in removing angiotensinase enzymes while allowing good recovery of renin. We have also found that the techniques recommended by Peart *et al.* (1966) are very effective but somewhat tedious.

The renin assay method is simple and sufficiently sensitive to measure renin activity in plasmas of all normal rabbits. The equipment, techniques and reagents required are not expensive. Time from blood collection to final bioassay of angiotensin need not exceed 8hr. when all procedures are done by a single operator. This is in contrast with the methods requiring partially purified renin and substrate (Lever *et al.* 1964; Lee *et al.* 1966), which may require 10–14 days for the assay of one plasma sample. A substantial decrease of elapsed time from blood collection to final assay of angiotensin would require a more sensitive means of measuring angiotensin I. As noted above, with current techniques it is difficult to measure angiotensin in concentrations less than 20ng./ml.

The renin activity unit proposed here has the usual disadvantages of first-order units. However, by following the routine assay procedures recommended, the calculation of renin activity does not require determination of the absolute amount of angiotensin released by plasma renin nor the actual angiotensin content of substrate in the reaction mixture. Instead, the angiotensin formed per unit time by plasma renin is compared by relative pressor potency to the angiotensin content of substrate. It is not necessary to assay against a standard reference solution of angiotensin. By not assaying against an external standard, the number of angiotensin assays is halved and the assay error is decreased.

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