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STUDIES ON THE PARTIAL ISOLATION OF HUMAN RENIN

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SUMMARY

Studies on the partial isolation of human renin have been described. We have observed that:

1. Thorough disruption of the cell membranes by an all glass homogenizer increases the yield of enzyme eluted from renal tissue.

2. QAE-Sephadex and calcium gel cellulose chromatography are valuable tools for the purification of renin. Gradient elution is superior in ion exchange chromatography of human renin.

The properties of the final preparation obtained (Fraction 9) are: mol. wt. 42 000; isoelectric point pH 5.25; $A_{280 \text{ m}\mu} : A_{260 \text{ m}\mu} = 2.1$; pH optimum for human substrate 5.5; hog substrate 7.5–8.5; and tetradecapeptide renin substrate 5.5. Specific activity 8.4 Goldblatt units per mg protein (by bioassay in the dog).

3. Evidence was obtained for the existence of considerable amounts of angiotensin I converting enzyme in human kidneys.

Problems concerning the standardisation of human renin have been discussed.

INTRODUCTION

Attempts to purify human renin have been made by several workers¹⁻⁵. The specific activity reported varies from 0.56 (ref. 1) to 33 (ref. 4) units/mg of protein. The specific activities obtained are lower than those reported for renin extracted from tissues of other mammals⁶⁻⁹. At least part of the difference is due to the small amount of renin which is extractable from human kidneys as described by HAAS *et al.*¹⁰.

The present study was undertaken with the objective of increasing the specific activity of human renin; and a comparative study of various extraction procedures

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has been performed. Several chromatographic techniques, heretofore not employed for the isolation of human renin, have been evaluated. A procedure has been developed which retains 16% of the biologically active material of the crude extract. The properties of the obtained material were determined.

EXPERIMENTAL PROCEDURES

Materials

DEAE-cellulose, Type 20 was obtained from Schleicher and Schuell. Sephadex QAE-A50, CM-C50, G-100 and G-200 superfine were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Calcium phosphate gel (30 mg/ml) was a gift of Dr. Massey and was prepared by the method of SWINGLE AND TISELIUS¹¹. Cellulose powder CF 2 was obtained from Whatman; Ampholine pH 4-6 from LKB Instruments Inc., Rockville, Md. The following standard proteins were obtained from Mann Research Laboratories for the estimation of the molecular weight: human γ -globulin (mol. wt. 160 000), bovine serum albumin (mol. wt. 67 000), ovalbumin (mol. wt. 45 000), chymotrypsinogen (mol. wt. 25 000) and myoglobin (mol. wt. 17 800). Bovine serum albumin (Fraction V) was obtained from Armour, lysozyme from Worthington (Freehold, N.J.). A standard preparation of human renin (Lot 13, 11-8-1965) was kindly provided by Drs. Haas and Goldblatt. A synthetic tetradecapeptide renin substrate (No. 107/71; H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-OH) was a gift of Dr. L. T. SKEGGS (see ref. 12). Hog renin plasma substrate was purchased from Pentex.

Methods

All procedures were carried out at 4°. Human kidneys were collected at autopsy and stored at -20°. After removal of fat and connective tissue, the kidneys were homogenized by use of an electric meat grinder, a Waring Blender and by 10 passes with an all glass homogenizer. The homogenate was centrifuged at 20 000 $\times g$ for 20 min (Sorvall Superspeed RC 2B, rotor GSA) and the supernatant saved. The amount of $(\text{NH}_4)_2\text{SO}_4$ used for various degrees of saturation was determined by use of a nomogram¹³.

Protein concentration was estimated either at 660 $m\mu$ by the method of LOWRY *et al.*¹⁴ or by measuring $A_{280\text{ m}\mu}$ in a Beckman DU spectrophotometer; recrystallized bovine serum albumin (Mann Research Laboratories) was used as a standard.

Pressure dialysis was performed as necessary with a Diaflo cell and UM 10 membranes (diameter 62 mm; Amicon, Lexington, Mass.) at 50-60 lb/inch² N₂.

Chromatographic procedures. Samples for chromatography were dialysed against three changes of the respective buffer for 24 h.

Ion exchange chromatography: DEAE-cellulose was used as described by SKEGGS *et al.*¹⁵. QAE-A50 and CM-C50 Sephadex were equilibrated with their respective buffer and used as described in the legends to Figs. 1 and 2. Gradient elution was according to PARR¹⁶.

Calcium phosphate gel was equilibrated with 1 mM SØRENSEN¹⁷ phosphate buffer (pH 6.8) and mixed with cellulose, stirred and degassed as described by MASSEY¹⁸. Consecutively a column of 4 cm \times 16 cm was packed under nitrogen at a pressure of 120 mm Hg. Specimens obtained from chromatography on QAE- or CM-Sephadex

were applied to the column under the same pressure, which produced a flow rate of 3 ml/min. Then the column was washed with 600 ml of 1 mM phosphate buffer (pH 6.8) and eluted with the same buffer at (a) 20, (b) 40, (c) 80 and (d) 160 mM in 0.4 M NaCl.

Gel filtration was carried out on columns, 2.5 cm × 90 cm previously coated with Desicote (Beckman), of Sephadex G-100 and G-200 superfine. The columns were equilibrated and run with 0.075 M Sørensen phosphate buffer (pH 7.5) containing 0.1 M KCl. The sample volume was 2 ml or less, the amount of protein loaded ranged from 4 to 48 mg. Flow rate was 3 ml/h. Usually fractions of 1.5 ml were collected.

Polyacrylamide gel electrophoresis was carried out with a Canalco Model 6 instrument at pH 8.6. Protein was stained with Coomassie blue obtained from Canalco.

Isoelectric focussing was performed with equipment from LKB Instruments Inc. (column size 110 ml, LKB 8121) in a sucrose gradient according to the manufacturers directions. 2% ampholine pH 4-6 was used. Electrofocussing was achieved at 800 V and 2 mA after 72 h.

Assay of renin activity. The renin activity was estimated by: (a) Bioassay in nephrectomized pentolinium treated rats as described by CONN *et al.*¹⁹ and PICKENS *et al.*²⁰. Infusion of 5% dextrose (2-4 ml/h) increased the sensitivity and extended the usefulness of the preparation. (b) Radioimmunoassay of angiotensin I and II as described by COHEN *et al.*²¹. All samples were measured in triplicate. (c) Bioassay in the dog. (d) Double isotope derivative technique for the determination of angiotensin²².

Substrates and incubation procedures applied for the indirect determination of renin activity by bioassay in the rat and by radioimmunoassay were as follows:

(a) Bioassay. Human substrate was prepared according to HAAS *et al.*²³ from pooled plasma of patients with low plasma renin activity. 300 μ l substrate were incubated at 37° with 100 μ l of unknown sample in a Dubnoff shaker for the time given. If necessary, dilutions of the unknown sample were made in orders of magnitude with a solution of 10% human substrate in phosphate buffer 0.05 M (pH 6.9). The generation of angiotensin was stopped by heating in a boiling-water bath for 10 min. Then 1 ml 0.15 M phosphate buffer (pH 7.5) was added and the denatured proteins removed by centrifugation. The supernatant was then assayed in the rat against a standard of angiotensin II (Ciba). The obtained values were corrected for a substrate blank and are expressed as ng angiotensin II generated per unit time.

Tetradecapeptide renin substrate was used as described by SKEGGS *et al.*²⁴. 0.5 nM were incubated with human renin for 30 min. Since tetradecapeptide renin substrate showed an intrinsic pressor effect, corrections for blank were made.

(b) Radioimmunoassay. Hog plasma renin substrate (1 nM) was used according to GOULD *et al.*²⁵ and incubated for 10 min. A dilution series of standard renin was included into each run of determinations.

8-Hydroxyquinoline (5 mM), sodium thioglycolate (50 mM) and EDTA (25 mM) was added to human plasma substrate (acid-citrate-dextrose) to inhibit "angiotensinases". 500 μ l of this solution was incubated for 10 min with 100 μ l of unknown or standard solution. Diisopropylfluorophosphate (4 μ l, diluted 3:7 in isopropanol) was added prior to incubation. The generation of angiotensin I was stopped by immersing the reaction tubes into an ice bath.

"Angiotensinase activity" was measured by incubation of 2500 pg of angiotensin I or II with 50 μ l of undiluted sample or blank in a final volume of 200 μ l of

TABLE I

EFFECT OF VARIOUS EXTRACTION PROCEDURES ON THE YIELD OF HUMAN RENIN

Procedure	n	Solvent	Tissue (kg)	Protein (mg/kg)	Biological activity (ng angiotensin II per 16 h)	
					per kg tissue ($\times 10^6$)	per mg protein ($\times 10^3$)
Meat grinder,						
Waring Blender	3	Distilled water	1.2-20	656- 1200	2.75- 98	3.5-150
+ Ethanol	4	0.15 M NaCl	0.6- 4.5	1570-14 500	5.8 -131	7.0- 30
+ DEAE-cellulose	4	Distilled water	0.8- 1.0	1174- 3000	17.4 - 56	9.9- 56
Glass homogenizer	1	0.15 M NaCl	0.067	15 500	670	44
	1	0.25 M sucrose	0.135	52 250	1060	20
	1	+ Ethanol	0.100	1 422	35	25

0.1 M Tris buffer (pH 7.5) containing 100 mg/100 ml lysozyme for 60 min. The reaction was stopped by heating in a boiling-water bath for 10 min.

Specific activity (biological activity/mg protein) was expressed as: (1) ng angiotensin II per 16 h (rat bioassay), (2) units referring to a standard preparation of human renin, and (3) dog units.

Stability and pH optimum of human renin were tested at various pH in 0.1 M citric acid-0.1 M Na_2HPO_4 buffer. The pH above 8.0 was adjusted with 0.1 M NaOH.

RESULTS

Extraction

Table I shows the effect of various extraction procedures. The yield of human renin was compared after treatment of the crude extract with 0.8 M NaCl at pH 2.8 and precipitation with $(\text{NH}_4)_2\text{SO}_4$. In order to precipitate most of the biologically active material available, a bracket of 15-60% saturation, corresponding to 10-40% (w/v) $(\text{NH}_4)_2\text{SO}_4$ was used. Similar yields of renin activity per kg tissue were obtained by simple homogenisation of the kidneys⁵ and by additional dehydration of the homogenate with ethyl alcohol²⁶. The use of DEAE-cellulose as used by SKEGGS *et al.*¹⁵ did not increase the yield of enzyme activity per kg tissue. However, if renal tissue previously minced by meat grinder and Waring Blender, and suspended 1:6 in 0.25 M sucrose or 0.15 M NaCl, was homogenized in an all glass homogenizer the yield of biologically active material increased considerably. Additional use of ethyl alcohol at this stage caused a loss of renin activity. A further increase of extractable renin could be obtained by repeated freezing (-70°) and thawing of the homogenate.

Ion exchange chromatography

Fig. 1 shows the results of chromatography of the $(\text{NH}_4)_2\text{SO}_4$ fraction on QAE-A50 Sephadex. The renin activity eluted with the second protein peak at pH 5.1-4.9. Samples loaded to this column with high biological activity were impossible to monitor by enzyme activity in the undiluted effluent. This effect was due to a high activity of angiotensinase for both angiotensin I and II. Dilution of the effluent 1:5 resulted in

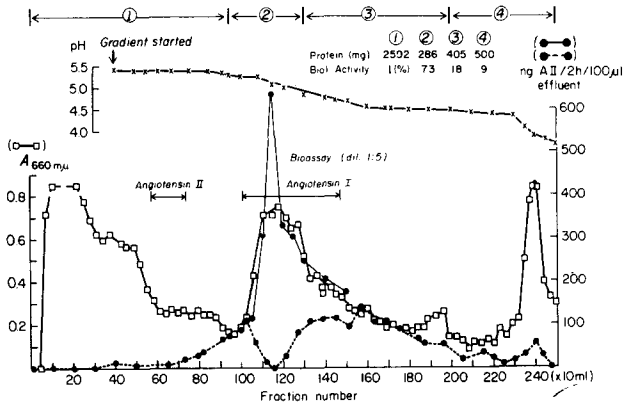


Fig. 1. Chromatography of the $(\text{NH}_4)_2\text{SO}_4$ fraction on a column, 2.5 cm \times 60 cm, of QAE-A5c Sephadex. The column was equilibrated and run with 0.025 M acetate buffer (pH 5.35) until the cationic proteins were eluted. Then a linear gradient was established using a cylindrical mixing chamber (1000 ml). Acetic acid 0.025 M containing 0.015 M NaCl, was added to the communicating reservoir. Renin activity was measured by bioassay (ng angiotensin II per 2 h per 100 μ l effluent). Angiotensin I and II generated were measured by radioimmunoassay. The amount of protein eluted and the relative distribution of renin activity of the pooled fractions is indicated.

an increase of the generation of angiotensin. The determination of angiotensin I and II by the more sensitive radioimmunoassay revealed two separate peaks for angiotensin I and II as generated by the diluted effluent and human plasma substrate. The peak of angiotensin I coincided with the biologically detectable renin activity. Angiotensin II however was mainly generated by the preceding fractions which eluted shortly after the cationic proteins, but clearly before the second protein peak. The yield of the chromatographed fractions of human renin ranged from 73 to 95%. The increase of specific activity was 4–13.6-fold.

CM-C50 Sephadex chromatography of the QAE-Sephadex fraction is shown on Fig. 2. Three protein peaks (pH 4.9; pH 5.35–5.6; and pH 6.4 with 1 M NaCl) were eluted. Human renin eluted with the descending slope of the second protein peak at

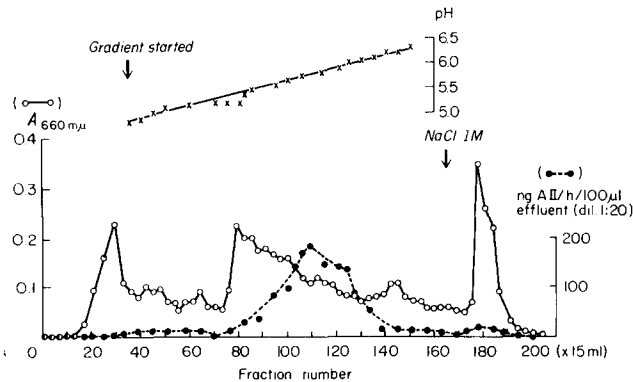


Fig. 2. Chromatography of the QAE-fraction on a column, 2.5 cm \times 60 cm, of CM-C50 Sephadex equilibrated with 0.1 M acetate buffer (pH 4.9). Elution was accomplished by (a) 0.1 M acetate buffer (pH 4.9), (b) linear gradient elution with 0.2 M, acetate buffer (pH 6.4) and (c) 1 M NaCl in 0.2 M acetate buffer (pH 6.4).

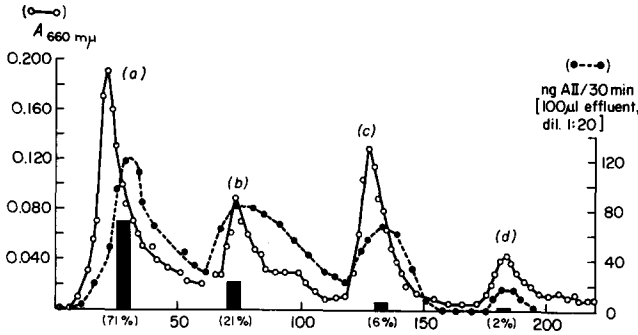


Fig. 3. Separation of human renin by calcium phosphate gel cellulose chromatography. Experimental details are given in *Methods*. The relative distribution of renin activity is shown by bars.

pH 5.7–6.0. The yield of this preparation was found to be between 50 and 106%. The purification achieved was 3.2–8-fold.

Calcium phosphate gel cellulose chromatography (Fig. 3)

This procedure was used for further purification of specimens obtained from chromatography on QAE- or CM-Sephadex. The bulk of the renin activity eluted with 20 and 40 mM phosphate buffer in 0.4 M NaCl. The distribution of renin activity between these two fractions was inconsistent. The optimal purification accomplished was 5.4–25-fold and depended on the amount of protein applied to the column. Smaller samples improved the purification, but decreased the yield slightly. Without the addition of 0.4 M NaCl renin eluted with 80 mM phosphate buffer.

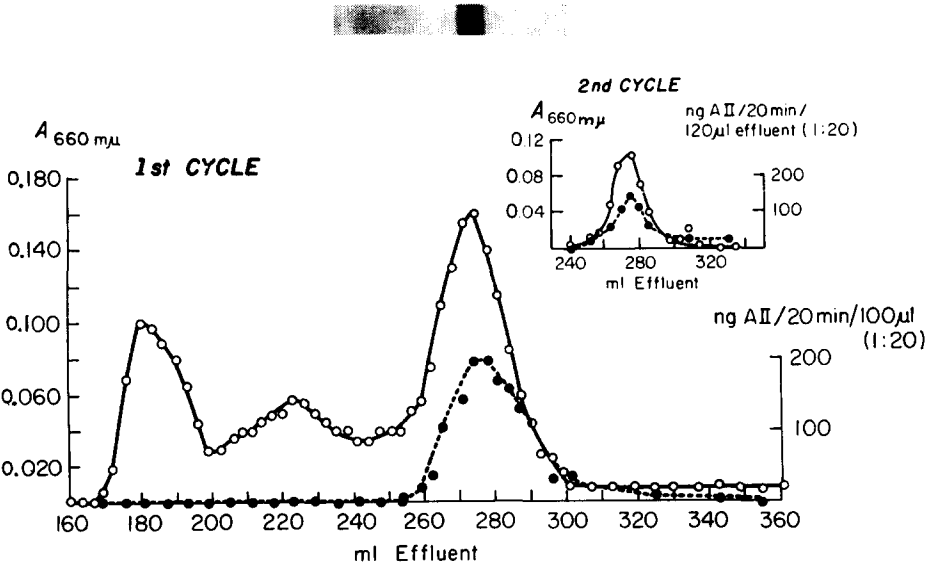


Fig. 4. Gel exclusion chromatography of human renin on Sephadex G-200. Polyacrylamide gel chromatography showed three distinct bands for the biologically active protein peak (top).

TABLE II

PREPARATION OF HUMAN RENIN FROM 5.75 kg OF RENAL TISSUE

The enzyme was extracted after glass homogenisation. The renin activity was estimated by radioimmunoassay for angiotensin I and compared with standard renin. Calciumphosphate gel (procedures 6–8) has been eluted with phosphate buffer (pH 6.8) in 0.4 M NaCl.

<i>Procedure</i>	<i>Protein (g)</i>	<i>Biological activity (units)</i>	<i>Specific activity (units/mg)</i>	<i>Yield (%)</i>	<i>Purification (-fold)</i>
1. Extraction	1260	4200	0.0033	"100"	—
2. pH 2.8 and 0.8 M NaCl	188	5000	0.026	119	7.8
3. (NH ₄) ₂ SO ₄	43.6	3835	0.088	91	26.6
4. QAE-Sephadex	2.9	3500	1.2	83	363
5. CM-Sephadex	0.8	3200	4.0	76	1212
6. 20 mM phosphate buffer	0.144	1320	9.1	31	2757
7. 40 mM phosphate buffer	0.108	1500	13.8	34	4184
8. 80 mM phosphate buffer	0.222	150	0.67	3.4	—
9. Sephadex G-200	0.028	672	16–24	16	4848–7272

Gel exclusion chromatography on Sephadex G-200 (Fig. 4)

An 1.7–8.1-fold increase of specific activity was accomplished by gel filtration on Sephadex G-200. This effect is inversely related to the specific activity of the applied sample. The recovery of the chromatographed protein solutions was 46–88%. 30–63% of the biological activity were recovered after the first cycle. Rechromatography reduced the yield to 24% and also impaired the specific activity. Renin activity eluted at 260–300 ml effluent. The peak of the enzyme activity was slightly shifted to the right of the protein even after rechromatography, suggesting the existence of various components of similar molecular weight.

Protocol for the partial purification of human renin

Table II summarizes the protein yields, the total units of renin activity recovered and the accumulated purification of the principal biologically active fractions obtained throughout the preparation. The overall yield of Fractions 6 and 7 was 65% of the extracted enzyme. Gel exclusion chromatography on Sephadex G-200 reduced the yield sharply to 16%. Gel filtration of Fraction 7 gave higher specific activities (16–24 units) than that of Fraction 6 (8–12 units). The angiotensinase activity of Fraction 9 was negligible. This fraction showed three bands of very similar mobility on polyacrylamide disk electrophoresis (Fig. 4), and cross-reacted immunoelectrophoretically with two distinct lines with antirenin prepared previously⁵.

Properties of partially purified human renin (Table III)

(1) Gel filtration. The molecular weight of Fraction 9 was determined as described by ANDREWS²⁷. As shown in Fig. 5 a linear relationship existed between the effluent values and the logarithm of the molecular weight of the proteins tested. From this graph the molecular weight of human renin was estimated to be 42 000.

(2) Isoelectric point. The isoelectric point of human renin was estimated by electrofocussing of 20–40 mg of Fraction 6 (Table II). The peak of renin activity was eluted in three experiments at pH 5.2–5.3 (Fig. 6). Gel filtration of the electrofocussed preparation on Sephadex G 100 was carried out to remove the low molecular amphi-

TABLE III

PROPERTIES OF THE PREPARATION OF HUMAN RENIN

Molecular weight (gel filtration)	42 000
Isoelectric point	pH 5.25
Optimal stability: (a)	pH 3.0 at 4°
(b)	-70°
(c)	4 M (NH ₄) ₂ SO ₄ at 4°
$A_{280} \text{ m}\mu : A_{260} \text{ m}\mu$	2.1
pH optimum with (a) human substrate	pH 5.5
(b) hog substrate	pH 7.5-8.5
(c) tetradecapeptide	pH 5.5
Specific activity (partially purified)	8.4 Goldblatt units/mg

line. This procedure resulted in a steep loss of renin activity and reduced the specific activity to 0.5-1.0 unit/mg.

(3) Stability. Carrier free material (1.10^{-3} unit) was stored in buffer of pH 3-9.5 (see *Methods*) for 2 weeks. Maximal recovery of 92% was found at pH 3, it declined with increasing pH to 49%. Storage of human renin for 2 months was optimal (recovery 97-109%) in 4 M (NH₄)₂SO₄ at 4° after addition of 10 mg bovine serum albumin per ml, or at -20° and -70° without carrier protein. The recovery was impaired (66%) by storage at +4° of both carrier free and carrier containing (10 mg bovine serum albumin per ml) material. Lyophilisation of Fraction 9 (see Table II) at -70° caused a complete loss of biological activity as measured by bioassay.

(4) The ratio of $A_{280} \text{ m}\mu : A_{260} \text{ m}\mu$ of Fraction 9 was 2.1.

(5) Fig. 7 shows the effect of time on the hydrolysis of both human and hog substrate. The maximal generation of angiotensin by $1.7 \cdot 10^{-3}$ dog unit (200 ng protein) was observed for human substrate after 60 min. For hog plasma substrate (1 nM) the generation of angiotensin had not yet reached a plateau after 2 h.

Fig. 8 demonstrates the effect of substrate concentration on the action of human renin. A clear substrate dependence of the velocity of hydrolysis is apparent for both human and hog substrate.

The effect of pH on the hydrolysis of various substrates by human renin is

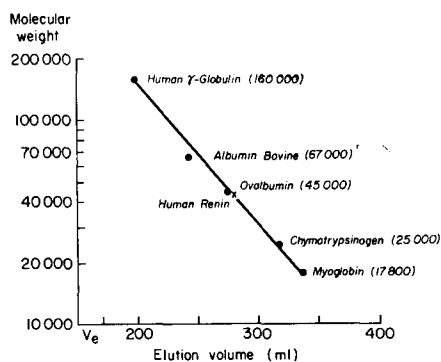


Fig. 5. Estimation of the molecular weight of human renin by gel-filtration on Sephadex G-200. Experimental details are given in the text.

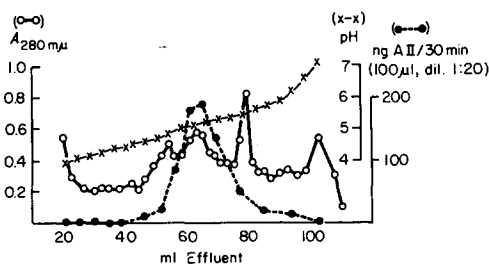


Fig. 6. Estimation of the isoelectric point of human renin ($pI = 5.25$) by electrofocussing.

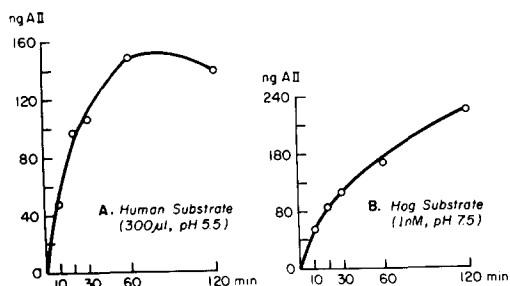


Fig. 7. Effect of time on the hydrolysis of human (A) and hog (B) plasma substrate by human renin (200 ng; 8.4 Goldblatt units/mg).

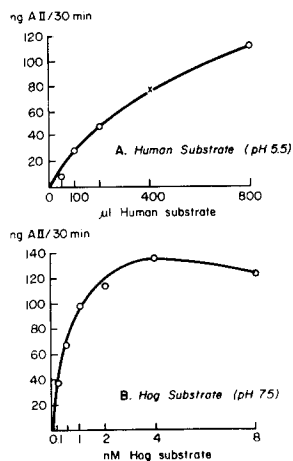


Fig. 8. Effect of substrate concentration (A, human; B, hog substrate) on the velocity of hydrolysis (human renin 200 ng; 8.4 Goldblatt units/mg).

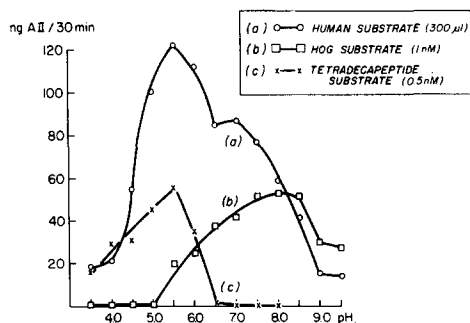


Fig. 9. Effect of pH on the hydrolysis of various substrates by human renin (200 ng; 8.4 Goldblatt units/mg).

demonstrated in Fig. 9. The pH optimum for the hydrolysis of human and tetradecapeptide renin substrate was found to be pH 5.5. Hog substrate was hydrolysed optimally at pH 7.5–8.5.

(6) Standardisation. The obtained preparations of human renin were evaluated *vs.* standard renin by various methods. The values for Fraction 9 were compared with some values reported in the literature (Table IV). Striking differences between the results of various procedures became apparent. The highest value of 37 units/mg was obtained using the rat bioassay technique for the evaluation of the produced pressor material. The lowest specific activity (5.9 units/mg) was found with a double isotope derivative technique for the determination of angiotensin. Human substrate prepared by the method of HAAS *et al.*²³ had the disadvantage that a very shallow slope (Δ angiotensin/ Δ renin concentration) was obtained. No parallel dose response curves of standard renin and unknown could be obtained with this substrate using both bioassay and radioimmunoassay techniques. Approximately parallel dose response curves of standard renin and unknown samples (Fig. 10) and similar values of specific activity

TABLE IV

SPECIFIC ACTIVITIES OF VARIOUS PREPARATIONS OF HUMAN RENIN

Reference	Direct measurements of renin activity		Indirect measurements of renin activity (compared with Goldblatt's standard)			
	Dog units	Cat units	Bioassay		Radioimmunoassay (angiotensin I)	
			Hog sub-strate	Human sub-strate	Hog sub-strate	Human substrate (S) or plasma substrate (P)
DEXTER <i>et al.</i> ¹	3.75-5.7* 0.56-0.86**	15 -23* 2.25-3.45**	—	—	—	—
HAAS <i>et al.</i> ²	0.93	—	—	—	—	—
FUKUCHI AND CONN ⁴	—	—	—	33	—	—
LUCAS <i>et al.</i> ⁵	—	—	—	—	—	5.0-12 (S)
Fraction 9	8.4	5.9***	31-37	24-27	15-21.4	16 -24 (P)

* Per mg N.

** Per mg protein.

*** Double isotope derivative technique for the determination of angiotensin²².

(Fraction 9: 15-24 units/mg) were obtained when human and hog plasma renin substrate were used for the generation of angiotensin I as measured by radioimmunoassay. However, these curves were not yet stoichiometric. Linearity of response with added enzyme was only obtained by the double isotope derivative technique for the determination of angiotensin generated, and by the bioassay in the dog (Fraction 9; 8.4 Goldblatt units/mg).

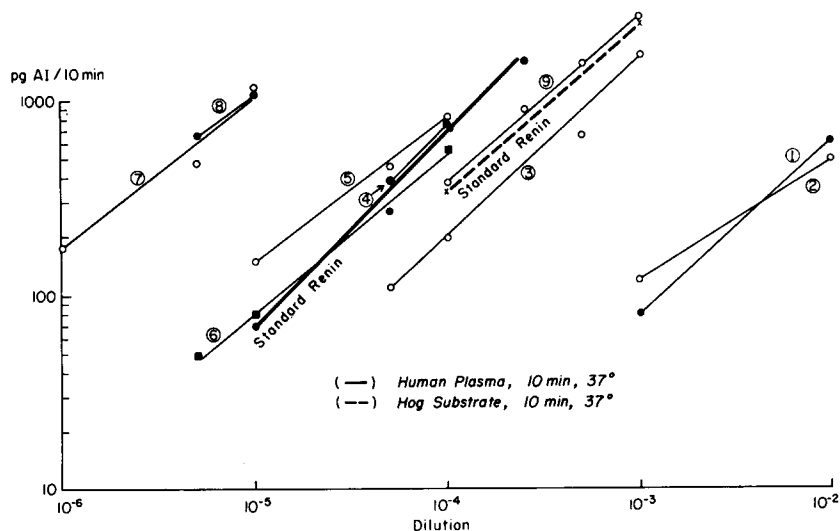


Fig. 10. Dose response curves for the standardisation of human renin. Dilution of standard renin *vs.* unknown Fractions 1-9 (see also Table II). —, human plasma substrate; ---, hog plasma substrate. Angiotensin I generated was measured by radioimmunoassay.

DISCUSSION

Thorough disruption of renal tissue by an all glass homogenizer improves the yield of renin activity extracted from human renal tissue. This effect is further enhanced by repeated freezing and thawing of the homogenized kidneys as also reported by OGINO *et al.*²⁸. By these means 730 units of renin activity extracted per kg tissue were obtained for the crude extract, which compares favorably with the 137 units reported by others².

The use of QAE-Sephadex and gradient elution from the ion exchange columns proved to be of additional advantage for the purification of renin. The simultaneous determination of angiotensin I and II as generated by the enzymes eluted from QAE-Sephadex showed different maxima for both products (Fig. 1). This pattern suggests the existence of considerable amounts of angiotensin converting enzyme in human kidneys and opens a new approach for further purification of this enzyme. A crude preparation of angiotensin I converting enzyme was obtained by SKEGGS *et al.*²⁹.

Chromatography of human renin on DEAE-cellulose (Fig. 11) as on QAE- and CM-Sephadex (Figs. 1 and 2) gave no evidence for the existence of various types of human renin as has been observed by SKEGGS *et al.*¹⁵ for hog renin. The elution of renin activity from calcium phosphate gel cellulose with both 20 and 40 mM phosphate buffer in 0.4 M NaCl is possibly due to the small difference in ionic strength of the eluting buffer, but does not prove the existence of various types of human renin.

Human renin, Fraction 9 as obtained after gel filtration showed three bands of very similar mobility on polyacrylamide gel electrophoresis. It seems likely that one of the three protein components represents human globin; *i.e.* hemoglobin with the heme removed and a molecular weight of 41 000–42 000 as described by ROSSI-FANELLI *et al.*^{30,31}. The estimated molecular weight confirms the value reported by WARREN AND DOLINSKY³² for crude human renin, which was prepared by the method of DEXTER *et al.*¹. The human enzyme appears in the lower range of the molecular weight estimated for hog renin by KEMP AND RUBIN³³, and differs from the estimated molecular weight of a renin like substance reported previously^{34,35}.

The observed pH optima of the obtained renin preparation with different substrates are the same as those described by GOULD *et al.*²⁵ for human renin. This

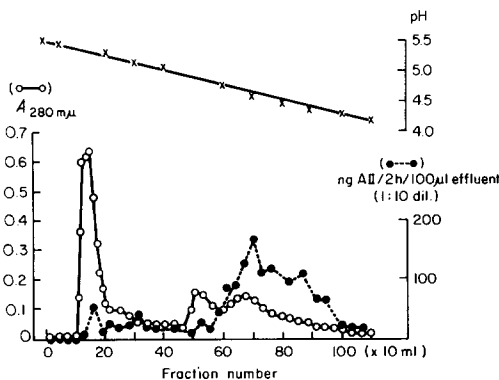


Fig. 11. Chromatography of human renin on DEAE-cellulose. Column 2.5 cm × 50 cm. Gradient elution pH 5.5–4.0 with 0.025 M acetate buffer.

provides clear differentiation between our preparation and pseudorenin as prepared by SKEGGS *et al.*²⁴, which hydrolyses tetradecapeptide renin substrate optimally at pH 4.5 and hog substrate at pH 5.

The specific activity of Fraction 9 varied over a wide range depending on the procedure used (Table IV). When compared on the basis of the reported respective specific activities of various preparations the relationship was improved. Similarly, BOUCHER *et al.*³⁶ and PICKENS *et al.*²⁰ had found considerable variations of angiotensin formation when a constant quantity of renin was added to different samples of plasma. This effect could be caused by varying amounts of "angiotensinase" in the substrate used.

The observed phenomena indicate that the values obtained represent only the apparent renin activity which is associated with the method applied. It appears that the result is influenced by a variety of circumstances such as the type and concentration of substrate(s), reaction products, activators and inhibitors of the enzyme system. The wide variation of the results suggests that various other compounds might be produced in addition to angiotensin I. Such substances could influence, either by pressor activity or by immunological cross reactivity with angiotensin I, both the results of the bioassay ("direct or indirect") and those of the radioimmunoassay for angiotensin I.

It seems important to emphasize that indirect measurements of renin activity using a standard preparation for comparison provide only a relative measure with which to follow the results of a purification procedure. It is therefore desirable to develop a direct assay for the determination of renin. In the meanwhile preparations should be tested "directly" in the dog before comparisons are made with other reported preparations.

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Biochim. Biophys. Acta, 221 (1970) 536-548