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THE RENIN-LIKE ENZYME OF RABBIT UTERUS

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SUMMARY

1. Means have been found for the assay of renin-like enzymes in simple aqueous extracts of rabbit uterus and kidney. These enzymes can be heated at 56°, at pH 6.0, for 30 min without losing activity. The same procedure reduces angiotensinase activities of kidney and uterine extracts by more than 90%. This reduction of angiotensinase activity is sufficient to allow assay of renin and the renin-like enzyme of uterus by their abilities to catalyze the release of angiotensin. This provides a basis for the determination of enzyme contents of kidney and uterus.

2. Further evidence has been obtained on the physicochemical properties of the renin-like enzyme of uterus. Using identical fractionation procedures, the renin-like enzyme of uterus and renin of kidney were purified more than 1400-fold. Either enzyme is adsorbed on DEAE-cellulose and can be eluted at pH 4.6 with 0.1 M NaCl or with 50 mM sodium phosphate–0.1 M NaCl at pH 7.0. These enzymes behave identically on Sephadex G-100, each appearing to have a molecular weight of 40 000 ± 3000. Both enzymes migrate in the pre-albumin region on starch gel electrophoresis at pH 8.6. Within the limits of this study, we found no basis for distinguishing the uterine enzyme from renin.

INTRODUCTION

Recently, GROSS *et al.*[1], found a renin-like substance in uteri of pregnant and non-pregnant rabbits. When injected intravenously, the substance caused a prolonged rise of arterial blood pressure, and when incubated with plasma it catalyzed the release of a heat-stable pressor substance like angiotensin. The uterine enzyme was precipitated by $(NH_4)_2SO_4$ at half-saturation and was not inactivated by moderate acidification. A subsequent study[2] showed that the specificity of the uterine enzyme is like

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that of renin. The product of the reaction of the enzyme with renin substrate partitioned on countercurrent distribution like angiotensin I and unlike angiotensin II. Angiotensin thus released was not degraded.

Others have confirmed that rabbit uterus contains a renin-like enzyme but estimates of the tissue content of enzyme vary widely[1,3,4]. There is, however, agreement that the content of enzyme in pregnant uterus is in great excess of that in non-pregnant uterus, suggesting that the enzyme may be important in the reproductive cycle. Studies aimed at determining any such role will require quantitative determinations of the enzyme at various stages of pregnancy. The present study was begun to find accurate means of measuring the content of the renin-like enzyme in pregnant and non-pregnant uterus and to extend the comparison of the uterine enzyme with renin.

MATERIALS AND METHODS

Substrate and enzymes

[Asn[1], Val[5]]-Angiotensin II was obtained from Ciba Pharmaceutical Company, Horsham, Sussex, Great Britain. Renin substrate was prepared from plasma of bilaterally nephrectomized rabbits by a method described previously[5]. Renin used as a standard in these studies was prepared from rabbit kidneys by a modification[5] of the method of SKEGGS *et al.*[6].

Preparation of tissue extracts

Kidney, liver, lung, spleen, thymus and submandibular salivary gland were collected from adult New Zealand Grey or White rabbits of either sex. Uterine horns were from non-pregnant rabbits and pregnant rabbits 25–29 days after mating. Tissues were frozen in a solid CO_2–acetone slurry, thawed at room temperature and then put through three more freeze–thaw cycles. Each tissue was minced in a meat grinder and then weighed. Water was added to give 2 ml/g of tissue mince. The suspension was stirred for 15 min and then centrifuged at 2000 rev./min, at 4°, for 30 min. The supernatants, called the aqueous extracts, were used in the experiments to be described.

Heating procedures

In preparation for assay, aqueous extracts were diluted with 0.1 M sodium phosphate buffer, adjusted to pH 6.0 or 7.4, to give a final protein concentration of 2–4 mg/ml. Aliquots were heated in a water bath at 20, 56, 58 or 60°. At 60° the bath temperature did not fluctuate more than $\pm$ 0.3°.

Assays

Angiotensinase activity was measured by the method described by JOHNSON AND RYAN[7]. Two types of renin assay were used. In the "direct" method, renin was measured by its ability to raise the mean arterial blood pressure of the pentolinium-treated, anesthetized rat[8]. A volume of unknown was found that gave the same pressure rise as 25 μl of the standard renin solution. In general, 25 μl of the standard raised mean arterial blood pressure by 10–15 mm Hg. For convenient calculation, it was decided arbitrarily that the standard solution contained 4 "direct" units/ml. "Indirect" renin assays were performed as described previously[5,9]. In brief, renin was measured in terms

of its ability to catalyze the release of angiotensin I, and angiotensin I thus released was assayed by its effect on rat blood pressure. Results of the "indirect" assays were expressed in the first order reaction units recommended previously[9].

Protein estimations and specific activity

Except where noted, protein was measured by absorbance at 280 nm. One protein unit was defined as the amount of protein required to give an absorbance of 0.100 at 280 nm, 1-cm light path. Specific activity was expressed as "indirect" renin units/protein unit.

Chromatography and electrophoresis materials

DEAE-cellulose (Whatman Grade 50) was cycled to the free base form. Some was stored in this form, as a moist pad, for use in purification Step 2. The remainder was equilibrated with 5 mM sodium phosphate buffer, pH 7.0, for use in Step 4. Sephadex G-100 and Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) were prepared by allowing the beads to swell in 2 mM Tris–HCl–0.15 M NaCl buffer, pH 7.4, for about 5 days. Starch (Connaught Laboratories, Toronto, Canada) was used as a 12% gel. After electrophoresis, starch gel segments were disrupted with a glass rod in 0.1 M sodium phosphate–0.15 M NaCl buffer, pH 6.0. Free solution was recovered by centrifugation at 2000 rev./min for 30 min.

RESULTS

Part I. Assay of renin and renin-like enzymes in aqueous extracts of uterus and kidney

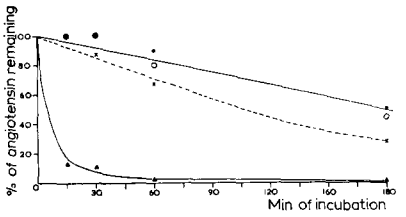
Previous studies have shown that rabbit renin is not inactivated when heated at 56° (see refs. 10, 11). Others have shown that some peptidase enzymes are inactivated completely by the same treatment[12,13]. These reports led us to determine whether a mild heating procedure could be used to inactivate angiotensinase enzymes while leaving renin and renin-like enzymes fully active. Success should provide a basis for measuring renin by its ability to catalyze the release of angiotensin.

Effects of mild heating on renin

We found that the stability of renin to heat depends upon pH. Results are shown in Table I (page 391). Samples of our renin standard, buffered at pH 6.0, were not inactivated when heated at 56 or 58° for up to 3 h. Half of the renin activity was lost during heating at 60° for 30 min. However, at pH 7.4, virtually all renin activity was lost on heating at 56°. Similar results were obtained by "direct" assay of renin in simple aqueous extracts of kidney and uterus.

Effects of heating on angiotensinase enzymes

Extracts of kidney and uterus were buffered at pH 6.0 and then heated as before. The clear supernatants obtained after centrifugation (2000 rev./min for 10 min) were incubated at 37° with [Asn[1], Val[5]]-angiotensin II, 500 ng/ml. Aliquots were taken at timed intervals to determine remaining angiotensin concentration. Reactions were stopped by heating the reaction mixtures at 100° for 3 min. Results obtained with kidney extract are shown in Fig. 1. Results were interpreted in terms of the time ($T_{50\%}$) required to reduce angiotensin activity by half[7]. $T_{50\%}$ of the control was 10 min.



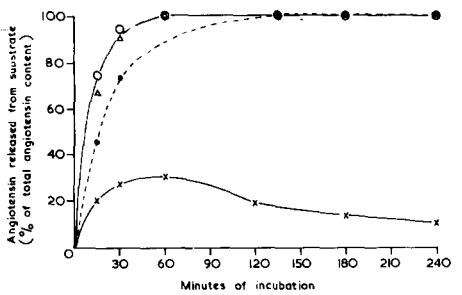


Fig. 1. Effect of mild heating on angiotensinase activity of kidney extracts. Kidney extract was diluted 1:5 with 0.1 M sodium phosphate buffer, pH 6.0, and then aliquots were heated (first incubation) at 20, 56, 58 or 60° for 30 min. After heating, the aliquots were diluted with phosphate buffer to give a final dilution of 1/100. [Asn^1, Val^5]-Angiotensin II was added to 500 ng/ml and these reaction mixtures were incubated (second incubation) at 37° for 180 min. Temperatures of legend refer to first incubation: ▲, 20°; ×, 56°; ○, 58° and ●, 60°.

Fig. 2. Conversion of renin substrate to angiotensin by heated and unheated kidney extracts. Three aliquots of kidney extract were diluted 1:5 in 0.1 M sodium phosphate buffer, pH 6.0. One aliquot was left at room temperature, another was heated at 56° and the third was heated at 58° (first incubation) for 30 min. 1 vol. of each aliquot was incubated (second incubation) at 42°, with 4 vol. of renin substrate (100 ng of angiotensin content per ml) for 240 min. Temperatures of legend refer to the first incubation: ×, room temperature; ○, 56°; △, 58°. The standard renin, ●, was left at room temperature.

$T_{50\%}$ of the extract heated at 56° was 105 min, indicating that less than 10% of the angiotensinase activity of the kidney extract survived being heated at 56°. Extracts heated at 58 or 60° contained even less angiotensinase activity, but none of the extracts was entirely free of angiotensinase activity. Extending the time of heat treatment to 180 min did not cause any greater inactivation.

Extracts of pregnant uterus were treated similarly. The control uterine extract contained less than 20% of the angiotensinase activity of control kidney extracts. The simple procedure of heating the uterine extracts at 56° for 30 min reduced angiotensinase activity by 98%.

The same treatment of other tissue extracts was much less efficacious. Extracts of liver, lung, spleen, thymus and submandibular salivary gland were tested. Their heated extracts contained sufficient peptidase activity to inactivate angiotensin at rates greater than 15 ng/min.

Indirect assay of renin in uterine and kidney extracts

The renin standard and uterine and kidney extracts were heated at 20, 56 or 58° for 30 min and were then incubated with renin substrate. The first experiments were performed using renin in a sufficiently high concentration to allow complete utilization of substrate within 4 h of incubation. In all experiments the concentration of renin substrate was less than 1000 ng of angiotensin content per ml, and its reaction with renin obeyed first order kinetics[5,9].

Results are shown in Figs. 2 and 3. The substrate preparation and the renin standard were each free of angiotensinase activity, thus it could be assumed that the rate of accumulation of angiotensin would equal the rate of angiotensin release and would be a measure of renin activity. Given a sufficient time for reaction the amount of angiotensin accumulated should equal the initial angiotensin content of substrate.

That this was the case is shown by Fig. 2. After 135 min the free angiotensin was equal to that expected from full conversion of substrate. Incubation for an additional 105 min did not result in any loss of angiotensin.

Results obtained by using the control (20°) kidney extract were in marked contrast. The angiotensinase activity of this extract was high, and the maximum amount of angiotensin accumulated did not approach the amount expected from full utilization of substrate.

The kidney (Figs. 2 and 3) and uterine extracts (Fig. 3) heated at 56 or 58° were known to contain some angiotensinase activity. In the dilutions used, the kidney extract was capable of inactivating [Asn[1], Val[5]]-angiotensin II at a rate of 2%/min. However, when these extracts were incubated with renin substrate, *plus* EDTA, dimercaprol and chlorohexidine gluconate[9], their incubation mixtures appeared to be free of angiotensinase activity. The amount of angiotensin accumulated was that expected from full conversion of substrate, and prolongation of the incubation to 240 min did not result in any loss of angiotensin. Indeed, prolonging the incubation to 24 h did not reduce angiotensin by more than 15%.

These results indicate that kidney and uterine extracts heated at 56° are sufficiently free of angiotensinase activity to allow assay of their renin contents by the indirect method. Further experiments were performed using kidney and uterine extracts in high dilutions: 1:1800 and 1:9000, respectively. These dilutions were selected on the basis of the apparent rates of reaction shown in Fig. 3. The conditions of incubation were such that no more than 10% of substrate was converted to angiotensin, thus allowing a precise measure of the initial rate of angiotensin release. Results are shown in Fig. 4. Corrected for dilutions, the renin activities of the kidney

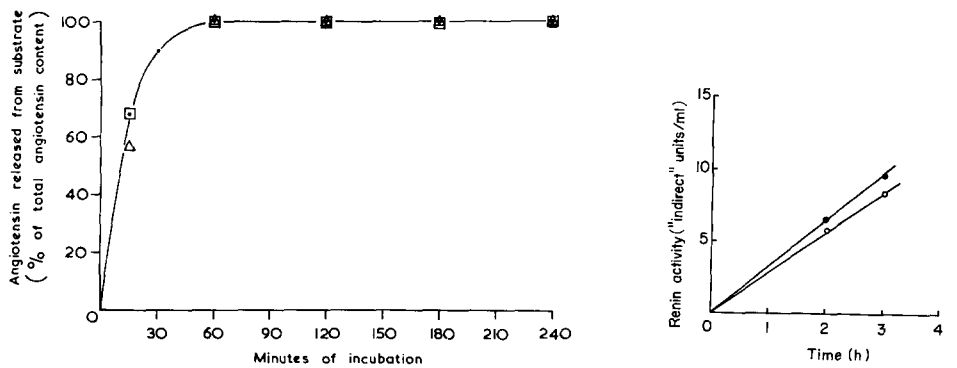


Fig. 3. Conversion of renin substrate to angiotensin by heated extracts of uterus and kidney. Extracts of pregnant uterus and kidney were diluted 1:5 in 0.1 M sodium phosphate buffer, pH 6.0, and then heated at 56° for 30 min. The extracts were then added to renin substrate such that the final dilution of kidney extract (△) was 1:25 and the final dilution of uterine extract (□) was 1:125. The renin standard (●) was not heated before incubation with renin substrate.

Fig. 4. Conversion of renin substrate to angiotensin by dilute extracts of kidney and uterus. Extracts of pregnant uterus and kidney were diluted 1:5 in 0.1 M sodium phosphate buffer, pH 6.0, and then heated at 56° for 30 min. These extracts were diluted again with phosphate buffer and then with renin substrate so that their final dilutions were 1:9000 and 1:1800, respectively. The renin substrate concentration at the beginning of incubation was 500 ng of angiotensin content per ml. ●, kidney extract; ○, uterine extract.

and uterine extracts were 5890 and 25 500 units/ml, or 380 and 2300 units/mg of protein (biuret method).

Replication of assays

The standard renin solution, three kidney extracts and seven uterine extracts were assayed in replicate. The difference between replicate determinations was 8%.

Part II. Partial purification of the renin-like enzyme of uterus

This study was begun to characterize the uterine enzyme and extend its comparison with renin. Renin itself is not fully characterized, but much is known about its behavior in various chromatography systems. Purification techniques used in this study were those previously shown of value for preparing hog renin[6,8]. However, since hog renin may differ from rabbit renin, we carried out a parallel purification of renin from rabbit kidney.

Purification steps

Except where noted, all procedures were carried out at 4°. Results shown in Tables II and III are of extracts of two pregnant uteri and twenty kidneys.

Step 1. Extraction with water. Tissues were put through two freeze–thaw cycles (frozen in an acetone–solid CO_2 slurry, thawed at 20°) and then minced in a meat grinder. The mince was suspended in water, 2 ml/g of tissue, and the suspension was frozen and thawed, stirred for 10 min, and then centrifuged at 2000 rev./min for 30 min. The supernatant was saved, and the precipitate was resuspended in the original volume of water. This procedure was repeated twice more. Finally the precipitate was resuspended in the original volume of water, frozen, thawed, homogenized in a blender at high speed for 1 min, and then centrifuged as before.

Step 2. First adsorption on DEAE-cellulose. The procedure was modified after that of SKEGGS *et al.*[6]. Aqueous extracts of uterus and kidney were diluted (3–5-fold) with water and then mixed with 0.1 vol. of toluene. The aqueous layer was recovered after centrifugation. Moist DEAE-cellulose, free base form, was added to give 15 g of DEAE-cellulose per g of protein (biuret). The suspension was stirred for 10–15 min

TABLE I

EFFECT OF MILD HEATING ON RENIN BUFFERED AT pH 7.4 OR pH 6.0

Aliquots of the standard renin solution, 4 "direct" units/ml, were adjusted to pH 7.4 or 6.0 with 0.1 M sodium phosphate buffer and were then heated for 30 min.

Sample	*pH*	*Incubation temp.*	*Renin activity (direct units/ml)*
Standard	7.4	20°	4
	7.4	56°	<0.4
	7.4	58°	<0.4
Standard	6.0	20°	4
	6.0	56°	4
	6.0	58°	4
	6.0	60°	1.5–2.5

TABLE II

PURIFICATION OF THE RENIN-LIKE ENZYME OF RABBIT UTERUS

Two pregnant uteri were prepared as described in the text. Protein was measured by absorbance at 280 nm, one protein unit having an absorbance of 0.100, 1-cm light path. Renin-like activity is expressed in "indirect" units.

Step	*Vol. (ml)*	*Protein concn. (protein units/ml)*	*Total protein*	*Enzyme concn. (indirect units/ml)*	*Specific activity (indirect units/protein unit)*	*Total enzyme (indirect units)*	*Recovery (%)*
1. Aqueous extract	180	23.4	4212	6 300	269	1 134 000	—
2. DEAE-cellulose pH 4.6, 0.1 M NaCl	500	1.5	750	1 400	933	700 000	62
3. $(NH_4)_2SO_4$ (520 g/l)	25	18.3	458	21 000	1 148	525 000	46
4. DEAE-cellulose pH 7.0, 50 mM sodium phosphate, 0.1 M NaCl	100	2.0	200	3 000	1 500	300 000	26
5. Sephadex G-100 (back-half)	20	0.08	1.6	9 460	118 000	189 200	16.6

and then filtered on a Buchner funnel with vacuum. The inactive filtrate was discarded. The DEAE-cellulose pad was resuspended in the original volume of water, and acetic acid was added to pH 4.6. The DEAE-cellulose pad was again collected by filtration, the inactive filtrate was discarded, and the pad was suspended in half of the original volume of water containing 0.1 M NaCl. The pH was adjusted to 4.6 with acetic acid. The suspension was stirred for 10 min and then filtered. This filtrate was saved, the pad was again suspended in 0.1 M NaCl, the pH was adjusted to 4.6, and the suspension was filtered. The last two filtrates were combined for further processing.

TABLE III

PURIFICATION OF RENIN OF RABBIT KIDNEY

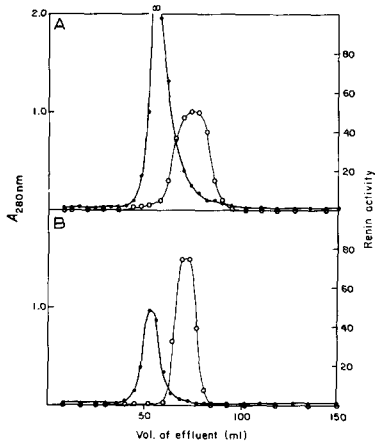
Twenty kidneys were prepared as described in the text. Other details are as indicated in Table II

Step	*Vol. (ml)*	*Protein concn. (protein units/ml)*	*Total protein*	*Enzyme concn. (indirect units/ml)*	*Specific activity (indirect units/protein unit)*	*Total enzyme (indirect units)*	*Recovery (%)*
1. Aqueous extract	500	50.8	25 400	7 200	139	3 600 000	—
2. DEAE-cellulose pH 4.6, 0.1 M NaCl	3200	1.0	3 200	610	610	1 952 000	54
3. $(NH_4)_2SO_4$ (520 g/l)	75	21.0	1 580	23 000	1 090	1 750 000	49
4. DEAE-cellulose pH 7.0, 50 mM sodium phosphate, 0.1 M NaCl	100	3.5	350	12 000	3 430	1 200 000	33
5. Sephadex G-100 (back-half)	20	0.18	3.6	17 500	97 000	350 000	9.7

Step 3. $(NH_4)_2SO_4$ precipitation. Solid $(NH_4)_2SO_4$ was added slowly, with constant stirring, to a concentration of 520 g/l. This mixture was left at 4° for 12–16 h and then filtered using Whatman No. 42 paper. The bulk of the precipitate was scraped off of the filter paper and was saved. The filter paper was washed exhaustively (10–15 times) with small volumes of 0.9% NaCl. Failure to wash exhaustively resulted in renin losses of 70–80%, suggesting a special affinity of renin for the filter paper. The visible precipitate and subsequent wash solutions were combined and then dialyzed, first against 4 l of water (6–12 h) and then against 8 l of 5 mM sodium phosphate buffer, pH 7.0 (12 h).

Step 4. Second adsorption on DEAE-cellulose. This step and the next were modified after the procedures of PEART *et al.*[8]. Moist DEAE-cellulose previously equilibrated with 5 mM sodium phosphate buffer, pH 7.0, was added to the nondiffusible solution obtained by Step 3 (20 g of DEAE-cellulose per g of protein). The suspension was stirred for 10 min and then filtered. The pad was washed by resuspending in twice the original volume of 50 mM sodium phosphate buffer, pH 7.0. The filtrate obtained from this wash contained up to 30% of the renin at a specific activity lower than that of the starting material (Step 3). This filtrate was not processed further. The DEAE-cellulose pad was resuspended in 50 mM sodium phosphate buffer, pH 7.0, containing, in addition, 0.1 M NaCl. The suspension was stirred for 10 min and then filtered. The filtrate contained renin with a specific activity 1.2–3.3 times greater than that of the previous step. Very little more renin was recovered from the DEAE-cellulose pad by repeating the last elution or by raising the NaCl concentration to 0.2 M. The final filtrate was ultrafiltered to about 2–3 ml.

Step 5. Chromatography on Sephadex G-100. The solution retained in the ultrafiltration sac was applied to a column (2.5 cm × 50 cm) of Sephadex G-100 equilibrated with 0.15 M NaCl in 2 mM Tris–HCl buffer, pH 7.4 (see ref. 14). Elution was carried out using the same buffer. Results are shown in Fig. 5. The renin peak was divided into front and back halves. Data for Step 5 in Tables I and III represent the back half.



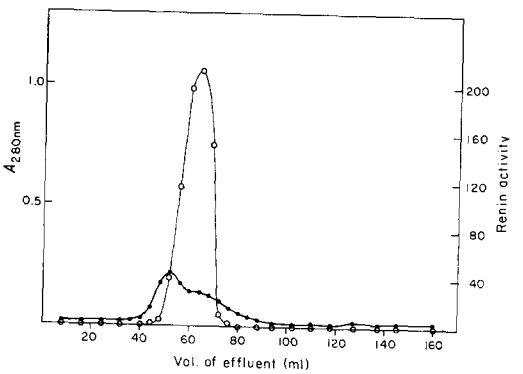


Fig. 5. Chromatography of renin (A) and the renin-like enzyme of uterus (B) on Sephadex G-100 (first cycle). Renin-like activity was estimated by direct assay. ●—●, protein ($A_{280\ nm}$); ○—○, renin-like activity.

Fig. 6. Chromatography (second cycle) of the uterine renin-like enzyme on Sephadex G-100.

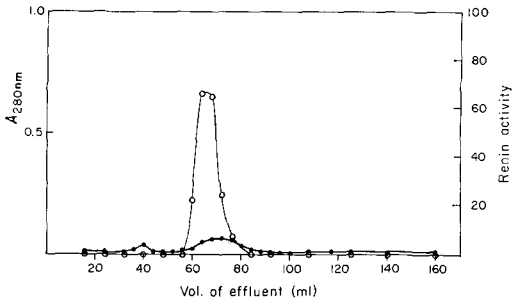


Fig. 7. Chromatography (third cycle) of the uterine renin-like enzyme on Sephadex G-100.

Chromatography on Sephadex G-200 (2.5 cm × 48 cm column) gave poorer resolution, the overlap of renin activity with the major protein peak being more extensive than that shown in Fig. 5. By starch gel electrophoresis (see Step 6) the major protein peak was identified as albumin.

The back half of the renin peak, obtained from the G-100 column, was ultrafiltered and then recycled on the same column. Results obtained with the uterine preparation are shown in Fig. 6. The back half of the second cycle was ultrafiltered and then cycled a third time. These results are shown in Fig. 7. The peak fraction contained the uterine renin-like enzyme at a specific activity 1700 times greater than that of the first aqueous extract. Purification of renin from kidney was slightly lower (1450-fold), owing largely to greater difficulty in removing albumin.

Step 6. Starch gel electrophoresis. Horizontal starch gel electrophoresis was performed as described by HOLLENBERG AND HOPE[15]. A small (4 mm × 4 mm) piece of Whatman No. 1 paper was soaked with the renin solution and then inserted into a slot, near the cathode, in the starch gel block. Electrophoresis was carried out for 4 h at pH 8.6 with a gradient of 10 V/cm. Human serum was included in every run. Bromophenol blue was added to serum to indicate the migration of albumin. After electrophoresis, the upper 1/4th of the block was removed and stained with Nigrosine (0.05%, w/v). Electrophoretic patterns are shown in Fig. 8. Under the conditions of

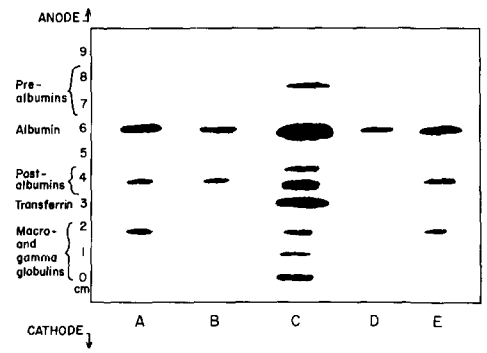


Fig. 8. Starch gel electrophoretic patterns of rabbit kidney and uterus extracts before and after chromatography on Sephadex G-100. Human serum was run for comparison. Columns: A, kidney extract, Step 4; B, kidney extract, Step 5; C, human serum; D, uterine extract, Step 5; and E, uterine extract, Step 4. Renin activity was always found 3–5 mm ahead of albumin and did not stain with Nigrosine. Its position did not correspond with the pre-albumin band of serum.

this experiment, serum albumin migrated 7 cm from the origin. Renin and the renin-like enzyme of uterus were always found as narrow bands in the pre-albumin region, 3–5 mm closer to the anode. In the concentrations used, neither enzyme could be located with Nigrosine. Their bands were located by bioassay. Although renin activity was found, protein was not measurable by absorbance at 280 nm, and we were unable to determine specific activity.

Estimation of molecular size

As shown above, renin and the renin-like enzyme of uterus eluted from Sephadex G-100 after albumin. The enzymes also eluted just after ovalbumin. The molecular weights of renin and the uterine enzyme were estimated[16] to have a mean value of 40 000 ± 3000. This estimate is in fair agreement with a previous study of the molecular weight of hog renin (43 000)[17].

Enzyme stability

In contrast to the apparent instability of highly purified hog renin[8], rabbit renin and the renin-like enzyme of uterus proved to be quite stable. Either enzyme, carried through Step 5, could be heated at 56°, at pH 6, for 30 min without loss of activity. Preparations of each enzyme have been stored at 4°, in sealed silicone-coated glass ampules, for over 1 year without loss of activity.

DISCUSSION

Our results confirm the original observations of TIGERSTEDT AND BERGMAN[10] that rabbit renin is stable at 56°. Its stability at 56° is dependent on pH, renin being stable at pH 6.0 but not at pH 7.4.

Whereas heating at 56°, pH 6.0, does not inactivate renin or the renin-like enzyme of uterus, it reduces angiotensinase activities of kidney and uterine extracts by more than 90%. The same treatment is much less efficient in reducing angiotensinase activities in extracts of tissues such as liver, lung and spleen. The poor results with liver are consistent with the recent observation[7] that rabbit liver contains a heat-stable carboxypeptidase capable of inactivating angiotensin II. This carboxypeptidase accounts for 30% of the total angiotensinase activity of liver extracts. Furthermore, other protease and peptidase enzymes are known to be heat-stable. Cathepsin C, for example, is not inactivated at 60° (see ref. 18).

The simple procedure of heating aqueous extracts at 56°, at pH 6.0, for 30 min allows an accurate assay of renal renin and the uterine renin-like enzyme by the indirect method. Previous studies of renin contents of these tissues have used partial-purification procedures of unproved reproducibility and/or direct assay methods of low precision and accuracy. By taking into account the efficiency of extraction, our procedure provides a basis for measuring the content of renin and renin-like enzymes in rabbit kidney and uterus.

Our results indicate that the renin-like enzyme of rabbit uterus is remarkably similar to renin of rabbit kidney. Either enzyme causes a prolonged rise of mean arterial blood pressure and releases a polypeptide like angiotensin I and unlike angiotensin II (ref. 2). Either enzyme is stable at 56°, at pH 6.0 but not at pH 7.4. Renin and the uterine enzyme behave identically in batch chromatography on DEAE-

cellulose using eluting solvents of decreasing pH and increasing ionic strength or neutral pH and increasing ionic strength. The apparent molecular weights are very similar, either enzyme eluting from Sephadex G-100 just after ovalbumin. Charge and size similarities are emphasized by results of starch gel electrophoresis. Both enzymes migrate toward the anode just ahead of serum albumin. Using the above procedures, with recycling on Sephadex G-100, the specific activities of the two enzymes are increased in parallel by more than 1400-fold. However, these characterizations are incomplete and we cannot conclude that uterine renin-like enzyme is renin.

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REFERENCES

1 F. Gross, G. Schaechtelin, M. Ziegler and M. Berger, *Lancet*, i (1964) 914.
2 J. W. Ryan and T. F. Ferris, *Biochem. J.*, 105 (1967) 16C.
3 T. F. Ferris, P. Gorden and P. J. Mulrow, *Am. J. Physiol.*, 212 (1967) 698.
4 J. Bing and P. Faarup, *Acta Pathol. Microbiol. Scand.*, 67 (1966) 169.
5 J. W. Ryan and J. K. McKenzie, *Biochem. J.*, 108 (1968) 687.
6 L. T. Skeggs, K. E. Lentz, J. R. Kahn and H. Hochstrasser, *Circulation Res.*, Suppl. II, 20–21 (1967) II-91.
7 D. C. Johnson and J. W. Ryan, *Biochim. Biophys. Acta*, 160 (1968) 196.
8 W. S. Peart, A. M. Lloyd, G. N. Thatcher, A. F. Lever, N. Payne and N. Stone, *Biochem. J.*, 99 (1966) 708.
9 J. W. Ryan, J. K. McKenzie and M. R. Lee, *Biochem. J.*, 108 (1968) 679.
10 R. Tigerstedt and P. G. Bergman, *Skand. Arch. Physiol.*, 8 (1898) 223.
11 E. M. Landis, H. Montgomery and D. Sparkman, *J. Clin. Invest.*, 17 (1938) 189.
12 E. Werle, in M. Schachter, *Polypeptides which Affect Smooth Muscles and Blood Vessels*, Pergamon Press, London, 1960, p. 119.
13 M. Schachter, in M. Schachter, *Polypeptides which Affect Smooth Muscles and Blood Vessels*, Pergamon Press, London, 1960, p. 232.
14 C. H. Bolton, J. R. Hampton and J. R. A. Mitchell, *Lancet*, ii (1967) 1101.
15 M. D. Hollenberg and D. B. Hope, *Biochem. J.*, 105 (1967) 921.
16 P. Andrews, *Biochem. J.*, 91 (1964) 222.
17 I. Rubin, H. Olesen and E. Kemp, *Acta Chem. Scand.*, 20 (1966) 2747.
18 H. H. Tallan, M. E. Jones and J. S. Fruton, *J. Biol. Chem.*, 194 (1952) 793.