A Comparison of the Properties of Renin Isolated from Pig and Rat Kidney

By MARTIN LAURITZEN, JENS JØRGEN DAMSGAARD, INGER RUBIN
and EDGAR LAURITZEN

Department of Biochemistry A, The Panum Institute, University of Copenhagen, DK-2200 Copenhagen, Denmark

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1. On isoelectric focusing, renin from rat kidneys showed three activity peaks with pI values at pH 5.0, 5.2 and 5.4 after a purification procedure involving differential centrifugation, acidification, chromatography on Sephadex G-75 and dialysis. 2. The preparation (purified 140-fold) was compared with a crude kidney extract in the absence and presence of 3M-urea by isoelectric focusing. The pattern of activity distribution was confirmed by these experiments and the content of isoenzymes in the three groups calculated. 3. Pig renin was prepared and compared with rat renin with regard to molecular weight, acid activation, behaviour on isoelectric focusing, immunogenicity and substrate affinity. 4. Extracts of rat kidney contained multiple forms of renin with mol.wt. between 39000 and 42000, whereas active pig renin had an approximate mol.wt. of 40000. Acidification of rat renal extracts did not increase the activity of renin, indicating the absence of an inactive form of renin in rat kidneys, whereas pig renin was activated by this procedure. Pig renin has isoelectric points at pH 4.6, 4.8, 5.05 and 5.2, significantly lower than for rat renin. The isoenzymes from the two species had no antigenicity in common, as shown by crossed immunoelectrophoresis or rocket immunoelectrophoresis. 5. The Michaelis constants for pig and rat renin were in the same range, 1×10^{-6} M, when rat renin substrate was used. The relative content of rat isoenzyme with pI in the pH ranges 4.9–5.1, 5.1–5.3 and 5.3–5.5 was approx. 20, 27 and 53% respectively. Purified pig renin prepared in two different ways had isoenzymes with pI in the pH regions 4.5–4.7, 4.7–4.9, 4.9–5.05 and 5.05–5.20 in the approximate proportions 14, 24, 28 and 29%.

Renin is an important enzyme in various tissues, since it catalyses the formation of angiotensin I from angiotensinogen. Heterogeneity of a purified preparation of renin was first described by Haas and co-workers, with the finding of two fractions in free-moving-boundary electrophoresis (Haas *et al.*, 1953b) and three forms in u.v. spectroscopy (Haas *et al.*, 1953c). Since then heterogeneity of renin from a variety of species has been demonstrated by different methods (Morgan & Leon, 1963; Skeggs *et al.*, 1967; Boyd, 1972; Skeggs *et al.*, 1972; Rubin, 1972).

Much confusion has been introduced with the findings of renin-like activity in extrarenal tissues, pseudorenins, and the isolation of zymogens of kidney renin in rabbit and pig (Leckie, 1973; Boyd, 1972). The present paper deals with active renin only, defining renin isoenzymes as kidney renins with a common enzymic and biological activity, but with differences in the physicochemical properties.

Our purpose was to investigate enzymically, physicochemically, immunologically and physiologically the properties of the enzyme and to establish the content of isoenzymes in the kidneys of normal hog and rat.

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Materials and Methods

Renin preparations

Rat renin. The animals (eight white Wistar female rats) were killed with a sharp blow on the neck and the kidneys were immediately removed, placed in ice-cold 0.45M-sucrose and homogenized in 4–8 vol. of sucrose in a Potter–Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 650g for 2½ min in a Sorvall centrifuge, type RC2-B. By this procedure renal lysosomes were kept in the supernatant, and maximum yield of renin was obtained. The supernatant was frozen at –70°C and thawed five times to liberate renin from the lysosomes (Ogino *et al.*, 1967). To compare the non-acidified renin with acidified renin, preparations were centrifuged at 6000g for 10 min and dialysed for 24 h against 3 × 5 litres of 1% glycine. The dialysed preparations were thereafter electrofocused. In all other cases (Rubin & Kemp, 1965) pH was adjusted to 3.6 for 20 min at 25°C with 0.2M-HCl and then re-adjusted to 7.1 by 0.2M-NaOH. To precipitate denatured proteins, the solution was centrifuged at 6000g for 10 min in a Christ Junior 15000 centrifuge. The supernatant was applied to the Sephadex G-75 column.

After Sephadex G-75 chromatography the eight fractions with highest specific activity were pooled,

concentrated sevenfold by vacuum dialysis and stored at 4°C. Pools from four to eight chromatographic runs were collected, dialysed and subjected to isoelectric focusing. All preparations were performed at 2–4°C.

Hog renin. Two different preparations of purified hog renin were used. One was bought from Pentex Biochemicals (Kankakee, IL, U.S.A.), prepared by the method of Haas *et al.* (1953a). Freeze-dried enzyme (250mg) was dissolved in 3.5ml of water. Renin activity was 28 Goldblatt unit/ml (see below) and the specific activity 0.33 Goldblatt unit/mg of protein. The preparation was subjected to Sephadex G-75 chromatography before dialysis and electrofocusing as described below. The other preparation was made by our own method (Rubin, 1972). It should be noted that acidification is included in both methods.

Activation studies of whole rat kidney extracts

A 0.5ml sample of crude rat kidney homogenate squeezed through cheesecloth was dialysed in 500ml of 0.05M-glycine/HCl/0.1M-NaCl buffer at the pH values 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0. After 20h the samples were re-adjusted to pH 7.4 by 0.1M-NaOH. Dialysis for another 20h at neutral pH against 0.5M-Tris/HCl/0.1M-NaCl followed.

A 0.5ml sample dialysed at pH 7.4 was used as a control. Precipitated proteins were centrifuged off at 6000g for 10 min in a Christ Junior 15000 centrifuge and the samples tested for renin activity and protein content.

Column chromatography and determination of molecular weight

Chromatography on Sephadex G-75 (Pharmacia Fine Chemicals, Uppsala, Sweden) was performed in 0.1M-Tris/HCl/1.0M-NaCl, pH 8.0, with 0.02% neomycin sulphate as preservative at 4°C.

The molecular weight of rat renin was estimated by the method of Andrews (1964). The following proteins were used as standards: bovine serum albumin, mol.wt. 67000; chymotrypsinogen A, mol.wt. 25000; cytochrome c, mol.wt. 12500 (all from Boehringer, Mannheim, Germany); ovalbumin, mol.wt. 45000 (Fluka, Buchs, Switzerland); Blue Dextran 2000 (Pharmacia Fine Chemicals) was used to determine the void volume. Calibration runs were performed before and after chromatography of unknown samples.

Isoelectric focusing

The isoelectric focusing was performed according to the manufacturers' instructions in a 110ml column (LKB 8101), cooled to 2°C with circulating

water, in a 1% solution of synthetic carrier ampholytes (Ampholine; LKB, Bromma, Sweden). Narrow-range pH gradients were prepared from pH 4–6 Ampholine. For hog renin a gradient from pH 4.5 to 5.5 was used, and for rat renin a gradient from pH 4.7 to 5.6 or from pH 4 to 6. All samples were dialysed against 1% glycine, pH 6.0, before isoelectric focusing. In two cases urea was added to the preparation to a final concentration of 3M on the isoelectric focusing column.

When the isoelectric focusing had reached a constant power after 1–2 days the experiments were maintained for another 1–3 days to sharpen the bands, although when urea was included the experiments were not maintained for more than 1–3h after constant power was reached. Samples were collected in 2.0ml fractions. pH was measured at 22–24°C and thereafter the fractions were kept at 2–4°C. Renin activity was tested in each fraction. Protein content was determined after 48h of dialysis, first with 2×2 litres of 0.9% NaCl and once against 2 litres of 0.05M-Tris/HCl/0.3% EDTA, pH 7.15, each dialysis taking 16h.

Antisera

Rabbit anti-(pig kidney) serum and rabbit anti-(rat semi-purified renin) serum were prepared as described by Warren *et al.* (1966) by using crude pig kidney homogenate or column-chromatographed rat renin as immunogens.

The rabbit anti-(angiotensin I) serum used in the radioimmunoassay was a generous gift of Dr. K. Poulsen (University Institute of Experimental Medicine, University of Copenhagen, Denmark).

Renin and angiotensin activity

Renin and angiotensin activity was measured either as a direct biological test (Skeggs *et al.*, 1953) in pentobarbital-anaesthetized rats treated with the ganglionic blocking agent, pentolon, or by using the antibody-trapping radioimmunoassay for renin as described by Poulsen & Jørgensen (1974).

Standard pig renin for the radioimmunoassay was from the Medical Research Council, Mill Hill, London N.W.7, U.K. (batch no. 65.119). Standard renin for the biological assay was a commercial preparation (Nutritional Biochemical Corporation, Cleveland, OH, U.S.A.). A correction was made in the calculations for the discrepancy between 1 NBC dog unit and 1 MRC Goldblatt unit. 1 Nutritional Biochemicals Corporation dog unit is 40% of 1 MRC Goldblatt unit. 1 MRC Goldblatt unit for porcine renin was defined in notes from Medical Research Council prepared June 1969. Standard angiotensin I was from Beckman (Geneva, Switzerland) and

standard angiotensin II was kindly supplied by CIBA (Basle, Switzerland).

Renin substrate was prepared from Fischer rats treated for 6 days with 0.25 mg of diethylstilboestrol subcutaneously (Nasjletti *et al.*, 1969), followed by bilateral nephrectomy for 24 h in order to increase the substrate concentration. The plasma was dialysed and acidified for 20 min at pH 3.6 before use. The average concentration of angiotensinogen varied between 3.39 and 4.33 μM expressed as angiotensin I. The determination of K_m of the rat isoenzymes with rat angiotensinogen was performed with a substrate concentration up to 5.12 μM .

Immunoelectrophoresis

The homogeneity of the peaks from column chromatography and isoelectric focusing was investigated by immunoelectrophoresis (Clarke & Freeman, 1966). The primary run comprised three identical agar strips run on the same glass plate covered with 1% agar (1–1.5 mm thick), in sodium barbital or Tris/barbital buffer, pH 8.6, I 0.025 mol/litre and 10 V/cm for 150 min.

The first strip was stained with Coomassie Blue; the second strip was cut in 0.5 cm slices, and renin activity was measured in each slice either by radio-immunoassay or directly on anaesthetized rats. The third strip was used for the secondary run, in which 0.2–2.0 ml of rabbit antisera was added to the agar and the electrophoresis was carried out for 22 h at 3 V/cm and the strip stained with Coomassie Blue (Weeke, 1973).

The antigenicity of the peaks was also investigated by rocket immunoelectrophoresis (Laurell, 1966), by taking advantage of the electrophoresis of antigens in antibody-containing agarose gel. Materials and treatment were the same as in crossed immunoelectrophoresis (Clarke *et al.*, 1966). The equipment used was an electrophoresis apparatus (Multiphor; LKB) cooled by circulating water at 2°C.

Polyacrylamide-gel electrophoresis

Electrophoresis was performed as described by Davis (1964) in a 7% (T) (total concentration of acrylamide, w/v), 2.6% (C) (proportion of the cross-linking agent) gel at pH 8.3, 2.5 mA for 3 h. Rods were prerun for 30 min to remove superfluous ammonium persulphate and acrylic acid. Protein was stained with Coomassie Blue, and renin activity estimated by slicing the gel in 1 cm strips, incubating with rat renin substrate, and testing the angiotensin formed by the direct biological assay.

Protein concentration

Protein concentration was measured by determining the difference in absorbance, $E_{224} - E_{236.5}$,

as described by Groves *et al.* (1968), with crystallized bovine serum albumin (BDH Biochemicals, Poole, Dorset, U.K.) as standard.

Results

General

The pressor substance isolated from rat kidneys was determined as a renin by the characteristic blood-pressure-elevation pattern produced on injection into the anaesthetized rat, by measuring the angiotensin I liberated from a rat plasma substrate, by the fact that the substance was non-diffusible through a dialysis membrane and with protein size.

Purification

The results and steps of the purification procedure are given in Table 1. Acid denaturation of contaminating proteins enhanced the specific activity fivefold. Though a decrease in renin activity of approx. 25% was observed (Table 1), this step still seems to be valuable in renin purification. Sephadex G-75 chromatography gave a fourfold purification, and a typical elution profile is shown in Fig. 1. The rather irregular peak of renin activity was preceded by one large peak of inactive protein, and followed by some non-diffusible yellow material that was greatly retarded on the column. The mol.wt. of rat renin, estimated from the elution volumes, was in the region 39000–42000. The shape of the renin activity peak was independent of the amount of protein applied, the elution rate and the use of acidification procedure.

On isoelectric focusing the different molecular forms showed different increments in specific activities. As shown in Fig. 2, inactive protein was evenly distributed along the pH gradient. Renin, on the contrary, focused in three activity bands at pH 5.0, 5.2 and 5.4, thereby enhancing its specific activity 1-, 2- and 7-fold respectively. The increase in specific activity in the four electrofocusing experiments with purified rat renin varied from 7 to 17 times in the purest fractions.

Isoelectric focusing

The results from eight electrofocusing experiments are summarized in Table 2. When a purified extract from rat kidneys was subjected to isoelectric focusing in the pH range 4.5–5.3, three distinct peaks of enzyme activities with maxima at pH 5.0, 5.2 and 5.4 were detected (Fig. 2; Table 2, run nos. I–IV).

To discover whether the heterogeneity of purified renin (Fig. 2) was due to artifacts produced during the purification procedure, protein-Ampholine or

Table 1. *Preparation of rat kidney renin*

The yields of enzyme activity are expressed as a percentage of the original activity, and purification factors are relative to the preceding step. The first three steps involve 16 kidneys from eight rats. The original material in this electrofocusing experiment comprised ultrafiltered fractions from six gel chromatographies (48 animals) in a total volume of 22 ml, a total enzyme activity of 73 Goldblatt units (MRC) and 49 mg of protein. The yield after isoelectric focusing is therefore expressed as the ratio of activity in the three purest peak activity fractions/total activity in the original material from 48 rats. The overall recovery in the electrofocusing experiment was, however, 80%.

	Protein (mg)	Activity (Goldblatt units)	Specific activity (Goldblatt units/ mg of protein)	Purification factor	Yield (%)
Before acidification	720	48.8	0.07	(1)	(100)
After acidification	100	35.5	0.36	5	72
After Sephadex G-75 chromatography and ultrafiltration	11.25	16.0	1.42	4	33
After electrofocusing:					
Peak 1, pH 5.0	1.80	2.39	1.33	1	2.5
Peak 2, pH 5.2	0.78	1.95	2.50	2	
Peak 3, pH 5.4	0.53	5.44	10.38	7

Fig. 1. *Purification of rat renin on Sephadex G-75*

Column dimensions were 100 cm × 5 cm². ●, Protein concentration; ▲, renin activity. The load was 13.5 ml, containing 27 Goldblatt units of renin and 93 mg of protein prepared from eight rat kidneys as described in the Materials and Methods section. The elution buffer was 0.1 M-Tris/HCl (pH 8.0)/1.0 M-NaCl. Fractions (3 ml) were collected. Fractions 69–84 were pooled and vacuum dialysed. For details see the text.

protein-protein interaction, crude extracts were prepared as described in the Materials and Methods section and run directly on the column.

The distribution pattern of renin activity was of the same character in four separate runs of crude rat kidney extract in the absence and presence of 3 M-urea, and the positions of the renin activity peaks were within the same pH range in all experiments. The difference in the position of peaks is within the experimental error of the pH measurements.

Further, when renin with pI in the pH ranges 4.9–5.1, 5.1–5.3 and 5.3–5.5 was isolated and refocused in three new pH 4–6 gradients, the renin activity peak was observed at pH 4.98, 5.25 and 5.33 respectively. But when the renin with pI 5.1–5.3

Fig. 2. *Isoelectric focusing of purified rat renin*

...., Ampholine gradient; pH 4.7–5.6: —, (histogram) renin activity; —, protein concentration. Purified renin was focused after collection of purified pools. The load was 73 Goldblatt units and 49 mg of protein from 48 animals. Ampholine concentration was 1% (w/v); renin activity was estimated and pH measured immediately after emptying the columns. Protein concentration was measured after 48 h of dialysis. A total of 78 fractions was collected, 2 ml fractions in glass nos. 4–22 and 58–74 and 1 ml fractions in glass nos. 23–57. Other details are given in the text.

isolated from a peak not so well-defined as the one shown in Fig. 2 was refocused, an additional minor peak appeared at pH 5.33, probably a combination of the following greater renin activity peak.

Thus the three explanations seemed unlikely and the microheterogeneity observed was considered to be a true property of rat renin.

In all cases the molecular forms with pI 5.2 and 5.4 were dominant whereas the molecular form with pI 5.0 seemed to be a minor component.

Studies on acid activation of crude extracts

The method of dialysing the sample against 1000 vol. of glycine/HCl buffer at 4°C for 20 h was

Table 2. Isoelectric focusing of rat (a) and pig (b) kidney renin

(a) The enzyme preparations were either crude kidney extracts or partially purified as described in the Materials and Methods section. The focusing was done in the absence or presence of 3M-urea. The distribution of renin activity in the different pH ranges is expressed as the percentage of total enzyme activity found between pH4.9 and 5.5. Activity outside this interval was negligible. (b) The enzyme preparations were either purified by our own method (Rubin, 1972) or processed through step 5 of the method of Haas *et al.* (1953a). The peaks were observed at the same pH values as has been described by Rubin (1972). Renin activity outside the intervals chosen was negligible.

Preparation	No. of animals	Run no.	pH ranges of gradient	pH of observed peaks	pH ...	Distribution of activity (%)			
						4.9-5.1	5.1-5.3	5.3-5.5	
(a) Purified rat renin	48	I	4.5-5.5	5.03, 5.25, 5.44		13	28	59	
	48	II	4.7-5.6	4.95, 4.98, 5.18, 5.38		15	26	59	
	24	III	4.5-5.5	4.94, 5.06, 5.24, 5.39		24	25	51	
	40	IV	4.5-5.5	4.92, 5.22, 5.31, 5.40		29	27	44	
Crude rat renin	4	V	4-6	4.92-5.02*, 5.19, 5.36		13	26	61	
	2	VI	4.7-5.5	5.15, 5.33		0	38	62	
	6	VII	4-6 (+urea)	4.60, 5.06*, 5.20, 5.46		12	25	63	
	8	VIII	4-6 (+urea)	4.89, 4.95-5.05*, 5.28, 5.40		6	18	78	
						Distribution of activity (%)±s.d.			
	No. of runs				pH ...	4.5-4.7	4.7-4.9	4.9-5.05	5.05-5.20
(b) Pig renin (Rubin, 1972)	4	IX-XII	4.3-5.5			14±3	24±3	28±6	29±5
Pig renin (Haas <i>et al.</i> , 1953a)	1	XIII	4.3-5.5			16	23	30	32
	1	XIV	4.3-5.5			10	20	38	32

* Shoulder, not peak.

Fig. 3. Crossed immunoelectrophoresis of two pig renin preparations

The electrophoresis was performed as described in the Materials and Methods section. (a) Protein pattern of 5µg of pig kidney extract analysed after Sephadex G-75 chromatography; renin activity is indicated by the arrow. (b) Protein pattern of 2µg of highly purified pig renin, pI4.9, analysed after electrofocusing. The upper part depicts the crossed immunoelectrophoresis. In the lower part the amount of renin in the individual 0.5cm slices is shown.

considered to be a gentle method for studying the effect of acid treatment on renin activity of the extract. Activity of the dialysed samples was decreased by 40-50% compared with the starting material and unaltered compared with the control sample which

had been dialysed for 40h at neutral pH. We suggest that the lack of an increase in renin activity on acid exposure indicates the absence of a protein-bound or inhibitor-bound form of renin in rat kidney homogenates.

Properties of two purified pig renin preparations

Sephadex G-75 chromatography of the Pentex preparation enhanced the specific activity fivefold; renin activity was eluted in a uniform peak with approx. mol.wt. 40000 as previously described (Rubin, 1972). The distribution of activity in isoelectric focusing is given at the bottom of Table 2. Crossed immunoelectrophoresis of pig renin against rabbit anti-(pig-kidney) serum is shown in Fig. 3. Chromatographed pig renin gave multiple peaks (Fig. 3a), whereas the purest pig isoenzyme from the isoelectric focusing gave only two precipitation peaks (Fig. 3b). Assuming that the amount of renin is equivalent to the area of the peak, the renin peak represents 35% of the cumulated area from both peaks (Clarke & Freeman, 1966).

Properties of the molecular forms isolated by isoelectric focusing

The molecular forms met the requirements mentioned under 'General'; they increased the blood pressure on injection into the anaesthetized rat in a similar manner to a pig renin standard, they liberated angiotensin I after incubation with rat renin substrate and they were isolated in the protein fraction with mol.wt. 40000. We therefore concluded that the molecular forms of renin were isoenzymes, and investigated whether the difference in surface charge was reflected in the kinetic and immunological properties of the enzymes.

The Michaelis constant (Table 3) with a rat renin substrate was of the same order of magnitude, 1×10^{-6} M, for the different isoenzymes; thus the

difference in isoelectric point was not significantly reflected in substrate affinities.

The isoenzymes were precipitated with rabbit anti-kidney serum, within the same species, suggesting that no important immunological part of the enzyme had been removed or destroyed during the preparation procedure. No cross-reaction took place between the two species.

Attempts to purify and investigate the isoenzymes by means of chromatography on Sephadex G-75 after isoelectric focusing were unsuccessful owing to the massive loss of renin activity; 90–95% of the renin activity was lost, the remaining activity being eluted as an irregular peak in the same position as the crude extracts.

Discussion

The results show that a high degree of heterogeneity of kidney renin molecules within the same species and in different species can be detected by isoelectric focusing. The heterogeneity observed within a species seems not to be due to artifacts or non-specific interactions. The distribution pattern of the enzyme would in such cases depend on the purity of the enzyme and the type of purification method. The distribution pattern of rat renin activity was similar, independent of the purity of the applied preparation and the use of the acidification step. In addition the distribution pattern was unaltered in the presence of 3M-urea, a concentration that is considered to abolish most protein-protein or protein-Ampholine interaction. Further, the position of renin activity peaks was confirmed by refocusing. If interactions had been important renin activity would have shifted either between the intervals chosen or to other pH values. Further, the purification procedure did not preferentially isolate any of the molecular forms.

The isoelectric points of rat renin differed by steps of approx. 0.2pH unit and were higher than those of pig renin. As a difference of 0.2pH unit in a protein molecule of this size suggests a difference of one charged group between the isoenzymes (Barrett, 1970), a very subtle difference in primary structure may distinguish the closely related iso-enzymes.

With the finding of 'big renin' in human pathology (Day & Luetscher, 1974; Day *et al.*, 1975) a protein-bound form of porcine renal renin (Boyd, 1974) and an inhibitor-associated form of renin in rabbit kidney (Leckie & McConnell, 1975) that are all sensitive to acids, it was surprising that no increase in renin activity was observed in rat renal extracts after exposure to acid. As the procedure for detection of such complexes was a commonly used one, the observation that renin activity decreased relative to the starting material and was unaltered relative

Table 3. *Michaelis constants for renin isoenzymes*

By using the antibody-trapping radioimmunoassay for renin the Michaelis constants for the reaction between rat and pig isoenzymes and rat renin substrate were determined. Rat or pig renal renin (5 μ l) was incubated with a serial dilution of plasma, pooled from nephrectomized rats, in the presence of anti-(angiotensin I) antibodies at pH 7.4 for 1 h at 37°C (Poulsen & Jørgensen, 1974). The Michaelis constants were calculated from a weighted Lineweaver-Burk plot as described by Wilkinson (1961); substrate concentrations are expressed as μ mol of angiotensin I/litre $\pm$ s.e.m. for one experiment. The total numbers of experiments are in parentheses.

Species	Isoelectric pH	K_m (μ M)
Rat	5.0	3.02 ± 0.55 (1)
	5.2	2.39 ± 0.31 (1)
	5.4	1.91 ± 0.24 (1)
Pig	4.9	1.39 ± 0.41 (3)
	5.0	1.33 ± 0.34 (3)
	5.1	1.45 ± 0.38 (3)

to the control indicated the absence of such complexes from rat kidney extracts. Still the possibility exists that the various forms of rat renin were derived from a common precursor by enzymic degradation in the kidneys, the precursor being unstable during the first steps of the preparation. It is also possible that the isoenzymes represent genetic variants arising from different genes in the same rat. At present the biochemical basis for the charge differences is unknown.

Rubin (1972) reported an increase in pig renin activity after acidification and Haas *et al.* (1953a) noted an increase in initial yield of renin activity after autolysis of a pig kidney extract. The subsequent purification procedures were quite different. However, when we compared the behaviour of renin processed through step 5 of the latter method with our own preparation, they were identical. Both were eluted in one uniform peak on Sephadex G-75 chromatography and both showed multiple forms on isoelectric focusing, with pI values in the same range.

A process analogous to that occurring during the activation of the proteinases of the digestive tract, splitting a peptide bond, may also occur during the activation process of the pig kidney extract. Bettelheim & Neurath (1955) described the occurrence of three forms of chymotrypsin after activation of chymotrypsinogen. If the activation of pig renin occurs by cleavage of peptide bonds near each other, the single uniform peak on Sephadex G-75 chromatography and the multiple forms on isoelectric focusing are not surprising, although the chemical basis for the charge difference is yet unknown.

With this hypothesis in mind it is noteworthy that Newsome (1969) observed only one peak of bovine renin after exposure to pH 2.6 during preparation on Sephadex G-100 chromatography, but four components on electrophoresis, and that Skeggs *et al.* (1972) obtained four peaks on DEAE-cellulose chromatography of human renin, all of which had mol.wt. 39000.

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References

- Andrews, P. (1964) *Biochem. J.* **91**, 222–233
 Barrett, A. J. (1970) *Biochem. J.* **117**, 601–607
 Bettelheim, F. R. & Neurath, H. (1955) *J. Biol. Chem.* **212**, 241–253
 Boyd, G. W. (1972) in *Hypertension 1972* (Genest, J. & Koiw, E., eds.), pp. 161–169, Springer-Verlag, Berlin
 Boyd, G. W. (1974) *Circ. Res.* **35**, 426–438
 Clarke, H. G. M. & Freeman, T. A. (1966) *Protides Biol. Fluids* **14**, 503–509
 Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404–427
 Day, R. P. & Luetscher, J. A. (1974) *J. Clin. Endocrinol. Metab.* **38**, 923–926
 Day, R. P., Luetscher, J. A. & Gonzales, C. M. (1975) *J. Clin. Endocrinol. Metab.* **40**, 1078–1084
 Groves, W. E., Davis, F. C. & Sells, B. H. (1968) *Anal. Biochem.* **22**, 195–210
 Haas, E., Lamfrom, H. & Goldblatt, H. (1953a) *Arch. Biochem. Biophys.* **42**, 368–386
 Haas, E., Lamfrom, H. & Goldblatt, H. (1953b) *Arch. Biochem. Biophys.* **44**, 63–68
 Haas, E., Lamfrom, H. & Goldblatt, H. (1953c) *Arch. Biochem. Biophys.* **44**, 79–93
 Laurell, C.-B. (1966) *Protides Biol. Fluids* **14**, 499–502
 Leckie, B. J. (1973) *Clin. Sci. Mol. Med.* **44**, 301–304
 Leckie, B. J. & McConnell, A. (1975) *Circ. Res.* **36**, 513–519
 Morgan, W. S. & Leon, H. A. (1963) *Exp. Mol. Pathol.* **2**, 317–322
 Nasjletti, A., Matsunaga, H. & Masson, G. M. C. (1969) *Endocrinology* **85**, 967–970
 Newsome, H. H. (1969) *Biochim. Biophys. Acta* **185**, 247–250
 Ogino, J., Matsunaga, M., Saito, N., Kiva, J. & Takayacu, M. (1967) *Jpn. Circ. J.* **31**, 1–8
 Poulsen, K. & Jørgensen, J. (1974) *J. Clin. Endocrinol. Metab.* **39**, 816–825
 Rubin, I. (1972) *Scand. J. Clin. Lab. Invest.* **29**, 51–59
 Rubin, I. & Kemp, E. (1965) *Abstr. FEBS Meet.* 2nd Abstr. no. A49
 Skeggs, L. T., Kahn, J. R. & Marsh, W. H. (1953) *Lab. Invest.* **2**, 109–114
 Skeggs, L. T., Lentz, K. E., Kahn, J. R. & Hochstrasser, H. (1967) *Circ. Res.* **20/21**, Suppl. 2, II-91–II-100
 Skeggs, L. T., Lentz, K. E., Kahn, J. R., Levine, M. & Dorer, F. E. (1972) in *Hypertension 1972* (Genest, J. & Koiw, E., eds.), pp. 149–160, Springer-Verlag, Berlin
 Warren, B., Johnson, A. G. & Hoobler, S. W. (1966) *J. Exp. Med.* **123**, 1109–1128
 Weeke, B. (1973) *Scand. J. Immunol. Suppl.* **1**, 37–46
 Wilkinson, G. N. (1961) *Biochem. J.* **80**, 324–332