Renin Excretion in Patients with Renal Disease

By Achille C. Pessino, M.D., Barry Hulme, M.D., Alessandro Rappelli, M.D., and W. Stanley Peart, M.D.

ABSTRACT

The renal handling of renin was studied in normal subjects and in patients with predominant tubular and glomerular damage. The renin in plasma and urine was assayed in the blood pressure preparation of the rat. Excessive renin excretion occurred only in patients with proximal tubular dysfunction and was not related to creatinine clearance, proteinuria or plasma renin levels. However, a significant correlation was found between renin clearance and clearances of other low molecular weight proteins which pass freely through the glomerular membrane and are normally reabsorbed by the tubules. All the evidence points to the important role played by tubular reabsorption in the control of renin urinary excretion.

ADDITIONAL KEY WORDS
chronic cadmium nephropathy
Fanconi syndrome
renin clearance
nephrotic syndrome
activated human pepsinogen

In their study in the rat, Rappelli and Peart (1) showed that renin, a protein with a molecular weight of 35,000 to 40,000 (2, 3) is filtered and subsequently reabsorbed by the proximal tubule. Lumbers and Skinner (4), studying the origin of renin in human urine, came to a similar conclusion and suggested that renin was subject to variable reabsorption. They also suggested that the tubular reabsorption of renin was similar to that of other proteins of low molecular weight.

It was therefore of interest to study the renal handling of renin in patients with renal diseases in which the main feature was proximal tubular dysfunction.

We have studied 13 normal, control subjects; four patients with Fanconi syndrome, one of whom had Wilson's disease; 13 patients with chronic cadmium nephropathy; one patient at different times after a cadaveric kidney transplant, and, in contrast, five patients with glomerular damage and heavy proteinuria.

Materials and Methods

The 13 normal subjects, 3 females and 10 males, were young volunteers, aged between 18 and 32, on an unrestricted diet and pursuing their normal daily activities.

Of the four patients with Fanconi's syndrome, patients 15 and 16 were sister and brother, aged 54 and 58, respectively. They and patients 14 and 17 showed the bony changes and typical biochemical abnormalities with hyperammoniuria and glycosuria, known to be present since early middle age.

Patient 18, aged 47, suffered from Wilson's disease, manifesting itself as weakness of the legs and unsteadiness of the limbs when the patient was 26. Hyperammoniuria, glycosuria and widespread osteomalacia were the main findings.

Patients 19 to 27 with chronic cadmium nephropathy were previously studied as part of a larger group by Adams et al. (5). They were known to have had proteinuria since 1948. Most of them showed hyperammoniuria and minor reductions in creatinine clearance. In patients 28 to 31, we had the opportunity of measuring only renin concentration in the urine. They were part of a different group of workers who contracted chronic cadmium nephropathy in the alkaline battery industry. Patient 32, a female aged 20, previously subjected to bilateral nephrectomy and maintained on hemodialysis, was studied during recovery from tubular necrosis after transplantation of a cadaveric kidney.

The five patients with nephrotic syndrome...
included two females and three males aged between 22 and 35. The renal biopsy showed in all cases the changes characteristic of chronic membranous glomerulonephritis.

**REIN ASSAY**

Excretion of renin was determined on samples of the 24-hour urine which had been collected at room temperature in bottles containing 200 mg of neomycin and stored at −20°C until assayed.

Renin in urine was measured by two methods: the first was a modification of the Skinner technique for plasma renin concentration (PRC) (6); the second employs ethylene diamine tetraacetic acid (EDTA) and 2,3-dimercaptosuccinic acid (BAL) to inhibit angiotensinase and plasma converting enzyme activities (7, 8).

**FIRST METHOD**

**Materials.**—Buffer A (pH 3.3; 0.10M): glycine, 0.05M; EDTA, 0.0051M; NaCl, 0.0949M; HCl, 0.01M. Buffer C (pH 7.45; 0.175M): NaH₂PO₄, 2-M₆O, 0.0122M; Na₂HPO₄, anhydrous, 0.0867M; EDTA, 0.001Mx; NaCl, 0.076M. Aprotinin (Trasylol FBA, Bayer Germany), 5000 units/ml; neomycin, 10 mg/ml; angiotensinamide (Hypertensin, CIBA), 0.1 μg/ml; and ox serum substrate (9);

One hundred milliliters of urine were dialyzed in Visking tubing (8/32) against buffer A at 4°C under negative pressure (−720 mm Hg). When the volumes were reduced to approximately 2 to 3 ml, dislay was stopped. After incubating at 32°C for 60 minutes, the concentrated urines were dialyzed overnight against buffer C at 4°C. After dialysis, the volumes were measured and the incubated mixture prepared adding 0.50 ml of aprotinin, 0.10 ml of neomycin, and 2.00 ml of ox serum substrate to each sample. The mixture was incubated at 37°C and assayed by the pressor response in the ganglion-blocked anesthetized rat (11). Ox serum substrate or human serum substrate incubated with an excess of human renin produced angiotensin to a concentration of 900 ng/ml and 963 ng/ml, respectively.

To prove the validity of the methods used, we first added human renin to the urine before and after concentration and assayed it by incubation with substrate as usual; the mean recovery was 70% with both methods in 10 experiments.

Angiotensin II was added to ultrafiltered urine to a final concentration of 100 ng/ml and incubated at 37°C to assess angiotensinase activity; after 2 hours, angiotensinase activity was absent with both methods. The results are not corrected for recovery of renin or "ase" activity and are expressed in arbitrary units/24 hours, one unit being the amount of renin that generated 1 ng of angiotensin • ml of incubate⁻¹ • hour of incubation⁻¹.

Both methods were employed in the assay of six urine samples. The difference between the two series of results was not significant (P > 0.40) (Table 1).

Increasing concentrations of activated human pepsinogen (Dr. A. P. Ryle, Edinburgh) were added to human serum substrate and incubated for 30, 60 and 120 minutes to see whether such a proteolytic enzyme could react with substrate under these conditions to produce pressor material. We were unable to detect any pressor response in the ganglion-blocked, anesthetized rat injected with such incubates prepared at pH 3.0 or 7.0 with or without BAL and EDTA present.

The proteolytic activity of this activated human pepsinogen was clearly demonstrated by the method of Northrup (12). At the end of the 24 hours of urine collection, blood samples (20 ml) for estimation of plasma renin activity were drawn without stasis from an antecubital vein after the subject had been resting in the sitting position for 20 to 60 minutes. The syringe

<table>
<thead>
<tr>
<th>Patient</th>
<th>Method 1 (units/ml)</th>
<th>Method 2 (units/ml)</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>12</td>
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</tr>
<tr>
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<tr>
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Circulation Research, Vol. XXVII, December 1970
Comparison of radioimmunoassay and bioassay of angiotensin 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Radioimmunoassay (ng · ml⁻¹ · hr⁻¹)</th>
<th>Bioassay (ng · ml⁻¹ · hr⁻¹)</th>
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<tr>
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</tr>
<tr>
<td>20</td>
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<td>4.5</td>
</tr>
</tbody>
</table>

contained 1 ml of 0.3M EDTA (pH 7.5) and 0.6 ml of 0.2M BAL according to the method of Boyd et al. (7). After separation of the plasma by centrifugation for 5 minutes, samples were incubated at 37°C without added substrate and assayed in the ganglion-blocked, anesthetized rat at 0, 30, 60, 120 minutes and 4 hours using angiotensinamide as standard. Angiotensinase activity in the plasma was nil after 2 hours of incubation.

On four occasions, radioimmunoassay of angiotensin 1 generation in the plasma was performed according to the method of Boyd et al. (13). The values obtained with both types of assay were similar, as shown in Table 2.

Proteinuria was determined by the biuret method (14). Serum and urine creatinine were determined by autoanalyzer using the technique of Chasson et al. (15).

Results
Renin was always present in measurable amounts both in plasma and in urine of normal control subjects (Table 3). Creatinine and renin clearances were determined from the formula

\[ U \times C \times V \]

where U represents the urinary creatinine in mg/100 ml and the urinary renin in arbitrary units, C the correction factor for urinary concentration, V the urine flow in ml/min, and P the plasma creatinine concentration in mg/100 ml and the plasma renin activity in arbitrary units, in the blood samples taken at the conclusion of the 24 hours of urine collections.

The mean creatinine clearance was 110.41 ± 33.9 (SD) ml/min and the mean renin clearance 0.254 ± 0.089 (SD) ml/min. The four patients with adult Fanconi syndrome and the patient with Wilson disease (Table 4) showed a very significant increase in renin excretion compared with normal subjects (P < 0.002). Repeated determinations performed on four subjects (14-16, 18) confirmed these observations. Only two of them had plasma renin activities higher than normal (patient 14 on the second occasion it was measured, and patient 17) (P < 0.15). The mean creatinine clearance was 34.1 ± 1.489 (SD) ml/min, the mean renin clearance 5.273 ± 3.46 (SD) ml/min. In these four

<table>
<thead>
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<th>Measurements in Normal Subjects</th>
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</thead>
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<tr>
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</tr>
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<td>---------</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>12</td>
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<tr>
<td>13</td>
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</tbody>
</table>

PRA = plasma renin activity, ng of angiotensin · ml⁻¹ · hr⁻¹; C<sub>P</sub> = renin clearance, ml/min; C<sub>Cr</sub> = creatinine clearance, ml/min.

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In the first two samples of urine collected from patient 32 during the recovery from tubular necrosis (Table 6), the amount of renin was comparable to the levels found in the patients with Fanconi syndrome and cadmium poisoning. Creatinine clearance was severely impaired at the time of the first two collections (16.7, 14.8 ml/min), but much improved (67.3 ml/min) during the last collection. In this urine sample, the amount of renin was only slightly elevated (1730 units/24 hours).

All the patients with nephrotic syndrome showed an increased excretion of renin in the urine (Table 7). In all the nephrotic patients, plasma renin activities were significantly higher than in the normal subjects, 4.34 ± 2.11 (SD) ng • ml⁻¹ • hour⁻¹ (P < 0.005).

Patients 36 and 37 with a severe reduction in glomerular filtration rate (27, 30 ml/min) also excreted the largest amounts of renin and showed very high renin clearances (2.5 to 1.8 ml/min). In contrast, the other three patients had normal creatinine clearances and the renin clearances were also normal. All these patients showed very heavy proteinuria: 13.76 ± 4.785 (SD) g/24 hours.

**Discussion**

The validity of the method used for the measurement of renin in the urine is support-
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ed by the high recovery of renin (70%) added to the urine before and after concentration and by the similar results obtained when both methods were employed in the assay of the same urine samples.

Croxatto and Croxatto (16) found that plasma globulins incubated with pepsin gave a product with pressor and vasoconstrictor properties, which they named pepsitensin. De Fernandez and Paladini (17) isolated a highly purified pepsitensin from ox plasma incubated with pepsin. They showed it to have the same specific pressor activity and the same amino-acid composition as synthetic valyl-5 angiotensin I. It was necessary therefore to see whether such a proteolytic enzyme could be present in the urine and react with substrate to produce the pressor material which had been generated in the urine samples. As we have already said, we were unable to detect any pressor response in the ganglion-blocked, anesthetized rat injected with human renin substrate incubated with activated human pepsinogen. There is no evidence that renin is excreted in the lower urinary tract and it seems unlikely that it is secreted by the proximal tubules (1).

Renin, which has a molecular weight of 35,000 to 40,000 (2, 11), should readily pass through the glomerular basement membrane, and it may be expected that relatively large amounts would appear in the urine. However this is not the case, since in normal subjects we found a mean renin clearance of only 0.254 ± 0.089 ml/min (Table 3).

Rappelli and Peart (1) showed that in rats infused with rat renin, most of the filtered renin was probably reabsorbed in the proximal tubules. Renin would thus behave like many other proteins with a molecular weight of less than 68,000 which normally would be reabsorbed in the proximal tubules. The same explanation may be applied in the case of the patient with evidence of recovering tubular necrosis following a cadaveric kidney transplant. Our observations would agree with the study by Sachs and Sachs (24), in which they described a pattern of proteinuria which is likely to have represented increased excretion of low molecular weight proteins.

In two of the patients with Fanconi syndrome (16 and 17) and in the patient studied after a cadaveric kidney transplantation, renin clearances, expressed as percent of creatinine clearances, were the highest found in our study: 30, 29 and 24, respectively. If we...
Measurements in Patients with Chronic Cadmium Nephropathy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Renin secretion (ng/ml/24 h)</th>
<th>PRA (ng • ml⁻¹ • hr⁻¹)</th>
<th>Diuresis (ml/24 h)</th>
<th>Cmin (ml/min)</th>
<th>CO (ml/min)</th>
<th>Cmin as % of CO</th>
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<tr>
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<td>2.5</td>
<td>500</td>
<td>1.77</td>
<td>58</td>
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<tr>
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<td>370</td>
<td>0.83</td>
<td>1800</td>
<td>3.14</td>
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<td>1.52</td>
<td>1500</td>
<td>1.76</td>
<td>59</td>
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<tr>
<td>30</td>
<td>390</td>
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<tr>
<td>31</td>
<td>365</td>
<td></td>
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</tbody>
</table>

Mean (±SD) 509 ± 180 1.63 ± 0.9 2.51 ± 0.9 64.4 ± 17.0 4.25 ± 2.27

Abbreviations same as in Table 3.

This table includes data taken from the paper by Adams et al. (5) on lysozyme, ribonuclease and uric acid clearances.

For renin (2, 11), we can compare the clearance of this protein with the clearances of other proteins described in the literature (25, 26).

![Figure 1](http://circres.ahajournals.org/)

FIGURE 1

Renin clearance expressed as percent of glomerular filtration rate (GFR) and plotted against molecular size of renin and other proteins in patients with insignificant tubular reabsorption.

Similar data on other proteins from the literature (25, 26).
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<table>
<thead>
<tr>
<th>(ml/min)</th>
<th>Cly as % of GFR</th>
<th>(ml/min)</th>
<th>Cly as % of GFR</th>
<th>Creatinine as % of GFR</th>
<th>Proteinuria (g/24 hr)</th>
<th>Aminoaciduria</th>
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<tr>
<td>0.209</td>
<td>0.213</td>
<td>0.17</td>
<td>0.173</td>
<td>25</td>
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<tr>
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<tr>
<td>0.239</td>
<td>0.412</td>
<td>3.941</td>
<td>6.785</td>
<td>28</td>
<td>0.193</td>
<td>+</td>
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</table>

28) (Fig. 1): Its clearance is consistent with those of other proteins with respect to its molecular size, assuming negligible tubular reabsorption.

All five patients with nephrotic syndrome had heavy proteinuria, but this was not the only feature which distinguished them from the other patients studied; all had the high plasma renin activities expected in patients with secondary hyperaldosteronism (27, 28). In the two patients with a severely impaired glomerular filtration rate, plasma renin levels were elevated even higher than the other three cases. With plasma renin levels higher than normal, the concentration of renin in the glomerular filtrate would also be increased and tubular reabsorption might be saturated.

However, in the three patients with normal creatinine clearances, we found renin clearances within the normal range. Our results are in accord with those of Lumbers and Skinner (4); these workers found normal renin clearances in normal subjects even when the plasma renin concentration was elevated to at least 2.5 times the control levels by sodium depletion.

In the two nephrotic patients with renin plasma levels raised 3.6 and 5.4 times the control levels and with severely reduced creatinine clearances, renin clearances were significantly increased. It is unlikely that low glomerular filtration rates might so raise the blood levels of renin that its concentration in the glomerular filtrate exceeds the usual reabsorptive capacity of the tubules. A more likely explanation is that their increased excretion reflects the tubular atrophy which is usual in advanced chronic renal disease. Similar increase in the elimination of low molecular weight proteins has been observed by Prockop and Davidson (29) and by Harrison (23).

In the patients with both glomerular and tubular damage (Table 7), we did not find any correlation between renin excretion and degree of proteinuria (P > 0.35); indeed three of our patients with heavy proteinuria showed normal renin clearances. It is not justified therefore to think that the increased renin excretion, even when found in patients with heavy proteinuria, is due to a competitive nonselective protein reabsorption by the tubules. Furthermore, our findings confirm the results of several other workers on the lack of correlation between the extent of proteinuria and the excretion of low molecular weight proteins (29, 30). This would indicate that a defect in glomerular filtration leading to proteinuria is not itself enough to increase renin excretion, but that it must be associated with a tubular defect as well.

All the evidence here points to the important role of tubular reabsorption in control of renin urinary excretion in man as in the rat.
### TABLE 6

**Measurements in Patient after Renal Transplantation**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Days after transplant</th>
<th>Renin excretion (units/24 hr)</th>
<th>PRA (ng · ml⁻¹ · hr⁻¹)</th>
<th>Diuresis (ml/24 hr)</th>
<th>C&lt;sub&gt;Cr&lt;/sub&gt; (ml/min)</th>
<th>C&lt;sub&gt;Cr&lt;/sub&gt; as % of C&lt;sub&gt;Cr&lt;/sub&gt;</th>
<th>Proteinuria (g/24 hr)</th>
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</table>

Abbreviations same as in Table 3.

### TABLE 7

**Measurements in Patients with Nephrotic Syndrome**

<table>
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<th>Patient</th>
<th>Renin excretion (units/24 hr)</th>
<th>PRA (ng · ml⁻¹ · hr⁻¹)</th>
<th>Diuresis (ml/24 hr)</th>
<th>C&lt;sub&gt;Cr&lt;/sub&gt; (ml/min)</th>
<th>C&lt;sub&gt;Cr&lt;/sub&gt; as % of C&lt;sub&gt;Cr&lt;/sub&gt;</th>
<th>Proteinuria (g/24 hr)</th>
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<td>6.200</td>
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</table>

Abbreviations same as in Table 3.
RENN EXCRETION

References


Renin Excretion in Patients with Renal Disease

ACHILLE C. PESELLA, BARRY HULME, ALESSANDRO RAPPELLI and W. STANLEY PEART

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