Renal Excretion of Renin in the Rat

By Alessandro Rappelli, M.D., and W. Stanley Peart, M.D.

ABSTRACT
The renal excretion of renin infused intravenously was studied in anesthetized rats. The renin in the urine was adsorbed onto DEAE cellulose and selectively eluted for separate assay on the rat blood pressure preparation. The influence of saline loading, furosemide, mercuric chloride, and maleic acid was determined by following the changes in renin excretion. Both mercuric chloride and maleic acid led to a sixfold increase in the amount of renin appearing in the urine. Among the various factors which might have led to this result, an effect on renal tubular reabsorption after glomerular filtration of the renin is the most likely.

ADDITIONAL KEY WORDS
furosemide  mercuric chloride  maleic acid

It is almost certain that renin is stored or made in the afferent arteriolar wall forming part of the juxtaglomerular complex and is released into the blood stream to appear in the renal venous blood (1). The molecular weight of renin is about 35,000 to 40,000 as judged by Sephadex gel filtration and ultracentrifugation (2-4). This means that unless the molecule has an unusual shape, it should readily pass the glomerular membrane to enter the filtrate, and further that it should do this immediately after release from the arteriolar wall into the blood. It would therefore be expected that relatively large amounts would appear in the urine unless other factors intervened, and while there has been little study of this point, a "renin-like enzyme" was found in human urine (5). Since previous observations in this laboratory suggested that very little renin appeared in the urine of rats receiving intravenous injections of renin for assay purposes, (W. S. Peart, unpublished observations), we have undertaken a fuller investigation of the renal excretion of renin. The amount of renin which appeared in the urine of anesthetized rats after intravenous infusion of a standard amount was measured, and since renal reabsorption of filtered renin was thought to be an important factor, the action of substances known to affect renal tubular reabsorption was also studied.

Methods

Materials
Rat renin was prepared by Dr. M. Cuthbert using saline extraction, ammonium sulphate precipitation, acid denaturation of impurities, and chromatography on DEAE cellulose (2). The final solution contained 80 units/ml in 0.9% sodium chloride solution. A unit is equal to the pig unit previously described (2); as a single injection it usually raises the blood pressure of the anesthetized rat preparation (6) by 5 mm Hg.
Angiotensin (Hypertensin, CIBA) standard solution 0.1 μg/ml in 0.9% sodium chloride.
Mercuric chloride was used as a 0.135% solution in water (1 mg/ml mercuric ion).
Maleic acid 0.1M in water adjusted to pH 7.4-Chloro-N(2 furfurylmethyl)5 sulfamoylsulfanylic acid (furosemide), (10 mg/ml water).

Experimental Procedure
Male white Wistar strain rats weighing between 200 and 300 g were anesthetized with pentobarbital, 0.1 ml/100 g ip, followed by smaller doses intravenously to maintain anesthesia. Intravenous infusions were given by a fine polyethylene catheter into the jugular vein, and the urine was collected by a polyethylene catheter inserted directly into the bladder through a suprapubic incision. All infusions were given at a rate of 0.25 ml/min by a motor-driven syringe.

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Circulation Research, Vol. XXIII, October 1968
Urine was collected separately during each of the following four periods and stored at 4°C: A. 0.9% sodium chloride for 1 hour  B. renin, 80 units/ml for 20 minutes (400 units)  C. 0.9% sodium chloride for 20 minutes  D. 0.9% sodium chloride for 20 minutes.

Recovery of Renin from Urine.—The volume of urine in each period was measured, and each sample was dialyzed (Visking 8/32 tubing) against 0.005M phosphate buffer, pH 7, at 4°C overnight. Each sample was applied to a separate column (0.6x8 cm) of DEAE cellulose equilibrated with the same buffer. The column was then washed successively with 6 ml of the same buffer, 0.025M phosphate buffer, pH 7, and finally 0.075M phosphate buffer, pH 7, in which the renin was eluted in 1-ml fractions. The ultraviolet absorption was read in a spectrophotometer at 280 m\textmu to give the approximate protein content (Fig. 1).

Measurement of Plasma Renin Levels during Infusion.—Three groups of three rats were treated as described above: (1) control, (2) mercuric chloride, 5 mg/kg 24 hours before infusion, (3) maleic acid 1 ml of 0.1M solution/kg, ip, 90 minutes before infusion. At midpoint of the renin infusion (10 minutes), 0.5 ml of blood was withdrawn slowly from the contralateral jugular vein into heparin. The plasma was separated by centrifugation and dialyzed overnight (Visking tubing 8/32) at 4°C against a large volume of distilled water. The renin in each plasma sample was assayed directly without further treatment on the anesthetized rat, as in the following paragraph. Because of the possibility that bleeding could cause a change in renal function, the

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**FIGURE 1**

DEAE cellulose chromatography of rat urine collected during renin infusion.

**FIGURE 2**

Direct assay on the rat blood pressure preparation of neat urine. Rat urine (U) compared with standard rat renin (R). Doses in milliliters.

**FIGURE 3**

The direct pressor effect of fractions E12 and E15 eluted after chromatography of urine on a DEAE column. There is the same type of pressure curve as with the standard rat renin (R). All doses in milliliters.

**FIGURE 4**

Indirect renin assay on the rat blood pressure preparation. The following mixtures were incubated at 37°C for 60 minutes and then assayed on the rat against a standard solution of angiotensin (A) containing 0.1 \textmu g/ml. R = 0.05 ml standard rat renin (4 units) + 0.45 ml phosphate buffer (0.075M, pH 7.0) + 2.0 ml ox substrate. E = 0.50 ml elution fraction (1 renin-containing fraction eluted after DEAE chromatography of urine) + 2.0 ml ox substrate. C (control) = 0.50 ml phosphate buffer (0.075M, pH 7.0) + 2.0 ml ox substrate.
animals used for measurement of renin plasma levels were not used for the urinary excretion studies.

Assay of Renin.—This was carried out on the individual fractions from the DEAE column by direct intravenous injection into the anesthetized rat (8); and the response of arterial blood pressure was compared with standard rat renin by a bracketing procedure (Figs. 2 and 3). The total excretion was expressed as percent of the total units infused.

That the material was renin was further shown by its ability to produce an angiotensin-like material when incubated with substrate (7) (Fig. 4).

Protein.—In addition to the ultraviolet absorption measurements, the protein content of the urine in each period was estimated by a turbidometric method (0.6 ml of 3% salicylsulfonic acid was added to 0.2 ml of urine and after 10 minutes read at 650 mg in a spectrophotometer).

Administration of Substances Affecting Tubular Reabsorption.—Mercuric chloride, 5 mg/kg (mercuric ion), was injected into six animals. In three, administration was 3 to 4 hours before the renin infusion and in the rest, 24 hours before.

Maleic acid was injected intraperitoneally (1 ml of 0.1 M solution/kg) 90 minutes before the renin infusion.

Furosemide was administered as a steady intravenous infusion by incorporating it in both the

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**TABLE 1**

Data on Urinary Function and Protein and Renin Excretion in All Animals

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Maleic acid</th>
<th>Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin (units)</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Protein (mg/ml)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% of total renin</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
</tbody>
</table>

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*Urine samples were pooled for renin assay.*

_Circulation Research, Vol. XXIII, October 1968_
Results

Recovery of Renin from DEAE Cellulose

The overall recovery, checked by adding standard renin to urine and taking it through the method, was about 60% ± 10%. This step was used because variable depressor responses vitiated the results when neat urine was used in the assays. This depressor effect was lost after chromatography, a procedure which also allowed concentration of the renin from larger volumes of urine.

Effects of Infusion

Normal Saline and Renin Infusions (Table 1, Fig. 5).—As usual, a diuresis occurred during the renin infusion and continued after its cessation. As previously reported (8), proteinuria occurred and rapidly returned to control levels. The excretion of renin was small and the mean for the six experiments was 2.92%, SD ± 1.83 (Tables 1 and 2).

Mercuric Chloride Followed by Renin Infusion in 3 to 4 or 24 Hours.—The diuresis was the same as in the controls except in one rat (no. 6), in which it was markedly in excess. The excretion of renin rose markedly in these animals and the mean for the six experiments was 17.48%, SD ± 10.42 (Tables 1 and 2).

Maleic Acid Followed by Renin in 90 Minutes.—The diuresis was similar to the control series, as was the proteinuria in response to renin, and the renin excretion was again high, with a mean of 18.16%, SD ± 7.99 (Tables 1 and 2).

Furosemide Given with the Saline and Renin Infusions.—The diuresis was very great compared to that in the other experiments, and the proteinuria with one exception (no. 3) was similar to the others. Urine flow was uniformly reduced during and after renin infusion. The renin excretion, however, was like that of the controls, with a mean of 2.5%, SD ± 1.82 (Tables 1 and 2).

Plasma Renin Levels during Infusion.—The plasma levels in the three groups of rats infused separately for this purpose are shown in Table 3. Although individual variation in the plasma levels is apparent, there is no consistent difference between the groups, which would not support the concept that higher levels in the rats treated with mercuric chloride and with maleic acid could explain the increased urinary excretion.

Significance of the Results.—Statistical treatment of the data (Table 2) showed that the excretion of renin in the groups treated with mercuric chloride and with maleic acid was significantly raised compared with controls (Student's *-test, P<0.01). There was no difference between the control and the furosemide-treated series, nor between the mercuric chloride and maleic acid groups.

Protein Excretion.—The usual increase in protein excretion occurred with infusion of renin in all the groups of rats in which it was measured, and for purposes of comparison, normal and furosemide-treated rats (six) were compared with the mercuric chloride- and maleic acid-treated rats (seven) (Tables 1 and 4). On such small numbers, it was not

<p>| TABLE 2 |
| Renin Excretion in All Animals as Percent of Total Number of Renin Units Infused |</p>
<table>
<thead>
<tr>
<th>Control</th>
<th>Mercuric chloride</th>
<th>Maleic acid</th>
<th>Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>14.5</td>
<td>26.0</td>
<td>3.0</td>
</tr>
<tr>
<td>2.0</td>
<td>37.5</td>
<td>15.0</td>
<td>5.0</td>
</tr>
<tr>
<td>2.5</td>
<td>18.5</td>
<td>17.5</td>
<td>2.5</td>
</tr>
<tr>
<td>0.0</td>
<td>16.0</td>
<td>28.0</td>
<td>0.0</td>
</tr>
<tr>
<td>4.5</td>
<td>6.7</td>
<td>6.0</td>
<td>3.5</td>
</tr>
<tr>
<td>5.0</td>
<td>13.7</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td><strong>2.92</strong></td>
<td><strong>17.48</strong></td>
<td><strong>18.16</strong></td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>±1.83</td>
<td>±10.42</td>
<td>±7.99</td>
</tr>
</tbody>
</table>

Control vs. mercuric chloride, P < 0.01; control vs. maleic acid, P < 0.01; control vs. furosemide, not significant; mercuric chloride vs. maleic acid, not significant.

<p>| TABLE 3 |
| Renin Units per Milliliter of Plasma at Midpoint of Infusion |</p>
<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Control</th>
<th>Mercuric chloride</th>
<th>Maleic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Circulation Research, Vol. XXIII, October 1968
thought worthwhile to carry out any further analysis but there was clearly no obvious difference between these groups.

Change in Urinary Flow with Renin Infusion.—With the exception of the furosemide-treated group, in which the flow fell, there was no obvious difference between the other three groups (normal, mercuric chloride- and maleic acid-treated) (Table 1).

Discussion

The results show clearly that the renal excretion of infused renin is small in the rat anesthetized with pentobarbital. Since the effects of mercuric chloride and maleic acid are mainly on the proximal tubule (9-11), and there is little or no effect on glomeruli (9, 12) and less on the distal tubule (11), it seems likely that these substances are acting on enzyme systems responsible for reabsorption of renin in the proximal tubule. The basic mechanism may therefore be the same as the effect of maleic acid on the reabsorption of amino acids and glucose (11, 13) and is reminiscent of the Fanconi syndrome produced by chronic cadmium poisoning in man (14-16). A striking feature of the latter condition is the "tubular proteinuria," in which proteins with a molecular weight of up to about 50,000 escape in the urine. The increased excretion of renin under the influence of mercury and maleic acid in the present experiments was accompanied by proteinuria, but this did not seem to be quantitatively different in most animals from the proteinuria produced by renin infusions in the control animals. In this latter type of proteinuria in normal animals, the proteins are mainly albumin (8), and in the present experiments there was no apparent correlation between the percentage of renin excreted and the degree of proteinuria (compare rat 6, control, and rat 4, mercuric chloride; rat 3, maleic acid, and rat 4, furosemide, in Table 1).

If mercuric chloride or maleic acid caused a change in the pore size of the glomerular membrane, it would be expected that proteins such as albumin, which are normally excreted in larger amounts by the rat (8), would also increase in amount. This was not the case (Tables 1 and 4). An alternative explanation would then be that mercuric chloride and maleic acid had a selective effect on the smaller pores of the glomerular membrane, allowing renin but not albumin to escape. While this is possible, it seems much less likely. Tubular excretion of renin is, of course, a possibility, but since maleic acid and mercuric chloride seem to act by damaging biochemical processes in the tubular cells (9-13), such excretion would be expected to decrease. If the renin levels in the plasma of the mercuric chloride- and maleic acid-treated groups rose to higher levels than those of the other groups because of a change in metabolic clearance, then renal clearance might rise. The separate experiments in which plasma levels of renin were measured in the different groups, while showing fairly wide individual variation, give no support to this possibility (Table 3). Similarly, if the glomerular filtration rate in the mercuric chloride- and maleic-acid treated groups rose much higher than in the control animals, renin excretion might rise. Renin in large doses always reduces the glomerular filtration rate in all animals, including the rat (1); judging by the similar effects on urine flow in the control, mercuric chloride-, and maleic acid-treated groups, it seems unlikely that there would be

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**Table 4**

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. rats</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal and furosemide</td>
<td>6</td>
<td>2.7</td>
<td>12.5</td>
<td>9.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Mercuric chloride and maleic acid</td>
<td>7</td>
<td>4.1</td>
<td>9.5</td>
<td>5.9</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Data derived from Table 1. A, B, C and D are periods of urine collection.
any great difference in the effect on filtration rate, although this could not be excluded absolutely. Since the increase in renin excretion in the mercuric chloride- and maleic acid-treated groups is sixfold, none of these possibilities seems as likely as interference with tubular reabsorption after filtration.

The present findings for renin in the rat resemble those previously reported for insulin clearance in patients with chronic cadmium poisoning (17), in whom insulin was cleared in the urine in large amounts in contrast to normal subjects. Together with the present findings, this supports the view that proteins with molecular weight of up to 50,000 are mainly reabsorbed in the proximal tubule (18). It is not known if this process serves any physiologic function, nor what the fate of the reabsorbed proteins may be. It could mean that if renin was stored in the cells into which it was reabsorbed, there would be a site with a high concentration. It is worth noting that earlier experiments in the rabbit did not show renin in the proximal tubules (19). The main tubular site from which renin has been extracted is the macula densa in the distal convoluted tubule (20), and there is some doubt about interpretation of the results (1). Nothing in the present data could positively preclude reabsorption at a site lower than the proximal tubule. The proposed great capacity of the kidney to reabsorb renin carries other implications. If it is stored, then the total amount of renin in the kidney should rise following its infusion, and if it is subsequently shown not to do so, then it would have to be either destroyed or returned to the circulation via the blood or lymph (21). It seems less likely to be destroyed since it is very stable in homogenates made in extracting renin from kidneys, and one method of increasing the yield consists of freezing and thawing with a preliminary autolytic step (22). Any destructive enzymes might, however, require an organized intracellular system to be effective, or might themselves be destroyed in the extraction process, so that argument is not conclusive. In our experiments, the persistence of renin excretion into the periods after the infusion was stopped suggests that the rate of removal by other tissues is slow under these circumstances, and this agrees with other evidence (23). The action of furosemide, which caused a very great diuresis, shows that a diuretic action alone is not enough to cause increased excretion of renin and that the sodium-reabsorbing systems in the proximal and distal tubules (24) would not be implicated in renin reabsorption. Two preliminary experiments with mersalyl (mersalyl injection B.P.), an organic mercurial diuretic with theophylline, showed no change in renin excretion; this again suggests that either a different enzyme system is responsible or that it has to be more markedly interfered with, as by the use of inorganic mercury. The final conclusion is that any procedures directed at the kidney in which renal arteriovenous renin differences are measured should take account of any possible interference with tubular reabsorption and loss of renin into the urine.

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