Chronic One-Kidney Hypertension in Rabbits

I. TREATMENT WITH KIDNEY EXTRACTS

By Leonard T. Skeggs, Joseph R. Kahn, Melvin Levine, Frederic E. Dorer, and Kenneth E. Lentz

ABSTRACT

An investigation into the mechanism that sustains the blood pressure in chronic one-kidney hypertension in rabbits was made using passive and active immunization with hog kidney extracts containing renin and with angiotensin antagonists. Seven hypertensive rabbits were passively immunized for extended periods with antiserum prepared in other rabbits. Antirenin levels were in the range of 1-3 units/ml. Control experiments demonstrated that antirenin concentrations of 1.0 unit/ml or more eliminated more than 99% of the pressor response to renin. There was no lowering of blood pressure that could be attributed to the antibodies. No decrease in blood pressure was observed in 13 experiments in 7 rabbits given the angiotensin II antagonist l-Sar-8-Ile-angiotensin II; infusion rates of 0.09-87 μg/min kg⁻¹ were used for periods of a few hours up to 3 days. A reduction in blood pressure occurred in 16 of 19 rabbits immunized directly with extracts containing renin with specific activities of 9.6-757 GU/mg. Plasma antirenin titers correlated poorly with reductions in blood pressure. The blood pressure of 22 rabbits given equal amounts of protein without renin was unaffected. It is concluded that the elevation of blood pressure in rabbits with chronic one-kidney hypertension is not dependent on circulating renin or angiotensin; rather, it results from the presence of renin in an extravascular location or from an unknown pressor substance.
renin. However, the effect of the antiserum was followed for only 40 minutes, and the actual titer of antirenin that was administered is difficult to ascertain and may have been very small.

A recent report has described the administration of anti-hog renin dog serum to hypertensive rabbits (11). No decrease in blood pressure was obtained in chronically hypertensive rabbits of the one-kidney type. The amounts of antirenin that were given were very small (6.4 units). Although plasma antirenin titers were not determined, the authors calculated that the maximum possible value was 0.02 units/ml. This level is far less than the 1-3 units/ml that Wakerlin (3) found necessary. The authors themselves stated that their level of antibody may not have been sufficient to lower the pressure of their one-kidney hypertensive rabbits (11).

The foregoing review indicates that immunization with heterologous renin preparations has lowered the blood pressure in hypertensive dogs, and one might expect that a similar immunization procedure would be effective in other species as well. If indeed this expectation is true, then blocking the renin-angiotensin system at some other point should be equally effective.

A review of the literature shows that several investigators have lowered the blood pressure of acutely hypertensive animals using anti-angiotensin antibodies, angiotensin antagonists, or converting enzyme inhibitors (12-16). Others have succeeded with animals having the chronic two-kidney form of hypertension (13, 14, 16-20), although several groups of workers have failed (14, 21-23).

Investigators who have worked with one-kidney hypertensive animals have been almost universally unsuccessful (13, 14, 16, 19, 21, 24, 25). The three exceptions are Gavras et al. (26), who lowered the pressure of one-kidney hypertensive rats after severe sodium depletion, Christlieb et al. (18), and Bing and Neilson (20). These last workers used very short-term infusions of an angiotensin antagonist in acute experiments in rats that were usually anesthetized. The use of anesthesia is a serious criticism which can be applied to all who have used angiotensin antagonists except for Bumpus et al. (16) and Johnson and co-workers (27), who did infuse dogs with chronic one-kidney hypertension for up to 72 hours with negative results.

Three groups of workers (21, 24, 25) have found anti-angiotensin II antibodies to be ineffective in hypertensive rabbits immunized before or after clipping or wrapping of their kidneys. Johnson and co-workers (28) have very recently reported blood pressure reductions in two of nine rabbits with chronic one-kidney hypertension as a result of a 30-minute infusion of angiotensin antagonist.

It is apparent that the mechanism sustaining high blood pressure in chronic one-kidney hypertension is still unknown. In addition, there is a serious contradiction in the literature which indicates that blocking renin lowers the blood pressure of one-kidney hypertensive animals but that neutralizing angiotensin II or preventing its formation from angiotensin I does not. The present communication describes the first of a series of experiments designed to resolve this contradiction and to discover the mechanism that is responsible for the elevation of blood pressure in chronic one-kidney hypertension in rabbits.

**Methods**

**MEASUREMENT OF BLOOD PRESSURE**

Blood pressure measurements in those experiments involving passive immunization with antirenin and infusion of angiotensin II antagonist were obtained by direct recordings from the lower aorta. Polyethylene catheters were implanted in the large vessel and brought out through the abdominal wall and beneath the skin to the back of the neck.

The catheters were prepared and surgically implanted using a slight modification of the method of Brooks and Muirhead (29). Heparinized saline (10 units/ml) containing phenylmercuric acetate (2.5 x 10^-5 M) as a preservative was infused at the very low rate of 0.01-0.025 ml/min to keep the tip of the catheter open. This flow did not affect the recording as long as a good pulse pressure was evident, nor was there any affect on blood pressure as judged by control periods lasting many days. Routine daily blood pressure measurements lasting only a few minutes were made on conscious rabbits while they were restrained but not cramped in specially built small boxes. Use of a MP 15 Micro pressure transducer and a Brush 220 recorder permitted simultaneous recording of the full pulse wave and the mean blood pressure.

A number of long-term experiments were conducted in 15 x 18 x 12-inch cages with the aortic catheter connected to a special swivel fitting at the top of the cage. This arrangement permitted continuous recording of the mean aortic blood pressure while the rabbit was allowed to move about and have ready access to food and water (B. Brooks and E. E. Muirhead, personal communication). Actual values for mean blood pressure were obtained by inspection of the record over 30- or 60-minute periods. Blood pressure measurements for those experiments involving direct immunization with kidney extracts were performed indirectly in the central artery of the ear using the capsule of Grant and Rothschild (30). Blood pressures were taken 5 days a week. The rabbits were placed in a cage at 40°C for 13 minutes prior to their blood pressure measurements. Five readings were made and their average recorded. Bias was eliminated by having two technicians alternate days on which a rabbit’s blood pressure was determined.
RENIN SYSTEM IN ONE-KIDNEY HYPERTENSION

PRODUCTION OF HYPERTENSION

Young male New Zealand white rabbits weighing between 2.2 and 2.6 kg and maintained on commercial rabbit pellets were used. After a control period of 1-3 weeks during which the normal mean or systolic blood pressure (60-75 mm Hg in either case) of the rabbits was established, a silver clip (having a gap of 0.023 inches) was placed on the left renal artery, and the right kidney was removed (31). Daily blood pressure values following this operation usually increased over a period of several weeks from their control level until a hypertensive level was obtained. No rabbit was considered hypertensive unless the blood pressure level was consistently 30 mm Hg higher than its average control value.

Most rabbits exhibited a well-sustained benign form of hypertension with a mean aortic or systolic ear blood pressure of about 100-110 mm Hg. An occasional rabbit suddenly exhibited a rapidly rising blood pressure and died within 2-3 weeks with the necrotizing arteriolitis characteristic of malignant hypertension. Such rabbits were easily identified and were not used.

SYNTHESIS OF 1-SAR-8-ILE-ANGIOTENSIN II

The angiotensin antagonist 1-Sar-8-Ile-angiotensin II (32) was synthesized by the solid-phase method as modified in this laboratory. After deblocking, it exhibited a single ninhydrin-positive spot on Whatman no. 1 paper in n-butanol-acetic acid-water solvents (1, 4, 5). It was purified by a 200-tube countercurrent distribution in the sec-butanol-0.01N HCl-2% NaCl system in which it formed a symmetrical band, indicating homogeneity with a distribution coefficient of 0.58. It was desalted on a 25 x 100-cm column of Biogel P2 in 0.1N acetic acid and lyophilized. Amino acid analysis was: Sar 1.14, Arg 1.07, Val 1.03, Tyr 0.94, His 0.92, and Pro 1.04. For use, the peptide was dissolved in physiological saline.

ASSAY OF ANTI-RENIN

Antirenin concentrations were determined by incubation of a series of samples of each antiserum with 1.0 ml of a 0.1M NaCl-0.05M sodium phosphate solution, pH 7.5, containing 0.02 Goldblatt units (GU)1 of rabbit renin. Control tubes which contained only renin in buffer were included in each set of determinations. After 15 minutes at 37°C, 1.0 nmole of semipurified hog renin substrate (~200 nmoles/g) in 1 ml of physiological saline was added and mixed; the incubation was then continued for an additional 30 minutes at 37°C. At the conclusion of this period, the pH was adjusted to 5.5, and the reaction was terminated by heating in a boiling water bath for 10 minutes. The samples were centrifuged, and the supernatant solution was assayed for angiotensin I by bioassay in the rat (33) or by radioimmunoassay. The volume of antiserum required to neutralize one-half (0.01 GU) of the rabbit renin was determined graphically. Antirenin concentrations were expressed in terms of the amount of renin neutralized. Thus, a unit of antirenin is equal to that amount which neutralizes 50% of 2 GU of renin. For determination of their antirenin concentration, blood samples from hypertensive rabbits were drawn in 2% ethylenediaminetetraacetic acid (EDTA) during the course of the experiments. The plasmas were separated and dialyzed for 24 hours against 1% NaCl containing 0.003M EDTA at pH 7 prior to assay. It was discovered during the latter part of the investigation that the dialysis step was unnecessary. The addition of 40 µl of a 0.3M solution of diisopropylfluorophosphate in isopropanol to the incubation mixtures as an angiotensiinase inhibitor was found to be advantageous.

PREPARATION OF RABBIT RENIN

The rabbit renin that was used in challenging doses in the hypertensive rabbits and in assaying antirenin was prepared from whole frozen rabbit kidneys by batchwise adsorption and elution from DEAE-cellulose (34). The final preparation was assayed in terms of the antirenin released from hog renin substrate, and its potency was calculated in terms of standard rabbit renin.

PREPARATION OF ANTI-RENIN

The extract was purified by a procedure that has been described in detail (35). The following steps were used in the purification: (1) acidification to pH 2.5, (2) fractionation with ammonium sulfate between the limits of 1.4 and 2.5M, (3) fractionation with acetone followed by dialysis, (4) batchwise adsorption on DEAE-cellulose followed by elution at pH 4.6, (5) fractionation with polyethylene glycol, and (6) adsorption and elution from kaolin. The final product was free of pseudorenin (35) and angiotenisinase and had a specific activity of 22 GU/mg.

Antibodies against this material were raised in young male New Zealand white rabbits by subcutaneous injections, three per week, over a period of 8 weeks. Each injection contained 112 GU. The initial injection was given with Freund’s complete adjuvant. The first injection of each succeeding week was given with incomplete adjuvant. At 10-day intervals following the initial 8-week course of injections, the rabbits were bled and then given single booster injections without adjuvant. All antisera having adequate potency were pooled, fractionated with ammonium sulfate between the limits of 1.0 and 2.0M, and dialyzed against a 0.17M NaCl-0.025M sodium phosphate solution, pH 7.5. The antirenin titer of several preparations varied from 15 to 21 units/ml assayed in terms of the amount of rabbit renin neutralized.

PREPARATION OF KIDNEY EXTRACTS

The cortex was sliced from whole hog kidneys (obtained fresh and frozen until they were used) and processed by a simple method devised by Haas et al. (36) for the preparation of renin. This method was modified for use in this laboratory and has been used by Hill et al. (7) in their passive immunization experiments in dogs. Briefly, an aqueous extract of coarsely ground kidney cortex was acidified to pH 1.6 for 10 minutes while the temperature was carefully held at 0°C. The pH was then raised to 6.2, and the insoluble material was removed by filtration. The pH was adjusted to 4.0, and the proteins precipitating between 0.75 and 2.0M ammonium sulfate

1 One Goldblatt unit of renin is that amount of the enzyme that is required to raise the mean blood pressure of a trained unanesthetized dog 30 mm Hg. We are indebted to Dr. Harry Goldblatt and Dr. Erwin Haas for the standardized hog and rabbit renin.

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were collected and dialyzed against distilled water. The precipitate forming on dialysis was discarded.

The crude preparation was adsorbed and eluted from DEAE-cellulose using batchwise techniques according to a method devised in this laboratory for the purification of hog renin (34). The product, after dialysis, was chromatographed on a DEAE-cellulose column (Whatman DE32, 5 x 100 cm). The column was equilibrated with 0.025M sodium acetate buffer having a pH of 5.50 and developed with a gradient to 0.25M NaCl in the same buffer. Renin was eluted in one component in contrast to the four forms that are obtained by DEAE-cellulose chromatography employing a pH gradient (34). The fractions containing the main renin component were pooled and concentrated by ultrafiltration. They were then passed through a pepstatin affinity column to remove as much of the renin but permitted most of the protein to flow through the column. Additional small amounts of protein were washed from the column along with minimal amounts of renin by further washing with the column buffer. This procedure removed all but trace amounts of renin by further washing with the column buffer containing 2M NaCl. After this rigorous washing, the renin fraction was eluted in a greatly purified state from the eluate by repeated ultrafiltration and dialution with water.

The remaining fractions which contained protein and minimum amounts of renin were also pooled and concentrated by ultrafiltration. They were then passed through a pepstatin affinity column to remove as much of the remaining renin as possible.

Affinity chromatography was conducted on a 1.2 x 25-cm column of immobilized pepstatin that was equilibrated with 0.01M sodium phosphate buffer, pH 7.5. Fractions (at the same pH and ionic strength) were applied to the column and washed through with column buffer. This procedure removed all but trace amounts of the renin but permitted most of the protein to flow through the column. Additional small amounts of protein were washed from the column along with minimal amounts of renin by further washing with the column buffer containing 2M NaCl. After this rigorous washing, the renin fraction was eluted in a greatly purified state by washing with 0.2M sodium phosphate buffer, pH 6.5, containing 4M urea. The urea was removed as rapidly as possible from the eluate by repeated ultrafiltration and dilution with water.

Fractions to be injected were made to contain 0.1M NaCl and 0.025M sodium phosphate buffer at pH 6.0. The final preparations were usually sterilized by filtration through a Millipore filter.

**Results**

**EFFECT OF PLASMA ANTIRENIN CONCENTRATION ON THE PRESSOR RESPONSE TO EXOGENOUS RENIN**

The three rabbits used in this experiment (Table 1) were hypertensive. Rabbit 3 had a mean aortic blood pressure over 100 mm Hg for 12 days, and the blood pressure of rabbits 1 and 2 had been above this level for 6 and 3 months, respectively.

Rabbit 1 received a single dose of 25 units of antirenin. Rabbit 2 was given two doses of the antibody; the first contained 312 units and produced a plasma level of 0.67 units/ml, and the second contained 625 units and produced a level of 1.47 units/ml. The experiment involving rabbit 3 continued for 7 days during which a total of 855 units of antirenin was given in four doses.

All of the rabbits responded to control doses of renin with large elevations in blood pressure that persisted for 2–4 hours. In marked contrast, the response to renin following the administration of antirenin was of very short duration and resembled the response to a small amount of angiotensin that might have been produced before total capture of the enzyme by its antibody. The experiments indicate that over 98% of the pressor response to an injected dose of renin is abolished when the plasma antirenin concentration lies between 0.1 and 1.0 unit/ml and that over 99% of the response is abolished when the plasma level of antirenin exceeds 1.0 unit/ml.

**EFFECT OF PASSIVE TRANSFER OF ANTIRENIN ON THE BLOOD PRESSURE OF HYPERTENSIVE RABBITS**

The results of this experiment in which hypertensive rabbits were given large amounts of antirenin over an extended period of time are presented in Table 2.

Rabbit 9 was given 750 units on the first day of

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**TABLE 1**

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Antirenin concentration (units/ml)</th>
<th>Response to renin (mm Hg x minutes)*</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 (Control)</td>
<td>5720</td>
<td>100.0</td>
</tr>
<tr>
<td>2</td>
<td>0 (Control)</td>
<td>6830</td>
<td>100.0</td>
</tr>
<tr>
<td>3</td>
<td>0 (Control)</td>
<td>2835</td>
<td>100.0</td>
</tr>
</tbody>
</table>

All rabbits had established hypertension. Blood samples were taken for determination of antirenin concentration after the pressor response to each challenging dose of renin was obtained. A challenge dose of 1.0 GU of rabbit renin was given rapidly through the aortic catheter. Mean blood pressure was recorded continuously while the rabbit was under partial restraint.

* The area that is generated when the increase in mean blood pressure above the control level is plotted against time.
The rabbits were hypertensive with a mean aortic blood pressure of 100 mm Hg or more for 10 to 50 days. BP = average mean blood pressure. BP for rabbits 4 and 9 is the average daily mean pressure derived from continuous recordings. BP for rabbits 6-8 is the daily mean pressure obtained by averaging one or more single observations made under partial restraint. Control BP values were determined from observations made immediately before the administration of antirenin. Experimental BP values were taken from the daily average BP measurements following administration of antirenin. Antirenin was given in divided doses during the course of the experiment to maintain an elevated plasma antirenin titer.

The experiment. On the ninth day, in an effort to raise the antirenin titer as high as possible, an exchange transfusion was conducted between the experimental rabbit and another rabbit which was normotensive but with a higher antirenin titer; 200 ml of blood was exchanged, 10 ml at a time, between the two conscious, lightly heparinized rabbits via their aortic catheters using two 10-ml syringes. The antirenin titer in the blood of hypertensive rabbit 9 thus reached 8.4 units/ml plasma.

The blood pressure of rabbit 7 decreased slightly on the third, fourth, and sixth days of the experiment, and the blood pressure of rabbit 8 was somewhat lower on the fifth, sixth, and eighth days. In neither case did the blood pressure fall into the normal range, nor was the pressure decreased consistently during either experiment. The blood pressures of rabbits 4-6 and 9 were either unchanged or even higher during the course of the experiment.

One additional rabbit (no. 2) (not shown in Table 2), which had been hypertensive for 82 days, was given a single dose of 312 units of antirenin. This dose of antibody resulted in a plasma level of 0.67 units/ml. Multiple blood pressure readings during the next 24 hours showed that the blood pressure remained essentially unchanged.

The results obtained in 13 experiments on seven rabbits using the antagonist 1-Sar-8-Ile-angiotensin II are summarized in Table 3. Infusion rates as low as 0.09 μg/min kg⁻¹ were completely effective in blocking a challenging dose of 2 nmoles of angiotensin II. Infusion rates much higher than this level were used for as long as 72 hours. In four short-term experiments, the infusion rates were extremely high. Rabbit 10 was infused at the rate of 83 μg/min kg⁻¹ for 3.9 hours. Under none of these conditions was a significant lowering of the blood pressure of these rabbits observed.

EFFECT OF DIRECT IMMUNIZATION WITH KIDNEY EXTRACTS ON THE BLOOD PRESSURE OF HYPERTENSIVE RABBITS

Treatment of hypertensive rabbits with 25 GU/day of the crude renin preparation resulted in significant reductions in blood pressure in 9 of 11 rabbits. Reductions in blood pressure were also obtained as a result of the injection of the kidney extract after batchwise treatment with DEAE-cellulose. One experiment in which extract containing 25 GU of renin of low specific activity was injected (from DEAE chromatography) is illustrated in Figure 1. In this instance, the slow development of hypertension, the reduction in blood pressure to normal during the injection period, and its subsequent return to a hypertensive level can be seen. A low antirenin titer developed during the injection period and disappeared soon after the injections were stopped. Rabbit 15 and the other rabbits that received kidney extract injections appeared healthy, did not develop abscesses at the site of the

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**Table 2**

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Length of experiment (days)</th>
<th>Antirenin given (units)</th>
<th>Plasma antirenin (units/ml)</th>
<th>Experimental BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Maximum</td>
<td>Minimum</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>600</td>
<td>1.40</td>
<td>0.33</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>822</td>
<td>1.82</td>
<td>0.13</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>900</td>
<td>2.90</td>
<td>0.35</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>900</td>
<td>2.20</td>
<td>0.54</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>1060</td>
<td>3.60</td>
<td>1.10</td>
</tr>
<tr>
<td>9</td>
<td>17</td>
<td>750</td>
<td>8.40</td>
<td>0.37</td>
</tr>
</tbody>
</table>

The rabbits were hypertensive with a mean aortic blood pressure of 100 mm Hg or more for 10 to 50 days. BP = average mean blood pressure. BP for rabbits 4 and 9 is the average daily mean pressure derived from continuous recordings. BP for rabbits 6-8 is the daily mean pressure obtained by averaging one or more single observations made under partial restraint. Control BP values were determined from observations made immediately before the administration of antirenin. Experimental BP values were taken from the daily average BP measurements following administration of antirenin. Antirenin was given in divided doses during the course of the experiment to maintain an elevated plasma antirenin titer.

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TABLE 3

Effect of the Angiotensin II Antagonist 1-Sar-8-Ile-Angiotensin II on the Blood Pressure of Rabbits with Experimental Hypertension

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Duration of infusion (hours)</th>
<th>Infusion rate (μg/min kg⁻¹)</th>
<th>Before infusion</th>
<th>After infusion</th>
<th>Control BP (mm Hg)</th>
<th>Change of BP during infusion (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5</td>
<td>79.6</td>
<td>1.0 65</td>
<td>1.0 0</td>
<td>125</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3.1</td>
<td>61.4</td>
<td>2.0 24</td>
<td>2.0 0</td>
<td>192</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>3.9</td>
<td>83.0</td>
<td>1.0 48</td>
<td>1.0 0</td>
<td>130</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>4.0</td>
<td>0.66</td>
<td>1.0 60</td>
<td>1.0 0</td>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3.8</td>
<td>87.0</td>
<td>1.0 64</td>
<td>2.0 0</td>
<td>130</td>
<td>-10</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>4.4</td>
<td>2.0 48</td>
<td>2.0 0</td>
<td>110</td>
<td>+5 to +10</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
<td>0.09</td>
<td>2.0 50</td>
<td>2.0 0</td>
<td>100</td>
<td>-10 to +10</td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>4.4</td>
<td></td>
<td></td>
<td>110</td>
<td>+5 to +10</td>
</tr>
<tr>
<td>11</td>
<td>24</td>
<td>3.7</td>
<td></td>
<td></td>
<td>98</td>
<td>-4</td>
</tr>
<tr>
<td>12</td>
<td>69</td>
<td>1.2</td>
<td>2.0 40</td>
<td>2.0 0</td>
<td>112</td>
<td>+30</td>
</tr>
<tr>
<td>8</td>
<td>21</td>
<td>2.4</td>
<td></td>
<td></td>
<td>122</td>
<td>0</td>
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<tr>
<td>13</td>
<td>20</td>
<td>1.2</td>
<td>2.0 56</td>
<td>2.0 0</td>
<td>100</td>
<td>+5 to +10</td>
</tr>
<tr>
<td>14</td>
<td>1.3</td>
<td>27</td>
<td>1.0 56</td>
<td>1.0 0</td>
<td>104</td>
<td>0</td>
</tr>
</tbody>
</table>

Rabbits had been hypertensive with mean aortic blood pressures of 100 mm Hg or more for 18–190 days. BP = average mean blood pressure. Blood pressure was recorded continuously from the aortic catheter. In many experiments, the values were verified by independent observations under restraint. Rabbits were free in their cages during the long experiments and were partially restrained during the brief experiments. Infusions of antagonist were given very slowly through the aortic catheter except for experiments of less than 8 hours during which infusions were given via the ear veins.

injections, and, as shown in Figure 1, did not lose weight.

The results from the injection of preparations obtained by chromatography on DEAE-cellulose and pepstatin affinity columns are shown in Table 4.

Rabbits that received the purified renin fractions were given daily injections that contained 25 GU of the enzyme. They were paired with other rabbits who received an approximately equal amount of nonrenin protein.

Preparations 2 and 7 as well as preparations 3 and 8 were derived from the same pepstatin affinity column chromatogram. Preparation 6 originated from the same DEAE-cellulose column chromatogram as did preparation 1 but was passed through a
TABLE 4

Effect of Direct Immunization with Extracts of Hog Kidney Cortex on the Blood Pressure of Hypertensive Rabbits

<table>
<thead>
<tr>
<th>Description of preparation</th>
<th>Maximum antirenin titer (units/ml)</th>
<th>Anti-hypertensive effect</th>
<th>Description of preparation</th>
<th>Rabbit</th>
<th>Maximum antirenin titer (units/ml)</th>
<th>Anti-hypertensive effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2.6 mg protein, 25 GU renin/dose</td>
<td>16</td>
<td>0.15</td>
<td>+</td>
<td>6, 2.6 mg protein, 0.002 GU renin/dose</td>
<td>35*</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>2, 0.1 mg protein, 25 GU renin/dose</td>
<td>24</td>
<td>1.67</td>
<td>++</td>
<td>7, 0.1 mg protein, 0.004 GU renin/dose</td>
<td>42†</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>3, 0.033 mg protein, 25 GU renin/dose</td>
<td>28</td>
<td>0.66</td>
<td>+++</td>
<td>8, 0.04 mg protein, 0.0014 GU renin/dose</td>
<td>48</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>4, 4 mg protein, 25 GU renin/dose</td>
<td>31†</td>
<td>0.13</td>
<td>-</td>
<td>9, 10 mg protein, 0.045 GU renin/dose</td>
<td>51</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>5, 6.5 mg protein, 25 GU renin/dose</td>
<td>33†</td>
<td>2.00</td>
<td>+++</td>
<td>10, 10 mg protein, 0.03 GU renin/dose</td>
<td>53</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>6, 2.6 mg protein, 25 GU renin/dose</td>
<td>17</td>
<td>0.16</td>
<td>+++</td>
<td>0.004 GU renin/dose</td>
<td>36*</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>18</td>
<td>0.09</td>
<td>+++</td>
<td></td>
<td>37</td>
<td>&lt; 0.05</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>0.66</td>
<td>+++</td>
<td></td>
<td>38</td>
<td>&lt; 0.05</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
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<td>++</td>
<td></td>
<td>39</td>
<td>&lt; 0.05</td>
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</tr>
<tr>
<td>21</td>
<td>0.50</td>
<td>-</td>
<td></td>
<td>40</td>
<td>&lt; 0.05</td>
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</tr>
<tr>
<td>22</td>
<td>0.11</td>
<td>+++</td>
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<td>41</td>
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<tr>
<td>23</td>
<td>0.55</td>
<td>-</td>
<td></td>
<td>42†</td>
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</tr>
<tr>
<td>24</td>
<td>33.30</td>
<td>++</td>
<td></td>
<td>43†</td>
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<tr>
<td>25</td>
<td>1.64</td>
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<tr>
<td>28</td>
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<tr>
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<td>0.66</td>
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<td>48</td>
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<tr>
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<td>+++</td>
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<tr>
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<td>0.13</td>
<td>-</td>
<td></td>
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<tr>
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<td>52</td>
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<tr>
<td>34†</td>
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<td>+++</td>
<td></td>
<td>53</td>
<td>&lt; 0.05</td>
<td>-</td>
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<tr>
<td>35*</td>
<td>0.051 GU renin/dose</td>
<td>55</td>
<td>&lt; 0.05</td>
<td></td>
<td>56</td>
<td>&lt; 0.05</td>
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Renin fractions 1, 4, and 5 were derived from renin peaks from DEAE-cellulose chromatography. Renin fractions 2 and 3 and nonrenin fractions (6-11) were purified by DEAE-cellulose chromatography followed by passage through a pepstatin affinity column. The antihypertensive effect was evaluated according to the following criteria: - = no effect, + = a significant decrease in blood pressure, ++ = a decrease in blood pressure which returned at least halfway to the preclipping control level, and +++ = a decrease in blood pressure to the preclipping level. Preparations were given subcutaneously 5 days each week for 5-8 weeks. Initial doses of preparations 2, 7, 3, and 8 were given with Freund's complete adjuvant. The first dose of each week thereafter was given with incomplete adjuvant. A group of six rabbits was given a course of injections of the 0.025M sodium phosphate-0.1M NaCl buffer, pH 6.0, that was used to prepare extracts for injection together with Freund's adjuvant. There was no effect on any of these rabbits' blood pressure, nor did an antirenin titer develop.

* Had been used previously to test a kidney extract with positive results.
† Had been used previously to test a kidney extract with negative results.

pepstatin affinity column to remove the last small amount of renin. Preparations 4 and 5 were renin peaks from two DEAE-cellulose column chromatograms, and preparations 9-11 were nonrenin fractions from DEAE-cellulose chromatography that were also passed through a pepstatin affinity column.

Significant reductions in blood pressure were obtained in 16 of 19 rabbits injected with the purified renin preparations. There was no reduction in the blood pressure of any of the rabbits receiving the nonrenin preparation.

Antirenin titers developed in all of the rabbits receiving the renin preparations; the magnitude of the titer ranged from very low (rabbit 18) to very high (rabbit 25). The titer correlated poorly with the antihypertensive effect. Significant titers did not develop in those rabbits receiving the nonrenin preparations.

The effect of highly purified renin preparation 3 on the blood pressure of rabbit 30 (see also Table 4) is illustrated in Figure 2. The decrease in blood pressure as a result of the injections occurred more gradually in this instance than it did with rabbit 15 (Fig. 1).

The length of time that elapsed before the blood pressure increased following a course of injections that lowered the blood pressure was variable, sometimes being as long as 1-4 weeks. In general, the length of time that elapsed before a blood
Effect of the injection of kidney cortex preparation 3 on the blood pressure of a rabbit (no. 30) with one-kidney hypertension. The daily subcutaneous injections contained 0.033 mg of protein and 25 GU of renin (specific activity 757 GU/mg). RN = right nephrectomy, and LC = left renal artery clipped. The concentrations of antirenin in the plasma and the body weights are given.

Discussion

We have discovered that the blood pressure of rabbits with chronic one-kidney hypertension can be lowered by direct injection of kidney extracts containing renin. However, 1-4 weeks of injections were required before the blood pressure lowering occurred, and a similar length of time elapsed after the last injection until the blood pressure began to rise again. On the basis of this observation, we assume that the blood pressure-lowering effect is due to the appearance of an antibody (elicited by an antigen in the extract that is injected) which neutralizes the pressor substance causing the hypertension.

Extracts containing renin with very high specific activities were effective in lowering the blood pressure. On this basis, one might decide that the elevated blood pressure in such rabbits was sustained by the renin-angiotensin system. However, immunization by passive transfer of large amounts of antirenin or treatment with angiotensin antagonist infused at high rates for long periods of time was completely ineffective in lowering the blood pressure of the hypertensive rabbits. Moreover, the plasma antirenin titers of the actively immunized rabbits correlated very poorly with the degree to which their blood pressures were lowered. In addition, the antirenin titers of the rabbits that were passively immunized were usually much higher, and yet no blood pressure lowering was observed.

It is clear that the elevation in blood pressure in these rabbits is not sustained by circulating renin or by the direct vasopressor action of angiotensin. If the renin-angiotensin system is responsible, it must exert its pressor effect in an indirect fashion, operating from a site such as the blood vessel wall where it might be inaccessible to antirenin and to the angiotensin antagonist. However, this explanation becomes more difficult to accept when one realizes that it requires that renin in such a location be accessible to antirenin formed directly in the animal but not to the antibody when it is administered passively.

2 Until very recently, the best preparation of hog renin that has been reported in terms of a recognized and well known unit was that of Haas et al. (38). The specific activity of their preparation was 780 GU/mg. The specific activity of our preparation 3 was 757 GU/mg. The purification of hog renin by means of a scheme similar to that used in the present work which also employs a pepstatin affinity column has been reported by Murakami and Inagami (39). Their product was said to be stable and pure, although the forms of renin (34) were not separated. It should be emphasized that the pepstatin affinity column is a remarkably effective tool but is not specific for renin, since it adsorbs pepsin and possibly other acid proteases as well.
RENIN SYSTEM IN ONE-KIDNEY HYPERTENSION

It is possible that the renin-angiotensin system is not involved in the maintenance of one-kidney hypertension in the rabbit. This event, it is necessary to assume that the blood pressure-lowering effect that we observed is due to immunization against some unknown pressor substance and not renin.

The renin used as antigen in the passive immunization experiments was prepared by a complex purification method (35) which could have eliminated the hypothetical pressor substance. However, it would be necessary to assume that the substance was not removed during the purification scheme used to prepare the antigen used in the direct immunization experiments.

One might easily inquire how the presence of an unknown pressor substance in the kidney could have been overlooked. This question might be answered best if the hypothetical substance were not pressor in the usual sense. It might act slowly over several days, causing an elevation in blood pressure resembling that which follows the application of a clamp to the renal artery of an experimental animal. A slow increase in blood pressure might suggest an indirect action involving several mechanisms and including, for example, an expansion in blood volume. Such an explanation would be consistent with the slow reduction in blood pressure over a period of 3-5 days that both Wakerlin (3) and Hill et al. (7) found following the passive transfer of "antirenin" into hypertensive dogs.

References
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