Chronic One-Kidney Hypertension in Rabbits

III. Renopressin, a New Hypertensive Substance

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SUMMARY The blood pressure of rabbits with chronic one-kidney hypertension can be lowered to normal by immunization with hog kidney cortex preparations that do not contain renin, thus providing evidence for a new factor essential for the maintenance of an elevated blood pressure. A search for the new factor has led to the discovery of a hypertensive substance which we have named renopressin. Subcutaneous injection of the new substance into normal rabbits produces a delayed, slow increase in blood pressure, and after a few days the development of a moderate hypertension which persists indefinitely. The response of the blood pressure to renopressin is totally unlike that to renin. While the pressor action of renin can be blocked by an angiotensin II antagonist, the hypertension caused by renopressin cannot. Renin can increase the blood pressure of hypertensive rabbits; renopressin has no effect. The blood pressure of hypertensive rabbits and of normal rabbits made hypertensive by injection of renopressin can be lowered to normal by passive immunization with the same antibody preparation. The evidence is consistent with the possibility that renopressin and the agent causing the elevation of blood pressure in hypertensive rabbits are similar or identical.

The evidence suggested that an unknown substance, not reative rabbits could be lowered to normal by direct immunization using a renin-free hog kidney cortex preparation. The first preparations that were used for this purpose contained renin. As a consequence the recipient hypertensive rabbits usually developed antirenin titers in their plasma. However, the titers that were produced did not correlate well with the reductions in blood pressure although large amounts of antibody were given and effective titers were maintained for a number of days.1

It was discovered that the blood pressure of hypertensive rabbits could be lowered to normal by direct immunization with preparations made from the cortex of hog kidneys.3 The first preparations that were used for this purpose contained renin. As a consequence the recipient hypertensive rabbits usually developed antirenin titers in their plasma. However, the titers that were produced did not correlate well with the reductions in blood pressure that were observed.

The renin then was completely removed from a hog kidney cortex preparation by passage through columns of immobilized antirenin.2 The antibody used for the purpose was identical to that which had been used unsuccessfully in the passive immunization experiments.

Thus it was found that the blood pressure of hypertensive rabbits could be lowered to normal by direct immunization using a renin-free hog kidney cortex preparation. The evidence suggested that an unknown substance, not renin, was present in the preparations that elicited a cross-reacting antibody which neutralized a factor essential for the maintenance of the blood pressure in hypertensive rabbits.7

As a result of these experiments we have conducted a search for such a factor in kidney cortex.

This search has led to the discovery of a new substance which increases the blood pressure of normal rabbits over a period of several days. The elevation in blood pressure, once established, appears to persist indefinitely. We have named the new substance renopressin.

Methods

EXPERIMENTAL ANIMALS

Young, male, New Zealand white rabbits initially weighing between 2.2 and 2.6 kg and maintained on commercial rabbit pellets were used.

Hypertension was produced in some of the rabbits by application of a silver clip to the left renal artery and removal of the right kidney in the same operation.2 Hyper-tensive rabbits that were used in experiments had exhibited a sustained form of benign hypertension for a period of at least 4 weeks. These methods have been described in detail.1,3 Systolic blood pressure (BP) was measured in the central artery of the ear of warmed rabbits.1,4

BLOCKADE OF THE RENIN-ANGIOTENSIN SYSTEM

The rabbits were warmed and several control blood pressures were obtained. Angiotensin II, 1 nmol, was injected into the marginal ear vein. Blood pressure measurements in the opposite ear were commenced immediately. Readings were made at 10-second intervals for 3 minutes during which time the blood pressure increased by 37-65 mm Hg (average, 53 mm Hg) and then fell to the approximate preinjection level. A solution containing [Sar1, Ile8]angiotensin II, 0.5 mg/ml, then was infused into...
the marginal ear vein at the rate of 0.025 ml/min for 30
minutes. The infusion rate in different experiments was
between 3.0 and 3.6 μg/min per kg. During this time the
effect of the blockade was determined by blood pressure
measurements made at 5-minute intervals. At the end of
30 minutes the blockade was tested by a second injection
of angiotensin II followed by similar blood pressure mea-
surements during a 3-minute period. Complete blockade
was obtained in all cases.

The synthesis of [Sar′,Ile′]angiotensin II has been de-
scribed.1

DETERMINATION OF PROTEIN

Protein was determined by an automated modification
of the method of Lowry et al.6

RENIN ASSAY

Renin was measured in terms of the amount of angi-
tensin I liberated at pH 7.4 from an excess of semipuri-
fied, angiotensinase-free hog renin substrate.7 The assay
of the angiotensin was performed in the rat8 and results
were calculated in terms of Goldblatt units (GU)* based
on a standard hog renin preparation.

METHODS FOR INJECTIONS

Preparations for subcutaneous injection were prepared
in 0.1 M sodium chloride-0.025 M sodium phosphate
buffer at pH 6.0 and were sterilized by passage through a
Millipore filter. Those preparations being tested for their
pressor activity were injected subcutaneously using a 1-ml
dose each day throughout the injection period. Rabbits to
be immunized were injected subcutaneously with 1-ml
doses 5 days a week. The initial injection of all prepara-
tions was given with an equal volume of Freund’s complete
adjuvant while the first injection of each subsequent week
was given with the incomplete adjuvant.

PREPARATION OF KIDNEY FRACTIONS

The starting material for all preparations was the crude
extract which was prepared from the cortex of rabbit or
hog kidneys that were kept frozen until used. The method
of extraction involved an acid treatment followed by am-
monium sulfate fractionation. All of the crude extracts
were fractionated further with acetone at low temperature
and in the presence of ammonium sulfate. The precipitates
were washed and dialyzed. The methods have been de-
scribed.1,2

Hog preparation H, obtained as the 33-50% acetone
fraction of the first batch. Preparation B, also from the
first batch, was taken from the 0-33% acetone fraction. A
portion of this latter fraction was chromatographed on a
pepsin affinity column. The major part of the material
was not adsorbed and passed directly through the column,
yielding preparation C, while the buffered 4 M urea eluate
became preparation D. The preparation and use of the
pepsin column has been described.2

Preparation E was derived from the 33-40% acetone
fraction of the second batch of rabbit kidney cortex. The
40-50% acetone fraction was chromatographed on a pep-
statin column. After application of the sample, the column
was thoroughly washed with 2 M NaCl in 0.1 M sodium
acetate buffer at pH 4. The adsorbant was removed from
the column and washed four times with 0.1 M acetic acid.
The acid eluate, containing the renin, was quickly neutral-
ized and dialyzed. The renin in the product, designated
preparation F, was purified 273-fold by the chromato-
graphic step and had a specific activity of 401 GU/mg.

The 33-50% acetone fraction of the third batch of
kidney cortex was chromatographed on an antirenin affin-
ity column. This fraction, containing 1,012 mg of protein
and 500 GU of renin, was passed through the column
three times in an effort to remove the renin completely.
The final product, preparation G, contained 587 mg of
protein and 3.1 GU of renin.

Rabbit renal medulla, lung, liver, and spleen were ex-
tracted and processed through acetone fractionation in
exactly the same manner as were the three batches of
rabbit kidney cortex. The 33-50% acetone fractions were
used for injection.

The specific activity of the renin in the kidney medulla
preparation was 0.01 GU/mg. The specific activity of the
renin in two corresponding preparations from kidney cor-

tex was 0.48 and 0.49 GU/mg. Renin was not detectable
in the spleen, liver, and lung preparations.

PREPARATION OF PLASMA GLOBULINS

Rabbit blood was collected in 0.1 volume of 2% ethyl-
endiaminetetraacetic acid (EDTA). The plasma was col-
lected and fractionated between 1.1 and 2.0 M ammonium
sulfate at pH 6.0. After dialysis the preparations were
prepared for injection in a solution of 0.1 M NaCl-0.025 M
sodium phosphate at pH 7.5. Preparations containing a
total of 1 g of protein were given in divided doses into the
marginal ear vein over a period of several hours.

Plasma globulins were prepared from hypertensive rab-
bits whose pressures had been lowered to normal by active
immunization with hog kidney cortex preparation K (Ab-
1) and preparation H (Ab-2). Globulins also were pre-
pared from normal rabbit plasma (NG).

ASSAY OF ANTIRENIN

Antirenin was measured in the plasma of the experi-
mental rabbits by the inhibitory effect of samples of the
plasma on rabbit renin as previously described.1

Results are given as antirenin units, which are equiva-
 lent to the Goldblatt units of rabbit renin neutralized. The
lowest concentration of antirenin that can be determined
reliably is 0.05 U/ml of plasma. The preparation of the

* One Goldblatt unit (GU) of renin is the amount of enzyme required to
raise the mean blood pressure of a trained unanesthetized dog 30 mm of
Hg.
RENOPRESSIN, A NEW HYPERTENSIVE SUBSTANCE/Skeggs et al.

The effect of the injection of rabbit kidney cortex preparations on the blood pressure of normal rabbits. Upper panel: preparation B was given to rabbit 564. Lower panel: preparation A was given to rabbit 568. The daily subcutaneous dose of preparation B contained 3.7 mg of protein and 0.04 Goldblatt units (GU) of renin; the dose of preparation A contained 5.3 mg of protein and 2.6 GU of renin. The elevations of blood pressure in both rabbits were unsuccessfully challenged by blockade of the renin-angiotensin system with /SarIleAngiotensin II at the times indicated by a, b, and c.

rabbit renin used in the assay of antirenin has been described.1

Results

THE RESPONSE IN NORMAL RABBITS

The effect of the subcutaneous injection of rabbit kidney cortex preparations A and B into two different normal rabbits is illustrated in Figure 1. A typical response was that of rabbit 564, shown in Figure 1 (upper panel), in which the blood pressure increased during the first 5 days of the injection period to reach a value of 82 mm Hg, or 20 mm Hg above the baseline value. During the subsequent 94 days the blood pressure remained on a plateau with an average value very close to 82 mm Hg even though injections ceased after 9 days.

Less frequently the blood pressure response resembled that of the rabbit 568, illustrated in Figure 1 (lower panel). The blood pressure began to increase 48 hours after the first injection and reached 104 mm Hg on the 4th day. The blood pressure remained above 95 mm Hg for the next 35 days. After this time it gradually fell, reaching a plateau at about 65 days which was maintained thereafter.

The effect of varying the dose of preparation A over a wide range is shown in Table 1. It will be seen that a number of days may be required before the maximum blood pressure is reached. Five of the rabbits attained maximum pressures of approximately 100 mm Hg. The pressures of all five of these rabbits declined to a plateau of about 80 mm Hg after 40 days. There appeared to be no discernible relationship between the size of the dose, the number of days that it was injected, and the blood pressure response.

Rabbits in which blood pressures have been elevated for extended periods as a result of the injection of kidney cortex preparations appear healthy and do not lose body weight. Microscopic examination of the kidneys from 10 of 15 rabbits in which blood pressures had been elevated for 32–50 days revealed marked hypertrophy and hyperplasia of the juxtaglomerular apparatus. The remaining five rabbits that had received similar amounts of the same preparations showed only an occasional hyperplastic juxtaglomerular apparatus.

THE RESPONSE IN CLIPPED HYPERTENSIVE RABBITS

As shown in Table 2, five clipped hypertensive rabbits were given two different dosages of the same preparation A from rabbit kidney cortex that was given to the normal rabbits of Table 1. None of the hypertensive rabbits responded with a significant change in blood pressure. In marked contrast, the six normal rabbits receiving the same dosages responded with pressure increases that ranged from 20 to 38 mm Hg and averaged 30 mm Hg.

THE EFFECT OF RENIN ON THE HYPERTENSIVE RESPONSE

As shown in Table 3, three preparations that contained 0.06 GU of renin or less per daily dose were effective in raising the blood pressure of normal rabbits. Preparation E contained less than 1 GU of renin per daily dose and was

Table 1: The Effect of Injecting Different Amounts of a Preparation* from Rabbit Kidney Cortex on the Blood Pressure of Normal Rabbits

<table>
<thead>
<tr>
<th>Amount injected (mg)</th>
<th>No. of days injected</th>
<th>No. of rabbits</th>
<th>Baseline BP (mm Hg)</th>
<th>Days to maximum BP</th>
<th>Maximum BP (mm Hg)</th>
<th>BP at 80 days (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>10</td>
<td>3§</td>
<td>64 ± 1</td>
<td>11</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>1.0</td>
<td>10</td>
<td>3</td>
<td>66 ± 1</td>
<td>15 ± 1</td>
<td>95 ± 10</td>
<td>81 ± 1</td>
</tr>
<tr>
<td>5.3</td>
<td>30</td>
<td>3</td>
<td>63 ± 1</td>
<td>12 ± 4</td>
<td>96 ± 7</td>
<td>81 ± 1</td>
</tr>
<tr>
<td>21.2</td>
<td>15</td>
<td>3</td>
<td>63 ± 1</td>
<td>9 ± 2</td>
<td>90 ± 10</td>
<td>81 ± 1</td>
</tr>
<tr>
<td>52.8</td>
<td>4</td>
<td>3</td>
<td>63 ± 1</td>
<td>11 ± 2</td>
<td>86 ± 4</td>
<td>81 ± 1</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD.

* Preparation A was the fraction of the extract of rabbit kidney cortex that precipitated between 33% and 50% acetone concentration and contained 0.3 Goldblatt units (GU) of renin/mg of protein.

† Given subcutaneously each day.

§ The average of 5 consecutive days' blood pressure readings.

¶ Two of the 3 rabbits injected with 0.2 mg did not respond.
also effective. In contrast to these findings, the injection of preparation F in daily doses containing as much as 10 GU of renin had no effect on the blood pressure of normal rabbits.

**THE EFFECT OF BLOCKADE OF THE RENIN-ANGIOTENSIN SYSTEM**

The infusion of the angiotensin II antagonist, [Sar\(^1\), Ile\(^8\)]-angiotensin II, did not lower the elevated blood pressure of the two rabbits of Figure 1. Similar infusions into the rabbits in the last three groups of Table 1 also were ineffective. The nine rabbits in these groups were tested during the first few days that their blood pressure was increased. Five of these rabbits were tested again 80 days after their first injection. Six other rabbits, in which blood pressures were elevated as a result of receiving different preparations and are not illustrated in Figure 1 or in Table 1, were also tested by blockade. In no case was a lowering of blood pressure observed on administration of the antagonist.

**THE RESPONSE OF NORMAL RABBITS TO CONTROL PREPARATIONS**

Preparations of rabbit kidney medulla, spleen, lung, and liver were each injected into three normal rabbits. Doses containing 5.3 mg of protein were given each day for 10-day periods. Although the same method was used for these preparations as was used for preparation A of Table 1 and Figure 1 (lower panel), there was no effect on the blood pressure of the rabbits.

A sample of preparation A was heated on a boiling water bath for 10 minutes. This material also had no effect on the blood pressure of three rabbits when a dose of 5.3 mg was given each day for 10 days.

Preparation C, the unadsorbed fraction from the pepstatin column, was not hypertensive in three rabbits when injected at the rate of 4.1 mg/day for 10 days. The 4 M urea eluate from the same column, preparation D, was active (Table 3).

**THE RESPONSE OF NORMAL RABBITS TO A HOG KIDNEY CORTEX PREPARATION**

As shown in Table 4, preparation H was tested for its effect at three dosage levels in three groups of rabbits. The blood pressures of those rabbits receiving a dose of 1 mg of protein each day were not affected; 0.5 mg/day of the same preparation was used to immunize clipped hypertensive rabbits. The three rabbits that received 5 mg/day responded with small blood pressure elevations of 8, 8, and 16 mm Hg. One of the three rabbits that were given 50 mg/day did not respond, whereas two others gave rises of 17 and 19 mm Hg. The blood pressure elevations that were observed in five of the nine rabbits in this experiment were maintained at a plateau after having once attained a maximum value.

**THE RESPONSE OF CLIPPED HYPERTENSIVE RABBITS TO PLASMA GLOBULINS**

Globulins were prepared from the pooled plasma of a group of eight hypertensive rabbits in which the blood

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**Table 2** The Effect of a Preparation* from Rabbit Kidney Cortex on the Blood Pressure of Clipped Hypertensive Rabbits

<table>
<thead>
<tr>
<th>Amount injected (mg)</th>
<th>No. of days injected</th>
<th>No. of rabbits</th>
<th>Normal precircling BP (mm Hg)</th>
<th>BP before injection (mm Hg)</th>
<th>Change in BP after injection (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.2</td>
<td>12</td>
<td>3</td>
<td>62 ± 1</td>
<td>99 ± 1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>5.3</td>
<td>16</td>
<td>2</td>
<td>63 ± 1</td>
<td>100 ± 0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD.

* Preparation A was the fraction of the crude extract of rabbit kidney cortex that precipitated between 33% and 50% acetone concentration and contained 0.5 GU of renin/mg of protein. Preparation A was also used in the normal rabbits of Table 1 and Figure 1 (lower panel).

† Given subcutaneously each day.

‡ The average of 5 consecutive days' blood pressure readings.

**Table 3** The Effect of Renin Content on the Blood Pressure Elevation Produced in Normal Rabbits by Several Preparations from Rabbit Kidney Cortex

<table>
<thead>
<tr>
<th>Renin injected (GU)</th>
<th>Protein injected (mg)</th>
<th>Description of preparation</th>
<th>No. of rabbits</th>
<th>Baseline BP (mm Hg)</th>
<th>Maximum BP (mm Hg)</th>
<th>Increase in BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04</td>
<td>3.7</td>
<td>B: 0-33% acetone precipitate of the crude extract</td>
<td>3</td>
<td>62 ± 1</td>
<td>85 ± 3</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>0.05</td>
<td>0.28</td>
<td>D: 0-33% acetone precipitate of the crude extract; chromatographed on pepstatin</td>
<td>3</td>
<td>65 ± 0</td>
<td>78 ± 7</td>
<td>13 ± 7</td>
</tr>
<tr>
<td>0.06</td>
<td>10.0</td>
<td>G: 33-50% acetone precipitate of the crude extract; renin removed on an antirenin column</td>
<td>3</td>
<td>62 ± 1</td>
<td>81 ± 1</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>0.89</td>
<td>5.0</td>
<td>E: 33-40% acetone precipitate of the crude extract</td>
<td>6</td>
<td>64 ± 1</td>
<td>80 ± 2</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>2.5</td>
<td>0.006</td>
<td>F: 40-50% acetone precipitate of the crude extract; renin purified on a pepstatin column</td>
<td>3</td>
<td>63 ± 1</td>
<td>61 ± 1</td>
<td>‡</td>
</tr>
<tr>
<td>5.0</td>
<td>0.012</td>
<td>F</td>
<td>3</td>
<td>61 ± 1</td>
<td></td>
<td>‡</td>
</tr>
<tr>
<td>10.0</td>
<td>0.024</td>
<td>F</td>
<td>3</td>
<td>62 ± 1</td>
<td></td>
<td>‡</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD.

* The amounts listed were injected subcutaneously each day for 10 consecutive days.

† An average of 5 consecutive days' blood pressure readings.

‡ Blood pressures did not increase above baseline.
pressure had been reduced to normal by direct immunization with preparation K that did not contain renin. This experiment was reported in a previous communication.\textsuperscript{2} Antirenin assays yielded negative results. As shown in Table 5, the passive intravenous transfer of this preparation (Ab-1) into three rabbits caused a significant lowering of blood pressure that required 8–10 days to return to the preinjection level. In one case the blood pressure fell to the normal preclipping level.

A second preparation of globulins (Ab-2) was derived from the plasma of a group of clipped hypertensive rabbits in which pressure had been lowered to normal by active immunization with preparation H. Antirenin assays of this preparation yielded the marginally significant value of 0.05 U/ml. Administration of these globulins to six rabbits was followed by decreases in blood pressure that in every case either attained or closely approached the normal preclipping level. From 5 to 9 days were required for the blood pressure to return to its preinjection value. The effect on blood pressure caused by the injection of globulin preparations Ab-2 and NG into one rabbit of this group is illustrated in Figure 2.

### THE RESPONSE TO PLASMA GLOBULINS OF RABBITS MADE HYPERTENSIVE WITH KIDNEY CORTEX PREPARATIONS

The rabbits made hypertensive with preparation A were treated with the same globulin preparations (Ab-1 and Ab-2) that were given to the clipped hypertensive rabbits of the preceding section and of Table 5. The results, presented in Table 6, show that both preparations were very effective, in most cases the blood pressure reaching the preinjection level. Globulins prepared from normal plasma (NG) did not have a significant effect on the blood pressure of the rabbits.

The record of one of the rabbits in this experiment is illustrated in Figure 3 and shows the fall in blood pressure to a normal level following the injection of a globulin preparation (Ab-2) and its return to the hypertensive preinjection level after an interval of 8 days. The injection of normal globulins (NG) was without effect.

### THE RESPONSE IN IMMUNIZED HYPERTENSIVE RABBITS

Twelve clipped hypertensive rabbits, one of which is illustrated in Figure 4 (left panel), were actively immunized with hog kidney cortex preparation H given in daily doses that contained 0.5 mg of protein and 0.1 GU of renin. The blood pressures of all 12 rabbits fell to normal. After several days during which blood pressures were normal, the injection of rabbit kidney cortex preparation A was initiated in six of the 12 rabbits. As illustrated by the example in Figure 4 (left panel), none of the six injected rabbits responded with an increase in blood pressure. The blood pressures of the uninjected immunized rabbits also remained unchanged during this time.

At the same time 12 normal rabbits were given preparation A in the same dosage for 10 days. All 12 rabbits

### Table 4

<table>
<thead>
<tr>
<th>Amount injected (mg)</th>
<th>No. of days injected</th>
<th>No. of rabbits</th>
<th>Baseline BP (mm Hg)</th>
<th>Days to maximum BP</th>
<th>Maximum BP (mm Hg)</th>
<th>BP at 44 days (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>17</td>
<td>3</td>
<td>62 ± 1</td>
<td>§</td>
<td>75 ± 5</td>
<td>74 ± 5</td>
</tr>
<tr>
<td>5.0</td>
<td>19</td>
<td>3</td>
<td>64 ± 0</td>
<td>25 ± 5</td>
<td>79 ± 1</td>
<td>81 ± 1</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD.

* Preparation H was the fraction of the extract of hog kidney cortex that precipitated between 33% and 40% acetone concentration and contained 0.2 GU of renin/mg of protein.

† Given subcutaneously each day.

† The average of 5 consecutive days' blood pressure readings.

§ The blood pressure did not increase.

| One rabbit did not respond.

### Table 5

<table>
<thead>
<tr>
<th>Source of globulin preparation</th>
<th>No. of rabbits</th>
<th>Normal preclipping BP (mm Hg)†</th>
<th>BP before giving globulins (mm Hg)†</th>
<th>Minimum BP after giving globulins (mm Hg)</th>
<th>Days to return to preinjection BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab-1: From clipped hypertensive rabbits immunized with preparation K‡</td>
<td>3</td>
<td>62 ± 1</td>
<td>97 ± 6</td>
<td>74 ± 10</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>Ab-2: From clipped hypertensive rabbits immunized with preparation H‡</td>
<td>6</td>
<td>63 ± 1</td>
<td>100 ± 1</td>
<td>65 ± 3</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>NG: From normal rabbits</td>
<td>6</td>
<td>62 ± 2</td>
<td>100 ± 4</td>
<td>97 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD.

* Given intravenously as described in the text.
† The average of 5 consecutive days' blood pressure readings.
‡ The plasma from which the globulins were prepared was collected during the time that the blood pressures of the clipped hypertensive rabbits had been reduced to normal by active immunization.
responded with an increase in blood pressure. The result found with one of the rabbits (rabbit 615) is illustrated in Figure 4 (right panel). The blood pressure increases for the entire group are given in Table 6 and range from 17 to 20 mm Hg.

Discussion

This investigation has demonstrated that the administration of certain extracts of kidney cortex to normal rabbits causes a delayed, slow increase of blood pressure and the establishment of a state of moderate, persistent hypertension. The elevation in blood pressure may be sustained for at least 94 days with no indication of a return to normal even though the administration of the extract was stopped on the 9th day (Fig. 1, upper panel).

The degree of hypertension that is established does not appear to be related to the size of the dose or the period of time over which it is given. It may be limited by an opposing or neutralizing factor in the preparation itself or perhaps by a controlling mechanism within the rabbit.

The hypertensive effect is not comparable to that of corticotensin⁹ or nephrotensin,¹⁰ substances of renal origin the pressor effect of which is characterized by an immediate onset and a very short duration.

The hypertensive effect is also totally unlike that of renin. Renin is pressor in hypertensive rabbits, whereas the new substance has no effect. Its action cannot be blocked with an angiotensin II antagonist, whereas that of renin can be eliminated completely. Preparations containing so little renin (0.04 GU) that the daily subcutaneous dose would produce an insignificant pressor response if injected intravenously, still produce the delayed, moderate, persistent hypertension. In contrast, the subcutaneous injection of a highly purified renin preparation containing as much as 10 GU of renin in each daily dose has no effect on blood pressure. The evidence supports the view that the hypertensive effect is not due to renin but to a new substance which we have named renopressin.

The hypertensive effect of kidney extracts has been observed previously. Thus, Masson et al.¹¹ have produced hypertensive vascular disease in the rat by chronic treatment with saline or water extracts of hog or rat kidneys.

Table 6  The Effect of Plasma Globulins* on the Blood Pressure of Rabbits Made Hypertensive with a Rabbit Kidney Cortex Preparation

<table>
<thead>
<tr>
<th>Source of globulin preparation</th>
<th>No. of rabbits</th>
<th>Normal baseline BP (mm Hg)†</th>
<th>BP before giving globulins (mm Hg)†</th>
<th>Minimum BP after giving globulins (mm Hg)</th>
<th>Days to return to preinjection BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab-1: From clipped hypertensive rabbits immunized with preparation K‡</td>
<td>3</td>
<td>63 ± 0</td>
<td>81 ± 1</td>
<td>64 ± 6</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>Ab-2: From clipped hypertensive rabbits immunized with preparation H†</td>
<td>6</td>
<td>62 ± 1</td>
<td>80 ± 1</td>
<td>63 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>NG: From normal rabbits</td>
<td>7</td>
<td>62 ± 1</td>
<td>81 ± 1</td>
<td>79 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD.

The rabbits in this experiment were injected subcutaneously with 1 mg (0.5 GU of renin) of preparation A each day for 10 days. The blood pressure increased in all rabbits, reaching a plateau between 3 and 17 days after the first injection. The blood pressures of all rabbits remained on this plateau until they were given globulins 15-30 days later.

* Given intravenously as described in the text.

† The average of 5 consecutive days' blood pressure readings.

‡ The plasma from which the globulins were prepared was collected during the time that the blood pressures of the clipped hypertensive rabbits had been reduced to normal by active immunization.
The authors believed that the hypertensive effect was due to renin and proposed a hypothesis to explain the means by which the renin-angiotensin system might produce sustained hypertension. More recently, Yamamoto et al. have demonstrated a "hypertensive-inducing potency" in rat kidney extracts administered to rats. These authors, however, could not find "definite evidence for the involvement of a renal substance(s) other than renin." Sen and Bumpus have extracted a compound from human urine that produces hypertension in normal rats.

It might appear inconsistent that the same hog renopressin preparation that was used to immunize clipped hypertensive rabbits (Fig. 4, left panel) also produced the persistent hypertension characteristic of renopressin when injected into normal rabbits (Table 4). However, the amounts of the preparation that were needed for an immunologic response may have been reduced by the use of Freund's adjuvant and were much smaller than were required to produce a hypertensive effect. Furthermore, all direct immunizations have been performed in hypertensive rabbits in which renopressin does not increase blood pressure (Table 2).

The evidence is consistent with the possibility that renopressin is responsible for the elevation in blood pressure in rabbits with one-kidney hypertension. Preparations containing rabbit renopressin have no effect in hypertensive rabbits in which pressures have been lowered to normal by active immunization (Fig. 4, left panel). This would suggest that the antibody that neutralizes the causative agent responsible for the hypertension also neutralizes the injected renopressin, and implies that the causative agent and renopressin must be similar or even identical.

Antibodies produced in hypertensive rabbits by immunization with hog preparations that contain renopressin not only lower the blood pressure of the rabbits in which they are produced but also lower the blood pressures of other hypertensive rabbits to which they are passively transferred (Fig. 2). (Our previous failure to lower the blood pressure of hypertensive rabbits by passive transfer may have been due to the absence of renopressin in the hog renin preparations that were used as the antigen for the production of the antiserum.) The antirenin that was produced in these experiments was used in immobilized form to remove renin from hog and rabbit kidney cortex preparations without removing renopressin. The same antibody preparations also lower the blood pressure of rabbits made hypertensive with rabbit renopressin (Fig. 3 and Table 6). Thus the antibodies must be directed both toward the substance that is responsible for the elevated blood pressure in the hypertensive rabbits and against the renopressin that elevated the blood pressure of the injected rabbits. This would indicate once again that the causative agent in the hypertensive rabbits and renopressin must be similar and possibly identical.

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References

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