Examination of the Relationship of Renin Release to Hypertension Produced in the Rabbit by Renal-Artery Constriction

By W. S. Peart, M.D., F.R.C.P., J. I. S. Robertson, M.B., B.Sc., M.R.C.P., and D. G. Grahame-Smith, M.B., M.R.C.P.

ALTHOUGH it is widely believed that renin release from the kidney is the mechanism responsible for the hypertension which follows renal-artery constriction, the evidence in favor of this view is, at best, inconclusive. We have earlier criticized some of the work purporting to demonstrate renin or angiotensin circulating in the blood in various forms of hypertension. In our view, adequate criteria for the identification of these substances have not been fulfilled. Conversely, experiments in which no evidence of circulating renin or angiotensin has been obtained have been indecisive, since it has not been demonstrated in those studies that the assay methods were sufficiently sensitive to detect the requisite quantities of renin or angiotensin. We, therefore, decided to re-examine the problem in hypertension produced in the rabbit by constricting the renal artery.

Rabbit renin may be easily detected and assayed by means of the pressor response obtained on its intravenous injection into the intact rat. For instance, by this technique, the renin contained in microscopic sections of rabbit kidney is readily detected. The rabbit is, moreover, the only animal in which it has been demonstrated that prolonged infusions of renin (or, indeed, any pressor substance) will produce sustained hypertension.

Summary of Previous Experimental Work Relating Renin to Hypertension in the Rabbit

Blacket et al. were able to produce sustained hypertension in the rabbit by infusions of renin continued for up to 18 days. The degree of elevation of blood pressure in these animals was limited to some 40 to 50 mm. Hg above the preinfusion level, and an increase in the infusion rate beyond this point produced a fall, rather than a rise, in arterial pressure. We, and several other workers, have found a similar limit in the height to which the blood pressure can be raised in the rabbit by renin infusion. Since considerably higher levels of blood pressure are found following renal-artery clamping in the rabbit, it appears that renin release alone cannot explain the phenomenon.

Pickering found that nephrectomy in the first week of renal-artery-clip hypertension usually, but not invariably, restored the blood pressure to normal; in hypertension of 7 to 15 weeks' duration, the blood pressure remained elevated in the short time the animal lived after nephrectomy. The implication here was that renin release might be responsible for hypertension in the first week after application of the clip; later, another mechanism was concerned, at least in part. Daniel et al. confirmed that the blood pressure remained elevated after total nephrectomy in rabbits with chronic hypertension. Blacket and Sellers studied the effect of removal of the renal-artery clip in rabbits with experimental hypertension of short and long duration. They found that the blood pressure was lowered in both groups, but that the return toward a normal pressure was slower in the animals with chronic hypertension. By contrast, Daniel et al. found that removal of the clip had little effect on the blood pressure of rabbits with hypertension of long duration. The animals used in the experiments by Daniel et al. had been hypertensive for longer periods than those of Blacket and Sellers, however, and were also shown radiologically to have a persistent deformity of the renal artery after removal of the clip.
Figure 1

Diagram of apparatus used in the earlier experiments. Renal-vein samples are collected via the wide-bore outer polyethylene catheter lying near the mouth of the renal vein. Infusions of renin are made through the inner fine nylon catheter passed up to the renal hilum.

Pickering, Prinzmetal, and Kelsall found that some rabbits with hypertension of short duration had greater concentrations of renin in the kidney than did normals; animals with chronic hypertension, by contrast, did not differ from unoperated rabbits in this respect. The renin concentration in the kidney does not, however, necessarily reflect the rate of renin release.

Evidence casting doubt on the significance of renin in relation to experimental hypertension in the rabbit is weighty. In this species, hypertension does not occur after renal-artery constriction unless the opposite kidney is removed. This suggested that if the hypertension is the consequence of renin release from the remaining kidney, unilateral nephrectomy might act by increasing the sensitivity of the animal to circulating renin. Blacket et al. demonstrated, however, that the sensitivity to infused renin is unaffected by the removal of up to two-thirds of the renal tissue, and several workers have found these experiments difficult to reconcile with the concept of renin release. Pickering and his colleagues have countered this by arguing that nephrectomy may alter the ratio between blood supply and demand in the ischemic kidney, and so increase the stimulus to renin release.

Prinzmetal et al. grafted kidneys from rabbits with hypertension following renal-artery constriction into other rabbits, including some which had had total nephrectomy performed. No elevation of the blood pressures of the recipient animals occurred.

Drury and his associates have produced the most compelling evidence contrary to the renin hypothesis. The repeated intravenous injection of renin into the rabbit leads to the diminution and final extinction of the pressor response. These workers abolished the pressor response to injected renin in this way in rabbits with both early and late hypertension and found that the blood pressure then reverted to its previously elevated level. Had renin been responsible for the hypertension in these animals, the blood pressure would have been expected to return to normal levels on extinction of the response to renin.

The present work was designed to attack the problem directly. Firstly, we attempted to determine whether or not renin, sufficient to raise the blood pressure of the rabbit, could be detected in the renal-vein blood at the prevailing rates of renal blood flow. Secondly, in rabbits rendered hypertensive by applying a clip to the renal artery, we examined the renal-vein blood for evidence of increased renin concentration.

Methods

Male rabbits of 1,500- to 3,500-Gm. weight were used. Routine blood pressures were measured from the central artery of the ear, using the capsule designed by Grant and Rothschild, the technique being as described by Pickering and Prinzmetal.

Experimental Hypertension

Experimental hypertension was produced by applying silver clips of various internal diameters, between 0.45 and 0.65 mm., to the left renal artery, and removing the right kidney; both procedures were carried out at one operation.
It is important to avoid handling the kidney at all stages in experiments of this nature; Govaerts and Verniory have rightly pointed out that manipulation of the kidney could itself lead to renin release. We applied the clip through a flank incision, the tendinous origins of the oblique abdominal muscles being separated from their attachments, and the long left renal pedicle being exposed extraperitoneally. At no stage was the kidney exposed or handled.

**Anesthesia**

Two methods were used. Some animals were given intravenous pentobarbitone sodium, others were given ether by inhalation. The type of anesthetic employed for each animal is stated in Table 1. Ether was used for all the rabbits in which direct measurement of renal blood flow was made. Approximately half of the control infusions of renin were made following pentobarbitone anesthesia, and half following ether.

**Sampling and Infusion**

Two principal techniques were used for obtaining renal-vein samples and for infusing renin into the renal venous blood. Essentially no difference was found in the results whichever method was employed. Care was taken to avoid handling the kidney at all stages. All rabbits were given 2,500 units of heparin at the outset, and further doses as required in the longer experiments.

1. In one group, anesthesia was induced and a polyethylene catheter of 2-mm. internal diameter was inserted into the right jugular vein. The catheter was passed via the right atrium into the posterior vena cava. A laparotomy was performed; the catheter was manipulated into the left renal vein and passed along the vein until the tip lay on the renal side of the main tributaries, usually a lumbar and a testicular vein. The catheter was then held in position by means of a ligature tied around the jugular vein. The tip lay free in the renal vein, permitting samples to be obtained as desired, while allowing renal-vein blood to flow past the catheter into the vena cava. Samples were taken off very slowly, to avoid drawing back caval blood, diluting the renal effluent, and so masking any pressor material which might be present. In some animals, as indicated, the entire sampling was performed under anesthesia; in others, the abdomen was closed and the rabbit was allowed to regain consciousness before collection. As a further and final maneuver in rabbits 6, 43, 52, 59, 78, 123, and 133, the sampling catheter was tied firmly in place in the renal vein under anesthesia and the entire renal-vein effluent then collected.

With this first method of renal-vein sampling, renin infusions were made into the renal end of the vein through an inner fine nylon catheter of 0.5-mm. bore passed up to the renal hilum, the samples being withdrawn as desired from the outer catheter (fig. 1). To facilitate adequate mixing, multiple fine holes were made in the tip of the infusion catheter. In the control infusions, the tip of the sampling catheter was placed in the renal vein close to the junction with the cava, so that the infused renin was further diluted by blood entering the renal vein from the lumbar and testicular tributaries. The sampling catheter was not tied into the renal vein in the infusion experiments, blood being permitted to flow freely into the vena cava.

2. In the later group of experiments, the entire
### Table 1

**Summary of Data Obtained in the Twenty-three Rabbits Hypertensive Following Unilateral Nephrectomy and Renal-Artery Clipping**

<table>
<thead>
<tr>
<th>Rabbit number</th>
<th>Duration clipping to sampling (days)</th>
<th>Duration of hypertension (&gt; 90 mm. Hg; days)</th>
<th>Ear capsule</th>
<th>Initial blood pressure (mm. Hg)</th>
<th>Final blood pressure (mm. Hg)</th>
<th>Blood pressure during sampling (mm. Hg)</th>
<th>Anesthetic</th>
<th>Final blood urea (mg. %)</th>
<th>Maximum possible renin content of renal-vein plasma (in terms of ml. standard renin/ml. rabbit plasma)</th>
<th>Renal blood flow (ml./min.)</th>
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<tbody>
<tr>
<td>6</td>
<td>22</td>
<td>17</td>
<td></td>
<td>75</td>
<td>134</td>
<td>134 — unrecordable</td>
<td>P</td>
<td>75</td>
<td>&lt; 0.05</td>
<td>—</td>
</tr>
<tr>
<td>29</td>
<td>36</td>
<td>21</td>
<td></td>
<td>72</td>
<td>132</td>
<td>132*</td>
<td>P</td>
<td>320</td>
<td>&lt; 0.08</td>
<td>—</td>
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<tr>
<td>37</td>
<td>24</td>
<td>8</td>
<td></td>
<td>71</td>
<td>114</td>
<td>114*</td>
<td>P</td>
<td>76</td>
<td>&lt; 0.03</td>
<td>—</td>
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<tr>
<td>43</td>
<td>71</td>
<td>45</td>
<td></td>
<td>63</td>
<td>115</td>
<td>110 — 115</td>
<td>P</td>
<td>100</td>
<td>&lt; 0.03</td>
<td>—</td>
</tr>
<tr>
<td>44</td>
<td>5</td>
<td>3</td>
<td></td>
<td>69</td>
<td>104</td>
<td>104*</td>
<td>P</td>
<td>304</td>
<td>&lt; 0.05</td>
<td>—</td>
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<td>120</td>
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<td>P</td>
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<td>&lt; 0.02</td>
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<tr>
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<td>68</td>
<td>56</td>
<td></td>
<td>76</td>
<td>134</td>
<td>105*</td>
<td>P</td>
<td>91</td>
<td>&lt; 0.03</td>
<td>—</td>
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<tr>
<td>59</td>
<td>520</td>
<td>161</td>
<td></td>
<td>73</td>
<td>115</td>
<td>110 — 115 C</td>
<td>+</td>
<td>E</td>
<td>87 &lt; 0.04</td>
<td>—</td>
</tr>
<tr>
<td>61</td>
<td>96</td>
<td>41</td>
<td></td>
<td>70</td>
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<td>100</td>
<td>&lt; 0.01</td>
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<tr>
<td>78</td>
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<td>13</td>
<td></td>
<td>87</td>
<td>119</td>
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<td>P</td>
<td>72</td>
<td>&lt; 0.02</td>
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<td>110 — 134</td>
<td>+</td>
<td>P</td>
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<td>—</td>
</tr>
<tr>
<td>111</td>
<td>38</td>
<td>8</td>
<td></td>
<td>68</td>
<td>112</td>
<td>96 — 108</td>
<td>+</td>
<td>P</td>
<td>293 &lt; 0.08</td>
<td>—</td>
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<td>123</td>
<td>54</td>
<td>17</td>
<td></td>
<td>78</td>
<td>98</td>
<td>94 — 98</td>
<td>P</td>
<td>91</td>
<td>&lt; 0.05</td>
<td>—</td>
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<tr>
<td>132</td>
<td>267</td>
<td>259</td>
<td></td>
<td>80</td>
<td>115</td>
<td>120 — 133 C</td>
<td>+</td>
<td>E</td>
<td>67 &lt; 0.08</td>
<td>17.0</td>
</tr>
<tr>
<td>133</td>
<td>17</td>
<td>12</td>
<td></td>
<td>72</td>
<td>100</td>
<td>102 — 120 C</td>
<td>+</td>
<td>E</td>
<td>138 &lt; 0.08</td>
<td>—</td>
</tr>
<tr>
<td>179</td>
<td>21</td>
<td>18</td>
<td></td>
<td>77</td>
<td>105</td>
<td>120 — 126 C</td>
<td>+</td>
<td>E</td>
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<tr>
<td>185</td>
<td>16</td>
<td>9</td>
<td></td>
<td>70</td>
<td>107</td>
<td>102 — 117 C</td>
<td>+</td>
<td>E</td>
<td>86 &lt; 0.04</td>
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<tr>
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<td>21</td>
<td>9</td>
<td></td>
<td>70</td>
<td>105</td>
<td>125 — 132 C</td>
<td>+</td>
<td>E</td>
<td>98 &lt; 0.04</td>
<td>5.0</td>
</tr>
<tr>
<td>199</td>
<td>61</td>
<td>38</td>
<td></td>
<td>59</td>
<td>140</td>
<td>100 — 130 C</td>
<td>+</td>
<td>E</td>
<td>247 &lt; 0.12</td>
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</tr>
<tr>
<td>202</td>
<td>10</td>
<td>3</td>
<td></td>
<td>81</td>
<td>106</td>
<td>50 — 105 C</td>
<td>+</td>
<td>E</td>
<td>60 &lt; 0.12</td>
<td>2.5</td>
</tr>
<tr>
<td>207</td>
<td>16</td>
<td>9</td>
<td></td>
<td>70</td>
<td>102</td>
<td>100 — 108 C</td>
<td>+</td>
<td>E</td>
<td>79 &lt; 0.08</td>
<td>2.5</td>
</tr>
</tbody>
</table>

C = Blood pressure by carotid catheter.

* = Some samples collected in conscious animals.

P = Pentobarbitone anesthesia.

E = Ether anesthesia.

* = Isolated readings at commencement only.
Assay of renal-vein plasma obtained from rabbit 187 during the infusion of renin sufficient to elevate the blood pressure 25 to 30 mm. Hg (see tables 1 and 2). Renal-vein plasma (P); standard renin (R). Doses in ml. Assay by effect on rat’s blood pressure. 0.1 ml. of renal-vein plasma contains less than 0.04 ml. of standard renin and more than 0.02 ml. The pressor effects of 0.1 ml. of plasma and 0.03 ml. of standard renin are equal. Note that the plasma causes a sharper rise in the rat’s blood pressure than does the standard renin, presumably due to the presence of angiotensin in the sample.

Renal-vein effluent was diverted through an external circuit as follows. The blood was carried from the renal-vein catheter through a vinyl drip chamber and returned to the animal by a second catheter inserted into the opposite jugular vein. A side branch permitted blood samples to be taken, and infusions of renin could be made into the system through the wall of a short length of silicone rubber in the circuit (see fig. 2). When a ligature had been tied around the catheter in the renal vein, the entire renal blood flow was carried through the external circuit, and direct measurement of the flow could be made either by timing the delivery of a measured quantity of blood, or by counting the drop rate and subsequently calibrating the system. When the circuit had been set up, the abdominal wound was closed and the animal was allowed to regain consciousness. It was found that this system could be maintained with the animal lightly restrained for up to 12 hours without appreciable fall in the renal blood flow or arterial pressure, and without the occurrence of undue hemolysis. Open ether was used as anesthetic in these experiments because of the speed with which the rabbits regained consciousness. The arrangement permitted renal-vein samples to be taken both in the resting state and during the infusion of renin at the same experiment, with simultaneous measurement of the renal blood flow in the fully conscious, intact animal. This system was capable of sustaining a unilateral renal blood flow of up to 28 ml. per minute and, therefore, did not seem to offer any undue resistance to flow. The circuit was not, however, intended to reproduce physiological conditions of renal blood flow, but to ensure that infusions of renin into renal-vein blood and the collection of renal blood from animals with hypertension were carried out under the same conditions, and were therefore comparable. This method of sampling was used in those rabbits for which renal blood flows are given in tables 1 and 2.

Blood-Pressure Measurement During Sampling

In some animals, measurement was made from the ear using Grant and Rothschild’s capsule. In others, a continuous record of the arterial pressure was made by means of an intracarotid polyethylene catheter connected to a mercury manometer. A side arm of this intracarotid catheter permitted carotid-artery samples to be obtained. Continuous blood-pressure measurement was used for all the renin-infusion experiments. The method of recording and the blood-pressure range during sampling are indicated in table 1 and are compared with the final presampling pressures obtained by the ear capsule. It is seen that there is good agreement between the two groups of readings, and with certain exceptions discussed in detail later, the rabbits remained

<table>
<thead>
<tr>
<th>Rabbit number</th>
<th>Blood pressure rise on renal infusion (mm. Hg)</th>
<th>Recovery from renal-vein plasma (ml.)</th>
<th>Renal blood flow (ml./min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>previously unoperated rabbits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>25</td>
<td>0.14</td>
<td>—</td>
</tr>
<tr>
<td>b</td>
<td>30–35</td>
<td>0.35</td>
<td>—</td>
</tr>
<tr>
<td>c</td>
<td>15–18</td>
<td>0.6</td>
<td>—</td>
</tr>
<tr>
<td>d</td>
<td>40–45</td>
<td>0.6</td>
<td>—</td>
</tr>
<tr>
<td>e</td>
<td>30–35</td>
<td>0.75</td>
<td>—</td>
</tr>
<tr>
<td>f</td>
<td>12</td>
<td>0.10</td>
<td>12–14</td>
</tr>
<tr>
<td>g</td>
<td>25</td>
<td>0.25</td>
<td>6.5</td>
</tr>
<tr>
<td>58</td>
<td>25–30</td>
<td>0.75</td>
<td>—</td>
</tr>
<tr>
<td>172</td>
<td>30</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>clipped, hypertensive rabbits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>132</td>
<td>20</td>
<td>0.12</td>
<td>17</td>
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<td>22–25</td>
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<td>187</td>
<td>25–30</td>
<td>0.30</td>
<td>5.0</td>
</tr>
<tr>
<td>199</td>
<td>22–25</td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Comparison of pressor effects of carotid-artery and renal-vein plasma samples from rabbit 179 (see table 1). Doses in ml. The effects of both the plasma samples are similar, and both are less than 0.01 ml. of standard renin.

hypertensive during sampling. The carotid values are generally rather higher than those from the ear, in agreement with the findings of Daniel et al. Collection of Blood Samples

The blood samples were taken into siliconed glass tubes cooled by immersion in ice water. These were centrifuged at 1,500 g at 2 to 4 C. for 25 minutes. The supernatant plasma was then pipeted into similar siliconed glass tubes and stored frozen to await assay.

Assay

Rabbit renin added to rabbit blood and taken through the procedure of plasma separation was recovered virtually without loss on assay of the plasma.

The assay was performed against an arbitrary standard solution of rabbit renin using the blood pressure of the anesthetized rat. This solution of renin was prepared from alcohol-dried rabbit kidney powder diluted so that the smallest volume which could conveniently be administered to the rat, 0.02 ml., contained the smallest quantity of renin which could consistently be recognized by the characteristic response (fig. 3). The same arbitrary standard was used throughout the present study.

Renin Infusion

The renin used for infusion was also prepared as described by Peart, and further purified by chromatography on diethyl-aminoethyl-cellulose. It was finally made up to the appropriate concentration in 0.9 per cent sodium chloride. Care was taken to ensure that this renin was not infected. An adjustable rate pump was used for renin infusion, the usual rates required to produce hypertension varying from 0.0125 ml./min. to 0.050 ml./min.

Results

Assay of Renal-Vein Plasma in Rabbits with Hypertension

Renal-vein samples were obtained from 23 animals with hypertension, at varying intervals after clipping. With two exceptions, to be discussed later, the pressor activity per ml. of plasma in all of these animals was less than 0.08 ml. of the standard renin preparation (table 1 and figs. 4 and 5). The sensitivity of the rats used in the assay varied considerably, and in the more sensitive preparations, it was possible to give a lower figure for the maximum quantity of renin present. It was unusual to find plasma samples devoid of pressor activity, whether drawn from control or hypertensive rabbits, from renal vein or other sites. Very often, however, the duration of action of these samples was briefer than that of renin (fig. 6). Since it was possible that at least some of the sharp-acting pressor effect might be due to angiotensin, formed by incubation during the process of sampling and separation of the blood, the plasma samples from 13 of the 23 hypertensive rabbits were incubated in a water bath for 30 minutes at 37 C. so as to obtain the maximum yield of angiotensin; the samples were then assayed against a standard angiotensin solution. In all instances, it was found that the quantity of sharp-acting material formed on incubation in this way was considerably less than that produced by incubating rabbit plasma containing our standard rabbit renin in a concentration equivalent to 0.08 ml. per ml. of plasma. In those cases in which the assay has been carried out in two ways, as above, the estimation which gave the greater value for renin content has been entered in table 1.

The incubation of rabbit plasma in siliconed glass tubes not infrequently resulted in the appearance of sharp-acting pressor material,
regardless of the site from which the sample was taken or whether the donor animal was hypertensive or not. Although we have, for the present purpose, assayed all these sharp-acting pressor responses as angiotensin, it is likely that in several cases a pressor artifact was introduced by incubation. Generally speaking, therefore, we have preferred to deal with unincubated plasma, assayed against renin. The figures given in table 1 refer to the maximum quantities of renin that might possibly be present in the samples; almost certainly, for the reasons given above, these figures overestimate the situation. Indeed, we found no convincing evidence of the presence of renin in any of the plasma samples taken from the rabbits with clip hypertension.

In 18 of the 23 hypertensive animals, control samples of blood were drawn, almost simultaneously with the renal-vein samples, from the posterior vena cava distal to the junction with the renal veins, the anterior vena cava, or the carotid artery. In all except three instances (rabbits 6, 199, and 202), no difference was found in either the height or shape of the response given by the control sample from that given by the renal-vein plasma (figs. 4 and 7). Incubation also showed no consistent difference between the control and renal-vein samples.

Samples Taken from Normotensive Rabbits

Plasma samples were taken for assay from the renal veins and control sites of 11 rabbits on which operations had been performed, but in which the blood pressures had remained normal. Similar samples were also taken from 19 unoperated, normotensive rabbits. It was unusual to find these plasma samples completely devoid of pressor activity on injection into the rat (fig. 6). Assay of the neat samples showed that, in all instances, these contained less than the equivalent of 0.08 ml. of the standard renin per ml. of plasma. Incubation of these samples for 30 minutes at 37 C. usually resulted in a small increase in short-acting pressor effect, but there was no significant difference between the samples taken from renal veins or other sites in this respect. All the samples taken from these normotensive rabbits fell, therefore, whether assayed neat against the standard renin, or, after incubation, against angiotensin, in the same range of pressor activity as did the samples taken from the hypertensive animals.

Samples Collected from Renal Veins of Rabbits Made Hypertensive by Infusion of Renin Into the Renal Vein

Renal-vein blood was collected from three groups of animals during the infusion of renin into the renal vein (table 2):
Figure 6
Pressor activity of renal-vein plasma obtained from a previously unoperated normal rabbit. Doses in ml.; plasma (P); standard renin (R). Assay on blood pressure of rat.

1. Seven previously unoperated normotensive rabbits were infused to give rises of arterial pressure varying from 10 to 50 mm. Hg. Simultaneous direct measurement of renal blood flow was made in two of these.

2. Rabbits 173 and 58, normotensive respectively four days and 94 days after renal-artery clipping, and with blood ureas of 61 mg. per cent and 75 mg. per cent, were infused to elevate the blood pressure 25 to 30 mm. Hg in each case. Simultaneous renal-blood-flow measurement was made in rabbit 173.

3. Four rabbits (nos. 132, 179, 187, and 199), hypertensive following renal-artery clipping, were given infusions of renin into the renal-vein blood after the initial renal-vein samples had been collected. Renal blood flow was measured in all of these, and the rate of infusion of renin was sufficient to raise the arterial pressure 20 to 30 mm. Hg above the already hypertensive level.

In each of these infusions, the renin was administered at a constant rate, and the arterial pressure was allowed to stabilize at that rate of renin infusion. Blood samples were not collected until the pressure had leveled off in this manner (fig. 8). Further, the experiment was not accepted as valid unless the animal was shown to respond to subsequent renin administration. These precautions were necessary to ensure that the renin was not infused at an inappropriately fast rate. The rate of renin infusion was, of course, varied from experiment to experiment, and in several instances an animal was given a number of infusions at different rates and with varying degrees of elevation of the arterial pressure.

In all three groups of infusion experiments, renin was readily detected in the renal-vein blood (figs. 3, 5, and 7). The lowest concentration of renin found in these samples was in an unoperated rabbit infused to give a blood-pressure rise of only 10 mm. Hg, that is, half the smallest blood-pressure rise achieved in the rabbits made hypertensive by clipping. In this case, the renal-vein plasma contained, in 0.25-ml. dose, between 0.01 ml. and 0.03 ml. of the standard renin, and the response was closely matched in height and shape by 0.02 ml. of the standard renin. Thus, the lowest concentration of renin found in all the control infusions was at the lower limit of the direct assay. Indirect assay, however, by incubation to form angiotensin, revealed, even in this instance, a distinctly greater renin concentration than the maximum possible in any of the samples taken from hypertensive rabbits or from unoperated rabbits not infused with renin. In all the other rabbits, which were infused at rates which gave hypertension more nearly comparable to that following clipping, renin was very easily detected in the renal-vein plasma (figs. 3, 5, and 7).

Direct Measurement of Renal Blood Flow
The blood flow from the left renal vein was measured by the direct method in 10 previously unoperated rabbits, one rabbit on which a renal-artery clip had been placed but which had remained normotensive, and seven animals with hypertension of varying duration.

Previously Unoperated Rabbits
The left renal blood flow of 10 previously unoperated rabbits varied between 6 ml./min. and 28 ml./min., with an average of 12.6 ml./min. In two animals of this group, technical
difficulties were encountered in setting up the circuit, and both were slightly hypotensive at the time the measurements were made. The lowest blood flow found in this group was in one of these two. These measurements of unilateral renal blood flow are less than would have been expected from the experiments of Hughes-Jones et al. using diodone clearance to measure the overall effective renal plasma flow. Some of the discrepancy may be a reflection of the inherent error of the clearance technique as a measure of renal blood flow, but it must be recognized that the anesthesia and operative procedures required to set up the bypass circuit used by us might, in themselves, be capable of reducing renal blood flow even when no obvious hypotensive episode had occurred.

**Rabbit Normotensive After Clipping**

In the one clipped, normotensive rabbit in which the renal blood flow was measured directly, this was found to vary between 9 ml. and 5 ml./min. before the infusion of renin.

**Rabbits with Hypertension (Table 1)**

In these seven animals, the renal blood flow was found to be generally a good deal lower than in the unoperated group. Rabbit 132, however, with chronic hypertension, had a renal blood flow of 17 ml./min. This animal was very big (4,420 Gm.), with a large remaining kidney (16.4 Gm.). The others, with early hypertension, had renal blood flows varying between 2 ml./min. and 7.5 ml./min. These were smaller rabbits (mean weight 2,550 Gm.), with a mean kidney weight of 12.4 Gm.

**Effect of Hypotension During Collection of Samples**

Table 1 shows that hypertension was generally well maintained during the collection of blood samples. There were certain exceptions, however, which merit consideration. Rabbit 199 had a blood urea of 247 mg. per cent and a blood pressure, as measured by the ear capsule, of 140 mm. Hg. Subsequent histological examination showed fibrinoid necroses in the arterioles of small and large intestines, and extensive mucosal and submucosal hemorrhages throughout the bowel. When the renal bypass circuit was first set up in this animal, the arterial pressure, as measured directly by means of a carotid catheter, was 100 mm. Hg. The renal artery proximal to the clip was seen to be pulsating vigorously, but there was no renal blood flow. No obstruction was present in any part of the circuit, and the kidney was not congested. As the animal recovered from the anesthetic, the blood pressure rose to the region of 120 to 130 mm. Hg, and with this the renal blood flow recommenced, settling to a steady 2 ml./min.

Rabbit 202 had milder hypertension, 106 mm. Hg by the ear capsule, and a blood urea only slightly raised at 60 mg. per cent. Technical difficulties were encountered in setting up the circuit, and the blood pressure was initially only 50 mm. Hg, as measured by the carotid catheter, at which time there was no renal blood flow. During the next five minutes, the blood pressure rose to 105 mm. Hg, when the renal blood flow started and continued at 2.5 ml. per minute.

These two animals indicate how critical is the general level of arterial pressure in governing the renal blood flow when a renal-artery constriction is present. A brief period of mild hypotension in each of these rabbits was sufficient to stop the renal blood flow. We have observed a similar phenomenon in renal-artery stenosis in man. Renal-vein plasma samples taken after the recommencement of the renal blood flow had, in both these rabbits, a greater pressor effect than plasma collected simultaneously from the carotid artery and, in both, fell outside the normal range.

In rabbit 6, the blood pressure fell to unrecordable levels during the sampling procedures, and again a greater pressor activity was found in renal-vein plasma than in plasma from the lower posterior vena cava. The pressor effect of this renal plasma was not, however, outside the normal range.

The increased pressor activity in the renal-vein plasma of these three rabbits must be interpreted with caution. In experiments in which we have deliberately clamped off the renal artery and then released the clamp, the renal venous blood has been found to contain
Figure 7

Comparison of effect on rat's blood pressure of plasma samples obtained from three rabbits. Dose, 0.1 ml. Posterior vena cava below renal veins (VC); renal vein (r). (A)-rabbit 78, and (B)-rabbit 43, both hypertensive following renal-artery clipping (see table 1); (C)-rabbit 58, which had remained normotensive after operation. Renal-vein sample collected from rabbit 58 during infusion of renin sufficient to elevate the arterial pressure 25 to 30 mm. Hg.

appreciable quantities of pressor material on intravenous injection into the rat. It is likely that the findings in the renal-vein plasma of rabbits 6, 199, and 202 are due, similarly, to acute interference with the renal blood flow at operation, and while renin may be released into the renal vein in these circumstances, the physiological significance of this remains doubtful.

Effect of Renin on Renal Blood Flow

The infusions of renin given in these experiments were found not to have marked effects upon renal blood flow, although in some instances, a reduction in blood flow occurred during the infusion. For example, the renal blood flow of rabbit (g) (table 2), previously unoperated, fell from 11 ml./min. to 6.5 ml./min. during a renin infusion sufficient to elevate the blood pressure 25 mm. Hg, and from 5 ml./min. to 4.5 ml./min. during a renin infusion which raised the pressure 12 to 15 mm. Hg, returning to the basal flow rate at the end of each of these infusions. By contrast, the renal blood flow of the unoperated rabbit (f) (table 2) was unaffected by renin infusions. The clipped, hypertensive rabbit 132 sustained a series of renin infusions, each producing a good rise in arterial pressure, without effect on renal blood flow. A single intravenous injection of renin sufficient to raise the blood pressure of a previously unoperated rabbit by 65 mm. Hg reduced the renal blood flow from 12 ml./min. to 6 ml./min., this returning to 12 ml./min. as the arterial pressure returned to normal. These findings are consistent with those of Hughes-Jones et al.20 who showed that the intravenous injection of renin in the rabbit produced a fall in diodone clearance.

Not surprisingly, the renal blood flow tended to fall in our experiments if repeated blood samples were taken from an animal.

Blood-Urea Levels in Rabbits with Hypertension

Most previous workers have indicated that hypertension produced in the rabbit by renal-artery clipping and unilateral nephrectomy is independent of renal failure; but we have been able to find only occasional examples in the literature6,19,22 of rabbits made hypertensive by the present method, and with unequivocally normal blood-urea levels.

Samples were taken for blood-urea estimation in 82 rabbits of the present series. In 25 animals, estimations were made before any surgical procedure had been carried out. In this group, the highest blood urea found was
Comparison of blood pressure (BP) with blood urea (121 estimations in 82 rabbits). Unoperated animals (open circles); unilateral nephrectomy and renal-arteryclip (crosses). Horizontal line at 90 mm. Hg; vertical line at blood-urea level of 55 mg. per cent.

55 mg. per cent, and all but two of the estimations were below 50 mg. per cent. After right nephrectomy and left-renal-artery clipping, blood-urea estimations were made at various times. Figure 9 shows the results obtained for blood urea plotted against the relevant blood-pressure readings. It will be seen that after operation, the animals fall into three categories. In one group of 16 estimations, both blood pressure and blood urea remained normal after operation. In a second group of 34 estimations, the blood-urea level was raised, but the blood pressure remained in the normal range. In the third group, consisting of 46 instances of hypertension, the blood-urea levels, with only one exception, were above the range found in unoperated animals. The lowest blood urea found in the rabbits with hypertension was 54 mg. per 100 ml., equal to the highest level found in the unoperated group. In no other instance was a blood urea found to be in the normal range in any rabbit with hypertension. Thus, post-operatively, the blood urea might be raised in the presence of a normal blood pressure, but the converse did not hold, hypertension being always associated with a high blood-urea level. In a number of animals, serial estimations were made of the blood urea, when it might be seen to rise with the arterial pressure. For instance, rabbit no. 123 had a blood urea of 31 mg. per cent before operation. Thirteen days later, when the blood pressure remained at the preoperative level, the blood urea was 60 mg. per cent. Fifty-four days after the renal-artery clip had been applied, and 17 days after the animal had been definitely hypertensive, the blood urea was 91 mg. per cent.

Our present experience, therefore, based upon 121 estimations of blood urea in 82 different animals, is that this form of hypertension in the rabbit is almost invariably associated with some impairment of renal function, as judged by the relatively crude test of blood-urea estimation. Since there is a possibility that the hypertension is in some way a consequence of the renal impairment, we may
note that after total nephrectomy, rabbits die before developing hypertension, but that removal of two-thirds of the kidney substance may be followed by hypertension and an elevation of the blood urea. 6

Discussion

The experiments described here have attempted to test the hypothesis that the hypertension which results in the rabbit from the removal of one kidney and the application of a clip to the opposite renal artery is due, at least at some stage, to release of renin in an active form from the kidney into the renal-vein blood. Renal-vein samples have been obtained from 5 to 529 days after the application of the clip, and with hypertension of from 3 to 259 days' duration. In none of these animals have we found evidence of renin release into the renal vein. Slight pressor effects have been found on injection of the renal-vein plasma samples into the rat, but these have not been outside the range encountered when plasma from normotensive control rabbits has been used. Further, there has been no significant difference in the pressor effect of renal-vein plasma and plasma obtained from another site in the hypertensive animals, with the exception of rabbits 6, 199, and 202 discussed herein. By contrast, renin infused into the renal veins of previously unoperated rabbits or in animals which maintained a normal blood pressure after operation, sufficient to raise the arterial pressure to comparable levels, was readily detectable, despite the fact that in the infused rabbits the renal blood flow was generally greater than in the clipped, hypertensive group. Furthermore, renin infused into the renal veins of clipped, hypertensive rabbits was always capable of raising the blood pressure further, and was in all instances easily detected.

We do not regard this work as constituting a final rebuttal of the renin theory in this connection, since the experiments were subject to certain limitations which should be carefully considered. Firstly, our sampling technique required the administration of a general anesthetic and laparotomy for the insertion of the renal-vein catheter. These procedures could have reduced the renal blood flow acutely, so that in the experiments in which renin was infused, it was found in unusually high concentration. Any systematic reduction in renal blood flow in our experiments would have occurred also in the clipped, hypertensive rabbits at sampling and, in fact, this group had generally lower renal blood flows than the rabbits used for the control infusions. A reduction in the renal blood flow in the clipped, hypertensive group would have increased the concentration of any renin present unless the procedure also inhibited renin release. This seems improbable in that the rabbits were hypertensive at the time of collection of the samples, and interference with the renal circulation would appear likely to stimulate, rather than inhibit, the release of pressor substances from the kidney, as was seen in rabbits 199 and 202.

A second possibility is that during the infusions, we were administering renin at an unnecessarily fast rate, and thus deceiving ourselves as to the adequacy of our assay technique. Rabbits rapidly become unresponsive to the repeated administration of large quantities of renin, and our present observations confirm the studies of Blacket et al. 8 who found that renin infusion raises the blood pressure only 40 to 50 mm. Hg, and that increasing the infusion rate beyond this maximum causes a fall rather than a rise in arterial pressure. In an attempt to meet this point, as already described, we accepted as valid experiments only those control infusions of renin in which the blood pressure rose to a plateau at a given dose rate and then remained steady, and in which the animal was also shown to be responsive to subsequent renin administration (fig. 8). The infusions given in the present experiments were, of course, short-term affairs, measured in hours rather than days. The work of Blacket et al. 8 had previously shown, however, that the sensitivity to renin was largely unaltered in infusions given for up to 18 days. Where any variation was noticed, the animals were more, rather than less, sensitive early in the infusion.
Thirdly, the assay technique used by us was not sufficiently sensitive always to detect renin in the peripheral blood of rabbits made hypertensive by renin infusion into the renal vein. It is, therefore, possible that after renal-artery clamping, renin was entering the circulation partly or wholly by a route other than the renal vein; for example, by way of the renal lymphatics and thoracic duct.

A further explanation might be that renin is released into the renal vein as an inactive precursor, which is then activated at another site. Active renin is, however, readily extracted from the kidney by very simple procedures.5

Our experiments in no way exclude, nor, indeed, were they designed to test, the possibility that renin is released from the kidney in small quantities and, in some way, initiates or potentiates a pressor mechanism other than the renin-angiotensin system.

Due weight should be given to these various objections. Negative findings almost invariably raise some doubts, and this is especially true in a controversial field, where emotion and sentiment may often outrun the data. Nevertheless, we may fairly conclude that the present experiments have produced nothing in favor of the renin hypothesis in its present form, and much which tends to refute it.

**Summary**

Plasma samples taken from the renal veins of rabbits hypertensive following renal-artery constriction were examined for evidence of increased renin content. No increase in the activity of the renal-vein samples was found as compared with plasma obtained from other sites in these rabbits, or with samples taken from unoperated animals. By contrast, renin infused into the renal veins of rabbits to give comparable elevation of the arterial pressure was, in all cases, detected. The experimental findings are discussed in relation to current views of the role of renin in experimental hypertension in the rabbit.

**Acknowledgment**

Technical assistance was provided by Mrs. N. N. Mendelsson, Miss G. Stean, Miss M. Tooley, Mr. A. Taylor, and Mr. D. Reder.

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Examination of the Relationship of Renin Release to Hypertension Produced in the Rabbit by Renal-Artery Constriction

W. S. PEART, J. I. S. ROBERTSON and D. G. GRAHAME-SMITH

doi: 10.1161/01.RES.9.6.1171

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/9/6/1171

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