The capacity of certain renal extracts to lower the blood pressure in experimentally induced hypertension has been described (1), but the study and use of such extracts has been hampered by the small yield obtained from renal tissue and the difficulty of concentrating this activity. The present paper reports an improved procedure for preparing active extracts and the purification of these to a form suitable for chemical study and for attempts at isolation of the active hypotensive principle.

**EXPERIMENTAL**

*Preparation of Renal Extract—* Fresh pork kidneys, immediately on removal from the body, are ground into acetone containing 1.6 ml. of concentrated HCl per liter, 0.9 liter of acetone being used per pound of kidney tissue. The active principle is rapidly destroyed after death since little or no activity is obtainable from kidneys that, although frozen promptly, are not processed immediately after slaughter of the animal. The active principle is also unstable in alkaline media and hence acid is added to the acetone. Heating also destroys the active principle and all distillations are therefore carried out in vacuo at a temperature not exceeding 50°. The finely ground suspension of tissue is shaken at intervals and allowed to stand overnight at 5°. It is then filtered through gauze and the residue reextracted twice overnight with 0.3 liter of a mixture of 2 volumes of acetone and 1 volume of water per pound of original kidney tissue.

The combined aqueous acetone extracts are filtered and concentrated in vacuo at a temperature not in excess of 50°. After chilling in the refrigerator overnight, the aqueous concentrate is again filtered. It contains 5.5 to 6.0 gm. of solid in a volume of approximately 1.5 gm. per pound of original kidney tissue. The crude extract thus obtained has a solid content of about 1.0 gm. per pound of original kidney tissue. It is assayed on hypertensive rats by mixing with the animals' food as described previously (1, 2). The equivalent of 1 to 2 pounds of kidney are required to lower the blood pressure of one rat significantly for a period of 2 to 4 days; the administration of the extract derived from 5 to 10 pounds of fresh kidney tissue results in a more profound and protracted decline in the blood pressure which does not return to its pretreatment level for 7 to 10 days. It is this prolonged decline in blood pressure, which reaches its maximum about 24 hours after the animals have completed ingesting the mixture of food containing the extract, that is characteristic of the hypotensive principle derived from renal tissue and distinguishes it from other hypotensive agents which may induce brief periods of hypotension with no latent period (3).

**Purification of Extract by Countercurrent Distribution—** Countercurrent distribution between water and sec-butanol has proved most useful in the purification of the crude extract obtained as just described. The extract derived from 40 pounds of kidney tissue in a volume of 300 ml. of sec-butanol is placed in the first upper 3 tubes (100 ml. in each tube) of a 30 tube Craig counter-current apparatus. An equal volume (100 ml.) of distilled water (saturated with sec-butanol) is placed in the 3 lower tubes and the extract distributed, withdrawing the top layers. The peak of activity is found in Tubes 20 through 29. Fractions in Tubes 1 through 9 and 40 through 59 are inactive and are discarded. The solid content of the various fractions is shown in Table I.

Redistribution of the active fractions (Tubes 20 to 29) obtained as described above leads to a further purification. For this purpose, the active aqueous extract concentrated to a volume of 100 ml. is placed in the first tube of the counter-current apparatus which is filled with sec-butanol and water. 30 top layers are withdrawn and replenished so that Tubes 0 through 29 contain both a top (butanol) and bottom (water) layer. This distribution showed three distinct peaks of solid content at Tubes 10, 20, and 28 with a possible fourth peak at 25. The

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**Table I**

*Solid content of various fractions of extract obtained from 40 pounds of kidney by first countercurrent distribution as described in text*

<table>
<thead>
<tr>
<th>Tube Nos.</th>
<th>Solid content</th>
<th>Tube Nos.</th>
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<tbody>
<tr>
<td>0-5</td>
<td>0.31</td>
<td>31-35</td>
<td>3.56</td>
</tr>
<tr>
<td>6-10</td>
<td>0.75</td>
<td>36-40</td>
<td>1.90</td>
</tr>
<tr>
<td>11-15</td>
<td>3.56</td>
<td>41-45</td>
<td>1.45</td>
</tr>
<tr>
<td>16-20</td>
<td>7.55</td>
<td>46-50</td>
<td>1.60</td>
</tr>
<tr>
<td>21-25</td>
<td>9.05</td>
<td>51-55</td>
<td>1.25</td>
</tr>
<tr>
<td>26-30</td>
<td>8.82</td>
<td>56-60</td>
<td>1.95</td>
</tr>
</tbody>
</table>
maximum activity was found in Tubes 30 through 39, Tubes 0 to 9 being inactive and Tubes 10 through 29 and 40 through 59 showing only traces of activity. The effect of administering the combined extract of Tubes 30 through 39 obtained from 40 pounds of kidney (total solid content, 0.5 gm.) to six hypertensive rats is shown in Fig. 1, which illustrates a typical assay. As a control, the absence of activity in Tubes 0 through 9 (solid content, 1.7 gm.) is also shown in Fig. 1.

**DISCUSSION**

The role of the kidney in the pathogenesis of hypertension is well established and the available evidence is compatible with the theory that this organ might elaborate an agent which would have an ameliorative effect in hypertensive disease (4). The use of renal extracts capable of reducing the blood pressure of hypertensive animals is not practical clinically because of the meager amount of active material obtainable from kidney tissue. Isolation, identification, and synthesis of the active agent is thus essential for progress in this field. The preparations described in the present paper offer a starting point for this goal.

Since we have not been able as yet to isolate the principle in renal extracts which is responsible for its hypotensive activity, any consideration of its probable chemical nature would be premature. The active principle is obviously not a protein since it is active when administered orally. Preliminary studies by paper chromatography indicate a mobility comparable to that of the smaller peptides. The active principle is non-volatile and passes through a collodion membrane. Further studies aimed towards isolation and identification of the active principle are under way.

**SUMMARY**

Methods are described for the preparation and concentration of renal extracts effective in lowering the blood pressure of hypertensive rats. By successive extractions with organic solvents and countercurrent distribution between sec-butanol and water, relatively purified preparations are obtainable.

**REFERENCES**

The Preparation of Renal Extracts Effective in Reducing Blood Pressure in Experimental Hypertension

James G. Hamilton and Arthur Grollman


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