STUDIES ON THE IDENTITY OF A VASCULAR PERMEABILITY FACTOR OF RENAL ORIGIN

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SUMMARY

1. The administration of crude renal extract to bilaterally nephrectomized rats causes an increase in vascular permeability to plasma proteins. This is associated with a fall in plasma volume. The active material resembles the enzyme renin in pressor activity, heat-lability, pH range of activity and molecular size.

2. To test the possibility that renin might be responsible, renin was extracted from rat renal cortical tissue using methods similar to those used for the purification of pig renin: these involved saline extraction, protein precipitation, freeze drying, ion-exchange and gel-filtration.

3. The final preparation had a pressor activity some 300 times that of the initial saline extract and gel-filtration suggested that the molecular size of rat renin is 40 000–50 000. Assay for pressor and vascular permeability activity at selected stages in the purification showed that both activities ran parallel and could not be dissociated. These results provide strong evidence that the vascular permeability factor is in fact renin.

4. Increase in vascular permeability after injection of semi-purified material could still be demonstrated after bilateral adrenalectomy, though the effect was reduced.

5. In all experiments in which vascular permeability was increased and in which blood pressure was measured, a considerable and sustained rise in blood pressure occurred. It is possible that the increase in blood pressure is causally related to the increase in permeability.

The injection of crude renal extract into bilaterally nephrectomized animals produces a syndrome of oedema, serous effusions and necrotic vascular lesions which resemble those found in malignant hypertension (Winternitz et al., 1940; Masson et al., 1956; Nairn, Masson & Corcoran, 1956; Asscher & Anson, 1963; Giese, 1963). Asscher & Anson (1963) have demonstrated that these changes develop concurrently with a fall in plasma volume, a rise in

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venous haematocrit and leakage of plasma protein from the vascular compartment and have also observed that the responsible substance or substances are localized in the cortex of the kidney. Giese (1964a) has since shown that a considerable and prolonged rise of blood pressure occurs under these conditions.

In an earlier publication (Cuthbert, Asscher & Jones, 1966), it was reported that the active material responsible for the increase in vascular permeability to plasma proteins resembled the enzyme renin in pressor activity, heat-lability, pH range of activity and molecular size. The pressor effect of renal cortical extract could not be dissociated from the permeability effect using preliminary procedures of renin purification. These results suggested that the vascular permeability factor in the cortex of the kidney is renin or some substance or substances with similar properties.

In the present work we have attempted to establish the role of renin in these circumstances by extracting the enzyme from rat renal cortical tissue using methods similar to those described for pig renin (Peart et al., 1966a) and have assayed the material for pressor and vascular permeability activity at selected stages of the purification.

An attempt has also been made to establish whether the increase in vascular permeability depends on the rise of blood pressure or is due to the direct effect of renin or angiotensin on blood vessel walls.

METHODS

Preparation of crude renal cortical extract

Homogenates of rat renal cortical tissues were prepared (Cuthbert et al., 1966), allowed to remain overnight at 4°C and then centrifuged at 10 000 g for 15 min. The supernatant was stored at −17°C.

Purification methods

Saline extraction and protein precipitation. A laboratory standard was prepared by saline extraction of rat renal cortical tissue, precipitation of renin with 40% ammonium sulphate, precipitation of impurities with 3% ammonium sulphate and freeze drying (Cuthbert et al., 1966). This material will be referred to as semi-purified renal extract.

Diethylaminoethyl (DEAE)-cellulose chromatography. Semi-purified renal extract was applied to columns of DEAE-cellulose (Peart et al., 1966a). The column was successively washed with 0.005 M phosphate buffer (pH 7.0) and 0.025 M phosphate buffer (pH 7.0), and renin eluted with 0.075 M phosphate buffer (pH 7.0). The procedure was carried out at 4°C.

Carboxymethyl (CM)-Sephadex chromatography. Material from DEAE-cellulose chromatography was applied to columns of CM-Sephadex (Peart et al., 1966a). The column was washed with 0.1 M acetate buffer (pH 4.9) and the renin eluted with 0.1 M acetate buffer (pH 6.0). The procedure was carried out at 4°C.

Sephadex G-100 gel filtration. Material from CM-Sephadex chromatography was applied to columns of Sephadex G-100 (Peart et al., 1966a) and eluted in 0.5 M phosphate buffer (pH 6.0), at room temperature.

Biogel P-60 gel filtration. Biogel P-60 (Biorad Laboratories, California) was equilibrated against 0.1 M phosphate buffer (pH 6.0) and packed in a column 200 × 2.5 cm under hydrostatic pressure. Material from gel filtration on Sephadex G-100 was applied to the column and eluted with the same buffer at room temperature.
Assay of renin

The increase in the specific activity of rat renin obtained from the stages of purification was followed by comparing the pressor activity of the various materials with that of a standard pig renin in anaesthetized rats treated with pentolinium (Peart, 1955). This standard was prepared from a single batch of pig renin taken through the first four stages of the purification method described by Peart et al. (1966a) and contained 80 units/ml. One such arbitrary unit is defined as that which produces on intravenous injection an average rise of blood pressure of 10–20 mm. The rat material was assayed against the standard pig renin on three separate occasions and the mean calculated. In some experiments, the purified rat material was incubated with an ox substrate, free of angiotensinase, to demonstrate the production of angiotensin. The method was similar to that described by Lever, Robertson & Tree (1964) for the assay of plasma renin.

Anti-renin

An anti-renin preparation was kindly supplied by Dr G. W. Boyd. The antisera had been raised in rabbits as described by Peart et al. (1966a) and the preparation used contained 2000–4000 units of anti-renin per ml. These units are defined in terms of inactivation of standard pig renin in the rat blood pressure assay. One unit of anti-renin was that which completely inhibited the pressor activity of one unit of pig renin.

Protein estimation

Protein was estimated either by the Folin–Lowry method (Lowry et al., 1951) with bovine albumin as standard, or by measuring the optical density at 280 mp. Specific activity was expressed in terms of units renin per mg protein.

Biological assay for vascular permeability activity

The effect of the various materials on vascular permeability was determined in groups of bilaterally nephrectomized rats. The assay is based on the changes in venous haematocrit which occur over the 24 hr period following intraperitoneal injection (Cuthbert, 1969).

Laboratory standard. The semi-purified freeze-dried material (Stage 2 of the purification) was used as a laboratory standard. Two batches were prepared which contained 280 and 390 units of renin/mg protein respectively.

Assay procedure. All experiments were carried out in female rats of a closely inbred Wistar strain, aged about 3 months and weighing between 180 and 240 g. The animals had both kidneys removed under ether anaesthesia; after recovery and within 3 hr of operation, each animal was given an intraperitoneal injection of isotonic saline, laboratory standard or purified material (2 ml/100 g body weight). Fluids were withheld from the time of nephrectomy and the animals killed approximately 24 hr after the injection. The venous haematocrit (0.05 ml tubes, Baird & Tatlock Ltd) was determined before and 24 hr after the injection.

The venous haematocrit falls progressively after bilateral nephrectomy and the mean value in animals receiving an injection of 0.9% saline (2 ml/100 g body weight) was $-5 \pm 0.9\%$ (mean for six animals $\pm$ SEM) of the initial value 24 hr after injection. Fig. 1 shows the effect of different doses of the standard material in groups of rats. The changes for doses of 1 mg/100 g or more were significant, and we observed that doses of 8 mg/100 g body weight produced a gross and sometimes fatal extravasation of fluid from the vascular compartment. Two doses
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of standard material, 1.0 and 2.0 mg/100 g body weight, were chosen because they appeared likely to lie on the logarithmic part of the dose/response curve.

Four groups of six rats were used in each assay. The animals for each group were selected at random from four litters. Two groups received the standard material in a dosage of 1.0 and 2.0 mg/100 g body weight respectively. The other two groups received equipressor doses of material recovered from one of the purification procedures. The test material was first assayed for renin content against pig renin and then the concentration adjusted so that the renin units in both standard and test material were equal when contained in the appropriate volume for injection. In some experiments, non-pressor material recovered from the purification procedures was also assayed for vascular permeability activity.

![Graph](image)

**FIG. 1.** The effect of semi-purified renal extract (standard material) on the venous haematocrit of rats injected within 3 hr of bilateral nephrectomy. Each column represents the mean change of venous haematocrit, 24 hr after injection, for a group of six animals. Vertical lines show the limits of the SEM.

**Measurement of blood pressure in unanaesthetized animals**

The changes in the blood pressure of conscious unanaesthetized rats following the intraperitoneal injection of crude or semi-purified renal extract or following the intravenous infusion of synthetic 5 valyl-angiotensin II amide ('Hypertensin'-Ciba) were measured by a modification of the method described by Weeks & Jones (1960). The polyethylene cannula was implanted in the abdominal aorta under ether anaesthesia between 24 and 48 hr before an experiment. During measurement of the blood pressure the cannula was connected to a Statham transducer and recording equipment.

For experiments in which synthetic angiotensin was infused, in addition a polyethylene
cannula with a silicone rubber ('Vivosil') tip was implanted in the superior vena cava or right atrium through the right external jugular vein under ether anaesthesia. The technique was a modification of that described by Weeks (1962). Synthetic 5 valyl-angiotensin II amide ('Hypertensin–Ciba, batch no. 96621) was dissolved in 0.9% saline and infused through the right heart cannula at rates of 0.05–0.2 μg kg⁻¹ min⁻¹ with a constant rate infusion pump (Palmer). The infusions were continued for approximately 23 hr so that each animal (weight 180–240 g) received a total of 12.5–66 μg angiotensin in a volume of 5–7 ml (approximately 0.02 ml kg⁻¹ min⁻¹).

RESULTS

Purification

The increase in the specific activity of rat renin and the recoveries obtained from each step of the purification procedure are shown in Table 1. The figures represent the totals for three batches of renal cortical tissue.

Table 1. Outline of steps in the purification of rat renin; the figures represent the combined totals for three batches of renal cortical tissue

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Protein</th>
<th></th>
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<th>Renin</th>
<th></th>
<th></th>
<th>Specific activity</th>
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<tr>
<td></td>
<td></td>
<td>Applied (g)</td>
<td>Recovered (g)</td>
<td>(%)</td>
<td>Applied (units × 10³)</td>
<td>Recovered (units × 10³)</td>
<td>(%)</td>
<td>(units/mg protein)</td>
<td></td>
</tr>
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<td>Extraction</td>
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<td>64.3</td>
<td>5.7</td>
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<td>1900</td>
<td>1200</td>
<td>63</td>
<td>35</td>
</tr>
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<td>3.46</td>
<td>6.4</td>
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<td>1200</td>
<td>63</td>
<td>350</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>0.63</td>
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<td>814</td>
<td>74</td>
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<td></td>
</tr>
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<td>0.1</td>
<td>28</td>
<td>683</td>
<td>363</td>
<td>53</td>
<td>3600</td>
<td></td>
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<td>0.032</td>
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<td>90</td>
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<td>0.0125</td>
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<td>176</td>
<td>133</td>
<td>77</td>
<td>10600</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Biogel P-60</td>
<td>0.025</td>
<td>0.0125</td>
<td>50</td>
<td>176</td>
<td>133</td>
<td>77</td>
<td>10600</td>
<td></td>
</tr>
</tbody>
</table>

Sephadex G-100 185 x 2.5 cm

![Graph](image)

**Fig. 2.** Step 5 in the purification of rat renin: Sephadex G-100 gel filtration. Fractionation of 10 mg of material from CM-Sephadex chromatography and 25 mg bovine albumin run separately. Fractions 5 ml. Optical density of fractions (○); distribution of renin in units/ml is shown by the interrupted line.
batches of rat renal cortical tissue. The first batch consisted of 195 g which was carried through steps 1-4; the second batch consisted of 272 g which was carried through steps 1-5 and the final batch consisted of 485 g which was carried through the complete purification procedure.

The results of fractionation of 10 mg of material from CM-Sephadex chromatography on a column of Sephadex G-100 is shown in Fig. 2. This produced an insignificant protein peak but the renin content of the individual fractions was determined and is represented in the figure by the broken line. 25 mg of bovine albumin was run separately and is represented by the continuous line. The positions of the albumin and renin peaks were almost identical to those obtained in a preliminary experiment in which albumin and semi-purified renal extract were run together on the same column.

**Identification of final preparation**

The identification of the final preparation as renin was established from the nature of the pressor response, by the production of angiotensin on incubation with renin substrate, and by its inactivation with anti-renin.

*Nature of the pressor response.* When small quantities of material from Biogel P-60 gel filtration were injected intravenously in the anaesthetized rat, the pressor response was in all respects similar to that seen with pig renin or semi-purified rat renin.

*Incubation with renin substrate.* One ml of material from Biogel P-60 gel filtration containing 20 renin units/ml was added to 4 ml of ox substrate in the presence of EDTA (final concentration 0.3 M) and the mixture incubated at 37° for 2 hr. Samples were taken at 0, 30, 60, 90 and 120 min and snap frozen at −20° while awaiting assay. Fig. 3(a) shows the increasing angiotensin-like activity of samples taken at 30, 60, and 90 min when small quantities were injected intravenously in the anaesthetized rat in comparison with synthetic 5 valyl-angio-
tensin II amide. The incubation product was found to be dialysable through Visking cellophane (24/32) and was heat-stable.

Natural rat angiotensin from the above experiment was partially purified by boiling at pH 5.5 and precipitated with absolute alcohol (3 volumes to 1 volume incubation mixture). The supernatant was then reduced to dryness by rotary film evaporation and dissolved in 0.05 M ammonium acetate buffer. The pH was adjusted to 7.5 with 0.1 N NaOH and the angiotensin incubated with a 4 mg/ml solution of trypsin (Seravac) in the same buffer at 37°C so that the ratio of trypsin to angiotensin was approximately 800:1 on a weight basis. The pressor effect of natural rat angiotensin was substantially reduced after a 2 hr incubation with trypsin and almost disappeared after a 14 hr incubation (Fig. 3b).

**Inactivation by anti-renin.** Pig renin containing 200 units/ml was incubated with a rabbit anti-pig renin preparation (activity 2000-4000 units/ml) in a series of doubling dilutions for 1 hr at 37°C. In a second series of doubling dilutions, material from Biogel P-60 gel filtration, also containing 200 units/ml, was incubated with the same anti-renin preparation for 1 hr at 37°C. Samples were stored at 4°C while awaiting assay. The pressor effects of pig renin and the rat material were obliterated by a 1:8 dilution and by undiluted anti-renin, respectively. The results of the lower dilutions are shown in Fig. 4. It was found that the concentration of anti-renin required to inactivate rat renin was 8 times that required to give a comparable effect with pig renin. The dose–response curves were almost parallel which suggests that similar mechanisms are involved in the inactivation of the two renin preparations.

**Stability of rat renin.** Rat renin was stable when in the crude state and up to stage 6 of the
purification. Crude renal extract was stable for at least 2 months when stored at $-20^\circ$ and after saline extraction, protein precipitation and freeze drying, no deterioration was noted on storage for at least 1 year at room temperature. Beyond step 2, rat renin appeared to be stable when stored in solution at $4^\circ$ in the presence of 10 mg/100 ml thiomersal for periods up to 3 weeks, but after gel filtration on Biogel P-60 losses occurred both on pressure dialysis at 400 mmHg and on storage at $4^\circ$.

**Biological assay for vascular permeability activity**

Biological assay was performed after the ion-exchange chromatography steps (DEAE-cellulose and CM-Sephadex) and after each of the gel filtration chromatography steps (Sephadex G-100 and Biogel P-60). The results of the three assays are shown in Table 2. There was no significant differences in the vascular permeability activity of the standard material and equipressor doses of the material recovered from purification procedure, as tested by four-point assay analysis (Schild, 1942; Holton, 1948).

<table>
<thead>
<tr>
<th>Assay 1. Standard</th>
<th>Standard 1 mg/100 g or Equipressor dose Test material</th>
<th>Standard 2 mg/100 g or Equipressor dose Test material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post CM-Sephadex</td>
<td>+2.3 ±1.1</td>
<td>+6.6±2.5</td>
</tr>
<tr>
<td></td>
<td>+0.3 ±2.0</td>
<td>+6.8±2.3</td>
</tr>
<tr>
<td>Assay 2. Standard</td>
<td>−0.75±1.75</td>
<td>+6.0±1.7</td>
</tr>
<tr>
<td>Post Sephadex G-100</td>
<td>+1.67±0.75</td>
<td>+6.6±2.45</td>
</tr>
<tr>
<td>Assay 3. Standard</td>
<td>+2.7 ±1.33</td>
<td>+7.7±2.54</td>
</tr>
<tr>
<td>Post Biogel P-60</td>
<td>+1.5 ±1.45</td>
<td>+7.2±2.4</td>
</tr>
</tbody>
</table>

No pressor or vascular permeability activity was detected in material eluted by 0.005 M and 0.025 M phosphate buffer (pH 7.0); by 0.01 M acetate buffer (pH 4.9), from CM-Sephadex, or in fractions eluted before the renin from Sephadex G-100 or Biogel P-60 gel filtration.

**Effect of crude renal extract on blood pressure and vascular permeability**

These observations are an extension of those previously reported (Cuthbert et al., 1966). A dose of crude renal extract was chosen which on the basis of previous results was expected to produce an increase in vascular permeability with a rise in the venous haematocrit in approximately half the animals. Five unanaesthetized rats in which aortic cannulae had been implanted received an intraperitoneal injection of crude renal extract in a dosage of 400 mg/100 g body
Renin and vascular permeability

weight within 3 hr of bilateral nephrectomy. Fig. 5(a) shows the substantial and prolonged rise of blood pressure, lasting approximately 8–9 hr, which occurred in three animals. In these animals there was a rise of venous haematocrit from 46, 42 and 41% before injection to 57, 52.5 and 49% respectively 24 hr after injection. In the remaining two animals there was a fall of venous haematocrit from 41.5 and 51% before injection to 34.5 and 43% respectively 24 hr after injection and there was a less marked but equally prolonged rise of blood pressure (Fig. 5b).

Three rats in which a mock operation had been performed without removing the kidneys also received crude renal extract (400 mg/100 g body weight) and the blood pressure changes were recorded (Fig. 6a). In contrast to the prolonged effects seen in nephrectomized animals, there was a less marked rise of blood pressure which returned to normal levels within 5–6 hr. The change of blood pressure which occurred in three nephrectomized rats following the intraperitoneal injection of 0.9% saline (2 ml/100 g body weight) are shown for comparison. Neither

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**Fig. 5.** (a) The marked rise in blood pressure in three conscious unanaesthetized rats which had received an intraperitoneal injection of crude renal extract (400 mg/100 g) within 3 hr of bilateral nephrectomy and in which a rise of haematocrit was found 24 hr after injection. (b) The less marked but equally prolonged rise of blood pressure in two nephrectomized animals showing no evidence of increased vascular permeability.
nephrectomized animals receiving saline injections nor animals which had undergone mock nephrectomy and received renal extract showed a rise in haematocrit or pleural effusions after injection.

Two rats in which aortic cannulae had been implanted, were injected intraperitoneally with semi-purified renal extract (step 2 of the purification) in a dosage of 2 mg/100 g body weight within 3 hr of bilateral nephrectomy. In both these animals the blood pressure changes were very similar to those shown in Fig. 5(a) and there was a rise of venous haematocrit from 38 and 37% before injection to 50 and 45% after injection.

![Graph showing blood pressure changes](image)

**Fig. 6.** (a) The changes in the blood pressure of three conscious unanaesthetized rats which had received an intraperitoneal injection of crude renal extract (400 mg/100 g) following mock nephrectomy. The rise of blood pressure is much less prolonged than that seen in nephrectomized animals. (b) The changes in the blood pressure in three animals which received intraperitoneal injections of 0.9% saline (2 ml/100 g) within 3 hr of bilateral nephrectomy.

**Effect of synthetic angiotensin on blood pressure and vascular permeability**

Table 3 shows the results of experiments in which 0.9% saline and synthetic 5 valyl-angiotensin II amide were infused intravenously in conscious rats. The infusions were begun within 3 hr of bilateral nephrectomy and continued for approximately 23 hr.

In two rats 0.9% saline was infused at a rate of approximately 0.02 ml kg⁻¹ min⁻¹ and in both these animals there was a fall of venous haematocrit and no pleural effusions were found. Four rats received infusions of synthetic angiotensin II amide 0.05 μg kg⁻¹ min⁻¹ and in none of these animals was any evidence of increased vascular permeability found, although
in one animal (rat 3) there was a rise in the venous haematocrit. In two of these animals the blood pressure was recorded by means of implanted aortic cannulae and the changes are shown in Fig. 7(a); the blood pressure rose 40–55 mmHg in the first hour and remained elevated 6–7 hr after commencing the infusion.

Synthetic angiotensin II amide \( \mu \text{g kg}^{-1} \text{ min}^{-1} \) 0.1 was infused in six rats, and all were found to have small pleural effusions (0.2–2 ml) at the end of the experiment, and in five of

<table>
<thead>
<tr>
<th>Rat</th>
<th>Haematocrit (% before infusion)</th>
<th>Haematocrit (% 24 hr)</th>
<th>Haematocrit change (%)</th>
<th>Pleural fluid (ml at 24 hr)</th>
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<tr>
<td>Saline 0.9%, 0.02 ml kg(^{-1}) min(^{-1})</td>
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<tr>
<td>1</td>
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<td>2</td>
<td>45</td>
<td>42</td>
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<tr>
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</table>

these animals there was a rise in venous haematocrit. In four animals (rats 9, 10, 11 and 12) the blood pressure was recorded by means of implanted aortic cannulae and the changes are shown in Fig. 7(b); in three of these animals (rats ○, ● and ■), in which a rise of venous
haematocrit was found at the end of the experiment, the blood pressure rose rapidly to 60–65 mmHg above the level before the infusion and remained elevated 40 mmHg or more for at least 7 hr. In rat ▲ there was a fall in the venous haematocrit and the rise in blood pressure was slightly less marked.

Histological examination showed necrotic vascular lesions in pancreatic tissue removed from all the animals in this group.

Fig. 7. (a) The changes in blood pressure of two conscious unanaesthetized rats which received an infusion of synthetic angiotensin II amide 0.05 μg kg\(^{-1}\) min\(^{-1}\) for approximately 23 hr following bilateral nephrectomy, and in which no evidence of increased vascular permeability was found. (b) The changes in the blood pressure of four animals which received 0.1 μg kg\(^{-1}\) min\(^{-1}\) synthetic angiotensin II amide. In rats ○, ● and ■ there was a rise of blood pressure more marked than in (a). Rat ▲ showed a fall of venous haematocrit; the others showed a rise.

In four rats which received infusions of synthetic angiotensin II amide 0.2 μg kg\(^{-1}\) min\(^{-1}\) there was a rise of venous haematocrit similar to that seen in the previous groups but small pleural effusions were found in only two of the animals. The blood pressure changes were not recorded but necrotic vascular lesions were found in pancreatic tissue removed from all four animals.
It therefore appeared that the infusion of synthetic angiotensin II amide in doses which produced a rise of blood pressure comparable to that produced by crude renal extract (Fig. 5a) also caused an increase in vascular permeability in nephrectomized animals, although the rise of venous haematocrit was less marked. In contrast, no rise in venous haematocrit or pleural effusions were found in animals infused with angiotensin II amide in doses which failed to produce a marked and sustained rise of blood pressure. We have not been successful in producing a sustained rise of blood pressure by the intravenous infusion of noradrenaline in anaesthetized rats.

**Experiments in adrenalectomized animals**

Experiments were performed on two groups of twelve rats to study the effect of adrenalectomy on the increase in vascular permeability when renal extract is injected into nephrectomized animals. The rats in one group were bilaterally nephrectomized and were injected within 3 hr of the operation. The rats in the other group were bilaterally nephrectomized and bilaterally adrenalectomized and were injected within 1 hr of the operation. Six animals in each group received semi-purified renal extract 1 mg/100 g and the remainder 2 mg/100 g body weight intraperitoneally. The changes in venous haematocrit in the nephrectomized animals were similar to those recorded in previous experiments (1 mg/100 g: +1.75±1.3 (SEM); 2 mg/100 g: +6.75±1.9) but the effect of semi-purified renal extract was significantly reduced (P<0.05) in the animals in which both nephrectomy and adrenalectomy had been performed (1 mg/100 g: 0.7±1.4; 2 mg/100 g: +2.25±1.9). There was no significant difference in the slopes of the dose/response relationships.

Replacement of adrenocortical hormone was attempted by the administration at the time of the operation, of corticosterone (Organon) 0.5 mg/100 g body weight subcutaneously or desoxycorticosterone glucoside (‘Percorten water-soluble’ Ciba) 1 mg/100 g body weight, but this did not have any influence on the change of venous haematocrit seen in the animals described above in which both nephrectomy and adrenalectomy had been performed.

**DISCUSSION**

Preliminary experiments performed by gel filtration on Sephadex G-100 and G-200 columns 70 cm in length with crude renal extract (Cuthbert et al., 1966) showed that fractions eluted at and immediately after the albumin peak had pressor and vascular permeability activity; this suggested that the molecular weight of rat renin is in the region of 40 000–70 000. Peart et al. (1966a) have found Sephadex G-100 to be superior to both Sephadex G-75 and Sephadex G-200 in the separation of pig renin from other proteins in the molecular weight range 30 000–100 000, and columns of Sephadex G-100, 185 cm in length, were used in the purification procedure described in the present work. Under these conditions, rat renin was eluted after most of the albumin had been eluted from the column; the molecular weight of rat renin, estimated from the elution volumes as described by Andrews (1964), was in the region 40 000–45 000. The final preparation was not as pure as that of pig renin described by Peart et al. (1966a) but it had a specific activity approximately 300 times that of the initial saline extract.

During purification, rat renin was noted to behave differently from pig renin in several respects. On DEAE-cellulose, rat renin was eluted by a phosphate buffer of lower molarity.
(0.05 M) than that required to elute pig renin and better separation of proteins was achieved by using 0.025 M phosphate buffer before eluting the rat renin with 0.075 M phosphate buffer. On CM-Sephadex, rat renin, in contrast to pig renin, was eluted by 0.2 M acetate buffer at pH 4.9 and 0.1 M acetate buffer was therefore substituted to permit adsorption on to the cation-exchanger. Behaviour of rat renin was comparable to that of pig renin on the gel filtration media. Paper electrophoresis showed that rat renin migrates more slowly than pig renin; this method did not prove to be practical for purification although Peart et al. (1966b) have reported good recovery of pig renin on paper electrophoresis. The differences in behaviour between the two species of renin are probably related to the charged groups on the protein rather than to differences in molecular size, since the elution volumes from Sephadex G-100 were identical; it seems likely that a difference in amino acid composition is responsible. In electrophoretic properties rat renin resembled human renin.

The object of the biological assay was to determine whether the renin-like pressor activity and the vascular permeability activity of renal extract ran parallel through the several stages of purification or could be dissociated. Assay of the material from the ion-exchange steps and from each of the gel filtration steps showed no significant difference in the vascular permeability activity of the standard and purified material when tested in equipressor doses; no pressor or permeability activity was detected in the other fractions eluted from the purification procedures.

Substances which under certain conditions are known to increase the permeability of blood vessels to plasma proteins include histamine and 5-hydroxytryptamine (Majno & Palade, 1961), bradykinin (Holdstock, Mathias & Schachter, 1957) and synthetic angiotensin II amide (Giese, 1961, 1964b). These substances, which are of low molecular weight, would be removed during purification unless strongly bound to plasma proteins. Kallikrein is an enzyme which has a molecular weight similar to that of renin (Moriya, Pierce & Webster, 1963) and we cannot exclude the possibility that small quantities of kallikrein might be carried through the purification, insufficient to affect the blood pressure but possibly affecting the permeability of blood vessels.

The vascular permeability factor in the cortex of the rat kidney resembles renin in pressor effect, cortical localization, heat-lability, pH range of activity and molecular size (Cuthbert Asscher & Jones, 1966). The present work has demonstrated that vascular permeability activity and renin content run parallel through successive stages of purification and provides strong evidence that the vascular permeability factor is renin itself.

One mechanism by which renal extracts or renin might increase vascular permeability is through the effect of an acute rise of blood pressure damaging blood vessel walls. Changes in the blood pressure seen in the three unanaesthetized nephrectomized rats receiving intraperitoneal injections of crude renal extract were very similar to those described by Giese (1964a) and in all these animals tissue oedema and vascular lesions were found, accompanied by leakage of protein from the vascular compartment. The rise of blood pressure was less marked but equally prolonged in two nephrectomized animals in which the same dose of extract failed to produce an increase in vascular permeability. It appears, therefore, that both the degree and the duration of the rise of blood pressure are important in determining the leakage from the vascular compartment.

Another possibility is that renin or angiotensin has a direct effect on the vascular endothelium, increasing its permeability to plasma proteins, which is independent of a rise of blood
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pressure. This mechanism may be responsible for the proteinuria which occurs after doses of renin and angiotensin which do not elevate the blood pressure (Sellers et al., 1952; Deodhar, Cuppage & Gableman, 1964). Our experiments with synthetic angiotensin II amide were performed to determine whether the infusion of angiotensin can cause significant leakage from the vascular compartment and to investigate the blood pressure changes which occur under these conditions. Small doses of angiotensin II amide which produced a modest but sustained rise of blood pressure had no detectable effect on vascular permeability. Higher doses which produced an initial rise of 60–65 mmHg and a sustained level of hypertension caused a rise of venous haematocrit, and effusions were found in the pleural cavity. The blood pressure was recorded in only a small number of animals infused with angiotensin II amide, but the rise of venous haematocrit was less marked than in animals injected with doses of crude or semi-purified renal extract which caused comparable changes of blood pressure. This might suggest that renin has some action on vascular permeability which is not shared by angiotensin but no definite evidence can be obtained until a pure renin preparation is available.

Bilateral adrenalectomy was found to reduce but not abolish the increase in vascular permeability which occurred when renal extract was injected into nephrectomized rats. The permissive role of the adrenal glands in this situation is not clear although the presence in the adrenal of other substances which may influence vascular permeability cannot be excluded. Sellers et al. (1952) and Deodhar et al. (1964) also found that bilateral adrenalectomy abolished the proteinuria induced by renin. Although no recordings of the blood pressure were made in the present experiments in adrenalectomized animals it is possible that the blood pressure in these animals did not rise following the injection of renal extract to the same extent as in bilaterally nephrectomized animals.

In conclusion, an increase in vascular permeability has been seen only in animals in which a substantial and prolonged rise of blood pressure occurred, whether this rise was induced by semi-purified renin or by synthetic angiotensin II amide. It seems likely that in the nephrectomized rat this acute rise of blood pressure is responsible for the increase in permeability of blood vessels to fluid and plasma proteins. This is supported by the observations of Byrom (personal communication) that pleural effusions and a raised venous haematocrit can occur in rats which develop severe hypertension following the constriction of one renal artery, the other kidney being undisturbed. Byrom (1954, 1963) has also demonstrated that in severe hypertension and hypertensive encephalopathy in rats, intense narrowing of cerebral and retinal arteries develop with conspicuous areas of dilatation. Giese (1964b) has shown that a similar pattern of alternating constricted and dilated segments develops in small mesenteric arteries when synthetic angiotensin II amide and other pressor substances are infused intravenously, notable in nephrectomized rats. Using a carbon tracer technique, Giese also demonstrated that focal areas of increased permeability developed predominantly in the dilated segments. Exactly how vascular permeability is increased under these conditions is uncertain, but if the blood vessel wall in the dilated segments is damaged by the overstretching of the fibres of the media, extravasation of plasma into and through the arterial wall could readily occur.

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