RENAL CONTROL OF CHANGES IN THE COMPLIANCE OF THE INTERSTITIAL SPACE: A FACTOR IN THE AETIOLOGY OF RENOPRIVAL HYPERTENSION

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SUMMARY

1. Measurements of plasma volume, haematocrit, extracellular fluid volume, blood pressure, venous pressure and interstitial tissue pressure were made in rats 4 days after unilateral nephrectomy. Measurements were repeated either 4 days after subsequent removal of the remaining kidney, or 4 days after anastomosis of the remaining ureter with the inferior vena cava. Extracellular fluid volume was expanded by giving 0.5% saline by mouth: in one series blood volume was expanded by injections of blood.

2. Plasma volume (PV) rose more after bilateral nephrectomy (BN), extracellular fluid volume (ECFV) more after unilateral nephrectomy and ureterocaval anastomosis (UNUCA). The PV/ECFV ratio was significantly higher after BN.

3. Blood pressure and venous pressure rose after BN but not after UNUCA. Interstitial tissue pressure (TP) rose more after BN in spite of greater expansion of interstitial fluid volume (IFV = ECFV – PV) after UNUCA. The ratio ΔIFV/ΔTP was several times less after BN than after UNUCA.

4. Interstitial space compliance was estimated by measuring changes in IFV and TP 10 min after a saline infusion (compliance = ΔIFV/ΔTP). After UNUCA there was little change from values obtained after unilateral nephrectomy alone; following BN compliance fell severalfold.

5. It is suggested that changes in compliance of the interstitial space may be brought about by a substance secreted by the kidney and represent a hitherto undetected mechanism by which plasma volume is maintained constant under different conditions of hydration. Changes in interstitial space compliance may also play a part in the aetiology of renoprival hypertension by raising tissue pressure, and thus venous pressure and cardiac output.

Key words: compliance of interstitial space, interstitial tissue pressure, renoprival hypertension, extracellular fluid volume, plasma volume, ureterocaval anastomosis.

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It has been demonstrated in this laboratory (Green, 1969) that, after expanding equally the blood volume of rats following (1) bilateral nephrectomy, (2) unilateral nephrectomy and anastomosis of the opposite ureter with the vena cava, and (3) unilateral nephrectomy alone, the presence of a kidney, whether excreting externally or not, is associated with the maintenance of normal blood pressure. This supports the suggestion of other workers that the normal kidney maintains normal blood pressure by some internal function as well as by excretion of sodium and water (Grollman, Muirhead & Vanatta, 1949; Kolff & Page, 1954; Floyer, 1955; Muirhead, Brown, Germain & Leach, 1970). Green (1969) also observed a different distribution of fluid between the vascular compartment and the interstitial space; the plasma/extracellular fluid (PV/ECFV) ratio was significantly higher after bilateral nephrectomy (BN) than after unilateral nephrectomy and ureterocaval anastomosis (UNUCA).

Ledingham & Pelling (1970) have shown in rats that hypertension after bilateral nephrectomy (renoprival hypertension) is associated with increased plasma volume and increased cardiac output; peripheral resistance remains normal or falls. Since expansion of the blood volume after unilateral nephrectomy and ureterocaval anastomosis does not cause a rise in blood pressure, either peripheral resistance must decrease or cardiac output must remain normal after this procedure. A possible explanation of the latter alternative is that after UNUCA the capacity of the venous side of the circulation is able to expand to accept the increased blood volume without causing a rise in central venous pressure and of cardiac output, but that after BN a rise in venous pressure occurs.

The rise of central venous pressure after blood volume expansion depends partly on the tone of the veins and venules and partly on the rise of interstitial pressure when increased capillary pressure causes more fluid to enter the interstitial space, i.e. the compliance of the interstitial space. If compliance falls (i.e. the same increase in interstitial fluid volume results in a greater rise of interstitial fluid pressure) after BN, but not after UNUCA, the proportion of total extracellular fluid in the interstitial space after BN will be reduced and that in the vascular compartment will be increased. This will increase central venous pressure, cardiac output, and blood pressure.

To discover if there is any change in the volume/pressure relationship of the interstitial space, the rise in interstitial fluid volume (IFV) and of tissue pressure (TP) in rats following BN and UNUCA was estimated. Changes in interstitial tissue pressure and in interstitial fluid volume following an infusion of saline after unilateral nephrectomy alone and after BN or UNUCA were also studied. Associated changes in blood pressure, plasma volume, extracellular fluid volume, haematocrit, tissue pressure and venous pressure were observed.

Some of these results have been published in abstracts (Lucas & Floyer, 1972b; Floyer & Lucas, 1972).

METHODS

Female Glaxo Wistar albino rats, weighing about 200 g, were used throughout the study. The animals were maintained at a temperature of 21°C on a diet of rat cubes (Dickensons) and tap water ad libitum unless otherwise stated.

Pressure measurements

Tissue pressure. This was measured using a modification (Floyer, 1966) of the technique of
Guyton (1963). Polythene capsules (3.5 cm × 1.5 cm), perforated by approximately 200 1-mm holes, were implanted subcutaneously between the thigh and abdominal wall. After 5–6 weeks these capsules become lined with tissue which grows through the holes, leaving a pool of fluid in the centre. Capsules were inserted at least 5 weeks before pressure measurements were made. Tissue pressure was measured by inserting a needle (internal diameter 0.325 mm) into the fluid through the skin and one of the holes. The needle was connected by a length of P.P. 50 polythene tubing (Portex Plastics) to a pressure transducer. The latter was a semi-conductor strain-gauge (Type S.E. 3/81; S. E. Laboratories Ltd) with a volume displacement of 0.01 mm³/100 mmHg. This was connected through a carrier amplifier (Type S.E. 423/2E) to an ultraviolet recorder (Type S.E. 2005). The drift of the system at atmospheric pressure was less than 0.1 cmH₂O over a period of 30 min. Amplification was adjusted so that 10 cmH₂O pressure represented full-scale (10 cm) deflection on the recording paper. Linearity over the range −20 to +20 cmH₂O was within 0.5% of full-scale deflection. This system was free from hysteresis and able to discriminate pressure changes of 0.1 cmH₂O. Zero reference point was always taken at the mid-point of the capsule. All pressure recordings were made with the animal prone and under light ether anaesthesia.

Venous pressure. Ideally, cardiac filling pressure should be estimated by measuring the transmural pressure in the right atrium. In the rat, the negative intrathoracic pressure and large respiratory pressure changes make this measurement difficult. As a compromise, we measured venous pressure in the inferior vena cava at about the level of the renal veins. These recordings were made from previously implanted cannulae, which consisted of a length of P.P. 25 polythene tubing tipped with 1 cm of Silastic medical-grade flexible tubing (0.05 cm internal diameter; 0.09 cm outside diameter; Dow Corning Ltd). The end of the polythene tubing was moulded into a tight semi-circle before the Silastic tubing was fixed on to it. The cannula was introduced into the lumbar vein and lay with its tip in the inferior vena cava between the openings of the two renal veins, pointing towards the heart. The other end of the cannula was passed through the posterior abdominal wall and ran subcutaneously to the back of the neck where it came out through the skin. The cannulae remained patent for several weeks and were used for venous pressure measurements, saline injections, and for blood sampling. Venous pressure measurements were made by connecting the venous cannula to the recorder described above. Measurements were made with animals prone and under light anaesthesia. Zero point for these pressure measurements was taken at the tip of the cannula; the level of this was found by X-raying six rats with implanted cannulae and is 1.7 (SD 0.3) cm above the surface on which the animal is lying.

Arterial blood pressure. This was measured by the apparatus described by Floyer (1951), a modification of the original method of Byrom & Wilson (1938). A further refinement was made by connecting the apparatus to pressure transducers to obtain a more accurate reading (Lucas, 1971). The tail plethysmograph was connected to the transducer–recorder system described above which was adjusted for maximum sensitivity (full-scale deflection 2 cmH₂O). The tail pressure cuff was connected to a similar system adjusted to give a full-scale deflection of 200 mmHg. Tracings from both pressure cuff and plethysmograph were recorded simultaneously on the same paper. The pressure cuff was inflated to above arterial pressure and allowed to fall slowly. A vertical line was drawn from the point on the reading from the plethysmograph at which the pressure began to rise; the blood pressure was taken from the point at which this line cut the recording from the pressure cuff. This method gives rapid
repeatable readings, since it is no longer necessary to watch the plethysmograph and the pressure cuff manometers simultaneously.

**Volume measurements**

*Plasma volume and extracellular fluid volume.* Plasma volume was measured by the Evans Blue (T1824) dye-dilution technique. Preliminary experiments showed that the mixing of dyes may not be complete until 25–30 min after injection, making it advisable to extend the period before sampling. To reduce errors due to a small amount of haemolysis we diluted plasma in 1% sodium carbonate instead of 0.9% saline and read the $E_{570}$.

Evans Blue (0.12 ml of a 1.5% w/v solution) was injected into the jugular vein from a micro-meter syringe, and 5 min later 0.4 ml of 10% (w/v) sodium thiocyanate was injected in the same way; 30 min later 0.3 ml of blood was removed from the opposite jugular vein through a dry heparinized hypodermic needle. The blood was put into a 0.5 ml Wintrobe haematocrit tube which was spun at 3000 rev./min for 20 min. The haematocrit was read and the plasma separated; 0.05 ml of plasma was made up to 5 ml with 1% sodium carbonate and the amount of Evans Blue present was determined by reading the $E_{570}$ in a Zeiss spectrophotometer (PMQ 11). A further 0.05 ml of plasma was diluted in 2 ml of distilled water and 2 ml of trichloroacetic acid. After being left for 10 min solutions were spun at 3000 rev./min for 20 min then 2 ml of a 5% (w/v) solution of ferric nitrate was added to 2 ml of the supernatant and the $E_{455}$ read. Erythrocyte and blood volume were calculated from the venous haematocrit and plasma volume, assuming total body haematocrit is $0.9 \times$ venous haematocrit (Chaplin Mollison & Vetter, 1955; M. A. Floyer, unpublished work).

**Chemical estimations**

Plasma sodium, potassium, chloride, bicarbonate, and urea were estimated from venous blood samples on an autoanalyser (Technicon). $P_{CO_2}$, $P_{O_2}$ and pH were estimated from 2 ml blood samples taken from the abdominal aorta just before killing the animals. The syringe was immediately plunged into ice-cold water and the blood pH, $P_{CO_2}$ and $P_{O_2}$ were determined by using a pH microelectrode, a Severinghaus-type $P_{CO_2}$ electrode, and a Clarke oxygen electrode (Radiometer).

**Surgical technique**

Nephrectomy was performed through a dorsal incision; the artery, vein and ureter were tied in a single ligature. Ureterocaval anastomosis was performed by a method modified from that of Floyer (1955). The ureter is dissected free and cleared for about 2 cm. The lower end of the cleared section is tied with a fine silk thread; the ureter is divided just below the ligature and the silk threaded into a needle. The right lumbar vein is tied about 1 cm from the vena cava and a small hole (2–3 mm) cut in it just proximal to this point. The needle is pushed through this hole into the lumbar vein, up the vena cava, and out at a point just below the left renal vein. The ureter is pulled into the lumbar vein and vena cava by means of the silk; the end is pulled out through the exit hole. The ligature is cut off and the upper part of the ureter pulled gently until the cut end slips back into the vena cava. Pressure with swabs for a few minutes controls bleeding.

The methods used for excluding animals with renal dilatation after this procedure are described in the Appendix.
Experimental procedures

After UNUCA rats drink more than after BN (Green, 1969), therefore we tried to ensure equal intake after both procedures by giving extra drinking saline (0.5%) by stomach tube to the BN animals. All rats were given, in addition, 50% (w/v) glucose by stomach tube in an attempt to limit the rise of serum potassium concentrations.

Series A. In this series blood volume and extracellular fluid volume were expanded after BN and after UNUCA as follows.

Day 1 Left nephrectomy.
Day 5 Baseline measurements of weight, plasma volume, haematocrit, extracellular fluid volume, blood pressure and tissue pressure.
Day 15 Right nephrectomy (twelve rats) and right ureterocaval anastomosis (nine rats).
Day 19 Final measurements (as on day 5).

From day 1 to 15 animals were given rat cubes and tap water ad libitum. After the second operation on day 15 they were offered a non-electrolyte, non-protein diet (cooking fat, glucose and starch) and had free access to 0.5% saline. They were also given, daily, 2.5 ml of 50% (w/v) glucose solution per 100 g body weight by stomach tube in divided doses. Each animal was given 2 ml per 100 g body weight of citrated rat blood and 2 ml per 100 g body weight of 0.9% saline daily in divided doses by intraperitoneal injection.

On day 15, a rat from the BN group was paired with one from the UNUCA group of approximately equal weight; each rat was kept in a separate cage, and the amount of 0.5% saline drunk each day was measured. The intake of the rat which drank least (usually the BN animal) was increased to equal that of the other by adding the appropriate volume of 0.5% saline to the glucose solution given by stomach tube. On a few occasions, two rats from the BN group were paired with two from the UNUCA; each BN animal received daily half the difference between the amounts drunk by the UNUCA pair and the BN pair.

On day 19, after the final measurements, the kidneys of the UNUCA animals were inspected and the wet weight/dry weight ratio was measured as described in the Appendix.

Series B1. In these animals extracellular fluid volume was expanded by allowing rats to drink saline; twenty-two rats were subjected to BN and twelve to UNUCA. Experimental procedure, diet, 50% (w/v) glucose by stomach tube and the procedure for ensuring equal intake of 0.5% saline were as for series A but no injections of blood or saline were given. Weight, haematocrit, blood pressure, tissue pressure, plasma volume, and extracellular fluid volume were measured on days 5 and 19 as in Series A.

Plasma Na⁺, K⁺, Cl⁻, HCO₃⁻, urea, and arterial Pco₂, Po₂ and pH were measured on day 19 as described above.

Series B2. In these animals, otherwise treated in the same manner as those in Series B1, an attempt was made to measure the compliance of the interstitial space after unilateral nephrectomy, and again after subsequent BN and ureterocaval anastomosis, by estimating changes in tissue pressure and interstitial fluid volume following rapid expansion of the latter after an infusion of 0.9% saline.

Twelve rats were subjected to BN and nine to UNUCA; the procedure was as for Series B1. Measurements were as for Series A and B1 except that plasma volume and extracellular fluid volume were not estimated. Venous pressure was measured through a cannula inserted into the inferior vena cava on day 1 at the same time as the left nephrectomy. On days 5 and 19 the
interstitial space compliance was estimated. Under light ether anaesthesia, warm (37°C) 0.9% saline (5 ml/100 g body weight) was infused steadily over 4 min through the cannula. Haematocrit, venous pressure and tissue pressure were measured before and 10 min after the end of the infusion. Preliminary experiments showed that all three values return to a steady level 10 min after infusion.

The expansion of interstitial fluid volume after expansion was assumed to be the volume of the saline infusion less the amount which remained in the plasma. Percentage plasma volume expansion after infusion can be calculated from changes in haematocrit, but the absolute increase can only be calculated if the plasma volume before infusion is known. It had been hoped to measure plasma volume just before the infusion on days 5 and 19 but preliminary experiments showed that if this was done on day 19 the animals became very ill after the subsequent infusion. Consequently, in Series B2 plasma and extracellular volumes were not measured. Since in all other respects Series B2 were treated the same as Series B1, the pre-infusion plasma volume of each rat in Series B2 was assumed to be equal to the mean volume for Series B1.

RESULTS

Detailed results are lodged with the Librarian of the Royal Society of Medicine, Wimpole Street, London, W.1, as Clinical Science Tables 73/1-4.

In Series A the weight increase from day 5 to day 19 was the same after BN as after UNUCA (5%). Although there was no change in haematocrit levels in either group, plasma volume rose significantly more after BN (34%) than after UNUCA (14%). There was, however, no significant change in extracellular fluid volume (26% and 28%). The PV/ECFV ratio increased significantly after BN (4-6%) and decreased after UNUCA (12%). Blood pressure rose after BN (28 mmHg) but fell slightly after UNUCA (10 mmHg). In spite of the equal expansion of ECFV, tissue pressure rose more after BN (4.4 cmH2O) than after UNUCA (1.3 cmH2O).

In Series B1 and B2 weight altered little after either procedure. Haematocrit readings in both series showed similar changes falling more after BN than after UNUCA, although the differences did not quite reach conventional significance. In Series B1 PV rose more after BN (24%) than after UNUCA (11%) but ECFV rose less after BN (16% compared with 31%). The PV/ECFV ratio rose after BN (7%) and fell after UNUCA (15%). Since erythrocyte volume fell, the total blood volume did not expand significantly in either group. Blood pressure rose after BN (17 mmHg) but fell after UNUCA (5 mmHg). Tissue pressure rose more after BN (2-2% compared with 1-0%) in spite of greater expansion of ECFV after UNUCA.

In Series B2 blood pressure showed similar changes (+23% compared with -10%). Tissue pressure rose more after BN (3-3 compared with 0-3 cmH2O). Venous pressure rose after BN (2-3 cmH2O) but fell insignificantly after UNUCA (0-8%).

In both Series A and B1 we have calculated the increase in interstitial fluid volume (IFV) per cmH2O rise in tissue pressure (∆IFV = ∆ECFV − ∆PV) after BN and after UNUCA (Table 1). This ratio is not a true expression of compliance but gives an indication of a change in compliance. Since in some rats tissue pressure did not rise, making this ratio infinite, we have calculated the average ratio for each group by finding the mean increase in interstitial fluid volume and dividing by the mean increase in tissue pressure (Table 1). The results are similar
TABLE 1. Volume/pressure relationship of interstitial tissue

<table>
<thead>
<tr>
<th>Series</th>
<th>Bilateral nephrectomy</th>
<th>Unilateral nephrectomy and ureterocaval anastomosis</th>
<th>T^2</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>ΔIFV/ΔTP (ml/cmH_2O)</td>
<td>n</td>
<td>ΔIFV/ΔTP (ml/cmH_2O)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>22</td>
<td>3.62</td>
<td>12</td>
<td>27.3</td>
<td>31.74</td>
</tr>
<tr>
<td>B1</td>
<td>12</td>
<td>3.40</td>
<td>9</td>
<td>16.8</td>
<td>21.34</td>
</tr>
<tr>
<td>A and B1</td>
<td>34</td>
<td>3.57</td>
<td>21</td>
<td>21.2</td>
<td>34.53</td>
</tr>
</tbody>
</table>

The above ratios were obtained by dividing the mean increase in interstitial fluid volume (IFV) by the mean increase in tissue pressure (TP) 4 days after BN or UNUCA. P values were obtained from an F test made on Hotelling's bivariant T^2 statistic (Snedecor & Cochran, 1968).

Fig. 1. Changes in interstitial fluid volume (IFV) plotted against changes in tissue pressure 4 days after BN (●) and UNUCA (○) (Groups A and B1 combined). The points for each group occupy significantly different areas. T^2 = 34.54, F = 16.94, P < 0.001 using Hotelling's bivariant T^2 statistic (Snedecor & Cochran, 1968).
for Series A and B1; in each the IFV/TP ratio after BN is five- to seven-fold less than after UNUCA.

In Fig. 1 the increase in interstitial fluid volume is plotted against the increase in tissue pressure for BN and UNUCA rats in Series A and B1 combined. Using Hotelling's bivariant $T^2$ test (Snedecor & Cochran, 1967) it is shown that the points from the BN rats occupy a significantly different area from those from the UNUCA animals ($P < 0.001$).

**Measurement of compliance by infusion (Series B2, Table 2)**

After unilateral nephrectomy alone (day 5) there was a slight fall in haematocrit and a slight rise in tissue pressure 10 min after the infusion in both groups. There was little change in venous pressure. Four days after the second operation (day 19) there was a marked difference in response; after UNUCA the changes were similar to those seen after unilateral nephrectomy

| Table 2. Infusion results, Series B2 |

<table>
<thead>
<tr>
<th></th>
<th>Bilateral nephrectomy ($n = 10$)</th>
<th>Unilateral nephrectomy and ureterocaval anastomosis ($n = 9$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Tissue pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cmH$_2$O)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>4.2</td>
<td>3.6</td>
</tr>
<tr>
<td>After</td>
<td>3.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Mean change</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Venous pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cmH$_2$O)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>5.2</td>
<td>5.2</td>
</tr>
<tr>
<td>After</td>
<td>5.2</td>
<td>5.2</td>
</tr>
<tr>
<td>Mean change</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>50.2</td>
<td>48.6</td>
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<tr>
<td>After</td>
<td>48.6</td>
<td>48.6</td>
</tr>
<tr>
<td>Mean change</td>
<td>1.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

The table shows mean tissue pressure, venous pressure and haematocrit before and 10 min after an intravenous infusion (5 ml of 0.9% saline 100 g body weight at 37°C given over 4 min). The baseline infusion was performed 4 days after unilateral nephrectomy and the final infusion 4 days after removal of the second kidney or ureterocaval anastomosis. n.s., Not significant.
### Table 3. Volume-pressure relationships of interstitial tissue following a saline infusion

<table>
<thead>
<tr>
<th></th>
<th>Increase in IFV (ml)</th>
<th>Increase in tissue pressure (cmH$_2$O)</th>
<th>Average compliance (mean IFV/mean tissue pressure) (ml/cmH$_2$O)</th>
<th>$T^2$</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>After unilateral nephrectomy</td>
<td>9</td>
<td>9.0</td>
<td>1.3</td>
<td>0.4</td>
<td>0.5</td>
<td>23</td>
</tr>
<tr>
<td>After subsequent ureterocaval anastomosis</td>
<td>8.9</td>
<td>1.4</td>
<td>0.4</td>
<td>0.7</td>
<td>22</td>
<td>0.081</td>
</tr>
<tr>
<td>After unilateral nephrectomy</td>
<td>10</td>
<td>9.7</td>
<td>1.2</td>
<td>0.6</td>
<td>0.5</td>
<td>16</td>
</tr>
<tr>
<td>After subsequent bilateral nephrectomy</td>
<td>8.2</td>
<td>1.4</td>
<td>1.7</td>
<td>1.3</td>
<td>47</td>
<td>0.081</td>
</tr>
</tbody>
</table>

Changes in interstitial fluid volume (IFV) and in tissue pressure following a saline infusion. Tissue pressure values are taken from Table 2 and interstitial fluid volume is calculated as described in the text. Average compliance values are obtained by dividing mean Δ IFV by mean Δ tissue pressure (see the text). $P$ values are obtained from an $F$ test made on Hotelling's bivariant $T^2$ statistic. n.s., Not significant.
alone, but after BN there was a significantly greater rise both of tissue pressure and of venous pressure, and a greater fall in haematocrit.

From the change in haematocrit after the infusion we estimated the percentage expansion of plasma volume, assuming that the erythrocyte volume and the total body/venous haematocrit ratio had remained constant. Since plasma volume was not measured in these animals, we assumed that the plasma volume in each rat was equal to the mean plasma volume of the appropriate group of rats in Series B1 which had been treated in the same manner. From this was calculated the absolute increase in plasma volume after the infusion for each animal. The increase in interstitial fluid volume was calculated as the infusion volume minus the increase in plasma volume (Table 2). Since plasma volume altered little, the error in calculating the interstitial fluid volume changes using these extrapolated plasma volume values is relatively small.

![Graph](image)

**Fig. 2.** Changes in interstitial fluid volume (IFV) and in tissue pressure 10 min after a saline infusion (5 ml/100 g body weight). (a) 4 days after unilateral nephrectomy (△) and 4 days after subsequent ureterocaval anastomosis (▲). Ellipses plotted with 95\% confidence limits for the two groups of points do not occupy significantly different areas. (b) 4 days after unilateral nephrectomy (△) and 4 days after subsequent bilateral nephrectomy (▲). Ellipses plotted with 95\% confidence limits for the two groups of points occupy significantly different areas (\(T^2 = 17.67, F = 8.346, P<0.001\)).

The compliance of the interstitial space is the expansion of interstitial fluid volume per cmH\(_2\)O rise in tissue pressure after the infusion. Since in some animals the tissue pressure did not rise, giving infinite compliance, we calculated the average compliance for each group by dividing the mean rise in interstitial fluid volume by the mean rise in tissue pressure. Table 3 gives the calculated mean increase in interstitial fluid volume, mean increase in tissue pressure and average compliance.

In Figs. 2(a) and 2(b) the calculated post-infusion rise in IFV is plotted against the rise in TP.
Renal control of interstitial space compliance

for each rat. It can be seen that there is little difference in changes in IFV, TP, or compliance following ureterocaval anastomosis compared with the results following previous unilateral nephrectomy (Fig. 2a). However, after bilateral nephrectomy there is less expansion of IFV but a greater rise of TP (Fig. 2b). This gives a fourfold fall in compliance (Table 3).

The significance of the compliance values is assessed by comparing the areas occupied by the points in Figs. 2(a) and 2(b). After ureterocaval anastomosis the area occupied is almost identical with that after unilateral nephrectomy. However, after bilateral nephrectomy the area differs significantly from that after unilateral nephrectomy ($P<0.001$; Hotelling’s bivariant T² test).

Fig. 3 shows changes in blood pressure 4 days after BN and UNUCA plotted against changes in venous pressure for Series B2 (measurements made before infusion). There is a rough correlation between BP and VP after BN but not after UNUCA.

Fig. 4 shows the relationship between changes in blood pressure and changes in tissue pressure for Series B1 and B2 combined. There is a rough correlation between BP and TP for both BN and UNUCA groups. The slope of the regression line for the BN animals is greater than that for the UNUCA but this difference is not significant.

Although variances do not differ significantly the intercepts are significantly different so the lines may not be considered identical (Documenta Geigy, 1970).

Fig. 5 shows the same relationship for Series A, in which blood volume expansion occurred.
Although the two sets of points are well separated there is no significant relationship between increase in tissue pressure and increase in blood pressure for either the BN or the UNUCA groups.

Electrolytes and blood gases

Although both groups showed marked acidosis and hyperkalaemia, there was no difference in blood pH, $P_{CO_2}$, $PO_2$, urea or electrolytes 4 days after BN or UNUCA. Serum sodium in the two groups did not differ significantly from values in normal rats. (Detailed results are lodged with the Royal Society of Medicine.)

DISCUSSION

The most striking results are the differences in interstitial tissue compliance between BN and UNUCA rats. There is little difference between the compliance measured after unilateral nephrectomy and that found after subsequent ureterocaval anastomosis. However, after bilateral nephrectomy there is a significant fall in compliance compared with values found in the same rats after unilateral nephrectomy alone (Table 3, Figs. 2a and 2b).
Although the ratio of the increase in interstitial fluid volume to the increase in tissue pressure 4 days after BN and UNUCA is not strictly a measurement of compliance, nevertheless the ratios so obtained are very similar to the compliance values following saline infusion.

It can be argued that the lower compliance of the BN rats is due to the fact that the pre-infusion tissue pressure was higher and that the tissues were therefore 'tighter'. Guyton (1965) has shown that compliance increases as tissue pressure rises near to atmospheric values; this makes the decrease in tissue compliance of the BN animals the more significant.

It is relevant to consider the changes in the interstitial space which might lead to a change in compliance. Recent evidence suggests that little, if any, fluid exists in the free state in the interstitial space but that it is bound in a gel. This gel consists of a network of collagen fibres around enmeshed molecules of hyaluronic acid and other glycosaminoglycans (Ogston, 1966; Laurent, 1970). Hyaluronic acid is a large molecule (mol. wt. $1 \times 10^6$ to $8 \times 10^6$) which consists of polysaccharide chains in the form of an expanded random coil, roughly spherical with a diameter of about 400 nm (4000 Å). This coil encloses a large amount of water; the hydrodynamic volume of the molecule in solution occupies about 1000 times the space of the unhydrated polysaccharide chain (Ogston & Stanier, 1951, 1953). There is evidence of entanglement of molecules of hyaluronic acid in solution at concentrations as low as 0.1% (Ogston, 1970). In a 1% solution, molecules show 80% overlap. Because of this the osmotic pressure of a solution of hyaluronic acid increases with concentration in a non-linear manner; a 1% solution has an osmotic pressure of 6.7 cmH$_2$O but that of a 2% solution is 24.5 cmH$_2$O (Loewi, 1961).
Snashall, Lucas, Guz & Floyer (1971) suggested that the sub-atmospheric pressure of the interstitial fluid is due to osmotic forces. They measured tissue pressure in rats by using a cotton wick introduced into the subcutaneous tissue through a wide-bore needle. Wicks soaked in saline measure sub-atmospheric pressures of the same order as those recorded by the capsule method. If the wick is removed, soaked in rat plasma or in 6–10% (w/v) bovine serum albumin solutions, and reintroduced, the recorded pressure remains the same. However, if the wick is soaked in hyaluronic acid solution of concentrations greater than 0.2%, higher pressures are recorded; in solutions of 1% or over the pressure rises above 1 atmosphere. Snashall et al. (1971) suggested that under normal conditions there is no free fluid in the interstitial space; if free fluid is introduced, the enmeshed hyaluronic acid molecules of the gel, supported by collagen fibres, act as a semipermeable membrane at the boundary zone between gel and free fluid. Osmotic forces are set up on account of the concentration of hyaluronic acid molecules in the gel, tending to attract fluid into it; equilibrium is reached when the hydrostatic pressure of the free fluid is sub-atmospheric by the same amount as the osmotic forces of the gel. Since protein molecules are small enough to diffuse through the hyaluronic acid molecule network (Laurent, Björk, Pietruszkiewicz & Persson, 1963), protein solutions are subjected to the same forces as saline. However, if the free fluid contains hyaluronic acid molecules which cannot diffuse through the boundary zone, osmotic forces are balanced. Any fluid which enters the interstitial space will be subjected to these forces, including fluid filtered from capillaries; in consequence the osmotic pressure of the collagen–hyaluronic acid gel must be a factor in determining the equilibrium across the capillary wall.

Guyton (1965) has shown that under normal conditions relatively small increases in the hydration of interstitial tissue result in a change of tissue pressure, i.e. the tissues have a relatively low compliance. When, with increasing hydration, tissue pressure rises to near atmospheric values the compliance suddenly rises, so that large amounts of fluid can enter the tissues with little change in pressure, and at this point oedema occurs. In addition Guyton, Scheel & Murphree (1966) showed that at the same point on the pressure–volume curve the resistance of tissues to the bulk flow of fluid, very high under normal conditions, decreases several hundred thousand fold. Presumably under conditions of normal hydration the hyaluronic acid gel is maintained in an unsaturated state, but as fluid continues to enter a point is reached at which the swelling pressure of the gel becomes equal to the solid tissue forces restricting swelling. The gel will no longer absorb water; any more fluid entering the interstitial space will remain free.

The compliance of the interstitial gel in the unsaturated state under conditions of normal hydration must depend upon the degree to which water entering changes the balance of forces within it. This must depend partly upon the size and degree of entanglement of the hyaluronic acid molecules. Changes in the chemical nature of the molecules may affect compliance; it is possible that such changes can be brought about by circulating substances. As we have observed marked differences in the compliance of the interstitial space following the removal of the kidneys we suggest that a substance coming from the kidney (or altered by the kidney) affects the chemical composition of hyaluronic acid and other glycosaminoglycans and changes the osmotic and swelling characteristics of the interstitial gel.

Speculating further, we propose that this represents a physiological mechanism. Under conditions of normal hydration a substance secreted by the kidney maintains the compliance of the interstitial tissues relatively high so that fluid can be stored in the tissues without undue rise in tissue pressure. When body water is depleted the secretion of this substance becomes less and
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Renal control of interstitial space compliance decreases, causing fluid to pass from the tissues into the circulation and thus maintaining adequate plasma volume. The kidney may maintain the blood volume constant by controlling both the excretion of salt and water and the movement of fluid between interstitial space and plasma, by secretion both of renin and of the substance we postulate.

If this is so it would be expected that partial renal artery constriction, after which the kidney behaves as if dehydration were present, would cause a decrease of interstitial space compliance. Floyer (1966) showed that interstitial space compliance measured by a saline infusion is lower in rats with hypertension after partial renal artery constriction than in normal rats. Lucas & Floyer (1972a) and J. Lucas & M. A. Floyer (unpublished work) showed that, 2 months after renal artery constriction, tissue pressure increases without an increase in interstitial fluid volume. Following removal of the constricting clip there is evidence of a rapid increase in interstitial space compliance, with passage of fluid from the plasma to the interstitial space.

We must now consider whether changes in the compliance of the interstitial space play a part in the elevation of blood pressure which occurs after bilateral nephrectomy, i.e. renoprival hypertension. When trying to predict the effect on blood pressure of changes in other parts of the circulation, great care is necessary in view of the many and complex factors involved. Time is one factor; although Green (1969) observed no rise in blood pressure after 4 days of 25% expansion of blood volume in rats with unilateral nephrectomy, rats with volume expansion of the same degree maintained for 1–2 weeks develop hypertension (M. A. Floyer, unpublished work). In addition, the mechanism by which the blood pressure is maintained may change with time; Conway (1963) showed that if the blood volume of dogs is expanded the blood pressure rises initially from increased cardiac output but that later peripheral resistance rises as well. A sounder theoretical study of circulatory haemodynamics can be made by computer simulation, making simultaneous adjustments for all known variable factors (Guyton, Thomas, Coleman, Bower & Granger, 1970).

Our studies were all made 4 days after BN or UNUCA; this time was chosen because 4 days after BN most rats have developed significantly raised blood pressure (Floyer, 1955). At this stage the rats have developed severe uraemic acidosis, and hyperkalaemia and it might be argued that this makes it difficult to draw any definite conclusions from the results. However, there was no significant difference between the pH, $P_{CO_2}$, $P_{O_2}$, urea and electrolyte values in the groups subjected to BN or UNUCA. Sodium concentrations in both groups were the same as those in normal controls. Moreover, recent studies (J. Lucas & M. A. Floyer, unpublished work) suggest that similar changes in interstitial space compliance are present 2 days after bilateral nephrectomy when the biochemical changes of uraemia are much less severe.

Ledingham & Pelling (1970) showed that after BN the blood pressure increase is associated with a rise of cardiac output and of plasma volume. In Series B2 the venous pressure rose following BN and there is a rough correlation between changes in venous pressure and changes in blood pressure (Fig. 4). This suggests that an increase of venous pressure, and thus of cardiac filling pressure, may be the cause of the increased cardiac output. In Series B1, although plasma volume increased after BN, the total blood volume changed little; erythrocyte volume fell. Assuming that changes in plasma volume in Series B2 are similar to those in Series B1, the increase in blood volume is unlikely to be the cause of the rise in venous pressure in Series B2. However, tissue pressure rose after BN. In Series B1 and B2 combined there is a rough correlation between increases in blood pressure and increases in tissue pressure after BN (Fig. 4). We suggest that the combination of increased interstitial fluid volume and reduced interstitial
tissue compliance after BN results in increased tissue pressure; this causes increased capillary pressure, venous pressure, cardiac output and blood pressure.

If failure of the blood pressure to rise after UNUCA is due solely to the greater tissue compliance and the consequent failure of tissue pressure to rise despite IFV expansion, the regression of blood pressure on tissue pressure after BN and UNUCA in Fig. 4 should be identical. Since these lines differ significantly, the difference between the behaviour of the blood pressure after BN and UNUCA must be due in part only to changes in tissue compliance. Other factors must also be responsible; the shunt effect of the kidney in lowering peripheral resistance may be one.

In Series A, in which blood volume did expand significantly, there is no correlation between increases in blood pressure and increases in tissue pressure after BN and after UNUCA (Fig. 5). Although venous pressure was not measured, it is possible that the extra plasma and erythrocytes increased venous pressure over and above the increment due to increased tissue pressure and that this blurred the relationship between BP and TP seen in Series B1 and B2. The question as to whether there is raised venous pressure (and cardiac output) following blood volume expansion after UNUCA is still unanswered.

Changes in plasma protein concentration and in capillary permeability to protein may play a part in determining the balance of extracellular fluid between plasma and interstitial space. Green (1969) and J. A. Green & M. A. Floyer (unpublished work) have shown that although after BN there is evidence of decreased permeability of the capillaries to globulin there is no significant change in the calculated osmotic pressure of plasma and of tissue fluids after BN and after UNUCA.

In patients with chronic renal failure maintained by intermittent haemodialysis small changes in fluid loading result in large changes in blood pressure. Dustan & Page (1964) show that in patients with chronic renal disease, both before and after bilateral nephrectomy, small changes in blood volume are associated with large changes in blood pressure. Following successful renal transplantation, similar changes in blood volume are associated with much smaller changes in blood pressure. A change in interstitial tissue compliance following renal transplantation may in part be responsible for this effect. Much work remains to be done on this subject.

APPENDIX

Validation of techniques of ureterocaval anastomosis

Since blood pressure rises after UNUCA if there is any degree of ureteric obstruction and renal distension (Floyer, 1955), it was necessary to recognize and exclude any rat in which this had occurred. After preliminary experiments, rats were accepted only if (1) there was no distension of the kidney, pelvis, or ureter after inspection, both before and after longitudinal section, and (2) the kidney wet weight/dry weight ratio did not exceed by more than 2 standard deviations the mean kidney wet weight/dry weight ratio of a group of rats subjected to unilateral nephrectomy alone. These criteria were tested by performing intravenous pyelograms (IVP) and measuring the size of the kidney, pelvis and ureter in vivo. This was done by injecting 1.5 ml of Hypaque 45 intravenously and taking X-rays enlarged twofold (macrograms). The images of the kidney, pelvis and ureter were measured with a ruler and the result divided by 2.

In twenty rats the left kidney was removed; 18 days later IVP was performed. The animals were killed and the right kidney was removed, sectioned longitudinally, blotted dry and weighed.
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TABLE 4. Measurement of right kidney, pelvis, and ureter after IVP

<table>
<thead>
<tr>
<th>Group</th>
<th>Diam. of ureter 7-5 mm below pelvis (mm)</th>
<th>Longitudinal diam. of kidney (mm)</th>
<th>Longitudinal diam. of pelvis (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>a (20)</td>
<td>1-0</td>
<td>0-07</td>
<td>13</td>
</tr>
<tr>
<td>b1 (5)</td>
<td>1-2</td>
<td>0-07</td>
<td>14</td>
</tr>
<tr>
<td>b2 (15)</td>
<td>-</td>
<td>-</td>
<td>19</td>
</tr>
</tbody>
</table>

Group a: 18 days after left nephrectomy. Group b1 and b2: 18 days after left nephrectomy and 4 days after right ureterocaval anastomosis. Group b1: no distension visible to the naked eye. Group b2: Distension of kidney, pelvis and ureter, visible to naked eye. Group b1 showed good concentration of dye in pelvis and ureter, but there was no concentration in group b2.

It was placed in a vacuum dessicator where it remained until the weight was constant (24 h). The wet weight/dry weight ratio was thus determined (Group a, Tables 4 and 5). A further twenty-four rats were also subjected to a left nephrectomy and the wet weight/dry weight ratio of the right kidney was determined 18 days later, but IVP was not performed (Group c, Table 5 and Fig. 1).

In twenty rats the left kidney was removed; 14 days later right ureterocaval anastomosis was

TABLE 5. Wet and dry weight and wet weight/dry weight ratios of right kidneys following various procedures

<table>
<thead>
<tr>
<th>Group</th>
<th>Wet weight</th>
<th>Dry weight</th>
<th>Wet weight/dry weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>SD</td>
<td>mg</td>
</tr>
<tr>
<td>VALIDATION SERIES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a (20)</td>
<td>979</td>
<td>102</td>
<td>265</td>
</tr>
<tr>
<td>c (24)</td>
<td>973</td>
<td>97</td>
<td>268</td>
</tr>
<tr>
<td>a and c (44)</td>
<td>976</td>
<td>99</td>
<td>266</td>
</tr>
<tr>
<td>b1 (5)</td>
<td>1305</td>
<td>45</td>
<td>325</td>
</tr>
<tr>
<td>b2 (15)</td>
<td>1610</td>
<td>218</td>
<td>243</td>
</tr>
<tr>
<td>d (6)</td>
<td>1587</td>
<td>86</td>
<td>220</td>
</tr>
<tr>
<td>EXPERIMENTAL SERIES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accepted (29)</td>
<td>1413</td>
<td>274</td>
<td>356</td>
</tr>
<tr>
<td>(Groups A, B and B2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rejected (26)</td>
<td>1527</td>
<td>331</td>
<td>212</td>
</tr>
</tbody>
</table>

All groups had a left nephrectomy 18 days previously. Groups a and c: left nephrectomy alone. Groups b1, b2 and experimental series: right ureterocaval anastomosis 4 days previously. Group d: right ureter ligated 4 days previously. Rats were accepted into the experimental series if there was no evidence of distention of pelvis and ureter visible to the naked eye, and if the wet weight/dry weight ratio was less than 4-31.
performed, and 4 days later an IVP was performed after which the animal was killed. The kidney and ureter were inspected carefully and the wet weight/dry weight ratio determined. In five of these animals the kidney was normal to inspection by the naked eye. In these animals IVP showed good concentration of dye in the pelvis and ureter, which differed little in size or appearance from those of rats after unilateral nephrectomy alone (Group b1, Table 4). In the remaining fifteen animals the kidney and ureter showed evidence of distension to the naked eye; IVP failed to outline the pelvis or ureter and the nephrogram effect showed the kidney to be enlarged (Group b2, Table 4).

In a further six rats the left kidney was removed; 14 days later the right ureter was ligated, then 4 days later the right kidney was removed and the wet weight/dry weight ratio measured. All these kidneys were markedly distended (Group d).

In our experimental series, UNUCA animals underwent right ureterocaval anastomosis 14 days after left nephrectomy, then 4 days later, after completion of all observations, the animals were killed; the right kidney was inspected and weighed wet and dry.

The kidney wet weight/dry weight ratios of all groups and of all rats on which UNUCA was attempted for the experimental series are shown in Table 5. The mean kidney wet weight/dry weight ratio for Groups a and c combined (unilateral nephrectomy only) was 3.67 (SD 0.32). Our arbitrary limit above which we excluded UNUCA rats from the experimental series was 4.31 (= mean + 2 SD). In four out of five of Group b1 rats (UNUCA with kidneys normal to the naked eye and with normal IVP) the wet weight/dry weight ratios were below 4.31; the fifth animal was only a little above. In Group b2 (UNUCA with distended kidneys to the naked eye and no concentration on IVP) the wet weight/dry weight ratio was well above 4.31 in all animals. This suggests that our criteria for accepting rats for the experimental series indicate that normal renal structure and function have been retained after UNUCA.

In thirty-two of fifty-five rats in which UNUCA was attempted, the kidney appeared normal to the naked eye, and twenty-nine of these thirty-two had wet weight/dry weight ratios below 4.31 and were accepted for the experimental series; three had no distension visible to the naked eye but had wet weight/dry weight ratios above 4.31 and were rejected. The remaining twenty-three had distended kidneys and a wet weight/dry weight ratio well above 4.31; they were also rejected. It is of interest that the dry kidney weight of rats after successful UNUCA is significantly greater than the dry kidney weight both of animals after unilateral nephrectomy alone and after ureteric ligation or unsuccessful UNUCA with obstruction. This increased hypertrophy following UNUCA has been described by Muirhead and his colleagues (Muirhead, Jones & Stirman, 1960).

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