The relation between the excretion of sodium and water and the perfusion pressure in the isolated, blood-perfused, rabbit kidney, with special reference to changes occurring in clip-hypertension

J. M. A. THOMPSON AND C. J. DICKINSON
Medical Unit, University College Hospital Medical School, London

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Summary
1. The sodium and water excretion rates of rabbit kidneys were studied when isolated and perfused at known pressure with blood from another normal anaesthetized rabbit. Studies at several different perfusion pressures confirmed that a small rise in perfusion pressure caused a large rise in sodium excretion and that the potential sodium-excreting ability of the isolated kidney was high. The curve obtained could be closely fitted by a quadratic equation which allowed an estimate to be made of the blood pressure below which no urine is formed, i.e. the 'theoretical perfusion pressure threshold'. For normal kidneys this was 55.4 mmHg.

2. A group of rabbits had a silver clip applied to the left renal artery and, 3–6 weeks later, the eight most hypertensive animals were selected to provide their kidneys for perfusion. Both kidneys were perfused simultaneously. The clip on the left renal artery was removed immediately before perfusion and the cannula placed distal to the stenosis in the post-stenotic dilatation. The function curves of these kidneys were compared with the curves obtained from normal kidneys.

3. The untouched kidney contralateral to the clip was found to require a significantly higher perfusion pressure (71.7 mmHg) for it to achieve a given sodium excretion rate and, surprisingly, the clipped kidney showed a similar functional change (76.4 mmHg). In other words the positions of both function curves were shifted though their slopes were not much changed.

4. Both kidneys in single-clip-hypertension appear to adapt or reset their sodium excretory behaviour. The resetting in the untouched kidney allows hypertension to be sustained without undue sodium loss. Aldosterone probably contributes little to the resetting. We infer, indirectly, that the normal kidney may, to a significant extent, restrain sodium excretion by virtue of its sympathetic innervation. We also opine that the kidney cannot be assigned fixed intrinsic functional properties on which a renal sodium-handling theory of long-term blood pressure regulation can be firmly based.

Key words: aldosterone, blood pressure, denervation diuresis, sodium excretion threshold, sympathetic nerves of kidney.

Introduction
Many mechanisms are known to play some part in blood pressure regulation. In terms of rapidity of action, the peripheral arterial baroreceptor reflexes appear to be the fastest-acting, but it has long been apparent that they are unlikely to be the mechanism by which blood pressure is stabilized over long periods of time. Baroreceptor resetting takes place relatively rapidly in several species, over a period of a few days (McCubbin, Green & Page, 1956; Ueda, Uchida, Yasuda & Takeda, 1966; Kreiger, 1970). The threshold for renin release also appears to be reset in human renal and essential hypertension.
(Kaneko, Ikeda, Takeda & Ueda, 1967; Kaneko, Ikeda, Takeda, Inoue, Tagawa & Ueda, 1968). It seems therefore unlikely that the control system constituted by renin release in response to a fall of renal perfusion pressure is one which the body could rely on for long-term blood pressure stabilization. The so-called volume receptors of the great veins and atria are presumably related to monitoring and control of circulatory filling, and hence indirectly to blood pressure regulation; but it appears that in heart failure, for example, the sensitivity of these receptors may be diminished (Greenberg, Richmond, Stocking, Gupta, Meehan & Henry, 1973) and their working range altered. None of these control systems therefore seems likely to account for long-term blood pressure stability.

There is growing interest in a general theory which places the intrinsic sodium handling characteristics of the kidney as the dominant factor in long-term blood pressure regulation (Borst & Borst de Gues, 1963; Ledingham & Cohen, 1964; Guyton, Jones & Coleman, 1973). As Guyton has repeatedly emphasized, this mechanism is unique in having potentially infinite gain over long periods of time. The theory runs as follows. The curve relating sodium excretion rate to perfusion pressure is very steep. A small change in perfusion pressure causes a very large change in the rate of sodium and water excretion, which in the long run acts as a powerful negative feedback stabilizer of systemic arterial pressure. Suppose, for example, the system in man is in balance at an average renal perfusion pressure of 100 mmHg and a sodium excretion rate of 150 mmol/day, balancing dietary intake. A small rise in average blood pressure, e.g. 1 mmHg, might increase the sodium excretion rate so that perhaps 2 mmol more sodium would be excreted per day. In the course of only 6 months this would add up to the net loss of 360 mmol of sodium, which would almost certainly be enough to bring the blood pressure perfusing the kidney back to its previous value.

It is very remarkable that this intrinsic functional behaviour of the kidney has been studied so little. Since the first observations of Richards & Plant (1922), only a few further measurements have been made (Selkurt, Hall & Spencer, 1949; Shipley & Study, 1951). The results of Selkurt et al. were presented by Borst (1966) in the now-familiar graphical format relating sodium excretion to perfusion pressure. Close inspection of these results, especially the low rates of sodium excretion obtained at normal perfusion pressures, casts some doubt on the adequacy of the experimental techniques available at the time. In Selkurt’s experiments the kidneys were not denervated and therefore potentially subject to influences other than renal perfusion pressure. The only other recent observations made (Thurau & Deetjen, 1962; Fourcade, Navar & Guyton, 1971) did not have as their prime object a quantitative characterization of the relationship. The time seemed ripe to re-examine the functional characteristics of the isolated perfused kidney using techniques now available for improving the conditions of such an experiment.

Another stimulus for the present investigation was the consideration that chronic hypertension can be established in several species by the application of a constricting clip to one renal artery, leaving the other kidney untouched. If the functional excretory characteristics of the contralateral kidney did not change after this procedure, it would appear impossible for hypertension to be sustained for long, since the untouched kidney, subjected to an increased perfusion pressure, would simply pour out sodium and water until the status quo was restored. The obvious way of accounting for the maintenance of hypertension after the application of a single clip is to suppose that aldosterone acts on the contralateral kidney. On the other hand, Dickinson & Yu (1967) were able to produce a progressive rise in systemic arterial pressure in the rabbit over a period of many days by the continuous infusion intravenously of small amounts of angiotensin, which were effective even in an adrenalectomized rabbit (Yu, 1966). They also noticed that blood pressure did not fall at once after the angiotensin infusion was stopped. This led us to consider the possibility that in addition to short-lived hormonal influences acting on the untouched kidney, there might be a true resetting of its sodium excretion threshold. We were concerned to establish whether a kidney, previously exposed to hypertension and perfused under standard conditions with blood from a normal donor animal, differed from normal in its functional characteristics. Our starting hypothesis (which now appears to have been only half correct) was that on the untouched side the excretion characteristics would be changed in such a way that sodium and water excretion would be restrained (i.e. the threshold would be raised) and that on the clipped side, where the effective perfusion pressure was reduced, the excretion curve would be reset in the opposite.
direction so that such a kidney perfused under standard conditions after removal of the clip would excrete sodium abnormally easily (i.e. the threshold would be lowered). The present investigation reports observations to test this hypothesis and to characterize accurately the perfusion pressure/sodium and water excretion characteristics of the isolated rabbit kidney.

Methods

Seventy New Zealand white rabbits, twenty-two male, forty-eight female, weight 1.8–4.5 kg (average 2.8 kg) were used. In each experiment, one lightly anaesthetized animal, intact apart from cannulation of artery and vein, acted as a standard blood-donor, and the other rabbit in each experiment provided either one or two kidneys for perfusion in an isolated system, with blood from the donor pumped to maintain a constant perfusion pressure.

Technique for isolated kidney perfusion

Preparation of the kidney donor rabbit. The rabbit providing the kidneys for perfusion was prepared as follows. Before the day of the experiment, blood pressure was taken twice weekly by the ear capsule method under standard conditions in a warm room. All animals were on a normal laboratory diet, with unrestricted access to water. General anaesthesia was induced by the intravenous injection of sodium pentobarbitone (30 mg/kg, supplemented by further doses of 5 mg/kg as required). This was followed by the intravenous administration of 100 i.u. of heparin. With the additional use of subcutaneous injection of local anaesthetic, if needed, a midline abdominal incision was made and the three main arteries to the alimentary tract were ligated. After the distal colon, oesophagus and porta hepatis were tied off the entire bowel was removed between the ties.

The renal arteries, veins and ureters were exposed and fine ligatures positioned but not tied. A fine glass cannula was inserted into the ureter 2 cm from the kidney and tied in place, and the distal ureter removed. The renal artery and vein were then individually cannulated without delay, Teflon cannulae being used (approximately 1 cm long with a minimum internal diameter of 2 mm). The whole kidney was removed to a saline bath maintained at 37°C. Perfusion with blood at a pressure of 60 or 70 mmHg from the blood donor (see below) was immediately begun and the ischaemia time recorded. This was between 3-5 and 12 min, and averaged 5-5 min. The second kidney was then cannulated in an identical fashion, also transferred to the bath and perfused. Wherever possible, both kidneys were used, but in some experiments technical problems prevented this, and in a number of experiments only one kidney was perfused. The kidneys and ureters were secured to prevent accidental movement and urine collections were made in pre-weighed glass containers.

Preparation of the blood-donor rabbit. On the evening before the experiment, ancord (Arvin) was given intravenously in a dose of 0.5 unit/kg. The dose was repeated the following morning. Anaesthesia was induced with a drug combination (Hypnorm) containing fentanyl citrate (0-12 nmol/ kg; 63 µg/kg) and fluanisone (5-6 nmol/kg; 2.0 mg/ kg), followed by halothane (Fluothane) and oxygen, and maintained with halothane, nitrous oxide (75%) and oxygen. The femoral artery was cannulated through a small groin incision, and the largest possible arterial cannula inserted. This cannula led to the extracorporeal circuit and pump, and blood returned from a large cannula into the external jugular vein. Throughout the experiment, for a period of up to 8 h, the blood-donor animal was allowed to breathe spontaneously and the level of anaesthesia kept just light enough that the animal remained still.

Perfusion circuit. The circuit consisted of a mercury manometer to measure the donor blood pressure, a variable-speed non-occlusive roller pump with variable by-pass resistance, another mercury manometer, somewhat damped, to measure mean renal artery perfusion pressure, a by-pass for the renal perfusion circuit containing a nylon-mesh filter, and a venous return line through which venous blood flowed back to the donor by gravity. This contained a direct-reading venous pressure manometer and also a horizontal graduated tube through which renal blood flow could be measured by timing the movement of a meniscus over a measured distance. Before the circulation was established 1000 i.u. of heparin was given intravenously to the blood donor, and the renal perfusion circuit clamped off. Blood was then circulated, at the donor animal’s blood pressure, through the pump by-pass, through the nylon mesh and back to the donor for 1½ h before the renal perfusion started. As soon as renal perfusion began, the nylon-mesh filter was
clamped off and removed from the circuit. All tubing was silicone rubber and connectors and cannulae were made of Teflon or siliconized polyethylene, with the exception of the ureteric cannulae, which were of glass.

Once perfusion had started, the perfusion pressure was held constant for about an hour to establish stable conditions and to allow the kidneys to recover from their initial ischaemia. Collections of urine at this pressure were made until the flow rate appeared to be steady. Each collection was of variable duration, depending on the urine flow rate; but, in every case, at least 0.3 ml of urine was collected from each kidney and, at the lower flow rates, this sometimes involved waiting between 10 and 20 min for each collection. The pressure was then raised in steps of 10 or 20 mmHg by adjusting the by-pass resistance. In most experiments, two successive increments of 10 mmHg pressure were used and these were followed by two further decrements of pressure back to the starting value. At each step-change in pressure, at least 30 min was allowed to elapse before urine collections were restarted, and no measurements were made until the urine flow rate appeared stable.

Production of renal artery clip-hypertension

Preparation of the renal-clip-hypertensive rabbits.

In eight animals, we were able to produce a sustained modest elevation of blood pressure by the application of a silver clip to one renal artery. Thirty-nine rabbits were anaesthetized with sodium pentobarbital, as described above for the kidney-donor animals, and, with a flank incision, a 0.5 mm internal diameter silver clip was applied to the left renal artery. Twice-weekly blood pressure measurements were then made as already described and the eight most hypertensive animals were selected for study. Two animals were submitted to sham operations at the same period (3–5 weeks) before the perfusion study was undertaken. These animals had the same anaesthesia and the left kidney was exposed and handled, but no clip was applied. On the day of the experiment, each kidney was removed for perfusion as already described above but, in the case of the clipped kidney, the clip was removed and an arterial cannula inserted into the post-stenotic dilatation of the renal artery distal to the site of the clip. The renal hypertensive, intact and sham-operated animals were studied randomly over the whole period, and not consecutively.

Chemical determinations

Urine weights were recorded by weighing the collection tubes before and after filling with urine and analyses for sodium and potassium were performed on an AutoAnalyzer (Technicon Instruments Ltd). In ten of the successful experiments, the secreted urine was at different times tested for the presence of glucose and protein by Clinistix and Albustix respectively but none was found in any experiment.

In three experiments, providing six individual kidneys, analyses of urinary creatinine were kindly undertaken for us by Dr S. Khattab, using the AutoAnalyzer (Technicon Instruments Ltd; NIIA method, with alkaline picrate) on deep-frozen specimens of urine. Because it was not our original intention to estimate glomerular filtration rate, we did not take any blood samples during these experiments, but made these observations retrospectively in some of the later experiments to help the interpretation of the results.

A record was kept of the rate of excretion of urine, and an appropriate volume of sterile fluid with approximately the expected electrolyte content (gauged by experience of previous experiments) was reinfused into the donor animal and perfusion circuit, to make good the fluid and electrolyte losses during the course of the experiment, which continued for 3–8 h.

Results

Sodium and water excretion by normal, blood-perfused kidneys

We tried to perfuse both kidneys simultaneously by using small recipient animals and large donor animals, so that we should be in a position to compare the left with the right kidney in clipped hypertensive animals. However, in four of the normal kidney-donor animals, only one kidney could be successfully perfused because of various technical failures, e.g. air and fibrin emboli and damage to vessels. We obtained sixteen kidneys from ten previously intact normal animals and four more kidneys from sham-operated control animals. The results from all the twenty normal kidneys are
Sodium and water excretion by perfused kidneys

presented in Fig. 1 together, since there was no systematic or detectable difference in results from intact or sham-operated animals nor from single- or double-kidney experiments.

Fig. 1 relates perfusion pressure to rate of sodium excretion. The data for each kidney are set out approximately in order of steepness of slope. This bore no detectable relation to the size of the kidney, but appeared to be associated chiefly with the success of the technical manoeuvres necessary for setting up the perfusion circuit. Many of the early experiments were failures because of air and fibrin embolism or too long an ischaemia time. In the successful experiments, as soon as blood flow had been re-established in the pump circuit, the kidney or kidneys gradually swelled over a half-hour period, until the standard size was achieved. In a few experiments (not reported in this paper) we watched the swelling process by placing the kidney in an oncometer, and in most cases stability both of urine flow rate and of renal size was reached at least within 20 min of a 10 mmHg change of renal perfusion pressure, sometimes sooner. If during an experiment the perfused kidney became ischaemic,

Fig. 1. All data from twenty individual perfused kidneys, relating sodium excretion to renal perfusion pressure. Each point represents one collection period. The curves shown are the curves of best fit calculated by the quadratic equation described in the text. The four experiments in the extreme right-hand column are from sham-operated animals. The rest are from normal animals.
even for a few seconds, there was a notable renal vasodilatation, which persisted for a much longer time, approximately proportional to the duration of ischaemia. Any change in perfusion pressure was followed by a changed rate of water and sodium excretion, which approached its final value asymptotically (see Fig. 2). The larger the change in pressure the longer the time to equilibrate and for this reason we have confined the results reported in this paper to maximum step changes of 20 mmHg. At high perfusion pressures or with sudden large pressure increments it was sometimes impossible to obtain a steady state in any reasonable time (Fig. 3).

**Perfusion of clipped and untouched kidneys**

*Results from clip-hypertensive animals.* The mean blood pressure measured by the ear capsule on the eight selected renal hypertensive animals was 105 ± 2.5 SEM, which can be compared with the ear clip pressures taken under the same conditions from the normal donors, which was 80.5 ± 3.8 SEM. The results of perfusing the left (clipped) kidney, after removal of the clip and with the catheter inserted into the post-stenotic dilatation, are shown in the lower part of Fig. 4, and the results for the simultaneously perfused untouched kidneys of the same eight animals are in the upper part of Fig. 4. It is apparent that, in general, the slopes representing the untouched kidney’s excretory function are on average steeper, and those of the previously clipped kidney are shallower than the slopes for normal kidneys shown in Fig. 1. However, there is in virtually every case an obvious displacement of both sets of curves towards the right, i.e. the threshold and position of the curve relating sodium excretion to perfusion pressure is shifted in such a
Sodium and water excretion by perfused kidneys

Fig. 4. Results of perfusion studies of sodium excretion in eight untouched kidneys from hypertensive animals (upper graphs) and eight clipped kidneys from the same animals (lower graphs). The curves, as in Fig. 1, are the curves of best fit calculated with the quadratic expression described in the text.

way that a much higher perfusion pressure (approximately 20 mmHg higher) was needed to induce the perfused kidney to release the same amount of sodium.

Mathematical analysis of results

We have attempted to characterize these curves and analyze the differences between them. Empirically the curves could with no notable exception be closely fitted by an expression of the general form

\[ Y = a \cdot (X-b)^c \]

in which \( Y \) represents sodium excretion, \( X \) is perfusion pressure, \( a \) is a constant describing the slope of the curve, \( b \) is another constant describing the
position of the curve which, if extrapolated, would pass through a 'theoretical perfusion pressure threshold' at which no urine would be secreted, and \( c \) is an exponent describing the degree of curvature.

We subjected the twenty normal results to computer-aided curve-fitting procedures to determine that value for exponent \( c \) which gave the smallest summed variance for the whole set of results. A value of 2.0 was found to give a closer fit for all the curves than could be obtained by using exponents of 1.9 or 2.1. To simplify the statistical analysis and to restrict the degrees of freedom we have therefore made the premise that the curve is most accurately fitted by a quadratic expression, and that the exponent \( c \) has a characteristic value of 2.0.

In these experiments we observed no tendency for the curves to flatten off at high pressures. Indeed, if anything we may have underestimated the flow rates at the higher pressures because in some cases it took a long time for stability of urine flow rate to be achieved. By a computer-aided curve-fitting procedure the best-fitting curve was obtained for each set of data. The calculated curves of best fit according to the formula are shown in Figs. 1 and 4 as continuous lines for each kidney studied. It is apparent that with very few exceptions the calculated curves fit the data closely.

For the pooled data from all twenty normal kidneys, including the four sham-operated normal animals and the sixteen kidneys from intact animals (which had the same functional characteristics), we have obtained values for constant \( a \) of 0.088 (±0.026 SEM) and for \( b \) 55.4 (±1.4 SEM). For the eight clipped kidneys, perfused after removal of the clip, the respective values for constant \( a \) were 0.044 (±0.008) and for \( b \) 71.7 (±4.1). For the untouched kidney, contralateral to the clip, the values of \( a \) were 0.091 (±0.016) and for \( b \) 76.4 (±3.6). For the four sham-operated animals, considered separately, \( b \) was 57.1 (±2.4), i.e. virtually the same as for the normal group as a whole.

Fig. 5 is an attempt to describe the probable position of the sodium excretion/perfusion pressure function curve for normal, clipped and contralateral-to-clip (untouched) kidneys. The shaded areas are obtained by using \( a, +1 \) SD, and \( b, -1 \) SD, at one side, and \( a, -1 \) SD, and \( b, +1 \) SD, on the other. By any statistical criterion which we have been able to apply (the simplest one of which is the analysis of difference between means) the values of \( b \) for both clipped and untouched (contralateral) kidneys were highly significantly different from the normal kidneys (\( P<0.001 \)), though the slopes (\( a \)) were not significantly different. There was a tendency for the contralateral kidneys to have a slightly steeper slope (i.e. a higher value for \( a \)) and the kidneys from the clipped side to have a lower value, but these results were not significantly different from normal. They are doubtless accounted for by the smaller size of the clipped kidney and by the larger size of the contralateral kidney.

Our original hypothesis was that the contralateral kidney would show a theoretical threshold reset upwards, that is in the direction actually observed in our experiments, and that on the side protected by the clip the threshold would move in the opposite direction. It was for this reason that originally we did not study normal animals which had previously been operated upon, because we guessed that each animal could act as its own control.
When we observed that the thresholds on both clipped and unclipped sides in the hypertensive animal were reset in the same direction, we studied four kidneys from otherwise normal sham-operated animals. These showed no significant or even suggestive difference from the others not similarly operated upon, and we have therefore included them in Fig. 1 and analysed all the data from normal kidneys together in Fig. 5.

Other observations made during perfusion experiments

Water excretion. Generally similar results were obtained when water excretion was used as the ordinate, but the results were very much less consistent than for sodium, even though the measurement of water excretion was, of course, more accurate than that of sodium. For water, the "theoretical excretion threshold" \((b)\) was 51.4 \((\pm 3.0 \text{ SEM})\) in normal animals. For the eight clipped kidneys it was 76.8 \((\pm 3.6; P < 0.001)\) and for eight untouched hypertensive kidneys it was 71.1 \((\pm 6.2; P < 0.001)\), i.e. both curves were significantly shifted to the right in the hypertensive animals. Results for six experiments in which sodium and water excretion are individually shown plotted against perfusion pressure are given in Fig. 6. There is clearly a more consistent relationship between sodium excretion and perfusion pressure than between water excretion and perfusion pressure, which we attribute to differing levels of circulating vasopressin in the blood coming from the lightly anaesthetized donor animal. The figures for potassium excretion showed a generally similar trend also. The curves are normalized to 100% for 90 mmHg perfusion pressure; in practice, sodium/potassium ratios varied between 1:2 and 48 (commonly about 12), but usually remained about the same during those experiments in which the potassium excretion was measured. There is clearly a wide scatter in the results, but a slight tendency for the excreted sodium to rise relatively more than the excreted potassium, thus raising the sodium/potassium ratio, as perfusion pressure is increased, probably because the distal tubular exchange mechanisms are overloaded.

Urinary creatinine excretion and inferences concerning changes in glomerular filtration rate. Results from six normal kidneys studied are shown in Fig. 6. At perfusion pressures between 70 and 80 mmHg, the excretion rate of creatinine rose sharply (though there was much individual variation) but the excretion rate was virtually unchanged as perfusion pressure was raised from 80 to 90 mmHg. This allows a reasonable inference that stability of glomerular filtration rate is achieved once perfusion pressure is at or above 80 mmHg, which in general accords with estimates in other species and in man. It is particularly notable that sodium excretion rises greatly as perfusion pressure is raised from 80 to 90 mmHg, suggesting that changes in tubular reabsorption are probably necessary to account for the changes.

Electrical stimulation of the renal pedicle. In a few instances, we concluded our experiment by stimulating one renal pedicle electrically with 5 V impulses of 10 ms duration once per second. This produced a prompt and dramatic fall in urine flow rate and a rapid reduction in the size of the stimulated kidney. This prompt reaction, and the invariably seen reactive vasodilatation after a brief period of ischaemia, suggests to us that even at the end of an experiment the renal vasculature was still fully capable of reacting to physiological stimuli.

Effects of aldosterone pretreatment of the perfused kidney. In four kidneys, perfused as described in the text, we prepared the rabbit by administering aldosterone (Aldocorten) by intramuscular injection, 3-50 nmol (0.125 mg) every 6 h for 2 days, concluding with a final injection shortly before the kidneys were removed for perfusion. Fig. 7 compares the pooled results with those from twenty normal kidneys. For the four aldosterone-treated kidneys \(a\) was 0.024 \((\pm 0.026 \text{ SEM})\) and \(b\) 47.2 \((\pm 3.6)\). The 'theoretical threshold' (i.e. the position of the curve relative to normal animals) is almost unchanged, but the curves are significantly less steep \((P < 0.01)\).

Discussion

Normal functional sodium and water excretion characteristics of the isolated perfused rabbit kidney

As the technical methods in this investigation were progressively improved and refined over several years, the results became more consistent and the remarkable ability of the isolated kidney to excrete sodium and water became apparent. The technical factors which contributed to this
Fig. 6. Composite results from six normal perfused kidneys showing the relation between renal perfusion pressure (70, 80 and 90 mmHg in all cases) and the excretion rates of creatinine, water, sodium and potassium. In some cases there was too little urine to analyse for creatinine and potassium, hence there are fewer results. Note the great consistency of the sodium excretion curve and the variability of water and potassium excretion. To enable comparison to be made all results are normalized to 100% for the 90 mmHg perfusion pressure collections. Vertical bars show 95% confidence limits of the mean values.

Fig. 7. Composite results for four aldosterone-pretreated kidneys, compared with the average curve from twenty normal kidneys (shaded area: see Fig. 5). The aldosterone curves are flatter, but their position is not significantly changed.

Improvement in experimental results were mainly:
1. Light halothane anaesthesia of the blood donor, which allowed in most cases an adequate blood pressure to be maintained in the donor, despite perfusion of some of its blood through the recipient kidney or kidneys, without evidence of gross release of vasopressin;
2. The use of siliconized tubing, which prevented the formation of small fibrin emboli;
3. Pretreatment of the blood donor before the experiment with Arvin, which also contributed to the same end;
4. Devising a technique of removal of the donor kidneys from the animal with only a few minutes of total ischaemia time, which usually allowed good function and stable conditions to be attained after about 30 min from the start of the experiment;
5. Leaving enough time, in some cases up to an hour, for a stable, unchanging urine flow to be attained at each level of perfusion pressure, which allowed the kidney to expand or contract and reach stable operating conditions.
conditions. We believe that these conditions have not previously been attained in experiments of this type. The importance of allowing enough time for stable conditions to be reached is evident from Fig. 2, in which it appears that stable operating conditions are reached asymptotically with a half-time of about 15 min.

Several striking features were apparent in the behaviour of the normal kidney perfused under the conditions of our experiments. First, we have never seen any evidence of flattening of the curve at high perfusion pressures. The rates of renal blood flow at perfusion pressures much above 100 mmHg were so rapid for kidneys in good condition that the donor animal was not able to sustain them. It was quite apparent that the maximal ability of a denervated and isolated perfused kidney in good condition to excrete sodium and water is very high indeed. The highest excretion rates observed in the best experiments would correspond, in the whole animal with two such kidneys perfused at 90 mmHg pressure, to a daily sodium excretion rate of at least 400 mmol. It is improbable that these high excretion rates were due to damage to proximal tubular reabsorptive mechanisms, since glucose was not found in the urine obtained even at these high flow rates.

When perfusion pressure was increased the kidney size did not increase immediately and the rate of sodium and water excretion changed only slowly. The attainment of the new steady state after 20 min or so at an increased pressure was accompanied by visible swelling of the kidney, which in a few experiments we confirmed by enclosing a kidney in an oncometer. The new steady state was achieved when the kidney was fully expanded. Under these circumstances electrical stimulation of the renal pedicle at frequencies of one per second produced a rapid shrinking of the kidney and a corresponding fall in the excretion rate of urine.

It was not our intention in the first instance to study glomerular filtration rate, but rather the overall behaviour of the kidney. The few observations made on the excretion rate of creatinine strongly suggest that under the conditions of our experiment glomerular filtration changed in parallel to the rate of sodium and water excretion up to a perfusion pressure of 80 mmHg, but that at higher pressures glomerular filtration rate remained constant while sodium excretion rate increased. We made a few crude observations on renal blood flow by single volume/timed collections, and in general renal blood flow appeared to change in parallel to rates of urine flow.

In conscious rabbits studied over long periods with indwelling catheters (Dickinson & Yu, 1967) it has been previously observed that the mean arterial pressure lies between 60 and 80 mmHg. At a perfusion pressure of 70 mmHg one would expect that the two kidneys together, if under no other influence, would be capable of excreting at least 80 mmol of sodium/day. Since this rate of excretion of sodium is far higher than is ever seen in rabbits on a normal laboratory diet, it is probable that, whenever the blood pressure is higher than 60 mmHg, normal rabbit kidneys in situ have their propensity for excreting sodium and water actively restrained by some mechanism. This could be normal tonic activity in sympathetic nerves, since most normally circulating hormones should have been present in our experiments. We observed during the course of the experiments that the blood pressure of the donor rabbit supplying the high flow rates necessary to maintain pressures of 90 mmHg or more in the perfused kidney arteries was often only 50 or 60 mmHg. It is possible that this low perfusion pressure or perhaps some other consequence of our perfusion conditions such as Arvin pretreatment, local release of prostaglandins, substance P or kallikrein (Mills, MacFarlane & Ward, 1974), accounts for the high rates of sodium excretion at relatively low pressures.

We are inclined, however, to interpret them as suggesting that the normal kidney perfused at mean blood pressures greater than 80 mmHg is under an important restraining influence from the sympathetic nervous system, which stops it excreting sodium and water at a high rate, and that this normal restraint may have been underemphasized in the past. It is, of course, obvious that other influences are available; otherwise, transplanted kidneys, which are inevitably acutely denervated, would be liable to pour out a massive volume of sodium and water, to the detriment of the recipient. However, restraint on sodium and water in such cases could come from increased sensitivity of the denervated kidney to circulating catecholamines from the donor, and it might be of some interest to observe the behaviour of autotransplanted kidneys in an animal deprived of both adrenal medullas. If our interpretation is correct such an operation might lead to extreme hypotension from sodium and water depletion. In 1951 Homer Smith reviewed the experi-
mental evidence for denervation diuresis and concluded that the phenomenon was an artifact produced by the release of the kidney from excessive sympathetic stimulation caused by anaesthesia, blood loss and surgical trauma (Smith, 1951). Later work has tended to refute the suggestion that there is no resting sympathetic tone in the renal nerves, and several authors have obtained evidence for a modest ipsilateral natriuresis in both anaesthetized and unanaesthetized animals after acute unilateral denervation (Kaplan, West & Forman, 1953; Kamm & Levinsky, 1965). Our experiments, though indirect and perhaps capable of being interpreted differently, suggest that the restraining influence of renal sympathetic tone could be important.

Implications for renal theories of long-term blood pressure regulation

Although our experiments have provided further evidence about the steepness of the curves relating sodium and water excretion to perfusion pressure, our inference concerning the restraining influence of the sympathetic nervous system suggests that it may be unrealistic to talk about the 'intrinsic functional behaviour' of the isolated kidney as the keystone of long-term blood pressure regulation. The experiments of Bianchi, Fox, Di Francesco, Dardi & Radice (1973) on the transmission of hereditary hypertension in rats, by grafting kidneys taken from a hypertensive strain, supply perhaps the best evidence so far of a renal factor in long-term blood pressure regulation. We only wish to make the point that if Bianchi's renal factor concerns resetting of the sodium and water excretion threshold, then either rat kidneys behave differently from rabbit kidneys, or there may be a direct renal influence acting upon the nervous system. It seems fair also to point out that since all blood pressure comparisons in Bianchi's experiments were made between rats with denervated kidneys, they cannot be taken to apply equally to experimental or clinical situations in which renal nerves are intact.

Adaptive changes in experimental clip-hypertension.

To allow single-clip-hypertension to be sustained it is obvious that some change in function must occur in the contralateral (untouched) kidney to prevent this kidney excreting large amounts of salt and water as the blood pressure rises. We expected that, if the blood pressure had been chronically raised as it was in these experiments, there might be a corresponding adaptive shift in the perfusion pressure/sodium excretion curve to the right with a rise in the threshold, so that a higher perfusion pressure would be necessary to excrete the same amount of sodium.

We were gratified to find this prediction of our hypothesis of resetting of sodium excretion threshold of the untouched kidney apparently justified. However, our collateral prediction—that the excretory behaviour of the clipped kidney, perfused after removal of the clip, with the cannula distal to the site of previous application of the clip, would be either unchanged or shifted towards the left—proved to be incorrect. On the contrary, although the slopes of the curves were slightly flatter, as might be expected by the reduced renal mass on the clipped side, the position of the curve and the sodium excretion threshold was also shifted to the right, if anything slightly more than on the untouched side.

This finding was most unexpected and was not compatible with our original hypothesis that resetting of the sodium excretion threshold might depend only upon the long-term ambient renal artery pressure. Instead it appeared much more compatible with some long-term hormonal or nervous influence acting upon both kidneys, producing either a very long-term functional or actual structural change in the kidneys. This change was still demonstrable 8 h after removing the kidneys from the animal and perfusion with blood from a normal donor. Although angiotensin would be the obvious candidate for such a hormone in a short-term experiment, its half-life is, of course, extremely short, and it would not be expected to persist for long enough. On the other hand, the prolonged effects of long-term angiotensin infusion at low rates (Dickinson & Yu, 1967) might have some parallel in our present series of experiments and suggest that angiotensin, once combined with its receptor, is capable of exerting a long-term effect entirely different in nature from its brief pressor effect, although it is more likely that the progressive response to angiotensin is due to baroreceptor resetting. Aldosterone naturally remains a possible explanation for the resetting of the sodium excretion threshold in our experiments, but although we have only carried out four observations with kidneys previously exposed to high concentrations of aldosterone for 2 days before, it appears from these experiments that the action of aldosterone over this period does not induce a change in sodium excretion performance in the recipient kidneys comparable with that we observed
in clip-hypertension. The flattening of the curves from aldosterone-pretreated animals may represent a true effect of aldosterone, but it is difficult without many more experiments to exclude this being due to slight technical imperfections in experimental technique. However, in clip-hypertension the 'theoretical threshold' for water excretion was reset almost exactly as for sodium. This seems unaccountable for by aldosterone action.

The slightly greater shift of threshold on the clipped side is certainly somewhat suggestive of a hormonal mechanism activated within the clipped kidney and affecting both itself and the contralateral one to a lesser degree. On balance we are inclined to suggest that angiotensin may be the responsible substance. Alternatively, the threshold on the untouched side might be reset by hypertension, per se, and on the clipped side by some different mechanism, e.g. a change in compliance resulting from long-term reduction of perfusion pressure. Although it seems unlikely, since the clip was removed before perfusion, the clipped kidney might have been secreting some material which was passing round the perfusion circuit to affect the opposite, untouched, kidney. It would require a large series of experiments to disprove this theory, but we think it is unlikely because the clip was removed before perfusion, and the perfusion pressure was the same for both kidneys.

Whatever the mechanism, the implication is strong that long-term and quite persistent change in sodium and water excretory behaviour may develop in the untouched kidney contralateral to a clipped one. By analogy similar changes may well be present in other forms of experimental hypertension and possibly also in essential hypertension; and resetting of this type needs to be considered as a potentially important adaptation, perhaps comparable with left ventricular and arterial medial wall hypertrophy, baroreceptor resetting and renin threshold resetting, by which hypertension can eventually be sustained despite its initial causes having diminished or disappeared.

Our inference concerning the normal importance of sympathetic nervous control of renal sodium and water excretion, and our observations on resetting of renal excretory behaviour after only a few weeks of clip-hypertension, make us doubtful whether it is reasonable to assume that this aspect of intrinsic renal function should still be accorded its present place as the most important long-term influence upon systemic arterial pressure. Infinite gain in a stabilizing mechanism can only be utilized if the components of the system are themselves stable. The perfusion pressure/sodium excretion characteristics of the isolated blood-perfused rabbit kidney are not intrinsically stable properties of the kidney.

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References


