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THE EFFECT OF INCREASING THE PLASMA MAGNESIUM CONCENTRATION ON RENIN RELEASE FROM THE DOG'S KIDNEY: INTERACTIONS WITH CALCIUM AND SODIUM

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SUMMARY

1. A denervated 'auto-transplanted' dog's kidney preparation was developed to study renin release into renal plasma and lymph. The function of the 'transplant' was compared with that of its partner. In the 'basal' state it had a similar rate of plasma and urine flow, Na, Ca, Mg and Cl excretion but a lower rate of glomerular filtration and K excretion and a lower urinary osmolality. In the 'basal' state the 'transplant' did not release renin into plasma, but invariably released it into lymph.

2. Infusions of $MgCl_2$ solutions into the renal artery which raised the renal plasma Mg concentration (P_{Mg}) by 0·1–2 m-mole.l.$^{-1}$ provoked a concentration-related increase in renin release into plasma. This was due to a rise in the veno-arterial renin difference and in the renal plasma flow rate. Blood pressure and Na excretion were unaltered.

3. In other experiments, an increase in P_{Mg} of 1·5–2·5 m-mole.l.$^{-1}$ was also found to increase renin release into lymph.

4. When the plasma Ca concentration was doubled by infusion of $CaCl_2$ into the renal artery, an increase in P_{Mg} of 1·5–2·5 m-mole.l.$^{-1}$ no longer increased renin release into plasma or lymph.

5. When the plasma NaCl concentration was raised by 8–15 m-mole.l.$^{-1}$ by infusion of hypertonic saline into the renal artery, $MgCl_2$ infusion failed to increase renin release until P_{Mg} was raised by more than 3 m-mole.l.$^{-1}$.

6. The results demonstrate that hypermagnesaemia stimulates renal renin release by a mechanism that is independent of the renal nerves, or of any changes in blood pressure or sodium excretion, but which is antagonized by concurrent hypercalcaemia or hypersalaemia. The possibility is discussed that Mg is reabsorbed from the tubular into the interstitial fluid where it antagonizes the action(s) of Ca on renin release from the juxtaglomerular cells.

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INTRODUCTION

Peart (1977) has suggested that an alteration in Ca flux across the juxtaglomerular cell membrane may be a final common pathway for many processes that control renin release. An inverse relationship between extracellular Ca concentration and renin release from the kidney has been shown. Thus infusions of $CaCl_2$ solutions into one renal artery of a dog reduces renin release from the kidney (Kotchen, Maull, Luke, Rees & Flamenbaum, 1974; Watkins, Davis, Lohmeier & Freeman, 1976). Moreover when Ca is removed from the Ringer solution perfusing a rat's isolated kidney, renin release is stimulated and is no longer inhibited by angiotensin II (Vandongen & Peart, 1974).

Extracellular Mg ions can antagonize certain of the actions of Ca on excitation–secretion or excitation–contraction coupling. Studies of these interactions at vascular smooth muscle cells led Altura & Altura (1974) to suggest that Mg competes with Ca for 'binding' sites at, or within the cell and may thereby modulate vascular tone. It has recently been shown that infusions of solutions of $MgCl_2$ into the renal arteries of dogs increased renin release (Churchill & Lyons, 1976). The present experiments were performed to establish the actions of Mg on renal resistance and renin release and to discern if they could be antagonized by increasing the Ca concentration.

Kotchen *et al.* (1974) observed that the addition of $CaCl_2$ to the diet of rats whose Na intake was restricted, reduced renin release and renal renin content. The Ca supplementation also reduced Na reabsorption. Thus they suggested that Ca might reduce renin release indirectly through raising the delivery of NaCl to the macula densa segment. An additional aim of the present experiments was to seek evidence for an interaction between Mg and Na on renin release by studying Na excretion during loading with $MgCl_2$ and observing whether any changes in renin release provoked by Mg could be antagonized by concurrent hypersalaemia.

A preliminary account of this work has been published (Wilcox, 1977).

METHODS

Preparation of the animals

Experiments were performed on adult greyhounds weighing $26{\cdot}0 \pm 4{\cdot}0$ kg (mean $\pm$ S.D.), anaesthetized with pentobarbitone sodium (30 mg kg^{-1} I.V. for induction and additional doses as required). The diet was not controlled except that food and water were withheld on the experimental day. The trachea was intubated and the dog was mechanically ventilated with a Starling pump. Ventilation was adjusted to maintain arterial blood p_{CO_2} between 32 and 38 mmHg. Blood pressure was recorded via a cannula inserted in one femoral artery and connected to a pressure transducer (Bell & Howell). A second cannula was inserted in the other femoral artery for obtaining blood samples. The right common carotid artery and jugular vein were approached by a mid line incision passing from larynx to sternum and dissected free. A pliable cannula was passed cephalad 3–5 cm up the jugular vein via a tributary. The vessels were ligated and transected 1–3 cm below the level of the larynx. Their free ends were passed through two stainless-steel tubes selected to have diameters similar to those of the vessels (Fig. 1). The ends of the artery and vein were everted over the outer aspects of the tubes and secured by ligatures. The tubes were screwed into sockets in a Perspex sheet which was later to form the front of a box constructed to house the transplanted kidney.

The abdomen was opened by a mid line incision. The right ureter was cannulated. The right kidney was not further disturbed and is referred to as 'untouched'. The left kidney was mobilized by cutting the peritoneum and supporting tissue between ligatures. Lymphatics caught in

ligatures became distended and were slit open. The renal vessels and proximal 15–20 cm of ureter were cleaned of all connective tissue and the lymphatics running on their surfaces slit open. It was important to dissect between the renal artery and vein where several lymphatics were usually found. Great care was taken to ligate all bleeding-points. The renal vessels and ureter were ligated and transected. The kidney was 'transplanted' from the abdomen to the neck. The cut ends of its vessels were pulled over the everted ends of the previously prepared neck vessels and secured by ligatures onto the stainless-steel tubes. The carotid artery was thereby anastomosed to the renal artery and the jugular vein to the renal vein whilst maintaining intima-to-intima vascular contact. The duration of renal ischaemia was 4–8 min. The free end of the ureter was ligated over a third stainless-steel tube which was also screwed into

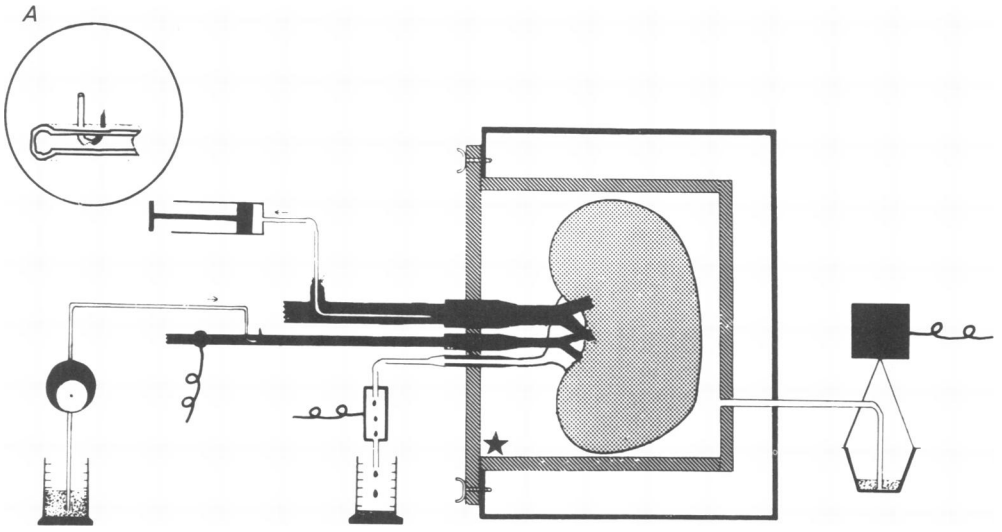

Fig. 1. Diagrammatic representation of the 'transplanted kidney' preparation. The star marks the site of the tip of the catheter used to aspirate renal lymph from the box. *A* is an enlargement of the needle inserted into the renal artery. For details see text.

a socket in the Perspex sheet. The kidney was supported on a coarse-mesh nylon net suspended from rods screwed into this sheet. It was housed in a Perspex box. The face supporting the kidney was fixed firmly onto the remainder by bolts, and the box was filled with light paraffin oil, B.P. An external chamber surrounding the top, side and rear surfaces of the box was circulated with water at 37 °C. The inner chamber containing the kidney had two outlets. One was used for filling it with paraffin oil, and was then clamped. The other was connected via a polythene tube to a beaker filled with paraffin and suspended on a level with the kidney in a sling attached to a force transducer (Dynamometer UFI). In this way, the transplanted kidney was housed within a rigid container maintained at blood temperature; any change in the volume of the contents did not cause an increase in pressure as paraffin was displaced from the box to the beaker. Lymph issuing from the cut lymphatics dropped through the nylon net and collected under the paraffin at the bottom of the box. It was aspirated every 30 min via a cannula, its volume measured and flow rate (l.f.r.) calculated. The rate of paraffin displacement recorded from the output of the force transducer provided an approximate visual record of the l.f.r. provided there was no change in renal volume. A cuffed flow transducer was placed around the carotid artery leading to the transplanted kidney. Blood flow rates were recorded by a Biotronics pulsed-logic flowmeter. Three to five centimetres distal to the flow transducer, the carotid artery was doubly transfixed by a hooked 18-gauge needle whose end was blocked and in which a new hole had been bored in the convexity. Through this needle, solutions were infused into the blood flowing to the kidney at a rate of 0·1 ml. min^{-1}.kg body $wt.^{-1}$, using a Sigmamotor peristaltic pump. A second Sigmamotor pump was used to infuse intravenously a solution composed of 67 % of 0·154 M-saline and 33 % of 1·10 M-mannitol at a rate of 0·04 ml. min^{-1} kg

body wt.$^{-1}$. To this were added the markers used to estimate glomerular fitration rate ([^{51}Cr]ethylenediaminetetra-acetic acid: EDTA) and renal plasma flow ([^{125}I] Na hippurate : Hippuran). These were obtained from Radiochemicals, Amersham, Bucks. The output of urine was registered by a dropmeter (Devices) and measured by timed collection.

Experimental protocol

Surgical preparation took 3–5 hr, during which the dogs received 500 ml. iso-oncotic (6 g. 100 ml.$^{-1}$) dextran (average molecular weight 110,000) in 0·154 M-saline (Dextraven 110, Fisons) intravenously. No observations were made for 1 hr after completion of surgery. Then followed a 'basal' period of four 15 min urine collections. During this time the transplanted kidney received 0·154 M-saline through the needle in its artery. Blood samples (15 ml.) were drawn from the femoral artery and the vein draining blood from the transplanted kidney at the beginning and end of this period. All blood samples were replaced by an equal volume of Dextraven 110. Renal lymph samples were obtained during the first and second half-hour periods.

The function of the transplanted kidney was assessed by reference to its 'untouched' pair using results from the last of the 15 min urine collection periods. The stability of the preparation was assessed by comparing values from samples obtained during the first and the last of the four 15 min urine collection periods. Following this 'basal' period four different types of experimental protocol were followed.

Series 1 (eleven experiments)

Solutions of $MgCl_2$ were made up in 0·154 M-saline to yield the following Mg concentrations: 0·013, 0·027, 0·053 and 0·13 M. Solutions of $MgCl_2$ were infused for 20 min periods through the needle in the artery supplying the transplanted kidney. In each experiment two $MgCl_2$ solutions of increasing concentrations were infused consecutively. Urine was collected for the last 15 min period of each infusion and systemic arterial and renal venous blood samples were taken in the middle of each urine collection. All blood samples were taken into cooled tubes and centrifuged at 0 °C. The plasma was separated and samples for subsequent renin assay were kept frozen at −50 °C.

Series 2 (seven experiments)

These followed the same protocol as in series 1, but the $MgCl_2$ solutions were 0·27, 0·60, 0·67, 1·0 and 1·33 M.

Series 3 (eight experiments)

Solutions of $MgCl_2$ were made up in 1·232 M-saline to yield the following Mg concentrations: 0·13, 0·27, 0·67, 1·0 and 1·33 M. These experiments followed the same protocol as those using 0·154 M-saline except that 1·232 M-saline was infused into the blood supplying the transplanted kidney for 60 min after the 'basal' period and before infusing an $MgCl_2$ solution.

Series 4 (five experiments)

A 0·133 solution of $MgCl_2$ in 0·154 M-saline was infused into the blood supplying the transplanted kidney. The solution was then changed to one with the same $MgCl_2$ concentration but with 0·25 M-$CaCl_2$ added, before returning to 0·154 M-saline alone. Each infusion was delivered for 35 min. Urine was collected during the last 15 min, blood samples were taken at the midpoint of urine collection, and renal lymph was collected over the periods 5–35 min.

Laboratory methods

Details of the analytical methods have been described previously (Nashat, Tappin & Wilcox, 1976). Na and K concentrations were measured with an EEL flame photometer; Ca and Mg concentrations were measured with a Unicam atomic absorption spectrophotometer; Cl was measured with a Corning Chloride Meter; osmolality was measured cryoscopically with an Advanced Osmometer. The pH, p_{CO_2} and p_{O_2} of arterial blood were measured with a Radiometer Blood Gas Analyser. Haematocrit was estimated as the mean of two measurements with a microhaematocrit centrifuge (Hawksley). The radioactivity of 2 ml. samples of arterial and renal venous plasma and urine was counted with a Packard Auto-Gamma counter. Renal plasma

flow (r.p.f.) was calculated from the clearance of Hippuran after correcting for its renal extraction. Values for renal blood flow rate (r.b.f.), unless otherwise specified, refer to those computed from the values of r.p.f. and the haematocrit. The electromagnetic flowmeter was used only to provide a visual record of blood flow during the experiments.

Assay for renin activity and preparation of renin substrate

The activity of renin in plasma and lymph was estimated *in vitro* by incubation of samples with excess substrate and measuring the rate of formation of angiotensin I (in p-mole.hr^{-1} $ml.^{-1}$) by radioimmunoassay (Boyd, Adamson, Fitz & Peart, 1969). Samples were thawed and then kept at 0 °C; a 0·2 ml. volume was added to 1·8 ml. renin substrate (see below). To this was added 0·2 ml. 0·3 M-EDTA (to inhibit the angiotensin-converting enzyme), 0·1 ml. 0·2 M-BAL and 0·02 ml. 0·3 M-8-hydroxyquinoline sulphate (to inhibit angiotensinases). The pH was then brought to 6·5 with 1 M-HCl (approximately 0·1 ml.). Two 1 ml. aliquots were taken into separate tubes. One was placed in boiling water for 90 sec to coagulate the protein and inactivate the renin. This sample, designated 'preincubation', was assayed for angiotensin I present in the sample before incubation. The other was incubated at 37 °C for 2 hr before protein coagulation ('post-incubation' sample). The tubes were centrifuged at 3000 rev/min for 10 min and a clear supernatant obtained for assay of angiotensin I. The angiotensin I formed in 2 hr was obtained by subtraction of the 'pre-incubation' from the 'post-incubation' value, and making allowance for renin activity present in added substrate. The interassay coefficient of variation of duplicate samples was 5 % (fifty samples).

The rate of renin release into plasma was calculated as the product of the r.p.f. and the renal venous less arterial plasma renin activity; the release into lymph was calculated as the product of l.f.r. and renal lymph less arterial plasma renin activity.

The addition of $MgCl_2$ to dog's plasma to raise plasma Mg concentration to 20 m-mole.$l.^{-1}$ or of $CaCl_2$ to raise plasma Ca concentration to 5 m-mole.$l.^{-1}$ did not affect the plasma renin activity or the recovery of added angiotensin I.

For preparation of renin substrate, a donor greyhound was given a diet supplemented daily with 10 g salt. On the 2 days before bleeding, it was given 120 mg propranolol (I.C.I., Ltd), added in two divided doses as a powder to the food. This ensured that the renin content of the substrate was very low (2–5 %) compared to that of the plasma from the experimental dogs. Under light pentobarbitone sodium anaesthesia, 350 ml. blood was withdrawn into 50 ml. tubes, each containing 0·2 ml. 0·3 M-EDTA. The plasma was separated by centrifugation, and renin substrate prepared by the method of Skinner (1967). The same dog was bled at 6–8 week intervals.

The plasma Mg concentration (P_{Mg}) of blood perfusing the 'transplanted' kidney was calculated from the following formula:

$$P_{Mg} = \frac{(\text{r.p.f.} \times V_{Mg}) + (U_{Mg}V)}{\text{r.p.f.}},$$

where r.p.f. = renal plasma flow; V_{Mg} = renal venous plasma Mg concentration and $U_{Mg}V$ = renal Mg excretion. Plasma Na and Ca concentrations were calculated similarly. All values for plasma concentrations of ions refer to those in blood perfusing the transplanted kidney.

RESULTS

'Basal' period

In thirty-six experiments, the function of the 'transpanted' kidney was contrasted with that of its 'untouched' partner at the end of the 'basal' period (Table 1). The two kidneys were quite comparable except that the 'transplant' had a lower g.f.r. (average 11 %) and a rather less consistent reduction in its urinary osmolality and K excretion. During the basal period, the renin release and the function of the 'transplanted' kidneys were studied over a 45 min interval (Table 2). There was a small (20 %) increase in mean rates of urine flow and Na and K excretion but the other variables did not change significantly. In particular, all the parameters used to

calculate renin release into blood or lymph remained stable. The renin activity of renal venous plasma did not differ significantly from that of arterial plasma, indicating that there was no net release of renin into the blood by the 'transplanted' kidney. In contrast, lymph renin activity was invariably greater than that of arterial plasma ($P < 0{\cdot}0005$).

TABLE 1. Comparison of basal function of 'transplanted' and 'untouched' kidneys

	Mean (±S.D.) value for 'transplanted' kidney	Mean (±S.D.) value for 'untouched' kidney	n	P
R.p.f. (ml. min^{-1})	160 ± 52	167 ± 69	36	N.S.
G.f.r. (ml. min^{-1})	37·4 ± 10·6	41·7 ± 13·4	36	< 0·0005
F.f. (%)	23·4 ± 6·4	25·0 ± 6·3	36	< 0·01
Vol. (ml. min^{-1})	0·97 ± 0·61	1·00 ± 0·72	36	N.S.
U_{osm} (m-osmole kg^{-1})	738 ± 360	800 ± 344	34	< 0·05
$U_{Na}V$ (μmole min^{-1})	40 ± 39	36 ± 32	35	N.S.
R/F_{Na}(%)	99·3 ± 0·8	99·3 ± 0·6	35	N.S.
U_kV (μmole min^{-1})	43 ± 20	49 ± 25	35	< 0·05
$U_{Mg}V$ (μmole min^{-1})	0·32 ± 0·30	0·40 ± 0·57	33	N.S.
$U_{Ca}V$ (μmole min^{-1})	0·21 ± 0·17	0·21 ± 0·14	32	N.S.
$U_{Cl}V$ (μmole min^{-1})	20 ± 25	16 ± 20	33	N.S.

Mean values (±S.D.) are shown for the 'transplanted' kidney for comparison with those obtained simultaneously for its 'untouched' pair at the end of the 'basal' period. Results shown are renal plasma flow, glomerular filtration rate, filtration fraction, urine flow rate, urine osmolality, Na excretion, fractional Na reabsorption, K excretion, Mg excretion, Ca excretion and Cl excretion; n = number of experiments; P = result of paired t test comparing pairs of 'transplanted' and 'untouched' kidneys; N.S. = not statistically significant ($P > 0{\cdot}05$)

Responses to Mg infusions

Series 1 (*see Table 3*). Infusions of lower concentrations of $MgCl_2$ solutions in isotonic saline raised P_{Mg} at the kidney by 0·1–2·1 m-mole.l.$^{-1}$. They led to some increase in arterial plasma renin activity (p.r.a.). There was, however, a much greater rise in renal venous p.r.a. and the renal V-A renin difference increased significantly ($P < 0{\cdot}0005$). There was also a rise in r.p.f. Consequently, renin release into plasma increased substantially. There were no changes in B.P., g.f.r., Na excretion or fractional Na reabsorption.

The increase in renin release was dependent upon the change induced in P_{Mg} ($r = 0{\cdot}48$; $n = 21$; $P < 0{\cdot}05$; Fig. 2). This was due to a steep rise in renal V-A renin difference with P_{Mg}. Although the infusions increased renal blood flow rate, the greatest changes in flow occurred at the lower rates of Mg infusions. Thus no relationship was found between changes in renin release and renal blood flow rate ($r = -0{\cdot}19$; $n = 21$; N.S.).

Series 2 (*see Table 3*). Infusions of solutions of high concentrations of $MgCl_2$ in isotonic saline increased P_{Mg} at the kidney to 7·17 ± 3·60 m-mole.l.$^{-1}$. There was a large increase in renal venous p.r.a. However, in this series there was also a large rise in arterial p.r.a., so that renal V-A renin difference did not differ significantly from that in series 1 ($t = 1{\cdot}34$; $n = 35$; N.S.). It is apparent from Fig. 2 that maximal

increments in renal *V-A* renin differences or in renin release into plasma were achieved by increments in P_{Mg} of about 0·5–2 m-mole.l.$^{-1}$.

These infusions reduced B.P. considerably. Renal plasma flow and g.f.r. also fell. An unchanged rate of Na excretion despite a large fall in the filtered load of Na indicated that the levels of both absolute and fractional Na reabsorption had declined.

TABLE 2. Stability of the function of 'transplanted' kidneys during basal period

	Starting value (mean ± S.D.)	Value 45 min later (mean ± S.D.)	*n*	*P*
B.P. (mmHg)	160 ± 24	161 ± 21	34	N.S.
A renin (p-mole.hr^{-1}.ml.$^{-1}$)	9·3 ± 6·4	9·5 ± 6·2	22	N.S.
V renin (p-mole.hr^{-1}.ml.$^{-1}$)	9·6 ± 7·2	9·8 ± 6·9	22	N.S.
V–A renin (p-mole.hr^{-1}.ml.$^{-1}$)	0·3 ± 2·1	0·3 ± 1·7	22	N.S.
R.p.f. (ml. min^{-1})	158 ± 56	156 ± 54	36	N.S.
R.r. plasma (p-mole.hr^{-1}.ml.$^{-1}$ ml.$^{-1}$ min^{-1})	101 ± 427	82 ± 362	22	N.S.
L renin (p-mole.hr^{-1}.ml.$^{-1}$)	17·7 ± 10·8	16·7 ± 9·3	17	N.S.
L–A renin (p-mole.hr^{-1}.ml.$^{-1}$)	7·2 ± 5·8	6·3 ± 5·1	17	N.S.
L.f.r. (ml. min^{-1})	0·116 ± 0·039	0·124 ± 0·036	17	N.S.
R.r. lymph (p-mole.hr^{-1}.ml.$^{-1}$ ml.$^{-1}$ min^{-1})	0·837 ± 0·723	0·867 ± 0·786	17	N.S.
G.f.r. (ml. min^{-1})	36·5 ± 10·5	37·6 ± 10·5	35	N.S.
F.f. (%)	23·2 ± 6·8	24·1 ± 6·7	35	N.S.
Vol. (ml. min^{-1})	0·81 ± 0·44	0·96 ± 0·60	37	N.S.
U_{osm} (m-osmole kg^{-1})	765 ± 342	741 ± 356	35	N.S.
$U_{Na}V$ (μmole min^{-1})	34 ± 31	40 ± 38	36	< 0·05
R/F_{Na} (%)	99·3 ± 0·7	99·2 ± 0·9	36	< 0·05
U_kV (μmole min^{-1})	38 ± 19	43 ± 21	34	< 0·0005
$U_{Mg}V$ (μmole min^{-1})	0·36 ± 0·37	0·32 ± 0·33	32	N.S.
$U_{Ca}V$ (μmole min^{-1})	0·21 ± 0·14	0·22 ± 0·18	31	N.S.
$U_{Cl}V$ (μmole min^{-1})	20 ± 25	21 ± 26	32	N.S.

Values obtained for the 'transplanted' kidney at the beginning of the basal hour are contrasted with those obtained 45 min later. During this period 0·154 M-saline was infused into the blood supply of this kidney. Values shown are mean blood pressure, arterial renin activity, renal venous renin activity, renal veno-arterial differences for renin activity, renal plasma flow, renin release into renal plasma, renal lymph renin activity, renal lymph-arterial difference for renin activity, renal lymph flow rate, renin release into renal lymph, glomerular filtration rate, filtration fraction, urine flow rate, urine osmolality, Na excretion, fractional Na reabsorption, K, Mg, Ca and Cl excretion; *n* = number of experiments; *P* = results of paired *t* test; N.S. = not statistically significant ($P > 0·05$).

Series 3. Infusions of solutions of high concentrations of $MgCl_2$ in hypertonic saline increased P_{Na} (mean values ± S.D.; m-mole.l.$^{-1}$) from 144 ± 3 to 155 ± 2 in systemic arterial plasma and to 171 ± 2 in plasma perfusing the experimental kidney. The P_{Mg} of plasma perfusing the experimental kidney increased to 6·5 ± 3·4 which was not significantly different from that of series 2 (unpaired $t = 1·0$; N.S.). There was a significant increase in renal *V-A* difference for p.r.a. (from 0·2 ± 2·0 p-mole.hr^{-1}.ml.$^{-1}$ to 2·4 ± 3·3; paired t = 3·8; $n = 18$; $P < 0·025$) and in renin release into plasma (from 5 ± 224 p-mole.hr^{-1}.ml.$^{-1}$ min^{-1} to 245 ± 423; paired $t = 2·96$; $P < 0·005$). Nevertheless, when compared with values from series 2 obtained during

TABLE 3. Responses to infusions of $MgCl_2$ in 0·154 M-saline at lower (series 1) and higher (series 2) concentrations

		Series 1			Series 2		
	Before (mean ± S.D.)	During $MgCl_2$ infusion (mean ± S.D.)	n	P	During $MgCl_2$ infusion (mean ± S.D.)	n	P
P_{Mg} (m-mole.l.$^{-1}$)	0·65 ± 0·14	1·43 ± 0·75	21	< 0·0005	7·82 ± 3·98	14	< 0·0005
L_{Mg} (m-mole.l.$^{-1}$)	0·86 ± 0·06	1·64 ± 0·96	6	< 0·05	—	—	—
B.P. (mmHg)	153 ± 17	151 ± 17	21	N.S.	123 ± 31	14	< 0·005
A renin (p-mole.hr^{-1}.ml.$^{-1}$)	6·2 ± 5·2	7·9 ± 5·6	21	< 0·05	17·6 ± 8·2	14	< 0·0005
V renin (p-mole.hr^{-1}.ml.$^{-1}$)	6·9 ± 5·7	12·8 ± 10·0	21	< 0·005	24·5 ± 12·5	14	< 0·0005
V–A renin (p-mole.hr^{-1}.ml.$^{-1}$)	0·7 ± 1·2	4·9 ± 5·4	21	< 0·0005	6·9 ± 3·3	14	< 0·0005
R.p.f. (ml. min^{-1})	172 ± 30	197 ± 52	21	< 0·01	137 ± 61	14	< 0·05
R.r. plasma (p-mole.hr^{-1}.ml.$^{-1}$ ml.$^{-1}$ min^{-1})	117 ± 194	955 ± 994	21	< 0·0005	943 ± 810	14	< 0·0005
G.f.r. (ml. min^{-1})	47 ± 5	46 ± 11	21	N.S.	28 ± 12	14	< 0·0005
F.f. (%)	27 ± 5	23 ± 4	21	< 0·0005	20 ± 6	14	< 0·0005
$U_{Na}V$ (μmole min^{-1})	47 ± 28	47 ± 41	21	N.S.	57 ± 78	14	N.S.
R/F_{Na}	99·3 ± 0·4	99·3 ± 0·7	21	N.S.	98·8 ± 1·1	14	< 0·05

Mean values (± S.D.) are shown for plasma and lymph Mg concentration at the transplanted kidney, mean blood pressure, arterial plasma renin activity, renal venous plasma renin activity, renal veno-arterial difference for plasma renin activity, renal plasma flow, renin release into plasma, glomerular filtration rate, filtration fraction, Na excretion and fractional Na reabsorption. Results refer to the experimental kidney. n = number of observations, P = results of paired t test comparing values before, with those during infusion of $MgCl_2$ solution at lower (series 1) or higher (series 2) concentrations; N.S. = not statistically significant ($P > 0·05$).

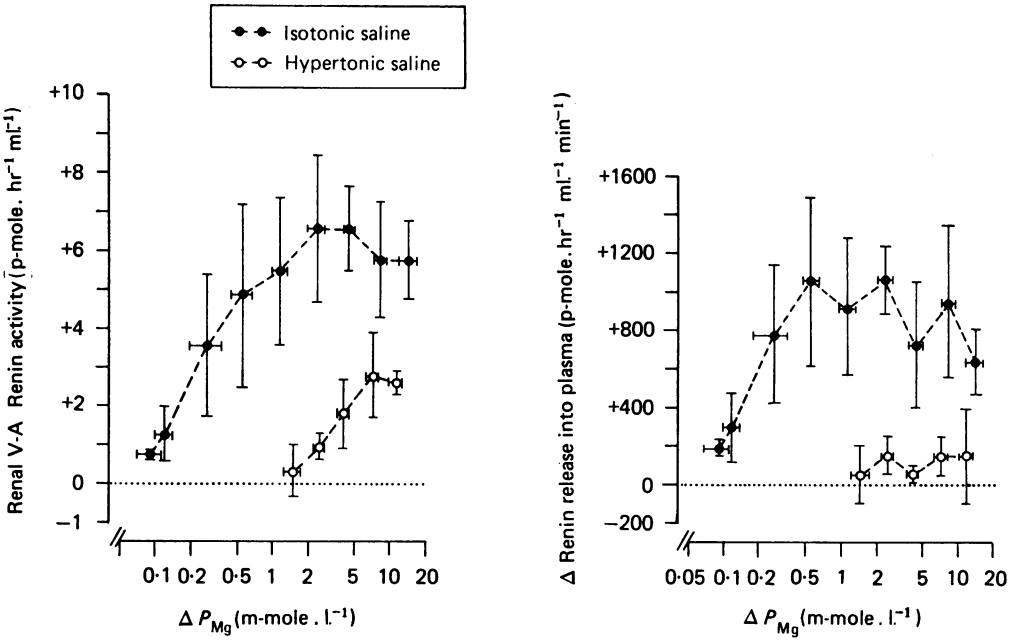

Fig. 2. Mean values (± S.E. of mean) for the changes in renal veno-arterial difference for renin activity (first panel) and in renin release into plasma (second panel) are displayed as a function of the increase in plasma Mg concentration (log scale) at the transplanted kidney. Filled circles refer to series 1 and 2 in which $MgCl_2$ solutions were infused in 0·154 M-saline and open circles to series 3 in which they were infused in 1·232 M-saline.

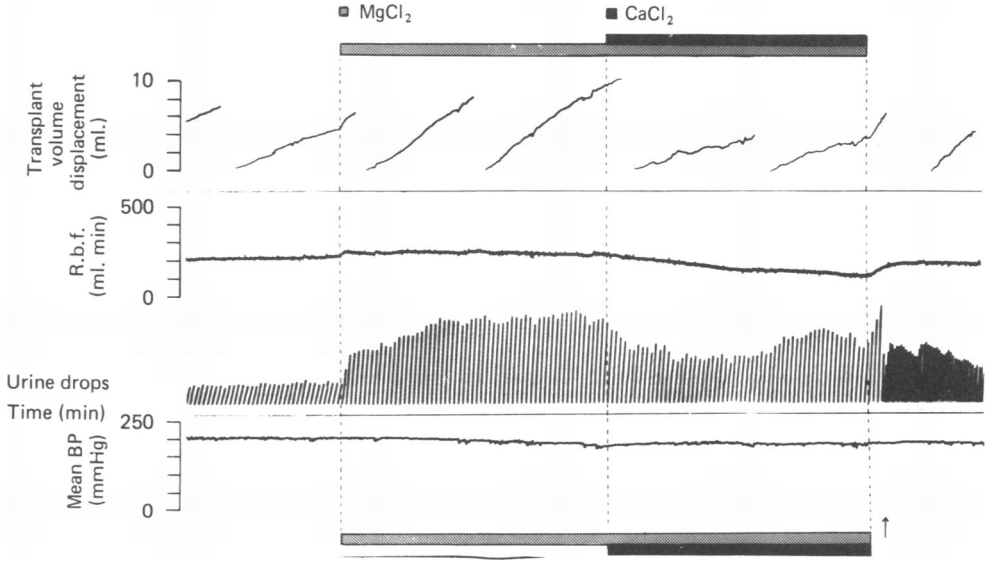

Fig. 3. Illustration of a record of one experiment from series 4. Saline (0·154 M) was infused into the renal artery throughout. During the period marked with cross-hatched bars, 0·133 M-$MgCl_2$ solution was added, and during that marked with filled bars, 0·25 M-$CaCl_2$ was also added. Records show the volume of paraffin displaced from the box housing the kidney, the mean renal blood flow, the mean blood pressure and the urine drop rate (the excursion of the drop recorder was reduced by half at the arrow).

isotonic saline infusions (Fig. 2), both the renal *V-A* renin difference and renin release into plasma were clearly reduced by the hypertonic saline at each level of P_{Mg}.

Series 4. The records of an experiment are shown in Fig. 3. In five experiments, infusions of 0·133 M-$MgCl_2$ solutions in isotonic saline increased the mean P_{Mg} at the experimental kidney to $2{\cdot}0 \pm 0{\cdot}5$ m-mole.l.$^{-1}$. As in series 1 there was again a significant increase in renin release into plasma due to rises in both renal *V-A* renin difference and in r.p.f. (Fig. 4). The additions of 0·25 M-$CaCl_2$ solutions increased the

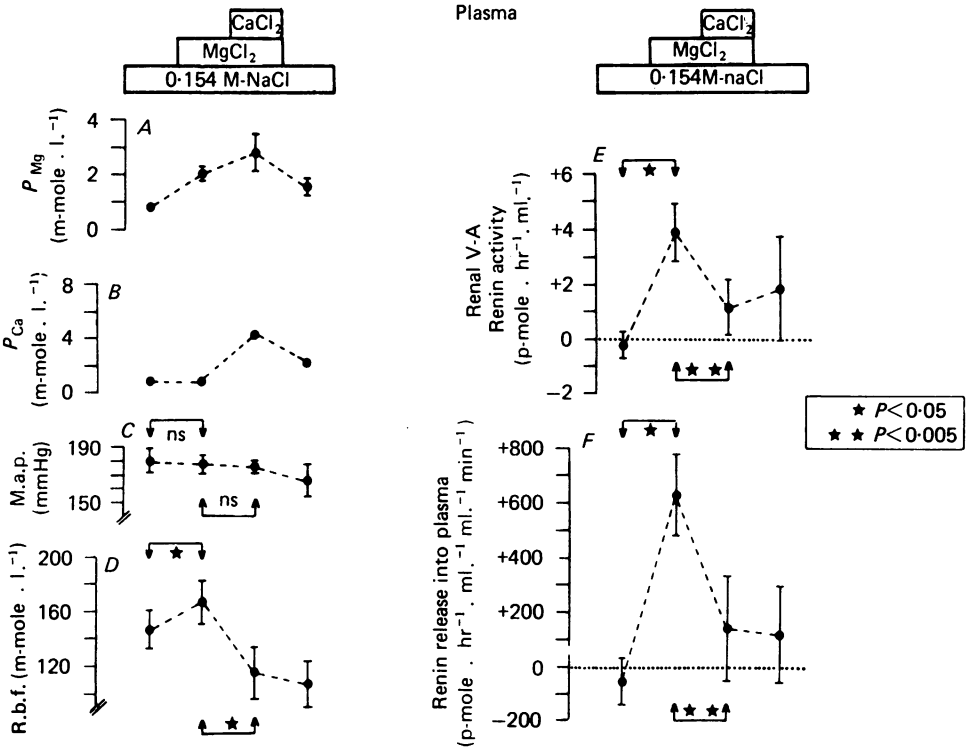

Fig. 4. Mean values (±S.E. of mean) for results from series 4. In each panel, the first value is recorded during infusions of 0·154 M-saline alone, the second during addition of 0·133 M-$MgCl_2$, the third during addition of 0·25 M-$CaCl_2$ and the fourth on return to saline alone. Thirty-five minutes separate each observation. *A*, plasma Mg concentration; *B*, plasma Ca concentration; *C*, mean arterial blood pressure; *D*, renal plasma flow; *E*, renal veno-arterial difference for renin activity; *F*, renin release into plasma. Arrowed brackets indicate values compared by paired *t* test. One star, $P < 0{\cdot}05$; two stars, $P < 0{\cdot}005$.

P_{Ca} at the kidney (mean ± S.D.) from $1{\cdot}8 \pm 0{\cdot}2$ m-mole.l.$^{-1}$ to $4{\cdot}3 \pm 0{\cdot}7$. This caused a reduction in r.p.f. and in renal *V-A* renin difference and reduced renin release into plasma to a value not significantly different from zero. There were no consistent changes in B.P.

The values relating to the renal lymph appear in Fig. 5. The magnesium and Ca concentrations of lymph followed those of plasma. The $MgCl_2$ infusions increased l.f.r. Lymph renin activity increased more than that of arterial plasma. Renin

release into lymph increased by an average of 940. Addition of $CaCl_2$ to the $MgCl_2$ infusions reduced l.f.r.; the *L-A* renin difference declined to a value not significantly different from zero ($t = 0{\cdot}8$; $n = 5$; N.S.).

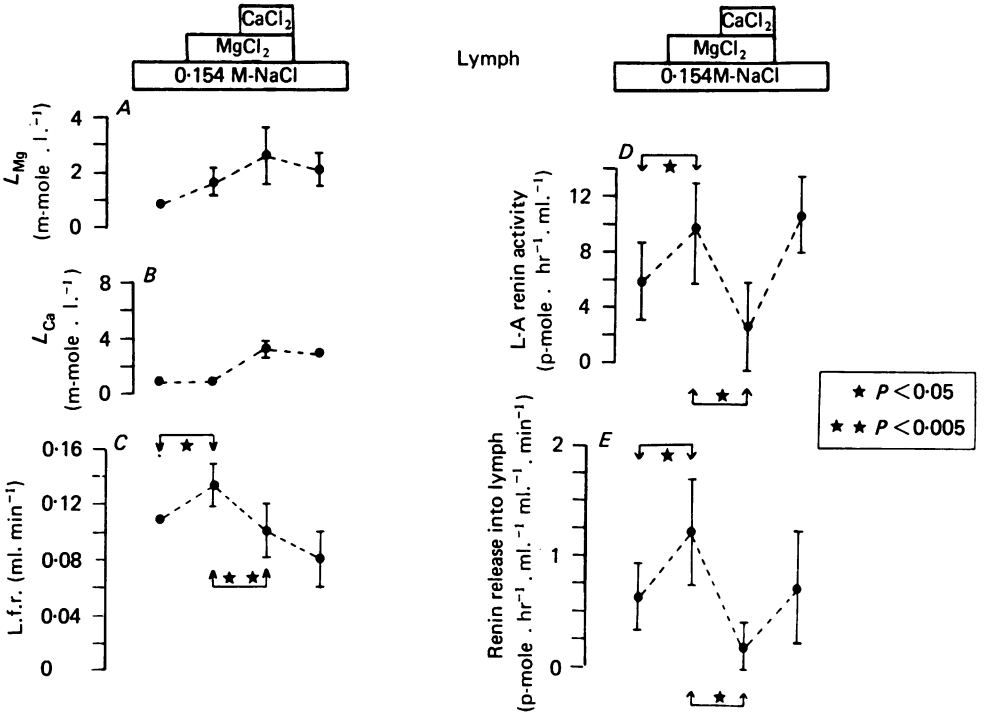

Fig. 5. Mean values (±S.E. of mean) for results from series 4. For explanations, see legend to Fig. 4. Panel A, lymph magnesium concentration; panel B, lymph calcium concentration; panel C, lymph flow rate; panel D, lymph-minus-arterial plasma renin activity; panel E, renin release into lymph.

DISCUSSION

The experimental method was adapted from that described by Reinhardt, Klose, Ellinghaus, Brechtelsbauer & Behrenbeck (1967) and Lichardus & Nizet (1972). The former authors transplanted a kidney across the neck vessels of another dog and studied its function after the animal had recovered from anaesthesia. At 5 hr or more after operation, the g.f.r. and maximum urinary osmolality of the transplant did not differ from the animal's own kidneys *in situ*. They concluded that the neck kidney was highly suitable for experimental work. In the present experiments, which were performed under continuous anaesthesia, only minor differences between the functions of the animal's 'transplanted' and 'untouched' kidneys were apparent at 1½–2 hr after operation. The exception was a lower g.f.r. of the transplant, averaging 11 % below that of its pair. All the parameters used in the calculations of renin release into blood and into lymph were stable over a 45 min observation period. There was no net release of renin into blood in the 'basal' state, perhaps as a consequence of renal denervation (Brubacher & Vander, 1968). These observations

demonstrate the suitability of the preparation for studying renin release. It has the advantage that, by allowing collection of the total output of renal lymph, a comparison can be made between renin release into blood and into lymph.

The results of this study confirm the work of Churchill & Lyons (1976) who showed that infusion of $MgCl_2$ solution into the renal artery of dogs led to a substantial increase in renin release. Recently, Fray (1977) has reported that addition of $MgCl_2$ to saline perfusing a rat's kidney *in vitro* stimulated renin release. These responses must be related primarily to the increases in Mg rather than to the increases in Cl concentrations, since the present experiments showed that increases in Cl concentration produced by $CaCl_2$ or NaCl were strongly inhibitory. There was a direct relationship between renin release and the increment in plasma Mg concentration up to 2 m-mole.$l.^{-1}$. Within this range of P_{Mg} the infusions produced no overt evidence of a systemic disturbance, and did not alter blood pressure.

As originally described by Lever & Peart (1962), the renin activity in renal lymph measured at the beginning of the experiments was found to be substantially greater than that measured simultaneously in plasma. Morgan & Gillies (1977), by use of micropuncture techniques, have found that renin released by the cat's kidney enters the blood stream distal to the efferent arteriole. They suggested that renin is secreted into the interstitial fluid and enters the circulation through the capillaries around the macula densa segment. This would explain why lymph, which drains directly from the interstitial space, has such a high renin activity. The outputs of renin into blood and lymph were both increased by $MgCl_2$ and both reduced by $CaCl_2$. The changes in renin release into blood cannot therefore be ascribed to altered capillary uptake. They must represent either altered secretion or altered interstitial degradation of renin.

Three main pathways have been proposed for renin secretion (Vander, 1967). One involves the stimulation of adrenoceptors, mainly of the beta-type, by noradrenaline released from renal sympathetic nerves or circulating adrenaline (Coote, Johns, MacLeod & Singer, 1972). In the present experiments the renal nerves were severed. Moreover, Mg normally inhibits catecholamine release from nerve or adrenal gland (Rubin, 1970). Thus, beta receptor stimulation is unlikely to have occurred during the Mg infusions.

A second pathway for renin release is held to be a fall in the load of NaCl at the macula densa segment of the tubule (Vander & Miller, 1964). However, direct evidence for the validity of this theory, or for the precise nature of any signal for renin release has not been forthcoming. $MgCl_2$ reduced glomerular filtration rate and fractional Na reabsorption only at the higher Mg concentrations (series 2), yet renin release was clearly increased at all concentrations. Mg can thus increase renin release without change in the over-all tubular load or handling of NaCl, although the possibility that it alters these factors locally at the macula densa segment cannot be excluded.

A third pathway for renin release is held to be a reduction in stretch (Tobian, 1967) or a dilatation (Eide, Loyning & Kiil, 1973) of the afferent arteriole. At the lower rates of $MgCl_2$ infusion, B.P. was unchanged; although there was a renal vasodilatation, renin release was not found to correlate with the changes in renal blood flow. During more prolonged infusions of $MgCl_2$, Churchill & Lyons (1976) found that

r.p.f. returns to previous levels, yet renin release persists. Moreover, Fray (1977) found that renin release from an isolated rat's kidney increased with perfusate Mg concentration without a change in perfusion pressure or flow rate. Thus a change in stretch or diameter of the afferent arteriole does not appear to be a prerequisite for Mg-induced renin release, although the hypotension characteristic of high rates of Mg infusion (series 2) may have contributed to the response at the highest levels of P_{Mg}.

The failure of existing theories to explain the action of Mg suggests that it may affect renin release by a direct action on the juxtaglomerular cells. Vandongen & Peart (1974) have suggested that renin release may be modulated by the concentration or flux of free Ca at juxtaglomerular cells. Using an isolated rat kidney preparation, they found that when Ca was removed from the perfusate, basal renin release was augmented and became unresponsive to the inhibitory actions of angiotensin II. La, which blocks trans-membrane movements of Ca, reduced basal renin release and blocked the stimulation of renin release by isoprenaline and glucogen (Logan, Tenyi, Peart, Breathnach & Martin, 1977). Perfusion with a solution containing an ionophore capable of translocating Ca across cell membrane reduced renin release (Fynn, Onomakpome & Peart, 1977). In contrast, however, Lestor & Rubin (1977), using an isolated cat's kidney preparation found that 'low Ca' perfusates did not significantly augment basal renin release. The infusion of Ca salts into the renal arteries of dogs has generally been reported to decrease renin release (Kotchen *et al.* 1974; Watkins *et al.* 1976; Kotchen, Galla & Luke, 1977; in one divergent report by Iwoa, Abe & Yamamoto (1974), intra-renal arterial infusion of $CaCl_2$ was found to increase renin release, but these experiments were unusual also in showing an increase in renal blood flow during hypercalcaemia. The close relationship observed between renal resistance and renin release (Eide *et al.* 1973) has led to the theory that common, Ca dependent mechanisms operate at renal vascular smooth muscle and juxtaglomerular cells (Vandongen & Peart, 1974). The tone of vascular smooth muscle is increased by Ca and decreased by Mg. From a study of the interactions between these two ions, Altura & Altura (1974) concluded that certain Mg 'binding' sites on vascular smooth muscle cells were completely exchangeable with extracellular Ca. Mg was believed to be very important in regulating the permeability, translocation and/or binding of Ca ions. By analogy, the opposite action of Mg and Ca on renin release could be understood if release were regulated by Ca flux at the cell membrane and this flux was antagonized by extracellular Mg. However, there is evidence that Mg can displace Ca from within smooth muscle cells (Altura & Altura, 1971). The present experiments do not decide whether Mg affects renin release by an action at or within the cell membrane.

In experiments with anaesthetized dogs, Shade, Davis, Johnson & Witty (1972) found that intrarenal-arterial infusions of hypertonic saline led to an abrupt reduction in renin release from intact kidneys but not from 'non-filtering' kidneys. They suggested that the responses of the intact kidneys were mediated by a change induced by the hypertonic saline at the macula densa. At this nephron segment renin-containing cells are in close apposition to tubule cells. Consequently, ions reabsorbed into the interstitial fluid might influence local renin release. Hypersalaemia has been shown greatly to curtail renal Mg reabsorption (Warren & Wilcox, 1977). The present

observations of inhibition of Mg-induced renin release that occurred during hypersalaemia might be understood as inhibition of Mg reabsorption from the tubular lumen into the interstitial fluid adjacent to the juxtaglomerular cells with a consequent reduction in the stimulus to renin release.

The stimulant actions of magnesium infusions on renin release were evident at a concentration sufficient to raise P_{Mg} by only 0·1 m-mole.l.$^{-1}$. This raises the possibility that variation in magnesium concentration at some intrarenal site may modulate renin release in certain physiological conditions. The reabsorption of magnesium is closely linked to that of Na during both acute and chronic changes in salt intake (Walser, 1973). These are circumstances that regularly induce changes in renin release. It is conceivable that the level of salt intake might regulate renin release by controlling the traffic of Mg ions through the interstitial fluid adjacent to the juxtaglomerular cells.

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REFERENCES

ALTURA, B. M. & ALTURA, B. T. (1971). Influence of magnesium on drug-induced contractions and ion content in rabbit aorta. *Am. J. Physiol.* **219**, 1698–1703.

ALTURA, B. M. & ALTURA, B. T. (1974). Magnesium and contraction of arterial smooth muscle. *Microvasc. Res.* **7**, 145–155.

BOYD, G. W., ADAMSON, A. R., FITZ, A. E. & PEART, W. S. (1969). Radioimmunoassay determination of plasma renin activity. *Lancet i*, 213–218.

BRUBACHER, E. S. & VANDER, A. J. (1968). Sodium deprivation and renin secretion in unanesthetized dogs. *Am. J. Physiol.* **214**, 15–21.

CHURCHILL, P. C. & LYONS, H. J. (1976). Effect of intrarenal arterial infusion of magnesium on renin release in dogs. *Proceedings of the Society for Experimental Biol. Med.* **152**, 6–10.

COOTE, J. H., JOHNS, E. J., MACLEOD, V. H. & SINGER, B. (1972). Effect of renal nerve stimulation, renal blood flow and adrenergic blockade on plasma renin activity in the cat. *J. Physiol.* **226**, 15–36.

EIDE, I., LOYNING, E. & KIIL, F. (1973). Evidence for haemodynamic autoregulation of renin release. *Circulation Res.* **32**, 237–245.

FRAY, J. S. (1977). Stimulation of renin release in perfused kidney by low calcium and high magnesium. *Am. J. Physiol.* **232**, F377–382.

FYNN, M., ONOMAKPOME, N. & PEART, W. S. (1977). The effects of ionophores (A23187 and RO2-2985) on renin secretion and renal vasoconstriction. *Proc. R. Soc.* B. **199**, 199–212.

IWAO, H., ABE, Y. & YAMAMOTO, K. (1974). Effect of intrarenal arterial infusion of calcium on renin release in dogs. *Jap. J. Pharmac.* **24**, 482–484.

KOTCHEN, T. A., GALLA, J. H. & LUKE, R. G. (1977). Effects of calcium on renin and aldosterone in the rat. *Am. J. Physiol.* **232**, E388–393.

KOTCHEN, T. A., MAULL, K. I., LUKE, R., REES, D. & FLAMENBAUM, W. (1974). Effect of acute and chronic calcium administration on plasma renin. *J. clin. Invest.* **54**, 1279–1286.

LESTER, G. E. & RUBIN, R. P. (1977). The role of calcium in renin secretion from the isolated perfused cat kidney. *J. Physiol.* **269**, 93–108.

LEVER, A. F. & PEART, W. S. (1962). Renin and angiotensin-like activity in renal lymph. *J. Physiol.* **160**, 548–563.

LICHARDUS, B. & NIZET, A. (1972). Water and sodium excretion after blood volume expansion under conditions of constant arterial, venous and plasma oncotic pressures and constant haematocrit. *Clin. Sci.* **42**, 701–709.

LOGAN, A. G., TENYI, I., PEART, W. S., BREATHNACH, A. S. & MARTIN, B. G. H. (1977). The effect of lanthanum on renin secretion and renal vasoconstriction. *Proc. R. Soc.* B. **195**, 327–342.

MORGAN, I. & GILLIES, A. (1977). Factors controlling the release of renin; a micropuncture study in the cat. *Pflügers Arch.* **368**, 13–18.

NASHAT, F. S., TAPPIN, J. W. & WILCOX, C. S. (1976). Plasma sodium concentration and sodium excretion in the anaesthetised dog. *J. Physiol.* **254**, 183–202.

PEART, W. S. (1977). The kidney as an endocrine organ. *Lancet ii*, 543–548.

REINHARDT, H. W., KLOSE, H. J., ELLINGHAUS, K., BRECHTELSBAUER, H. & BEHRENBECK, D. W. (1967). Ein Nierenpräparat (sog. Halsniere) zur Erleichterung Komplizierter Funktionsuntersuchungen. Homotransplant am narkotisierten Hund. *Pflügers Arch.* **297**, 71–88.

RUBIN, R. P. (1970). The role of calcium in the release of neurotransmitter substances and hormones. *Pharmac. Rev.* **22**, 389–428.

SHADE, R. E., DAVIS, J. O., JOHNSON, J. A. & WITTY, R. T. (1972). Effects of renal arterial infusions of sodium and potassium on renin secretion in the dog. *Circulation Res.* **31**, 719–727.

SKINNER, S. L. (1967). Improved assay methods for renin 'concentration' and 'activity' in human plasma. *Circulation Res.* **20**, 391–402.

TOBIAN, L. (1967). Renin release and its role in renal function and the control of salt balance and arterial pressure. *Fedn Proc.* **26**, 48–54.

VANDER, A. J. (1967). Control of renin release. *Physiol. Rev.* **47**, 359–382.

VANDER, A. J. & MILLER, R. (1964). Control of renin secretion in the dog. *Am. J. Physiol.* **207**, 537–545.

VANDONGEN, R. & PEART, W. S. (1974). Calcium-dependence of the inhibitory effect of angiotensin on renin secretion in the isolated perfused kidney of the rat. *Br. J. Pharmac.* **50**, 125–129.

WALSER, M. (1973). Divalent cations; physicochemical state in glomerular filtrate and urine and renal excretion. In *Handbook of Physiology*, section 8, ed. ORLOFF, J. & BERLINER, R. W., 2 pp. 555–586. Washington, D.C.: Am. Physiol. Soc.

WARREN, R. L. & WILCOX, C. S. (1977). The excretion of magnesium and calcium by the dog kidney during an acute increase in the plasma sodium chloride concentration. *J. Physiol.* **268**, 12–13*P*.

WATKINS, B. E., DAVIS, J. O., LOHMEIER, T. E. & FREEMAN, R. H. (1976). Intrarenal site of action of calcium on renin secretion in dogs. *Circulation Res.* **39**, 847–853.

WILCOX, C. S. (1977). Renin release from the dog's kidney during hypermagnesaemia. *J. Physiol.* **275**, 25–26*P*.