THE ACTION OF ANGIOTENSIN II ON THE RAT KIDNEY

by

J.P.M. FINBERG

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The Medical Unit,
St. Mary's Hospital Medical School,
London W.2.

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ABSTRACT

Flow dynamics in superficial tubules of the rat kidney have been investigated during diuresis induced by intravenous infusion of angiotensin II (0.5 μg/kg/min). During the diuretic phase, inulin clearance was reduced, proximal and distal lissamine green transit times were markedly prolonged, and dye appeared as a very concentrated bolus in distal tubules. Flow rate in superficial tubules may therefore be reduced during angiotensin diuresis, indicating that the diuresis may take place through deeper nephrons. In some angiotensin infused rats, distal tubular calibre was found to increase far more than the level observed at the same degree of diuresis induced by osmotic diuretics, noradrenaline and chlorothiazide, and in these cases, proximal tubular calibre was sometimes also markedly increased, indicating that a degree of internal hydronephrosis may have developed. In experiments without marked distal tubular distension, proximal tubular calibre was generally reduced by angiotensin.

An *in vitro* preparation of the circular smooth muscle surrounding the tip of the papilla of the rat kidney has been developed, which showed a marked increase in tone on administration of angiotensin. The anatomical situation of this muscular tissue showed that by constriction it could cause an increased resistance to flow in the collecting ducts, and hence a degree of outflow obstruction.

The kidney showed a dose-dependent contraction on

injection of angiotensin, but the size of the whole kidney during continuous infusion of angiotensin could be reduced or increased, depending on the extent of diuresis, the degree of vasoconstriction and the presence of internal hydronephrosis.

Blood distribution studies using intravenous injection of Indian ink and thioflavine S, indicated a marked occlusion of capillary filling in the sub-cortex and outer medulla during angiotensin infusion. Possible ways by which a reduction in peritubular capillary blood flow could affect sodium reabsorption have been suggested.

CONTENTS

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INTRODUCTION

The renin-angiotensin system

Angiotensin II, an octapeptide, the formation of which has been described in many mammalian species, is produced by the action of renin on a plasma substrate according to the scheme shown below (Fig. 1.1).

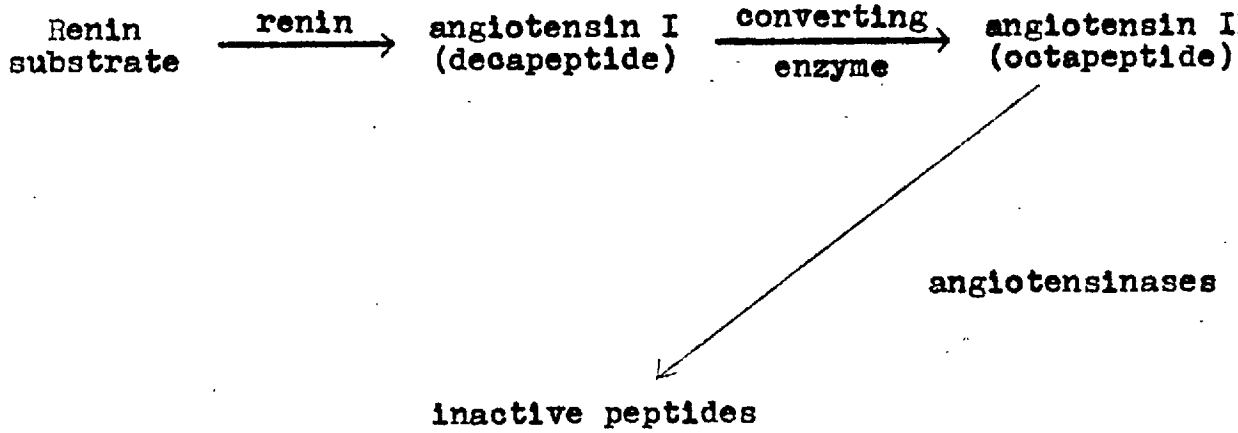


Fig. 1.1. The renin-angiotensin system

A detailed description of the production, separation and assay of the various components of the system is outside the scope of this work, but may be found in several major articles (Page & Bumpus, 1961; Haas & Goldblatt, 1963; Peart, 1965).

Renin itself is an enzyme, first described by Tigerstedt & Bergmann (1898), which is produced in the vascular pole of the glomerulus (Cook & Pickering, 1962) and probably in the granulated cells of the juxtaglomerular

apparatus (Tobian, 1962; Hartroft, 1963; Faarup, 1968; Cook, 1968). The composition of angiotensin I, the initial product of the reaction of renin with substrate, was determined almost simultaneously by Elliot & Peart (1956) and by Lentz, Skeggs, Woods, Kahn & Shumway (1956). Skeggs, Kahn & Shumway (1956) described the occurrence in plasma of converting enzyme, which converts angiotensin I to the octapeptide angiotensin II; they prepared angiotensin II from horse angiotensin I and showed it to have the sequence asp-arg-val-tyr-ileu-his-pro-phe (Skeggs, Lentz, Kahn, Shumway & Woods, 1956).

Naturally occurring human (Arakawa, Nakatani, Minohara & Nakamura, 1967) and hog (Bumpus, Schwarz & Page, 1957) angiotensin has the same structure as that of the horse, but bovine angiotensin differs in that position 5 is occupied by valine (Elliot & Peart, 1956). The commercially available form, valine^5 angiotensin II amide, has been used in this study. The isoleucyl-5 and valyl-5 forms of angiotensin II have equivalent biological activity, as do the aspartyl and asparaginyl forms (Gross & Turrian, 1960). Sambhi & Barrett (1967) found tachyphylaxis to the pressor effect in the rat of large intravenous injections of the free acid but not the amide, which is the only reported biological difference between the two forms. Throughout this text, in the description of other worker's results, the administration of angiotensin refers to the use of synthetic material; where earlier investigators have used preparations from naturally occurring material, this

has been referred to as angiotonin or hypertensin, but such work has largely been excluded from this introductory review, since impure preparations may have been contaminated with other vasoactive substances.

Angiotensin II is the most potent pressor substance known, being 10 times more active than noradrenaline (on a weight basis) in raising the blood pressure of the unanaesthetised dog on single injections (Gross & Bock, 1962). Comparison of pressor potency with noradrenaline on other preparations may yield an even greater ratio of activity (Page & Bumpus, 1961). The powerful vasopressor properties of angiotensin have implicated it in the pathogenesis of renal hypertension. In 1934, Goldblatt, Lynch, Hanzal & Summerville reported the production of persistent hypertension in dogs following constriction of the renal artery. Subsequently, Skinner, McCubbin & Page (1964) have shown that renin activity in renal venous blood is elevated by small reductions in renal perfusion pressure, too slight to affect renal blood flow (RBF). Other workers have reported increased renin secretion (Lever & Peart, 1962; Vander & Miller, 1964) and angiotensin generation in systemic blood (Regoli & Vane, 1966) following local reduction of renal perfusion pressure. Tobian (1962) has proposed that the juxtaglomerular cells in the afferent arteriole could act as stretch receptors, responding to a decreased stretch of the arteriolar wall with an increased secretion of renin.

Participation of the renin-angiotensin system in sustaining the high blood pressure of many forms of human hypertensive disease is unlikely. Peripheral blood renin (Brown, Davies, Lever & Robertson, 1964) and angiotensin (Boyd, Landon & Peart, 1969) concentrations are not always elevated in hypertension due to renal arterial or parenchymal disease, and are only consistently elevated in the malignant phase of hypertension. Angiotensin may, however, interact with hormonal or neural systems, or play a role in initiating hypertension which is then maintained by some other mechanism.

Apart from the effect of reduced perfusion pressure, renin secretion can be elevated experimentally by stimulation of the renal nerves (Vander, 1965) infusion of catecholamines into the renal artery (Vander & Miller, 1964; Wathen, Kingsbury, Stouder, Schneider & Rostorfer, 1965), haemorrhage [redacted] (Brown, Davies, Lever, Robertson & Verniory, 1966; Hodge, Lowe & Vane, 1966) or depletion of body sodium content (Veyrat, de Champlain, Boucher & Genest, 1964; Fasciolo, de Vito, Romero & Cucchi, 1964; Brown, Davies, Lever & Robertson, 1964). The potent action of angiotensin in stimulating the secretion of aldosterone in the dog and human (Genest, Nowaczynski, Koiw, Sandor & Biron, 1960; Laragh, Angers, Kelly & Lieberman, 1960; Ganong, Mulrow, Boryczka & Cera, 1962) has implicated it in the mechanism of salt conservation. A reduced sodium load

delivered to the macula densa site (Gross, Brunner & Ziegler, 1965) or an effective reduction in blood volume as a result of sodium depletion (Tobian, 1960) may stimulate renin secretion, which, through release of aldosterone, may enhance the reabsorption of sodium by the distal tubule. Contrary to this view, Thurau & Schnermann (1965) have suggested that angiotensin may be generated from juxtaglomerular cells in response to a high sodium concentration at the macula densa site, thus reducing the individual nephron glomerular filtration rate (GFR) by afferent arteriolar constriction. This scheme awaits the proof that angiotensin can be generated at a sufficiently high rate to cause the effects observed.

Angiotensin is unlikely to play a role in the autoregulation of renal blood flow, since moderate to perfect autoregulation could still be demonstrated during renal arterial infusion of renin or angiotensin, sufficient to cause an increase in renal vascular resistance (Belleau & Earley, 1967). Autoregulation of RBF also occurred normally in salt-loaded dogs, in which the renal content of renin would have been markedly depleted.

The precise role of the renin-angiotensin system in the regulation of sodium balance is far from clear. Recent work indicates that animals immunised to angiotensin II still respond normally to dietary salt restriction and overload (Peart, 1969). Apart from an action through aldosterone,

angiotensin itself exerts complex effects on sodium excretion. In normal human subjects, angiotensin infusion causes antinatriuresis (De Bono, Lee, Mottram, Pickering, Brown, Keen, Peart & Sanderson, 1963), but in hypertensive patients this response is reversed to a natriuresis (Nijensohn, 1957; Brown & Peart, 1962). Angiotensin infusion also causes either antinatriuretic or natriuretic responses in animals depending on the experimental conditions. These responses are seen before any aldosterone released by the infusion would have had time to participate in the effect. The present study was carried out to investigate the diuretic response to angiotensin, which appears to be in contradiction to its suggested role in salt conservation. The investigation has been confined to the rat, since the diuretic response can consistently be evoked in this species, and it was decided to explore the effect thoroughly in a single species rather than to compare responses between several species.

Pharmacological actions of angiotensin

Angiotensin possesses a wide and interesting spectrum of pharmacological activity, a consideration of which is necessary in evaluating the action on the kidney.

Vascular smooth muscle is caused to contract *in vitro* by low concentrations of angiotensin. Helically cut strips of resistance vessels from dogs and rabbits contract in response to angiotensin, with the exception of dog renal resistance vessels (Bohr, Goulet & Taquini, 1961). On isolated blood

vessels of sheep, dogs and cats, tachyphylaxis occurred on repeated administration of angiotensin, which could be reversed by addition of plasma, a semi-purified angiotensinase preparation or Dowex 50, which is capable of adsorbing angiotensin (Khairallah, Page, Bumpus & Turker, 1966). Tachyphylaxis to the constrictor effect of angiotensin did not develop on the rabbit aorta, which was found to contain a high concentration of angiotensinase. Khairallah et al. concluded that angiotensin tachyphylaxis was due to continued receptor site occupation.

In the intact animal, blood flow through any particular region during angiotensin infusion is dependent on the balance between the increase in perfusion pressure and the rise in vascular resistance. Mandel & Sapirstein (1962) found that in rats, the kidneys were the only region showing a consistently reduced flow fraction during pressor infusions of angiotensin, other areas showing a constant or increased blood flow despite an increased vascular resistance. A number of reports indicate interaction between angiotensin and sympathetic nervous activity during angiotensin induced vasoconstriction. Scroop & Whelan (1966) studied human subjects with absence of sympathetic innervation to the upper limbs, or following phenoxybenzamine or bretylium in normal subjects, and found that complete abolition of the vasoconstrictor response to angiotensin in the hand was caused by sympathectomy. A direct effect on the blood vessels was observed on close

arterial administration at a far higher concentration than that reaching the blood vessels after intravenous administration.

Vasoconstrictor responses to angiotensin, but not to noradrenaline, were markedly reduced following acute sympathectomy in in situ perfused dog hind-limbs (Zimmerman, 1962). Angiotensin potentiated the vasoconstrictor responses to sympathetic stimulation in the skin (Zimmerman & Gomez, 1965) and cat spleen (Benelli, Della Bella & Gandini, 1964), but vasoconstriction produced by noradrenaline was also potentiated in these experiments. Angiotensin may block the re-uptake of neurotransmitter, permitting a larger amount of noradrenaline to act on vascular smooth muscle following nerve stimulation (Zimmerman and Gomez, 1965). However, potentiation of one vasoconstrictor substance by another is difficult to relate to a physiological or biochemical mechanism.

Angiotensin constricts smooth muscle of many origins apart from the vascular system, and direct as well as indirect stimulant actions have been found in intestinal and other smooth muscle preparations. The contractions of rabbit and guinea pig ileum were found by Robertson & Rubin (1962) to be potentiated by anticholinesterase substances and inhibited by atropine and Botulinum toxin, indicating an effect through the parasympathetic nervous system. The isolated vas deferens is not caused to contract by angiotensin (Benelli et al., 1964) but responses to sympathetic nervous stimulation are markedly increased. Isolated ventricular

myocardium shows a positive inotropic response to angiotensin, which is apparently a direct action, since it was not affected by prior reserpinisation, or treatment with a sympathetic β-receptor antagonist (Koch-Weser, 1965).

The angiotensin induced increase of peripheral sympathetic activity may be due to facilitating release of noradrenaline from nerve endings (Benelli et al., 1964) and angiotensin may deplete isolated blood vessels of noradrenaline (Distler, Liebau & Wolff, 1965), but does not potentiate the release of noradrenaline from sympathetically stimulated isolated cat spleen (Thoenen, Hurlimann & Haefely, 1965). The pressor responses to tyramine and the ganglion stimulating agent DMPP (1,1'-dimethyl-4-phenyl piperazinium iodide) in the anaesthetised dog are also potentiated by infusion of angiotensin and crude renin, but only in the presence of an intact sympathetic nervous system (McCubbin & Page, 1963).

A direct ganglion stimulant effect is not the mechanism of the potentiation of autonomic transmission referred to above, but angiotensin has been shown to stimulate directly the superior cervical ganglion (Lewis & Reit, 1965) and the stellate ganglion (Aiken & Reit, 1968) of the cat. In addition, Bickerton & Buckley (1961) showed in cross circulation experiments in dogs that angiotensin possesses a central pressor effect; later, Severs, Daniels, Smookler, Kinnard & Buckley (1966) observed a sympathetically mediated pressor response on infusion of angiotensin into the

lateral ventricle of the cat. Aars & Akre (1968) observed an initial reduction followed by an increase in renal nerve activity in laparotomised rabbits, during infusion of a pressor dose of angiotensin, but concluded that increased sympathetic nerve activity, as monitored in the renal nerve, was unimportant in causing the stable pressor response. A marked pressor effect was observed by Akinkugbe, Brown & Cranston (1966b) following infusion of a small amount of angiotensin into the vertebral artery of rabbits.

A summation of central pressor effect, ganglion stimulant action and facilitation of sympathetic transmission could account for the gradual rise in blood pressure which occurs on the infusion of an acutely sub-pressor dose of angiotensin in rabbits and dogs over several days (Dickinson & Lawrence, 1963; McCubbin, De Moura, Page & Olmsted, 1965).

The stimulation of aldosterone secretion by angiotensin referred to above (p. 10) has been shown in dogs to be a direct action on the adrenal gland, since a marked increase in urinary aldosterone output occurred on infusion into the adrenal artery of a dose which caused a much smaller effect on intravenous infusion (Ganong et al., 1962). In the rat, however, stimulation of aldosterone secretion by physiological amounts of angiotensin has not been demonstrated. Angiotensin did not stimulate the production of aldosterone by rat adrenal glands incubated in vitro (Glaz & Sugar, 1962). Dufau & Kliman (1968) observed an increase in aldosterone

and corticosterone secretion 20 min after the intravenous injection of a huge dose (250 µg) of angiotensin in anaesthetised rats. Such an effect could well be due to secondary changes in plasma sodium and potassium concentrations, rather than a direct effect of the peptide on the adrenal cortex.

Angiotensin also stimulates the release of catecholamines from the adrenal medulla, following direct injection into the coeliac artery of eviscerated cats (Feldberg & Lewis, 1964), as indicated by contractions of the nictitating membrane. Release of adrenaline and noradrenaline from the adrenal glands of anaesthetised dogs is increased following intravenous infusion of angiotensin, with a greater effect on adrenaline (Peach, Cline & Watts, 1966). The release of adrenaline causes a marked hyperglycaemic response in the rabbit, which is abolished by adrenalectomy (Akinkugbe, Brown & Cranston, 1967).

Effect of angiotensin on renal function in various species

Renal function may be affected by angiotensin through a variety of mechanisms resulting from the range of physiological actions indicated above. Thus it is necessary to consider a direct effect on the renal vasculature, together with the modifying effect on function of a rise in blood pressure. Renal blood flow may also be affected by a potentiation of renal nervous impulse traffic, or by a sympathetic central or

ganglionic stimulation. Catecholamines and corticosteroids may be released from the adrenal gland during angiotensin infusion, and contribute to the renal effect. In addition, there may be a direct effect on tubular reabsorptive mechanisms, and finally, an effect on the smooth muscle of the urinary tract may modify the observed response.

The total renal action may show a species variation, and may also be affected by the presence of an anaesthetic agent, or modified by the physiological status of the animal. The dose administered will also determine the final effect, because of the balance between the various types of action described above.

a) The human

In the normal human, angiotensin causes a profound inhibition of urine flow and electrolyte excretion at pressor and sub-pressor doses (Bock & Krecke, 1958; De Bono et al., 1963; Gill, Barbour, Slater & Bartter, 1964; Ames, Borkowski, Sicinski & Laragh, 1965; Jones, Barraclough, Perriello & Marsden, 1967). Fairly large doses of 4 to 5 µg/min in the series of De Bono et al. (1963) caused profound antidiuresis, however Louis & Doyle (1965) were able to administer huge doses of up to 1.5 µg/kg/min in one patient with terminal carcinoma, and found doses of 0.4 µg/kg/min and above to cause diuresis with natriuresis.

The antidiuretic response is accompanied by a fall in p-amino-hippurate clearance (C_{PAH}) in every case, and by a

reduction in inulin clearance (C_{IN}) in the subjects of Gill et al. (1964) and De Bono et al. (1963). Angiotensin reduces both free-water clearance (C_{H_2O}) and osmolar clearance (C_{OSM}) in normal, hydrated subjects (Gill et al., 1964; Jones et al., 1967). The fall in C_{H_2O} together with the reduced sodium excretion, indicates that a smaller amount of sodium is reaching the cortical diluting segment (Gill et al., 1964) in subjects undergoing mild water diuresis, probably as a result of the reduced GFR; although an enhancement of proximal solute reabsorption must also be considered here.

In contrast to the effect in normal humans, angiotensin infusion causes a diuretic and natriuretic response in patients with renal hypertension and in primary hyperaldosteronism (Del Greco, 1961; Brown & Peart, 1962) as well as in cirrhosis with ascites (Laragh, Cannon, Bentzel, Sicinski & Meltzer, 1963; McCloy, Baldus, Summerskill & Maher, 1966; Schroeder, Shear, Sancetta & Gabuzda, 1967). The diuretic response can be produced with pressor or sub-pressor doses (Brown & Peart, 1962) and is accompanied by inconsistent changes in GFR. However there is a tendency for GFR to reduce less than in normal subjects, and increases in inulin or creatinine clearances were reported in some patients in the studies of Brown & Peart (1962) and Del Greco (1961). Schroeder et al. (1967) found that diuresis could be elicited in the face of an increase or decrease in GFR, in patients with cirrhosis of the liver. In patients with proven renal

artery stenosis (Brown, Matthew & Robertson, 1964) angiotensin generally causes a diuretic response in both kidneys, although occasional patients may show an antidiuretic response in both kidneys, or just in the stenosed kidney.

When angiotensin was infused together with aldosterone in normal subjects (Louis & Doyle, 1966) an antidiuresis still resulted. However, Mills & Barkham (1962) were able to elicit a diuresis in normal subjects in the escape phase of mineralocorticoid infusion. A diuretic response can still be obtained in bilaterally adrenalectomised patients (McCloy et al., 1966), although the usual response is an antidiuresis in adrenalectomised patients and those suffering from Addison's disease (Statius Van Eps, Zurcher-Mulder & De Vries, 1963; Biron, 1964).

Thus in a variety of human conditions characterised by hyperaldosteronism, angiotensin causes an increased elimination of salt and water. A diuretic and natriuretic response also occurs following severe dietary salt restriction (Louis & Doyle, 1966). Secondary hyperaldosteronism and cirrhosis with ascites have been shown to be accompanied by a reduced vasoconstrictor and pressor response to angiotensin (Johnston & Jose, 1963; Laragh et al., 1963; Kaplan & Silah, 1964) and reduced vascular responsiveness may favour the appearance of a tubular natriuretic component.

The antidiuretic effect of angiotensin is probably a result of the vascular effects; it is not mediated by a release

of vasopressin, since it occurs also in patients suffering from diabetes insipidus (Del Greco, 1962; De Bono et al., 1963) and differs from the antidiuretic response to pitressin in that urinary osmolar concentration is reduced instead of elevated. It is also not mediated by aldosterone secretion in the acute experiments described here, because no time lag exists before the appearance of the effect, and because potassium excretion is not increased.

(b) The dog

In normal unanaesthetised dogs, a dose dependency of the renal effects has been noticed by several workers. Lameijer, Soghikian & de Graeff (1966) observed an antidiuretic response to low doses, i.e. below 0.01 µg/kg/min, whereas larger doses of 0.06 to 0.13 µg/kg/min caused natriuresis with increased tubular rejection of sodium. The higher doses used by Lameijer and co-workers would have caused an antidiuretic response in the experiments of Cannon, Ames & Laragh (1966) and Levitin, Lehmann, Pigeon, Warren & Goldenberg (1963). The normal antidiuretic response to low doses of angiotensin in conscious dogs is converted to diuresis by pentobarbitone anaesthesia (Levitin et al., 1963; Cannon et al., 1966). Louis & Doyle (1965) found that in anaesthetised dogs, infusion of 0.03 µg/kg/min caused antidiuresis, rise in blood pressure and fall in GFR, whereas a larger dose of 0.25 µg/kg/min caused a similar rise in blood pressure with diuresis and an increase in GFR. Schmid (1968) was able to correlate GFR changes with

the appearance of a diuretic or antidiuretic response; a reduction in GFR of more than 5% was associated with antidiuresis and reduced sodium excretion, whereas an increase greater than 5% was associated with natriuresis; the latter response could only be evoked in renal hypertensive dogs.

The antidiuretic response in the dog is due to reduced filtered load of sodium, however the natriuresis cannot be accounted for solely by an increased GFR, according to Lameijer et al. (1966) and Schmid (1968), since sodium clearance per unit GFR increases. Lindheimer, Lalone & Levinsky (1967) showed that increases of GFR per se does not markedly increase sodium excretion unless the extracellular fluid volume is simultaneously expanded. In the dog as in the human, angiotensin produces a predominantly natriuretic response in renal hypertensive animals (Schmid, 1968) or following chronic fluid retention, hyperaldosteronism and ascites which can be produced experimentally in dogs by thoracic vena cava constriction (Cannon et al., 1966; Porush, Kaloyanides, Cacciaguida & Rosen, 1967). However a diuretic response to angiotensin does not occur during short term infusion of aldosterone (Healy, Suszkiw, Dennis & Schreiner, 1966).

Reversal of antidiuretic to a diuretic response may be produced in the dog in circumstances in which the renal vasoconstrictor effects are blunted, as in pentobarbitone anaesthesia (Levitin et al., 1963), constriction of the vena cava (Porush et al., 1967), or where the renal

vasoconstrictor effect is antagonised pharmacologically by vasodilatation with acetylcholine (Earley & Friedler, 1966), or by treating with reserpine or guanethidine (McGiff, 1967). However the diuretic response is always accompanied by a decrease in RBF. Corcoran & Page (1940) originally observed an increase in GFR with a reduction in RBF following renin and angiotonin infusion. Such a balance of renal effects indicates predominantly efferent arteriolar vasoconstriction and the diuresis could be accounted for by the increased GFR together with the rise in blood pressure.

An increase in renal perfusion pressure within the range of autoregulation results in diuresis and natriuresis (Selkurt, 1951; Shipley & Study, 1951). Thurau & Deetjen (1962) showed that medullary blood flow increases with an increase in perfusion pressure, although cortical blood flow remained constant, resulting in a diminished counter-current exchange capacity of the vasa recta (see Introduction to Chapter IV, p.149). Papillary sodium content is reduced by elevated perfusion pressure (Selkurt, Womack & Dailey, 1965) resulting in a wash-out of the medullary osmolar gradient, and a decreased passive diffusion of water into the medullary interstitium from the descending limb of the loop of Henle and from the collecting ducts.

Evidence that the diuretic response to angiotensin in the dog is dependent on an increase in systemic pressure was

provided by Cannon et al. (1966). In their experiments, a normally pressor and diuretic intravenous dose produced a weak and inconsistent natriuresis when infused directly into the renal artery. A lower dose which was not pressor when infused intra-arterially did not increase sodium excretion unilaterally. Earley & Friedler (1966) were able to cause a unilateral vasodilatation by intra-arterial infusion of acetylcholine, and an intravenous dose of angiotensin now caused diuresis on the vasodilated side but antidiuresis on the non-vasodilated side, which led them to conclude that the diuresis was dependent on an increased systemic pressure. Porush et al. (1967) however, managed to obtain a unilateral natriuresis following intra-arterial infusion, in dogs with constriction of the vena cava and ascites, of a dose of angiotensin which did not appear to produce systemic effects. The diuresis was accompanied by a significant decrease in tubular reabsorption of free water (TC_{H_2O}) which may be due to decreased reabsorption of sodium from the medullary portion of the ascending limb of the loop of Henle (Seldin, Ekinoyan, Suki & Rector, 1966). Angiotensin infusion increased the sum of C_{H_2O} and sodium clearance (C_{Na}) indicating an increased proximal tubular rejection of solute and an increased delivery of filtrate to the distal tubule.

In a study using stop-flow clearance techniques, Vander (1963) infused angiotensin into the renal artery, and found that a low dose (6 to 12 mμg/kg/min) which did not affect systemic

blood pressure, inhibited DISTAL sodium reabsorption. [redacted] Larger doses (28 to 75 mμg/kg/min) which did produce a systemic effect, resulted in unilateral natriuresis when GFR was unchanged or increased, but antidiuresis when GFR was reduced. Vander concluded that angiotensin possessed an inhibitory effect on distal sodium reabsorption, although its effect on sodium excretion depends on the extent of change in GFR.

The results of clearance experiments in the dog show that angiotensin natriuresis is accompanied by decreased proximal and distal sodium reabsorption. The diuresis cannot be entirely explained by the pressor effect, since the degree of sodium loss greatly exceeds that produced by pressure induced diuresis (Cannon et al., 1966); nor can it be explained solely by the increase in filtered load. The absence of a unilateral natriuretic effect in the experiments of Cannon et al. (1966) led them to conclude that a critical haemodynamic alteration, caused by a combination of pressor and vasoconstrictor effects, provokes a tubular natriuresis. The work of Porush et al. (1967) and Vander (1963), however, suggests that angiotensin may also possess an effect on intrinsic reabsorptive capacity in the dog.

(c) Rabbits and rats

In rabbits and rats, the diuretic rather than antidiuretic response is the more usual effect. Pickering & Prinzmetal

(1940) reported that administration of renin to rabbits resulted in a copious increase in water and solute excretion. Subsequently, Langford & Pickering (1965) reported a dose-dependency of the effect; low doses of angiotensin (0.2 µg/kg/min) elicited antidiuresis and large doses (1 µg/kg/min) produced increased urine flow, sodium excretion and proteinuria in conscious rabbits. Akinkugbe, Brown & Cranston (1966a) similarly described a diuresis and natriuresis on infusion of 1 µg/kg/min intravenously in water loaded rabbits. This dose of angiotensin, however, is large enough to produce a diuretic response in dog and man. Barraclough (1965) drew attention to the necessity of comparing dose effects on a body weight basis, and was able to elicit antidiuretic responses in water loaded rabbits to low doses of angiotensin (0.0001 to 0.01 µg/kg/min). As in the human, the diuretic response to angiotensin persists following adrenalectomy with maintenance on salt diet (Akinkugbe, Brown & Cranston, 1967).

The administration of renin and of large amounts of angiotensin to rats was also noticed to produce diuresis with marked proteinuria (Addis, Barnett, Boyd & Ureen, 1949). Diuresis to renin was noted by Masson, Corcoran & Page (1950) following daily subcutaneous injections. Renin administration was accompanied by a reduction in body weight and kidney weight in unilaterally nephrectomised, saline and protein fed rats. Sellers, Smith, Goodman & Marmorston (1951) found that renin

diuresis was accompanied by little change in creatinine clearance; indeed Addis et al. (1949) postulated that the proteinuria to renin might be due to efferent arteriolar constriction with increase in filtration fraction. The mechanism of proteinuria is not clear, but might well be explained by changes in capillary pressure. Renin causes increased vascular permeability in the lungs and other territories (Cuthbert, Asscher & Jones, 1966) which could be the result of increased cellular permeability or alteration in the balance of capillary and interstitial pressure.

The proteinuria, as well as the diuretic effect of renin, is almost certainly mediated through angiotensin production. Angiotensin generally produces an initial antidiuretic effect before diuresis commences (Barraclough, Jones & Marsden, 1967) and because of its very short biological half-life, must therefore be given in continuous infusion if the diuretic effect is to be revealed. Thus Croxatto, Barnaffi & Passi (1952) did not observe diuresis after the intraperitoneal injection of hypertensin; Schroder, Meyer-burgdorff, Rott & Brahms (1961) observed an antidiuretic response during water diuresis in the rat, following intravenous injection of a huge dose of 1 μg, although a diuresis was sometimes seen in saline infused animals.

In a number of studies, pure synthetic angiotensin has been shown to produce diuresis and natriuresis in the rat.

The renal response, however, may be modified by the pre-existing level of solute excretion in the rat (Croxatto et al., 1952) as it is in the rabbit (Langford & Pickering, 1965). Peters (1963) observed diuresis and natriuresis in saline-infused, conscious rats at 0.04 to 0.2 µg/kg/min. The diuresis was accompanied by reduction in C_{PAH} but little change in C_{IN}, and occurred at a sub-pressor dose. Subsequently, Regoli, Bonjour & Peters (1966) found that the natriuretic effect of 0.25 µg/kg/min angiotensin II was most apparent during intravenous infusion of isotonic saline, and was attenuated or absent in dehydrated or non-diuretic rats. In water diuresis in the rat (Bonjour, Peters, Chomety & Regoli, 1967) doses of 0.05 to 0.25 µg/kg/min caused diuresis and natriuresis, whereas doses between 0.002 and 0.05 µg/kg/min did not produce an effect significantly different from the changes occurring in control experiments. The diuretic effect persisted following anaesthesia with pentobarbital, but could not be elicited in ethanol anaesthetised rats.

In contrast to the results reported by Peters' group, Barraclough (1965) and Barraclough, Jones & Marsden (1967) were able to detect an antidiuretic response at 0.00005 to 0.005 µg/kg/min in conscious rats infused at a high rate with normal or hypotonic saline. Larger doses, from 0.005 to 0.5 µg/kg/min, produced an initial antidiuresis of short duration followed by a diuresis.

According to Barraclough, Perriello, Marsden & Jones (1967) the antidiuretic effect of doses of 0.0005 to 0.005 µg/kg/min was absent, or converted into diuresis, in salt depleted rats. Salt loaded, pentobarbital anaesthetised rats showed either no effect or an increase in sodium excretion, but a predominantly antidiuretic response was never observed. Malvin & Vander (1967) also observed variations in the renal response to angiotensin with the salt-balance of the animal. An increased sodium excretion occurred in rats infused with isotonic saline, but not in those infused with dextrose, or kept on salt-free diet. Bonjour, Regoli, Rooh-Ramel & Peters (1968) and Regoli et al. (1966) found that dehydrated or non-diuretic rats did not show a diuretic response to angiotensin, but that a fluid infusion did effect the appearance of a diuresis. A minimum of 3 ml. of isotonic saline, or 19 ml. of hypotonic dextrose (0.17 M) had to be infused before a diuretic response consistently appeared, and in nephrectomised rats it was confirmed that isotonic saline infusion was more efficient in expanding extracellular fluid (ECF) volume than hypotonic infusion (Bonjour et al., 1968). Thus a certain degree of ECF volume expansion is necessary before the diuretic response to angiotensin is elicited, but the pre-existing degree of sodium excretion also determined the response; the increase in fractional excretion of Na was found to be a linear regression on the pre-existing degree of tubular sodium rejection (Bonjour et al., 1968).

In addition to ECF expansion, vascular reactivity may change in these varied conditions of salt balance, and the abolition of the antidiuretic response to low doses of angiotensin by salt depletion (Barraclough, Perriello, Marsden & Jones, 1967) may be a reflection of such an altered vasoconstrictor response.

The normal diuretic response to renin in rats was suppressed by adrenalectomy with maintenance on 1% NaCl, and reversed to antidiuresis in adrenalectomised rats drinking water (Croxatto et al., 1952). The diuretic response to angiotensin was absent 7 days after adrenalectomy in rats drinking 1% NaCl (Gross et al., 1964), and was restored by treatment with aldosterone and prednisolone (Gross, Schaechtelin, Brunner & Peters, 1964). This effect was shown by Peters (1964) to be produced by prednisolone but not aldosterone alone.

When renal hypertension is induced in the rat by unilateral renal artery constriction, the diuretic effect of exogenous angiotensin is present in the "clamped" kidney, but absent in the opposite, untouched side (Peters, 1965; Borkowski, Howards & Laragh, 1965). Renin concentration is elevated in the clamped kidney and depleted in the contralateral kidney, but the diuretic effect of angiotensin is present in rats made hypertensive by treatment with cortexone and salt, in which renal renin concentration is reduced almost to zero

(Gross et al., 1964). Renal renin content alone is therefore not the modifying factor in the renal action of angiotensin.

The diuretic and natriuretic action of angiotensin in rodents cannot be explained by an increase in filtered load. Thus Pickering (1964) found a constancy of creatinine clearance and of glucose excretory capacity (TmG) in rabbits during renin diuresis. In the rat, GFR may be little changed during angiotensin diuresis, although PAH clearance is always reduced, leading to a marked increase in the filtration fraction (C_{IN}/C_{PAH}) (Peters, 1963; Gross et al., 1964; Barraclough, Jones & Marsden, 1967). However, Malvin & Vander (1967) found that inulin clearance was reduced at a range of doses of angiotensin which produced both reduction and increase in sodium excretion in saline loaded rats. Thus at 0.02 to 0.06 µg/kg/min, sodium excretion and GFR were reduced; at 0.08 to 0.1 µg/kg/min, equivocal effects resulted, whereas at 0.2 to 1.0 µg/kg/min, sodium excretion was increased with reduction in GFR. Bonjour & Malvin (1969) were able to correlate the reduction in sodium excretion during infusion of a low dose of angiotensin with the fall in C_{IN} and C_{PAH}. During the diuretic response to a higher dose, however, no correlation could be demonstrated between the degree of natriuresis and the change in blood pressure, C_{IN} or C_{PAH}.

Bonjour et al. (1967) observed no change in C_{H_2O} with angiotensin diuresis in rats undergoing water diuresis. They inferred that angiotensin acts mainly to increase delivery from

the proximal tubule without affecting distal reabsorption. In some conditions, particularly during ethanol anaesthesia, a vasopressin-like depression of C_{H_2O} with antidiuresis was observed. In this study, it was also shown that diuretic infusions of angiotensin were not accompanied by any change in medullary or cortical tissue composition of water, electrolytes or urea, so that the diuresis was not due to a "washout" of the medullary osmolar gradient (see p.23).

Using the occlusion time technique, Leyssac (1964, 1965b) inferred an inhibitory effect of angiotensin on proximal tubular sodium reabsorption; proximal tubules were observed to collapse at a slower rate following aortic occlusion when angiotensin had been injected immediately before. However, this technique probably reflects changes in bulk fluid transport rather than specific alterations in sodium transporting capacity. In a micropuncture study, Horster, Nagel, Schnermann & Thurau (1966) were unable to detect any effect of angiotensin on proximal tubular split drop reabsorption time, or on sodium and water flux in microperfused loops of Henle. In their study, angiotensin was added to the microperfusion fluid, or was infused intravenously in conditions which were stated not to affect urine volume flow or sodium excretion. Urine flow was also not markedly affected during intravenous infusion of a large dose of angiotensin in the micropuncture investigation of Lowitz, Stumpe & Ochwadt (1969). These workers also observed no effect on

proximal or distal split-drop reabsorption following addition of a high concentration of angiotensin to the tubular fluid, although perfusion of peritubular capillaries with angiotensin was found to prolong distal split-drop reabsorption. In the capillary perfusions, however, the authors mentioned that proximal tubules were occasionally seen to collapse following the micro-infusion of angiotensin (2.5 µg/ml.), which indicates that some of the dose was reaching glomerular arterioles, and that the effects observed could have been due to general haemodynamic alterations rather than a specific effect on reabsorptive capacity.

Angiotensin does not inhibit sodium transport in isolated amphibian skin or bladder (McAfee & Locke, 1967) or in isolated perfused rabbit proximal tubules (Burg & Orloff, 1968). An initial report that sodium flux out of isolated rat kidney cortex slices was inhibited by low concentrations of angiotensin (Leyssac, Lassen & Thayssen, 1961) could not be confirmed (Bojesen & Leyssac, 1965). Few reports of the effect of angiotensin in isolated enzyme systems exist; renal carbonic anhydrase activity is unaffected (Healey & Douglas, 1968) but glucose-6-phosphate dehydrogenase activity may be stimulated after huge doses of angiotensin, which was interpreted to be a result of inhibition of the enzyme pathway normally concerned with sodium reabsorption (Capelli, 1967) in keeping

with an earlier theory of Gross et al. (1964). Angiotensin had no effect on the Na-K activated adenosine triphosphatase system in isolated rabbit cortex and medulla (Bonting, Canady & Hawkins, 1964).

Angiotensin infusion in the rat may produce antidiuresis or diuresis. The antidiuresis is accompanied by a reduction in GFR, whereas the diuresis occurs in the face of an increased filtration fraction, reduced RBF and even reduced GFR. The diuresis may be elicited with sub-pressor doses, and is dependent on the sodium balance and ECF volume of the animal. It is due to increased tubular sodium rejection, but an effect of angiotensin on sodium flux in vitro cannot be demonstrated. A true species difference in the renal response to angiotensin does not occur, since both antidiuretic and diuretic effects can be obtained in rat, rabbit, dog and man depending on the dose administered.

In the present study, changes in proximal and distal tubular flow rates were examined during angiotensin diuresis in the rat. Angiotensin appeared to produce a degree of internal hydronephrosis, which prompted an investigation of its effect on smooth muscle in the upper urinary tract. In addition, some effects of angiotensin on renal blood flow distribution in the rat have been investigated.

CHAPTER I

Proximal and distal tubular flow dynamics during angiotensin diuresis

INTRODUCTION

Microscopic examination of the surface of the living rat kidney shows a field composed of a mass of short convolutions belonging mainly to the proximal tubule, surrounded by peritubular capillaries. The first investigators to make such observations on the living mammalian kidney (Edwards & Marshall, 1924) used phenolsulphonphthalein injection to differentiate proximal and distal tubules. Subsequently, Steinhausen (1963) introduced the dye lissamine green, which is filtered at the glomerulus, and when injected intravenously as a concentrated bolus, may be observed first in proximal tubular fluid and later in distal tubules.

The time of passage of the lissamine green bolus from glomerulus to approximately 60% of the length of the proximal tubule (proximal transit time) can be accurately measured; however, the passage time through a known length of distal tubule cannot be so measured. The variability in length of the loops of Henle belonging to superficial nephrons is uncertain, and although Schnermann (1968) found them to be of relatively constant length, the data of Walker & Oliver (1941) show almost a twofold variation. In addition, the portion of the distal tubule which appears on the surface can vary between 10% and 95% of the length of the distal convolution (see e.g. Lassiter, Gottschalk & Mylle, 1961).

Angiotensin causes marked changes in RBF and GFR, and in this investigation, the results of these changes on lissamine green transit time in relation to urine flow have been determined. From measurements of transit time and tubular diameters, it was hoped to obtain estimates of changes in proximal and distal bulk flow. Because of the errors involved in these measurements, small changes in bulk flow cannot be detected in this way, but the gross quantitative and qualitative changes observed during angiotensin infusion have enabled some conclusions to be drawn about superficial nephron flow during the diuretic phase.

Changes in proximal tubular diameter have been implicated in causing changes in intrinsic reabsorptive capacity. Gertz, Mangos, Braun & Pagel (1965) showed by micropuncture experiments in the rat, that total proximal transtubular reabsorption varied in proportion to the square of the luminal radius. These results were confirmed by Rector, Brunner & Seldin (1966) who postulated that saline diuresis resulted from a reduction in the ratio of tubular volume to nephron GFR ($\frac{\pi r^2 d}{Vo}$). Subsequently, Koch, Aynedjian and Bank (1968) demonstrated that decreased proximal solute reabsorption during carotid artery occlusion in the rat was associated with an increase in the $\frac{\pi r^2 d}{Vo}$ ratio, and emphasised the importance of renal interstitial pressure in regulating reabsorption. Changes in proximal tubular volume, however, must still be

considered to play a role in reabsorptive rate in some circumstances. Earley & Friedler (1966) postulated that transmission of perfusion pressure to peritubular capillaries may result in changes in interstitial volume and pressure, which may cause reciprocal changes in tubular radius. They suggested that such a change may be responsible for the diuresis caused by angiotensin in vasodilated dog kidneys.

As a result of these implications of tubular radius in the regulation of sodium reabsorption, it was decided to measure changes in tubular radius directly during angiotensin diuresis. No reports have so far appeared in the literature of changes in transit time or tubular radius during angiotensin infusion. In addition, changes in GFR occurring under the conditions of these experiments (reported in detail by previous workers) have been recorded and confirmed. The relationship between distal tubular diameter and urine flow has been investigated in angiotensin and other types of diuresis over a range of values of urine flow.

The diuretic phase of infusion in the presence of a reduced GFR was of particular interest in this investigation, and so a large dose of angiotensin was used which consistently evoked a diuretic response in rats given a small isotonic saline load.

METHODOLOGY

Diuretic experiments have been concluded as quickly as possible to exclude the effect of depletion of ECF, or of aldosterone released by angiotensin.

Experiments have been carried out (a) in non-diuretic rats given 3 ml. Ringer's solution during surgical preparation, and (b) in rats undergoing mild saline diuresis, in which Ringer's solution was infused continuously throughout the experiment at 0.06 ml./min in addition to the 3 ml. load. These animals were used in clearance determinations.

Preparation of animals

Male Wistar rats weighing 250 to 350 g were anaesthetised with [redacted]* Inactin (100 mg/kg) intraperitoneally. A further 5 mg Inactin was administered intravenously at completion of surgery, and anaesthetic did not generally have to be administered throughout the rest of the experiment. The animal was placed on a heated stage which maintained rectal temperature at 36° to 37°C and the trachea was cannulated to permit aspiration of mucus.

Lissamine green was injected via a silicone rubber catheter in the right jugular vein. Drugs and infusions were given through polythene catheters in the femoral veins. Blood pressure was measured from a carotid or femoral artery using a Sanborn pressure transducer (type 267B) coupled to a Sanborn

* Sodium 5-ethyl-5-(1-methyl propyl)-2-thiobarbiturate

recorder. Blood samples were collected from a carotid artery into small plastic tubes containing 2 to 3 mg solid disodium edetate, and immediately mixed thoroughly by vibration.

The left kidney was exposed via a longitudinal 2 cm flank incision and gently freed from fatty attachments and the adrenal gland. The kidney was covered with warm light liquid paraffin, which was replaced at intervals throughout the experiment, and placed in a shaped resin holder which fitted it closely. The holder was clamped to the stage (Fig. 1.1), and the kidney surrounded with loosely packed cotton wool. The ureter was gently separated, and cannulated with a 5 to 6 cm length of fine polythene tubing (pp 10), of which the tip was passed to the level of the renal hilum. Three ml. of warm Ringer's solution were administered intravenously during preparation.

The animal table was placed on the modified stage of a Leitz trinocular microscope fitted with an 'Ultropak' illuminator (Figs. 1.2, 1.3). The kidney surface was observed through an intact capsule, using a X11 Ultropak objective fitted with a dipping cone, which made contact with the liquid paraffin bathing the kidney.

Photomicrographs of the renal surface for measurement of tubular diameters, were obtained using a 'Robot' automatic transport camera, and Kodak High Speed Ektachrome colour transparency film. The kidney surface was illuminated with a

Fig. 1.1. Perspex animal table, warmed by circulation of water at 39°C. Shaped resin kidney holder, and support for ureteral catheter in position. Actual size, 20 x 18 cm.

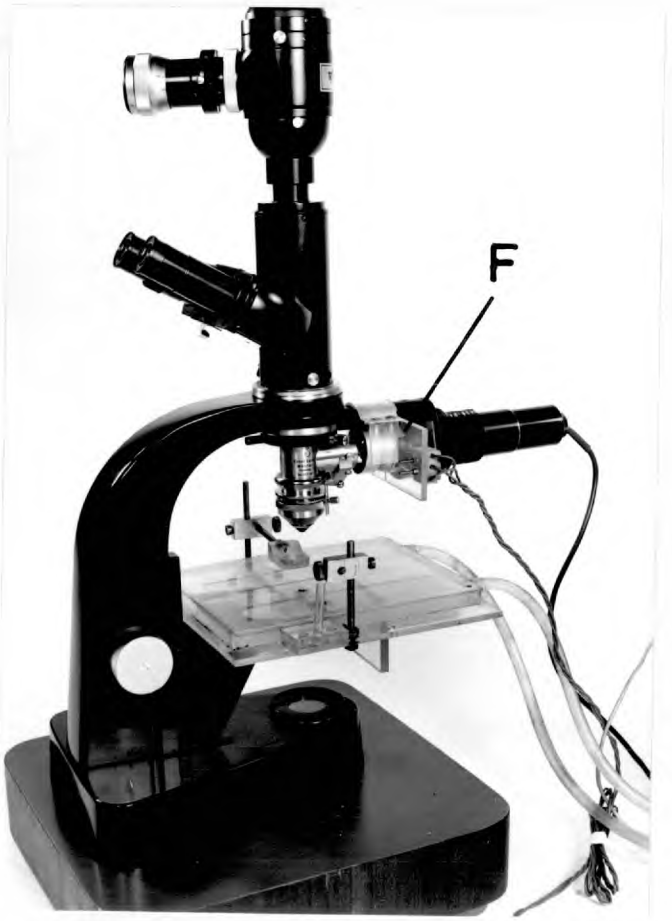


Fig. 1.2. Apparatus for incident-light observation of kidney surface. Leitz microscope with 'Ultropak' attachment, and electronic flash tube in light path (F).

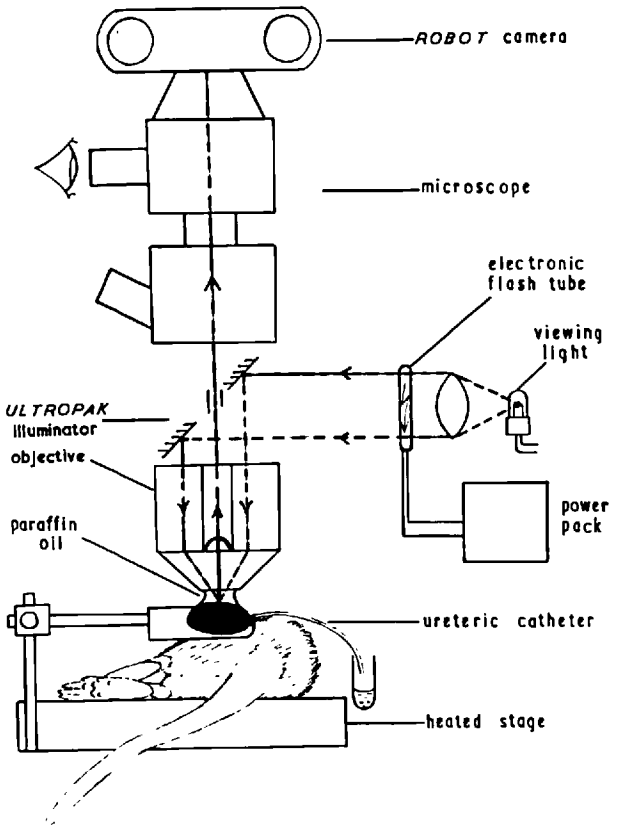


Fig. 1.3. Diagrammatic representation of light path in the Ultropak illuminator and microscope.

rapid-charging electronic flash unit, of which the tube was placed in the Ultropak light path (Figs. 1.2, 1.3). The kidney surface was scanned for a clear, well-defined area of tubules, and the late parts of proximal convolutions identified, as being those clustering around star-shaped branching capillaries (Gertz et al., 1965).

Urine was collected under liquid paraffin in small plastic tubes, and the urinary volume determined by weighing, without making correction for specific gravity. The end of the ureteral catheter was kept 2 to 3 cm below kidney level to assist flow through the catheter.

Rats were rejected if (a) respiration was hindered by excessive mucus secretion, (b) the kidneys showed evidence of infection, (c) renal tubules were obviously dilated at the start of the experiment, or (d) lissamine green transit times were excessively prolonged in the distal tubules with irregular dye clearance in proximal tubules. Such experiments amounted to only 5% of the total number performed.

Experimental Protocols

(a) Non-infused rats

Following an equilibration period of 1 hr after completion of the preparation, control urine collections of 5 or 10 min were commenced for 30 min, and control transit times and photomicrographs obtained. Diuretic administration was then commenced, and transit times and photomicrographs

repeated when urine flow had stabilised (i.e. successive urine collections not more than 10% different). Infusions administered were angiotensin (0.5 µg/kg/min at 0.06 ml./min), noradrenaline (0.5 µg/kg/min at 0.06 ml./min), mannitol (5% solution at 0.22 ml./min for 20 min) or Ringer's solution at 0.06 ml./min.

In some experiments, angiotensin was given 20 to 30 min after the completion of noradrenaline or mannitol infusions, and in these cases the figures for pre-infusion urine flow refer to values obtained 10 to 15 min before commencement of angiotensin infusion.

(b) Saline-infused rats

Changes in GFR were measured as inulin clearance, since inulin is reabsorbed or secreted by the rat nephron to negligible extent (Marsh & Frasier, 1965; Baumann, Oelert, Rumrich & Ullrich, 1965). Animals were prepared as above, and plasma and urine blanks obtained. Inulin was given as a priming dose (100 mg/kg intravenously) followed by a sustaining infusion, which delivered 2 mg/min in Ringer's solution at 0.06 ml./min. Control urine collections were commenced 1 to 1½ hr later, when plasma inulin concentration had reached a stable level, and blood samples (0.3 ml.) obtained at 10 min intervals. Each blood sample was immediately replaced with the same volume of donor rat blood (freshly drawn)

containing inulin and heparin.

Diuretic infusions were angiotensin (0.5 µg/kg/min), noradrenaline (0.5 µg/kg/min) and chlorothiazide (2 mg/kg intravenously followed by 2 mg/kg/hr) which were administered in Ringer's solution at 0.06 ml./min. Dextrose (20% in distilled water) was given at 0.11 ml./min or 0.22 ml./min. Urine and plasma samples, transit times and photomicrographs were obtained during periods of stable urine flow.

Inulin clearance was calculated from the standard equation:

$$C_{IN} = \frac{U_{IN} \times V}{P_{IN}}$$

where U_{IN} is the inulin concentration in urine, V is the urine flow rate and P_{IN} refers to the plasma inulin collection midpoint value, obtained from a graph of plasma inulin concentration with time.

Since inulin was being infused continuously, changes in total inulin clearance were reflected by changes in plasma inulin concentration. Unilateral clearances were measured from urine and plasma inulin concentrations in this study, since changes in the mobilised and untouched kidney could not be assumed to be identical.

Determination of transit time

Transit times were determined by a modification of the method of Gertz et al. (1965).

A constant volume bolus of about 0.03 ml. of 10% lissamine green in isotonic saline was injected suddenly, and times recorded by marking on a kymograph. In diuretic experiments with dextrose and mannitol, the bolus size was increased to 0.05 ml. to permit good resolution of the tubular lumen.

The time between dye injection and appearance of a deep green flush in the superficial renal capillaries is termed capillary appearance time (I), and is a measure of the passage time of blood from the central venous site to post-glomerular capillaries. Filtration of the dye bolus occurs simultaneously with its appearance in capillaries, and colouration of successive proximal convolutions is next seen. The time between capillary appearance and colouration of the last of a group of late proximal convolutions is termed proximal transit (passage) time (II), and is a measure of flow velocity along to 50 to 60% of the length of the proximal tubule.

Distal passage time has been recorded as the average between time at the late proximal convolution, and appearance of dye in the earliest (III) and latest (IV) distal convolutions visible in the microscope field. These times give an indication of flow velocity in the loop of Henle together with a variable length of distal tubule.

Thus proximal transit time is given by (II - I), and distal transit times by (III - II) and (IV - II). The results

were expressed as a ratio of experimental to control values. Control transit times were those immediately preceding the test infusion, or refer to the mean of two or more determinations in control periods, where these were not greatly different.

Measurement of tubular diameters.

Transparencies of the renal surface were projected onto a white screen and the luminal areas of proximal and distal tubules traced onto white paper. The tracings were cut out and weighed. The ratio between the paper weights in diuretic and control periods ($\frac{W2}{W1}$) is equal to the ratio of the tubular diameters, and the square of the ratio is equal to the ratio of cross sectional areas, and hence contained volume, assuming that length of the convolution does not change (Rector et al., 1966). The results were expressed as a percentage change, using the expression: $\%$ change $= \left[\left(\frac{W2}{W1}\right)^2 - 1\right] \times 100$.

The proximal tubular lumen could be resolved whether dye was contained or not, since the brush-border provides a well-defined white line around the lumen. However the distal tubular lumen could only be resolved when containing the dye bolus.

Three tracings were generally made of each proximal convolution in control and diuretic periods, and at least 4 proximal convolutions were traced in each film experiment. Two or more distal convolutions were traced, but not repeatedly.

since the change measured was far greater than that occurring in proximal tubules.

The diameter of distal convolutions was measured with calipers from transparencies obtained during distal dye transit, at least 10 separate measurements being obtained for each value of diameter quoted. The measurements were converted into microns by reference to a micro-scale photographed and projected at the same magnification.

Chemical determinations

Sodium and potassium concentrations in urine were measured by flame photometry using a Unicam SP90 atomic absorption spectrophotometer, with reference to external standards.

Inulin was determined in deproteinised plasma and in urine by the method of Heyrovsky (1956) modified for a urine sample of 0.01 ml. and plasma sample of 0.1 ml. Dextrose and lissamine green did not interfere with the determination.

Lissamine green clearance was measured in 3 rats before and during the infusion of angiotensin (0.5 µg/kg/min). Lissamine green was given as a priming dose of 5 mg/kg followed by a sustaining infusion delivering 2 mg/min. Urine and blood samples were collected as described for inulin clearance determination. Plasma was diluted in normal saline, and urine was diluted in water. Lissamine green was estimated colorimetrically by absorption at 625 mµ.

Drugs used

All solutions were made up immediately before use.

Inactin: supplied by courtesy of Messrs. Byk-Gulden Ltd., Constanz, Germany.

Lissamine green: G.T. Gurr Ltd.

Angiotensin: Hypertensin (Ciba).

Noradrenaline: Levophed (Bayer).

Chlorothiazide: Saluric (Merck, Sharp and Dohme).

'Ringer' solution contained the following milliequivalents of ions per litre: Na^+, 147; K^+, 4.2; Ca^{++}, 4.4; total Cl^-, 155.4.

RESULTS

(a) Control parameters

In non-infused experiments, urine flow generally tended to increase to a stable level between ½ and 1 hr after completion of surgical preparation. Such an increase in urine flow has been observed previously in anaesthetised rats prepared for micropuncture (Gottschalk & Mylle, 1956), and may possibly indicate a gradual diminution of the effect of antidiuretic hormones released during anaesthesia and surgery. In preliminary experiments, it was confirmed that the administration of Ringer's solution during surgery brought inulin clearance and urine flow to near normal values, and resulted in fairly short lissamine green transit times which were otherwise excessively long, as described by Rector et al. (1966). The action of intravenous fluid replacement is uncertain; it may replace extracellular fluid lost as a result of surgery, as suggested by Rector, or it may represent a saline load which superimposes an increased sodium excretion rate on a depressed value.

Control urine flow ranged from 14 to 13[illegible] µl./min and sodium excretion ranged between 0.1 and 2.2 µeq/min in non-infused experiments. Angiotensin and noradrenaline were administered in Ringer's solution at 0.06 ml./min, and infusion of vehicle alone was associated with a further slight increase in urine flow and sodium excretion (Fig. 1.4, Table 1.7).

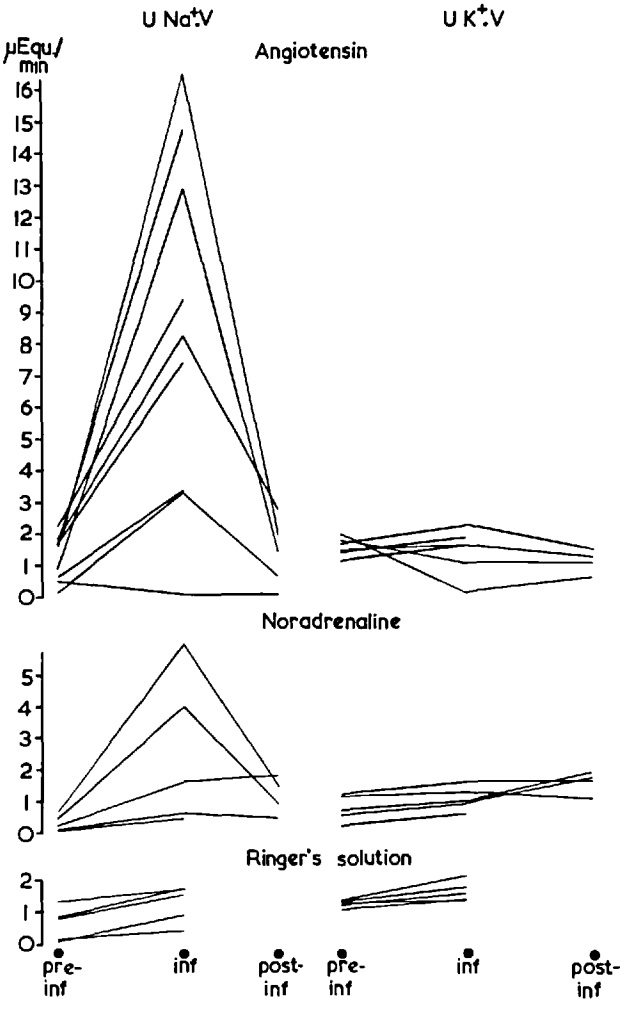


Fig. 1.4. Changes in sodium (U Na^+.V), and potassium (U K^+.V) excretion in mobilised left kidney during infusion of angiotensin and noradrenaline at 0.5 µg/kg/min, and Ringer's solution at the same rate (0.06 ml./min).

Each line represents one animal. Values refer to mean of determinations on two or more 5 or 10 min collection periods. 'Pre-inf.' refers to 30 min control period, 'inf.' refers to period between 20th and 30th min of infusion and 'post-inf.' refers to period between 5th and 15th min after terminating infusion.

In 4 control experiments, inulin clearance and sodium and potassium excretion were measured during continuous infusion over 3 hr. Inulin clearance tended to increase from 1 to 1½ hr after commencement of infusion, and showed a tendency to increase slightly thereafter. Sodium and potassium excretion showed no change over prolonged infusion (Table 1.1).

Mean blood pressure in control periods ranged between 110 and 165 mmHg with an average of 135 mmHg. Thus in many experiments, systolic blood pressure was in excess of 150 mmHg. This rather high blood pressure may have been due to the use of a barbiturate anaesthetic.

Transit time of lissamine green tended to decrease during control experiments (Table 1.7), particularly distal transit time. Tubular diameters during control experiments infused at a low rate with Ringer's solution did not alter significantly (Table 1.7).

(b) Diuretic experiments with noradrenaline, mannitol, chlorothiazide and hypertonic dextrose

These experiments will be considered together, since the changes occurring in measured parameters were similar.

Noradrenaline produced a moderate diuresis and increase in sodium excretion with no change in potassium excretion (Fig. 1.4). The diuresis and natriuresis produced by noradrenaline in non-infused experiments was somewhat less than that due to

angiotensin, although in saline-infused experiments, a greater increase in urine flow was produced (Fig. 1.5). The pressor effect of noradrenaline was less than that produced by angiotensin at the same dose level (Fig. 1.6). The level of diuresis produced by the other diuretic agents was chosen to match that produced by angiotensin and noradrenaline.

Noradrenaline and mannitol, in non-infused experiments, produced a moderate increase in urine flow and in distal tubular volume. The changes observed in proximal tubular volume were in general within those observed in the control group, although a significant decrease occurred in one experiment with noradrenaline. Proximal and distal transit times showed no change in the mannitol group, but in the noradrenaline group, both decreased as in the controls (Tables 1.3 and 1.6). In all the experiments with diuretic substances other than angiotensin, the lissamine green density in distal tubular transit appeared reduced, and the duration of the colour wave was shortened (i.e. individual distal tubules remained coloured for a shorter period of time).

In clearance experiments, the increases in urine flow produced by all diuretics were greater than in the non-infused experiments, and this was associated with a moderate increase in kidney size, and a variable increase in distal tubular volume. Distal tubular volume showed an increase dependent upon the level of diuresis, and increased over 100% at a urine flow in

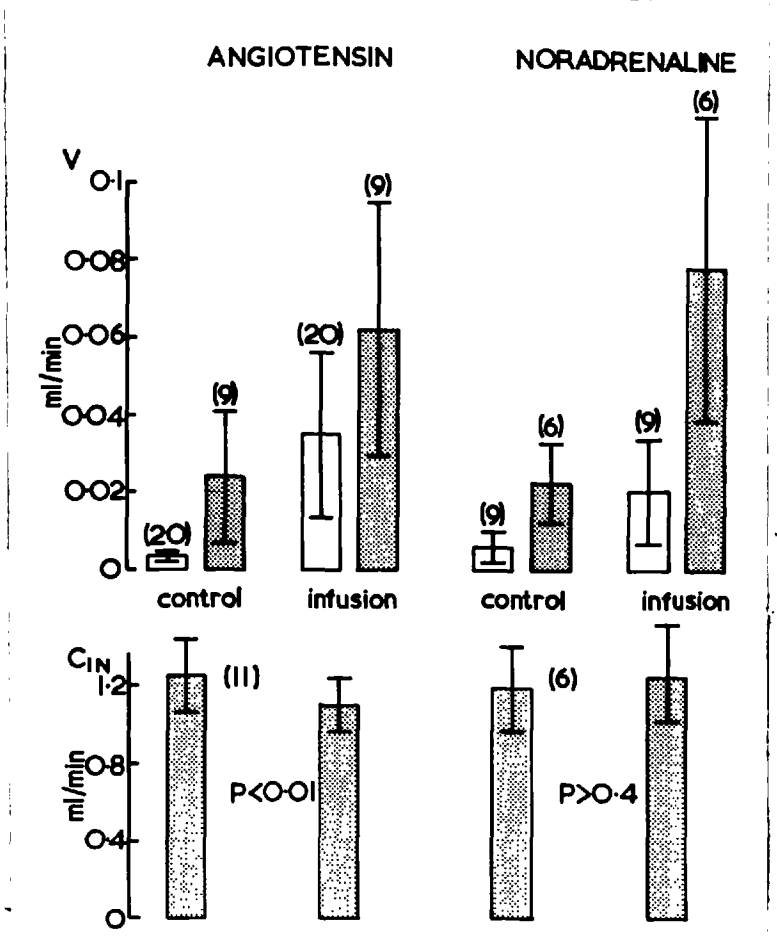


Fig. 1.5. Effect of infusion of angiotensin and noradrenaline (0.5 µg/kg/min) on urine flow (V) and inulin clearance (C_{IN}) in mobilised left kidney.

Urine flow results obtained between 20th and 30th min of infusion. Inulin clearance results obtained between 5th and 30th min of infusion.

□ = group without sustaining infusion.

▒ = infused with Ringer's solution at 0.06 ml./min.

Mean data shown ± S.D.

Figures in brackets refer to numbers of animals per group.

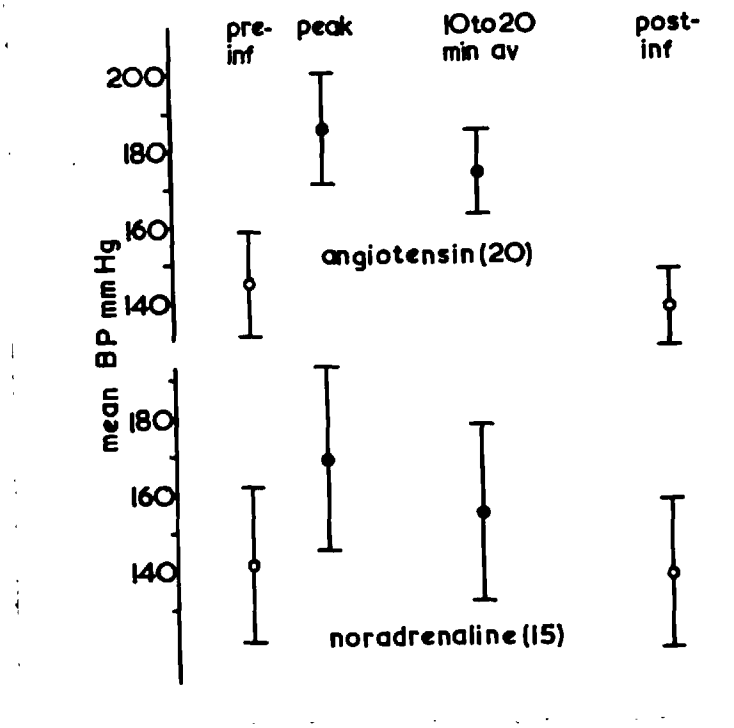


Fig. 1.6. Changes in mean arterial pressure during infusion of angiotensin and noradrenaline (0.5 µg/kg/min).

Average values in 10 min before infusion (pre-inf); highest value immediately following commencement of infusion (peak); average values between 10 and 20 min of infusion and average value in 5 min following termination of infusion (post-inf).

Mean values shown ± S.D.

Figures in brackets refer to numbers of animals per group.

the order of 0.1 ml./min. Proximal tubular volume showed a slight increase, and in one experiment in the dextrose group (No. 52) in which a considerable increase in distal tubular volume was recorded, proximal tubular volume also increased considerably. When urine flow was increased further to between 0.13 and 0.23 ml./min by the infusion of hypertonic dextrose at a faster rate, a consistently elevated proximal tubular volume was recorded (Table 1.4).

Inulin clearance was unchanged or (in one experiment) increased in the noradrenaline group, but in some experiments in the dextrose and chlorothiazide groups, significant decreases occurred (Tables 1.3, 1.4 and 1.5). When examined by a paired t-test on the combined data for the groups, the mean change occurring during dextrose infusion was significant at the 95% level ($P = 0.01$ to 0.001) whereas that occurring during noradrenaline and chlorothiazide infusion was not. Proximal and distal transit times tended to increase slightly in the dextrose and chlorothiazide groups, but the increase could not be related to a reduction in inulin clearance. Transit times were almost invariably reduced in the noradrenaline group (Fig. 1.7).

(c) Angiotensin

Commencement of angiotensin infusion always produced a brief period of cessation of urine flow for 1 to 2 min, which was often followed by a period of reduced flow (Fig. 1.8).

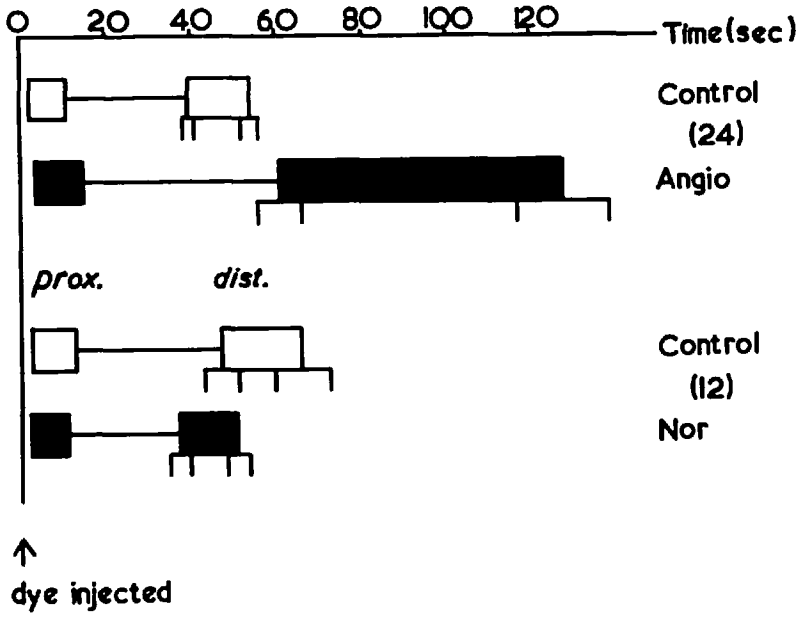


Fig. 1.7. Lissamine green proximal (prox.) and distal (dist.) transit times during infusion of angiotensin and noradrenaline (0.5 µg/kg/min).

Results shown from all experiments in which, during stable diuresis, urine flow ranged between 0.02 and 0.08 ml./min (angiotensin) and 0.01 and 0.08 ml./min (noradrenaline).

Control values obtained in period immediately preceding infusion. Standard error of mean shown, where greater than 1 sec. Figures in brackets indicate numbers of experiments.

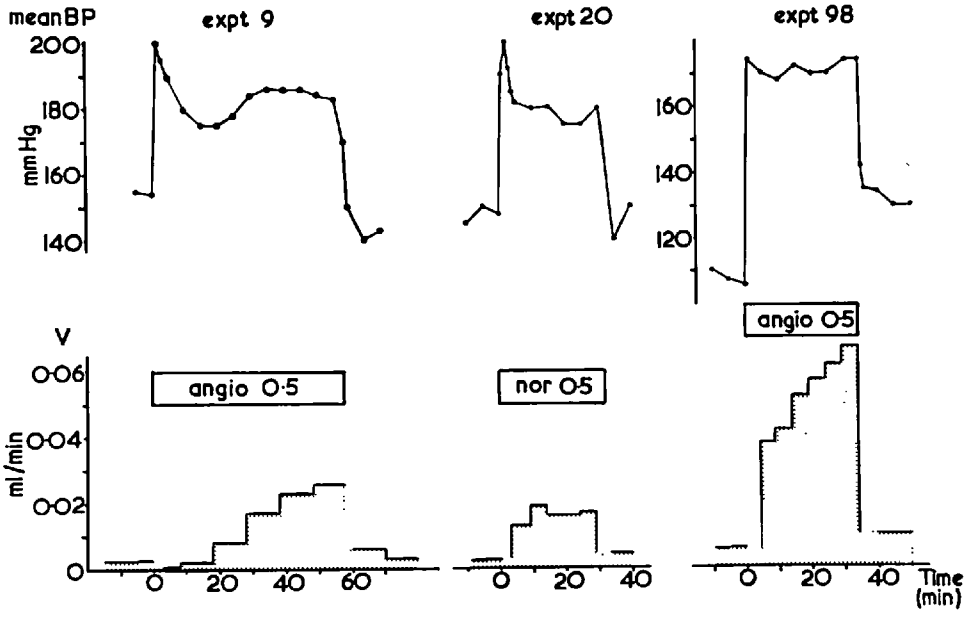


Fig. 1.8. Changes in mean arterial blood pressure (BP), and urine flow of mobilised left kidney (V) in 3 experiments during infusion of angiotensin (angio) and noradrenaline (nor) at 0.5 µg/kg/min. Experiments 9 and 20 without sustaining infusion; experiment 98 infused continuously with Ringer's solution at 0.06 ml./min.

Subsequently, urine flow increased to diuretic levels at 10 to 20 min after infusion. The antidiuretic period was in general more pronounced in non-infused rats than in saline-infused animals, and urine flow was increased to greater levels in the latter group (Figs. 1.5, 1.8). Sodium excretion was greatly increased by angiotensin, although potassium excretion was little changed (Fig. 1.4). In occasional experiments, urine flow as well as sodium and potassium excretion was decreased throughout the entire period of infusion of angiotensin. Such anti-diuretic experiments constituted only 5% of the total. The increases in urine flow and sodium excretion were in no way correlated with the degree of blood pressure rise produced, or with the absolute elevation of blood pressure.

On cessation of infusion, blood pressure fell to control levels or below (Figs. 1.6, 1.8). Urine flow decreased less rapidly than blood pressure, generally remaining elevated for 5 min or so after blood pressure had returned to control levels, but subsequently returned to pre-infusion levels.

Simultaneously with the rise in blood pressure due to commencement of angiotensin infusion, a reduction in kidney size and a marked paling of the surface was observed. This was shortly followed by a visible decrease in proximal tubular diameter and frequently a collapse of the majority of proximal tubules occurred. This brief phase was followed by return of capillary circulation, reopening of proximal tubules and

return to near control diameters in about one min. Following the reopening of proximal tubules, lissamine green was often observed to reappear in dark green colour in distal tubules, although no further dye had been injected intravenously. Neither a reduction in kidney size, nor collapse of proximal tubules was ever observed with the onset of noradrenaline infusion (0.5 μg/kg/min).

Throughout the period of angiotensin infusion, the kidney surface appeared pale. Kidney size increased following the reopening of proximal tubules to a level which was below, above or on a par with the post-infusion size, as judged by the degree of focussing of the microscope stage necessary on terminating the infusion (Table 1.10).

Throughout the antidiuretic period, proximal and distal tubular diameters appeared normal or reduced, but both values increased on commencement of diuresis. In the ascending diuretic phase, distal tubules appeared distended, and frequently showed transient dark green colouration with lissamine green. Proximal tubules also showed increased diameter, and the brush border appeared more sharply defined.

As observed in all diuretic experiments, distal tubular diameter increased with increasing urine flow. However in experiments with a low level of diuresis, a much more profound distension of distal tubules sometimes occurred with angiotensin than was ever seen with the other diuretic

substances, as can be observed by comparing experiments in which urine flows increased to a comparable extent (Table 1.2, Fig. 1.9). This distal tubular distension was sometimes, but not invariably, correlated with an increased proximal tubular volume, and kidney size always increased. In experiments where distal tubules were overdistended, all visible distal convolutions on the surface showed the effect, whereas occasionally, only isolated distal tubules appeared distended, probably due to blockage of the nephron distally with a cast or crystal.

In other angiotensin experiments, distal tubular volume increased to a similar degree as observed with the osmotic diuretics, chlorothiazide or noradrenaline, and in these experiments, proximal tubular volume was significantly decreased, and kidney size was generally decreased or normal throughout the diuretic period (Tables 1.2, 1.10).

The measurement of increases in tubular volume gave a result which could only be referred to control experiments in which urine flow was increased to a comparable extent by other methods. The measured increase also depended on the pre-existing level of diuresis. Therefore distal tubular diameter was measured directly from the film experiments, and the results of all measurements in noradrenaline, chlorothiazide and osmotic diuresis, as well as in preinfusion periods, were plotted against urine flow in Fig. 1.10. When the values were averaged between 5 ranges of urine flow, a linear relation between

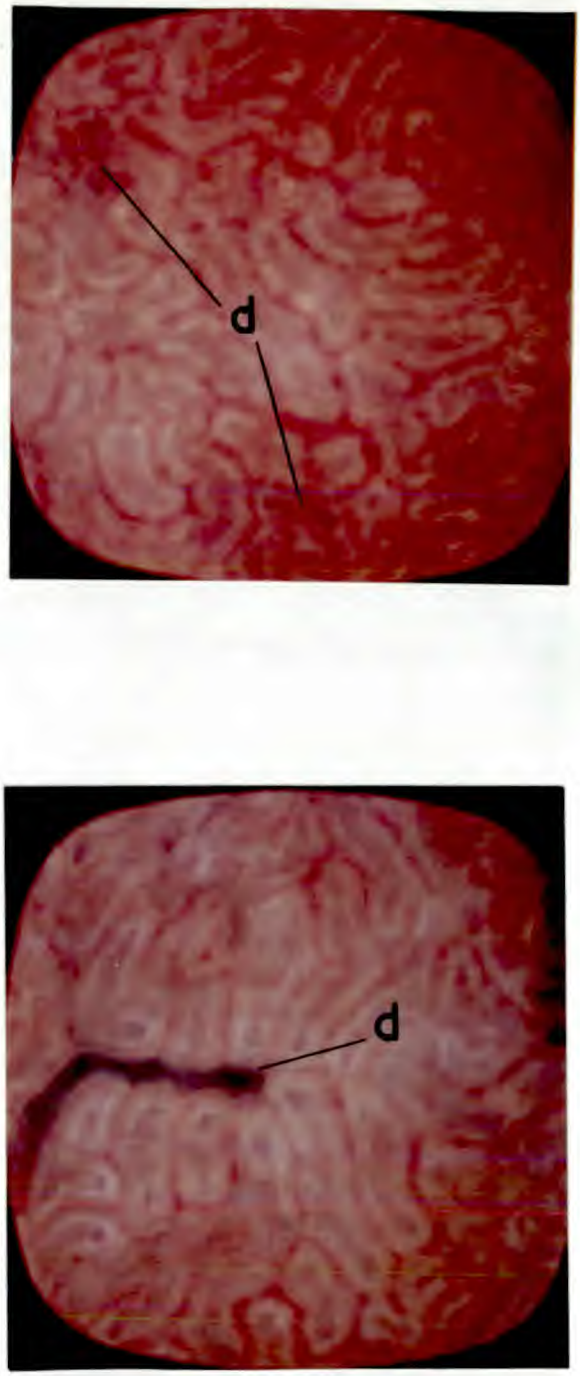


Fig. 1.9. Appearance of kidney surface during chlorothiazide diuresis (above) at 0.042 ml./min, and angiotensin diuresis (below) at 0.047 ml./min. Passage of lissamine green through distal tubules (d). From original Ektachrome transparency, X140.

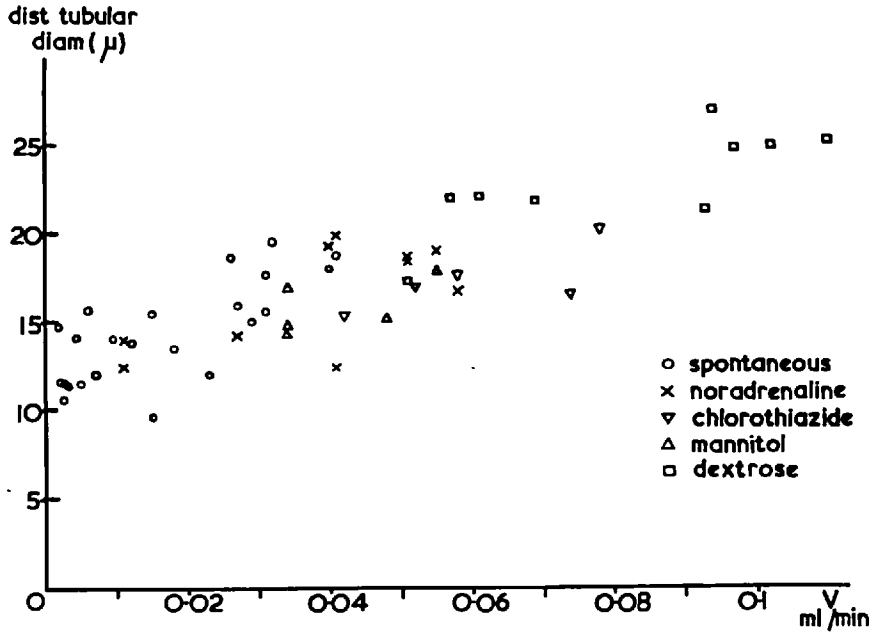


Fig. 1.10. Graph of distal tubular diameter against simultaneously measured urine flow (V). Results during pre-infusion periods (spontaneous) and during stable diuresis induced by infusion of noradrenaline (0.5 μg/kg/min), chlorothiazide (2 mg/kg/hr), mannitol (5% solution at 0.22 ml./min for 20 min) and dextrose (20% solution at 0.11 or 0.22 ml./min).

distal tubular diameter and urine flow was obtained at urine flow rates above 0.01 ml./min, and up to 0.1 ml./min, which was the greatest level of diuresis for which measurements were available (Fig. 1.11). Distal tubular diameter presumably increases further with urine flow, to a limiting value which was not attained under the conditions of these experiments.

During angiotensin diuresis, however, this relationship was lost, and distal tubular diameter was often significantly greater than in other diuretic experiments at the same urine flow, whereas sometimes it was equal to or less than the expected value (Fig. 1.11).

The observed changes in proximal tubular volume are also easier to relate to changes in distal tubular diameter than distal tubular volume.

The changes in proximal tubular volume were plotted against the simultaneously measured distal tubular diameter for diuretic experiments with noradrenaline, chlorothiazide, mannitol and dextrose (Fig. 1.12). A modest increase in proximal tubular volume was generally noted.

When the results for angiotensin diuretic experiments were similarly arranged (Fig. 1.13) it could be seen that a slight increase in distal tubular diameter was generally accompanied by a reduced proximal tubular volume. When distal tubular diameter increased above about 20 μ, however, proximal tubular volume also increased.

In addition to tubular volume, marked changes in lissamine green transit times were observed. Following injection of the

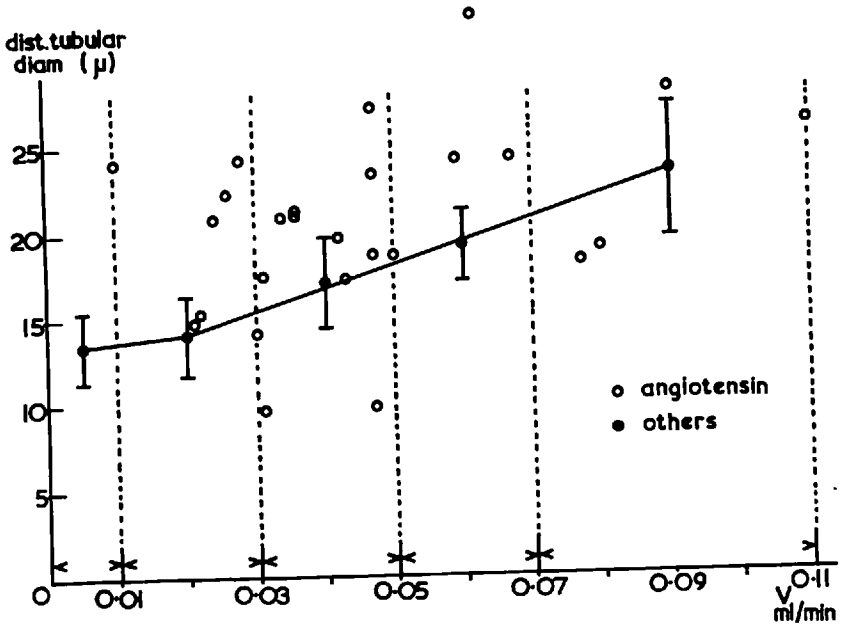


Fig. 1.11. Graph of distal tubular diameter against simultaneously measured urine flow (V) during stable diuresis induced by angiotensin infusion at 0.5 μg/kg/min (open circles).

Averaged results from all other diuretic experiments presented in Fig. 1.10 shown together with S.D. (closed circles).

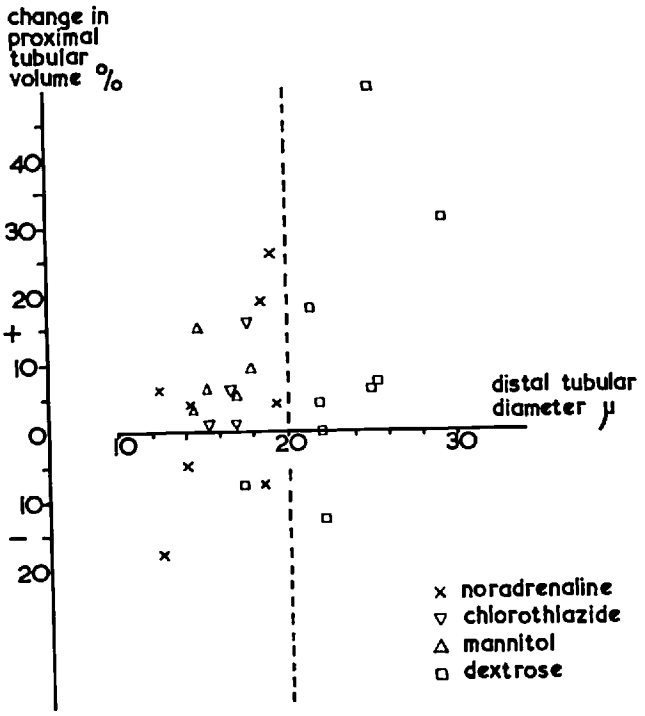


Fig. 1.12. Changes in proximal tubular volume plotted against simultaneously measured distal tubular diameter, at all levels of diuresis induced by infusion of noradrenaline (0.5 μg/kg/min), chlorothiazide (2 mg/kg/hr), mannitol (5% solution at 0.22 ml./min for 20 min) and dextrose (20% solution at 0.11 or 0.22 ml./min).

Arbitrary line of identity (dotted) drawn through distal tubular diameter of 20 μ.

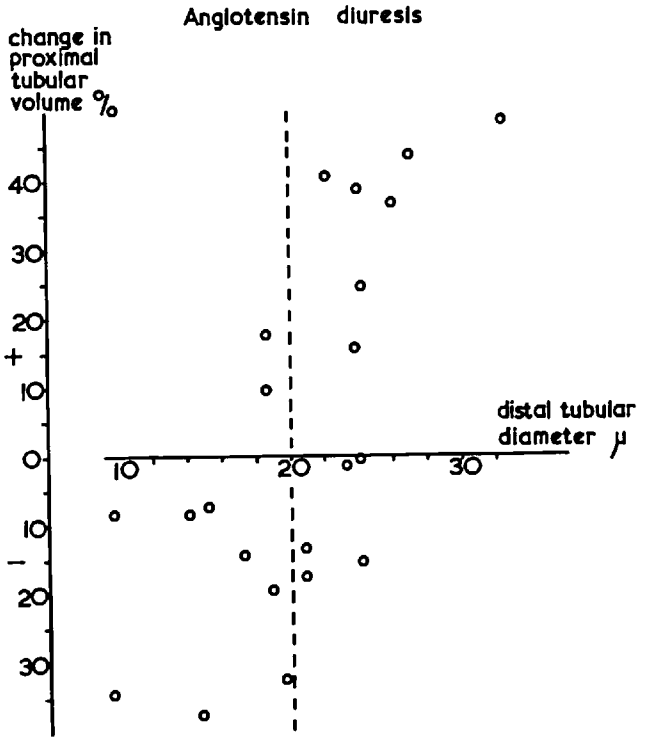


Fig. 1.13. Changes in proximal tubular volume plotted against simultaneously measured distal tubular diameter, at all levels of diuresis induced by angiotensin (0.5 μg/kg/min).

Arbitrary line of identity (dotted) drawn through distal tubular diameter of 20 μ.

dye bolus, a much slower filling of capillaries was observed than in control periods, giving rise to an increased capillary appearance time (Table 1.8). Proximal transit time was also prolonged during angiotensin diuresis, but the most pronounced change was in distal transit time, where the appearance time of both early and late distal tubules was greatly prolonged, so that in some cases, distal tubules were appearing coloured 4 or 5 min after injection of the bolus (Table 1.2, Fig. 1.7). Transit times were prolonged in antidiuretic and diuretic phases, and in experiments with normal and increased distal tubular diameter, although the greatest prolongations occurred in experiments with tubular distension. During angiotensin infusion, lissamine green appeared in high density in distal tubules (Fig. 1.9), and individual tubules remained coloured for long periods of time, sometimes for several minutes, giving the appearance of a greatly reduced flow rate. Lissamine green concentration in distal tubules appeared to be in higher concentration than that in ureteral urine at this time.

Because of the appearance of an increased intratubular lissamine green concentration, the effect of angiotensin on lissamine green clearance was examined. Lissamine green clearance in 3 experiments averaged 0.35 ml./min, and during the infusion of angiotensin at 0.5 μg/kg/min, the clearance was in every case reduced, to a mean value of 0.32 ml./min (Table 1.9). Plasma lissamine green concentration in these experiments was observed to rise during angiotensin infusion.

The increase in proximal and distal transit times could not be correlated with whole kidney GFR. Thus although inulin clearance was in general reduced by angiotensin, in one experiment (48) it was increased, but transit times were also prolonged in this case. The change in inulin clearance during angiotensin infusion was significant ($P = 0.01$ to 0.001) at the 95% confidence level.

Table 1.1. Consecutive inulin clearance and electrolyte excretion data in 4 control experiments

Expt.	Time after onset of inulin infusion (hr)	V(ml./min)	UNa x V μeq/min	UK x V μeq/min	C_{IN} ml./min
10/7/67	1 - 1½	0.048	4.91	1.59	0.61
	1½ - 2	0.031	4.68	1.63	0.81
	2 - 2½	0.025	4.46	1.47	0.87
	2½ - 3	0.022	4.23	1.29	0.89
13/7/67	1 - 1½	0.015	2.84	1.91	1.21
	1½ - 2	0.023	5.03	2.01	1.32
	2 - 2½	0.036	5.08	1.79	1.31
	2½ - 3	0.041	5.96	2.02	1.37
20/7/67	1 - 1½	0.023	2.57	0.75	1.03
	1½ - 2	0.020	2.31	0.68	1.06
	2 - 2½	0.021	2.78	0.83	1.12
	2½ - 3	0.023	3.41	0.82	1.14
24/7/67	1 - 1½	0.026	2.18	1.63	1.09
	1½ - 2	0.023	2.29	1.46	1.18
	2 - 2½	0.026	3.55	1.32	1.31
	2½ - 3	0.025	3.99	1.28	1.25

In each period, data refer to mean of 4 to 6 (5 min) urine collections.

Table 1.2. Changes in tubular volume and transit times during infusion of angiotensin (0.5 µg/kg/min).

(a) Not infused with saline

Experiment No.	V (ml./min)		Tubular volume change (%)		Transit time ($\frac{exp.}{control}$)		
	Control	Experiment	Proximal	Distal	Proximal	Early distal	Late distal
5	0.0025	0.022	+39	+141	1.71	3.10	5.69
6	0.0064	0.047	+44	+156	1.00	1.30	2.10
7	0.0027	0.029	-15	+212	1.29	1.57	2.22
8	0.0017	0.026	+41	+309	1.48	1.75	2.48
9	0.0024	0.023	+33	-	1.46	2.30	3.24
16*	0.0074	0.0011	- 6	- 25	1.46	1.95	1.84
19*	0.0068	0.043	-14	+269	1.00	1.35	2.94
57**	0.0068	0.034	-13	+ 50	1.00	1.34	1.53
58**	0.0019	0.022	- 7	+ 83	0.61	1.21	1.10
59*	0.0028	0.036	-17	+443	0.77	1.37	2.24
62	0.0032	0.078	-19	+365	1.13	1.78	2.23
67	0.0057	0.047	- 8	0	1.44	1.44	2.28
68	0.0014	0.063	-13	+ 38	1.22	1.50	2.40
71	0.0037	0.030	- 8	+ 76	1.37	2.53	3.02
79	0.0047	0.047	- 1	+247	1.17	1.41	5.54
92	0.0024	0.050	+10	+110	1.33	2.00	3.58
Mean	0.0039	0.037	+ 2.9	164.9	1.21	1.74	2.78
S.D.	0.0021	0.018	22.8	138.6	0.29	0.52	1.27

Control urine flow = mean of 3 collection periods before infusion; experimental value = urine flow at time of tubular volume measurement.
* after previous infusion of noradrenaline. ** after previous mannitol infusion.

Table 1.2. Changes in tubular volume and transit times during infusion of angiotensin (0.5 µg/kg/min)

(b) Continuous saline infusion at 0.06 ml./min.

Experiment No.	V (ml./min)		Tubular volume change (%)		Transit time ($\frac{exp.}{control}$)			C_{IN} (ml./min)	
	Control	Experiment	Proximal	Distal	Proximal	Early distal	Late distal	Control	Experiment
43	0.031	0.066	+25	+123	1.50	1.75	2.47	1.26	1.15
47	0.013	0.17	+16	+237	1.38	1.55	2.96	1.17	0.99
48	0.054	0.24	+63	+129	1.29	1.84	>2.50	1.10	1.26
50	0.018	0.11	+37	+137	1.39	0.82	1.59	1.26	1.17
53	0.013	0.052	+18	+295	1.43	1.22	2.64	1.51	1.26
77	0.044	0.096	- 4	+109	3.11	1.42	2.07	0.85	0.86
98	0.0053	0.060	+49	+452	2.02	3.40	3.92	1.23	1.06
99	0.025	0.033	-34	- 1	1.62	1.70	2.54	1.19	0.86
101	0.029	0.023	-37	- 30	1.00	1.10	1.63	1.44	1.21
102	0.0063	0.043	-32	+129	1.19	1.40	2.00	1.28	1.09
104	0.0090	0.059	0	+518	1.62	1.36	4.30	1.45	1.05
Mean	0.022	0.086	+ 9.2	+191	1.60	1.60	2.61	1.25	1.09
S.D.	0.016	0.066	34	172	0.57	0.67	0.91	0.18	0.14

Table 1.3. Changes in tubular volume and transit times during infusion of noradrenaline (0.5 µg/kg/min)

(a) Not infused with saline

Experiment No.	V (ml./min)		Tubular volume change (%)		Transit time ($\frac{exp.}{control}$)		
	Control	Experiment	Proximal	Distal	Proximal	Early distal	Late distal
12	0.0022	0.010	-	-	0.78	0.60	0.53
13	0.0071	0.041	+ 6	+ 9	1.00	0.87	1.00
14*	0.0036	0.027	+ 4	+ 3	0.78	0.63	0.73
16	0.0074	0.040	+ 4	+ 9	0.77	0.73	0.69
19	0.0068	0.011	- 5	+20	0.67	0.55	0.41
20	0.0026	0.016	+ 9	+95	0.88	0.72	0.84
21	0.012	0.035	-10	+32	0.85	0.73	0.68
59	0.0028	0.011	-18	+20	0.91	0.91	0.76
82	0.013	0.040	-	+69	0.93	0.97	0.98
Mean	0.0064	0.026	- 1.4	+32.1	0.84	0.74	0.74
S.D.	0.0040	0.014	9.9	32.8	0.10	0.14	0.19

(b) Continuous saline infusion at 0.06 ml./min

								C_{IN} (ml./min)	
								Control	Experiment
44	0.019	0.12	-	-	0.94	1.15	1.17	1.34	1.41
49	0.016	0.054	+26	+73	0.95	0.70	0.79	0.92	0.91
83	0.016	0.051	-	+106	0.95	1.18	1.28	0.93	0.91
84	0.020	0.051	+19	+ 7	0.55	0.90	0.94	1.38	1.29
85	0.023	0.049	- 8	+ 6	0.89	0.77	0.80	1.25	1.52
55	0.042	0.079	-	-	0.95	1.00	0.98	1.48	1.50
Mean	0.023	0.049	+12	+48	0.87	0.95	0.99	1.22	1.26
S.D.	0.010	0.021	18	50	0.16	0.20	0.20	0.24	0.28

* Infused at 2.0 µg/kg/min.

Table 1.4. Changes in tubular volume and transit times during infusion of 20% dextrose (saline infused at 0.06 ml./min)

(a) Dextrose infused at 0.1$\frac{1}{8}$ ml./min

Experiment No.	V (ml./min)		Tubular volume change (%)		Transit time ($\frac{exp.}{control}$)			C_{IN} (ml./min)	
	Control	Experiment	Proximal	Distal	Proximal	Early distal	Late distal	Control	Experiment
51	0.033	0.091	+18	+105	1.20	1.00	1.32	1.43	1.34
52	0.033	0.10	+50	+338	1.23	1.19	1.83	1.30	1.30
54	0.032	0.11	+ 6	+173	1.19	0.96	1.42	1.24	1.14
72	0.028	0.064	+ 4	+101	1.19	1.60	2.10	1.55	1.51
74	0.015	0.058	0	+ 37	0.83	1.13	1.36	1.66	1.57
75	0.035	0.11	+ 7	+126	1.00	0.80	1.91	1.47	1.31
76	0.027	0.061	-13	+52	1.14	1.60	2.39	1.03	0.96
Mean	0.029	0.085	+10.3	+133	1.11	1.18	1.76	1.38	1.30
S.D.	0.007	0.023	20	101	0.14	0.31	0.41	0.21	0.21

(b) Dextrose infused at 0.22ml./min

34	0.014	0.15	+20	+140	1.24	1.04	1.00	1.37	1.28
36	0.022	0.23	+31	+180	1.58	0.91	1.40	1.39	1.24
38	0.024	0.13	+33	-	0.87	0.63	0.84	-	-
41	0.024	0.22	+32	+199	1.00	0.92	1.19	1.05	1.08
35	0.037	0.26	-	-	1.53	1.42	2.18	1.65	1.64
Mean	0.024	0.20	+29	+173	1.24	0.98	1.32	1.36	1.31
S.D.	0.008	0.05	6	30	0.31	0.29	0.52	0.25	0.24

Table 1.5. Changes in tubular volume and transit times during chlorothiazide diuresis (2 mg/kg i.v. + infusion delivering 2 mg/kg/hr) - saline infused at 0.06 ml./min

Experiment No.	V (ml./min)		Tubular volume change (%)		Transit time ($\frac{exp.}{control}$)			C_{IN} m . min	
	Control	Experiment	Proximal	Distal	Proximal	Early distal	Late distal	Control	Experiment
42	0.023	0.071	+ 6	+66	1.30	1.14	1.03	1.41	1.14
46	0.025	0.054	+ 1	+12	1.00	1.00	0.98	1.11	1.09
93	0.009	0.042	- 2	+89	1.12	0.83	1.21	-	-
94	0.009	0.044	+ 1	+69	1.00	1.17	1.06	1.29	1.18
95	0.015	0.060	+16	+86	1.00	1.04	1.03	1.28	1.24
96	0.051	0.077	-	+13	1.00	0.94	1.00	1.12	1.14
Mean	0.022	0.058	+ 4.4	+56	1.07	1.02	1.05	1.24	1.16
S.D.	0.016	0.014	7.1	35	0.12	0.13	0.08	0.13	0.06

Table 1.6. Changes in tubular volume and transit times during mannitol diuresis (5% mannitol infused intravenously at 0.22 ml./min for 20 min) - no saline infusion

Experiment No.	V (ml./min)		Tubular volume change (%)		Transit time ($\frac{exp.}{control}$)		
	Control	Experiment	Proximal	Distal	Proximal	Early distal	Late distal
57	0.0068	0.055	+ 9	+ 30	1.31	0.82	0.97
58	0.0019	0.032	+ 3	+ 53	0.72	1.07	0.88
60	0.0026	0.026	- 7	+103	1.00	1.00	0.95
*		0.048	+ 6	+180	1.20	1.26	1.23
64	0.0046	0.034	+15	+ 41	0.92	0.81	0.82
65	0.0032	0.034	+ 5	+ 91	1.17	1.00	0.90
Mean	0.0038	0.038	+ 5.2	+ 83	1.05	0.99	0.96
S.D.	0.0019	0.011	7.3	55	0.22	0.17	0.14

* after further 20 min mannitol infusion

Table 1.7. Changes in tubular volume and transit times in control rats, following infusion of Ringer's solution at 0.06 ml./min for 20 min.

Experiment No.	V (ml./min)		Tubular volume change (%)		Transit time ($\frac{exp.}{control}$)		
	Control	Experiment	Proximal	Distal	Proximal	Early distal	Late distal
18	0.0084	0.014	+ 10	+ 33	0.82	0.89	0.73
30	0.0025	0.0027	+ 5	- 5	0.90	0.76	0.80
31	0.0033	0.0083	0	- 17	0.93	0.91	0.81
100	0.0078	0.013	- 1	- 11	0.91	0.72	0.77
103	0.0100	0.0086	- 4	+ 14	0.85	0.83	0.73
106	0.0069	0.011	- 6	+ 18	0.88	0.71	0.93
Mean	0.0065	0.0096	+0.67	+5.3	0.88	0.80	0.79
S.D.	0.0030	0.0041	5.9	19	0.04	0.09	0.07

Table 1.8. The effect of angiotensin and noradrenaline infusion (0.5 µg/kg/min) on lissamine green capillary appearance time

(a) Angiotensin			(b) Noradrenaline		
Experiment No.	Capillary appearance time (sec)		Experiment No.	Capillary appearance time (sec)	
	Control	Experiment		Control	Experiment
5	2	3	12	2	1
6	2	3.5	13	2	2
7	3	4	14	2	2
8	3	4	16	2	2
10	2	3	19	2	1
16	2	3	20	2	2
19	1.5	2.5	21	2	2
22	2	3	59	2	2
57	2	2	82	2	1.5
Mean	2.2	3.1		2	1.7

Capillary appearance time = time from intravenous injection of dye bolus to appearance of lissamine green in capillaries of renal surface.

Table 1.9. Changes in lissamine green clearance during angiotensin infusion (0.5 µg/kg/min)

Experiment	V (ml./min)		Lissamine green clearance (ml./min)	
	Control	Experiment	Control	Experiment
A	0.0024	0.010	0.40	0.37
B	0.023	0.044	0.32	0.30
C	0.0016	0.0082	0.33	0.24

Figures for urine flow and lissamine green clearance refer to mean of 3 collection periods.

Table 1.10. Changes in kidney size and superficial tubular size during angiotensin diuresis

Experiment No.	V (ml./min)		Distal tubular diameter during angiotensin infusion (μ)	Change in proximal tubular volume during angiotensin infusion (%)	Change in kidney size on terminating infusion*
	Control	Experiment			
101	0.032	0.021	15	-37	increase
86	0.002	0.013	-	-	increase
91	0.002	0.018	-	-	increase
124	0.001	0.03	21	+29	decrease
78	0.007	0.035	-	-	increase
118	0.005	0.035	17	- 1	decrease
80	0.002	0.04	-	-	no change
102	0.007	0.043	20	-32	no change
120	0.005	0.043	20	-	decrease
79	0.005	0.047	23	- 1	no change
92	0.002	0.047	19	+10	decrease
104	0.01	0.055	24	0	decrease
98	0.006	0.067	33	+49	decrease
87	0.006	0.075	-	-	decrease
121	0.008	0.094	25	+61	decrease
77	0.04	0.10	28	- 4	decrease
119	0.002	0.10	-	-	decrease

- signifies no measurements available.

* as observed by degree of coarse focus adjustment on microscope stage necessary to maintain sharp image. In all cases, kidney size decreased on commencing infusion.

DISCUSSION

This investigation revealed marked differences between the effects of angiotensin on renal function and tubular flow characteristics, and those of the other diuretic agents examined.

It was found that large increases in urine volume flow caused marked tubular dilatation with all substances investigated but that at low urine flow rates, a differential effect of angiotensin could clearly be demonstrated. Two experimental protocols, in non-diuretic and in mildly saline diuretic rats, were therefore adopted.

The use of Inactin as anaesthetic permitted a level of resting urine flow similar to that found in conscious rats, which could be readily increased by diuretic agents. In preliminary experiments, pentobarbitone and urethane were found to produce a much lower level of diuresis. Inactin enables the production of a water diuresis in the rat and has been recommended for experiments on urine flow in anaesthetised rats (Wirz, 1957).

Noradrenaline was found to produce a diuretic effect on infusion at 0.5 µg/kg/min, which was considerably more pronounced in animals undergoing mild saline diuresis. This observation is in keeping with the known diuretic properties of catecholamines in unanaesthetised rats (Dexter & Stoner, 1952; Green & Sim, 1961).

Diuresis elicited by angiotensin infusion (0.5 ng/kg/min) was associated with a greater excretion of sodium than that following noradrenaline infusion. Similarly, angiotensin has a greater natriuretic tendency than noradrenaline in cirrhotic patients, following infusion of equipressor amounts of the two substances (Laragh et al., 1963).

It is difficult to draw conclusions about the diuretic potency of angiotensin compared to noradrenaline from these results, since the dose of angiotensin used produced a greater rise in blood pressure than that produced by noradrenaline. The effects of the two substances on the kidney are greatly different, since angiotensin produced a reduction in GFR, which noradrenaline did not. The two substances may also affect the vasculature supplying superficial and deeper glomeruli in a different way. The studies with noradrenaline show that other pressor substances may also increase urine flow and electrolyte excretion, but the degree of diuresis produced could not be related either with angiotensin or noradrenaline to the extent of the pressor response. Similarly, Bonjour & Malvin (1969) could find no correlation between the degree of natriuresis produced by angiotensin infusions in anaesthetised rats, and the increase in blood pressure; indeed, diuresis can be elicited in the rat by sub-pressor doses of angiotensin (Bonjour et al., 1968). However, under the conditions of the present experiments, mean systemic pressure

was sometimes elevated to levels above 180 mm Hg by the pressor agents, so that pressure diuresis must be considered as contributing to the effects observed. Pressure diuresis has not been demonstrated to occur in the rat, apart from the observation in the isolated, perfused rat kidney (Tobian, Coffee, Ferreira & Meuli, 1964) that a high perfusion pressure, ~~elevates distal delivery, fluid volume, and therefore~~ may inhibit distal tubular reabsorption of sodium.

In this investigation, diuresis produced by 0.5 µg/kg/min angiotensin was accompanied by a significant reduction in GFR, as observed by Malvin & Vander (1967) in conscious rats. Bonjour & Malvin (1969) found a significant reduction in GFR in anaesthetised rats infused at 0.25 µg/kg/min, but doses in the order of 0.1 to 0.2 µg/kg/min may produce little or no change in GFR (Peters, 1963; Malvin & Vander, 1967).

The initial blanching of the kidney and collapse of proximal tubules observed on commencing the angiotensin infusion is strongly indicative of afferent arteriolar constriction, and is similar to the effect reported by Leyssac (1965a) following the intravenous injection of up to 25 ng angiotensin. Leyssac also observed a reduction in proximal intratubular pressure following angiotensin injections. Such an afferent arteriolar constriction could provide a ready explanation for the reduction in GFR of the whole kidney. The subsequent reopening of proximal tubules despite continued angiotensin

infusion may indicate a tachyphylaxis of the smooth muscle of the afferent arteriole to the effect of angiotensin, but tubular perfusion may also have been modified by further changes in blood pressure, or other readjustments within the kidney.

A similar tachyphylaxis of the renal vasculature of the frog was observed by Wakim, Root & Essex (1941) following topical application of an angiotensin preparation. The first application produced blanching followed by engorgement of glomeruli, whereas repeated doses produced only engorgement. The infusion of large doses of angiotensin in dogs produced a rise followed by a return of blood pressure to near normal levels (Louis & Doyle, 1965) which may also result from a decline in the response of arteriolar smooth muscle to continued presentation of the agonist.

The initial afferent arteriolar vasoconstriction to angiotensin, and reduction in GFR, is presumably responsible for the preliminary antidiuretic period. Subsequently, a vascular adjustment occurs so that afferent and predominantly efferent arteriolar constriction takes place, as indicated by the rise in filtration fraction (Bonjour & Malvin, 1969); urine flow and sodium excretion may be increased or decreased while GFR remains reduced.

When diuresis was produced by noradrenaline, chlorothiazide or osmotic diuretics, distal tubular volume was observed to increase, and when measured directly, a linear relationship was

found between distal tubular diameter and urine flow above 0.01 ml./min. Gottschalk (1958) noticed that at low urine flow in the rat kidney, distal tubular diameter could be increased without an increase in intratubular pressure. The distal tubule is in a partially collapsed state at low urine flow, and a gradient exists between capillary pressure and distal intratubular pressure. By contrast, the proximal tubule is well-distended, and pressure transmission across its wall is efficient, resulting in an equality of pressures in proximal tubules and peritubular capillaries (Gottschalk & Mylle, 1956). When urine flow was increased further by osmotic diuretics, Gottschalk & Mylle (1957) found that distal intratubular pressure increased resulting in an increase in proximal tubular pressure, so that at high urine flow, pressures in proximal and distal tubules and peritubular capillaries were elevated and equivalent. The main resistance to flow in the nephron at high urine flow rates is therefore located in the collecting ducts.

The increase in proximal tubular diameter at high urine flow observed in the present study is in keeping with the known elevation in proximal tubular hydrostatic pressure during osmotic diuresis (Gottschalk & Mylle, 1957; Koch, Dume, Krause & Ochwadt, 1967). Tubular diameter is dependent on the transtubular pressure gradient and on the elasticity of the tubule wall; thus:

$r = \beta P + \alpha$ (Bossert & Schwartz, 1967).

where r = tubular radius,

p = transmural pressure gradient, β and α are constants.

A primary increase in proximal tubular pressure will result in a transient pressure gradient and therefore tubular dilatation. Transmission of intratubular pressure to the peritubular capillaries results in a rise in capillary pressure.

The distal tubule may be sufficiently distensible to permit tubular dilatation without a rise in intratubular pressure as suggested by Gottschalk (1958) although the elevation in pressure may have been too small to be detectable by the method used. The linear rise in distal tubular diameter with urine flow reported here resembles the linear relationship between distal tubular pressure and urine flow (Gottschalk & Mylle, 1957); although tubular diameter and pressure may not always be related due to the limitation imposed by the renal capsule.

The graph of distal tubular diameter with urine flow (Fig. 1.10) shows that distal tubules would remain open at zero urine flow, as has been observed by Steinhausen, Iravani, Schubert & Taugner (1963) following supra-renal aortic occlusion.

In the majority of diuretic experiments with angiotensin, proximal tubular volume was reduced, which was most probably due to the fall in GFR. Partial occlusion of the renal artery, or of the aorta above the renal artery, has been used by many

workers to investigate the effect of a reduced GFR on renal tubular function. Wahl, Liebau, Fischbach & Schnermann (1968) observed a reduction in superficial proximal tubular diameter with renal artery occlusion in diabetes insipidus rats. Baines, Gottschalk & Leyssac (1968) however, find that renal tubular diameter is initially reduced following a fall in GFR, but that after 30 to 60 min, proximal tubular diameter returns to the control level before clamping.

Many inaccuracies surround the measurement of proximal tubular radius from photomicrographs of the renal surface. The proximal tubular wall is not even, but contains cavities and sacculations, resulting in an uneven diameter. The limit of the cell border is also difficult to determine from photographs, because of the presence of the refractile brush border. Such difficulties in measurement may be in part responsible for the disagreement between Wahl, Nagel, Fischbach & Thurau (1967) and Baines et al. (1968) on the relationship between proximal tubular radius and spontaneous variations in GFR. In this study, changes in proximal tubular size were obtained by tracing the lumen. This does not give an absolute measurement of tubular radius, but an integrated measurement of the entire tubular lumen is made.

The distal tubular lumen, however, is much more even, and absolute measurements of diameter are more reproducible. The reduction in proximal tubular volume observed during angiotensin infusion is probably similar to the initial phase

of reduced tubular diameter after renal artery compression described by Baines et al. (1968). A reduced proximal tubular volume was associated with a normal or reduced distal tubular volume. In some diuretic experiments with angiotensin, however, distal tubular diameter was greatly increased at low levels of urine flow. The fact that in such experiments an increase in proximal tubular volume frequently occurred, and that kidney size was increased, indicated that intratubular pressure was probably elevated. Intratubular pressure was not measured in these experiments, however, and so a primary decrease in interstitial pressure in causing tubular dilatation must be considered possible. However in experiments with distended tubules, the kidney appearance strongly resembled the change observed after ureteral compression, which causes an increase in kidney size, distension of proximal and distal tubules and prolongation of lissamine green transit times (Rector et al., 1966). Thus a degree of internal hydronephrosis apparently develops in some rats during angiotensin infusion, which must be due to an increase in resistance to flow in the collecting ducts, since the ureter was cannulated up to the level of the renal hilum. A possible mechanism for this change, viz. by constriction of smooth muscle in the renal pelvis, is considered in the next chapter.

Proximal tubular and peritubular capillary pressure remains constant over a wide range of spontaneous variation in blood pressure (Gottschalk & Mylle, 1956; Thurau & Wober,

1962). Koch et al. (1968) observed an increase in proximal tubular pressure and tubular dilatation when systemic pressure was elevated by bilateral carotid artery occlusion and vagotomy in the rat. However in their experiments, carotid occlusion resulted in a large increase in urine flow and GFR which would be expected to cause a rise in intratubular pressure. The changes in tubular diameters during angiotensin infusion, therefore, are unlikely to be due to the increased arterial pressure alone.

Proximal tubular intrinsic reabsorptive rate has been shown to be related to r^2 (Gertz et al., 1965; Rector et al., 1966). However in assessing the role of tubular size in reabsorption, the ratio $(\frac{\pi r^2 d}{Vo})$, or tubular volume per unit nephron GFR, has to be determined by micropuncture techniques (Brunner, Rector & Seldin, 1966). Therefore in the present experiments where proximal tubular volume was reduced during angiotensin infusion, it is not possible to conclude whether the ratio $(\frac{\pi r^2 d}{Vo})$ was altered. However in the experiments in which proximal tubular volume was increased, there was almost certainly a great increase in $(\frac{\pi r^2 d}{Vo})$, which would be expected to result in an enhanced proximal tubular reabsorption. Earley & Friedler (1966), on the basis of experiments on vasodilated dog kidneys, postulated that diuresis caused by pressor agents was due to a transmission of the systemic pressure to peritubular capillaries and hence to the interstitium. The elevated interstitial pressure was presumed to result in an inhibition of

proximal reabsorption, possibly by tubular compression. Later, Martino & Earley (1968) found that renal capillary pressure was transiently increased following angiotensin infusion, which was suggested to set up an increased accumulation of interstitial fluid and hence to reduce reabsorption.

The finding of an increased proximal tubular volume during angiotensin diuresis in the present investigation demonstrates that a reduction in proximal tubular calibre *per se* cannot be the mechanism leading to an inhibition of reabsorption. In addition, a transient increase in proximal and distal tubular diameter was always noticed at the onset of angiotensin diuresis, which presumably occurred secondary to a large increase in flow delivered to the collecting ducts. Therefore an elevated proximal tubular pressure transmitted to the peritubular capillaries may have caused the increased capillary pressure observed by Martino and Earley.

The profound prolongation of proximal and distal transit times caused by angiotensin resembles the effect of renal artery constriction. When renal perfusion pressure is reduced sufficiently to cause a fall in GFR, proximal transit time is prolonged in non-diuretic rats (Gertz *et al.*, 1965; Brenner, Bennett & Berliner, 1968) but not in rats undergoing a high rate of saline diuresis (Rector *et al.*, 1966). In addition, Landwehr, Schnermann, Klose & Giebisch (1968) and Baines *et al.* (1968) have observed an increased lissamine green distal appearance time following renal artery constriction.

The prolongation of capillary appearance time observed could be explained by a reduction in cardiac output. However cardiac output has been shown by Mandel & Sapirstein (1962) to be unchanged following the infusion of angiotensin at 0.5 μg/kg/min. Hence the effect was probably due to the reduction in RBF, which would cause an increase in the mean transit time of a dye bolus.

In the experiments with diuretic substances other than angiotensin, transit times were reduced and lissamine green density in distal tubules was diminished as would be expected during an increase in tubular flow rate. The prolongation of transit times noticed at high urine flow was probably due to tubular distension, although slight reductions in GFR may have participated in this effect.

During angiotensin diuresis, however, distal transit time was in many cases increased considerably more than the increase in distal tubular volume recorded at the same level of urine flow, indicating that distal tubular volume flow rate was lower than in the pre-infusion period. Comparison of transit time and cross sectional area changes in this way can only yield an approximate estimate of flow rate changes, since the transit time refers to loop of Henle passage as well as the distal tubule, and loop distension cannot be estimated. However the profound increases in transit time in experiments where tubular volume was not greatly increased (e.g. Nos. 67, 68 and 71 in Table 1.2) lend greater significance to the presumed

reduction in distal flow rate. In addition, individual distal convolutions sometimes remained coloured for several minutes emphasising the greatly reduced flow velocity.

The qualitative observation of an increased lissamine green density in distal tubules during angiotensin diuresis, while lissamine green clearance for the whole kidney was reduced, was strongly indicative of an increased reabsorption of tubular fluid. The reappearance of green dye in distal tubules following the onset of angiotensin infusion (p.61) was probably due to the concentration of tubular fluid in the loop of Henle and distal tubule during a period of stasis.

Since urine flow was increased at a time when superficial distal nephron flow rate was apparently reduced, the diuresis caused by angiotensin must result from an increased flow rate in nephrons situated in the deeper parts of the cortex. The alternative, that dilution of tubular fluid takes place in the collecting ducts, is so unlikely as to be considered impossible.

In superficial nephrons, the diminished flow velocity caused by angiotensin may possibly lead to further concentration of tubular contents.

Velocity of flow of tubular fluid is a parameter which is difficult to relate directly to reabsorption; however Ullrich, Kramer & Boylan (1961), in an analysis of counter-current exchange, note that reduction in flow velocity would be

expected to lead to enhanced reabsorption in the loop of Henle. Gertz et al. (1965) found a linear relationship between proximal transit time and reabsorption, and formulated an equation relating them:

$$\ln \left(\frac{TF}{P}\right)_{IN} = K.T,$$

where $\left(\frac{TF}{P}\right)_{IN}$ is the ratio of tubular fluid to plasma inulin concentration, K is the reabsorptive rate constant, and T is the transit time.

In experiments where reduction in GFR is accompanied by a prolongation of proximal transit time, proximal $\left(\frac{TF}{P}\right)_{IN}$ is increased indicating enhanced reabsorption (Brenner, Bennett & Berliner, 1968; Landwehr et al., 1968; Wahl et al., 1968).

The relationship between linear flow velocity, tubular volume and reabsorption was studied by microperfusion of single rat proximal tubules (Wiederholt, Hierholzer, Windhager & Giebisch, 1967). Reabsorption was related directly to tubular volume but not to linear flow velocity; the effect of increments in linear flow velocity in reducing reabsorption was obscured by the simultaneous increase in tubular volume. However, when flow rate was increased without change in tubular volume, resulting in an increased linear flow velocity of fluid, fractional reabsorption was observed to decrease.

Ureteral occlusion results in an increased proximal

transit time, which was found by Rector *et al.* (1966) to be associated with an enhanced reabsorption, although Brenner *et al.* (1968), in a later study, observed little change in proximal $(\frac{TF}{P})_{IN}$ ratio in the same situation. Technical errors in fluid collection as a result of the increased intratubular pressure could have caused the high values of Rector *et al.*

The results from micropuncture experiments indicate an important role of tubular volume and transit time in reabsorption. In many experimental manoevres, however, reabsorptive capacity may be determined largely by changes in peritubular factors which may obscure the effects of changes in tubular radius or flow velocity. Partial renal venous occlusion, for example, leads to a reduction in absolute reabsorption without change in tubular radius, but as a function of renal plasma flow (Lewy & Windhager, 1968).

The distribution of total kidney GFR in the rat has recently been shown to vary markedly during salt deprivation, and following the administration of vasopressin (Horster & Thurau, 1968). Renal artery occlusion in the dog has also been shown to result in a greater reduction in superficial than in total GFR (Liebau, Levine & Thurau, 1968). In view of the known diversity of individual nephron GFR, it is quite likely that angiotensin could cause a profound reduction in superficial nephron GFR, but a less marked reduction, or even an increase, in juxta-medullary nephron GFR. It is therefore impossible to

predict changes in transit times in all nephrons from those observed in the most superficial tubules. In those nephrons in which reabsorption is enhanced, a prolongation of transit time may assist this effect. The increase in tubular volume observed in superficial nephrons, however, is more likely to be reflected throughout the whole kidney, since it appears to be due to an increased collecting duct resistance.

Angiotensin diuresis is due to a reduction in tubular reabsorption, which may occur in spite of an increased tubular volume. Other peritubular factors which might influence reabsorption during angiotensin infusion will be considered in Chapter IV.

CHAPTER II

Situation and function of smooth muscle of the renal pelvis - response of isolated pelvis muscle to angiotensin and other drugs

INTRODUCTION

The observation of an over-distension of distal tubules during angiotensin diuresis, strongly indicated that resistance to flow in the collecting ducts was elevated by angiotensin, resulting in an increased intratubular pressure. Dilatation of tubules could result from an increased filtration fraction, causing elevated peritubular capillary oncotic pressure, and decreased interstitial volume and pressure (Lewy & Windhager, 1968). However, the increase in total kidney volume in those experiments showing over-distension of tubules was suggestive of an elevated intratubular pressure.

Increased resistance to flow in the medullary region of the rat kidney was observed by Lewy & Windhager (1968) following renal vein occlusion. This manoevre resulted in marked dilatation of occasional distal tubules on the renal surface, and those tubules which showed over-distension were found to have an increased intratubular pressure. Thus an increased blood volume of the kidney could cause compression of collecting ducts by dilated medullary blood vessels, or interlobar and arcuate veins and arteries. However, kidney blood volume is reduced during angiotensin infusion, as shown by the reduction in kidney size (Chapter III). An increase in medullary blood flow, or in medullary interstitial pressure, could also result in compression of collecting ducts, but medullary blood flow appears to be reduced by angiotensin, as

shown by tracer injections of vascular markers (Chapter IV).

An alternative possibility in causing collecting duct compression appeared as a result of the observation that the papilla of the rat kidney is in intimate contact with surrounding musculature (Fig. 2.1). The papilla of young rats, exposed by removing the upper part of the ureter, can be seen to exhibit rhythmic circular movements. Steinhausen (1964) noticed rhythmic contractions of musculature at the base of the exposed hamster papilla; a corresponding rhythmical flow of tubular fluid could also be detected in some of the superficial collecting ducts.

The existence of intrarenal smooth muscle was noted by Henle (1866) who postulated that circular muscle fibres situated near the base of the papilla could exert a milking action on the papillary contents. A milking action was also ascribed to the calyceal muscle by Muschat (1926).

The role of intrarenal smooth muscle in assisting the expulsion of urine from the kidney is difficult or impossible to prove, due to the inadequacy of the investigative techniques available. In the rat kidney, however, circular smooth muscle fibres can clearly be seen to surround the apex of the papilla. Since angiotensin possesses potent smooth musole stimulant properties, an isolated preparation of the intrarenal smooth muscle of the rat has been developed, to enable the study of the action of angiotensin and other drugs on contraction.

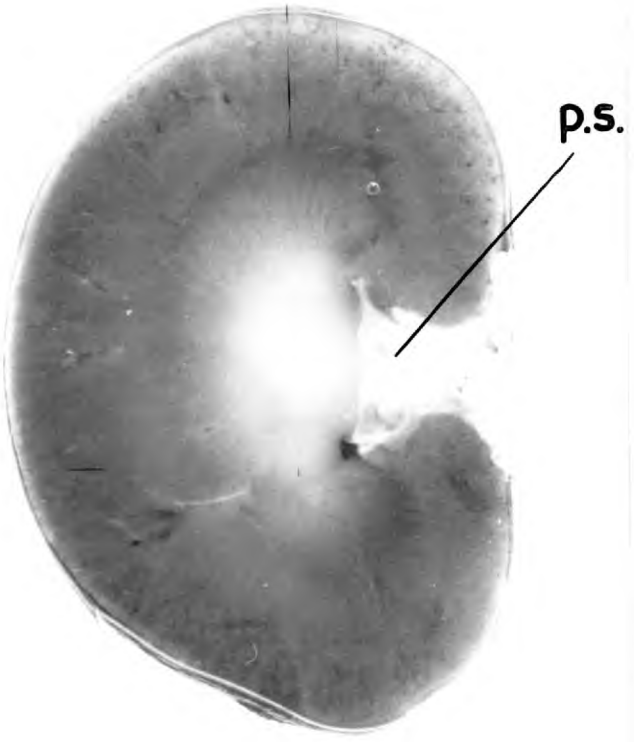


Fig. 2.1. Rat kidney hemisected to show the pelvic septum (p.s.) containing muscular tissue, overlying the tip of the papilla. X4.

The rat kidney is unipapillate, and no true renal pelvis exists, the apex of the papilla being in close apposition to the origin of the ureter. The papilla is, however, surrounded by a pelvic space of complex structure (Pfeiffer, 1968). The term pelvis muscle (Pfeiffer, 1968) or pelvic septum (Sheehan & Davis, 1959) will be used to describe the tissue surrounding the apex of the papilla and appearing as an anatomical extension of the ureter.

METHODS

Rats (250 to 300 g) were killed by a blow on the head, and a kidney removed. The pelvic septum was exposed by a razor cut which hemisected the kidney in the sagittal plane, passing through the sinus renalis to one side of the papilla (Fig. 2.1). The bulk of the parenchyma was then separated by a transverse cut, and the papilla removed. A fine scissor point was passed into the pelvic cavity and so to the ureter, and the pelvic septum divided at one point, thus enabling the muscular portion to be opened out, and remaining renal parenchyma and ureteric tissue trimmed off.

Using a fine atraumatic suture (Mersilk, 7.0) the muscle strip was suspended in an organ bath (15 ml. capacity) and attached to a calibrated Statham isometric strain gauge (± 0.15 oz. maximum load). Contractions were recorded on a Sanborn direct writing recorder. The preparation was placed in Locke's solution, gassed with 95% O_2 + 5% CO_2, at 38°C, and given a resting tension of 50 mg. Following a 30 min equilibration period, drugs were added in a 3 min cycle allowing a 30 sec contact time.

In 4 rats anaesthetised with Inactin, side arm free flow hydrostatic pressure was recorded from a ureteric catheter inserted up to the level of the hilum of the exposed left kidney. The catheter was made of ppl0 tubing attached to

a pp30 T-piece, and the free end was kept in a saline reservoir, the surface of which was 1 to 2 cm below the renal pelvis. Pressure was measured from the side arm of the T-tube with a Sanborn 267B pressure transducer coupled to a Sanborn recorder.

Drugs

l-noradrenaline and l-adrenaline as acid tartrate: the amounts mentioned in the text refer to the base. Isoprenaline sulphate, tyramine sulphate, nicotine acid tartrate, 5-hydroxytryptamine creatinine sulphate, acetylcholine chloride: the amounts refer to the salts. Phentolamine as Rogitine (Ciba). The catecholamines contained 1 in 100,000 ascorbic acid.

Composition of Locke's solution

NaCl, 9 g; $CaCl_2$, 0.2 g; KCl, 0.42 g; $NaHCO_3$, 0.3 g; glucose, 1 g; distilled water to 1 litre.

RESULTS

The isolated pelvis muscle commenced spontaneous contractions immediately on suspension in Locke's solution. The amplitude of contractions was generally 20 to 50 mg at a rate of 10 to 18 per min. Contractions generally declined in rate, and became less regular, after 1½ to 2 hr.

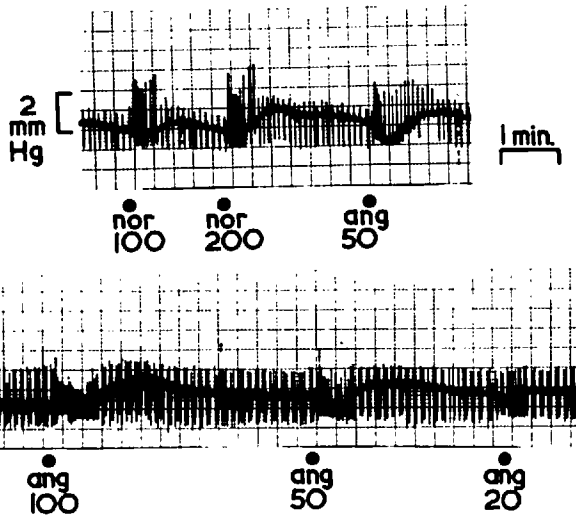
Angiotensin caused a marked increase in tone, and generally reduced the amplitude of spontaneous contractions. Adrenaline produced a similar response, but tended to increase the amplitude of spontaneous contractions. Both substances increased the rate of spontaneous activity. Noradrenaline caused mainly an increased rate and amplitude of spontaneous activity, with only a slight increase in resting tension (Figs. 2.2, 2.3). Because of the spontaneous activity of the preparation, and the variability in response to the agonists of a single muscle strip, the effect was impossible to quantitate accurately. However, in 13 out of 15 preparations in which the same doses of angiotensin and adrenaline were used, angiotensin was equally or slightly more potent than adrenaline in increasing the tone, on a weight basis. There was a great variation in responsiveness of different muscle strips to the agonists, both in sensitivity and in maximum tension developed, but sensitivity to adrenaline and angiotensin in different preparations ran parallel.

Fig. 2.2.

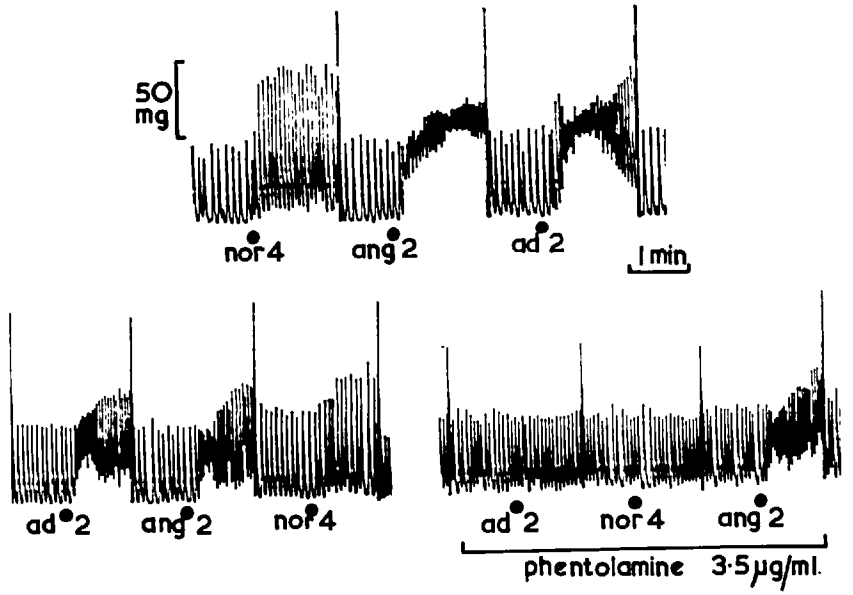
A. Pressure fluctuations recorded from side arm of ureteric catheter passed up to hilum of left kidney of anaesthetised rat. Increased rate of pressure fluctuations following intravenous injection of noradrenaline (nor), 100 and 200 ng, and angiotensin (ang) 20, 50 and 100 ng.

B. Contractions of circular component of rat renal pelvis muscle in Locke's solution at 38°C, gassed with 95% O_2, 5% CO_2. (2 experiments). Responses to noradrenaline (nor) 4 µg, angiotensin (ang) and adrenaline (ad), 2 µg. In right-hand portion of bottom record, phentolamine was present in bath fluid at 3.5 µg/ml. Bath capacity, 15 ml.

A. Ureteric Catheter Free-Flow Pressure



B. Isolated Pelvis Muscle – Circular Component



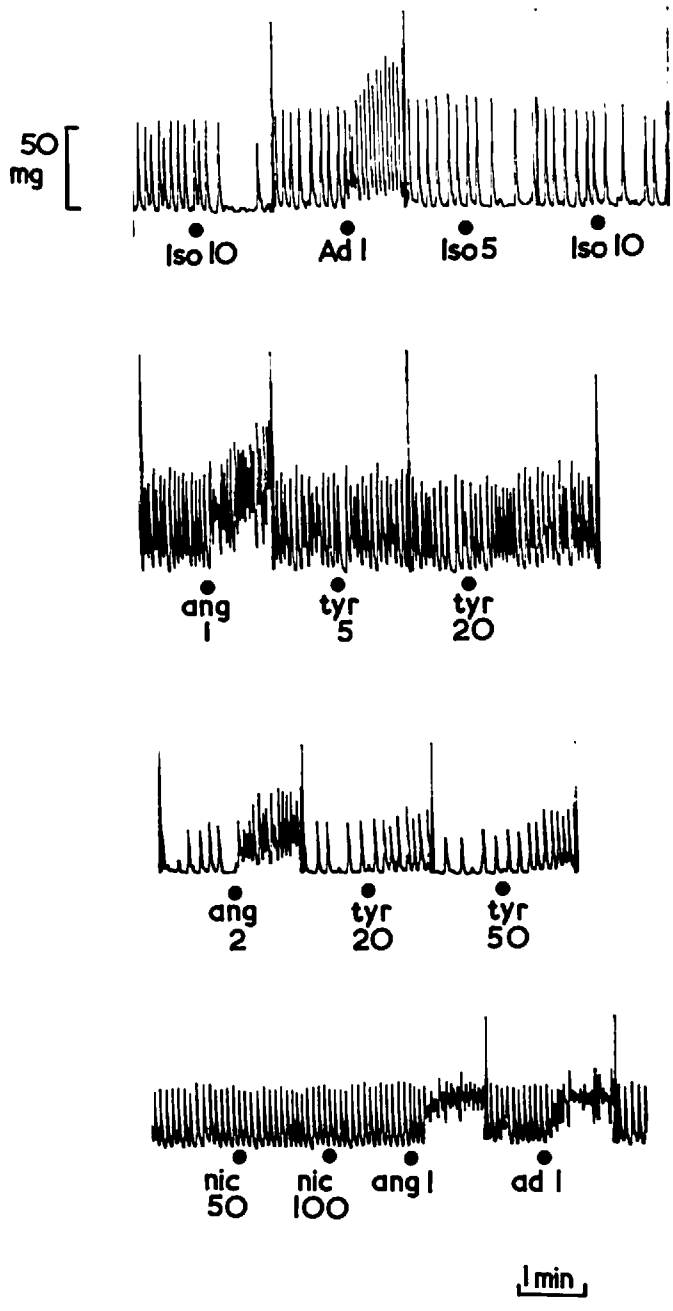


Fig. 2.3. Contractions of circular component of rat renal pelvis muscle in Locke's solution at 38°C, gassed with 95% O_2, 5% CO_2. Responses to isoprenaline (Iso), adrenaline (ad), angiotensin (ang), tyramine (tyr) and nicotine (nic). Figures indicate amount of drug in µg added to bath (15 ml.). 4 experiments.

Most preparations showed a response to angiotensin at 13 ng/ml. but the increase in tone produced varied between 5 and 50 mg. Occasional preparations showed only a poor response to angiotensin (67 ng/ml.) The most sensitive preparation produced a distinct rise in tone to 1.3 ng/ml. of angiotensin.

Both the increase in rate of contraction, as well as the rise in tone, produced by adrenaline and noradrenaline, were prevented by phentolamine (3.5 µg/ml.) added to the bath 2.5 min before the catecholamines. The response to angiotensin was unaffected by this dose of phentolamine (Fig. 2.2B).

Isoprenaline produced either no effect on the pelvis muscle, or an inhibition in the rate of spontaneous activity when given in high dose (0.67 µg/ml.). Nicotine, acetylcholine and 5-hydroxytryptamine produced no increase in tone or spontaneous activity at concentrations up to 6.6 µg/ml. Histamine produced a slight increase in the tone of 1 out of 5 preparations, and tyramine produced a slight increase in the rate of contraction in 3 out of 4 preparations.

A comparison between the effects of these various agonists in increasing tone of the muscle strips, estimated as the average increase in resting tension, is shown in Table 2.1. The doses of angiotensin, noradrenaline and adrenaline selected all produced submaximal responses. Responses of muscle strips to isoprenaline, tyramine and nicotine are shown in Fig. 2.3.

The rate of spontaneous contractions of the isolated

pelvis muscle was similar to the rate of pressure fluctuations recorded from a ureteric catheter during free flow in anaesthetised rats. Angiotensin injected intravenously in a dose (20 to 100 ng) which produces marked renal vasoconstriction in the rat (see Chapter IV) caused a decrease in the free flow pressure, and an increase in the rate of pressure fluctuations. Noradrenaline, however, caused an increase in the rate of pressure fluctuations, and a transient rise in free flow pressure (Fig. 2.2A).

Table 2.1. Approximate increase in resting tension (mg) of isolated rat pelvis muscle to challenge doses of various agonists

Doses refer to amount of drug (µg) added to bath (15 ml. capacity).

Expt.		Angio-tensin	Adrenaline	Nor-adrenaline	Isoprenaline	Tyramine	Histamine	5HT	Nicotine
1	Dose (µg)	1	1	1 10					
	Response (mg)	25	20	12.5 25					
2	Dose (µg)	1	1	1					
	Response (mg)	75	50	20					
3	Dose (µg)	1	1	1					
	Response (mg)	25	25	5					
4	Dose (µg)	1	1	1	10				
	Response (mg)	35	10	5	inhibition				
5	Dose (µg)	1	1	2			10 50	50 100	
	Response (mg)	10	15	10			10 15	slight inhibition	
6	Dose (µg)	0.2	1	1					5 100
	Response (mg)	25	25	15					no effect
7	Dose (µg)	1	1	2					50 100
	Response (mg)	25	25	15					no effect
8	Dose (µg)	1	1						
	Response (mg)	5	12.5						
9	Dose (µg)	1	1	1					
	Response (mg)	50	50	17.5					
10	Dose (µg)	1	1						
	Response (mg)	35	30						
11	Dose (µg)	0.5	1			20	10		
	Response (mg)	15	20			Incr. rate	no effect		
12	Dose (µg)	1	1		5 10	20	100		
	Response (mg)	50	35		inhibition	no effect	no effect		
13	Dose (µg)	0.2	0.5		10 20	5 10	10 50		
	Response (mg)	30	25		no effect	Incr. rate	no effect		
14	Dose (µg)	2	2		20	20 50	10 50		
	Response (mg)	7.5	10		no effect	Incr. rate	no effect		

"Inhibition" signifies reduction in rate of spontaneous contractions.

"Incr. rate" indicates an increase in rate of spontaneous contractions with insignificant increase in resting tension.

DISCUSSION

The role of intrarenal smooth muscle in assisting the expulsion of urine from the kidney must vary greatly between species, owing to the structural diversity of the renal pelvis and calyces. In addition, the inadequacy of the techniques available for investigating this problem has led to a variety of opinions on the interpretation of the results.

Muschat (1926) following one observation on haematuria in man, postulated that constriction of circular muscle around the papilla could cause venous congestion, and could possibly be responsible for reflex anuria. By making serial sections of the calyces, he found the smooth muscle to be oriented as a single shallow spiral, giving the appearance of two circular bands joined by an oblique strip. Muschat (1928) studied the pressure developed in single pig calyces suspended *in vitro*; the calyx was found to expel its entire contents every 30 sec. Subsequently (1929) he studied strips of spiral muscle from pig calyces *in vitro*, which showed spontaneous contractions at a rate of 10 to 12 per min, and found adrenaline to cause a marked rise in tone of the preparation. These observations by Muschat appear to be the only previous studies on the contractions of intrarenal smooth muscle *in vitro*.

Narath (1951) in a much larger investigation of human calyces, found that the circular muscle does not generally

exist as an intact band around the papilla. Narath proposed that longitudinally oriented muscle bundles were able to protect the canaliculi from reflux, by elevating the fornix against the ducti Bellini during contractions of the pelvis. Kiil (1957), however, found that intrapelvic pressure never increased greatly during contraction of the pelvis, and argued against the necessity for such a mechanism.

In the rat kidneys examined, the tip of the papilla was always observed to be in contact with the surrounding pelvis muscle, with the exception of one or two heavier rats in which the pelvis muscle lay just below the tip of the papilla. In the young rat, the papilla projects into the proximal part of the ureter, and is thus surrounded by a greater amount of muscular tissue, which may play an important role in expelling urine from it. The situation of pelvis muscle in contact with the papillary tip has been noticed in dissected kidneys from 3 rodents (rat, hamster and jerboa) which have a long papilla, suggesting that the "milking" action of the smooth muscle may assist urine expulsion from the long collecting ducts.

In fixed specimens, shrinkage artefacts cause an increase in the apparent space between papilla and surrounding muscular septum (Fig. 2.4) but the appearance of the smooth muscle as an intact circular band can easily be seen in the region of the papillary tip. The circular muscle is

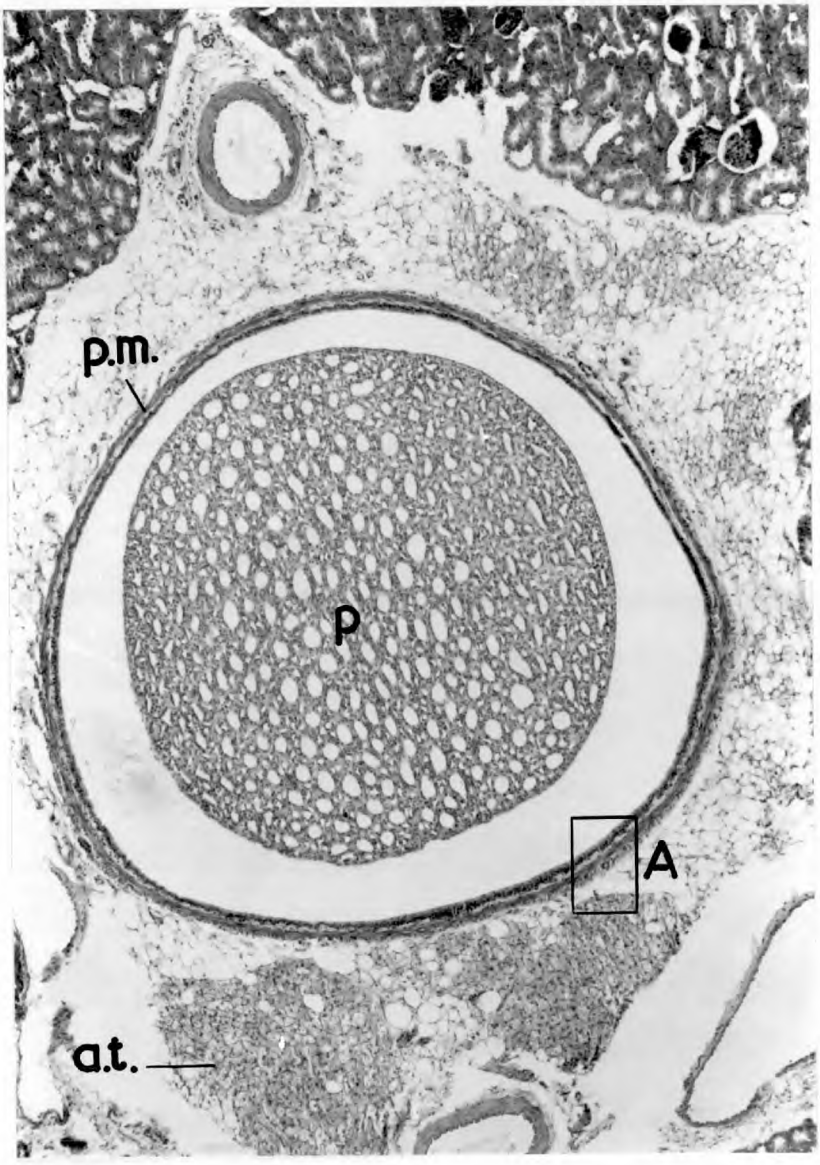


Fig. 2.4. Transverse section of rat kidney, about 0.6 mm from tip of papilla.
p = papilla, p.m. = pelvis muscle, a.t. = adipose tissue of sinus renalis.
H & E stain, X64.

surrounded by oblique and longitudinally oriented bands (Fig. 2.5), and is loosely attached to the renal parenchyma by fatty tissue.

More than 2 mm from the tip of the papilla, the intact circular band appears disrupted, as the crescentic border of the pelvic septum is approached, and oblique or longitudinal muscle bands become predominant, arising from the mucosa overlying the interlobar arteries and veins. The pelvic mucosa receives a plentiful blood supply from the interlobar arteries (Moffat & Fourman, 1963), and so its vascular supply would not be hindered by sectioning of the upper ureter.

The ureteric free flow pressure tracings demonstrate the continuous activity of the pelvis muscle *in vivo*. The free flow pressure is dependent on the rate of urine flow in the catheter and bears no relation to the tone of the musculature. However, the rate of pressure fluctuations *in vivo* corresponds closely to the rate of spontaneous contractions seen *in vitro*. In addition, the increase in rate of contractions produced by angiotensin and noradrenaline was seen both *in vitro* and *in vivo*.

The contractions of isolated pelvis muscle described here all refer to the circular component. When the muscle strip was suspended so as to record the longitudinal component, similar spontaneous contractions, but of a smaller amplitude, were recorded. Continuing contractions have been observed by incident-light microscopy, in the pelvic musculature of a

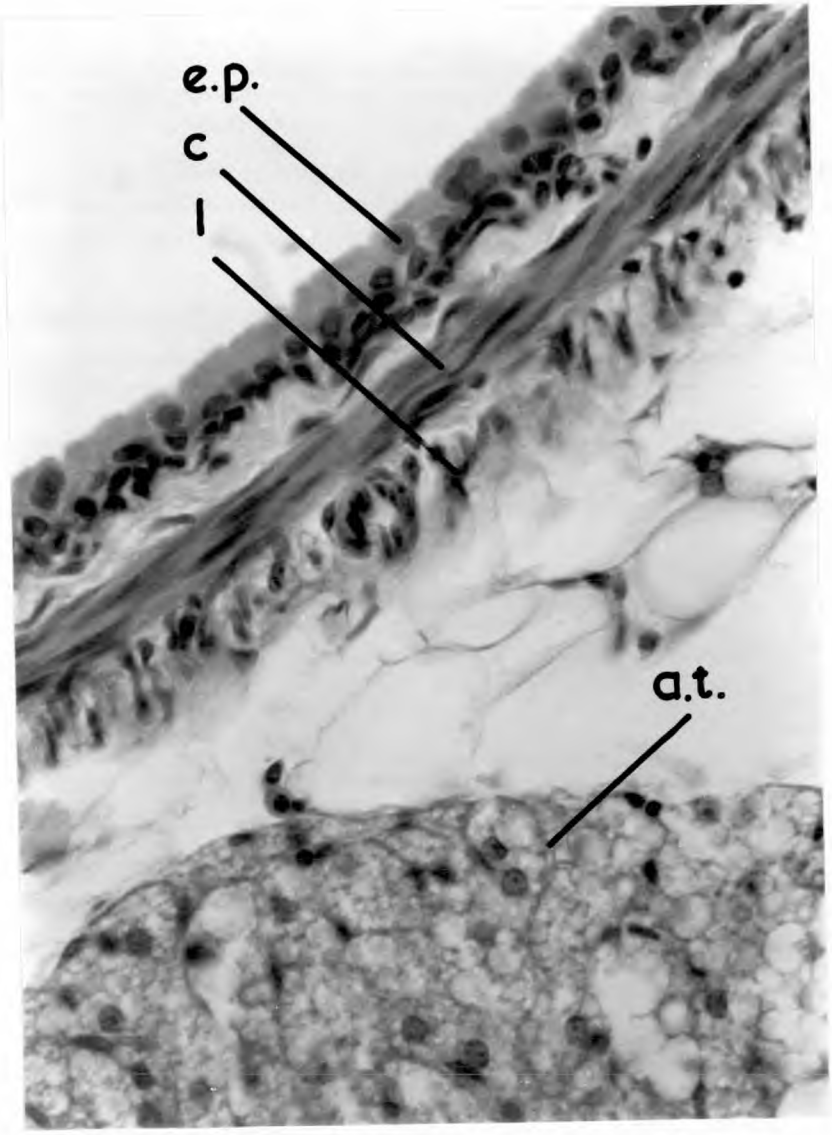


Fig. 2.5. High-power view of region A outlined in Fig. 2.4. e.p. = epithelium, c = circular smooth muscle, l = longitudinal smooth muscle, a.t. = adipose tissue. X1,000.

hemisected rat kidney placed in Locke's solution at 38°C, and appear to be a combination of circular and longitudinal components. It is possible that such contractions assist in expelling urine from the papilla during life. The situation of the circular pelvis muscle around the tip of the papilla is an ideal one for causing outflow obstruction, since contraction of the circumference of the papilla would result in a reduction in the radius of all collecting ducts, and hence a marked increase in their resistance to flow.

In the present investigation, angiotensin and adrenaline were found to contract the circular muscle, whereas noradrenaline produced only a weak rise in tone. The actions of the catecholamines appeared to be solely on α adrenergic receptors, since they were completely prevented by phentolamine, and since isoprenaline had no stimulant effect on this tissue. Thus the rat renal pelvis muscle is similar to the rabbit uterus (Ahlquist, 1948) in that adrenaline is a more potent stimulant of α receptors than noradrenaline.

The effect of angiotensin appears to be due to direct stimulation of smooth muscle cells. Nicotine produced no contraction of the isolated pelvis muscle, reflecting the absence of ganglion cells from this area (Gruber, 1933; Notley, 1969). Tyramine, which is a potent stimulus to the release of noradrenaline from sympathetic post-ganglionic nerve endings, also produced no increase in tone; thus the contraction produced by angiotensin was unlikely to be due

to stimulation of sympathetic ganglia, or to the release of catecholamines.

A constriction of the circular pelvis muscle could explain the raised distal intratubular pressure during angiotensin diuresis in the rat (Chapter I). The variability in responsiveness of the smooth muscle to agonists could explain the fact that this phenomenon was not invariably observed on angiotensin infusion, although variables in setting up the in vitro preparation may have caused an apparent range of sensitivity. Slight differences in the anatomy of the pelvis may also contribute to the variability observed.

Contraction of the pelvis muscle could be responsible for the observation by Von Mangos & Braun (1967), that infusion of adrenaline in rats was accompanied by an increased pressure gradient between distal tubule and ureter, indicating an increased resistance to flow in the medullary region of the kidney. The level of urine flow attained during adrenaline infusion (1.5 to 3.0 µg/min), however, was not specified, and so a comparison with the results presented in Chapter I is not yet possible. An attempt to correlate pelvis muscle contractility with outflow obstruction was made by examining the response to angiotensin of the pelvis muscle isolated from rats which had shown distal tubular overdistension on angiotensin infusion. However an increased sensitivity could not be demonstrated in this group, and the muscle strips

prepared from animals which had been anaesthetised, showed a poorly maintained spontaneous activity.

Thus although it cannot be proved that an increased pelvis muscle tone could elevate collecting duct flow resistance, yet the available evidence strongly indicates the possibility of such a mechanism. These results also draw attention to the necessity of considering urinary tract flow dynamics, when the action of substances with potential smooth muscle stimulant properties is examined in the intact kidney.

CHAPTER III

Changes in kidney volume during intravenous and intra-arterial infusion of angiotensin

INTRODUCTION

During observation of the renal surface *in vivo*, the onset of angiotensin infusion was seen to be accompanied by marked reduction in kidney size, blanching, and collapse of some or all of the visible proximal convolutions. Subsequently, during the diuretic phase of infusion, kidney volume was seen to be reduced or increased depending on the level of urine flow, and the presence or absence of internal hydronephrosis (Table 1.10). Noradrenaline, in contrast, produced no detectable change in kidney volume at the beginning of an infusion. The marked difference observed between the actions of angiotensin and noradrenaline on kidney volume, prompted a quantitative analysis of these effects, using a plethysmographic technique. The variations in kidney volume during continuous infusion of angiotensin have also been followed plethysmographically.

The significance of changes in kidney volume is discussed later in this Chapter. The acute reduction observed following angiotensin administration is interpreted to largely indicate a reduction in RBF, and has been utilised to investigate the effects of intra-arterially as opposed to intravenously administered angiotensin.

Angiotensin has been shown to cause vasoconstriction in the kidney following administration into the renal artery of the

dog (Page & McCubbin, 1953; del Greco & Page, 1961; Zimmerman, Abboud & Eckstein, 1964), cat (Barer, 1961) and rabbit (Akinkugbe, Brown & Cranston, 1966a). Evidence exists, however, that part of the renal vasoconstrictor effect of angiotensin may be mediated through the sympathetic nervous system, since the fall in RBF caused by an intravenous dose of angiotensin could be abolished by renal denervation, and by treating with guanethidine, bretylium and hydralazine (McGiff & Fasy, 1965). The mammalian kidney is profusely innervated with post-ganglionic, sympathetic nerve fibres (Smith, 1956) and in the rat these have been shown to supply afferent and efferent arterioles (Barajas, 1964; Wagermark, Ungerstedt & Ljungqvist, 1968). Stimulation of the sympathetic nerves supplying the kidney causes a reduction in blood flow, as in most other organs with sympathetic innervation.

In the cat, renal vasoconstriction can be caused by stimulation of specific areas of the brain (Pappenheimer, 1960). Angiotensin may possess a central stimulant action, since administration into the lateral ventricle of the cat (Severs et al., 1966) or vertebral artery of rabbits and dogs (Dickinson & Yu, 1967; Scroop & Lowe, 1968) has been shown to cause a rise in systemic blood pressure by amounts which would not be pressor if infused intravenously. Thus the renal vasoconstriction caused by angiotensin could be due to central stimulation of sympathetic vasoconstrictor neurones

(although the central pressor effect observed by Scroop & Lowe in the dog was apparently mediated through the parasympathetic nervous system). The observation by Bohr & Uchida (1967) that isolated dog renal arterioles did not respond with a constriction to direct application of angiotensin (up to 1.0 μg/ml.) adds further weight to this possibility, although subsequently, Walter & Bassenge (1969) have reported a stimulant effect on similarly prepared strips of larger canine renal arterioles at 0.1 μg/ml.

Administration of angiotensin into the renal artery results in an effect localised to the infused kidney, as shown by the absence of a systemic pressor effect (Akinkugbe, Brown & Cranston, 1966a and b); indeed, the kidney is one of the chief organs which take up and inactivate angiotensin (Bumpus, Smeby, Page & Khairallah, 1964). It was therefore decided to determine whether angiotensin can exert a vasoconstriction on close-arterial administration into the kidney of the rat, since a direct effect on the renal vasculature of this species has not been demonstrated, apart from the observations of Skeggs, Kahn & Shumway (1956) in isolated, saline-perfused kidneys at room temperature. Thurau & Schnermann (1965) have postulated an intra-renal feedback mechanism for regulating sodium excretion, whereby angiotensin, released in response to increased sodium concentration at the macula densa, could reduce GFR by constriction of the afferent arteriole. This hypothesis

assumes that angiotensin is capable of causing a direct vasoconstrictor effect in the rat kidney. Experiments in this Chapter have been carried out to test this assumption.

METHODOLOGY

A. Construction of plethysmograph chamber

The chamber was constructed from a block of resin (Margros Penler embedding resin) in which a large rat kidney had been embedded (Fig. 3.1). The block was shaped, and cut in half with a fine toothed circular saw, the kidney was removed and the cavity enlarged. The bottom part was mounted on a brass rod for attachment to the animal stage (Chapter I) and the top was fitted with ports for filling with oil, and screws for clamping together both portions. A cavity 5 x 4 mm was filed in the lower portion to take the renal vessels and ureter, and this trough, as well as the surfaces between both portions, was liberally coated with soft paraffin to effect waterproofing.

B. Recording of changes in kidney volume

Rats (200 to 300 g) were anaesthetised with Inactin and given 1 ml./100 g Ringer's solution intravenously during preparation as in Chapter I. The trachea was cannulated, and drugs were administered *via* jugular or femoral venous cannulas. Blood pressure was recorded from a femoral or carotid artery, using a Sanborn transducer and recorder. The animal was placed on the warmed stage, the left kidney exposed from a flank incision and placed in the lower part of the plethysmograph chamber. In most experiments, the ureter was cannulated with

Fig. 3.1. Rat kidney plethysmograph chamber, with brass rod for attachment to animal stage.

ppl0 polythene tubing, which passed up to the hilum of the kidney, for the measurement of urine flow rate.

The top of the chamber was fixed in position, and the cavity filled with warm silicone oil, and connected to a separation chamber with PVC tubing. Changes in kidney volume were transmitted by the oil to a column of coloured water, and recorded by the change in meniscus of the water column in a fine 0.1 ml. calibrated pipette, placed horizontally and level with the kidney. Readings were taken every 1 or 2 min, and at the peak of a change in kidney volume. Following intravenous injections, the change in kidney volume was recorded as the difference between the pipette reading immediately preceding the injection, and that at the peak of the response.

C. Intra-arterial infusion of angiotensin

Angiotensin was infused into the renal artery of the rat by two methods.

(1) Aortic cannula: The kidney was exposed by a flank incision, and the aorta and vena cava ligated immediately below the renal artery and vein. Arterial branches immediately opposite the renal artery were ligated. The left carotid artery was exposed, tied distally and clamped proximally. A puncture hole was made in the arterial wall with a No. 16 hypodermic needle, and a ppl0 cannula introduced, and passed down the aorta until the tie was reached. The cannula was securely tied in place

at the carotid artery, and the kidney placed in the plethysmograph for recording of volume changes as above.

The cannula was infused continuously with saline containing heparin (100 U/ml.) from a syringe pump, at 0.04 to 0.05 ml./min. Blood pressure was recorded with the Sanborn transducer and recorder, as inflow pressure proximal to the ppl0 cannula, to avoid bilateral carotid ligation. This method of blood pressure recording produced a pulsatile pressure trace, with a mean pressure 2 to 3 mmHg higher than systemic pressure because of the inflow resistance. Sudden changes in systemic pressure were damped on the inflow pressure trace, however mean inflow pressure during infusions always correlated closely with directly measured blood pressure (obtained by clamping the infusion tubing). Pressure was monitored as the ppl0 cannula was introduced, and in this way, entry into the renal artery, which was indicated by a sudden drop in inflow pressure, could be avoided.

Angiotensin infusions were given at the same rate as the saline infusion, from a paired syringe in the pump, connected to the infusion tubing by a polythene T-piece (Fig. 3.2). Distribution of the infusate was always checked at the end of an experiment by infusing concentrated lissamine green solution. Only those experiments in which dye immediately entered the kidney in high concentration were considered valid intra-arterial infusions.

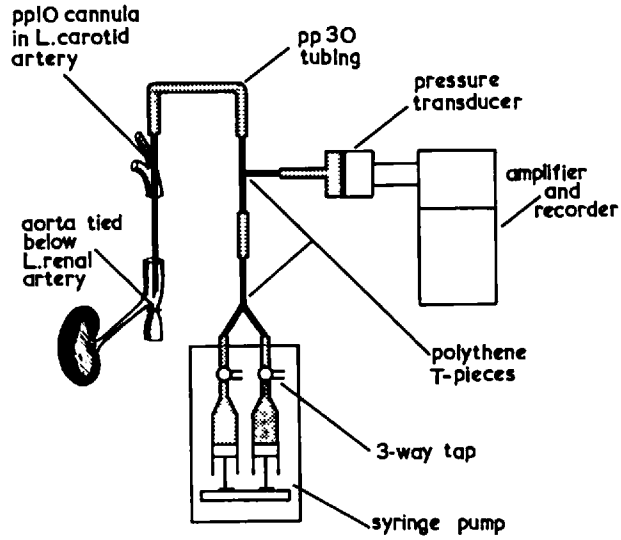


Fig. 3.2. Method of administering intra-arterial infusions by aortic cannula. Polythene T-pieces were constructed from pp30 tubing, in order to present a low dead space. Connections between polythene tubing made with 23 gauge hypodermic needle tubing, except ppl0 cannula which was welded into pp30 tubing.

(2) Indwelling renal artery cannula: In 3 experiments, the renal artery was cannulated and infused directly without tying the aorta. The cannula was constructed from a finely drawn out polythene tube, of external diameter 0.15 mm, which was introduced into the testicular artery. This artery has a variable origin, but frequently arises immediately opposite the left renal artery. Using a stereoscopic dissecting microscope, the cannula was introduced into the testicular artery, and passed across the aorta and into the renal artery. The position of the cannula in the renal artery was checked by post-mortem examination, and in all cases, the cannula had projected 1 to 3 mm into the artery. Ischaemia of the kidney was never observed in this operation, since at the origin of the renal artery, the internal diameter is wide in relation to the cannula used.

The cannula was infused continuously at 0.04 ml./min with heparinised saline, and angiotensin was administered at the same rate, by switching the infusion to a paired syringe on the pump.

RESULTS

The oncometer and recording system resisted leakage during periods of slight negative pressure, and kidney volume reductions of more than 0.15 ml. have been recorded; however, apparent kidney volume tended to increase in experiments where the neighbouring blood vessels had been manipulated in preparation for intra-arterial infusions, and heparin had been infused. In these cases, blood and extracellular fluid were often observed to accumulate inside the oncometer. The kidney always appeared uniformly vascularised and no signs of compression or ischaemia were observed. Pressure changes were rapidly transmitted to the water in the recording pipette, and the meniscus often showed a fine pulsation at arterial pulse frequency.

In these experiments, the volume of the kidney containing blood varied between 1.0 and 1.2 ml.

Intravenous injection of angiotensin produced a dose-dependent shrinkage of the kidney, coinciding with the rise in systemic pressure. Using three moderate doses of angiotensin, a linear relationship could be demonstrated between response and log dose, on the blood pressure and kidney volume (Fig. 3.3). In 6 experiments, responses to noradrenaline and angiotensin were compared in the same rats. In these experiments, several responses to each substance were determined together in order

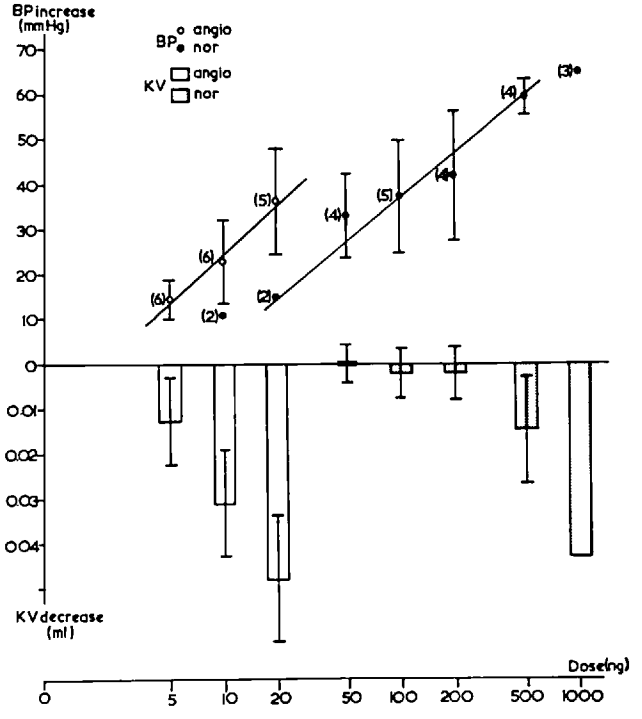


Fig. 3.3. Changes in mean blood pressure (BP) and kidney volume (KV) induced by intravenous injections of angiotensin and noradrenaline in 6 experiments. Numbers of animals in each dose group shown in brackets by blood pressure data.

to avoid "washing in" the injected dose with saline, to keep the dose volume as low as possible. A portion of the results of a single experiment is shown in Fig. 3.4, and the entire results are illustrated graphically in Fig. 3.3.

Noradrenaline was found to have approximately 1/4 to 1/5th of the pressor potency of angiotensin on a weight for weight basis, but was much less potent (1/90 to 1/100th) in reducing kidney volume. Pressor and renal vasoconstrictor responses to angiotensin occurred at approximately the same threshold dose, however doses of noradrenaline of 50 ng and below, which produced strong pressor responses, caused no reduction in kidney volume. Consistent renal vasoconstriction was only seen at very large doses of 0.5 and 1.0 μg of noradrenaline. Adrenaline was also found to cause little change in kidney volume below doses of 100 ng.

In 5 experiments, angiotensin was infused intravenously at 0.5 μg/kg/min, and additionally at 1.0 μg/kg/min in 2 of these. The onset of angiotensin infusion was always marked by a profound and rapid decrease in kidney volume, ranging from 0.05 to 0.14 ml. (for the 0.5 μg/kg/min dose level); subsequently, kidney volume increased gradually throughout the infusion, and rose sharply on terminating the infusion (Fig. 3.5). A sharp rise in urine flow rate during the angiotensin infusion was accompanied by a similar rapid increase in kidney volume, and when urine flow reached a level of about 0.04 ml./min, there was only a slight or equivocal change in kidney volume on

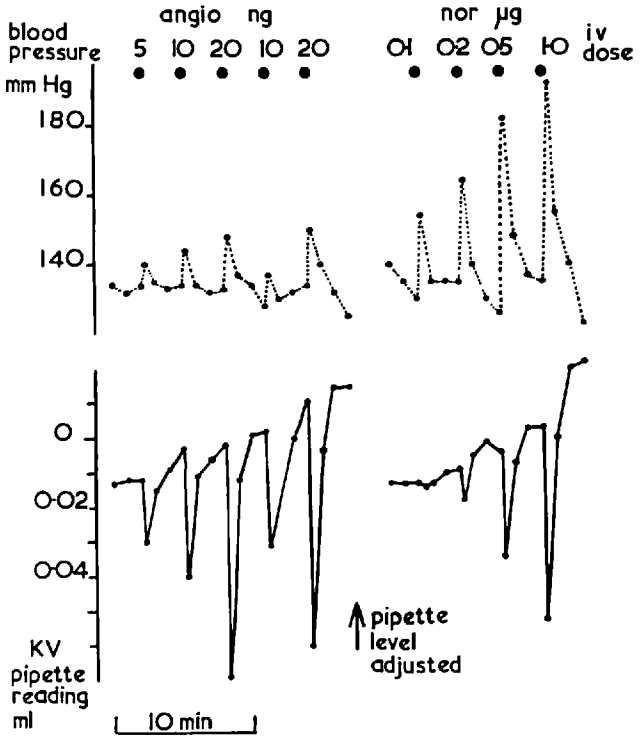


Fig. 3.4. Portions of the results of a single experiment, showing the changes in mean blood pressure and kidney volume (KV) recorded, following the intravenous injection of angiotensin (5, 10 and 20 ng) and noradrenaline (0.1, 0.2, 0.5 and 1.0 µg).

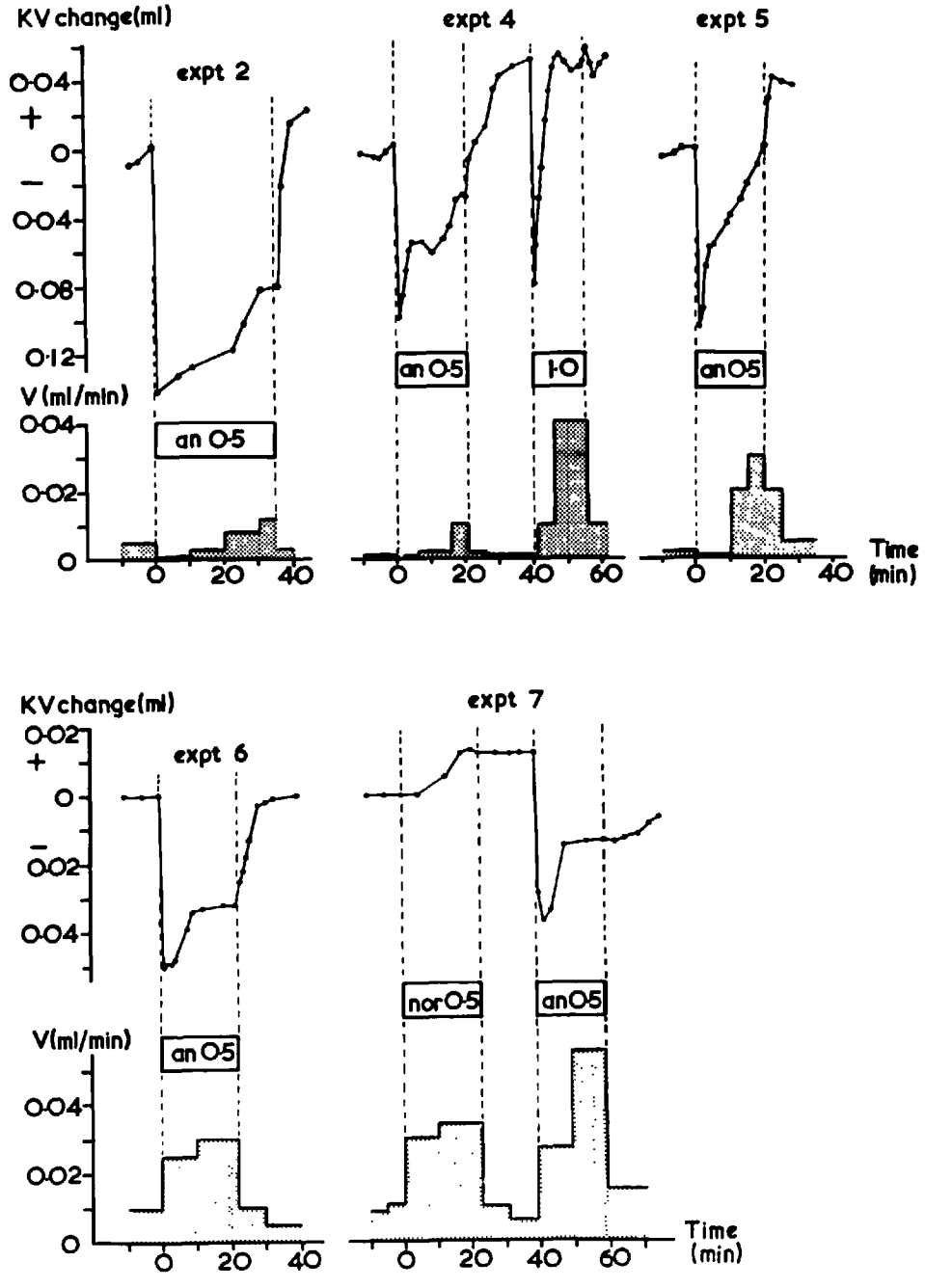


Fig. 3.5. Changes in kidney volume (KV) and urine flow rate (V) in mobilised left kidney during intravenous infusion of angiotensin (an) and noradrenaline (nor). Dose level of infusion shown in μg/kg/min. Volume of kidneys containing blood varied between 1.0 and 1.2 ml.

terminating the infusion (Fig. 3.5, experiment 4). Conversely, a low level of urine flow throughout the angiotensin infusion was accompanied by a persistent, profound depression of kidney volume (Fig. 3.5, experiment 2). Apparent kidney volume as registered by the plethysmograph generally increased slowly throughout these infusion experiments, but the sudden increase in kidney volume on terminating the angiotensin infusion could be easily distinguished from this change.

Noradrenaline infused at 0.5 µg/kg/min for 10 min produced in one experiment a slight increase and in another a slight decrease, in kidney volume, in the order of 1.5 to 3.5% of the total volume of the kidney.

Angiotensin was found to be more potent in reducing kidney volume on intra-arterial than on intravenous administration. In testing the intra-arterial vasoconstrictor potency, the drug was infused at a slow rate (0.04 to 0.05 ml./min) for 5 or 10 min, and the difference between control pipette reading and peak reduction was recorded, since it was felt that bolus injection in the small mixing space might largely by-pass the renal artery. In these experiments, prolonged infusions were not administered, since apparent kidney volume tended to increase from leakage of fluid, which would have made analysis of the results of infusion experiments difficult. However angiotensin infusion caused immediate reductions in kidney volume as in the previous experiments.

Because of the increased amount of surgery performed on these animals, and the aortic ligation, diuretic studies were not performed, since these factors would have modified the diuretic response to angiotensin infusion.

In 3 experiments in which angiotensin was infused in the thoracic part of the aorta, a similar or greater effect on kidney volume resulted as on intravenous infusion, with a slight reduction in pressor response (Table 3.1). When the tip of the arterial cannula was located near the left renal artery, intra-arterial infusion was 2 to 3 times more potent in causing vasoconstriction, and the effect on blood pressure was greatly reduced, but still present. Pressor responses were impossible to measure accurately in these animals, since rhythmic swings in blood pressure linked to respiration occurred, but intra-arterial infusions definitely caused delayed pressor responses in some animals. This was attributed to the fact that not all of the infusion was introduced into the kidney, and some presumably reached the general circulation after passing through peripheral vascular beds.

In 3 experiments, infusions were successfully made directly into the renal artery, and in these cases, angiotensin was found to be 4 to 5 times more potent in causing vasoconstriction than on intravenous administration (Table 3.1). Systemic pressor responses were largely absent following intra-arterial administration, although in one experiment a slowly developing

pressor response occurred to 0.1 µg/kg/min angiotensin. This could indicate that some of the infusate had reached the general circulation, but could also be due to release of pressor amines from the adrenal medulla, as found by Page & McCubbin (1953) during intra-arterial infusion of nicotine in the perfused dog kidney. Dye injections showed that the intra-renal artery infusion perfused the left kidney and adrenal in high concentration, and the kidney volume changes showed that a greater proportion of the infusion reached the kidney by this method than by aortic cannulation. Dye solution infused directly into the renal artery during life resulted in segmental filling of the kidney, so that the whole kidney was eventually coloured, but some areas coloured before others. This was presumably the result of streaming in the renal artery.

Table 3.1. Blood pressure and kidney volume changes following intravenous and intra-arterial infusions of angiotensin

	Blood pressure change (mm Hg)							Kidney volume change (ml.)						
	iv. infusion				ia. infusion			iv. infusion				ia. infusion		
Dose (ug/kg/min)	0.05	0.1	0.2	0.5	0.05	0.1	0.2	0.05	0.1	0.2	0.5	0.05	0.1	0.2
Arterial cannula in thoracic aorta		+12 (3)				+8.7 (3)			-0.017 (3)				-0.022 (3)	
Arterial cannula opposite renal artery	+6 (1)	+14 (6)	+20 (1)	+37 (6)	+5 (2)	+4 (5)		-0.006 (1)	-0.021 (6)	-0.037 (1)	-0.089 (6)	-0.017 (2)	-0.063 (5)	-0.07 (1)
S.D.		9.2		6.1		3.7			0.01		0.034		0.019	
Cannula in renal artery	+5 (3)		+18 (2)	+39 (3)	0 (3)	+10 (1)	0 (1)	-0.003 (3)		-0.046 (2)	-0.087 (3)	-0.053 (3)	-0.103 (1)	-0.098 (1)

Changes shown represent average values during 5 min infusion for blood pressure, and maximum initial value for kidney volume.
Figures in brackets indicate numbers of experiments.
Standard deviation shown of mean of 4 or more experiments.

DISCUSSION

Angiotensin has been shown by Peters (1963) to reduce the renal clearance of PAH in the rat. This has recently been found to occur without any change in the renal extraction ratio of PAH (Bonjour & Malvin, 1969), and therefore indicates a fall in renal plasma flow. Mandel & Sapirstein (1962) also observed a decreased RBF in the rat and a decreased flow fraction of Rb^{86} to the kidney. The profound effect of angiotensin on RBF has been apparent in almost every study determining its effects on the kidney. Because of the small size of the rat kidney, a direct determination of blood flow by flowmeters is, with presently available techniques, difficult. The changes in kidney volume observed here offer additional evidence of the profound renal vasoconstriction produced by angiotensin in the rat.

A fall in RBF will cause a reduction in kidney size by reducing the renal blood volume; but in addition, afferent arteriolar constriction causing a reduction in GFR would lead to reduction in proximal tubular diameter and a further fall in kidney volume. The changes observed with angiotensin injection, therefore, are not the result solely of blood flow reduction.

Apart from reduction in RBF, kidney volume could possibly be decreased by changes in urine flow or lymph flow. However

the reduction in kidney volume observed here immediately following angiotensin injection occurred far more quickly than any conceivable effect on lymph or urine flow.

A decrease in renal blood volume could result from constriction of capacitance venous vessels, in the absence of any marked change in blood flow. This was unlikely to be the cause of the angiotensin induced effect, since in a variety of studies, angiotensin has been shown to be a poorer venoconstrictor than noradrenaline on a weight for weight comparison (Haddy, Molnar, Borden & Texter, 1962; Gross & Bock, 1962; Rose, Kot, Cohn, Freis & Eckert, 1962), but can produce constriction in small veins from monkey, cat and dog limbs (Emerson, Hinshaw & Brake, 1965).

The results obtained on kidney volume changes with angiotensin and noradrenaline are in contrast to those reported by Folkow, Johansson & Mellander (1961) on the cat hind limb. Angiotensin and noradrenaline both caused a reduction in limb blood flow, however noradrenaline produced a much greater reduction in limb volume than angiotensin. It was concluded that receptors to angiotensin were located mainly in smooth muscle of arterioles, whereas those to noradrenaline were present in arteriolar and venous (capacitance) vessels. In view of the results of Folkow et al. it was surprising that noradrenaline produced insignificant changes in kidney volume unless given in large amounts, which may indicate that venous

receptors to noradrenaline are largely absent from the rat kidney. The reduction in kidney volume caused by large doses of noradrenaline, was seen to be accompanied by blanching of the kidney surface when viewed with the incident-light microscope, and was therefore due at least in part to constriction of arterioles.

In contrast to the relatively small renal vasoconstrictor action of noradrenaline compared to angiotensin in the rat, Page & McCubbin (1953) observed a much greater effect of noradrenaline and adrenaline than of angiotensin on the *in situ* blood perfused dog kidney. Noradrenaline has been shown by others to cause vasoconstriction in dog (McGiff & Aviado, 1961; Langston, Guyton, de Poyster & Armstrong, 1962; Zimmerman *et al.*, 1964; Auckland, 1968) and human (Barnett, Blackett, Depoorter, Sanderson & Wilson, 1950; Smythe, Nickel & Bradley, 1952; Laragh *et al.*, 1963) kidneys. In the human, Laragh *et al.* (1963) observed that angiotensin and noradrenaline, in amounts producing similar increases in blood pressure, also caused similar reductions in C_{PAH}. Published investigations in the rat of the action of noradrenaline on renal function, have so far not included any estimation of the changes induced in RBF. The results shown here indicate that the renal vasculature of the rat may respond less effectively to noradrenaline than that of the dog and man, since doses of noradrenaline required to produce renal vasoconstriction

caused very large increases in blood pressure. Proof of this species variation in the response to noradrenaline would involve an investigation of the action of noradrenaline on RBF in conscious rats.

The action of angiotensin on kidney volume determined in the present work, appears to be in contradiction to observations made by earlier workers in dog kidneys. Saline extracts of pig kidneys, which produced larger pressor responses, and can be considered as crude preparations of renin, on intravenous injection into dogs produced an increased kidney volume and diuresis (Bingel & Claus, 1910; Friedman, Abramson & Marx, 1938; Merrill, Williams & Harrison, 1938). Renin injection produced a preliminary reduction in kidney volume followed by an increase, in most of the experiments of Friedman et al. (1938). However in some cases, an increased kidney volume occurred without any previous reduction (Bingel & Claus, 1910; Friedman et al., 1938). In all the experiments of Merrill et al. (1938) renin injection produced a reduction in RBF. The effect of crude renin preparations on kidney volume was markedly different from that of tyramine, which always produced a reduction in RBF and kidney volume (Merrill et al., 1938).

Crude renin preparations and angiotonin produced an increased inulin extraction and a decreased PAH clearance in the dog kidney explanted under the skin of the flank (Corcoran & Page, 1940a and b), indicating efferent arteriolar

constriction. The increased kidney volume produced by renin in the dog was ascribed by Merrill et al. (1938) to efferent arteriolar constriction resulting in increased glomerular size, although it is likely that increased proximal tubular volume would have been a greater factor in increasing kidney size in their experiments. These early results in the dog are in conflict with results of later investigators using pure synthetic angiotensin, which produced either no change or a decrease in inulin clearance in normal, unanaesthetised dogs (Lameijer et al., 1966; Schmid, 1968). It is most likely that impurities in the crude renal extracts were responsible for the discrepancy, either modifying the angiotensin effect or themselves producing the changes observed.

The increased GFR produced by the crude renal extracts would have resulted in an increased tubular volume and diuresis, which may have obscured the tendency of kidney volume to fall due to the reduced RBF. The results obtained here in the rat are in agreement with an afferent arteriolar constriction by angiotensin, since both kidney volume and GFR were reduced. The diuresis produced by angiotensin in the rat results in an increased volume of the distal, and possibly also proximal, part of the nephron (see Chapter I), which causes an increased kidney volume. For this reason, only the rapid initial reduction in kidney volume can be ascribed to a change in blood flow.

Angiotensin was observed to be capable of causing a reduction in kidney volume throughout a period of increased urine flow (Fig. 3.5). In these cases, the reduction in RBF presumably outweighed the increased tubular content of urine. In any infusion experiment with angiotensin, the net change in kidney volume will depend on the degree of reduction in RBF, the degree of diuresis, and the presence of internal hydronephrosis due to outflow obstruction.

Infusion of angiotensin close-arterially to the kidney by intra-aortic administration was initially found to occasionally produce no reduction in kidney volume. However this was the result of a variable amount of the infused dose reaching the kidney, due to streaming effects in the aorta, which could result in most of the dose entering a small branch artery. When the infusion initially perfused the kidney, there was always an immediate reduction in kidney volume, which was of greater magnitude than that caused by the same dose given intravenously. Renal artery infusions also resulted in inadequate mixing of infusate and blood, so that one pole of the kidney was initially infused in higher concentration. For this reason, an exact comparison of the effect of intra-arterially and intravenously administered angiotensin was not possible, but it was observed that the effect of the intra-arterial dose was about 4 to 5 times as great as the intravenous

dose. Allowing for inadequate mixing in the renal artery, it is therefore apparent that the renal vasoconstrictor effect of an intravenous dose of angiotensin in the rat can be entirely accounted for by a direct action on the renal vasculature, and it is unnecessary to implicate as indirect neural mechanism *via* the brain.

These experiments do not, however, prove that the vasoconstriction was caused solely by the effect of the drug on arteriolar smooth muscle, since the kidneys were innervated. In anaesthetised laparotomised cats, constant electrical activity has been demonstrated in the renal nerve (Astrom & Crafoord, 1968), and most probably also exists in rats under the conditions of these experiments. Angiotensin given intra-arterially has been shown to potentiate the renal vasoconstrictor response to low frequency renal nerve stimulation in the dog (Zimmerman & Gisslen, 1968) and so the renal vasoconstriction produced by angiotensin could conceivably be mediated through the potentiation of the effect of sympathetic tone to the arterioles. However in view of the profound nature of the renal vasoconstriction caused by angiotensin, and the relatively weak effect of exogenous noradrenaline, it is considered more likely that the angiotensin effect was due to a direct action on blood vessels. Proof of this conclusion would involve an investigation of the action of angiotensin on the denervated rat kidney, which was

considered outside the scope of this work, since the kidney can only be denervated with certainty by transplantation.

These observations are therefore in agreement with other reports of a unilateral renal vasoconstriction following close-arterial administration of angiotensin. Page & McCubbin (1953) were able to assess the presence of a centrally mediated vasoconstriction by cross-perfusing dog kidneys in situ from donor dogs. In their experiments, angiotensin administered systemically to the recipient in pressor amounts, caused no vasoconstriction in the recipient kidney, indicating that a central sympathetic stimulation to the kidney did not occur. However evidence exists that angiotensin may cause vasoconstriction through stimulation of the sympathetic nervous system in other territories (Scroop & Whelan, 1966), and participation of such a mechanism in the renal response to angiotensin must always be considered; the rat may well respond differently from the dog in this respect. McGiff & Fasy (1965) have shown that drugs paralysing transmission of impulses from sympathetic nerves, and in particular guanethidine, may inhibit renal vasoconstriction due to exogenous angiotensin. This antagonism, however, could only be demonstrated immediately following administration of a large dose of guanethidine. Sympathetic neurone blockade by guanethidine is normally prolonged, although a short lasting ganglion blocking effect follows intravenous administration of the drug (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960).

In two experiments in rats, guanethidine was administered in divided doses of up to 30 mg/kg, and was found to produce a short-lasting inhibition of the renal vasoconstrictor response to intravenously infused angiotensin (0.1 μg/kg/min), but responsiveness returned after 20 to 30 min. Such brief antagonism may well be due to non-specific antagonism between the two drugs. In the experiments of McGiff & Fasy (1965), abolition of responses to renal nerve stimulation by the sympathicolytic drugs was not demonstrated, nor was the control experiment performed of examining the interaction between guanethidine and angiotensin in denervated kidneys. However renal denervation alone prevented the vasoconstrictor effect of intravenous angiotensin, suggesting either a participation of the renal nerves in the normal response, or an alteration in vascular reactivity in this acute condition.

In conclusion, angiotensin produced a profound reduction in kidney volume on close-arterial administration in the innervated rat kidney, which was suggestive of, but did not prove, a direct vasoconstrictor effect of the drug on renal blood vessels. In other species, previous workers have not conclusively demonstrated participation of the renal nerves in the renal vascular response to angiotensin, but its known effect of causing central sympathetic stimulation makes such a mechanism conceivable.

CHAPTER IV

Intrarenal distribution of blood flow during angiotensin infusion

INTRODUCTION

Blood flow rate in the renal medulla is an important factor influencing the degree of urine concentration. Counter-current exchange of solute in the vasa recta maintains the hypertonicity of the medullary interstitium which is built up by counter-current multiplication in the loops of Henle (Wirz, Hargitay & Kuhn, 1951; Wirz, 1953). Berliner, Levinsky, Davidson & Eden (1958) indicated the importance of medullary blood flow rate in maintaining the osmotic gradient in the papilla, and hence in affecting the extent of back-diffusion of water in the collecting ducts. Studies by Thurau & Deetjen (1962) showed that medullary blood flow is not autoregulated, and suggested that increased medullary blood flow could account for the diuresis due to elevated arterial pressure.

Angiotensin possesses both vasoconstrictor and pressor properties, and therefore could obviously be capable of causing profound changes in medullary blood flow rate. Daniel, Prichard & Ward-McQuaid (1954), in an angiographic study in rabbits, observed that renin injection caused ischemia of the renal cortex, but medullary blood flow appeared to be well-maintained. Carriere, Thorburn, O'Morchoe & Barger (1966), measuring Kr^{85} eflux rate to estimate cortical and medullary blood flow, mentioned that angiotensin, noradrenaline and adrenaline caused reduction in cortical but not medullary

blood flow, although no results were presented. Aukland (1968), however, using a polarographic technique, found that adrenaline, noradrenaline and angiotensin infusions reduced total renal and outer medullary blood flows to the same extent.

In this study, the results are reported of the investigation of gross blood flow changes during angiotensin infusion by two techniques which indicate morphologically the intrarenal blood flow distribution. Kahn, Skeggs & Shumway (1950) reported the effects of various drugs, including renin and hypertensin, on Indian ink distribution in rabbit kidneys. Renin and hypertensin were found to cause an increased density of glomerular filling, which was attributed to efferent arteriolar constriction. The present experiments confirmed these observations, and extended them to regional blood flow changes in the rat kidney. The technique of Indian ink injection has been refined in order to indicate regional capillary filling under the conditions obtaining during life, avoiding as far as possible the gross physiological changes occurring after injection of this toxic substance.

The other technique used here involves injection of the fluorescent dye thioflavine S, which stains the walls of the blood vessels through which it passes (Schlegel, 1949). Using this compound, it was hoped to demonstrate any occlusion of vasa recta filling, such as was demonstrated by Fourman & Moffat (1964) following the administration of antidiuretic

hormone.

Using these injection techniques, it was also hoped to investigate any change in the distribution of blood between superficial and deeper glomeruli in the cortex, which may have indicated an increased blood supply and GFR in juxtamedullary glomeruli during angiotensin diuresis (Chapter I).

METHODOLOGY

Male Wistar rats weighing 200-300 g were anaesthetised with Inactin (100 mg/kg intraperitoneally). During surgical preparation, 1 ml./100 g Ringer's solution was administered intravenously as in Chapter I. The trachea was cannulated, tracer substances were administered by a jugular vein cannula, and drugs and infusions were given by a femoral vein cannula. In some experiments, blood pressure was measured from a carotid artery using the Sanborn transducer and recorder. No attempt was made to keep body temperature constant with warmed animal tables.

The left kidney was exposed *via* a flank incision and covered with warm mineral oil. In angiotensin infusion experiments, the kidney was placed in a shaped holder and the ureter cannulated to measure urine flow. In control experiments, the kidney was either similarly placed in a holder, or simply left exposed on the flank. No difference was observed in tracer distribution as a result of placing the kidney in the shaped holder.

After completion of surgery, an equilibration period of 1½ hr was allowed before infusing angiotensin (0.5 μg/kg/min). The vascular markers were injected at varying times after the commencement of angiotensin infusion, or in control kidneys, between 1½ and 2 hr after the completion of surgery.

Indian ink injection

Initial attempts to inject the ink intra-arterially into the aorta via the left carotid artery produced a variable degree of filling of the kidney due to streaming and inadequate mixing in the aorta. Consequently, the ink (undiluted "Reeves" Indian ink) was injected rapidly into the right jugular vein in a dose of 0.1 ml./100 g body weight, and the kidney was removed by cutting across the pedicle at various intervals up to 7 sec after the injection. The kidney was hemisected, rinsed in saline, and 2 to 3 drops of saline were placed on the cut surface, which was covered with a cover-slip and examined with the Ultropak microscope and an X4 objective. Photomicrographs of the cut surface were made on High Speed Ektachrome colour film with a Watson camera.

In some experiments, a loose tie was placed around the aorta just above the level of the left renal artery, and blood pressure was monitored from a femoral artery. Indian ink was injected 5 min after partial occlusion of the aorta, sufficient to reduce femoral blood pressure to 60 to 80 mm Hg.

In a further series of experiments, carbon distribution was examined microscopically by transmitted light in cleared sections of the kidney. Indian ink was injected and the kidney removed as above. The kidney was allowed to drain of blood, a shallow section of the dorsal surface removed, and the kidney

was fixed in formol-saline. Frozen sections of the fixed kidneys were cut at 200 μ, dehydrated in 70% followed by 95% ethanol, and cleared overnight in Beechwood Creosote. Sections were examined using a low-power objective, and scored according to the content of carbon black in various areas.

The peritubular capillary plexuses of the cortex, subcortex and outer medulla were graded on a 5-point scale. The score of 5 represented the greatest degree of filling observed in the series, which was seen in a control kidney, and the score of 1 indicated a virtual absence of carbon, apart from occasional scattered particles, in the area. The degree of filling of glomeruli and vascular bundles was graded on a 3-point scale. A score of 3 for glomeruli indicated a full and even filling of the capillaries of all glomeruli, 2 represented a less even but dense filling, and 1 indicated light filling of similar density to that in the peritubular plexus. A score of 3 for the vascular bundles indicated dense black filling of all bundles in the section, whereas lower scores indicated a progressively lighter filling.

Sections were coded and examined 'blind' on 4 occasions.

Thioflavine S

In solution, thioflavine S shows a variable degree of polymerisation. The high molecular weight polymers are

formed mainly in concentrated solution, and these diffuse less than the lower molecular weight substances, and provide a sharper outlining of the vascular system (Moses, Emery & Schlegel, 1951). Accordingly, "vasoflavine" was prepared as described by Moses et al. (1951) by dissolving thioflavine S (obtained from G.T. Gurr Ltd., London) in water (10% w/v) and evaporating the supernatant to dryness. The residual solid ("vasoflavine") produced a considerably reduced absorption peak at 360 mμ when compared with thioflavine S, indicating the presence of higher molecular weight polymers (Fourman & Moffat, 1967).

Kidneys were removed 5 sec after intravenous injection of 0.2 ml. vasoflavine solution (8% in saline), hemisected and immediately frozen in liquid nitrogen. Frozen sagittal sections were cut at 50 to 60 μ and carefully mounted in glycerol. The sections were observed using transmitted blue light from a high pressure mercury lamp with a Leitz Orthoplan microscope and appropriate U.V. suppression filters. Photomicrographs were taken immediately with a Leitz automatic microscope camera. The preparations could be observed for up to 30 min, after which time diffusion of the dye noticeably increased the background fluorescence, and decreased the contrast of fluorescence of blood vessels.

Comment on methods used

The injection of tracer substances was an attempt to

illustrate the pattern of blood flow existing during life, and so only the initial filling phase was investigated, since both tracer substances caused profound reductions in blood pressure, which would have modified the distribution pattern over a longer period.

Following the intravenous injection of Indian ink, a slight pressor effect usually occured, followed by a pronounced depressor response, commencing 5 to 7 sec after completion of the injection (Fig. 4.1). Vasoflavine produced a rapid fall in blood pressure, to a maximally reduced level 8 to 10 sec after injection. Coincident with the depression of blood pressure produced by vasoflavine, a blanching of the exposed kidney was often observed, which could have been the result of hypotension or vasoconstriction. Subsequently, blood pressure rose after 0.5 to 2 min to a level comparable to or below that in the pre-injection period (Fig. 4.1).

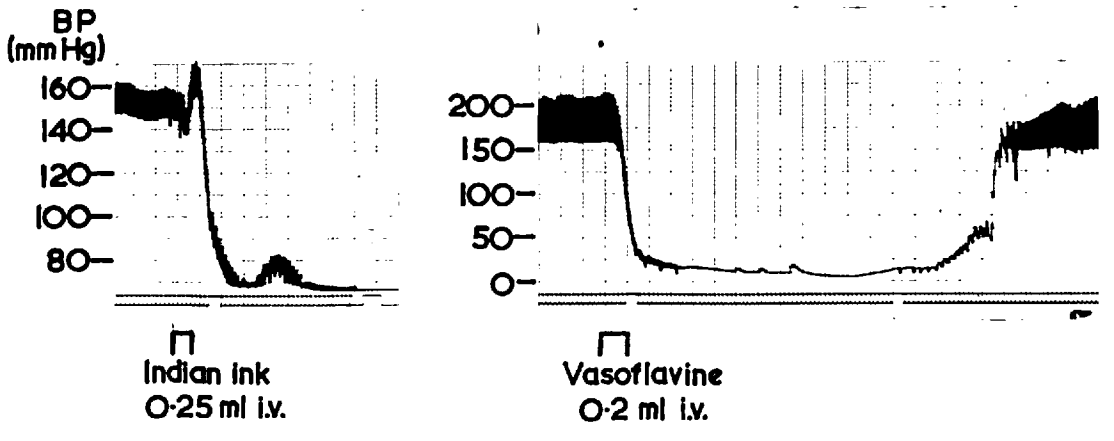


Fig. 4.1. Systemic blood pressure (BP) of anaesthetised rat during intravenous injection of 0.25 ml. undiluted Indian ink (left), and 0.2 ml. of saturated vasoflavine solution (right). Time trace, 1 sec.

By removing kidneys 5 sec after injection of tracer substances, the depressor response was largely avoided, and good filling of cortical and outer medullary blood vessels was obtained. Fourman & Moffat (1967) allowed a delay of 30 sec after injection of thioflavine S, before beginning removal of the kidney, to permit filling of medullary blood vessels. However, mean medullary circulation time in the rabbit is only 24 sec (Grangsjo, Ulfendahl & Wolgast, 1966) and is presumably shorter in the smaller rat kidney. The 5 sec removal time employed in the present experiments permitted adequate filling of outer medullary blood vessels, but the inner medulla was only sparsely filled. Observations have been largely confined to the outer part of the medulla. Fourman & Moffat (1967) believed thioflavine S to be largely devoid of toxic effects; however, the transient depressor response observed here could only have been noticed during continuous blood pressure monitoring which they do not appear to have done. The sample of dye used in the present study also differed from that described by Fourman & Moffat (1967) in (a) exhibiting a secondary absorption peak below 275 mμ at a concentration of 0.002%, and (b) not showing any yellow fluorescence in solutions of 0.01%. Indeed, the material used in the present study did not exhibit marked fluorescence in solution at any concentration, but produced a bright yellow

fluorescence when adsorbed on filter paper, dialysis tubing etc. Thus the appearance of fluorescence appeared to be dependent on binding of the compound to some adsorbing surface, and it is probably incorrect to attribute the colour of fluorescence produced in blood vessels to the attainment of any specific dye concentration.

The fluorescence in blood vessels after vasoflavine injection may be affected by local conditions of pH or osmolarity, or by the degree of uptake and binding of dye by the vessel wall. Results of fluorescence patterns can therefore only tentatively be ascribed to blood flow rates. The bright fluorescence in cryostat sections mounted in glycerine was not observed when sections had been immersed, even for a very short time, in absolute alcohol. Thus no attempt was made to dehydrate or fix the sections since it was felt that a variable degree of quenching of fluorescence may have resulted. The degree of fluorescence shown by the sections was not quantitatively estimated, because variations in section thickness, and plane of section, would have affected the measurement far more than dye concentration.

RESULTS

Control urine flows and the extent of angiotensin diuresis were comparable to those in the non-infused group investigated in Chapter I. Urine flow before angiotensin administration ranged from 0.001 to 0.005 ml./min, and angiotensin (0.5 µg/kg/min) produced a period of reduced flow lasting from 5 to 10 min followed by diuresis ranging from 0.015 to 0.06 ml./min.

For descriptive purposes, reference will be made here to 4 zones of the kidney, corresponding to those described by Moffat & Fourman (1963).

(a) Cortex: the region containing all the glomeruli of the kidney.

(b) Sub-cortex: The zone lying immediately below the cortex, which has a pale appearance and contains no glomeruli. This zone consists mainly of the terminal portions of proximal convoluted tubules, and has a pectinate shape (Sternberg, Farber & Dunlap, 1956).

(c) Outer medulla: this zone contains vascular bundles, and has a pink appearance macroscopically.

(d) Inner medulla: the pale region extending to the tip of the papilla.

The distribution of carbon particles was the same when

observed in unfixed hemisected kidneys, or in cleared sections. The gross distribution pattern in unfixed kidneys is shown in Table 4.1, and the scored data in Table 4.2.

Indian ink injection in control animals, was followed by intense blackening of the kidney at 1 to 2 sec, which increased to maximum density at about 3 to 4 sec and thereafter rapidly declined. Kidneys removed at +3 to 5 sec showed the greatest degree of filling with carbon particles in the capillaries of the cortex. Glomeruli showed partial filling, with carbon appearing as loose aggregates of particles. Control kidneys demonstrated an even and dense filling of the capillary networks of cortex, sub-cortex and outer medulla, with a slightly greater filling of the outer cortex (Table 4.2, Figs. 4.2, 4.3), but the vascular bundles in the outer medulla showed up as dense black groups of vessels. Whereas in capillaries the carbon particles were singly dispersed, in vasa recta many particles appeared together in long columns. The inner medulla contained Indian ink particles even when the kidney was removed only 3 sec after intravenous injection. When the removal time was lengthened to 7 sec, the inner medulla was found to be filled to a greater extent with carbon, but the capillary plexus in the cortex and outer medulla now contained fewer particles. The glomeruli, however, contained a greater density of Indian ink as the removal time was increased (Table 4.1).

Fig. 4.2. Low power, incident-light photomicrograph of cut surface of control kidney, from rat injected intravenously with Indian ink 4 sec before cutting the renal pedicle.

A. Showing even dispersion of carbon particles in capillaries of cortex and sub-cortex, and partial filling of glomeruli (gl.).

B. Filling of capillary plexus and vascular bundles (v.b.) in outer medulla.

From original Ektachrome transparency. X 39.

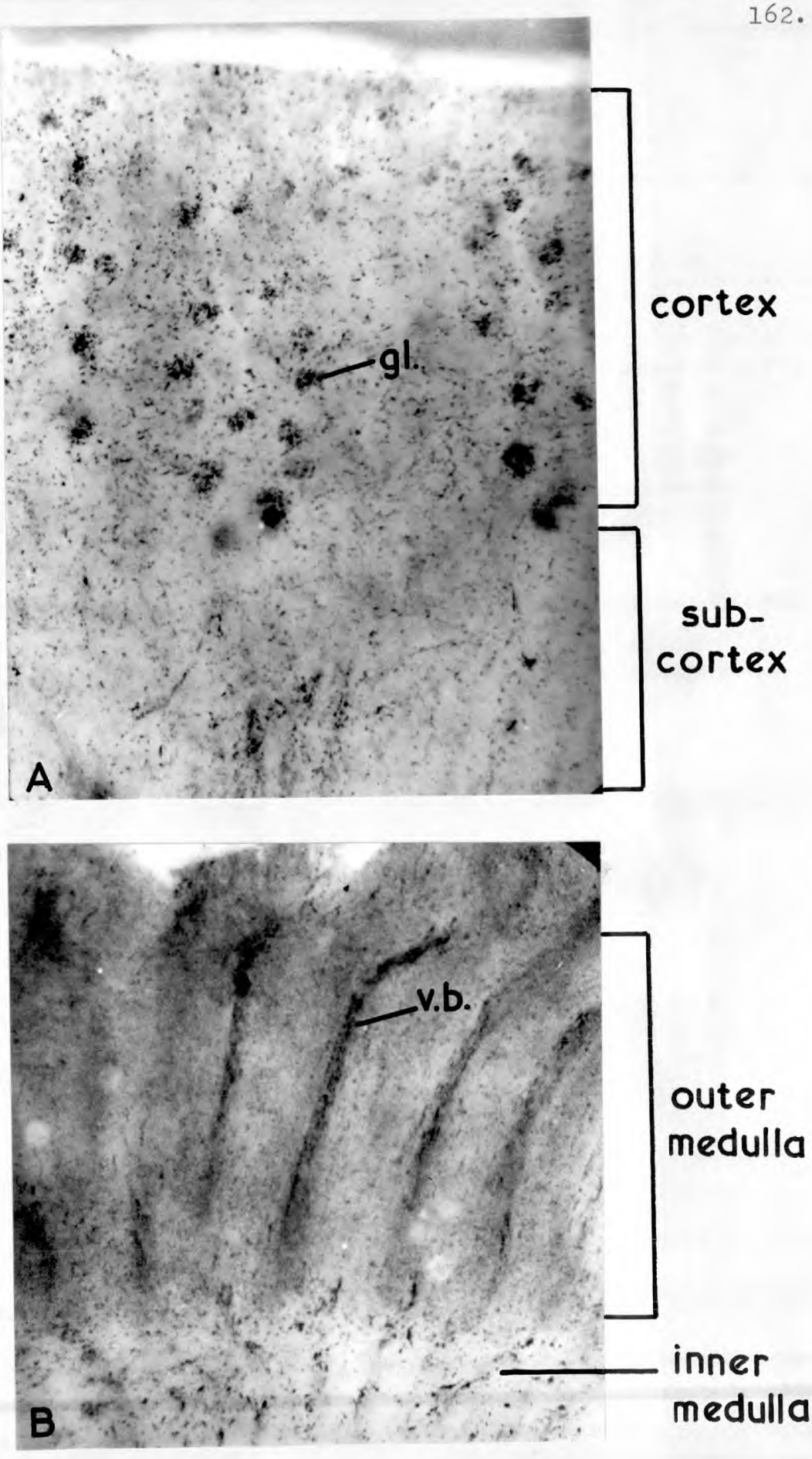
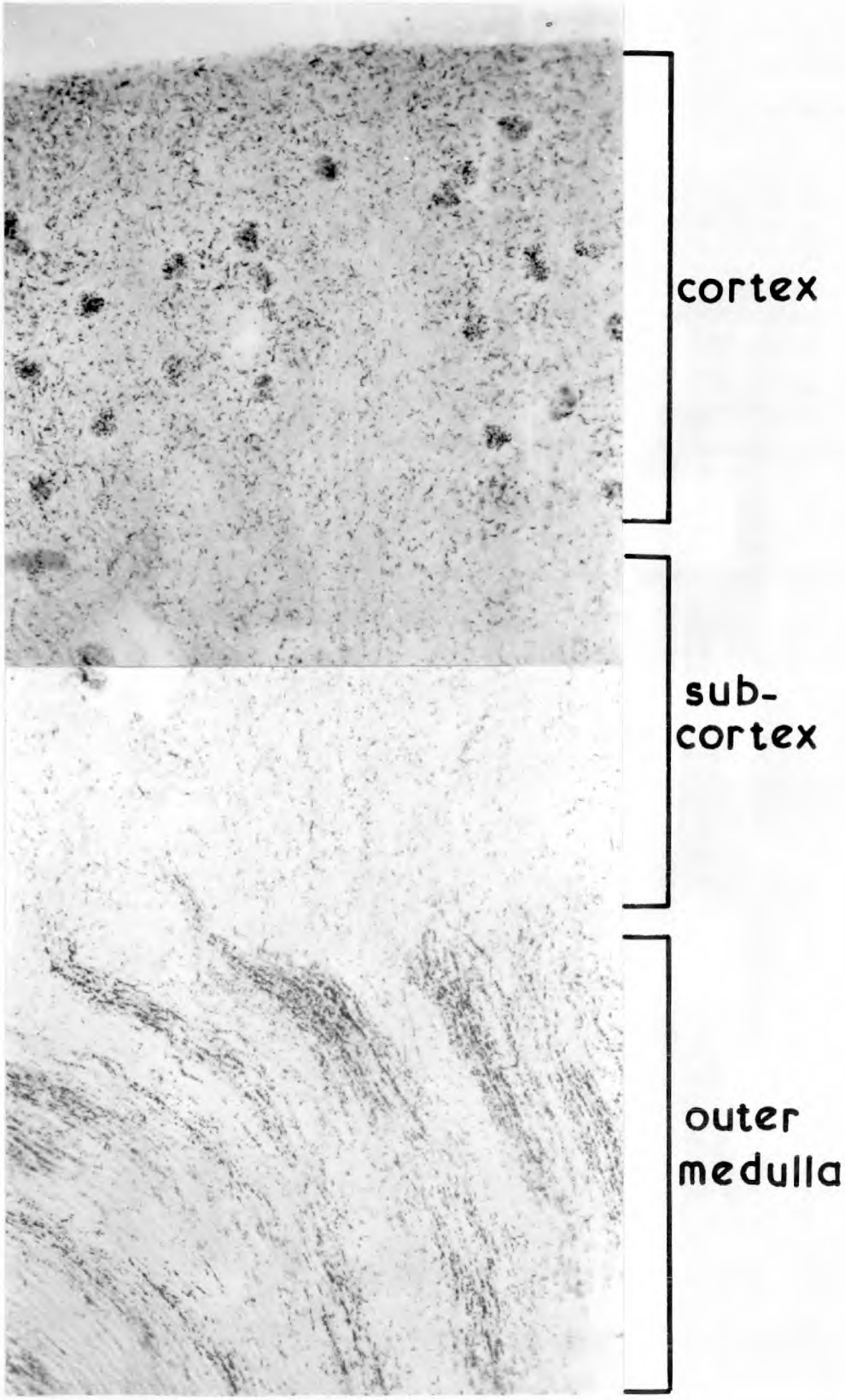

gl.
cortex
sub-
cortex
A
v.b.
outer
medulla
inner
medulla
B

Fig. 4.3. Low-power photomicrograph of cleared section (200 μ thick) of control kidney, from rat injected intravenously with Indian ink, 4 sec before cutting renal pedicle. Even dispersion of carbon particles in capillary plexus of cortex, sub-cortex and outer medulla (*c.f.* Fig. 4.2). X 48.


cortex
sub-
cortex
outer
medulla

Kidneys from animals which had been infused with angiotensin, showed a dramatically altered pattern of filling with carbon particles. The kidney was observed to fill much more slowly with Indian ink, so that the maximum carbon concentration was reached in 4 to 6 sec, and was less intense than in controls.

A marked alteration in the distribution of Indian ink particles was apparent when the cut surface of the hemisected kidney was examined with the naked eye. In control kidneys, the cortex appeared uniformly pale, with a greater density of carbon in the outer medulla. In angiotensin-infused kidneys, however, the outer cortex showed as a dark rim, contrasting strongly with the pale subcortical area. In the outer medullary zone, vascular bundles appeared as dark streaks against a pale background (Fig. 4.4).

Low power incident light examination revealed that the darker appearance of the outer cortex was due to the very dense filling of the glomerular capillaries, whereas the peritubular capillaries were filled to an equal or lesser extent than controls (Fig. 4.5, Table 4.1). It was a characteristic feature of angiotensin-infused kidneys that the glomeruli appeared as dense round bodies due to the well-filled capillaries (Fig. 4.5, 4.6). The capillary plexus of the cortex generally filled evenly with carbon particles, but

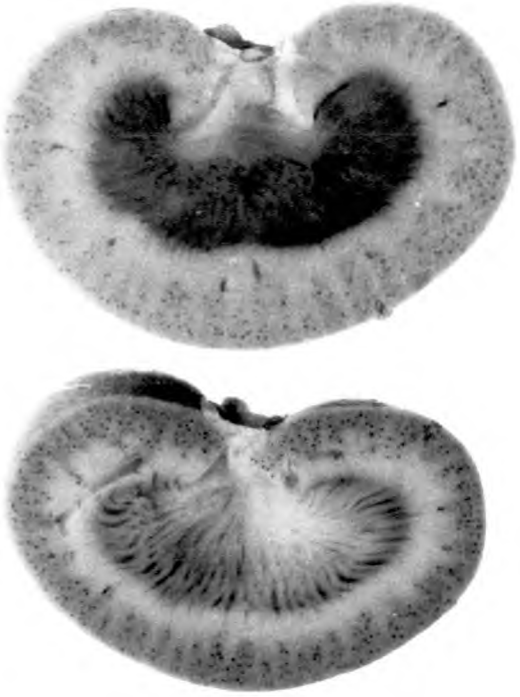


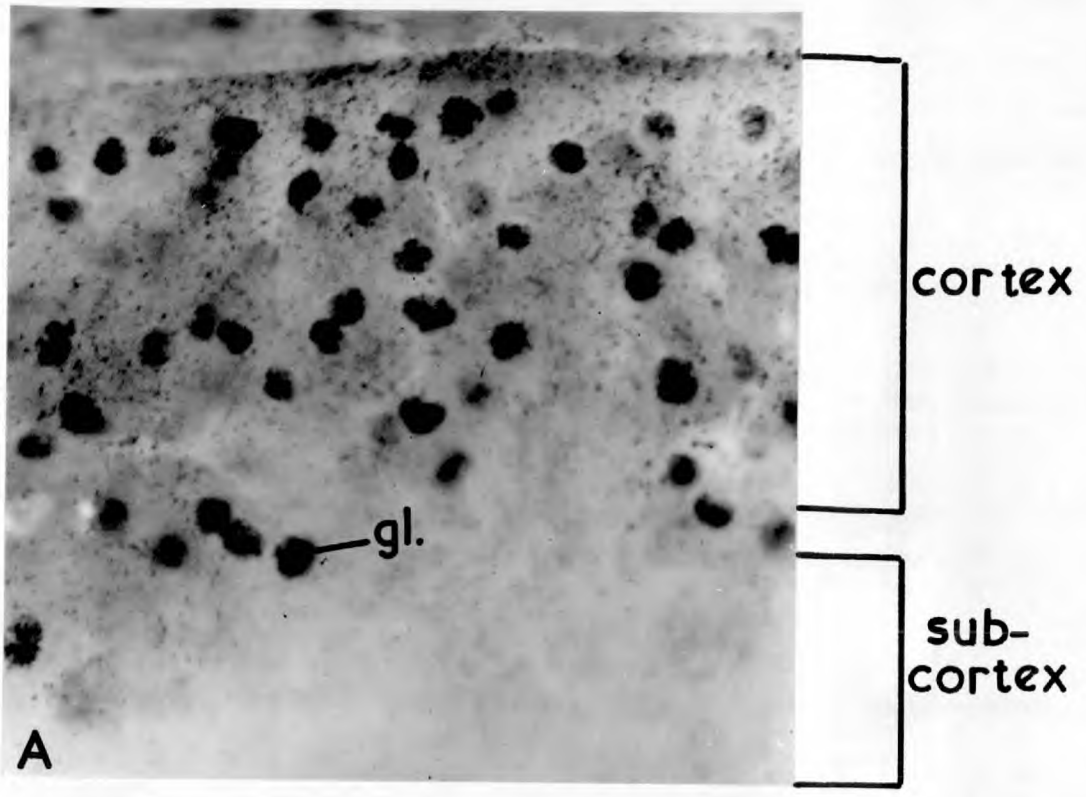
Fig. 4.4. Kidneys removed from rats injected intravenously with Indian ink.
Above: control.
Below: during infusion of angiotensin at 0.5 µg/kg/min.
X 4.

Fig. 4.5. Cut surface of left kidney, from rat injected intravenously with Indian ink 4 sec before cutting pedicle. Angiotensin infused intravenously at 0.5 µg/kg/min. Urine flow of left kidney: 0.008 ml./min.

A. Showing dense and even filling of glomeruli (gl.) with carbon particles, and virtual absence from sub-cortical zone.

B. Showing absence of carbon particles from capillary bed of outer medulla, and presence in vascular bundles (v.b.).

From original Ektachrome transparency, X 39.


cortex
gl.
sub-
cortex
A

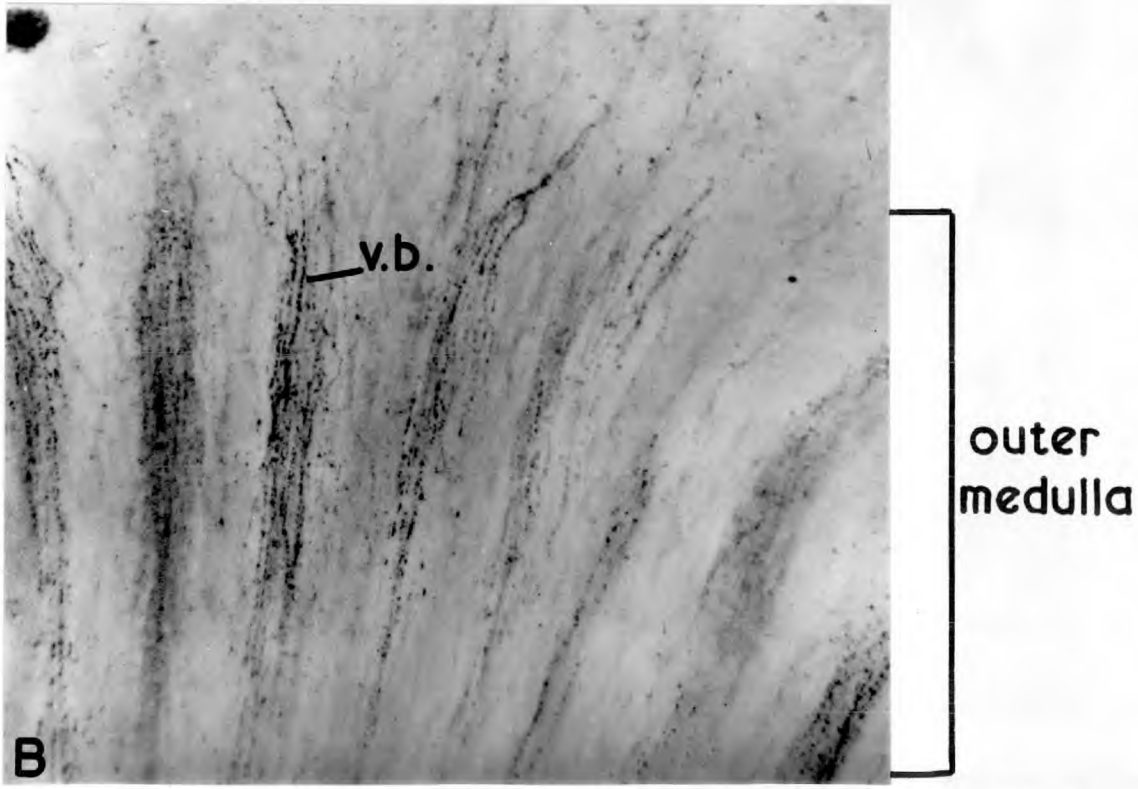
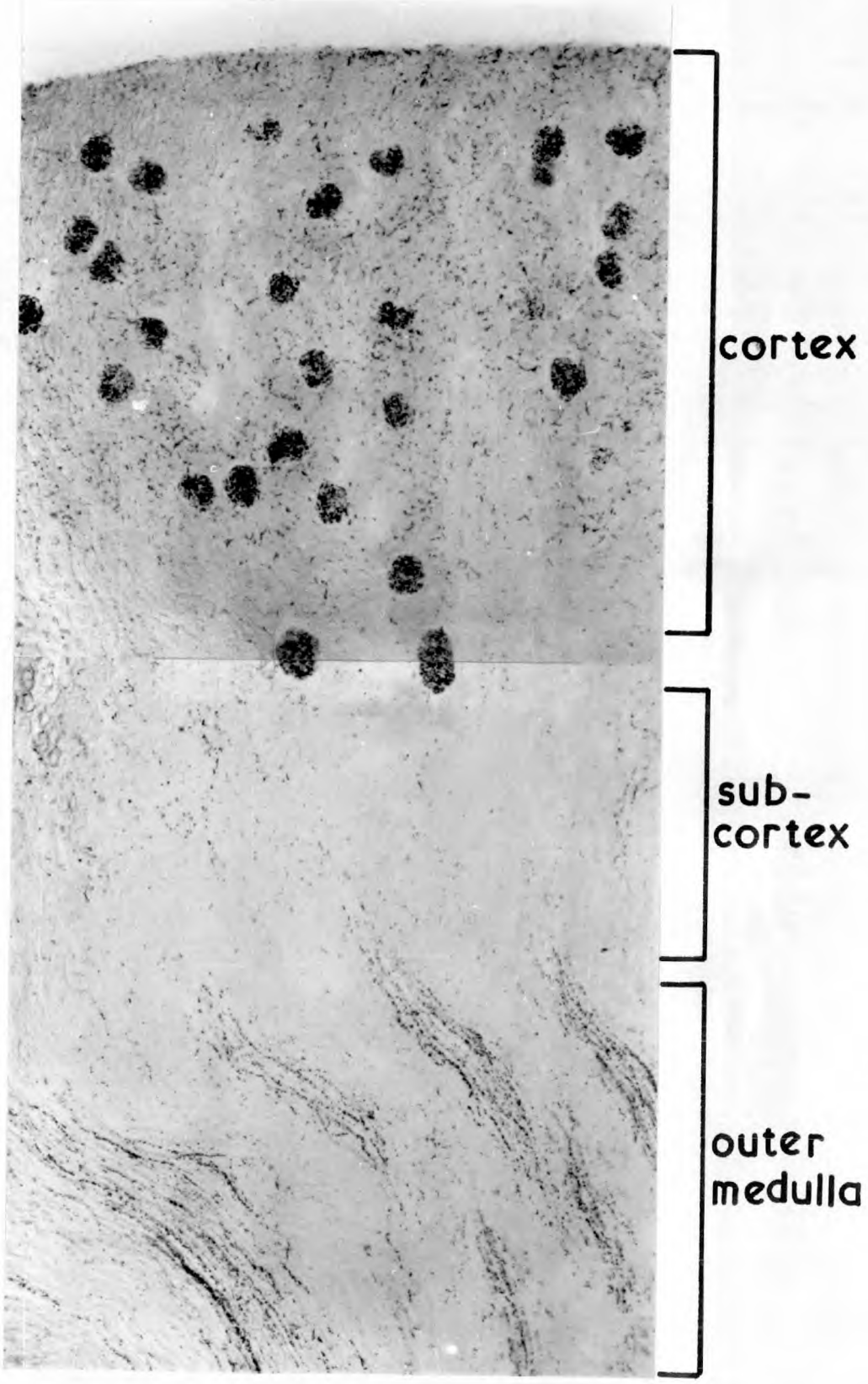

v.b.
outer
medulla
B

Fig. 4.6. Low-power photomicrograph of cleared section (200 μ thick) of left kidney, from rat infused intravenously with angiotensin (0.5 μg/kg/min), and injected intravenously with Indian ink 5 sec before cutting pedicle. Urine flow of left kidney: 0.01 ml./min. Dense filling of glomeruli in all parts of cortex with carbon particles, and very low degree of filling of capillaries of sub-cortex and outer medulla. Patchy, irregular filling of peritubular capillaries in cortex, with some clear areas (e.g. top left-hand region of photograph). X 48.


cortex
sub-
cortex
outer
medulla

some angiotensin-treated kidneys showed a marked patchiness of filling, so that dense black glomeruli could be seen surrounded by clear areas of virtually unfilled capillaries (Fig. 4.6).

In marked contrast to the capillaries of the cortex, however, the sub-cortical capillaries of angiotensin treated rats were largely devoid of carbon particles (Table 4.1, 4.2, Figs. 4.5, 4.6). The very pale appearance of the sub-cortex enhanced the pectinate shape of this zone (Fig. 4.4).

The virtual absence of carbon from the sub-cortex extended to the capillaries of the outer medulla, although the vasa recta always contained carbon particles (Table 4.2). The vascular bundles stood out sharply against a pale background when the cut surface was examined (Fig. 4.5) or against clear zones in the sections (Fig. 4.6), but filled to a lesser degree in controls (Table 4.2).

The inner medulla filled more slowly than the remainder of the kidney, in keeping with the known low blood flow of this area. In control rats the inner zone contained some ink particles even after a 3.5 sec removal time, whereas 3 out of 9 of the angiotensin-treated kidneys contained no ink particles at all in the inner zone of the medulla, and the remainder only very few particles, showing a great reduction in blood flow to this area.

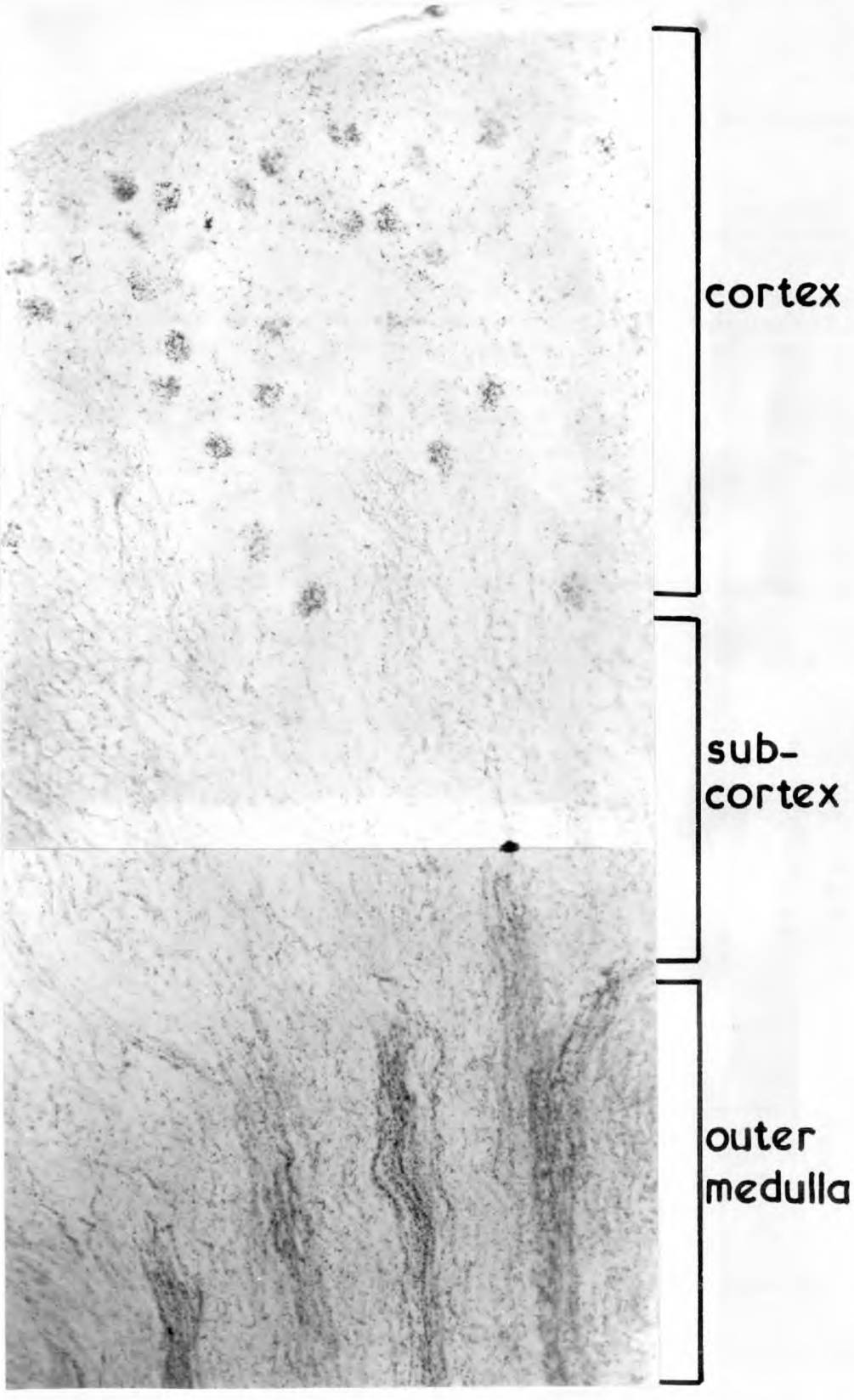
The pattern of dense glomerular filling and virtual absence

of filling in sub-cortex and outer medulla was seen in 4 out of 8 angiotensin treated rats whose kidneys were fixed and sectioned (Table 4.2). In the other 4 rats of this group, the glomerular filling was less dense, and the reduction of filling intensity in sub-cortex and outer medulla was less spectacular. These latter 4 experiments were performed in very hot weather, when temperatures in the animal rooms reached 29.5°C, and the high ambient temperature may well have modified vascular responsiveness.

The dense glomerular filling in angiotensin-treated kidneys, was seen even when the kidneys were removed only 4 sec after injection of Indian ink. When the removal time was increased to 6 to 7 sec, the degree of filling in sub-cortex and outer medulla was not enhanced, although the degree of filling of the inner medullary zone increased. The dense filling of glomeruli appeared to be uniform throughout the cortex; no gradation could be observed between superficial and juxtamedullary glomeruli.

Constriction of the aorta sufficient to produce a femoral artery perfusion pressure of 70 to 80 mm Hg, resulted in a slower filling of the mobilised kidney with carbon than in controls. The cortex of these kidneys contained a lower concentration of Indian ink particles, although the sub-cortical plexus was well filled (Fig. 4.7, Table 4.2). Assessment of

Fig. 4.7. Low-power photomicrograph of cleared section (200 μ thick) of left kidney, from rat injected intravenously with Indian ink 4 sec before cutting renal pedicle. Aorta constricted above left renal artery sufficient to produce femoral arterial blood pressure of 72 mm Hg. Adequate filling of capillaries of sub-cortex and outer medulla with carbon particles. X 48.


cortex
sub-
cortex
outer
medulla

the carbon content of vascular bundles was found difficult apart from gross changes. Aortic constriction generally produced little reduction in the degree of filling of vascular bundles and capillary plexus of the outer medulla, but sometimes reduced capillary filling to the extent seen in angiotensin treated animals (Table 4.2).

Similar changes induced by angiotensin in the degree of capillary filling in the outer medulla were observed in kidneys which had been injected with thioflavine S (Table 4.3). Sections from these kidneys showed a bright yellow fluorescence in glomeruli and vascular bundles and a weaker, yellow-green fluorescence of the tubules. In angiotensin-infused rats, the gross appearance of fluorescence in the cortex was similar to control rats. Glomeruli appeared fluorescent in outer and inner cortex as did the vascular bundles in the outer medulla; however the overall fluorescence in the outer medulla appeared less than in control rats, and this was found to be due both to a reduced degree of filling of individual vascular bundles, and to a reduction in the amount of fluorescence between the bundles. In control kidneys, fluorescent blood vessels could be observed between the tops of vascular bundles in the outer medulla, whereas in angiotensin-diuretic kidneys, only the vasa recta themselves appeared fluorescent, and the area between the vascular bundles appeared dark and devoid of

fluorescence (Fig. 4.8).

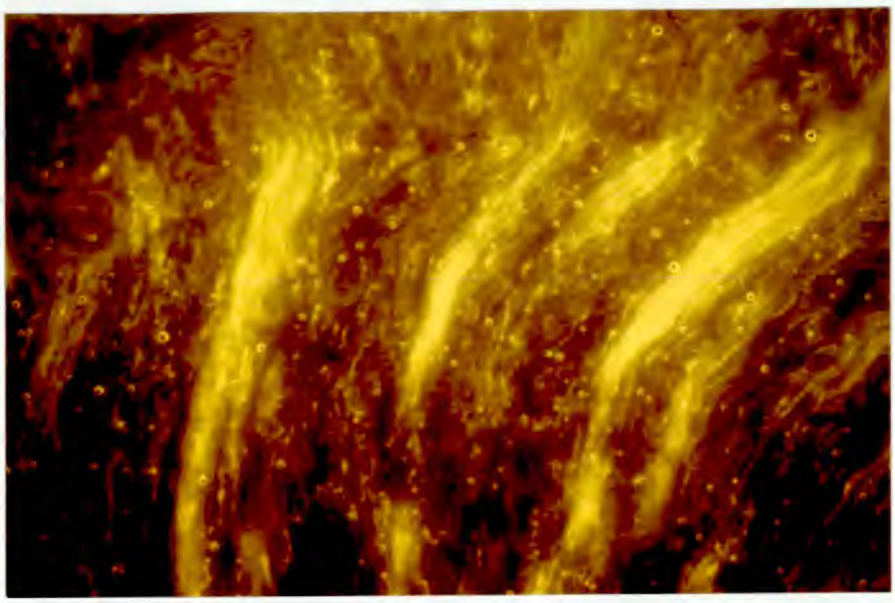
As with the distribution of Indian ink particles, the pattern of fluorescence of blood vessels was essentially the same at all levels of diuresis induced by angiotensin. In the Indian ink injected group, in addition to rats undergoing angiotensin diuresis, 3 animals were sacrificed after only 5 min of infusion, during the antidiuretic phase of angiotensin administration. The pattern of ink distribution in diuretic and antidiuretic kidneys was the same (Table 4.1).

Fig. 4.8. U.V. fluorescence photomicrograph of frozen sections of left kidneys from rats injected intravenously with a saturated solution of thioflavine S. Region of outer medulla.

Above: Control.

Below: During intravenous infusion of angiotensin at 0.5 µg/kg/min; urine flow of left kidney, 0.05 ml./min. Marked fluorescence in vascular bundles, but not in capillary bed.

Glycerin mount. X 55.



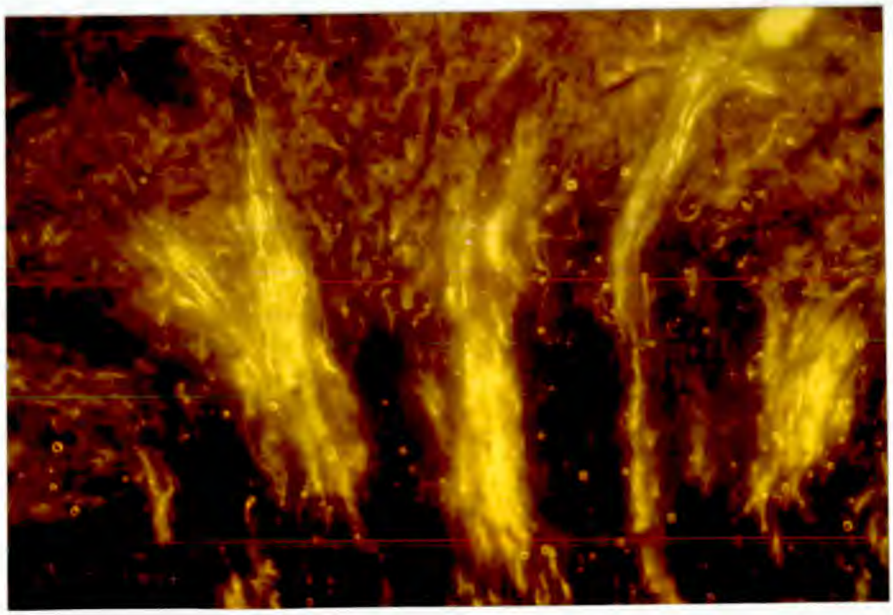


Table 4.1. Zonal distribution of carbon particles observed on examination of cut surface of hemisected left kidney, following intravenous injection of Indian ink

(a) Controls

Removal time (sec)	Degree of filling with carbon particles				
	glomeruli	cortex	sub-cortex	vascular bundles	outer medulla
5	Poor	++	+	++	+
3.5	"	+	+	++	+
3.5	"	+	+	+	++
4	"	+	+	+	+
7	Good	+	+	+	+
5	"	+	+	++	+
4	"	+	+	+	+
7	"	+	+	+	+
4	"	+	+	++	+
5	"	+	+	+	+

(b) Angiotensin infusion (0.5 µg/kg/min)

Removal time (sec)	Length of infusion (min)	Urine flow (ml./min)	Degree of filling with carbon particles				
			glomeruli	cortex	sub-cortex	vascular bundles	outer medulla
5	5	<0.002	complete	+	-	+	-
5.5	5	<0.002	"	+	-	+	-
4	5	0.002	"	+	-	+	-
4	20	0.035	"	+	-	+	-
4	20	0.025	"	+	-	+	-
4	20	0.01	"	-	-	-	-
4	30	0.008	"	+	-	+	-
7	20	0.023	"	+	-	+	-
5	15	0.05	"	+	±	±	-

Removal time = time allowed between completion of intravenous injection of Indian ink and excision of kidney.

Degree of filling: ++ = large amount
+ = small amount
- = virtual absence of particles.

Table 4.2. Regional variations in density of filling with carbon particles observed in cleared sections of left kidney following intravenous injection of Indian ink

(a) Controls

Removal time (sec)	Degree of filling with carbon particles				
	glomeruli	cortex	sub-cortex	vascular bundles	outer medulla
5	1	2	1.7	2	2.6
5	2	5	3	2.9	1.3
4.5	1	2.5	2	2	2.3
4	1.5	3.3	2.5	2.9	1.9
4.5	1.3	2.2	1.3	2	3
3.5	1.5	3.2	2.2	2	2.5
Mean:	1.4	3.0	2.1	2.3	2.3

(b) Aortic constriction

Removal time (sec)	Perfusion pressure (mm Hg)	Degree of filling with carbon particles				
		glomeruli	cortex	sub-cortex	vascular bundles	outer medulla
5	74	2	3.8	2	2	1
5	78	1	2.3	1.8	2	3.5
4.5	75	2	2.8	2.5	2	2.5
4	72	1.3	2.3	3	2	3.3
5	73	1	2	2	2	2.5
5	70	1.8	2.3	2.3	2	4.3
Mean:		1.5	2.6	2.3	2	2.9

(c) Angiotensin (0.5 µg/kg/min)

Removal time (sec)	Length of infusion (min)	Urine flow (ml./min)	Degree of filling with carbon particles				
			glomeruli	cortex	sub-cortex	vascular bundles	outer medulla
5	12	0.03	3	3	1	1.9	1.1
5	25	0.01	3	2.7	1	2	1.3
5	20	0.05	3	3	1	2	1
5	25	0.02	2	3.8	2	2	2
5	20	0.09	2	2.7	1.7	2	2
4.5	25	0.05	2	4	2.2	2	1.2
4	12	0.03	2	3	1.5	2	1.5
5	25	0.025	3	3	1	2	1
Mean:			2.5	3.2	1.4	2	1.4

Removal time = time allowed between completion of intravenous injection of Indian ink and excision of kidney.

Degree of filling: glomeruli, 3 = complete, 1 = poor.
vascular bundles, 3 = maximum, 1 = poor.
capillaries, 5 = maximum, 1 = virtual absence of particles.

Table 4.3. Fluorescence in left kidney following intravenous injection with thioflavine S in control rats, and during infusion with angiotensin

Controls			Angiotensin, 0.5 µg/kg/min			
Removal time (sec)	Appearance of fluorescence in: cortex	outer medulla	V (ml./min)	Removal time (sec)	Appearance of fluorescence in: cortex	outer medulla
5	All glomeruli fluorescent.	Strong fluorescence in all vascular bundles and in capillary bed.	0.11	7	All glomeruli fluorescent.	All vascular bundles fluorescent, but less than controls. Capillary bed not fluorescent.
5	"	"	0.11	5	"	"
5	"	"	0.03	5	"	"
5	"	"	0.05	5	"	"
5	"	"	0.02	5	"	"

Removal time = interval allowed between completion of thioflavine S injection and excision of kidney.

V = urine flow rate in mobilised left kidney.

DISCUSSION

Control rats showed a consistent pattern of filling of the kidneys with Indian ink and fluorescent dye. The glomeruli did not fill densely with ink but did show an even, bright fluorescence as observed by Fourman & Moffat (1967) following thioflavine S injection. Fourman & Moffat ascribed this difference between the degree of glomerular filling by the two markers, to post-mortem artefacts leading to a redistribution of ink particles. However it seems reasonable that the glomerular capillaries normally should be filled by the particulate tracer to about the same degree as the post-glomerular capillaries, whereas the fluorescence of glomeruli is probably a result of staining of the basement membrane.

Angiotensin causes constriction of both afferent and efferent glomerular arterioles in the rat kidney, but the efferent constriction is relatively greater, as shown by the rise in filtration fraction (see Introduction, p.31). The increased filling of glomeruli by carbon during angiotensin infusion, is probably the result of efferent arteriolar constriction, as remarked by Kahn et al. (1950), but it is less certain how the effect is actually produced. Constriction of the efferent arteriole may effectively trap the particles, or increased viscosity of blood in the glomerular capillaries due to the elevated filtration fraction may slow their passage.

Additionally, since angiotensin infusion maintains a near normal GFR in spite of a greatly reduced RBF, the glomerular filtration pressure must increase, and this increased filtration pressure may impact the particles onto the capillary wall. The increased glomerular filling in controls left a longer time before removal of the kidney could be due to further trapping of particles in the capillary labyrinth, or to an effect of hypotension. The increased glomerular filling caused by angiotensin was not due to a slowing of the filling phase, since aortic occlusion, which reduced renal perfusion pressure to levels shown by others (Rector et al., 1966) to cause marked reduction in GFR and presumably in RBF, did not reproduce the effect.

The number of particles trapped in any capillary bed must be dependent on particle size and shape as well as on blood flow. The Indian ink particles used, ranged in size from about 0.1 to about 5 μ diameter, and were of irregular shape. When a very fine carbon suspension was used, consisting of 0.1 μ and smaller diameter particles*, the renal cortex of control rats appeared devoid of carbon after sectioning the kidney and washing, although the kidney had appeared dense black at the moment of removal. An angiotensin-infused rat, however,

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* Sterling 'R'; Cabot Carbon Ltd., Stanlow, Cheshire.

treated with the same finely dispersed carbon preparation, showed the typical dense glomerular filling although the peritubular capillaries were left largely unfilled (Fig. 4.9). These observations with finely divided carbon showed that particles could be washed out of the peritubular capillaries with residual blood, but not out of the glomerular capillaries of angiotensin-treated kidneys. These capillaries showed an even dispersion of particles rather than a packing together, which suggested that the particles were impacted on the capillary wall. However it is equally possible that the relatively greater efferent than afferent arteriolar constriction resulted in an increased glomerular capillary volume, slowing of flow velocity and hence increased carbon content.

An increased density of carbon particles in capillaries could equally well be due to an increase or a decrease in blood flow, depending on the time of kidney removal, since the former would cause an increased delivery of indicator, whereas the latter would result in a slowing of the injected bolus. For this reason, the kidneys were removed at a brief time interval, when the ink concentration in the kidney was at, or approaching, maximum. Under these conditions, the reduced degree of filling in sub-cortical and outer medullary capillaries produced by angiotensin was almost certainly the result of a decreased blood flow rate.

The appearance of a reduced capillary filling in sub-cortex

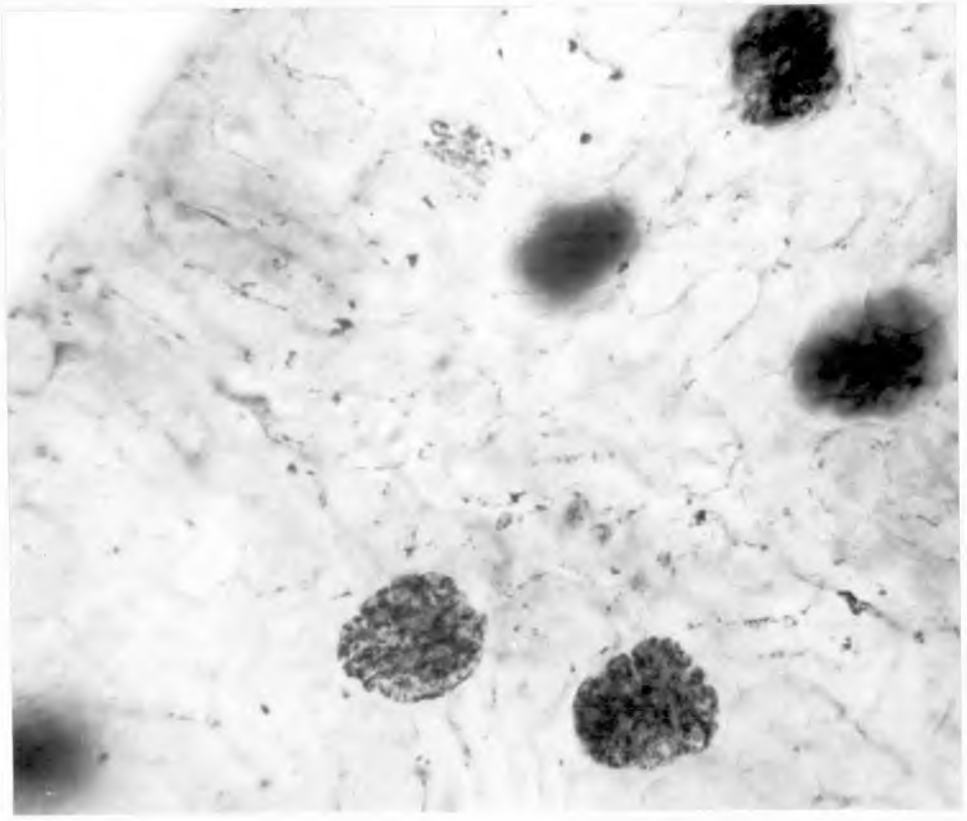

Fig. 4.9. Cleared section (200 μ thick) of rat kidney, removed 5 sec after intravenous injection of fine carbon suspension (Sterling R), during intravenous infusion of angiotensin at 0.5 μg/kg/min. Even, dense, filling of glomerular but not peritubular capillaries with carbon particles. X 80.

and outer medulla with angiotensin, was associated with a reduction in the total carbon black content of cortex and medulla. However angiotensin appeared to specifically affect the capillary plexus, since aortic occlusion caused a general reduction in total Indian ink content of the kidney to about the same degree, but maintained an even dispersion of particles between capillaries and vasa recta.

Since the amount of ink in various zones of the kidney could not be measured accurately, it could not be concluded that the apparently specific effect of angiotensin was not due to a reduction in the total amount of ink in the kidney. Aortic constriction was performed in order to reduce the total RBF, and hence the total ink content of the kidney, but this manoeuvre may not reduce medullary blood flow as much as angiotensin.

Aortic constriction was applied for only 5 min, in the hope that the amount of angiotensin generated in the general circulation would be negligible. However the possibility exists that angiotensin generated intrarenally could have caused the reduced capillary filling in the outer medulla observed in one animal of this group. The results using fluorescent dye injection confirmed the appearance of reduced capillary filling during angiotensin infusion by a different technique, lending weight to the observation.

The reduced capillary filling produced by angiotensin, indicates that the blood supply to capillaries of the outer

cortex may be anatomically separate from that to the sub-cortex and outer medulla.

Moffat & Fourman (1963) described the vascular pattern of the rat kidney, and showed that capillaries of the sub-cortical zone arise from juxtamedullary glomerular efferent vessels, and from branches of the vasa recta bundles. The vasa recta bundles also give off vessels which supply the capillary plexus of the outer medulla. Fourman & Kennedy (1966) believed the capillary plexuses of cortex and medulla to be continuous, since administration of vasopressin to rats resulted in a lack of filling of vasa recta bundles with fluorescent dye, whereas the outer medullary capillaries contained fluorescent material. Vasopressin was thought to constrict the juxtamedullary efferent arterioles, preventing filling of vasa recta but allowing the capillaries in the medulla to fill from the cortex.

Rollhauser, Kriz & Heinke (1964), however, produced anatomical evidence for an independence of the capillary blood supply to cortex and medulla. These authors described 3 zones of the medulla which have independent capillary blood supplies, from juxtamedullary efferent arterioles. These zones correspond to the sub-cortex, outer medulla and inner medulla described above. In addition, Rollhauser et al. differentiated the capillary supply to the cortical labyrinth from that to the so-called medullary rays of the cortex (which

correspond to the projections of the sub-cortex into the cortex). According to this view, blood can only reach the inner medulla from the vasa recta bundles, and not from the capillary plexus of the outer medulla.

The present results are consistent with a constricting effect of angiotensin on the vessels supplying the capillaries of sub-cortex and outer medulla, but permitting flow to occur through the vasa recta bundles. The capillaries of the outer cortex are wider than those of the sub-cortex, and the vessels supplying them are efferent arterioles, which may well be wider than the branch vessels supplying the sub-cortical and outer medullary plexuses. Thus angiotensin may produce a more complete occlusion of the vessels supplying the capillary beds of the sub-cortex and outer medulla. The results obtained by Fourman & Moffat (1967) with thioflavine S injected during vasopressin administration are difficult to interpret, since these workers used the very long time of 30 sec before removing the kidney after dye injection. The apparent absence of vasa recta filling in their experiments could therefore be the result of a rapid transit of the bolus and diffusion of dye out of the vessel walls. However the fluorescence of blood vessels injected with thioflavine S also depends on the extent of dye uptake by the vessels, and on the extraction of dye during tissue fixation processes, as used by Fourman and Moffat (1967). For these reasons, the present study of

intrarenal blood flow distribution has been accomplished by a combination of dye and carbon black injection, since the latter substance does not diffuse out of blood vessels.

The present work is in keeping with the results obtained by Aukland (1968) in the dog kidney, showing that outer medullary and cortical blood flows were reduced to about the same extent by angiotensin. Thus increased medullary blood flow is unlikely to be the cause of angiotensin diuresis in either species.

The intrarenal redistribution of blood flow produced by angiotensin may offer some explanation of its natriuretic effect. A reduction in capillary blood flow to areas of renal tubules could result in a reduction of sodium reabsorption by two mechanisms: (a) a virtual shut-down of capillary flow would reduce the removal of reabsorbate by the peritubular fluid, and (b) a degree of anoxia of tubular cells may result in an inhibition of the active reabsorption process.

In the first case, an increase in interstitial volume would result from accumulation of fluid in intercellular spaces, leading to an increased passive back diffusion of sodium and possibly inhibition of the active sodium pump. Such a mechanism was proposed by Lewy & Windhager (1968) to explain the reduction in proximal tubular reabsorptive capacity in the rat kidney following partial renal vein occlusion. Tubular reabsorptive capacity was found to decrease proportionally to

the reduction in renal plasma flow following venous occlusion, but in control periods, reabsorptive capacity was directly related to filtration fraction. The authors suggested that sodium ions were actively transported into the intercellular spaces followed by passive movement of water, but that reabsorbate then moved into the capillary lumen according to the balance between hydrostatic and oncotic pressures across the capillary wall. The rise in filtration fraction caused by angiotensin would lead to an increased oncotic pressure of blood in the capillaries, tending to enhance reabsorption from the interstitium, but this effect might be offset by a drastic reduction in peritubular removal of reabsorbate. Increased accumulation of fluid in the interstitium would elevate interstitial pressure; evidence that an increased hydrostatic pressure to the serosal cell surface can inhibit active sodium reabsorption has been produced in the isolated frog skin by Nutbourne (1968). The effect was postulated to be due to a reduction in the eflux of reabsorbed sodium from exits in intercellular pores.

In the outer medulla, peritubular capillaries and ascending vasa recta are the only routes for removal of fluid entering the interstitium from descending vasa recta and collecting ducts (Marsh, 1969). A decrease in blood flow to these areas could therefore reduce removal of solute from the inner medulla and so reduce papillary osmolality, or permit

interstitial volume expansion resulting in a decreased net solute reabsorption as above. Such an effect could outweigh the tendency for papillary osmolality to increase, secondary to reduced vasa recta blood flow. Bonjour et al. (1967), however, could detect no change in medullary sodium, potassium or urea concentrations following angiotensin diuresis in hydrated rats.

Net sodium reabsorption by the kidney has been shown to be related to oxygen consumption over a range of values of GFR and reabsorption (Thurau, 1964). In the isolated frog skin, a constant relationship exists between oxygen uptake and sodium transport (Zerahn, 1956), and so a reduction in the oxygen supply to actively reabsorbing tubular cells would be expected to reduce sodium transport.

The intra-arterial infusion of inhibitors of aerobic metabolism has been shown to cause a unilateral natriuresis in the dog (Herms & Malvin, 1963; Fujimoto, Nash & Kessler, 1964) although the precise role of oxygen in reabsorptive mechanisms cannot be proved in vivo. Studies on the metabolism of isolated slices of renal cortex and medulla, have shown a high rate of aerobic metabolism in the cortex, but a largely anaerobic metabolism in the inner medulla (Kean, Adams, Winters & Davies, 1961; Lee, Vance & Cahill, 1962). However, in the medulla of the dog kidney, oxidative pathways were calculated to furnish twice as much energy as glycolysis, so that the

availability of oxygen to the medulla may play a critical role in active sodium reabsorption (Bernanke & Epstein, 1965). Oxygen tension is low in the outer medulla of the rat kidney (Leichtweiss, Lubbers, Weiss, Baumgartl & Reschke, 1969) which may be due to a low blood flow, or to shunt diffusion of oxygen across the tops of the vasa recta (Levy & Sauceda, 1959).

In the sub-cortical zone, the ischaemia produced by angiotensin would affect primarily the terminal portions of proximal convoluted tubules, which are the major constituents of this area (Sternberg, Farber & Dunlap, 1956). In the outer medulla, the ascending limbs of the loops of Henle would be rendered anoxic. Both these affected areas are the sites of a high level of active sodium reabsorption; angiotensin might therefore be expected to reduce sodium reabsorption in the proximal tubule and loop of Henle. Weinstein & Klose (1969) however, reported that cyanide infused into the renal artery of the rat suppressed proximal reabsorption, but did not affect reabsorption in more distal parts of the nephron.

Kjekshus, Aukland & Kiil (1969) found that angiotensin infusion in dogs (2 μg/min) reduced sodium reabsorption and oxygen consumption by the kidney to the same degree. This is consistent with a reduced oxygen supply reducing sodium

transport, or with a primary inhibition of reabsorption reducing oxygen uptake secondarily.

The observations on intrarenal blood flow distribution produced by angiotensin also yield a possible explanation for the decreased flow during a diuretic phase of angiotensin infusion observed in superficial tubules (Chapter I). The proximal and distal convolutions of superficial nephrons lie closely coiled near the renal surface, according to the camera lucida drawings presented by Walker & Oliver (1941), and therefore receive the highest blood flow during angiotensin administration. These segments of the superficial nephrons may therefore be subjected to the greatest reabsorptive activity, while reabsorption may be inhibited in deeper nephrons. According to this view, it is not necessary to invoke a redistribution of GFR between superficial and deeper glomeruli, in order to explain the effects observed in Chapter I.

Angiotensin diuresis may result from a specific reduction in blood supply to areas of renal tubules with a high reabsorptive capacity, while GFR is maintained at a reasonably high level. Aortic constriction, which could reduce tubular perfusion to a similar extent, also causes a large reduction in GFR which might result in almost complete reabsorption of the filtrate. The diuretic effect of angiotensin therefore may result from its efferent arteriolar constriction, and particularly from occlusion of branch vessels supplying areas

of the capillary plexus.

The degree of natriuresis produced by angiotensin cannot be correlated with the degree of reduction in RBF, as indicated by PAH clearance (Bonjour & Malvin, 1969). However, the degree of natriuresis may depend on the balance between changes in GFR and RBF produced by angiotensin, as well as on the extent of inhibition of proximal and distal tubular reabsorption.

CONCLUDING REMARKS

Angiotensin may affect renal function through a number of different mechanisms, and the effects of prolonged administration may differ from the acute response investigated here.

During a diuretic phase of administration, angiotensin appeared to produce a reduction in superficial nephron flow rate, as evidenced by the delayed appearance of a concentrated lissamine green bolus in distal tubules. This observation provides indirect evidence that the diuresis is produced through deeper nephrons, either by a tubular mechanism affecting only these nephrons, or by an increase in GFR in deeper nephrons which is balanced by a marked reduction in superficial nephron GFR, resulting in a net reduction for the whole kidney. The only other possibility, if all nephrons behave as the superficial ones, is that distal tubular fluid is diluted in the collecting ducts, which is regarded as extremely unlikely.

The observations presented in Chapters I and II provide indirect evidence that angiotensin may cause a degree of internal hydronephrosis by constriction of circular pelvis musculature around the tip of the papilla. This effect could simply be a pathological response to large amounts of angiotensin, or could represent a primitive, vestigial mechanism of urine flow regulation. A comparative study of the

situation, and responsiveness to drugs, of the pelvis musculature of other species, might substantiate the possibility of such a mechanism. Spasm of the circular pelvis muscle might produce an ischaemia of the tip of the papilla, and could be a contributory factor in drug-induced papillary necrosis.

The profound variations in tubular size and total kidney size observed during angiotensin infusion, make a primary role of tubular geometry in causing the diuresis unlikely, and emphasise that an inhibition of the reabsorptive capacity of the tubular epithelium must have occurred. In the study of Lowitz et al. (1969), no prolongation of split-drop reabsorption times was observed during intravenous infusion of angiotensin. However, in their experiments, an infusion of 0.5 μg/kg/min angiotensin caused a rise in blood pressure of only 4 mm Hg, and no change in GFR, sodium or potassium excretion, indicating that the vascular responsiveness of the animals used was markedly different from those used in the present study, possibly due to different conditions of anaesthesia.

Since an inhibitory effect of angiotensin in isolated sodium transport systems has never been demonstrated, it is tempting to relate the diuretic effect to the marked changes in intrarenal blood flow distribution observed in Chapter IV. The diuresis is suggested to result from the specific effect of angiotensin in reducing blood supply to areas of tubules, while

maintaining total kidney GFR at a near-normal level. This study offers no information on changes in interstitial fluid volume and pressure, which are crucial factors in investigating such a mechanism. Total kidney volume increases during angiotensin diuresis, but this effect is related to changes in tubular calibre, renal blood volume and the presence of internal hydronephrosis, and cannot be attributed to a primary increase in interstitial pressure. The question of whether a reduction in oxygen supply to areas of tubules could reduce their reabsorptive capacity requires further knowledge of the oxygen requirements of different areas of the rat kidney.

These experiments offer no explanation for the alteration in renal response to angiotensin produced by extracellular fluid volume expansion, except to suggest that alterations in responsiveness of the renal vasculature may play a role. The reversal of antidiuretic to diuretic response produced in the human by certain pathological conditions, appears to be associated with a change in the normal reduction of total GFR and RBF, and may be due to a different mechanism from that suggested above.

It is hoped that the work described in this thesis may further understanding of the mechanism of the renal response to angiotensin, and may assist in ascertaining the role played by this hormone in sodium and fluid balance of the organism.

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