STUDIES OF THE HORMONAL CONTROL OF RENAL FUNCTION IN NORMAL MAN AND IN TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS.

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ABSTRACT OF THESIS (Regulation 3.5.10)

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Title of Thesis Studies of the hormonal control of renal function in normal man and in Type 1 (insulin-dependent) diabetes mellitus.

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Angiotensin II (ANGII) has profound effects on renal and systemic haemodynamics and renal tubular sodium handling. A pathophysiological role has been proposed for ANGII in causing alterations in renal function in the early stages of human Type 1 (insulin-dependent) diabetes mellitus (IDDM). The studies in this thesis have examined the effect on renal function of acute changes in renin-angiotensin system (RAAS) activity induced by low dose ANGII infusion in normal man and patients with IDDM.

In preliminary studies the effects of low dose ANGII infusion on whole kidney renal function were defined in normal man and in IDDM. The renal haemodynamic response to ANGII was normal in IDDM patients, but whole kidney tubular sodium retention occurred in IDDM in parallel with a reduced suppression of plasma renin activity after dietary sodium loading compared to control subjects.

The utility of lithium clearance as an indirect marker of tubular sodium handling was then assessed. Several problems in interpreting renal haemodynamic data after lithium pretreatment were identified in normal man and in IDDM, but supplementary studies indicated that lithium clearance remains of value as a marker of tubular sodium handling in IDDM. The data indicate that enhanced proximal tubular reabsorption of sodium is associated with a blunted proximal antinatriuretic response to ANGII infusion in IDDM, the severity of this abnormality correlating with the level of chronic glycaemic control. The urinary concentrating response to ANGII infusion is also abnormal in IDDM. In separate studies insulin did not affect lithium clearance or interact intrarenally with angiotensin II; insulin treatment could not therefore itself account for the abnormal proximal tubular function found in the diabetic subjects.

These results support the hypothesis that increased renal proximal tubular retention of sodium in stable uncomplicated Type 1 diabetes is an acquired functional defect, related to the severity of the diabetic metabolic abnormality. This hyperreabsorption of sodium appears to be an 'appropriate' renal response which is necessary for the maintenance of glomerulotubular balance; the phenomenon occurs irrespective of whether or not glomerular hyperfiltration is present, and may be mediated by increased activity of the endogenous intrarenal RAAS.

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Finally and most importantly, none of the work would have been successful without the enthusiastic cooperation that I received from all the normal subjects and diabetic patients who took part in the studies.

DECLARATION.

The studies described in this thesis were performed while I was working in the Medical Renal Unit of the Royal Infirmary, Edinburgh, between December 1988 and October 1991. I received technical assistance which has already been acknowledged. I was entirely responsible for the planning and execution of the studies, and for all the data analysis. The composition of the thesis is also my own work. This thesis has not previously been submitted in candidature for a degree or diploma to any institution.

DAVID W. EADINGTON

January 29th 1992.

CHAPTER ONE.

THE CONTROL OF RENAL SODIUM HANDLING IN NORMAL MAN.

"The kidney presents in the highest degree the phenomenon of 'sensibility', the power of reacting to various stimuli in a direction which is appropriate for the survival of the organism; a power of adaptation which almost gives one the idea that its component parts must be endowed with intelligence."

E.H. Starling, 1909.

1.1 THE IMPORTANCE OF SODIUM BALANCE.

An appropriately filled extracellular fluid (ECF) space and an adequate plasma volume delivering oxygen to cells at an optimal rate and pressure are fundamental requirements for normal tissue metabolism. The sodium ion plays a central role in the regulation of ECF volume. The tonicity of the ECF is maintained within strict limits, and water equilibrates across cell membranes according to osmotic gradients, so that ECF volume is determined by the total osmolar content of the extracellular fluids. Sodium is actively excluded from the intracellular space, and sodium salts comprise more than 90% of the osmolar content of the ECF, making the ECF sodium content the major determinant of interstitial fluid and plasma volume. Plasma volume in health is maintained within narrow limits, and a complex hierarchy of interacting physical and hormonal systems has evolved in order to preserve sodium balance. Disturbance of these mechanisms in disease states leads to abnormal sodium homeostasis.

Sodium balance exists when dietary sodium intake and sodium excretion are equal. The preservation of normal ECF and plasma volume requires the maintenance of appropriate levels of sodium excretion in the face of potentially wide variations in sodium intake, and the pivotal role of the kidney in achieving this has been recognized for many years (Starling, 1909). Important

insights into the renal regulation of sodium balance in humans first came from balance studies (McCance RA, 1935; Strauss et al, 1958). These showed that when an individual in balance on a low sodium diet changes to a diet containing 150 mmol/day, urinary sodium excretion increases over 3-5 days until sodium output matches the new input. Attainment of this new steady state involves small increases in weight, total exchangeable sodium, plasma volume and venous pressure, changes which are reversible when sodium intake is reduced. Sodium balance is therefore normally maintained around a notional 'set point', arbitrarily defined as the amount of sodium chloride in the body when an individual is in balance on zero salt intake (allowing for small obligatory losses in sweat and faeces) (Hollenberg, 1980). An individual on a 10 mmol sodium diet will thus excrete an extra sodium load, even if only a small additional amount is given, while the detection of a sodium deficit will lead to retention of sodium by the kidney until the 'set point' is regained and sodium balance restored.

Sodium balance is maintained by a feedback mechanism with afferent sensing limbs and a range of coordinated effector responses. Under resting conditions about one quarter of the cardiac output enters the kidneys, which each day filter more than 25 mols of sodium (about eight times the total exchangeable body sodium content). Against this background of high turnover, small changes

in the filtered load of sodium or in tubular reabsorption produce rapid and substantial changes in sodium excretion.

Understanding of the mechanisms controlling renal sodium excretion followed the development of methods for studying renal function first in animals and later in man (reviewed by Smith, 1951). Newer *in vitro* techniques have continued to expand knowledge of the control of glomerular and tubular haemodynamics, tubular transport, tubular transport and the electrical events which accompany it, and the effects of hormones and drugs on the kidney. However, a problem remaining in such studies is the identification of those phenomena which are physiologically relevant, particularly when studies of normal kidney function are extended to disease states in which renal sodium handling is abnormal. Traditional *in vivo* human renal physiology still contributes useful information under these circumstances.

1.2 AFFERENT MECHANISMS.

The maintenance of a normal ECF volume is essential, and volume sensors are present in both the venous and the arterial circuits, and within the kidney itself, to provide information on the "effective volume" or "fullness" of the circulation, ie the relationship of plasma volume to the capacitance of the vascular space.

1.2.1 Extrarenal Sensors.

1.2.1.1 Intrathoracic Low Pressure Volume Receptors.

The distensible low pressure venous compartment is well suited for detection of small changes in circulating blood volume. A variety of neural receptors which alter their discharge rate according to changes in mechanical stretch and in transmural pressure have been found in the great veins and in the cardiac chambers (Gauer et al, 1970). In the cardiac atria two classes of mechano-receptor are found, Type A receptors discharging during atrial systole independently of atrial volume (Arndt et al, 1971), and Type B receptors which discharge during atrial filling and are sensitive to changes in atrial volume (Paintal, 1973). Studies in the conscious dog show the effectiveness of this volume receptor reflex in controlling sodium and water excretion (Goetz et al, 1970). Ventricular receptors may also modulate renal sodium excretion since stimulation of right ventricular afferent nerves causes a prompt natriuresis (Wennergren et al, 1976). Juxtapulmonary capillary (J) receptors have been found in the interstitium of the lung (Paintal, 1973), an ideal situation to detect early interstitial oedema.

Afferent signals from these receptors travel along cranial nerves 9 and 10 to hypothalamic and medullary centres where inputs from volume and osmotic receptors

can theoretically interact. Evidence that intrathoracic blood volume does affect sodium excretion comes from studies utilising manouevres which increase venous return such as tilting (Hulet and Smith, 1961), zero gravity (Gauer and Henry, 1976), negative pressure respiration (Gauer et al, 1954) and head-out water immersion (Epstein et al, 1978), all of which are accompanied by a natriuretic response. These experiments, however, do not prove that the response is mediated by a neural pathway. The importance of neural afferent input is supported by the abolition of the natriuresis accompanying volume expansion after cervical vagotomy (Atkins and Pearce, 1959). However, other workers have not detected any change in the natriuretic response to volume expansion after either cardiac denervation or cervical vagotomy (Knox et al, 1967; Gilmore and Zucker, 1978). It therefore seems that intrathoracic volume receptors do exist and may modulate sodium excretion, but other factors must also operate to sense the effective volume of the ECF space.

1.2.1.2 Arterial Volume Receptors and Baroreceptors.

The arterial portion of the circulation is smaller and less distensible than the venous compartment. The existence of arterial volume receptors was first suspected after the observation in man that closure of a traumatic atrio-ventricular fistula increased diastolic

pressure and caused natriuresis despite a fall in right atrial pressure and cardiac output, and without any measurable changes in glomerular filtration rate or renal blood flow (Epstein et al, 1953). In these circumstances natriuresis occurs because the kidney responds more to the increase in effective filling of the arterial tree than to the decreased filling of the venous system. The carotid sinus baroreceptor (Guyton et al, 1952) is a likely candidate for the sensor; traction on the baroreceptor in unanaesthetised rats leads to natriuresis (Keeler, 1974), while bilateral carotid ligation is antinatriuretic (Prosnitz, 1977), the reflex being dependent on an increase in renal sympathetic nerve activity.

1.2.1.3 Central Nervous System Volume Receptors.

The possibility of intracerebral interaction between stimuli arising from extracranial 'volume' and osmotic receptors has been mentioned. Intracranial receptors monitoring sodium balance also exist; an increase in cerebrospinal fluid sodium concentration promptly increases sodium excretion (Passo et al, 1975), as does infusion of a small amount of hypertonic saline into the carotid artery (Thornborough et al, 1973). It is not known if these receptors play a physiological role in sodium homeostasis.

1.2.1.4 Hepatoportal Volume Receptors.

The notion that the liver may modulate renal excretion of salt and water is plausible, since it is ideally placed to detect changes in sodium absorption from the gut into the portal vein. Experimental support for hepatic sensing is provided by the greater natriuresis seen after infusion of hypertonic saline into the portal vein than after systemic infusion (Daly et al, 1967), and the larger increment in sodium excretion when a sodium load is given orally rather than intravenously (Carey, 1978). These observations have not been confirmed in all studies (Obika et al, 1981).

1.2.2 Intrarenal Sensors.

The initial evidence for a separate intrarenal volume sensor came from studies of the isolated perfused kidney preparation, in which an increase in perfusion pressure suppressed renal renin and angiotensin II formation (Tobian et al, 1959). The demonstration of this relationship in the non-filtering kidney established the existence of a renal afferent arteriolar baroreceptor located in the juxtaglomerular apparatus (Blaine et al, 1970). Intrarenal mechanoreceptors that sense changes in subcapsular and interstitial pressure have also been identified (Burnett and Knox, 1980).

1.3 EFFERENT MECHANISMS.

The kidney responds to physical, neural and hormonal stimuli, and urinary sodium output is the net result of the combined activities of each input. The physiological importance of a particular mechanism under different conditions, and its interaction with other systems, is frequently not clear.

1.3.1 PHYSICAL FACTORS.

1.3.1.1 Glomerular Filtration Rate and Glomerulotubular Balance.

During ECF volume expansion whole kidney glomerular filtration rate increases (Osgood et al, 1978), but the natriuresis of volume expansion persists when the filtered load of sodium is held constant or even reduced (DeWardener et al, 1961). However if glomerular filtration rate, and hence the filtered load of sodium, is increased by manoeuvres other than volume expansion there is little if any change in fractional sodium excretion (Lindheimer et al, 1967). The occurrence of these proportional changes in the tubular reabsorption of sodium which maintain constancy of sodium excretion in the face of alterations in glomerular filtration rate, glomerulotubular balance, was suspected many years (Walker et al, 1941) before its confirmation (Giebisch et al, 1964). The phenomenon prevents changes in glomerular filtration rate, which cause large changes in the filtered load of sodium, from destabilising sodium

balance. The existence of glomerulotubular balance has been confirmed in whole animal studies, in the isolated perfused kidney preparation, and by micropuncture experiments (Reineck et al, 1985). The renal tubules, especially the proximal segment, play a major role in the process.

1.3.1.2 Peritubular Capillary Starling Forces.

About 60% of the filtered load of sodium is reabsorbed in the proximal tubule. Reabsorption of fluid in this segment is for all practical purposes iso-osmotic, sodium entering the tubular cell from the urinary space across the apical membrane by passive transfer down an electrochemical gradient. Its transfer is linked to the transport of other ions and solutes in both electrogenic and electroneutral processes. The active transport of sodium across the basolateral membrane of the proximal tubular cells is mediated by the Na^+-K^+-ATPase complex. The final stage of reabsorption, uptake of fluid from the interstitium into the peritubular capillaries, is determined by the passive Starling forces which govern all capillary transport systems. Fluid flux may be simplistically represented as:

$$\text{Flux} = K_f(\pi_c - \pi_i) - (P_c - P_i)$$

where π_c and π_i are the oncotic pressures and P_c and P_i the hydrostatic pressures in the capillary lumen and the

interstitium respectively, and K_f is the ultrafiltration coefficient, a measure of capillary permeability. The postglomerular capillary plasma has a high oncotic pressure following filtration of albumin-free fluid into the urinary space, while efferent arteriolar resistance reduces postglomerular capillary hydrostatic pressure. The rate of sodium uptake therefore depends on the balance between these two opposing forces, which normally favours reabsorption of fluid and sodium in the proximal tubule (Knox et al, 1983), and to a lesser extent on the plasma flow rate which determines the rate of delivery of oncotically active particles. Peritubular oncotic pressure is also important, and a direct relationship has been shown between proximal sodium reabsorption and the peritubular oncotic pressure (Brenner et al, 1969), although other studies did not confirm this (Holgreve and Schrier, 1975). When glomerular filtration rate alters without a change in renal plasma flow, peritubular capillary oncotic pressure varies in parallel with the filtration fraction, the fraction of plasma water extracted from plasma during glomerular filtration. A fall in glomerular filtration rate reduces the filtration fraction and tends to reduce fractional tubular sodium reabsorption, maintaining glomerulotubular balance.

The importance of Starling forces is not incompatible with the simultaneous occurrence of active sodium reabsorption in the proximal tubule, and both

processes are accommodated by the "pump-leak" model of reabsorption, in which alterations in the physical forces influence the rate of uptake of reabsorbate from the interstitial space. If the balance of physical forces favours fluid rejection, interstitial pressure will increase, and fluid will leak back into the tubular lumen across the tight junctions (Reineck et al, 1985). The interdependence of active and passive processes is further demonstrated by the abolition of the influence of peritubular oncotic pressure on proximal reabsorption of sodium after inhibition of active sodium transport by ouabain (Green et al, 1974).

The disruption of glomerulotubular balance by volume expansion with saline is mediated by a reduction in fractional proximal sodium reabsorption. Intravenous saline loading reduces plasma protein concentration and thus plasma oncotic pressure, while plasma expansion with hyperoncotic albumin produces a smaller increase in sodium excretion than an equivalent degree of expansion induced with saline (Levy and Levinsky, 1972), further supporting the importance of changes in peritubular forces. Although the volumes of saline and albumin used to change plasma oncotic pressure in these studies are unphysiological, this does not rule out a physiological role for physical forces under normal conditions.

Glomerulotubular balance is also disrupted by changes in renal haemodynamics through changes in the

filtration fraction, which is influenced by glomerular efferent arteriolar tone and can modulate both peritubular capillary oncotic and hydrostatic pressures. Factors increasing renal blood flow usually reduce the filtration fraction and facilitate sodium excretion, and reflex efferent vasodilatation with a reduction in filtration fraction may partly account for the phenomenon of pressure natriuresis which will be discussed later. The peritubular physical forces can thus be modified both by haemodynamic and tubular events.

1.3.1.3 Renal Nerves.

The kidney is innervated by postganglionic adrenergic vasomotor fibres, which travel with the main renal artery and terminate on all arterial components, particularly the afferent arterioles in the region of the juxtaglomerular apparatus, and on both proximal and distal tubules (Barajas, 1978). The renal nerves may modulate sodium excretion by more than one mechanism. Changes in plasma volume modulate peripheral sympathetic outflow: in the dog a 15 mmHg increase in left atrial pressure reduced renal nerve activity by 40% and increased sodium excretion by 80% (Prosnitz and DiBona, 1978), and this response was abolished by cervical vagotomy. There is also an inverse relationship between pulmonary artery pressure and renal nerve activity (Thames, 1982). Direct renal nerve stimulation reduces

GFR and renal plasma flow via vascular alpha adrenergic receptors, and concomitant renin release may contribute to this. Sodium excretion may also be reduced in the absence of any haemodynamic changes by low level nerve stimulation, and micropuncture studies confirm that this is a tubular effect (Bello-Reuss et al, 1976), also mediated by alpha receptors (Chan, 1980). Renal nerve stimulation activates the renin-angiotensin and prostaglandin systems, but blockade of these with saralasin and indomethacin does not attenuate the effects of renal nerve stimulation (Zambraski and Di Bona, 1976).

The phenomenon of denervation natriuresis is well documented in anaesthetised animals, but studies on conscious dogs have failed to confirm that denervation affects sodium output (Smith, 1951). Most evidence suggests that the renal nerves are of minor importance under normal conditions (Gottschalk, 1985), but under some circumstances a physiological role may become apparent, as when renally denervated dogs fail to conserve sodium when placed on a low sodium diet (DiBona and Sawin, 1983), a phenomenon also exhibited by patients with idiopathic autonomic neuropathy (Bartter et al, 1959).

1.3.1.4 Medullary Blood Flow, Interstitial Solute Composition, and Distal Tubular Reabsorption.

Alterations in medullary blood flow can alter the solute composition and hypertonicity of the medullary interstitium. This may be important in the regulation of sodium transport in the medullary and papillary portion of the collecting ducts (Osgood et al, 1978), for example during volume expansion (Stein et al, 1976). A number of hormones have definite effects on renal sodium handling predominantly through a distal tubular action (i.e aldosterone, corticosteroids, the atrial natriuretic peptides, vasopressin), but the interactions between medullary haemodynamic effects and these hormonal factors are less well delineated than is the case in the proximal tubule. It is clear however that most parts of the distal nephron exhibit load dependency of reabsorption to maintain glomerulotubular balance, and the coupling of the transport functions of the various nephron segments by changes in medullary interstitial solute composition (Knepper and Burg, 1983) means that the amount of sodium finally excreted in the urine depends more on distal tubule and collecting duct function than on events occurring in the proximal tubule (DeWardener, 1978).

1.3.2 HORMONAL FACTORS.

Many hormones alter renal sodium handling in acute experiments, but a more restricted number have a proven physiological role in normal man. This overview will concentrate on the renin-angiotensin-aldosterone system (RAAS), whose actions are examined in the studies which follow. Other systems will be mentioned mainly to highlight their interactions with the RAAS.

1.3.2.1 Renin-Angiotensin-Aldosterone Axis.

It is almost one hundred years since an extract from a fresh rabbit kidney was shown to have pressor activity (Tigerstedt and Bergman, 1898). The active agent in this extract was called *renin*, but its major role as a homeostatic regulator was recognised only much later (Goldblatt, 1934). The basic elements of the renin-angiotensin-aldosterone system (RAAS) are shown in Figure 1.1. Renin is formed by the processing of its precursor prorenin in the juxtaglomerular cells. These are specialised myoepithelial cells at the distal end of the afferent arteriole which are apposed to an area of specialised cells in the early distal tubule of the same nephron called the macula densa. This juxtaglomerular apparatus (JGA) contains all the elements needed to exert feedback control on glomerular function at the single nephron level. One of the principle functions of the RAAS is the maintenance of normal glomerular filtration,

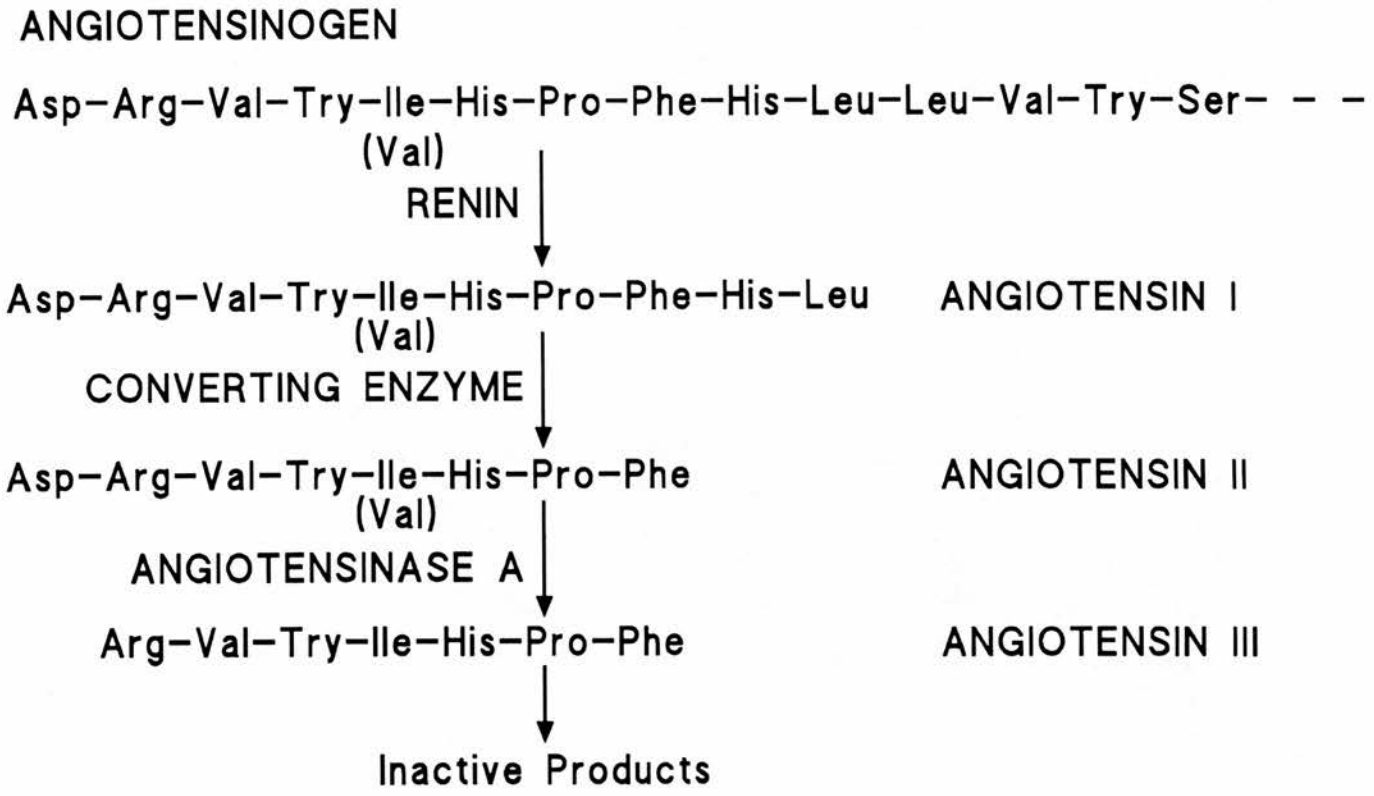

Figure 1.1: Components of the renin–angiotensin system.

sodium balance and blood pressure during periods of salt depletion or reduced renal perfusion pressure. The prevailing state of sodium balance is therefore an important determinant of the activity of the system, and the inverse relationship between dietary sodium intake and the endogenous concentrations of renin and angiotensin II (ANGII) (Hollenberg et al, 1972) is teleologically appropriate, since as dietary sodium intake falls the RAAS is progressively more activated and capable of a prompt response if sodium homeostasis is threatened.

1.3.2.1.1 Renin.

The proteolytic enzyme renin acts on the liver-derived alpha-2-globulin angiotensinogen to yield the decapeptide angiotensin I (ANGI). This has little physiological activity, but is metabolised by a converting enzyme to the potent octapeptide ANGII. ANGII is hydrolysed by angiotensinases to the heptapeptide ANGIII, which has some physiological activity but is less important because of its short biological half-life.

Four inputs are accepted as particularly important in modulating the secretion of renin from the JGA (Davis and Freeman, 1976):

1. A reduction in renal perfusion pressure stimulates renin secretion through the intrarenal baroreceptor in the juxtaglomerular apparatus. Prostaglandins are able

to stimulate renin release through the same mechanism (Dunn, 1979).

2. Sympathetic nerve stimulation and circulating catecholamines increase renin release via the beta-adrenergic receptors on juxtaglomerular cells.

3. Angiotensin II produces feedback inhibition of renin release (Vander and Geelhoed, 1965), the 'short-feedback loop'.

4. Changes in macula densa chloride transport affect renin release, modulating feedback-mediated changes in renal haemodynamics which stabilize glomerular filtration rate, a process called tubuloglomerular feedback (see Chapter 1.5).

1.3.2.1.2 Angiotensin II.

Angiotensin II (ANGII) is the most significant effector peptide of the RAAS. It is a potent constrictor of vascular smooth muscle, systemic vasoconstriction increasing effective ECF volume, peripheral vascular resistance and arterial blood pressure. A renal vasoconstrictor effect of ANGII is apparent at both afferent and efferent glomerular arterioles (Steinhausen et al, 1990; Edwards, 1983), but there is controversy as to which site of action is more important physiologically. In micropuncture studies ANGII increases afferent arteriolar resistance only when infused at doses which also increase blood pressure in

the rat (Myers et al, 1975), the dog (Hall and Granger, 1986b) and the rabbit (Edwards, 1983), suggesting that afferent constriction is a secondary 'myogenic' response, and *in vitro* dose response curves demonstrate a 10^4 fold greater sensitivity of the efferent arteriole to exogenous ANGII when compared to the afferent (Steinhausen et al, 1990). However, peritubular ANGII infusion causes a prompt reduction in stop flow pressure when afferent arterioles retain their normal structural relations, implying predominantly preglomerular vasoconstriction under these conditions (Mitchell and Navar, 1987).

Support for a 'mixed' afferent/efferent site of action for ANGII *in vivo* also comes from a theoretical analysis of the changes in segmental renal resistances accompanying vasoconstrictor stimuli (Smith, 1951). When renal plasma flow is reduced by a 'pure' efferent vasoconstrictor, GFR rises because increases in efferent arteriolar resistance elevate intraglomerular capillary pressure and hence the filtration fraction. A 'pure' afferent constrictor reduces GFR in parallel with renal plasma flow without changing the filtration fraction. Finally, equal changes in afferent and efferent resistance maintain GFR as renal plasma flow drops, the effect of falling renal perfusion on GFR being exactly compensated by the rise in intraglomerular pressure. This last analysis is most compatible with the known

action of ANGII to produce a minimal fall in GFR and a modest rise in filtration fraction when renal perfusion is compromised, an action which is apparent even when myogenic afferent vasoconstriction is prevented by administering ANGII intrarenally (Johnson and Malvin, 1977). It is therefore very likely that ANGII affects both afferent and efferent arteriolar tone under physiological conditions.

Many vasoconstrictors, including ANGII, are capable of inducing glomerular mesangial cell contraction (Yoshimoto et al, 1990). The speculation that the contractile properties of the mesangium may be linked to modulation of the glomerular capillary ultrafiltration coefficient K_f through changes in the filtering surface area has recently been demonstrated *in vivo* (Fogo et al, 1990). ANGII-induced changes in K_f (Blantz et al, 1976) therefore provide a further mechanism by which ANGII may modulate GFR without directly affecting intrarenal resistances. The capacity for ANGII to affect structure-function relationships within the kidney is also reflected in its effects on mesangial macromolecular trafficking (Singhal et al, 1990), cell growth and hypertrophy (Berk et al, 1989), and extracellular matrix accumulation (Homma et al, 1990), topics which are beyond the scope of this review.

ANGII affects renal sodium excretion by several distinct but interconnected mechanisms. The importance

of these direct effects outweighs the indirect effects of ANGII-stimulated aldosterone release from the adrenal cortex (Laragh and Sealey, 1973). Sodium excretion during ANGII infusion shows a biphasic dose response pattern, with antinatriuresis at low infusion rates but increased sodium excretion at high doses (Barraclough, 1965). Interpretation of these studies is complicated by large and variable changes in glomerular filtration rate and renal plasma flow, but intrarenal infusion of angiotensin II produces the same biphasic response, so that systemic 'pressure-natriuresis' is not the only explanation (Waugh, 1972). The physiological relevance of the natriuretic action of ANGII at high doses is questionable because the circulating levels produced far exceed those attained by endogenous production (Johnson and Malvin, 1977), but the increase in whole kidney filtration fraction produced by physiological doses of ANGII increases capillary oncotic pressure and reduces peritubular capillary hydrostatic pressure, both changes favouring an increase in sodium reabsorption at the proximal tubule (Mujais, 1986).

ANGII can also directly promote proximal tubular sodium reabsorption independent of haemodynamic effects (Johnson and Malvin, 1977; Harris and Young, 1977; Schuster et al, 1984). A bimodal effect is again apparent, with inhibition of sodium reabsorption at high concentrations and enhanced transport at lower levels

which may relate to the way in which ANGII couples to its receptors (Douglas et al, 1990). ANGII affects epithelial function directly by occupying receptors on the cell surface or indirectly by presynaptic receptors which control catecholamine release from nerve terminals on the cell. ANGII receptors exist on both luminal and basolateral surfaces of proximal tubular cells (Brown and Douglas, 1982; Brown and Douglas, 1983), which are also richly innervated, giving the potential to use both mechanisms.

The majority (85%) of sodium reabsorption in the proximal tubule is linked to either bicarbonate or chloride. Changes in sodium chloride transport occur mainly through presynaptic receptors on renal nerves (Liu and Cogan, 1989). ANGII predominantly affects bicarbonate rather than chloride transport, via epithelial cell receptors which enhance the activity of the Na^+-H^+ antiporter, the major proximal tubular acidification mechanism. This is achieved by reducing the K_m of the antiporter rather than by changes in V_{max}, utilising inhibition of tubular adenylate cyclase as the second messenger (Liu and Cogan, 1989). Additionally, stimulation of glucose-linked sodium cotransport by ANGII has recently been described (Garvin, 1990); this is likely to be only a small component of total proximal sodium reabsorption in the normal kidney, but may become more significant when the filtered load of glucose is

increased as in diabetes.

The bipolarity of ANGII signalling means that both filtered and intravascular ANGII potentially have physiological activity in the proximal tubule. The ubiquitous presence of angiotensinogen mRNA in glomerular and tubular cells, the capacity to endocytose interstitial renin, and the ready availability of converting enzyme makes local ANGII generation at various intrarenal sites likely (Levens et al, 1981), especially since intrarenal ANGII concentrations far exceed circulating levels (Mendelsohn, 1979). Thus autocrine, paracrine and endocrine mechanisms are all conceivable for the control of proximal tubular function and the regulation of intrarenal haemodynamics by ANGII (reviewed by Carey, 1988).

ANGII regulation of proximal sodium chloride transport does not exist in isolation, but affects downstream tubular transport elements as well as glomerular haemodynamics. The coordinated linkage of filtered solute and proximal tubular reabsorption has been described already, and the glomerular and tubular actions of ANGII give it obvious potential as a mediator of this response. As well as resetting glomerular vascular resistances and K_f, ANGII modifies two other factors which independently affect proximal tubular sodium transport, neurogenic tone (via the presynaptic receptors) and peritubular protein concentration (by

changing the filtration fraction).

Abundant ANGII binding sites are found in the medulla where ANGII may modulate medullary blood flow (Cupples et al, 1988), but the physiological importance of ANGII as a regulator of distal renal sodium and water excretion remains uncertain and is probably limited in comparison to its effect on proximal tubular events. Loop of Henle sodium chloride reabsorption exhibits good but not perfect load dependence, and increased ANGII activity may also modulate sodium transport in the loop by constricting the medullary circulation.

At the cellular level ANGII receptor numbers on glomeruli are directly regulated by the circulating ANGII level, with an increase in receptor density when plasma ANGII is low, and a fall in density when plasma ANGII is increased. Up- or down-regulation of receptor numbers with increasing or decreasing dietary sodium intake is not accompanied by any change in receptor affinity (Bellucci and Wilkes, 1984). A 50% fall in ANGII receptor density on human platelets occurs within 15 minutes of starting an infusion of ANGII, and is maintained for up to 2 hours (Moore et al, 1984). This is not an artefact due to receptor occupancy. Following a change in dietary sodium intake an altered receptor density is detectable after 2 days, concomitant with the time scale of changes in plasma ANGII, which reaches a new stable level after 6 days (Moore et al, 1984), and an

increased ANGII receptor density accounts for the enhanced sensitivity to exogenous ANGII seen in the renal vasculature after sodium loading. The effect of the plasma ANGII concentration on receptor density is tissue-specific, the adrenal glomerulosa showing the opposite pattern to that described above (Gordon et al, 1983). The effect of dietary sodium or plasma ANGII on proximal tubular ANGII receptor density and affinity has not yet been reported.

Many individual tissues have the capacity to synthesize ANGI and ANGII, and the final response probably represents a combination of the reaction to circulating and locally generated ANGII, components which may be separately regulated (Samani, 1991). The concentrations of circulating renin and ANGII are not therefore necessarily an accurate reflection of the activity of the system in a particular tissue. All components of the RAAS are also present in the brain, are unable to cross the blood-brain barrier, and probably operate independently of the systemic axis. However, application of ANGII to the cerebral ventricles produces a systemic pressor response in the rat (Al-Barazanji and Balment, 1990), which may be partly due to facilitated sympathetic outflow, providing another mechanism by which the RAAS can maintain glomerular filtration while encouraging sodium retention.

1.3.2.1.3 Aldosterone.

Aldosterone secreted from the adrenal gland promotes sodium retention in the distal tubule, acting on the Na^+-K^+-ATPase system in the cortical collecting segment (Gross, 1974). The circulating ANGII level is an important regulator of aldosterone production, reflected in the inverse relationship between dietary sodium intake and the circulating level of both hormones (Best et al, 1971). Adrenal sensitivity to exogenous ANGII also increases as dietary sodium intake falls (Gordon et al, 1983). The reduction of aldosterone levels in untreated Addisons disease contributes to salt wasting, but these patients maintain sodium balance on fixed physiological replacement doses of glucocorticoid despite wide variations in dietary sodium intake (Rosenbaum et al, 1959). Saline loading causes a natriuresis despite exogenous mineralocorticoid excess (DeWardener et al, 1961; Singer et al, 1991), and chronic mineralocorticoid administration causes only transient sodium retention, followed by the phenomenon of "mineralocorticoid escape" (Relman and Schwartz, 1952; August, 1958). These findings all suggest that the maintenance of sodium balance in healthy humans is not solely dependent on the capacity to regulate the circulating level of aldosterone.

1.3.2.2 Dopamine.

Dopamine is the precursor molecule for the synthesis of the catecholamines noradrenaline and adrenaline. Interest in its renal effects was stimulated by the observation that intravenous infusion of pharmacological doses of dopamine increases renal plasma flow, glomerular filtration rate, and sodium and water excretion in both dog and man (McDonald et al, 1964). The existence of a highly specific renal vascular receptor (DA_1) for dopamine was subsequently confirmed, and dopamine receptors are now classified as DA_1, present at the vascular receptor and in the renal proximal tubule, and DA_2, found in the post-ganglionic sympathetic neurone, stimulation of which leads to emesis and the inhibition of prolactin release (Goldberg, 1984).

The amount of dopamine in urine is ten to twenty times greater than the urinary noradrenaline concentration, while plasma dopamine concentrations are much lower than noradrenaline, suggesting that dopamine is synthesized within the kidney (reviewed by Lee, 1987). A physiological role for endogenous dopamine in renal sodium handling is suggested by the increase in renal dopamine synthesis which follows both acute and chronic sodium loading (Ball et al, 1978), the parallel falls in urinary DA output and sodium excretion following chronic dopa decarboxylase inhibition (Ball et Lee, 1977), and the blunting of saline-induced natriuresis after

carbidopa administration in the dog (McClanachan et al, 1985) or dopaminergic blockade in man (Coruzzi et al, 1986). However, carbidopa does not influence the natriuretic response to acute saline loading in normal man (Jeffrey et al, 1989). The sensing mechanism for dopamine formation is unknown but may depend on the filtered load of sodium and chloride. Frusemide increases urine dopamine output (Kuchel et al, 1978), but inhibition of dopamine synthesis does not modify the natriuretic response to frusemide (Jeffrey et al, 1987), and the increased renal plasma flow following frusemide administration is not dopamine dependent.

The interactions between dopamine and other intrarenal hormone systems are relatively unexplored, but there is some evidence that dopamine interacts with the RAAS at several levels. The changes in responsiveness of plasma aldosterone to exogenous ANGII infusion which accompany changes in dietary sodium intake are modulated by dopamine infusion (Drake and Carey, 1984; Connell et al, 1987) and by dopaminergic blockade with metoclopramide (Gordon et al, 1983). The dopaminergic prodrug gamma-L-glutamyl-L-dopa (gludopa) increases urine dopamine excretion several hundred-fold and reduces plasma renin activity despite causing natriuresis and a negative sodium balance (Worth et al, 1985), suggesting that high intrarenal concentrations of dopamine may directly inhibit renin release. Finally, acute

angiotensin converting enzyme inhibition tends to increase the rise in urine dopamine excretion produced by frusemide, although the natriuretic response to frusemide is not modified (MacDonald et al, 1990), suggesting that changes in intrarenal ANGII levels within the physiological range can modulate dopamine production.

Evidence of a physiological role for dopamine in the kidney is complemented by abnormalities of dopamine production in pathological states. Dopamine disappears from the urine in patients with chronic renal failure when the plasma creatinine exceeds 600 umol/l (Itskovitz and Gilberg, 1981), and patients with chronic renal failure do not increase urinary dopamine excretion in the normal way when given added dietary sodium (Casson et al, 1983). Secondly, when Caucasian patients with essential hypertension are given added dietary sodium, urine dopamine output paradoxically falls for two days before rising towards the baseline values (Harvey et al, 1984), in contrast to the prompt and sustained increase seen in normotensive controls. The failure to mobilise dopamine in response to sodium loading is likely to be a precursor to and not a consequence of hypertension because the normal positive correlation between urine sodium excretion and urine dopamine is absent in the normotensive relatives of hypertensive patients (Saito et al, 1986), and in normotensive black individuals (Critchley et al, 1987) who respond in the same way as

Caucasian hypertensives. This genetically determined 'dopamine defect' may contribute to the high incidence of essential hypertension in negroid populations in developed countries.

1.3.2.3 Prostaglandins.

Renal prostaglandins such as PGI_2 (prostacyclin), PGF_2, PGE_2, PGD_2 and thromboxane A_2 are potential modulators of renal haemodynamics and sodium excretion (reviewed by Dunn, 1979). Prostaglandin synthesis is regulated by angiotensin II, noradrenaline, vasopressin and bradykinin. PGE_2 and PGI_2 are vasodilators which antagonize the vasoconstrictor effects of ANGII (Aiken and Vane, 1973), TxA_2 (Anderson et al, 1976), and catecholamines (Henrich et al, 1978a).

Prostaglandin production is compartmentalised within the kidney, the principal synthetic sites being the cortical arterioles (PGI_2), and the glomerular mesangial cells, medullary interstitial cells and collecting ducts (PGE_2). This anatomical segregation, and the problems of assaying compounds with short biological half-lives, has hampered attempts to assign physiological roles to the various compounds, but they are believed to function more by modulating other systems than as primary determinants of renal function. In salt replete unstressed humans endogenous prostaglandins do not affect renal plasma flow or glomerular filtration rate, but when the RAAS is

activated (by impaired renal perfusion or low dietary sodium intake) inhibition of prostaglandin synthesis produces clinically significant reductions in both renal blood flow and GFR (Henrich et al, 1978b; Muther et al, 1981).

Prostaglandins exert a potentially pro-natriuretic influence by several distinct mechanisms. Antagonism of ANGII-mediated efferent arteriolar vasoconstriction reduces the filtration fraction and so reduces proximal tubular sodium reabsorption. Prostaglandins also reduce medullary interstitial tonicity by increasing medullary blood flow (Solez et al, 1974), reducing medullary urea accumulation (Shimizu et al, 1969) and inhibiting sodium reabsorption in the cortical collecting tubule (Stokes and Kokko, 1977). PGE_2 may also decrease sodium reabsorption in the medullary thick ascending limb (Stokes, 1979). However, pharmacological doses have been used in most studies, and exogenous administration cannot reproduce the specific intrarenal localisation of endogenous prostaglandins. Studies of the effect of changing sodium balance on urinary prostaglandin production have given confusing results, with salt loading increasing, not affecting or even reducing prostaglandin excretion in man and in animals. This may reflect the difficulty of trying to use measurements of total urinary prostaglandin excretion to detect small changes in intrarenal prostaglandin production.

1.3.2.4 Kallikrein-Kinin System.

Renal kallikrein is a serine protease which acts on kininogen yielding the biologically active kinins bradykinin, lys-bradykinin (kallidin), and met-lys-bradykinin. These compounds are rapidly inactivated by kininases present in both proximal and distal tubules (reviewed by Scicli and Carretaro, 1986). All the components of the kallikrein-kinin system exist within the kidney, and the activity of the system is usually inferred from measurements of the urinary excretion of kallikrein, although the relationship of this parameter to intrarenal kinin release is poorly defined. Because of the apparent restriction of kinin synthesis to distal tubular elements (Orstavvik and Inagami, 1982), the relevance of intrarenal kallikrein to the regulation of renal haemodynamics and tubular function has been questioned. However, the recent demonstration of kallikrein-like granules and kallikrein mRNA in the glomerular peripolar cell provides a mechanism by which locally formed kallidin may gain access to the glomerular vasculature and exert direct effects on renal haemodynamics (Xiong et al, 1989).

The kallikrein-kinin system has a reciprocal stimulatory relationship with the renal prostaglandins. Kinins stimulate synthesis of phospholipase A_2, releasing arachidonic acid, while phospholipase A_2 can activate membrane bound renal kallikrein, and prostaglandins also increase urinary kallikrein excretion (Nasjletti and

Malik, 1979). The rise in kallikrein excretion during low dietary sodium intake (Margolius et al, 1974) supports the hypothesis that prostaglandins and kinins act synergistically to oppose the antinatriuretic and vasoconstrictor effects of ANGII during salt depletion. The conflicting evidence that acute intravenous salt loading may increase kallikrein synthesis must be interpreted in the light of the potential artefactual effect of distal tubular 'washout' by the associated diuresis (Grez et al, 1982). Intrarenal infusion of kinins produces vasodilatation and natriuresis (Fadem, 1982), and the administration of anti-kinin antibodies to saline-expanded rats decreases sodium excretion (Grez, 1974).

The kallikrein system interacts with the RAAS in several ways. Urinary kallikrein activates prorenin *in vitro* (Sealey et al, 1978) and bradykinin infusion stimulates renin release *in vivo* (Flamenbaum et al, 1979). Aldosterone is a powerful stimulus for renal kallikrein synthesis (Margolius et al, 1976). The kinin degrading enzyme kininase II is identical to angiotensin converting enzyme, so that increased kinin levels may contribute to the effects of this class of drugs. This question, and the physiological role of the kallikrein system in sodium homeostasis, may be answered by studies with the newly available specific kinin antagonists.

1.3.2.5 Other Hormonal Factors.

There is experimental evidence for a distinct but unidentified low molecular weight natriuretic hormone involved in renal salt handling. Cross circulation experiments in the dog (Buckalew and Nelson, 1974) indicate the existence of a transferable hormonal factor, which has also been found during volume expansion in normal man (Epstein et al, 1978) and in patients with chronic renal failure (Bourgoignie et al, 1974). The factor may derive from the hypothalamus, is a normal constituent of plasma, and inhibits sodium transport at the nephron. Its action involves the inhibition of Na^+-K^+-ATPase, and the same factor has been implicated in the pathogenesis of essential hypertension, where compensatory overactivity of the putative substance increases sodium excretion by the kidney (DeWardener and MacGregor, 1983).

Other substances affecting intrarenal haemodynamics and tubular sodium transport in experimental studies include the atrial natriuretic peptides, serotonin, neuropeptide Y, adenosine, histamine, glucagon, parathyroid hormone, calcitonin and insulin. With the exception of ANP, the evidence that any of these has an important influence on renal sodium handling in healthy humans is highly speculative. Some are potentially more important in disease states, as will be discussed later in the case of insulin.

1.4 RENAL-BODY FLUID FEEDBACK CONTROL OF ARTERIAL BLOOD PRESSURE.

Changes in arterial pressure and renal perfusion pressure influence the renal excretion of sodium and water, to provide a negative feedback control on ECF volume, a phenomenon known as 'pressure-natriuresis' (Thompson and Dickinson, 1976). This feedback loop (Figure 1.2) operates through the neurohumoral controls described above to regulate blood pressure both acutely and in the long term. According to this viewpoint long-term blood pressure control is dictated primarily by renal excretory function; persistently abnormal renal haemodynamics or tubular reabsorption initiate a resetting of the pressure-natriuresis mechanism so that sodium balance is restored, but only at the expense of hypertension. Changes in cardiac output or total peripheral resistance are not considerd to be as a primary 'cause' of hypertension, but merely a means of adjusting blood pressure to the level required for the prevailing renal excretory capacity.

An opposing view is that alteration of the pressure-natriuresis mechanism is a secondary consequence of an increase in arterial pressure (Omvik et al, 1980). However, the observation that transplantation of a kidney from a normotensive strain of rat into a genetically hypertensive recipient reduced the recipient's blood pressure to normal (Bianchi et al, 1974) indicates that

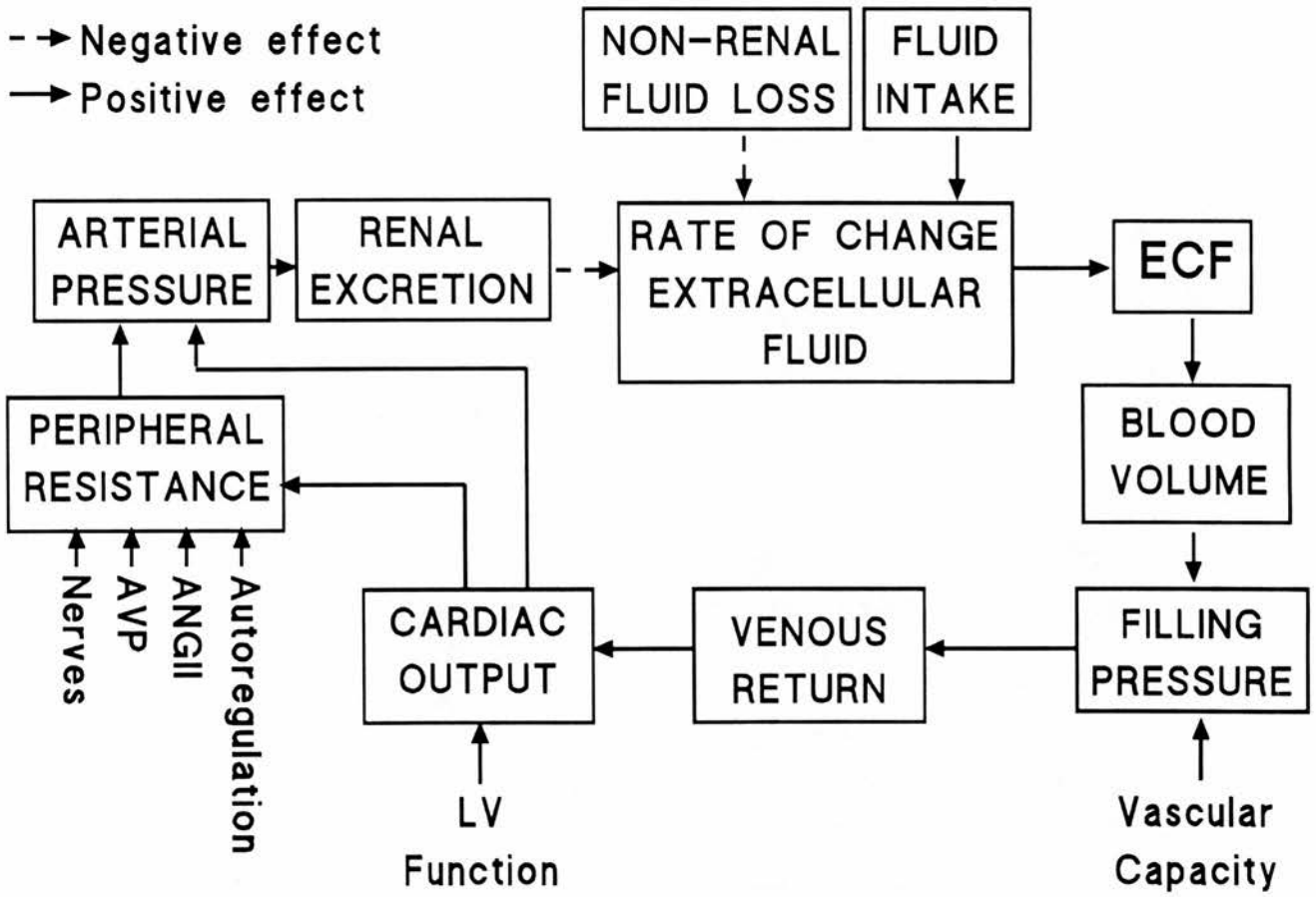

Figure 1.2: Renal–body fluid feedback system.

this is not the case. Experiments in the conscious dog with chronic antinatriuretic influences such as mineralocorticoid excess (Hall et al, 1984a) or ANGII infusion (Hall et al, 1984b) also support the central importance of pressure-natriuresis; when a rise in renal perfusion pressure was prevented mechanically, relentless sodium retention occurred and blood pressure rose without plateauing until cardiovascular collapse ensued. When renal perfusion pressure was then allowed to move in parallel with systemic blood pressure there was a prompt escape from sodium retention and a fall in arterial pressure. Thus at least in these experimental situations chronic hypertension is an essential compensatory response to a primary inability of the kidney to excrete appropriate amounts of sodium and water at normal arterial pressure.

The acquisition of mechanisms to conserve sodium probably conferred an evolutionary advantage when man inhabited only salt-poor areas. Migration to temperate habitats and the introduction of meat into the diet produced a steady increase in dietary sodium intake, and intrarenal mechanisms have evolved in parallel to allow the excretion of large salt loads in order to maintain sodium balance. Economic and social forces in the developed world today encourage the consumption of a diet with a relatively high sodium content, and a positive association has been consistently found between the

prevalence of essential hypertension in a population and its dietary sodium intake (Intersalt, 1988). The hypothesis has been advanced that all populations contain individuals who through genetic variability are less able to excrete dietary sodium loads efficiently, and that in an indigent population which is acculturated to a low salt diet there are likely to be more individuals who are genetically 'sodium-retainers'. If this sub-group of the population is suddenly exposed to an increased dietary salt intake, sodium balance can be maintained only by increasing plasma volume and blood pressure in order to produce a pressure-natriuresis. The high incidence of volume-dependent hypertension in American Negroes in comparison to American Caucasians may be an example of this phenomenon (Brier et al, 1991).

Renal abnormalities that cause hypertension commonly increase the ratio of tubular sodium reabsorption to GFR. This can be due to primary reductions in GFR (ie chronic renal failure), increased preglomerular resistance (Goldblatt hypertension), decreased K_f (glomerulo-nephritis), or a primary increase in tubular reabsorption (antinatriuretic hormones). As well as secondary compensations which may obscure the initial abnormality, circulatory changes due to increased arterial pressure subsequently develop. Attempts to define any postulated renal defect must therefore involve the study of subjects during the earliest stages of the disorder.

1.5 INTEGRATION OF THE RENAL RESPONSE: RENAL AUTOREGULATION AND TUBULOGLOMERULAR FEEDBACK.

Three separate mechanisms operate to maintain the stability of glomerular filtration in normal man. The classical 'myogenic' response buffers glomerular filtration rate against sudden changes in systemic arterial pressure by causing reflex changes in afferent arteriolar tone. The renin-angiotensin system is activated primarily when renal perfusion pressure falls, maintaining GFR via an increase in efferent arteriolar resistance. The existence of a third system, the tubuloglomerular feedback mechanism (TGF) (Figure 1.3), was first suspected after the demonstration that injection of a solution with a high sodium chloride concentration into the early distal tubule caused the proximal tubule to collapse, implying a large fall in glomerular filtration rate (Thurau and Schnermann, 1965). Micropuncture studies have confirmed that individual nephrons have a feedback mechanism which operates on the afferent arteriole to stabilize GFR at normal systemic arterial pressure (Navar et al, 1980; Schnermann and Briggs, 1985; Wright and Briggs, 1979).

Although mostly studied in the rat, the existence of TGF has been confirmed in the dog (Bell et al, 1978) and in the isolated human kidney (Schnermann et al, 1977b). The response exhibits nephron heterogeneity, with juxta-medullary nephrons having a greater maximal response

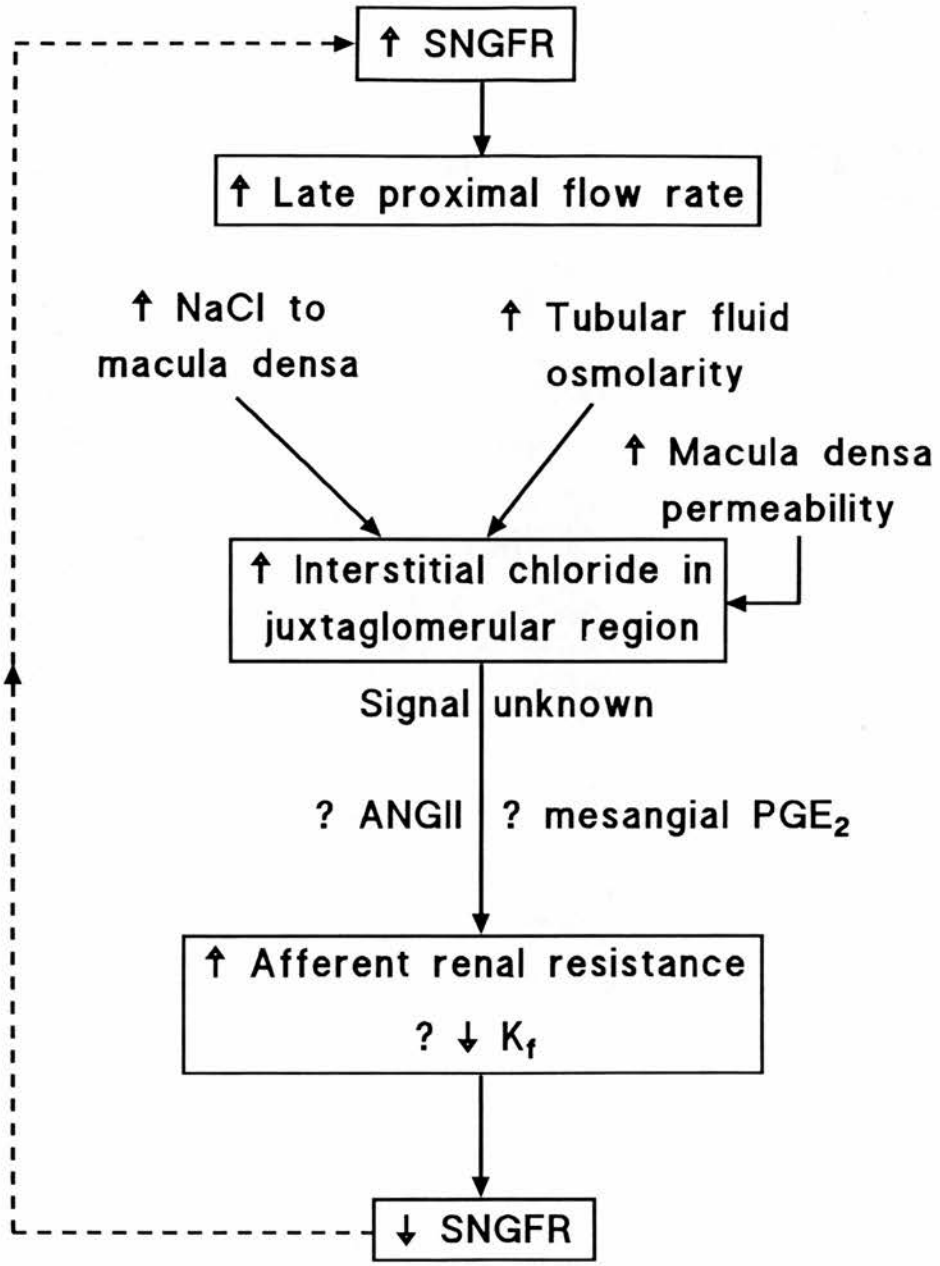

Figure 1.3: Simplified outline of proposed operation of the tubuloglomerular feedback system.

and a greater sensitivity to changes in tubular flow rate than superficial nephrons (M≟ller-Suur et al, 1983). About 50% of the maximal response occurs within a physiological range of tubular flow rates in the rat (10-20 nl/min), and the maximal sensitivity to changes in tubular flow rate is also found within this span (Schnermann and Briggs, 1985).

1.5.1 Afferent Mechanism.

A change in the composition of fluid reaching the macula densa is essential to elicit a TGF response, since changes in flow rate alone induced by mannitol have no effect (Thurau and Schnermann, 1965). Sodium chloride concentration in the early distal tubule is flow-dependent, the change in tubular fluid composition between the end of the thick ascending loop of Henle and the macula densa being greatest at low flow rates. The change in single nephron GFR is inversely related to the macula densa NaCl concentration between 15-60 mM, and the overall sensitivity of the system has been estimated to be about 0.5 $mlGFR.min^{-1}.mM.[Na^{+}]^{-1}$ (Schnermann et al, 1976).

The TGF response is independent of tubular fluid osmolarity at constant NaCl concentrations, and proximally acting diuretics such as carbonic anhydrase inhibitors do not affect responsiveness (Wright and Schnermann, 1974). However, inhibition of sodium

chloride transport in the thick ascending loop of Henle with either diuretics or metabolic inhibitors does block the TGF response (Wright and Schnermann,1974), as does replacement of chloride in the perfusion fluid with a variety of other anions (Schnermann et al, 1976). This suggests that changes in macula densa transport activity rather than changes in luminal NaCl concentrations initiate the subsequent vascular response, a hypothesis which has recently received direct experimental support (Ito and Carretero, 1991).

1.5.2 Effector Mechanism.

The glomerular vasoconstriction produced by the TGF response is consistently localised to the afferent arteriole (Schnermann et al, 1980). Most functional studies report a fall in filtration fraction and intraglomerular pressure which is also consistent with an afferent response (Wright and Briggs, 1979). Conflicting evidence showing a constant intraglomerular pressure during high tubular flow rates (Ichikawa, 1982), suggesting 'balanced' afferent-efferent vasoconstriction, may reflect differences in the range of tubular flow rates studied.

TGF responsiveness is very sensitive to changes in volume status. Acute infusion of isotonic saline (Persson et al, 1979b) or isotonic plasma (Ploth et al, 1978) suppresses feedback activity, and responsiveness is

preserved during volume depletion or hypotension (Kaufman et al, 1982; Bell et al, 1979). Changes in TGF after chronic stimulation follow the same pattern, with a high dietary sodium intake suppressing feedback responses (Dev et al, 1974) and sodium and water depletion (Persson et al, 1982) and ureteric occlusion (Morsing and Persson, 1991) accentuating the activity of the system. Resetting of TGF involves unknown mechanisms. An increase in net interstitial pressure is common to the situations in which the response is suppressed (Persson et al, 1979a), and this might be translated into changes in juxtaglomerular cell volume. Luminal factors have also been proposed (Blantz et al, 1982; Haberle and Davis, 1984).

1.5.3 Mediating Mechanisms.

No single mediator of the TGF pathway has been firmly established. Circulating ANGII is not likely to be relevant: hypertonic saline infusion (increasing distal tubular flow rate of sodium) depresses plasma renin activity despite causing renal vasoconstriction (Nashat et al, 1976), and hypotension increases renin release (Davis and Freeman, 1976) despite renal vasodilatation which is at least partly feedback-mediated (Schnermann and Briggs, 1981).

A 'paracrine' role for ANGII formed locally in the JGA is a more plausible suggestion, because perfusion of

the loop of Henle with saline increases JGA renin activation (Thurau et al, 1972) only when TGF is active, and not when feedback responses are blocked (Wright and Schnermann, 1974). TGF responses are also reduced in kidneys depleted of renin by sodium loading (Schnermann et al, 1975) or by creation of a contralateral renal artery stenosis (Ploth et al, 1977). However, the restoration of TGF activity after acute volume depletion in sodium-expanded rats without any change in renal renin content (Moore and Mason, 1983) emphasises that the above results show only parallel shifts in the two systems and do not prove a direct causal role for intrarenal ANGII. Furthermore, both ACE inhibitors (Ploth et al, 1979) and the ANGII receptor-blocking agent saralasin (Ploth et al, 1982) reversibly reduce TGF responsiveness, but can not abolish the response completely even at high doses, further suggesting that ANGII is a modulator rather than a mediator of the response. Blockade of renal prostaglandins with indomethacin reduces TGF activity only in salt replete states, and does not modulate responsiveness in salt-depleted animals when TGF is most activated. Renal nerves have no direct influence (Schnermann et al, 1977a), but may act indirectly through stimulation of the renin-angiotensin system (Stowe et al, 1979).

The evidence favouring adenosine as a local mediator is more convincing: TGF is blocked by administration of

theophyllines (Osswald et al, 1980) either from the vascular or the luminal side, and by adenosine deaminase (Osswald et al, 1982). Dipyridamole, which increases extracellular adenosine concentrations, augments TGF (Osswald et al, 1980). The working hypothesis that adenosine is formed as an intracellular messenger in response to macula densa NaCl transport is also compatible with a modulatory role for ANGII because adenosine infusion does not cause renal vasoconstriction in renin-depleted kidneys (Osswald et al, 1975).

1.5.4 Tubuloglomerular feedback and renal autoregulation.

Renal blood flow is constant over a wide range of systemic arterial pressures in normal man. Tubulo-glomerular feedback activation may contribute to this regulation because glomerular filtration rate falls in parallel with blood pressure when TGF is interrupted in the dog (Navar et al, 1980). In the rat whole kidney GFR and distal SNGFR are better preserved against changing arterial pressure than proximal GFR (Ploth et al, 1977), and this autoregulatory capacity is also dependent on an intact TGF loop since autoregulation is absent after frusemide treatment or in the hydronephrotic kidney when TGF is inactivated (Morsing and Persson, 1991).

The observed fall in GFR during a reduction in blood pressure after TGF blockade is smaller than would be

expected on the basis of theoretical considerations (Moore et al, 1980), implying that an alternative pathway continues to contribute autoregulatory capacity. This may relate to intrinsic myogenic reactivity (Gilmore et al, 1980) which is responsible for up to half of the change in renal vascular resistance in the higher pressure range. The time course of autoregulatory resistance changes has two phases (Young and Marsh, 1981). The first phase is complete in three seconds, before a change in tubular flow could have been transmitted to the macula densa, and is therefore believed to be myogenic in origin. The second phase is slower, with a time course compatible with a feedback-dependent mechanism. Both phases account for about 50% of the total resistance change.

In the subnormal pressure range, the fraction of autoregulation attributable to TGF falls progressively with decreasing blood pressure (Schnermann et al, 1984), and hormonal factors become relatively more important. An ANGII-mediated increase in efferent arteriolar vasoconstriction and prostaglandin-mediated afferent arteriolar vasodilatation both serve to augment glomerular filtration pressure and maintain GFR in the face of reductions in renal perfusion pressure (Schnermann et al, 1984).

CHAPTER TWO.

THE KIDNEY IN DIABETES MELLITUS.

2.1 THE NATURAL HISTORY OF DIABETIC NEPHROPATHY.

The occurrence of proteinuria in diabetic patients is not a recent observation (Cotunnius 1770; Rollo 1798), but the ominous prognosis for patients with this diabetic complication was not apparent until insulin therapy allowed the prolonged survival of patients with Type 1 (insulin-dependent) diabetes. Pathological definition of nodular and diffuse hyaline masses in glomeruli (Kimmelstiel and Wilson, 1936; Fahr, 1942) was followed by recognition that death from kidney failure in Type 1 diabetic patients is more likely in subjects of long duration, especially those with a young age of onset of diabetes (Mann et al, 1949), and diabetic nephropathy was soon established as one of the specific microangiopathic complications of diabetes (Lundbaek, 1954).

The peak prevalence of diabetic nephropathy has been reported as about 40% of Type 1 diabetic patients after 40 years of diabetes (Knowles et al, 1974; Deckert et al, 1978; Borch-Johnsen et al, 1985). During the 1970's the mean time from the appearance of clinical renal disease to renal death was only seven years (Deckert et al, 1981a), but many diabetic patients never develop nephropathy, and the proportion of patients affected is falling steadily (Krolewski et al, 1985; McNally et al, 1990) for reasons which remain obscure. The clustering of cases within families (Deckert et al, 1981b; Seaquist et al, 1989), and the increased incidence in certain

racial groups (Cowie et al, 1989) suggests that genetic influences are involved. This is likely to bias attempts to examine the influence of environmental factors on the development of nephropathy.

The association in individual diabetic patients between nephropathy and the other microangiopathic complications of diabetes emphasises the importance of the diabetic state *per se* as a predisposing factor in the development of diabetic complications. The incidence and prevalence of nephropathy are both increased in patients with poor cumulative chronic glycaemic control (Pirart, 1978); the incidence in this study also correlated with the quality of control in the year before the examination, whatever the overall degree of control in preceding years. The important relationships between arterial blood pressure and the development of nephropathy will be discussed later.

Proteinuria in Type 1 diabetic patients is usually due to diabetic nephropathy, but about 10% of cases have alternative or associated glomerular diseases (Lynn et al, 1988). The annual incidence of proteinuria peaks between 15 and 20 years diabetes duration, and it is rare to find new cases after more than 30 years of diabetes (Mogensen et al, 1989; Drury et al, 1989). Established diabetic nephropathy progresses at a variable but usually inexorable rate to end stage renal failure if the patient survives long enough. The incidence of end stage renal

failure due to diabetes in Britain exceeds 10 per million per year (Joint Working Party, 1988), and about 15% of patients receiving renal replacement therapy have diabetes, usually Type 1 disease. Diabetic patients with renal disease suffer a greatly increased morbidity and mortality from macrovascular disease, especially younger patients whose relative mortality is up to 100 times that of age and duration-matched non-proteinuric diabetic patients (Borch-Johnsen et al, 1985); more of these patients die from myocardial infarction and stroke than from uraemia.

Current understanding of the natural history of diabetic nephropathy is based on cross-sectional studies. Three distinct phases have been defined in the evolution of diabetic kidney disease. The first stage comprises functional and pathological changes including increased glomerular filtration rate, nephromegaly and intermittent microalbuminuria (Keen et al, 1981). These changes often regress if meticulous diabetic control is achieved. In the second stage small amounts of protein (predominantly albumin) become detectable in the urine, initially intermittently and later persistently. Microalbuminuria is an indicator that early diabetic nephropathy is present (Berglund et al, 1987; Chavers et al, 1989), and is strongly predictive of the development of overt proteinuria, by which time there is a significant relationship between structural kidney damage and

impairment of function (Viberti et al, 1982). During this period further thickening of the glomerular basement membrane and mesangial matrix occurs, and mesangial cell expansion progresses (Steffes et al, 1989). Arteriolar hyalinosis leads to glomerular occlusion, and glomerular filtration rate begins to fall in parallel with the loss of filtration surface, although still within the normal range. Hypertension develops and accelerates progression to the third stage of overt clinical nephropathy with frank proteinuria and nephrotic syndrome, worsening hypertension, and a declining glomerular filtration rate.

Morphometric studies have emphasised the central role of mesangial expansion in early glomerular hypertrophy (Steffes et al, 1989). Mesangial expansion causes glomerular damage only when it is proportionately greater than the increase in glomerular size, by adversely affecting glomerular capillary density and filtration surface area (Mauer et al, 1984; Ellis et al, 1986). However, the relationship between structural damage and impairment of renal function in diabetes is poorly defined. Morphological glomerular damage is present in >90% of Type 1 diabetic patients after 20 years of diabetes (Thomsen, 1965), but most of these patients have normal kidney function. Interstitial fibrosis is also a prominent feature of established diabetic nephropathy (Thomsen et al, 1989). This tubular damage may be more important as a determinant of the

effect of structural damage on glomerular filtration rate than has previously been considered (Pinter and Atkins, 1991), as it is in patients with non-diabetic glomerular disease (Risdon et al, 1968).

Structural changes leading to nephron loss are clearly important in the later stages of diabetes, but patients at risk of clinical nephropathy cannot be identified by the detection of early structural changes alone. Alterations in GFR after changes in metabolic control occur over too short a time scale to be directly related to changes in filtration surface area (Kroustrup et al, 1977), stressing the predominance of functional factors in the early stages of diabetes. Much attention has therefore been directed towards delineating renal functional changes in the diabetic kidney which precede the development of clinical renal disease.

2.2 RENAL HAEMODYNAMICS IN EARLY TYPE 1 DIABETES.

Many studies have confirmed the initial observation (Cambier, 1934) that glomerular filtration rate (GFR) is increased in some patients with early Type 1 diabetes (Stalder and Schmid, 1959; Ditzel and Schwartz, 1967; Mogensen et al, 1971a; Christiansen et al, 1981a; Wiseman et al, 1984). The proportion of patients with a GFR which is statistically abnormally high, termed glomerular

hyperfiltration, depends on the subgroup studied and the population from which they are drawn. It cannot be determined whether a GFR within the 'normal range' in a diabetic patient is always higher than that individual's 'pre-diabetic' GFR; the unimodal rather than bimodal distribution of GFR in the uncomplicated diabetic population suggests that this may be so.

2.2.1 Determinants of increased GFR in diabetes.

Most of the experimental data in this area comes from animal models of diabetes, and the prospective studies needed to test the relevance of renal functional changes in early human diabetes to the development of clinical nephropathy are notable by their scarcity. Because of the problems inherent with prolonged follow-up and repeated investigations, and recognition that the aetiology of diabetic nephropathy is multifactorial, definitive data is not likely to be forthcoming on a large scale.

Of the four determinants of GFR (Brenner et al, 1977), renal plasma flow (RPF) and glomerular transcapillary hydraulic pressure (ΔP_{GC}) have been most studied in diabetes. Increased single nephron RPF in insulin-treated hyperglycaemic diabetic rats results from reductions in intrarenal resistances; afferent arteriolar resistance is reduced more than efferent resistance, and mean glomerular hydraulic pressure is elevated (Hostetter

et al, 1981a; Jensen et al, 1981). The ultrafiltration coefficient K_f is normal in the rat (Hostetter et al, 1981a). Glomerular hyperfiltration in the rat is therefore apparently due to a combination of increased glomerular flow and increased intraglomerular capillary pressure.

Effective renal plasma flow (ERPF) and GFR correlate well in human Type 1 diabetes (Mogensen et al, 1973; Christiansen et al, 1981a; Puig et al, 1981; Hannedouche et al, 1990a), and renal plasma flow in Type 1 diabetes has been reported as elevated (Mogensen et al, 1971a; Christiansen et al, 1981a), suggesting that at least some of the increase in GFR is plasma flow dependent. However, other studies have found normal (Ditzel et al, 1972; Hannedouche et al, 1990a; Jenkins et al, 1990; Fioretto et al, 1991) or even reduced (Stalder and Schmid, 1959) ERPF in Type 1 diabetic patients with and without increased GFR. In the more recent studies these results were certainly not due to underestimation of ERPF by the formation of a PAH-glucose adduct in glycosuric urine (Dalton et al, 1988), so that other factors must also be involved.

GFR correlates with RPF in both diabetic and control subjects (Hannedouche et al, 1990a), but in most studies GFR adjusted for ERPF remains increased in diabetes, and this increase in the filtration fraction (GFR/ERPF) has been interpreted as an indication that elevated

intraglomerular pressure may independently contribute to the rise in GFR in human Type 1 diabetes (Mogensen et al, 1976a). Intraglomerular pressure can not be measured directly in humans, and interpretation of the filtration fraction as an equivalent measurement is only valid if both oncotic pressure and the ultrafiltration coefficient K_f are unchanged. Oncotic pressure is normal in early human Type 1 diabetes, as judged by plasma protein concentration (Hommel et al, 1990), and arteriolar oncotic pressure is not different in diabetic and control rats (Hostetter et al, 1981a). However, the increased glomerular filtration surface area in human Type 1 diabetes (Kroustrup et al, 1977) correlates with GFR in patients with and without nephropathy (Hirose et al, 1980; Osterby et al, 1988), and could augment GFR simply by increasing K_f independently of haemodynamic factors (Premen et al, 1988). Filtration fraction data in human studies must always be interpreted very cautiously.

2.2.2 Mechanisms of GFR elevation.

An increase in GFR occurs only during moderate hyperglycaemia: a blood glucose greater than 16 mmol/l in human diabetes leads to a normal or reduced glomerular filtration rate (Mogensen et al, 1971b; Wiseman et al, 1984), and severely hyperglycaemic animals have normal GFR and normal intraglomerular pressure (Hostetter et al, 1981a; Michels et al 1981). Acute increases in blood

glucose following either oral ingestion or intravenous infusion of glucose (mean increase 11 mmol/l) have either shown minimal effects on GFR and ERPF (Mogensen et al, 1971c; Jenkins et al, 1989), or have produced small increases both in normal subjects and in diabetic patients with hyperfiltration (Christiansen et al, 1981b; Wiseman et al, 1987). GFR in the diabetic rat is normalised by insulin treatment in some (Jensen et al, 1987; Tucker et al, 1991) but not all studies (Bank et al, 1988). Acute reduction of blood glucose from hyperglycaemic to normal levels by bolus insulin injection reduces GFR and ERPF in diabetic patients (Mogensen et al, 1978). However, the elevated GFR in human diabetic patients at the time of diagnosis is only partially normalised by one week of insulin treatment, before there has been any change in renal size (Christiansen et al, 1982), and only if euglycaemia is achieved and not if hyperglycaemia is maintained (Christiansen et al, 1981c). This suggests that the quality of longer term glycaemic control is an important modulator of intrarenal haemodynamics in addition to the effects of acute changes in blood glucose on glomerular filtration rate.

Vascular beds throughout the body tend to show a decreased resistance to blood flow in early Type 1 diabetes (Parving et al, 1983c), implying that a generalized diabetes-related impairment of autoregulation

exists which interacts with local factors. Abnormalities of vasoregulatory hormones have obvious potential as mediators of the renal haemodynamic effects of chronic hyperglycaemia. PGE_2 production by mesangial cells from diabetic rats is increased (Schambelan et al, 1985), and is normalized by insulin administration (Kreisberg and Patel, 1983). Enhanced production of vasodilatory prostaglandins has been confirmed in human Type 1 diabetes (Esmatjes et al, 1985; Fioretto et al, 1991). Prostaglandins have most significance as regulators of renal vascular tone when vasoconstrictor hormones are activated, but the glomerular contractile response to ANGII, noradrenaline and vasopressin remains normal in experimental diabetes (Barnett et al, 1987). The absence of any change in GFR after cyclooxygenase inhibition in early human Type 1 diabetes (Christiansen et al, 1985) also argues against a primary role for vasodilatory prostaglandins in initiating hyperfiltration.

Urinary kallikrein is reduced in untreated rats with streptozotocin-induced diabetes (Jaffa et al, 1987), but is increased above control values when insulin treatment is given, urinary excretion then correlating with GFR and ERPF (Harvey et al, 1990). However, the effects of acute infusion of the kallikrein inhibitor aprotinin are conflicting (Bank et al, 1988; Harvey et al, 1990), and the role of kinins remains unclear.

A possible role for endogenous atrial natriuretic

peptide (ANP) rests on the parallel increases in plasma ANP and GFR during moderate hyperglycaemia both in the diabetic rat (Ortola et al, 1987) and in human diabetes (Laragh and Atlas, 1988). Infusion of a specific anti-ANP antiserum reduces GFR in hyperfiltering diabetic rats (Ortola et al, 1987), while ANP infusion in normal rats raises glomerular capillary pressure (Dunn et al, 1986). Plasma ANP correlates with the quality of glycaemic control in human diabetes (Bell et al, 1989), but systemic infusion of low dose ANP in normal man slightly decreases GFR and ERPF despite causing natriuresis and suppression of plasma renin activity and ANGII levels (Cottier et al, 1988), effects which differ from those of poorly controlled diabetes.

Plasma ANGII concentrations and plasma renin activity tend to be reduced in early diabetes, which combined with the reduction in ANGII receptor density in diabetic glomeruli (Ballermann et al, 1984) might suggest a permissive role for a suppressed renin-angiotensin system in causing hyperfiltration. However, the decrease in ANGII receptor density does not prevent the renal vascular response to exogenous ANGII (Bank et al, 1988), and non-renal vascular beds actually show an increased responsiveness to exogenous ANGII (Christlieb, 1976). Moreover, baseline renal plasma flow and the response of renal plasma flow to acute angiotensin converting enzyme inhibition both correlate with the prevailing level of

glycaemic control (Jenkins et al, 1990). This has been interpreted as implying that intraglomerular pressure is elevated by ANGII-mediated efferent arteriolar constriction in parallel with worsening hyperglycaemia.

In diabetic rats acute hyperglycaemia and glycosuria separately and additively blunt the tubuloglomerular feedback (TGF) mechanism causing relative hyperfiltration (Blantz et al, 1982). However, in chronically hyperglycaemic rats either normal (Seney and Salmond, 1988) or increased activity of the TGF response has been described (Pollock et al, 1991; Tucker et al, 1991). The feedback response curve is reset so that the TGF system only partially restores the elevated GFR towards normal, a higher than normal distal tubular flow then coexisting with a higher than normal GFR. Persistence of TGF activity in chronic diabetes is also shown by the blunting of the hyperfiltration response after partial control of blood glucose with insulin, and by the finding that the increased renal blood flow and decreased renal resistance coincident with the elevation in GFR do not occur if TGF is blocked (Woods et al, 1987). The relevance of these findings to human diabetes is unknown.

2.2.3 Glomerular Hyperfiltration as a Potential Initiator of Renal Injury.

The suggestion that glomerular hyperfiltration may contribute to the development of diabetic nephropathy

arose from the observation that morphological changes of diabetes in a patient with unilateral renal artery stenosis were confined to the nonstenosed kidney (Berkman and Rifkin, 1973). Nephron destruction in non-diabetic renal disease often progresses despite removal of the initial injury (Schimamura et al, 1975; Hostetter et al, 1981b). Single nephron GFR in surviving nephrons rises as an adaptive response due to increases in single nephron capillary flow and transglomerular hydraulic pressure. This response may be maladaptive (Anderson and Brenner, 1989) since increases in capillary flow and intra- glomerular pressure accelerate the development of proteinuria and progressive glomerular injury in healthy surviving nephrons (Brenner et al, 1985). These nephrons in turn will sclerose and cause whole kidney GFR to decline further. The occurrence of 'glomerular hypertension' has now been documented in a wide range of experimental nephropathies (Anderson and Brenner, 1989).

The possible importance of glomerular hypertension as an initiator of diabetic nephropathy has been vigorously championed by Brenner, and is supported by experimental work in the rat showing that manoeuvres which aggravate the haemodynamic effects of early diabetes also accelerate glomerular injury (O'Donnell et al, 1986; Zatz et al, 1985). The reduction of filtration surface area following glomerular tuft occlusion will reduce whole kidney K_f, which in turn will increase

transglomerular pressure further at constant renal plasma flow unless efferent arteriolar resistance is reduced. It is therefore very important that intraglomerular hypertension and its sequelae are prevented in the diabetic rat by reducing dietary protein intake (Zatz et al, 1985) or by giving the angiotensin converting enzyme inhibitor enalapril which reduces efferent arteriolar tone and intraglomerular pressure (Zatz et al, 1986).

The hyperfiltration hypothesis elegantly describes events in the diabetic rat, but there are problems in extrapolating the theory to human diabetes. Firstly, the streptozotocin-diabetic rat develops focal glomerular sclerosis, a lesion histologically different from human Type 1 diabetes, and similar but milder histological changes are a normal aging phenomenon in the non-diabetic rat (Bras, 1969). This led to the suggestion that progressive renal disease in the rat following induction of glomerular hyperfiltration is an exaggerated form of a species-specific phenomenon (Bovee et al, 1979; Watnick et al, 1988). Recent data refutes this hypothesis however (Bourgoignie et al, 1987; Novick et al, 1991), and the discrepancy between the rat and larger species is probably related more to the severity of the insult applied, the time scale of follow-up, and possibly to the initial nephron number (Fine, 1991) than to a fundamental difference between species.

The second problem lies in proving a causal relation

between glomerular hypertension and glomerular injury. One study has suggested that increasing intraglomerular pressure initiates renal injury only in diabetic rats with hypertension, and not in rats with diabetes or hypertension alone (Bank et al, 1987). Furthermore, glomerular hyperfunction induced by ureteric diversion (without nephrectomy) does not by itself promote the glomerular hypertrophy which is a necessary precondition for the development of glomerular sclerosis (Yoshida et al, 1989).

Short-term studies in diabetic patients have shown that dietary protein restriction can reduce albuminuria (Cohen et al, 1987) and slow the progression of diabetic nephropathy (Evanoff et al, 1987). However, longer term studies, some prospective, have not agreed whether an early increase in GFR is (Mogensen, 1986; Rudberg, 1991) or is not (Lervang et al, 1988; Messent et al, 1991) a risk factor for nephropathy independently of glycaemic control and blood pressure. Other factors which are of possible relevance to the expression of nephropathy include basement membrane glycosylation (Spiro et al, 1976), the effects of systemic hypertension (Mogensen et al, 1976b; Viberti et al, 1985), abnormal capillary permeability to macromolecules (Parving, 1975), and genetic susceptibility (Seaquist et al, 1989). The failure to conclusively confirm that an elevated GFR independently predicts the development of diabetic

nephropathy is therefore perhaps not surprising, and may have been caused by a type II statistical error due to heterogeneity within the relatively small groups of patients studied.

The variety of abnormalities that have been described in the diabetic kidney make it almost inevitable that for any individual patient the development of nephropathy is multifactorial. The rest of this review will concentrate on just one important area, describing the changes in the relationships between renal and whole-body sodium homeostasis and systemic blood pressure which precede and accompany the development of nephropathy.

2.3 HYPERTENSION AND NEPHROPATHY IN DIABETES MELLITUS.

Systemic hypertension often complicates both Type 1 and Type 2 diabetes and is an important risk factor for the development of microangiopathy. Elevation of blood pressure shortens the time interval between the onset of diabetes and the occurrence of nephropathy (Hasslacher et al, 1985), and accelerates the rate of decline of glomerular filtration rate once nephropathy is established (Mogensen, 1982; Parving et al, 1983a). Slightly elevated systolic blood pressure has been found in some diabetic patients in some (Moss, 1962; Cruickshanks et al, 1985; Hasslacher et al, 1985) but not

other (Kaas Ibsen et al, 1983) cross-sectional studies, and only a very small increase in diastolic pressure has been reported (Tarn and Drury, 1986). Two large and important studies have recently argued persuasively firstly that blood pressure is not increased in uncomplicated diabetes (Norgaard et al, 1990), and secondly that elevation of urinary albumin excretion precedes the rise in blood pressure during the development of incipient nephropathy (Mathiesen et al, 1990). The evidence therefore suggests that at most only small increases in blood pressure occur in diabetic patients without complications. An increase in arterial pressure into the hypertensive range is a response to the development of incipient or overt nephropathy, occurring before kidney function is impaired. The speed with which hypertension develops is evident from the finding that more than 50% of young Type 1 diabetic patients with incipient or established nephropathy with a normal serum creatinine already have a diastolic blood pressure $\geq$95mmHg (Parving et al, 1983a).

Transmission of systemic hypertension to the glomerular microcirculation increases glomerular capillary tuft pressure in various models of hypertensive kidney disease (Andersen et al, 1986), including SHR rats with streptozotocin-induced diabetes (Bank et al, 1987) and normotensive rats with insulin-treated diabetes (Hostetter et al, 1981a; Jensen et al, 1986). Such

increases in pressure may be more injurious to the enlarged glomerular capillaries found in the diabetic kidney since the wall tension created for a given transmural pressure gradient is proportional to vessel size (Fries et al, 1988). This may account for the clinical observation that an accelerated rate of decline of glomerular filtration rate can be detected in hypertensive diabetic patients at levels of blood pressure which would not cause nephron failure in non-diabetics with essential hypertension (Parving et al, 1983b).

The causes of hypertension at different stages of diabetes are uncertain, but effective antihypertensive treatment reduces microalbuminuria and the rate of decline of GFR in hypertensive patients with overt nephropathy (Mogensen, 1982; Parving et al, 1983b; Parving et al, 1987, Parving et al, 1988; Bjorck et al, 1986; Parving et al, 1989a), and improves the renal prognosis (Parving and Hommel, 1989b). A beneficial effect of antihypertensive treatment is also apparent in both hypertensive and normotensive patients with incipient nephropathy (Marre et al, 1988; Mathiesen et al, 1991). It is controversial whether any particular antihypertensive therapy confers a specific advantage; various drug regimes are being compared to determine whether lowering of blood pressure within the 'normal' range can modify or prevent the development of

nephropathy in normoalbuminuric diabetic patients.

The relationship between blood pressure and nephropathy in diabetes is complex. Increased blood pressure does not *cause* diabetic nephropathy, but a rising blood pressure is apparent soon after the development of incipient nephropathy, and undoubtedly accelerates the progression of nephropathy. Two hypotheses have been postulated to explain the available data. First, diabetes may cause metabolic and hormonal changes which lead by separate pathways to both kidney damage and hypertension. Alternatively, a genetic predisposition to essential hypertension may exist and act as an additional risk factor in the presence of diabetes, promoting kidney damage. The debate over the possible value of a parental history of essential hypertension or an increase in red cell sodium-lithium countertransport activity as a predictor of the risk of nephropathy is beyond the scope of this discussion (Viberti et al, 1987; Jensen et al, 1990; Walker et al, 1990). However, in considering possible causes for the unusually early appearance of rising blood pressure in young subjects with normal kidney function, abnormalities of renal sodium handling and cardiovascular reactivity are present in Type 1 diabetes and may be of relevance.

2.4 SODIUM HOMEOSTASIS IN DIABETES.

Patients with diabetic nephropathy develop sodium and water retention early in the course of their chronic renal failure, and some patients require dialysis solely to relieve salt and fluid overload before symptoms of uraemia have developed. 'Volume-dependent' hypertension results at least partly from expansion of the extracellular fluid volume due to defects in the renal excretion of sodium and water (Hamlyn and Blaustein, 1986). Extracellular fluid volume and cardiac output are maintained within relatively normal limits at the expense of an increase in blood pressure and hence renal perfusion pressure in the chronic phase of the disease (see Chapter 1.4). Patients with uncomplicated diabetes already have an increased exchangeable body sodium pool (Weidmann et al, 1979), and definition of the relations between abnormal sodium homeostasis, other factors regulating blood pressure control, and the development of hypertension and diabetic nephropathy therefore has practical as well as academic importance.

Many studies of sodium handling in diabetes have been performed upon heterogenous patient groups, or have used inappropriate criteria when defining the presence of hypertension (a blood pressure cutoff of $\leqslant$160/90 mmHg which is 'normotensive' in a non-diabetic 60 year old by WHO criteria should not be regarded as normal in a Type 1 diabetic population mostly aged from 20 to 50 years).

Some important studies were completed before the assessment of medium term glycaemic control by means of glycated haemoglobin assays became available, and these could not determine whether relationships exist between exchangeable sodium and chronic glycaemic control. Many studies have also not differentiated patients with Type 1 (insulin-dependent) from Type 2 (non-insulin-dependent) diabetic patients, in whom the causes and the consequences of raised blood pressure may not be the same. These sources of potential bias will be highlighted as necessary.

2.4.1 Exchangeable Sodium.

Cross-sectional studies have shown that patients with diabetes have an increased exchangeable body sodium pool (TENa) (de Chatel et al, 1977; Weidmann et al, 1979; Beretta-Piccoli et al, 1982a; O'Hare et al, 1985; Feldt-Rasmussen et al, 1987). The greatest increases occur in patients with incipient or frank nephropathy (O'Hare et al, 1985; Feldt-Rasmussen et al, 1987), but the excess of extracellular sodium, which in most studies is about 10% greater than control groups, is apparent in normotensive and hypertensive patients, across all age groups and in both genders, and in both Type 1 and Type 2 diabetic subgroups. Despite overall sodium retention, plasma volume is normal in normotensive and even low in some hypertensive diabetic subjects (de Chatel et al,

1977; Beretta-Piccoli et al, 1982a). This contrasts with the situation in uncomplicated essential hypertension in which TENa and plasma volume are normal (Lebel et al, 1974; Schalekamp et al, 1974).

O'Hare et al (1985) found a positive correlation between TENa (increased to a mean of 118% of control values) and mean arterial pressure in 16 patients with overt diabetic nephropathy, and Weidmann et al (1985) reported a similar finding among 124 unselected diabetic patients, the closest correlation being between systolic blood pressure and TENa in patients with high blood pressure (>90 mmHg diastolic). In 17 hypertensive Type 2 diabetic patients studied before and after six weeks treatment with a diuretic (chlorthalidone), TENa fell from an initial value of 109% of the control value to the same level as the control group, while blood pressure fell from 165/93 to 145/82 mmHg over the same period (Weidmann et al, 1979). This suggests a link between increased total body sodium and the rise in arterial pressure in these patients, although a fall in blood pressure due solely to a weak venodilator effect of chlorthalidone can not be excluded. Conversely, patients with Type 2 diabetes changing from a low to a high sodium diet retained more sodium than nondiabetic controls (Tuck et al, 1990), although only hypertensive patients (either diabetic or control) showed any rise in blood pressure during sodium loading.

Experimental evidence of a defect in renal sodium handling in Type 1 diabetes has come from analysis of the renal responses to head-out water immersion and intravenous saline infusion. The natriuretic response to both stimuli in a group of uncomplicated diabetic patients was blunted by about 50% when compared with a carefully matched control group (Roland et al, 1986; O'Hare et al, 1986). The absence of changes in glomerular filtration rate in these experiments (albeit assessed using creatinine clearance) suggested that abnormal tubular function caused the altered renal sodium handling rather than a haemodynamic abnormality. The site of this defective sodium handling could not be determined, but several groups have recently used the lithium clearance method as a means of studying tubular sodium handling in more detail in diabetic patients. These studies have consistently shown an increase in fractional proximal sodium reabsorption in both Type 1 and Type 2 diabetic patients (Mbanya et al, 1989; Nosadini et al, 1989; Hannedouche et al, 1990a; Trevisan et al, 1990), sodium balance being maintained by a reduction in fractional distal tubular sodium reabsorption.

2.4.2 Mechanisms of Sodium Retention.

2.4.2.1 Sodium-glucose cotransport.

Mildly raised blood levels of glucose and ketone bodies, as found in treated diabetes, increase the filtered load of these substances entering the proximal tubule and undergoing active reabsorption in the proximal tubular cell. This reabsorption is accompanied by sodium reabsorption in an approximately 1:1 ratio by distinct cotransport mechanisms (Ullrich, 1976; Crane, 1977). Glucose reabsorption also changes the transtubular osmotic pressure gradient to favour sodium and water reabsorption by increasing solvent drag. In the rabbit isolated proximal tubule, perfusion with 5.5 mM D-glucose augments sodium reabsorption by up to 25% (Burg et al, 1976). This mechanism is insulin-independent (Harris et al, 1986) and is demonstrable within four days of the onset of STZ-induced diabetes in the rat (Kumar et al, 1988). Mild hyperglycaemia with blood glucose concentrations below the T_m for glucose therefore promotes excess proximal sodium reabsorption; more severe metabolic decompensation during periods of very poor control leads to a glycosuric osmotic diuresis, net sodium losses, hypovolaemia and decreased blood pressure (Atchley et al, 1933; McCance and Lawrence, 1935).

2.4.2.2 Insulin.

The pathophysiological effects of insulin on renal sodium handling may also be relevant in diabetes. Miller and Bogdonoff (1954) showed that normal subjects undergoing solute or water diuresis had a reduced sodium excretion when given insulin. This effect is independent of the sodium-retaining effect of hypoglycaemia (Patrick et al, 1989). DeFronzo used clamp techniques in the dog to show that sodium retention is due to insulin alone and not to hyperglycaemia (DeFronzo et al, 1976), and suggested that excess sodium reabsorption occurs in the distal nephron. The plasma concentrations of insulin used were far higher than would normally occur in Type 1 diabetes, but suppression of endogenous insulin release in normal subjects by somatostatin infusion increases sodium excretion (DeFronzo et al, 1978), although an effect due to other hormonal changes can not be excluded.

Insulin may enhance sodium reabsorption through stimulation of the renal tubular Na^+-K^+-ATPase (Jorgensen, 1980; Moore, 1983) and the Na^+-H^+ antiporter (Moore, 1983; Mahnensmith and Aronson, 1985), or by amplifying the action of aldosterone on Na^+ and K^+ transport (Fidelman and Watlington, 1984). The effect has been observed only acutely in humans (Saudek et al, 1974; DeFronzo et al, 1975; Skott et al, 1989a; Gans et al, 1991a). Chronic insulin infusion in the non-diabetic dog leads to sodium retention followed by an "escape"

phenomenon similar to that occurring after mineralocorticoid administration (Hall et al, 1990).

Mansell et al. (1990) studied the renal response to acute hyperinsulinaemia in normal subjects by infusing insulin (or vehicle) at stepped doses reproducing 'physiological' plasma free insulin concentrations during a natriuresis produced by head-out water immersion. They interpreted the absence of a change in urinary sodium excretion during hyperinsulinaemia as indicating that insulin has no physiological role in the control of renal sodium handling.

Trevisan et al (1990) studied the effect of hyperinsulinaemia on atrial natriuretic peptide release and the natriuretic response to isotonic volume expansion in normal and Type 1 diabetic subjects. In the diabetic group the natriuretic response to intravenous saline loading was impaired as expected, mainly due to greater rates of proximal sodium reabsorption (assessed by lithium clearance). Infusion of insulin into the normal subjects for 24 hours, at doses reproducing the levels seen at baseline in the diabetic group, increased proximal tubular sodium reabsorption and reduced urinary sodium excretion, with no evidence of a separate distal tubular effect. Insulin infusion increased plasma ANP levels at baseline, but impaired the normal increase in ANP secretion seen during saline loading. The authors concluded that insulin primarily increased proximal

tubular sodium reabsorption.

The contrasting results of these two studies may reflect different experimental designs, in particular the differing durations of insulin infusion. The effects of insulin on renal sodium handling may be mediated indirectly by changes in other intrarenal hormone systems rather than by a direct insulin-receptor interaction within the kidney, and it is interesting that euglycaemic insulin infusion stimulates noradrenaline release (Rowe et al, 1981), and raises plasma renin activity and plasma ANGII levels (Trovati et al, 1989), providing further mechanisms which may promote proximal tubular sodium retention.

2.4.2.3 Physical and hormonal factors.

Intravascular oncotic pressure may be reduced in some hypoalbuminaemic nephrotic diabetic subjects due to accompanying reduced albumin synthesis (Ejarque et al, 1959), and increased microvascular permeability to macromolecules (Parving, 1975) will facilitate elevation of transcapillary microvascular pressure in the presence of hypertension, favouring a shift of fluid and sodium to the extracellular space, which may be further aggravated by increased tissue avidity for sodium following changes in collagen composition (Francis et al, 1974). This tendency to intravascular hypovolaemia may result in a finite degree of sodium retention as an 'appropriate'

renal response, leading to a new steady state with a restored blood volume at the cost of increased ECF sodium and interstitial volume (de Chatel et al, 1977).

Plasma atrial natriuretic peptide levels are increased in Type 1 diabetic subjects with incipient or clinical nephropathy (Sawicki et al, 1988), and this increase correlates positively with the quality of glycaemic control in uncomplicated patients (Bell et al, 1989). The response of plasma ANP to acute saline loading is enhanced in some (de Chatel et al, 1986) but not all (de Chatel et al, 1986; Haak et al, 1986) normotensive diabetics. These results in themselves may simply reflect the presence of ECF expansion due to sodium retention, but impairment of the natriuretic response to exogenous atrial natriuretic peptide has also been described in diabetic subjects with (Liebermann et al, 1991) and without nephropathy (Nosadini et al, 1991), suggesting that end-organ responsiveness is genuinely abnormal.

Finally, a genetic defect in renal sodium handling in diabetes has been suggested, based on the parallel between the increased Na^+-Li^+ red cell countertransport seen in some Type 1 diabetic patients (Krolewski et al, 1988; Mangili et al, 1988) and the postulated enhanced activity of the proximal tubular brush border Na^+-H^+ exchanger (Weder, 1986). This hypothesis awaits experimental verification.

2.5 CARDIOVASCULAR REACTIVITY IN DIABETES AND DIABETES-ASSOCIATED HYPERTENSION.

Variations in cardiovascular responsiveness to vasoactive stimuli are important in blood pressure regulation, and in normal man blood pressure responses to noradrenaline (NE) and ANGII are inversely related to their basal blood levels (Chinn et al, 1972; Philipp et al, 1978). In stable uncomplicated diabetes, plasma total catecholamines (Christensen, 1972), plasma and urinary noradrenaline and adrenaline (de Chatel et al, 1977; Beretta-Piccoli et al, 1979; Christensen, 1979; Feldt-Rasmussen et al, 1987), plasma active renin, ANGII, and plasma aldosterone (de Chatel et al, 1977; Christlieb, 1976; Beretta-Piccoli et al, 1979; Beretta-Piccoli et al, 1981a; Fernandez-Cruz et al, 1981; Ferriss et al, 1982; Feldt-Rasmussen et al, 1987; Tuck et al, 1990) are all normal or sometimes low with respect to age and/or urinary sodium excretion. Noradrenaline spillover and clearance rates are also normal (Beretta-Piccoli et al, 1982b).

Hypertension in diabetic patients is therefore not initiated primarily by activation of the sympathetic nervous system or the RAAS, but even normal plasma levels of these effector hormones could be regarded as inappropriately high in the presence of concomitant sodium retention (Weidmann et al, 1985). Furthermore, in stable diabetic patients pressor responsiveness to

exogenous NE is often exaggerated relative to plasma levels (Barany, 1955; Christlieb et al, 1976; Weidmann et al, 1979; Christensen, 1979; Beretta-Piccoli et al, 1981b), the dose needed to elevate mean arterial pressure by 20mmHg being less than half that required in normal subjects. This hyperresponsiveness is independent of age, duration of diabetes, diabetic complications, or the presence of hypertension (Weidmann et al, 1979; Beretta-Piccoli et al, 1981b).

Pressor responsiveness to exogenous ANGII also exceeds a physiological adaptation to circulating renin and ANGII levels in some diabetic patients, the dose of ANGII needed to raise MAP by 20mmHg being reduced in nonazotaemic diabetic patients despite no parallel decrease in plasma renin activity (Weidmann et al, 1985; Tuck et al, 1990). This abnormality is a function of the diabetic state *per se*, being apparent in normotensive diabetic subjects without microvascular complications (Drury et al, 1984), and is reflected in an inappropriate increase in ANGII binding sites on diabetic platelets (Mann et al, 1989), although this has not been a consistent finding (Connell et al, 1986). It is also interesting to note that improved glycaemic control in Type 2 diabetes decreases plasma ANGII levels and lowers blood pressure (Sullivan et al, 1980).

The hyperreactivity to exogenous NE and ANGII seen in normotensive diabetic patients differs from the

pattern in the normotensive offspring of essential hypertensive families, who are hyperreactive only to NE (Bianchetti et al, 1984). Sodium retention has been suggested to play an important role as a contributor to abnormal vascular hyperreactivity in diabetes. Removal of excess body sodium by diuretic treatment restored NE responsiveness to normal without changing pre-infusion plasma NE levels, implying an improvement in the initially disturbed relationship between NE reactivity and the prevailing sympathetic nerve activity (Weidmann et al, 1979) . The same diuretic therapy also reduced pressor responsiveness to ANGII but failed to normalize the relationship between ANGII reactivity and plasma renin activity, the vasodepressor effect of reduced body sodium on pressor reactivity to ANGII being opposed by the increased circulating renin following diuretic treatment. The hypothesis that relative 'inappropriate' systemic vasoconstriction exists at any given level of total body sodium in diabetes is also supported by the failure of dietary sodium restriction to normalize pressor reactivity to ANGII in hypertensive Type 2 diabetic patients (Tuck et al, 1990).

Vascular reactivity to both NE and ANGII is in part calcium-dependent (Trost and Weidmann, 1987). Calcium channel blockade (with nitrendipine) in Type 2 diabetic subjects with mild hypertension markedly reduced blood pressure and improved vascular hyperreactivity to both NE

and ANGII without changing body sodium content, consistent with a decrease in vascular tone due to reduced cytosolic Ca^{2+}. Morphological alterations in resistance vessels develop early in the course of diabetes (Blumenthal et al, 1965), and even mild vasculopathy may enhance reactivity to pressor agents. Hypertension of any aetiology may also be complicated by secondary hypertrophy of the vascular wall as a complementary mechanism perpetuating the initial abnormal milieu (Folkow, 1971). Autonomic reflex arc interruption by diabetic autonomic neuropathy also promotes exaggerated pressor responsiveness (Reid et al, 1990), and this may be relevant in some patients with established nephropathy and associated hypertension and autonomic neuropathy who develop the constellation of low plasma renin and aldosterone levels with severe hyperkalaemia, the syndrome of hyporeninaemic hypoaldosteronism (Weidmann et al, 1973; DeLeiva et al, 1976).

CHAPTER THREE.

AIMS AND METHODS.

3.1 AIMS OF THE WORK.

Interacting physical and hormonal systems regulate renal sodium handling and total body sodium content, glomerular haemodynamics and systemic blood pressure. Alterations in both renal sodium handling and glomerular haemodynamics have been described from the early stages of Type 1 diabetes, changes which may be relevant to the later development of hypertension and diabetic nephropathy. The determinants of these early changes have been examined in detail in experimental animal models of Type 1 diabetes, but much less information is available from *in vivo* human studies to establish the relevance of animal data to diabetes in man.

The renin-angiotensin-aldosterone system (RAAS) is one of the most powerful hormonal systems controlling intrarenal haemodynamics and tubular reabsorption of sodium. Abnormalities of the systemic actions of the effector hormone angiotensin II occur in early diabetes; the aim of the studies in this thesis was to determine whether the intrarenal actions of ANGII are also changed in early diabetes by comparing dynamic renal responses to acute changes in RAAS activity in normal man and patients with uncomplicated Type 1 diabetes. The validity of lithium clearance as a marker of tubular sodium handling was evaluated systematically with the intention of studying abnormal renal sodium handling in diabetes in more detail.

3.2 ETHICAL APPROVAL.

The subjects studies were either healthy volunteers or patients with Type 1 diabetes attending the Department of Diabetes of the Royal Infirmary for long-term supervision of their condition. All the protocols were approved as ethically acceptable by the Lothian Health Board Ethics of Medical Research Sub-Committee for Medicine and Clinical Oncology. Subjects gave fully informed consent after a careful explanation of the nature and purpose of the investigation concerned had been given.

3.3 EXPERIMENTAL PROTOCOLS.

3.3.1 Experimental changes in RAAS activity: angiotensin II infusion or ACE inhibition?

It is important when studying ANGII-mediated events *in vivo* to select the most physiologically appropriate intervention. RAAS components are localized within the kidney in different nephron subpopulations and within different anatomical parts of the nephron (Hollenberg, 1984; Levens et al, 1981), and intrarenal concentrations of renin and ANGII far exceed plasma levels (Mendelsohn, 1979). Systemic infusion of ANGII does not reproduce the normal pattern of intrarenal ANGII generation, and its effects may differ from those following endogenous intrarenal ANGII generation, which for example may

produce local concentrations of ANGII sufficient to constrict the efferent arteriole or directly stimulate proximal tubular reabsorption of sodium, but without affecting nephron sites with a higher threshold or spilling over into the systemic circulation. The effects of systemic infusion of ANGII on cardiac output, sympathetic activity and systemic blood pressure (Scroop and Lowe, 1969) may also produce independent and confounding effects on renal haemodynamics and natriuretic responses.

Recent studies have therefore often adopted the alternative strategy of removing endogenous ANGII using inhibitors of angiotensin converting enzyme (ACE), an approach which assumes that converting enzyme inhibition produces a balanced reduction in plasma and tissue ANGII concentrations. Converting enzyme is not specific for ANGI and is identical to the enzyme kininase II which inactivates bradykinin, so that by attenuating intrarenal kinin degradation ACE inhibition introduces the possibility of separate effects due to potentiation of the intrarenal actions of kinins. Infusion of ANGII after ACE inhibition may confirm that the effects of ACE inhibition are due to removal of ANGII rather than to accumulation of kinins, but reintroduces all the problems of ANGII infusion and may complicate rather than clarify the issue.

Because of the potential non-specificity of ACE

inhibition, systemic ANGII infusion has been used as the intervention in the majority of the present studies. Vascular reactivity to ANGII, especially the renal haemodynamic response, is extremely sensitive to changes in sodium intake through inverse changes in ANGII levels and tissue ANGII receptor numbers (Hollenberg et al, 1972). The protocols therefore aimed to achieve low baseline levels of endogenous ANGII through controlled increases in dietary sodium intake before the study. This allowed infusion of low doses of ANGII which produced increments in plasma ANGII levels within the physiological range while minimising the systemic actions of ANGII. The sodium-loading phase lasted 5-7 days in order to allow a new level of stable whole body sodium balance to be established before the acute study.

3.3.2 Experimental design.

The renal and systemic effects of infusion of angiotensin II (ANGII) have been examined under various circumstances in different groups of subjects. The basic clearance protocol is outlined in Figure 3.1; any deviations from this protocol are specified as necessary in individual experimental chapters.

Female subjects were not studied because of the practicalities of urine collection and the potential for variations in sodium balance during the menstrual cycle. Normal volunteers were healthy normotensive (<140/90

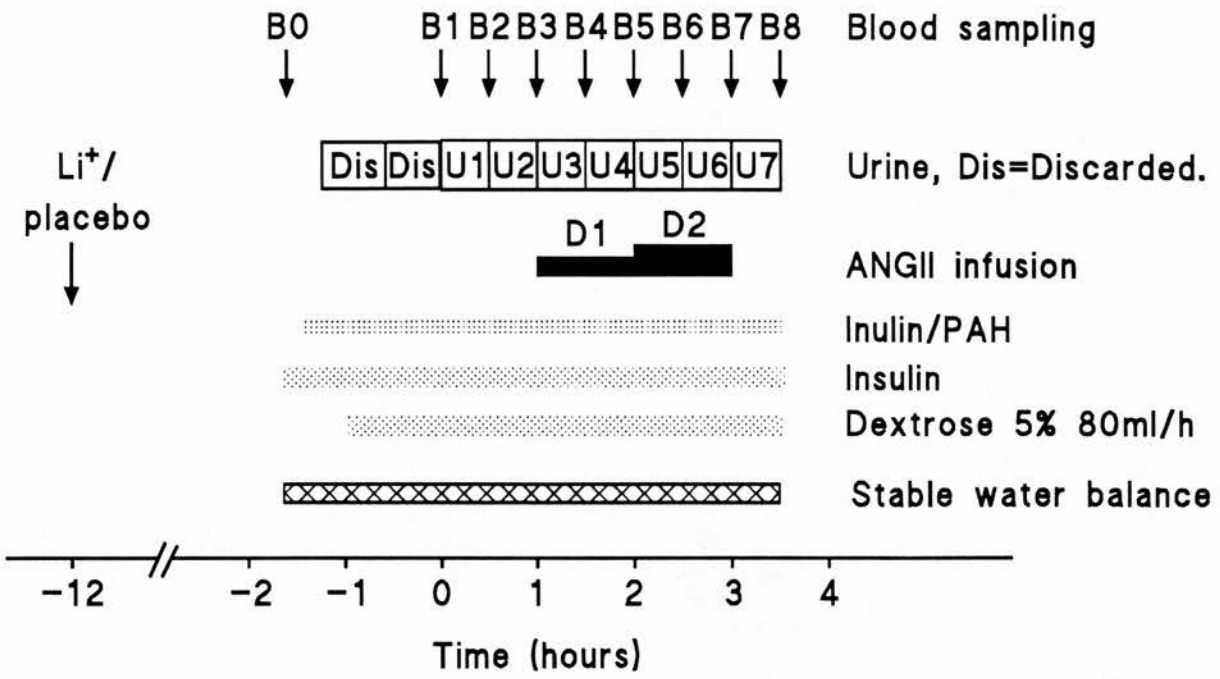

Figure 3.1: Outline of experimental protocol used for clearance studies in diabetic patients, (insulin/dextrose omitted in normal subjects).

mmHg, diastolic phase V) males aged 18-45 years on no medication. The diabetic patients had Type 1 (insulin-dependent) diabetes defined on the basis of age at diagnosis and mode of presentation, which included the presence of ketonuria at diagnosis indicating an absolute need for insulin therapy. They were taking no medication other than insulin, and were treated with at least two injections of unmodified (soluble, regular) and isophane (NPH) insulins daily ('conventional regime'). Some were using a 'basal-bolus' insulin regime (unmodified insulin before each meal, supplemented by an evening dose of intermediate-acting insulin). In order to study only the 'functional' changes associated with diabetes, patients were screened to exclude those with any evidence of diabetic tissue damage: this included a detailed review of clinical records, a full physical examination including direct ophthalmoscopy through dilated pupils, and measurement of urinary albumin excretion in at least two overnight urine collections. Objective tests of cardiovascular autonomic function were not performed, but no subject had any symptoms or signs of diabetic autonomic neuropathy. All subjects were in good health when studied with no recent episodes of hypoglycaemia or other metabolic upset.

Subjects avoided xanthine-containing drinks, alcohol and tobacco for at least 12 hours before each study. Urine was collected for 24 hours before each study to

document dietary sodium intake. Studies were performed after an overnight fast in the Clinical Research Laboratory starting at about 0800h, when a Teflon cannula (Venflon) was inserted into a large forearm vein in each arm. The subjects were supine except when passing urine. They drank 500 ml of tap water between 0700h and 0730h, and a further 600 ml of iced water over a twenty minute period after arriving at the hospital. The bladder was emptied at about 0830h, and after this and all subsequent urine collections the subjects drank an appropriate volume of water, adjusted according to the volume of urine passed and the volumes of fluid infused and blood sampled, so as to maintain a stable positive water balance. This hydration regime produced a near-maximal diuresis at baseline: maximal diuresis was avoided because of the possible effects of very high urine flow rates on the renal handling of *para*-aminohippurate (Smith, 1951).

A sterile solution of Val_5-ANGII amide (Hypertensin, Ciba, Hoddesdon, UK) 5μg/ml was prepared in the Royal Infirmary Pharmacy, and 30μg aliquots stored in glass ampoules at -70°C. This stock solution was thawed slowly on the study day and diluted to a concentration of 0.2μg/ml in 0.15M sodium chloride before infusion. Effective renal plasma flow and glomerular filtration rate were estimated by measuring the renal clearances of sodium *para*-aminohippurate (PAH) and Polyfructosan-S

(Inutest) respectively. Priming doses of PAH (0.45g) and Inutest (3.5g) were infused over 15 mins, followed by a maintenance infusion of PAH (8.3g/l) and Inutest (10g/l) in 0.15M sodium chloride at 120 ml/h. After a 75 minute equilibration phase blood samples were withdrawn at the beginning and end of accurately timed 30 minute clearance periods. Two baseline periods preceded two urine collections during infusion of ANGII at each of two doses, with a final collection after the ANGII infusion was stopped. Blood pressure and heart rate were monitored every 10 minutes throughout the study using an automated sphygmomanometer (Sentron, CR Bard Inc., Lombard, IL, USA). A 25 mmHg rise in mean arterial pressure from baseline during ANGII infusion was predetermined as the maximum safe increase, above which the ANGII infusion would be stopped immediately. This safety limit was never exceeded.

Sample collection.

Blood was collected before each voiding for measurement of PAH, Inutest, electrolytes, creatinine and haematocrit. Samples for PRA, plasma aldosterone (ALDO) and plasma ANGII were drawn at baseline and before each change in ANGII infusion dosage. Samples were collected into chilled heparinized tubes stored on ice, except those for ANGII estimations which were collected (total volume 10ml) into chilled plastic tubes containing 0.5ml phenanthroline 0.025M/EDTA 0.125M. After centrifugation

at 4°C, aliquots of plasma for hormonal assays were stored at -70°C. Assays of renal haemodynamic markers and electrolytes were performed within 48 hours of the study.

3.4 ANALYTICAL METHODS.

3.4.1 Renal Haemodynamics: the concept of 'clearance'.

Many of the technical advances which have given novel insights into renal structure and function *in vitro* are practically and ethically inapplicable to human experimentation. Hypotheses generated in animal models can often still only be tested in man using the long-established techniques of whole kidney clearance methodology (Smith, 1951).

The renal 'clearance' of a substance is defined as 'the volume of plasma from which all of a substance can be removed by the kidney in unit time'. This is an artificial concept physiologically since the kidney actually removes a much smaller proportion of the substance concerned from *all* of the plasma passing through it, but it gives a useful measure of excretory capacity. Renal haemodynamic parameters are measured by estimating the clearance from the blood of infused marker substances which are chemically inert in the circulation. After a loading dose and a period of equilibration to ensure that net extrarenal tissue uptake of the infused

marker is zero, the rate of infusion is assumed to be equal to the rate of urinary excretion; the clearance is calculated using the formula $U_x/P_x * V/T$, where U_x and P_x are urinary and plasma concentrations of the marker and V/T is the urine flow rate.

A suitable marker for glomerular filtration rate must be excreted only by the kidney, and after free filtration at the glomerular barrier must not undergo secretion or reabsorption in the tubules. Inulin is a multimeric polymer of fructose with a molecular weight of approximately 3500 which possesses these properties, and is the standard against which new methods are compared. The principal requirement in measuring renal plasma flow is for a marker which is completely extracted from the blood in one passage through the kidney; the weak acid *para*-aminohippurate (PAH) fulfils this criterion. The proportion of PAH in renal arterial blood excreted during a single passage through the kidney (the Extraction Ratio) varies between species, and in normal humans is between 90 and 95%. Incomplete extraction of PAH is mainly due to the shunting of blood through the inert perirenal fat and the capsule, and recirculation of this small proportion of PAH makes the measured clearance a slight underestimate of true renal plasma flow. This systematic source of error is acknowledged by using the term *effective* or *estimated* renal plasma flow (ERPF) when presenting results. Fortunately the error has minimal

physiological importance because most shunting occurs through structures which do not contribute to glomerular filtration.

Two important assumptions are made when using PAH clearance as an estimate of renal plasma flow. The first is that the Extraction Ratio is the same for all participants in the study, and the second that PAH extraction is not affected by an experimental intervention. Changes in the renal extraction ratio for PAH during ANGII infusion may invalidate the use of PAH clearance as a measure of renal plasma flow (Gassee et al, 1976), but in the dog this effect is only important at high doses of ANGII (40 $ngkg^{-1}min^{-1}$ intrarenally), when the extraction ratio changes from 0.76 to 0.86. It is therefore unlikely that the low doses of ANGII infused in the present studies (up to 2.5 $ng.kg^{-1}min^{-1}$ intravenously) significantly affected the renal extraction of PAH. Similarly, the extraction ratio for PAH in patients with uncomplicated Type 1 diabetes does not differ from normal controls (Nyberg et al, 1982).

3.4.1.1 Glomerular Filtration Rate.

Glomerular filtration rate was estimated by measuring the clearance of Polyfructosan-S (Inutest, Laevosan, Linz, Austria). The assay used a chemical method (Dawborn, 1965) in which Inutest is hydrolysed to fructose and then linked to indolylacetic acid to form a

coloured adduct. Urine samples were diluted 10 to 50-fold before analysis, plasma samples were analyzed without dilution.

Reagents.

i) A stock solution of Inutest (1000 mg/dl in distilled water) was diluted to produce standard solutions with concentrations from 5-50 mg/dl.

ii) Concentrated hydrochloric acid (conc. HCl, s.g. 1.18).

iii) 0.5M hydrochloric acid. 174ml of conc. HCl was made up to 4000ml in distilled water.

iv) 3-indolylacetic acid (3-IAA). 0.25g of 3-IAA was added to 50ml 80% ethanol.

Procedure.

A semi-automated technique was used (Technicon Autoanalyzer, Technicon Instruments Corp., Tarrytown, NY). Aspirated sample (0.4 ml/min) was mixed with 0.5M HCl (1.8 ml/min) and air (0.8 ml/min, to encourage mixing), and heated (10 mins, 60^{o}C) to hydrolyse Inutest to fructose. Protein was removed by dialysis, and protein-free dialysate (0.3 ml/min) mixed with conc. HCl (2.3 ml/min), 3-IAA (0.1 ml/min), and air (0.6 ml/min). The colour was produced by heating this mixture for 8 minutes at 60^{o}C. The intensity of colour (proportional to inulin concentration) was estimated spectrophotometrically (by absorbance at 520nm), and recorded graphically. Standards were run before and after every

assay run to confirm the validity of the standard curve throughout the procedure, and unknown samples read from the standard curve by interpolation.

3.4.1.2 Effective Renal Plasma Flow.

Effective renal plasma flow was estimated as the renal clearance of *para*-aminohippurate (PAH). This was assayed by a modification of a chemical method (Harvey and Brothers, 1962) which diazotises the amino- group in PAH to produce a coloured adduct. Urine samples were diluted before analysis 50 or 100 fold to produce concentrations lying within the standard curve. Plasma samples were analyzed without dilution.

Reagents.

i) A solution of 0.25ml of 20% PAH (Merck, Sharp and Dohme) in 100ml of distilled water (50 mg/dl), was prepared every two months, and stored in darkness at $4^{o}C$. Standard PAH solutions were prepared by dilution to produce concentrations ranging from 0.5 to 5.0 mg/dl.

ii) Sodium nitrite. 0.25g $NaNO_2$ in 100ml concentrated hydrochloric acid, diluted to 1000ml in distilled water.

iii) Ammonium sulphamate. 3.25g in 1000ml distilled water.

iv) N-(1-napthyl)ethylenediamine hydrochloride (NEDDC). 1.05g in 200ml 95% ethanol, diluted to 1000ml with distilled water.

Procedure.

The same semi-automated apparatus was used as described for inulin. Sample was aspirated (0.6 ml/min), diluted with 0.15M sodium chloride (0.8 ml/min), and aerated (0.8 ml/min). This mixture passed through a 'cuprophane' dialyzer, transferring PAH but excluding larger molecules containing amino- groups such as proteins. After diazotisation with sodium nitrite (0.6 ml/min), excess nitrite was destroyed by the ammonium sulphamate (0.32 ml/min), and the colour produced by coupling with NEDDC (0.32 ml/min). A complete cycle lasted 30 minutes. After removing the air the intensity of the colour was measured in a spectrophotometer (absorbance, 550nm) and recorded graphically. Standards were run at the beginning, middle and end of each series to correct for baseline drift. A standard curve was constructed, and the PAH concentration in samples read from this by interpolation.

3.4.2 HORMONAL ESTIMATIONS.

3.4.2.1 Plasma Renin Activity.

Blood was collected into chilled tubes containing sodium EDTA as anticoagulant, centrifuged immediately at $4^{o}C$, and separated plasma stored at $-70^{o}C$ until assay. Plasma renin activity (PRA) was measured using a commercial kit by radioimmunoassay (RIA) of angiotensin I

(ANGI) generated under standard conditions (SB-REN-2, CIS, Gif-sur-Yvette, France) (Haber et al, 1969). The assay involved two stages:

i) generation of ANGI in plasma samples under standard conditions (renin being the rate limiting step for the production of ANGI),

ii) single antibody solid phase (coated-tube) RIA in two aliquots of the sample, one incubated at 37^{o}C and the other non-incubated (sample blank). RIA involved competition between unlabelled ANGI (sample/standard) and radiolabelled ANGI for binding sites in the solid phase. The concentration of labelled ANGI on the solid phase after incubation was inversely proportional to the sample concentration.

Procedure.

1. Angiotensin I generation.

500µl of sample, 10µl of phenylmethylsulfonyl fluoride ethanol (a converting enzyme inhibitor), and 50µl of buffer (pH 6) were added in turn to a non-coated generation tube in an ice-bath. After mixing, 200µl was transferred to a second generation tube which was incubated for 4-5 hours in a thermostatically controlled water bath. This long generation time was necessary because PRA levels in the samples were generally low after the period of dietary sodium loading before the study. After incubation the tubes were immediately placed in an ice-bath.

2. Radioimmunoassay.

All assays were performed in duplicate. 50ul of sample, sample blank or standard (0-50 ng/ml) and 500ul of [^{125}I] ANGI were added to an antibody-coated tube, and incubated for 12-18 hours at room temperature. The liquid contents of the tube were carefully aspirated, and the residual radioactivity counted with a gamma counter.

Calculation.

For each standard ANGII concentration the ratio B/B_0 was calculated as: $\frac{\text{standard mean counts}}{\text{'zero standard' mean counts}} \times 100\%$

A standard curve was constructed by plotting B/B_0 against ANGI concentration (ng/ml). The concentrations of ANGI in an incubated sample (37^{o}C) and its corresponding non-incubated sample blank (4^{o}C) were read directly from the calibration curve, and PRA calculated as;

$$PRA = \frac{[ng(37oC) - ng(4^{o}C)] \times 1.12}{\text{generation time (hours)}} \quad ngANGI.ml^{-1}h^{-1}$$

The antibody used was highly specific for ANGI, with cross-reactivity against ANGII of <0.2%. The lower detection limit of the assay was 0.2 ng/ml (equivalent to a PRA of about 0.04 $ngANGI.ml^{-1}h^{-1}$). The intra- and interassay coefficients of variation were 7% and 8% respectively.

3.4.2.2 Plasma Angiotensin II.

Extraction of plasma samples was followed by a single antibody radioimmunoassay using a modification of

the method developed and validated in the MRC Blood Pressure Unit, Western Infirmary, Glasgow (Morton and Webb, 1984). The antibody (R6B4) has a cross-reactivity with ANGI of 0.5%; because of the low plasma renin activity and ANGI levels after sodium loading, which fell further during ANGII infusion due to the short feedback loop, a correction for ANGI was not applied to the plasma ANGII results.

Reagents.

i). Converting enzyme inhibitor for sample collection. 4.65g EDTA dissolved in 100ml distilled water was mixed with 0.45g c-phenanthroline dissolved in 1ml absolute alcohol, and stored at 4°C.

ii). Assay buffer. 1.51g tris (hydroxymethyl) methylamine [2-amino-2(hydroxymethyl)propan-1,3-diol (tris)] dissolved in 250ml distilled water, mixed with 150ml 0.05M hydrochloric acid, with 0.1% sodium azide, and stored at 4°C.

iii). Charcoal suspension. A suspension of 0.6% activated charcoal (Sigma C-5260), 0.06% dextran, and 0.04% gelatin in 0.1M phosphate buffer pH 7.4 was stored at 4°C.

iv). Radiolabel. The radiolabel was synthesized using a chloramine-T iodination procedure, purified by anion exchange column chromatography, and stored at -20°C until use.

Procedure.

1. Sample collection.

9.5ml of blood was collected into a chilled plain tube containing 0.5ml of phenanthroline/EDTA, transported on ice, centrifuged immediately (20 min, 4°C, 3000g), and plasma stored at -70°C until extraction.

2. Extraction of plasma samples.

Samples were thawed at 4°C. Columns (Sep-Pak C18, Millipore, Milford, Mass., USA) were pre-washed with 5ml methanol and 5ml distilled water. 5ml of plasma (volume accurately noted) was then applied to the column, and washed with 5ml distilled water. ANGII was eluted by passing through 2.2ml 80% methanol, the eluate collected into polypropylene tubes (LP4), and evaporated to dryness under an air stream in a sample concentrator at 40°C (3-4 hours). The dried extract was then resuspended in 0.5ml of assay buffer (pH 7.4) containing 0.1% bovine serum albumin.

3. Radioimmunoassay.

The antibody was made up in assay buffer and used at a final dilution of 1:300,000, which bound 60-70% of the radiolabel in the zero standard tube. Assays were performed in duplicate. Radiolabel, standards and quality controls were all made up in assay buffer. 50µl of standard/QC/sample, 100µl antibody, and 100µl radiolabel (final concentration about 5000cpm) were added in turn to LP3 tubes, and incubated overnight at 4°C.

Free and bound radiolabel were separated by adding 0.6ml of charcoal suspension at 4°C, centrifuging (30 min, 4°C, 1720g), and aspirating the supernatant (bound fraction). The charcoal pellet was then counted for two minutes (LKB gamma-counter). A standard curve was constructed using standards between 0 and 10.24 ng/ml; quality controls and unknown samples were read from this by interpolation.

Calculation.

The ANGII assay value (ng/ml) was multiplied by 0.5 (resuspended in 0.5ml), then by 10/9.5 (dilution during sample collection), and divided by the volume of plasma applied to the Sep-Pak column to give ANGII per ml of plasma. All samples from each study were analysed in one batch, with an intraassay coefficient of variation of 11%. The interassay c.v. was 10%; one batch of samples (see Table 7.2) gave results which were consistently much higher than expected. The reasoning that this is likely to represent an assay problem rather than a real difference is discussed in a footnote to the Table.

3.4.2.3 Aldosterone.

Blood was collected into plain tubes, centrifuged promptly, and separated serum stored at -70°C until assay. Aldosterone was measured by a single antibody solid phase radioimmunoassay (RIA) (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA) without an extraction step (Tan et al, 1978).

Procedure.

All samples were analyzed in duplicate. 200ul aliquots of sample or standard (0-1200 pg/ml) were added to antibody-coated tubes, followed by 1 ml of [^{125}I]-aldosterone. After gentle mixing the tubes were incubated for 3 hours at 37^{o}C, and the liquid contents then thoroughly aspirated. Residual radioactivity was counted for one minute in a gamma counter.

Calculation.

For the standards the binding (mean counts) was expressed as a percentage of maximum binding (the zero standard): a plot of 'Percent Bound' against 'Concentration' on logit-log paper yielded a straight line. Aldosterone concentrations in unknown samples were read from this line by interpolation.

The antibody used had negligible cross-reactivity against other steroid hormones or plasma constituents. The lower detection limit of the assay was 16 pg/ml, and the intra- and interassay coefficients of variation were 5% and 8% respectively.

3.4.2.4 Urine Free Dopamine.

About 80% of dopamine in urine is conjugated, mainly as the sulphoconjugate, but free dopamine is believed to be the physiological moiety. Urine samples were therefore extracted to bind free dopamine, which was then eluted. Free dopamine was measured by HPLC with

electrochemical detection, using epinine (N-methyl dopamine) as internal standard.

Urine samples.

Urine (20ml) was collected into Universal containers containing 0.4 ml 5M HCl, giving a final pH <3 to prevent oxidation of free dopamine. Samples were stored at -40^{o}C before assay.

Reagents.

i) Standards were prepared by diluting a stock solution of dopamine HCl (Sigma) (50mg in 50 ml M HCl) first 1:99 with distilled water and then 1:49 with 0.01M HCl, producing a final dopamine concentration of 200 ng/ml. The internal standard epinine (Sigma) was prepared by diluting a stock solution (50 mg in 50 ml of M HCl) first 1:9 with distilled water and then 1:199 with 0.01 M HCL to produce a final concentration of 500ng/ml.

ii) The HPLC solvent contained citric acid monohydrate (5.75 g); sodium acetate trihydrate (6.80g); sodium hydroxide (2.40g); 1-octane sulphonic acid, sodium salt (0.10g); acetic acid (1.05 ml); and di-sodium EDTA (0.10g) made up to one litre with deionised water, pH 5.2. Solvent was filtered through a 0.22 µm membrane (Millipore) and methanol 10% (v/v) added prior to use. Dissolved oxygen was removed by bubbling helium through the mixture.

iii) Aluminium hydroxide (Brockman grade 1, BDH) was pre-activated by heating at 200^{o}C for 2 hours and stored

at 100°C. Activated alumina was washed with 0.5M EDTA before use.

Procedure.

Extraction of dopamine from urine.

The extraction process used the method of Anton and Sayre (1964). Undiluted urine was used. 5 ml of test sample and 0.5 ml of internal standard were added to 0.5 g of washed activated alumina, and buffered to pH 8.3 with 0.5M Tris. After mixing and centrifugation (1000g, 2 min, 4°C), the supernatant was syphoned off, and the deposit washed three times with distilled water. Dopamine was eluted by addition of 2 ml of 0.2M acetic acid. Tubes were shaken for 10 minutes at 37°C and centrifuged at 4°C. The supernatant was removed and stored at -40°C. All samples were processed and analysed in duplicate.

Dopamine standards were prepared in the range 5-50 ng/ml, and 0.5 ml of the internal standard, 0.5 µg/ml, was added to 5ml of each standard concentration. All standards were extracted by the same procedure as test samples, and were frozen at -40°C. Fresh standards were prepared every month. The recovery of dopamine from the extraction process was 69.7(SD 0.5%) compared against unextracted standards.

Analytical system.

Each assay consisted of test samples, duplicate standards, run at the beginning and end of each batch,

and two quality control samples. 5 µl were injected automatically into a solvent stream delivered at 1 ml/min to a Waters 4 µm, Novapak C18 HPLC column under radial compression. The column effluent stream was delivered to an electrochemical detector (Waters 460), at a potential of 0.4 volts, in oxidative mode, with background current approximately 0.5 mA. Output signals were passed to a computerised integrator which measured the area under the curve of peaks of interest.

Calculation.

The integrated area of each standard peak was divided by the area of its internal standard. The duplicated standard ratios were averaged and a standard curve plotted against the known concentrations, the plot providing a straight line relationship. Test sample ratios were read from the curve and urine dopamine expressed in nmol/l. The sensitivity of the assay was 5 ng/ml. Intra- and interassay co-efficients of variation were 4% and 6% respectively.

3.4.3 Miscellaneous.

Sodium and potassium concentrations in plasma and urine were measured by emission flame photometry (Corning 435, Ciba Corning Diagnostics) with lithium as internal standard. Osmolality was measured by freezing point depression (3MO Osmometer, Advanced Instruments Inc., Mass., USA). Creatinine, total protein and albumin were

measured by standard chemical methods using a Gemstar autoanalyser. Urinary albumin concentration was estimated by an immunoturbidimetric assay (Albusure QNT, Cambridge Life Sciences, Cambridge) (Spencer and Price, 1985), urinary and plasma glucose by the glucose oxidase method, and glycated haemoglobin by an electrophoretic method (Read et al, 1980). Plasma free insulin was assayed by double antibody radioimmunoassay (Soeldner and Stone, 1965); samples from diabetic subjects were extracted with polyethyleneglycol (PEG) 6000 immediately after sampling to remove the fraction of insulin bound to anti-insulin antibodies in diabetes (Nakagawa et al, 1973). The intraassay c.v was 8%. Serum converting enzyme activity was estimated by measuring the *in vitro* rate of hydrolysis of a synthetic analogue of ANGI, N-(3-{2-furyl}acrolyl)-L-phenylalanylglycylglycine, using a spectrophotometric assay (Maguire and Price, 1985).

3.4.4 Lithium estimation.

Two methods were assessed for the estimation of lithium concentrations in serum and urine. Urinary lithium assay by flame emission photometry has conventionally been regarded as unreliable because of variable background interference by sodium and potassium (internal standards for measuring serum lithium by emission photometry contain sodium 140mM and potassium 5mM as background 'blanks'). Lithium estimation was

therefore initially attempted by flameless atomic absorption spectrophotometry (Perkin Elmer 272, Beaconsfield, Bucks.), using 10µl samples without dilution or deproteination. Surprisingly, in this system the absorbance for any given lithium concentration was markedly affected by the background sodium concentration. The pattern of interference suggested that sodium was suppressing detection of the signal produced by the much lower concentration of lithium (Figure 3.2).

The validity of emission photometry was therefore re-examined (IL943, Instrument Laboratories, Warrington) using caesium chloride 1.50mM as internal standard. 'Blank' values for a range of sodium and potassium concentrations alone and in combination were measured by aspiration directly into the flame after predilution into CsCl 1.5mM (Figure 3.3). The interference produced by both cations was independent of lithium concentration, and the total background 'blank' was accurately predicted by an additive model (Figure 3.4), with no evidence of separate indirect interference beteen sodium and potassium. The data for each lithium concentration was therefore pooled to provide separate regression equations for background sodium and potassium (Figure 3.5).

Samples collected after pretreatment with lithium 750mg were diluted approximately 100-fold by an automated sampler before entering the flame. At this dilution the correction factors for the differing sodium (urine

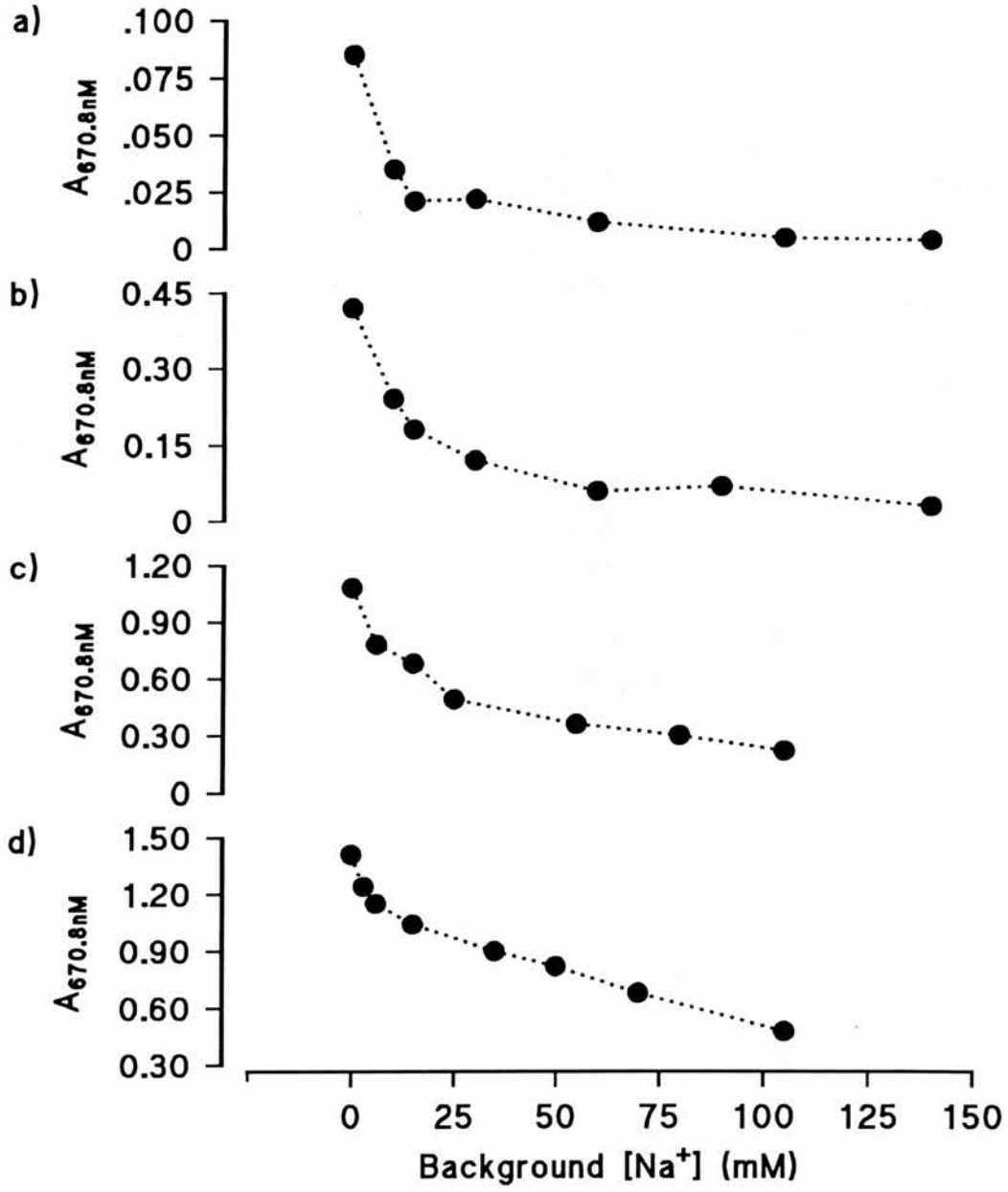

Figure 3.2: Effect of background sodium on $Absorbance_{(Li)}$ for a) 10μM, b) 50μM, c) 200μM and d) 500μM lithium. A is shown in arbitrary units.

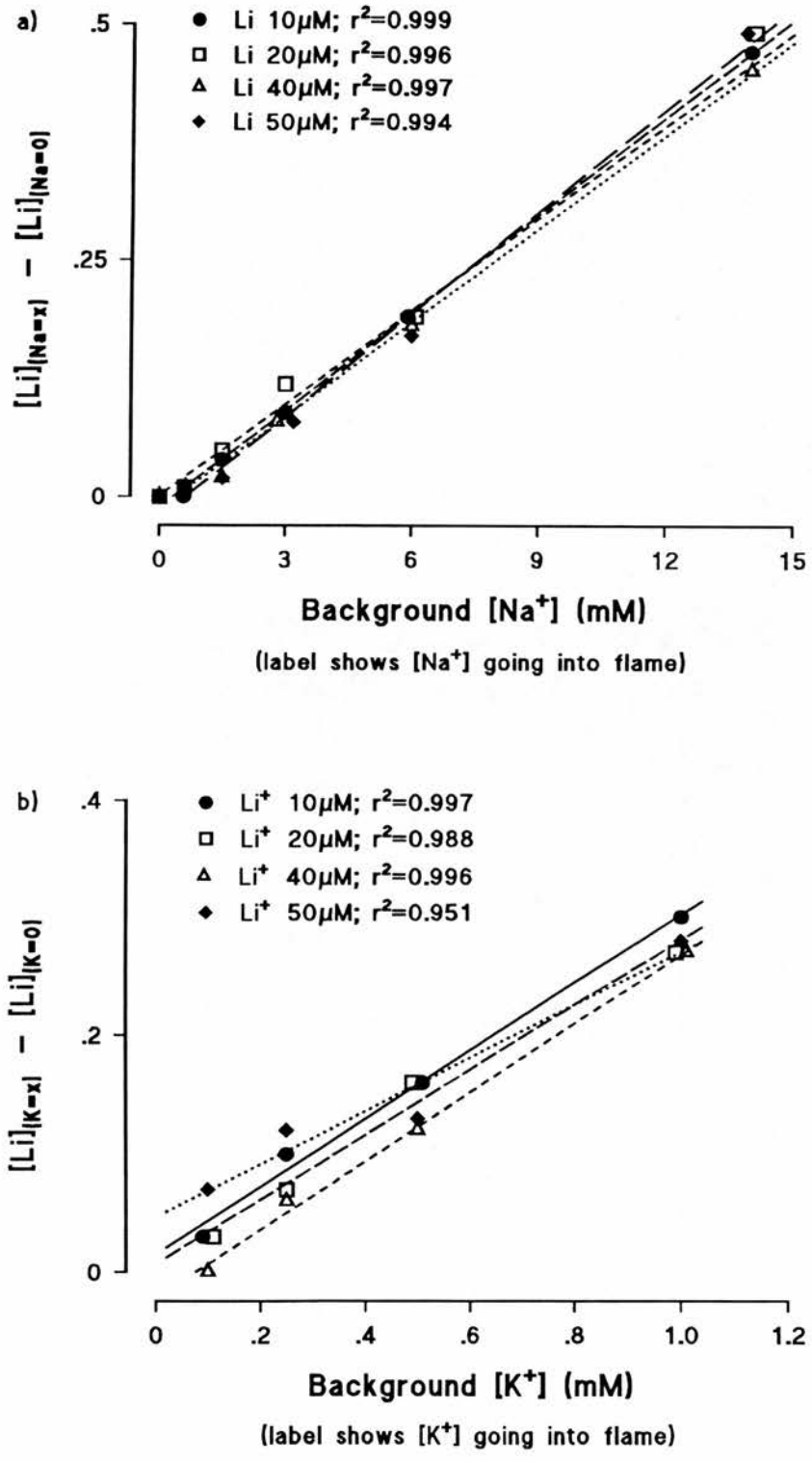

Figure 3.3: Effect on apparent lithium concentration of varying background concentrations of a) sodium and b) potassium.

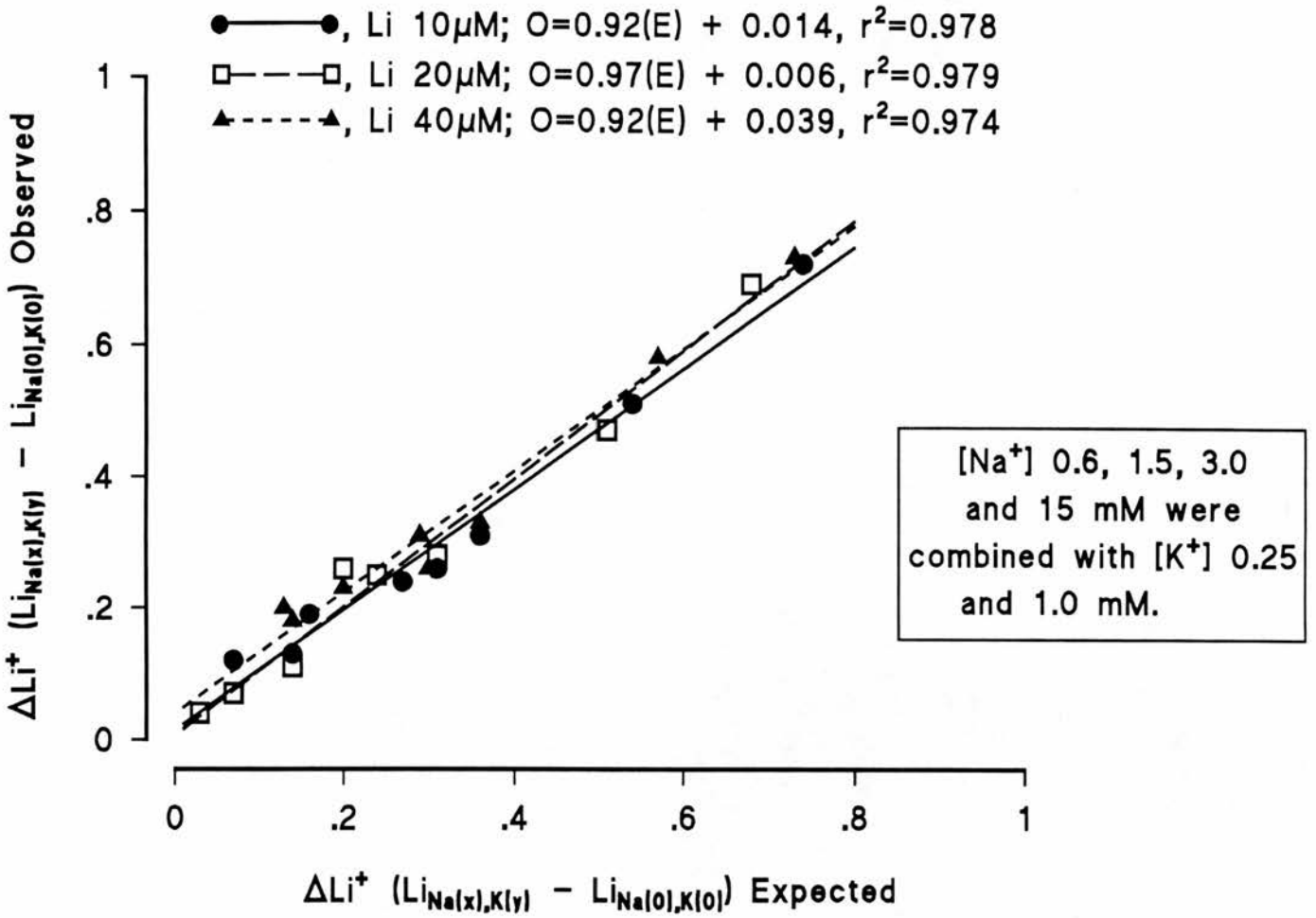

Figure 3.4: Observed interference (Y–axis) for various combinations of background sodium and potassium. The X–axis value indicates the expected ΔLi^+ based on the data in Figure 3.3, assuming that blanks for Na^+ and K^+ add together without interaction.

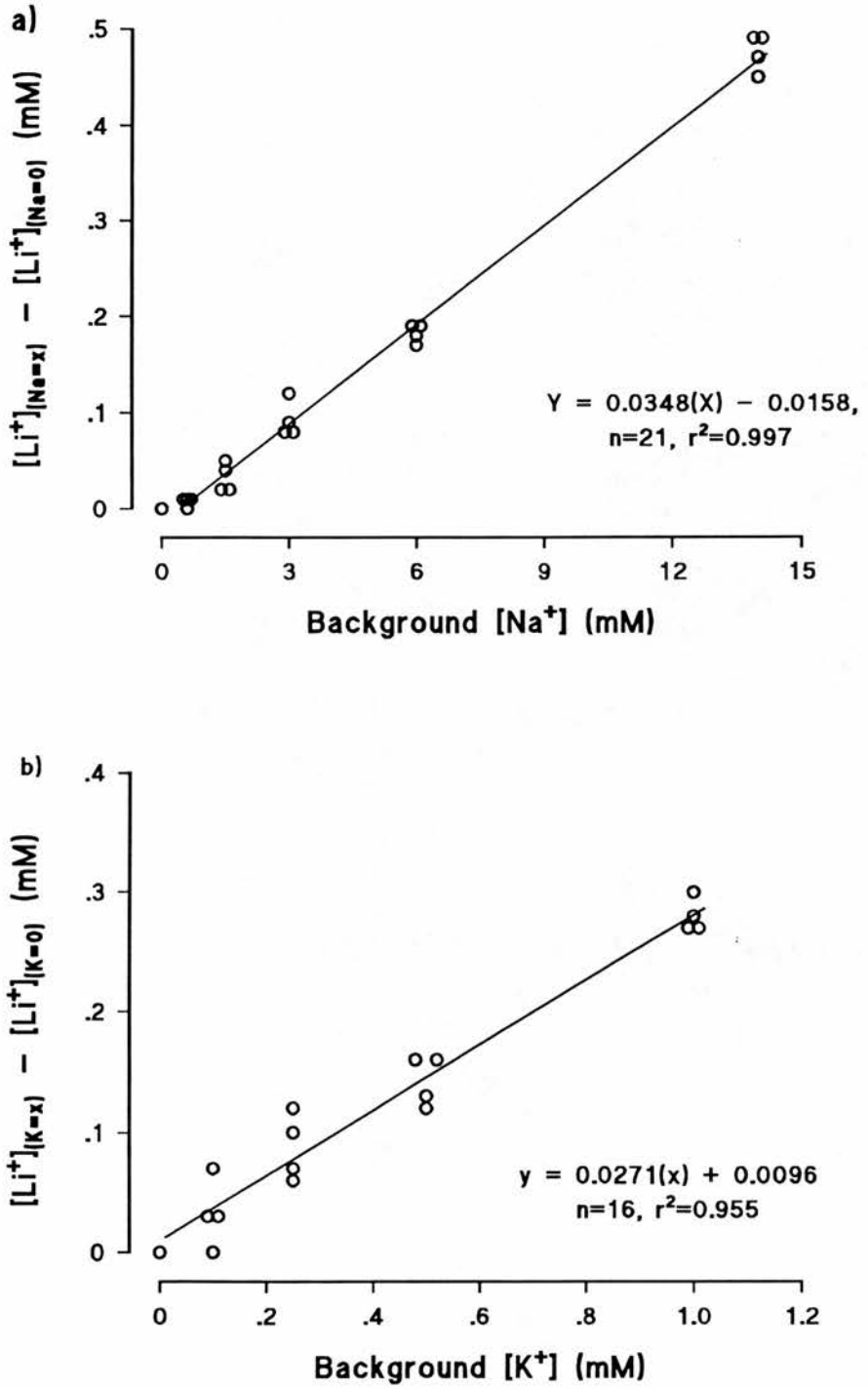

Figure 3.5: Regression of 'error' in lithium estimation due to background a) sodium and b) potassium concentration. Data for four concentrations of lithium (Fig 3.3) has been pooled.

0.1-0.2mM, serum 1.4 mM) and potassium concentrations (urine 0.05-0.1mM, serum 0.05mM) were very small and were constant throughout the study within each subject (see Figure 3.5). These very minor errors were therefore ignored when calculating lithium clearance.

Samples collected after lithium 250mg were diluted manually 1:10 into 1.65mM caesium chloride (0.2778 g/l), producing a final caesium concentration of 1.50mM as above: this sample was then aspirated into the flame without further dilution. By avoiding the large dilution produced by the automated sampling procedure the overall sensitivity of the assay was increased about 9-fold. Serum lithium concentrations of 0.10mM could thus be measured to three decimal places with an intraassay coefficient of variation of 2%. However, because the sample was diluted only 11-fold there was a greater disparity in background interference between serum and urine samples (Na^+; urine 1-2mM, serum 13mM: K^+; urine 0.5-1.0mM, serum 0.5mM). These results were therefore corrected using the equations in Figure 3.5 before calculating lithium clearance. The need to apply a correction for sodium and potassium concentrations probably makes these results less reliable than those obtained after lithium 750mg.

Samples from each study were always assayed together to prevent interassay variation from having any effect on the lithium clearance results (U*V/P).

3.5 CALCULATIONS AND STATISTICAL ANALYSES.

Renal clearance was calculated using the formula U_x*V/P_x, U_x and P_x being the urinary and plasma concentrations of the substance x, and V the urine flow rate. P_x was the mean of the plasma level at the beginning and end of the clearance period concerned. Fractional clearance was defined as (Clearance/GFR)*100%.

Mean arterial pressure (MAP) was estimated as diastolic pressure plus one third of the pulse pressure. Effective renal vascular resistance (ERVR) was estimated by the formula:

$$(MAP-10) \times \frac{(1-Hct)}{ERPF} \times 72269 \text{ dynes.sec.cm}^{-5}$$

which assumes a constant renal venous pressure of 10mmHg.

Statistical analyses were performed on an IBM/PC microcomputer using Statistical Package for Social Sciences software (SPSS/PC+ version 2.0). The results are presented as mean(sem) unless otherwise stated. In all the studies (with the exception of Chapter 9), results from the two baseline periods were not significantly different for any variable, and the mean of these periods was therefore used in the analyses. The influences of group, ANGII infusion, and drug treatment were assessed by two-way analysis of variance with repeated measures correction (SPSS/PC+ MANOVA; Snedecor and Watson, 1987). This procedure allows for the non-independence of successive data points in experiments

involving repeated measurements on a subject on one occasion, or on more than one occasion after different treatments. Data sets were always tested for inequality of variances, and when this was detected the number of degrees of freedom was adjusted using the Greenhouse-Geiser Epsilon to give a more conservative significance level. T-tests (two-tailed) were paired or unpaired as appropriate, and were corrected when necessary for multiple comparisons (Bonferroni's method). In many of the Figures which illustrate differences between whole response curves for either groups or treatments, time interactions are not listed and only summary F ratios and P values for group interactions are quoted, without using subsequent t-tests. This simplifies the presentation of data and avoids the attribution of particular (often artificial) significance to specific timepoints. Correlation between variables was tested by univariate (least squares) or multivariate linear regression analysis. Non-parametrically distributed data was log-transformed before analysis, or was analysed by the Kruskal-Wallis test (a generalized analysis of variance for non-normally distributed data) followed by Wilcoxon 'Z' or Mann-Whitney `U` tests. A value for $2P<0.05$ was accepted as statistically significant.

CHAPTER FOUR.

THE ACTIONS OF EXOGENOUS ANGIOTENSIN II IN NORMAL MAN.

4.1 INTRODUCTION.

The physiological actions of angiotensin II (ANGII) and its interactions with other renal hormone systems were reviewed in Chapter 1. This preliminary study was designed firstly to determine whether the expected physiological actions of ANGII on the kidney could be demonstrated in normal man when ANGII is administered systemically in very low doses. Secondly, because the intrarenal actions of ANGII are the opposite of the effects attributed to endogenous dopamine (DA), which may interact with the RAAS at several levels, the effect of inhibition of endogenous DA synthesis on the renal response to ANGII infusion was also examined to determine whether there is any intrarenal interaction between the RAAS and DA.

4.1.1 Protocol.

Six healthy subjects (mean age 32 years, range 27-39 years) were studied on two occasions at least one month apart. Their normal diet was supplemented with sodium chloride 200 mmol/day ('Slow Sodium', Ciba, Horsham) for five days before each study to suppress endogenous ANGII and to augment intrarenal DA levels. Renal responses to infusion of ANGII at 0.5 and 1.0 ng.kg bodyweight^{-1}.min^{-1} were determined using the clearance protocol outlined in Chapter 3. On the 'active' day the subjects took carbidopa (Merck, Sharp, and Dohme, Hoddesdon, Herts,

U.K.) by mouth, 100mg at 0700h and 50mg at 1000h, to suppress intrarenal DA production. Neither ANGII infusion nor carbidopa caused any side effects.

4.2 RESULTS.

Mean arterial pressure rose minimally during ANGII infusion on both days (control: 91(2) to 94(3) mmHg, P=NS; carbidopa: 89(2) to 95(1) mmHg, P<0.02). Heart rate did not change during ANGII infusion (data not shown). Plasma ANGII levels were low at baseline (Table 4.1), and the increment in ANGII levels during infusion was not affected by carbidopa. Plasma renin activity and plasma aldosterone were suppressed at baseline and did not change significantly during ANGII infusion on either day. Plasma sodium and potassium concentrations and urinary potassium excretion did not change during ANGII (data not shown).

Carbidopa did not change baseline GFR, or the small but significant fall in GFR that occurred during ANGII infusion on both days (Fig 4.1a). There was a consistent elevation in ERPF after carbidopa pretreatment during the baseline period and during ANGII (Fig 4.1b), but the reduction in ERPF during ANGII was the same on both days. Filtration fraction rose to the same extent during ANGII on both days (control: 18.4(0.6) to 21.9(1.9) %, P<0.02; carbidopa 15.8(1.3) to 18.6(1.4) %, P<0.01).

Urinary dopamine excretion (Fig 4.2) was suppressed

Table 4.1: Plasma renin activity (PRA), plasma angiotensin II (ANGII), and plasma aldosterone (ALDO) in six normal subjects during ANGII infusion with and without carbidopa pretreatment.

		B1	B2	ANGII($ng.kg^{-1}.min^{-1}$) 0.5	1.0
PRA ($pmol ANGI.h^{-1}ml^{-1}$)	Control	180 (100-290)	110 (80-230)	80 (20-140)	90 (50-300)
	C/Dopa	150 (120-270)	190 (120-300)	100 (70-250)	180 (50-200)
ANGII (pg/ml)	Control	3.1(0.5)	3.2(0.7)	9.8(1.2)[a]	12.1(0.7)[b]
	C/Dopa	2.4(0.5)	3.1(0.3)	8.6(1.6)[a]	18.4(3.5)[a,c]
ALDO (ng/dl)	Control	-	1.5(1-5)	1.8(1-3)	2.2(1-3)
	C/Dopa	-	1.8(1-5)	1.7(1-4)	2.1(1-4)

Data are mean(sem) or median(range). B1 and B2, baseline periods. [a], $P<0.01$; [b], $P<0.005$ vs baseline. The group [c] contained an unexplained outlier (30 pg/ml, included in analysis).

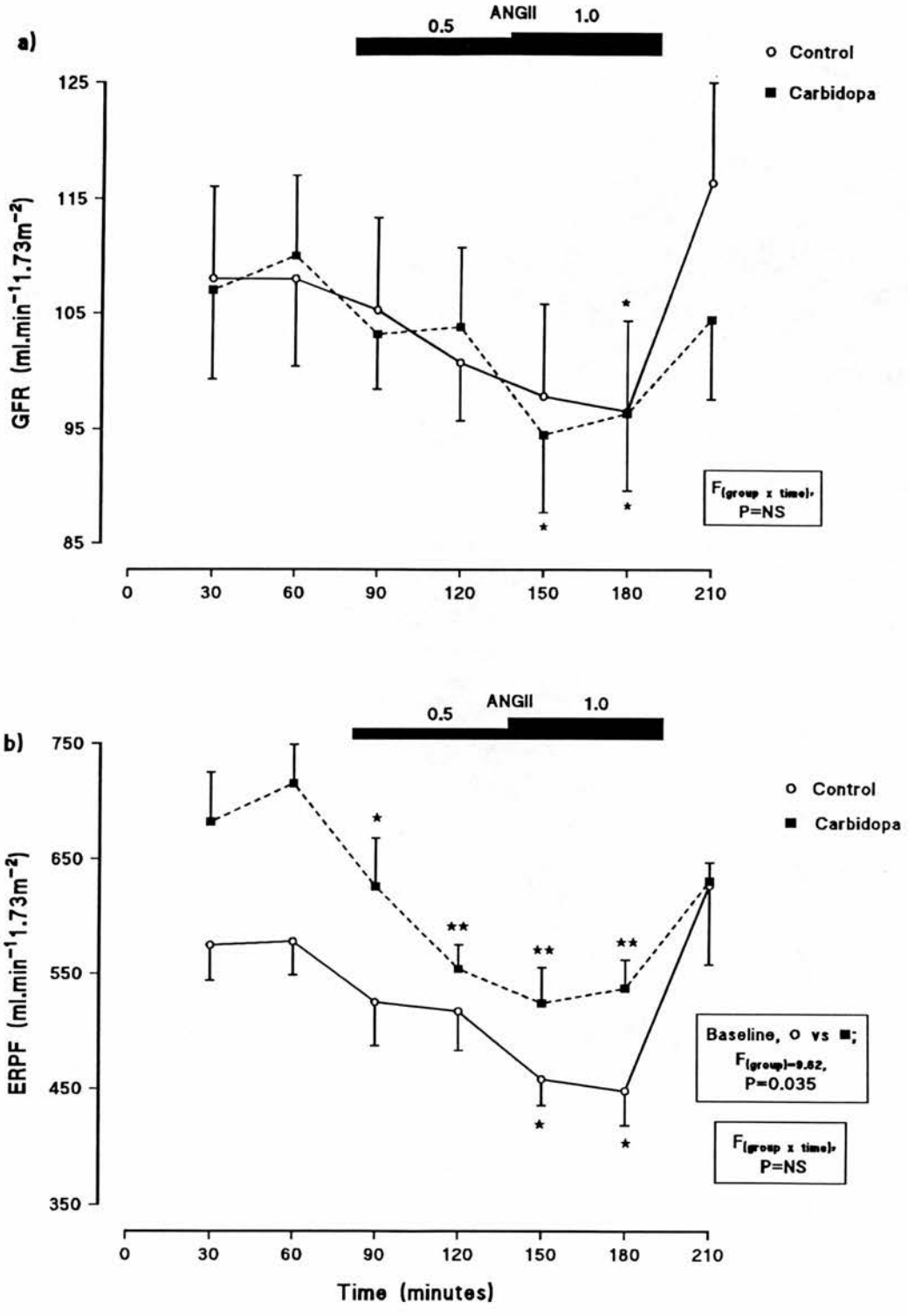

Figure 4.1: a) glomerular filtration rate and b) effective renal plasma flow in 6 normal subjects during ANGII infusion with (■) and without (o) carbidopa. Mean(sem). ⋆ P<0.05, ⋆⋆ P<0.01 vs baseline.

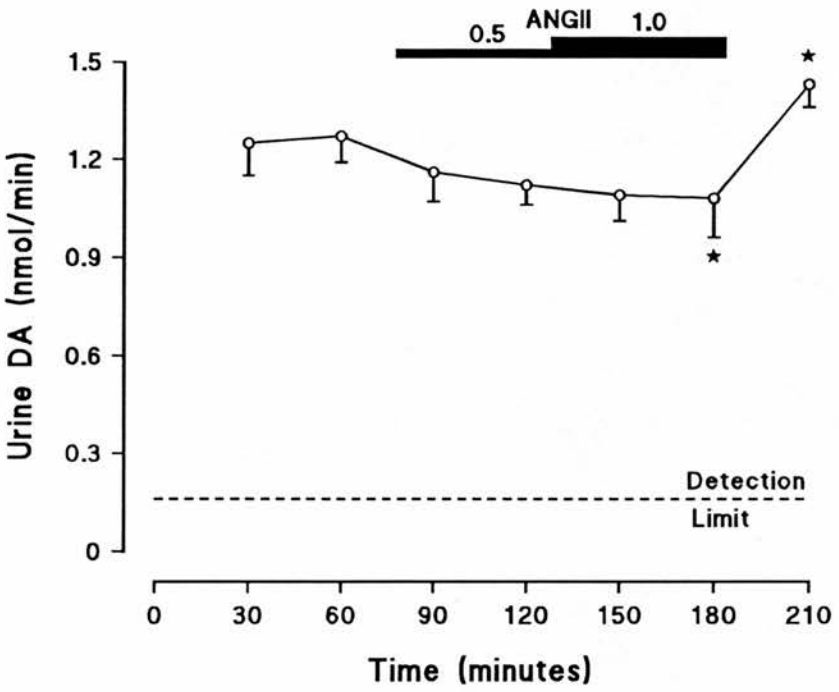

Figure 4.2: Urine dopamine (DA) excretion in 6 normal subjects during ANGII infusion without carbidopa pretreatment.
Mean(sem). MANOVA; $F_{[time]}$=3.55, P=0.007. ★, P<0.05 vs baseline.
DA excretion was below the detection limit after carbidopa.

suppressed to undetectable levels (<0.16 nmol/min) in each subject throughout the study after administration of carbidopa. On the control day urine DA fell in all subjects during ANGII infusion (1.23(0.10) to 1.08(0.08) nmol/min, P<0.05), with a rebound in excretion above the baseline level during the recovery period afer the ANGII infusion had been stopped (P<0.05).

Urine flow rate (Figure 4.3) fell to a similar extent during ANGII on both days (control 7.1(0.3) to 4.7(0.7) ml/min, P<0.05; carbidopa 9.2(1.1) to 5.5(0.9) ml/min, P<0.01). Urinary sodium excretion was similar during the 24 hours before both study days (control 370(37) mmol, carbidopa 335(27) mmol). Baseline absolute sodium excretion (Fig 4.4a) was not influenced by carbidopa (control 468(68) µmol/min, carbidopa 423(56) µmol/min), and the antinatriuretic response to ANGII was not modified by carbidopa pretreatment, either in terms of absolute (Figure 4.4a) or fractional excretion of sodium (Figure 4.4b). Cumulative sodium excretion was the same on both days (total excretion: control 66(8) mmol, carbidopa 64(9) mmol). The sodium load infused during the study was approximately 70 mmol on both days, hence sodium balance was stable throughout both studies.

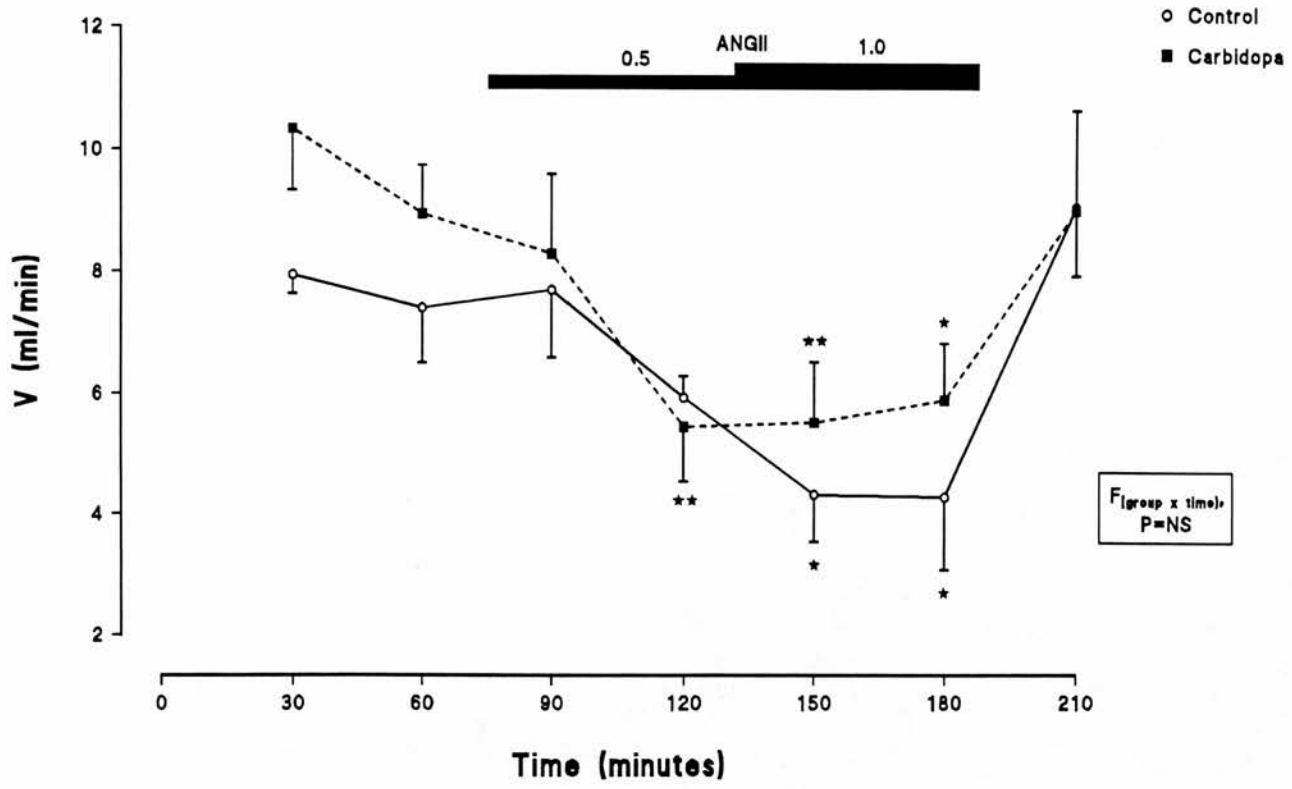

Figure 4.3: Urine flow rate (V) in 6 normal subjects during ANGII infusion with (■) and without (○) carbidopa pretreatment.
Mean(sem). ⋆, P<0.05; ⋆⋆, P<0.01 vs baseline.

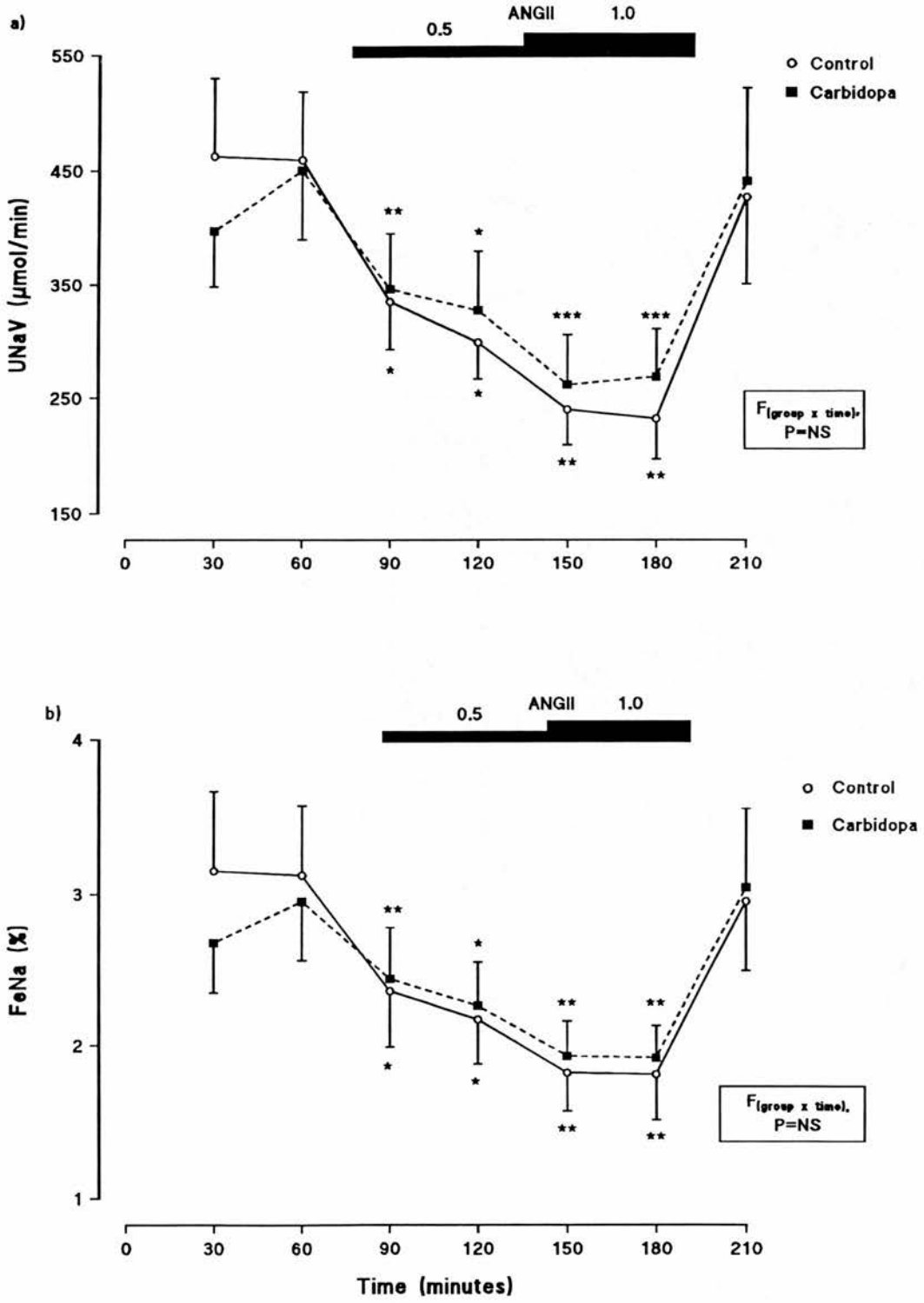

Figure 4.4: a) absolute (UNaV) and b) fractional sodium excretion (FENa) in 6 subjects during ANGII infusion with (■) and without (○) carbidopa. Mean(sem). ⋆ P<0.05, ⋆⋆ P<0.02, ⋆⋆⋆ P<0.01 vs baseline.

4.3 DISCUSSION.

This study confirms that physiological increments in plasma ANGII concentrations which induce minimal effects on the peripheral circulation cause renal vaso-constriction, antinatriuresis and antidiuresis in normal man. The rapidity of onset of the changes in sodium and water excretion, the absence of changes in urinary potassium excretion, and the attenuated response of aldosterone to exogenous ANGII all suggest that the response to ANGII was independent of the sodium-retaining effect of aldosterone (Ames et al, 1965). The fall in glomerular filtration rate and filtered load of sodium during ANGII reduced total tubular reabsorption of sodium, but the fraction of the filtered load which was reabsorbed was increased. This effect of ANGII may be predominatly localised to the proximal tubule (Hall et al, 1986a; Seidelin et al, 1989), but it is not possible from this data to determine whether the mechanism promoting increased sodium reabsorption is a direct tubular action of ANGII, a change in peritubular capillary haemodynamics, an altered distribution of intrarenal blood flow, or a combination of these factors. However, the doses of ANGII used in this study have no effect on intrarenal blood flow distribution in the dog (Johnson and Malvin, 1977).

Urinary dopamine (DA) excretion is a sensitive marker of intrarenal DA synthesis (Brown and Allison,

1981), and the fall in urinary DA during ANGII infusion and the rebound above baseline during the recovery phase indicate real changes in the renal levels of the hormone. Similarly, the suppression of urinary DA to undetectable levels after carbidopa administration indicates that renal DA synthesis was effectively prevented. Such acute inhibition of DA synthesis did not affect basal sodium excretion in this study, as previously reported (Jeffrey et al, 1989). One explanation of the discrepancy between these results and previous studies is that dopamine may modulate the activity of other natriuretic or antinatriuretic systems, rather than directly influencing tubular sodium handling itself. This is the postulate which was examined in the present study by inhibiting the formation of endogenous dopamine, and under these conditions there was no direct interaction between intrarenal DA and the renal actions of ANGII. The direct negative feedback effect of exogenous ANGII on renin release precludes any conclusions regarding the influence of renin release on DA synthesis.

The fall in urine DA during ANGII infusion did not affect the renal response to ANGII, but is nonetheless interesting. Changes in urine DA were qualitatively similar to the changes in water excretion, but were probably not causally related since urine DA is unaffected by a pure water diuresis (Casson and Lee, unpublished data). Variations in urine DA also

paralleled the changes in absolute and fractional sodium excretion during the study. Since acute inhibition of intrarenal DA synthesis did not affect urinary sodium excretion at baseline or during ANGII infusion, the correlation between urine DA and sodium excretion suggests if anything that renal tubular sodium handling may exert a modulatory effect on DA synthesis rather than *vice versa*.

If the reduced proximal tubular sodium delivery due to the fall in GFR during ANGII infusion led to reduced absolute proximal sodium reabsorption, despite the increase in fractional proximal sodium reabsorption which ANGII produces (Seidelin et al, 1989), then the present data would be consistent with the notion that proximal tubular reabsorptive capacity in some way regulates the synthesis of DA in the proximal tubular cells. Such a hypothesis does not discount a physiological role for dopamine in regulating sodium handling in some circumstances, but could explain why several studies in addition to this one have failed to show any influence of dopaminergic inhibition or blockade on a variety of interventions causing both natriuresis and antinatriuresis.

The renal haemodynamic response to ANGII was unchanged after pretreatment with carbidopa, but ERPF was consistently elevated on the carbidopa day. This finding was unexpected as inhibition of a putative vasodilator

might be expected to accentuate the renal vasoconstrictor effect of ANGII and to reduce ERPF. Pooling of the data for plasma PAH (n=54) and urinary PAH excretion (n=48) shows that the urinary excretion of PAH was unchanged by carbidopa (control 375(20) mg, carbidopa 349(13) mg; P=NS), but that the plasma PAH level was reduced (control 2.69(0.09) mg/dl, carbidopa 1.95 (0.05) mg/dl; P<0.0001, unpaired t-test). The increase in calculated PAH clearance after carbidopa is therefore attributable to the use of a reduced denominator in the clearance formula U*V/P. PAH and carbidopa both contain an amino group, but the peak heights of the PAH standard curve were unaffected by the presence of carbidopa over a wide range of PAH and carbidopa concentrations, and pure carbidopa was not detectable by the PAH assay system, so that an *in vitro* artefact was not involved. In any event such an interaction would have tended to increase rather than decrease the plasma PAH concentration. The speculation that the presence of carbidopa may alter the renal extraction ratio of PAH is also untenable since ER is about 0.95 in man (Nyberg et al, 1982) and the potential for an increase in ER (<5%) can not account for the 25% difference in the plasma PAH levels between the study days. The explanation for the difference in ERPF is therefore not clear, but does not affect the main conclusion of the study, as carbidopa did not affect the fall in renal plasma flow which occurred during ANGII.

In conclusion, this experiment confirmed that the known actions of angiotensin II described in previous human and animal studies can be reproduced in normal man by infusion of a very low dose of ANGII which produces plasma ANGII concentrations within the physiological range. The results additionally show that dopamine plays little part in regulating the renal response to physiological concentrations of ANGII. Dopamine inhibits noradrenaline release from sympathetic ganglia and nerves via DA2 receptors (Goldberg, 1984), and it would be relevant to establish whether dopamine inhibition or DA2 receptor blockade modifies the renal response to other antinatriuretic stimuli such as noradrenaline infusion. The mechanism for the reduction in urinary DA excretion which occurs during ANGII infusion is not clear.

CHAPTER FIVE.

THE EFFECT OF LITHIUM ON RESPONSES TO EXOGENOUS ANGIOTENSIN II IN NORMAL MAN.

5.1 LITHIUM IN RENAL PHYSIOLOGY.

Micropuncture techniques used in animal studies are inapplicable to human renal physiology, and the contributions of proximal and distal tubular sites to overall sodium and water reabsorption can not be measured directly. Various markers of proximal tubular sodium delivery in man, such as uric acid and phosphate clearances, have been found to be unsuitable. Analysis of tubular function during maximal water diuresis is also unreliable because of the existence of "backflow" of water across the collecting duct even in the absence of vasopressin (Bartoli et al, 1983). Use of the renal clearance of the monovalent cation lithium as an estimate of proximal tubular sodium rejection has therefore aroused great interest.

In normal subjects given a single oral dose of lithium, diuretic drugs with a predominantly proximal tubular action increased the clearances of sodium and lithium equally, while diuretics with an action on the distal nephron (amiloride, spironolactone, triamterene) affected lithium clearance minimally or not at all (Thomsen and Schou, 1968; Thomsen et al, 1969; Thomsen and Leyssac, 1986). Fractional lithium excretion was similar to existing estimates of fluid delivery into the loop of Henle based on data from experiments performed using the maximal water diuresis method, and was independent of GFR, leading Thomsen to suggest that

lithium clearance provides a quantitative measure of fluid and sodium delivery from the proximal tubule (Thomsen, 1984).

The validity of this postulate depends on three assumptions. Firstly, lithium should be reabsorbed in the proximal tubule in the same proportion as sodium and water. Secondly, lithium should be neither reabsorbed nor secreted distal to the late proximal tubule. Thirdly, lithium should have no pharmacological effects on renal function in the doses used. If these criteria are fulfilled the urinary clearance of lithium is equal to the proximal tubular delivery of sodium (and to proximal water delivery since proximal reabsorption of sodium is iso-osmotic). The equations describing tubular sodium handling then require the measurement only of urinary sodium and lithium clearances, and glomerular filtration rate:

$$\text{Absolute proximal sodium reabsorption} = (GFR - C_{Li}) \times P_{Na}$$

$$\text{Fractional proximal sodium reabsorption} = 1 - (C_{Li}/GFR)$$

$$\text{Absolute distal sodium reabsorption} = (C_{Li} - C_{Na}) \times P_{Na}$$

$$\text{Fractional distal sodium reabsorption} = 1 - (C_{Na}/C_{Li})$$

where GFR is glomerular filtration rate, and C_{Na} and C_{Li} are the urinary clearances of sodium and lithium. Similar expressions allow calculation of segmental water handling.

Early micropuncture studies (Hayslett and Kashgarian, 1979; Thomsen et al, 1981; Shirley et al, 1983) supported the validity of lithium clearance as a proximal marker, and numerous clinical studies have tested the method in man by comparing lithium clearance with measurements of sodium excretion made during maximal water diuresis, or by studying the effects of diuretics on renal lithium handling (Thomsen 1990, refs 16-28). Some of this data questions the validity of lithium clearance, especially in sodium-depleted states. Thomsen himself showed (Thomsen, 1977) that lithium clearance decreased relatively more than maximal urine flow rate in rats with diabetes insipidus during low sodium intake, and that the urinary lithium level became less than the plasma lithium concentration, indicating that lithium was being reabsorbed against its concentration gradient, an improbable event in the proximal tubule as lithium can diffuse freely via the paracellular pathway in the electrically 'leaky' proximal tubular epithelium (Holstein-Rathlou, 1990). Amiloride-sensitive 'post-proximal' lithium reabsorption occurs during severe sodium restriction in rats (Kirchner, 1987) and dogs (Boer et al, 1987), suggesting that the phenomenon occurs in the late distal tubule or the collecting duct where amiloride-sensitive channels are most prominent (Brown, 1989). Transport of lithium does not depend upon Na^+-K^+-ATPase (Dunham and Senyk, 1977), and lithium

therefore theoretically could not be reabsorbed through 'tight' epithelia such as the distal renal tubule. It has thus been suggested (Holstein-Rathlou, 1990) that sodium restriction induces amiloride-sensitive lithium transport due to the insertion of sodium channels into the apical surface of distal tubular cells (Bridges, 1989), such channels being activated by the high levels of aldosterone found in sodium-depleted animals, and possibly also by vasopressin (Boer, 1988a). Human studies have not conclusively demonstrated amiloride or spironolactone-sensitive lithium reabsorption during low dietary sodium intake (Roos et al, 1985; Boer, 1988b; Atherton et al, 1987; Bruun et al, 1987), but it is nonetheless tacitly accepted that sodium repletion is a prudent precondition for valid interpretation of human lithium clearance data.

There is also debate as to whether the increased lithium clearance induced by intravenous frusemide (Atherton et al, 1987), an effect which is also more easily demonstrated after sodium depletion, shows that lithium can be carried by the Na-K-2Cl cotransporter in the thick ascending limb, or simply reflects the weak carbonic anhydrase activity of frusemide which could directly increase delivery of sodium from the proximal tubule. Ethacrynic acid, which lacks carbonic anhydrase activity, also increases lithium clearance but to a smaller extent than frusemide, suggesting that some

lithium reabsorption (estimated at about 10% of the filtered load) occurs in the loop of Henle (Boer et al, 1990). The validity of lithium clearance is also dubious during osmotic diuresis (Skott et al, 1987) and after prostaglandin synthetase inhibition (Rabelink et al, 1989), and during vasopressin-induced antidiuresis (Kirchner, 1989). Despite these shortcomings lithium clearance remains the only method available for analyzing segmental renal sodium reabsorption in humans, albeit approximately, and delineation of its reliability in different experimental situations and diagnostic groups is an important prerequisite for the application of the method to the study of disease states.

The controversy as to how 'post-proximal' handling of lithium affects the validity of lithium clearance has overshadowed evidence that lithium interacts pharmacologically with intrarenal hormone systems even at the low plasma levels produced by single doses. Lithium causes a natriuresis when given acutely (Murphy et al, 1969; Shirley et al, 1991), but attenuates the natriuretic responses to the dopamine prodrug gamma glutamyl-L-dopa (Jeffrey et al, 1988), to saline infusion (Jeffrey et al, 1989), and to atrial natriuretic peptide (Freestone et al, 1990). Lithium also increases plasma renin activity (Jeffrey et al, 1988; Jeffrey et al, 1989), and inhibits the distal tubular action of mineralocorticoids (Stewart et al, 1987; Stewart et al, 1988).

The dose of lithium used for analyzing tubular sodium handling in normal man is usually 300-500mg (Girbes et al, 1990; Seidelin et al, 1989), but doses of 600-750mg have usually been given in studies of sodium handling in diabetes (Hannedouche et al, 1990; Trevisan et al, 1990). It is not known whether these higher doses of lithium interact with the RAAS at the level of the proximal tubule. Because the effects of angiotensin II (ANGII) on renal sodium handling are mediated mainly at proximal tubular level, this study examined renal and systemic responses to ANGII infusion in normal man after pretreatment with two different doses of lithium in a placebo controlled study.

5.1.1 Protocol.

Ten subjects, mean age 30.7 (SD 3.3) years (range 27-45 years) and body mass index 22.7(1.2) kg/m^2, were studied on two occasions at least one week apart. Six of these subjects also completed the protocol on a third occasion. Their normal diet was supplemented with sodium chloride 100mmol/day ('Slow Sodium') for five days before each study. The subjects collected urine from 2100h until 0700h on the day before the study, and at 2100h took lithium carbonate 750mg (Camcolit, Norgine, Oxford) (Li750, n=10), lithium 250mg (Li250, n=6), or placebo control (C, n=10), in single blind random order. Renal and systemic responses to infusion of ANGII at doses of 1.25 and 2.5 ng.kg $bodyweight^{-1}min^{-1}$ were studied.

5.2 RESULTS.

5.2.1 Hormonal responses to angiotensin II (Table 5.1).

There were no untoward effects due to either ANGII or lithium during any of the studies. Plasma ANGII concentrations were comparable at baseline on all three study days when levels were low after the period of sodium loading, and during ANGII infusion. Lithium did not affect baseline PRA or the fall in PRA during ANGII infusion. Plasma aldosterone (ALDO) was higher at baseline after Li750 than after C ($P=0.03$) or Li250 ($P=0.02$), but the increment in ALDO during ANGII (all days $P<0.01$) was similar on each day (ΔALDO: C 223(59), Li250 223(43), Li750 214(52) pmol/l).

5.2.2 Systemic responses to angiotensin II (Table 5.2).

Baseline heart rate was similar before each study, and did not fall during ANGII infusion (data not shown). Mean arterial pressure (MAP) was significantly higher at baseline after Li750 had been given ($P=0.014$); this difference was due to a rise in systolic blood pressure ($P<0.02$) rather than the very small change in diastolic pressure. Because of this increase in baseline MAP, the rise in MAP during ANGII was significantly reduced after Li750 ($P<0.02$), with an attenuated increase in both systolic and diastolic blood pressures. The pressor response to ANGII after Li250 was not significantly

Table 5.1: Plasma hormone responses to ANGII infusion in ten normal subjects in presence or absence of lithium.

		Baseline	ANGII($ng.kg^{-1}min^{-1}$)	
			1.25	2.5
PRA	C	590(350-690)	-	220(130-320)[a]
(pmol AI.	Li250	510(290-690)	-	340(180-620)
$ml^{-1}h^{-1}$)	Li750	510(280-940)	-	240(110-490)[a]
ALDO	C	134(22)[c]	-	357(72)[b]
(pmol/l)	Li250	126(21)[c]	-	349(55)[b]
	Li750	185(19)	-	399(57)[b]
ANGII	C	6.1(1.2)	10.4(2.1)	21.3(3.9)[b]
(pg/ml)	Li250	8.8(1.6)	13.8(3.7)	25.2(6.7)[b]
	Li750	5.7(1.1)	11.6(3.4)	16.0(3.1)[a]

Mean(sem) or median(1st-3rd quartiles).

PRA, plasma renin activity: ALDO, aldosterone: ANGII, angiotensin II. C, control; Li250, lithium 250mg; Li750, lithium 750mg. [a], $P<0.05$; [b], $P<0.01$ vs baseline: [c], $P<0.05$ vs Li750.

Table 5.2: Blood pressure responses during angiotensin II infusion in ten normal subjects in presence or absence of lithium.

		Baseline	Change from baseline (mmHg)		
			ANGII ($ng.kg^{-1}min^{-1}$)		R
			1.25	2.5	
SBP	C	124.1(2.2)	7.7(2.2)[b]	13.1(3.4)[a]	4.7(2.2)
	Li250	127.8(4.5)	3.1(5.1)	9.6(5.2)	-1.1(4.8)
	Li750	128.9(2.2)[e]	1.5(2.7)	5.4(2.3)[c,d]	1.7(2.0)
DBP	C	72.6(1.8)	8.9(1.6)[a]	13.1(2.0)[a]	3.7(2.1)
	Li250	74.4(2.0)	7.6(1.2)[c]	11.5(1.7)[b]	-1.5(1.7)
	Li750	74.7(1.6)	6.1(1.4)[b]	9.7(2.0)[b]	1.7(1.6)
MAP	C	89.5(1.9)	7.8(1.7)[b]	12.2(2.4)[a]	2.4(1.6)
	Li250	92.2(2.7)	6.1(2.3)	10.8(2.8)[c]	-1.4(3.1)
	Li750	93.1(1.7)[e]	4.3(1.8)[c]	8.2(1.8)[b,d]	2.0(1.6)

Mean(sem). R, recovery period. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; C: control; Li250: lithium 250mg; Li750: lithium 750mg. [a], $P<0.01$; [b], $P<0.02$; [c], $P<0.05$ vs baseline: [d], $P<0.05$; [e], $P<0.02$ vs control.

different from control. Haematocrit did not differ between the three study days, and did not change during ANGII (C 42(1)% to 41(1)%, Li250 40.5(1)% to 40(1)%, Li750 41(1)% to 40.5(0.5)%).

5.2.3 Renal haemodynamics.

Baseline GFR and ERPF were not changed by the administration of lithium (Figure 5.1a,b). ANGII infusion produced a dose-dependent fall in ERPF and a proportionately smaller fall in GFR, leading to a rise in filtration fraction (Figure 5.1c). Li250mg did not modify the renal haemodynamic response to ANGII (data is omitted from the Figures for clarity). The absolute changes in ERPF and GFR during ANGII infusion were smaller after Li750 but were not significantly different from C: however, the rise in filtration fraction during ANGII 2.5 $ng.kg^{-1}min^{-1}$ was reduced after Li750 (Figure 5.1c, 5.2a) (MANOVA test of interaction between treatment and change in FF with time, P=0.01). Baseline effective renal vascular resistance (ERVR) was not affected by either dose of lithium, but the increase in ERVR during ANGII was smaller after Li750 than C (Li750 5530(270) to 9230(450), C 5140(255) to 10235(550) $dynes.sec.cm^{-5}$; MANOVA test of interaction between treatment and change in ERVR with time, P=0.03) (Figure 5.2b).

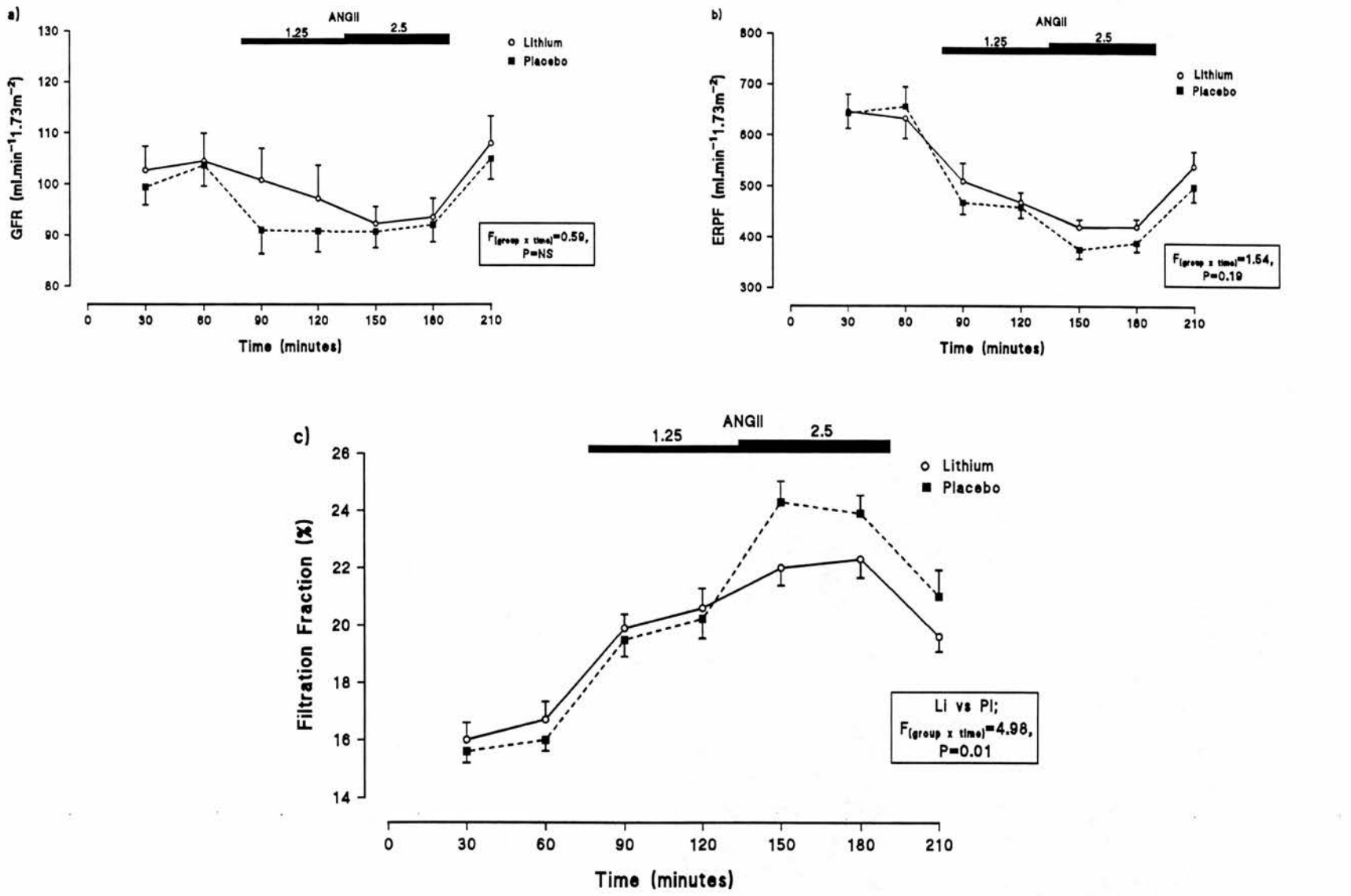

Figure 5.1: a) glomerular filtration rate, b) effective renal plasma flow and c) filtration fraction in ten normal subjects during ANGII infusion. Mean(sem). All parameters changed significantly during ANGII, P values and Li250 data have been omitted for sake of clarity.

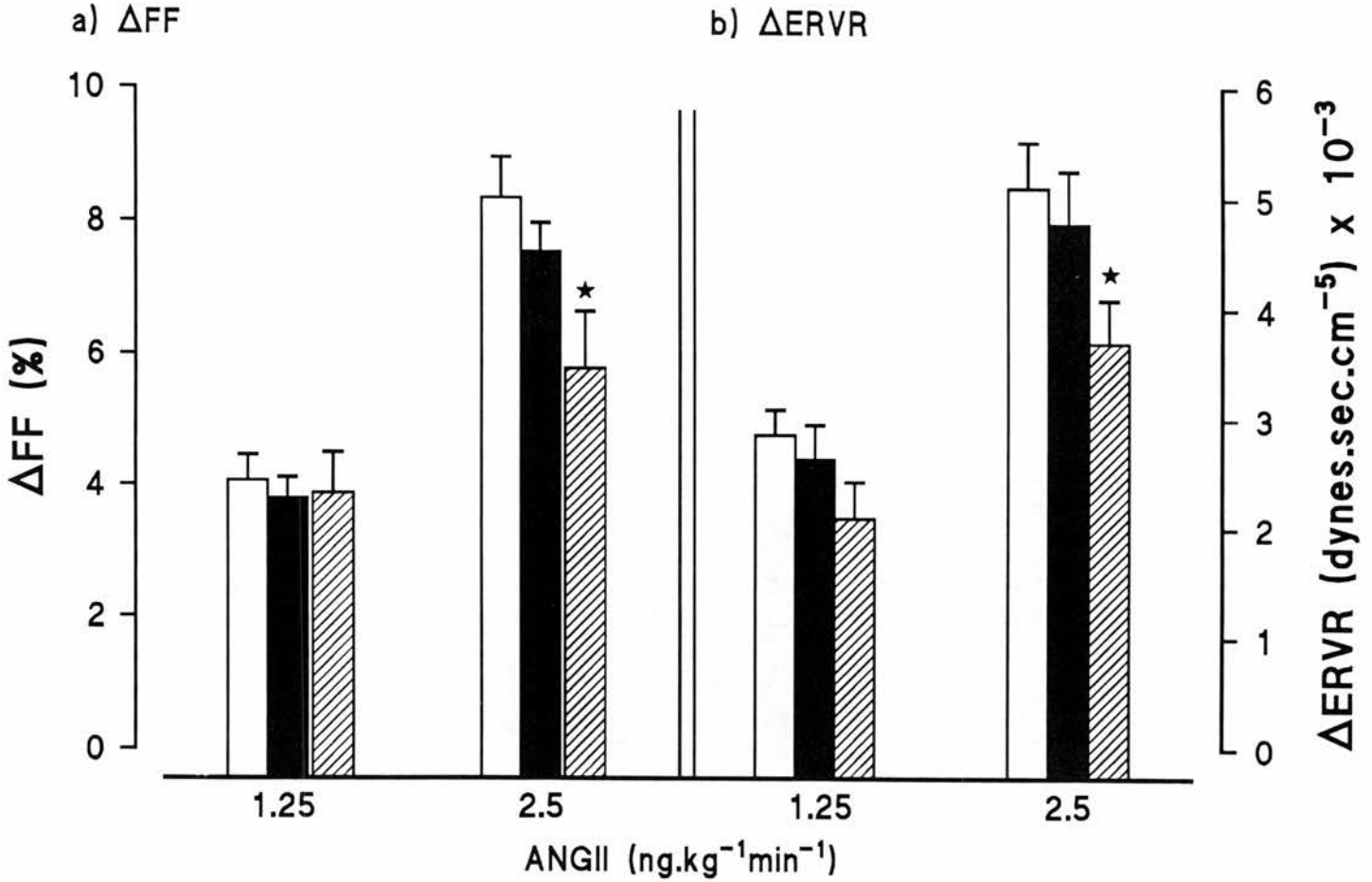

Figure 5.2: Increase in filtration fraction (FF) and renal vascular resistance (ERVR) in ten normal subjects during ANGII infusion after placebo control □, lithium 250mg ■, and lithium 750mg ▨. ★ P<0.05 vs placebo. Mean(sem).

5.2.4 Electrolyte excretion.

The volume and sodium content of the overnight urine collection increased after lithium (Na^+: Li750 131(21) mmol, Li250 96(14) mmol, C 79(10) mmol, Li750 vs C P=0.007; volume: Li750 0.81(0.13)l, Li250 0.60(0.08)l, C 0.50(0.06)l, Li750 vs C P=0.021). Significant changes in overnight urine potassium excretion and osmolality were not detected after lithium (K^+; Li750 27.8(4.1) mmol, Li250 18.9(2.2) mmol, C 20.9(2.3) mmol: osmolality; Li750 610(78), Li250 751(120), C 780(85) mOsm/kg). Baseline urine flow rate and the antidiuresis which occurred during ANGII infusion were not affected by the presence of lithium (Li750, 12.2(0.9) to 4.4(0.7) ml/min, P<0.001; Li250, 12.7(0.6) to 4.4(0.8) ml/min, P<0.005; C, 13.1(1.0) to 5.3(0.6) ml/min, P<0.001). The fall in urine flow rate during ANGII was accompanied by a rise in urine osmolality on each day (Li750 110(11) to 225(30) mOsm/kg, Li250 104(10) to 219(41) mOsm/kg, C 115(10) to 168(17) mOsm/kg, all P<0.02).

Baseline sodium excretion and the antinatriuretic response to ANGII were indistinguishable after Li250 and C. Despite the overnight natriuresis after Li750, the urinary excretion rate of sodium (UNaV) was not increased at baseline after Li750 (Fig 5.3a). Cumulative sodium excretion during ANGII infusion was higher after Li750 than C (67.6(3.5) vs 55.7(2.8) mmol; P<0.05). Although UNaV tended to be higher during ANGII after Li750

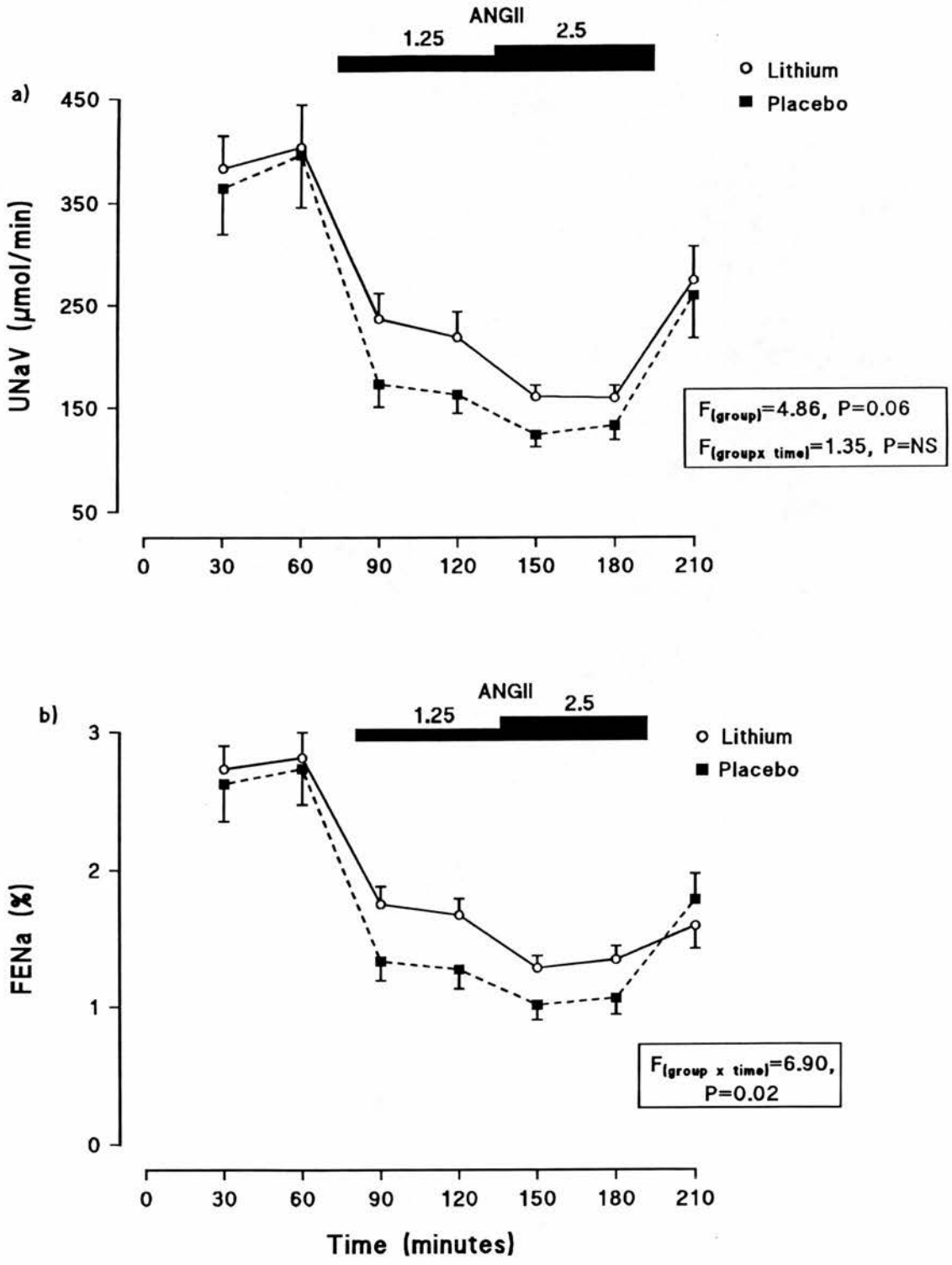

Figure 5.3: a) absolute and b) fractional sodium excretion in ten normal subjects during ANGII infusion after placebo and lithium 750mg. Mean(sem). Li 250mg data is omitted for clarity.

(P=0.065), the absolute fall in UNaV during ANGII was not significantly reduced after Li750 (165(21) vs 206(30) umol/min). Baseline fractional sodium clearance (FENa) was not increased after either dose of lithium, but the minimum level to which FENa fell during ANGII was slightly but significantly higher after Li750 (2.73(0.24) to 1.34(0.08)%) than after C (2.69(0.26) to 1.01(0.11)%; MANOVA test of interaction between treatment and change in FENa with time, P=0.02), implying attenuation of the whole kidney tubular antinatriuretic response to ANGII after lithium pretreatment.

5.2.5 Lithium clearance data (Table 5.3).

Serum lithium concentrations twelve hours after the 750mg and 250mg doses were 0.29(0.02) mmol/l and 0.10(.01) mmol/l respectively. FE_{Li} fell during ANGII (P<0.01), suggesting an increase in fractional proximal reabsorption of sodium. In addition the ratio C_{Na}/C_{Li} also fell during ANGII (P<0.05), implying an increase in fractional distal tubular sodium reabsorption. Both the baseline data and the ANGII-induced changes in lithium clearance were similar after both doses of lithium.

Table 5.3: Sodium and lithium clearances during angiotensin II infusion in normal subjects after pretreatment with lithium 250mg (n=6) and lithium 750mg (n=10).

		Baseline	ANGII($ng.kg^{-1}min^{-1}$)		R
			1.25	2.5	
C_{Na} (ml/min)	Li250	2.4(0.2)	1.3(0.2)[c]	1.0(0.1)[d]	1.8(0.3)
	Li750	2.8(0.2)	1.6(0.2)[c]	1.2(0.1)[d]	2.0(0.2)
C_{Li} (ml/min)	Li250	29.4(2.1)	16.5(0.9)[c]	14.9(0.9)[d]	20.3(1.2)
	Li750	31.5(2.1)	20.7(2.0)[c]	17.8(1.7)[d]	25.8(2.5)
FE_{Li} (%)	Li250	28.3(2.0)	17.8(1.2)[b]	16.9(0.9)[c]	21.1(1.1)
	Li750	31.1(2.0)	21.3(2.3)[c]	19.7(1.9)[d]	25.2(2.9)
C_{Na}/C_{Li} (%)	Li250	8.3(0.4)	8.1(1.2)	6.6(0.8)[a]	8.7(1.0)
	Li750	9.2(0.9)	8.9(1.3)	7.1(0.8)[b]	8.3(1.3)

Mean(sem). R, recovery period. C_{Na}, sodium clearance; C_{Li}, lithium clearance; FE_{Li}, fractional lithium clearance.

[a], P<0.05; [b], P<0.02;[c], P<0.01; [d], P<0.005 vs baseline.

5.3 DISCUSSION.

A precondition for applying lithium clearance to the analysis of tubular sodium handling is that an inert dose of lithium should be used, and this study aimed to establish whether lithium pretreatment modifies renal responses to angiotensin II. The two doses of lithium studied encompass the range used in published studies, most having used between 300mg and 600mg. A 250mg dose of lithium had no measurable effect on responses to ANGII, but after a 750mg dose overnight sodium excretion was increased, systemic and renal haemodynamic responses to ANGII were slightly altered, and there was a suggestion that the tubular antinatriuretic response to ANGII was attenuated.

Equality of daytime sodium excretion before each study was not documented, but the difference in overnight sodium excretion after lithium 750mg was consistent (mean 52 mmol) and similar to the results of a recent controlled study using a 600mg dose of lithium (48 mmol, Shirley et al, 1991), making a systematic difference in dietary sodium intake very unlikely. Two possibilities for the natriuresis which occurs after a single dose of lithium are either antagonism of the distal tubular action of aldosterone (Stewart et al, 1987), or inhibition of proximal tubular sodium reabsorption. The proportionate increases in sodium and water excretion and the absence of a fall in potassium excretion after

lithium are more compatible with a proximal than a distal action, as previously suggested (Shirley et al, 1991; Hecht et al, 1978).

Sodium intake was increased before each study to minimise the potential impact of a natriuretic effect of lithium on vascular reactivity to ANGII mediated through changes in total body sodium (Hollenberg et al, 1972). Since a significant natriuresis did occur, further control studies would be required to establish whether the observed differences in renal responses to ANGII after pretreatment with lithium 750mg were a direct effect of lithium or due to the relative sodium depletion which followed lithium pretreatment. This uncertainty does not however negate the validity of the present investigation, which was designed only to determine whether renal responses to ANGII are different after lithium pretreatment.

Despite the overnight natriuresis, baseline sodium excretion in the clearance study was not increased after lithium 750mg, in contrast to some (Jeffrey et al ,1988; Jeffrey et al, 1989; Freestone et al, 1990) but not all previous studies (Hannedouche et al, 1990b). UNaV was higher in eight out of the ten subjects after Li750, but the greater variance in UNaV after sodium loading makes small differences harder to demonstrate. Nonetheless, the normal renal response to acute sodium loss is sodium retention, and the absence of a reduced baseline UNaV

after lithium in itself suggests that lithium was still exerting a natriuretic effect. The absence of the previously reported rise in PRA after Li750 probably reflects the relatively larger analytical errors when measuring low levels of PRA in sodium-loaded subjects; the increased plasma aldosterone after Li750 argues that RAAS activation did occur.

The small fall in GFR and the modest increase in filtration fraction during ANGII seen after placebo indicates a balanced increase in pre- and postglomerular arteriolar resistances (Smith, 1951), consistent with the consensus from *in vitro* studies that ANGII increases both afferent and efferent glomerular arteriolar tone at doses within the physiological range (Steinhausen et al, 1990). The smaller increase in renal vascular resistance during ANGII infusion after lithium 750mg was only partly attributable to the reduced rise in MAP during ANGII which followed the lithium-induced change in baseline blood pressure, and suggests that the renal vasoconstrictor response to ANGII was genuinely blunted after lithium. Attenuation of the ANGII-induced rise in filtration fraction after lithium may be interpreted as indicating that the balance of pre- and post-glomerular resistances moved towards afferent dominance (Smith, 1951), but the data can not determine whether the reactivity of one or both of the major resistance segments was primarily affected.

Reports of increased blood pressure after both short term and prolonged lithium administration do exist (Gross et al, 1990; Johnstone et al, 1990; Meltzer and Sealey, 1990), but it was surprising to find that a single 750mg dose of lithium could cause a consistent, albeit small, increase in mean arterial pressure. The absolute level of MAP during ANGII infusion was the same after Li750 and placebo (Table 5.2), and it could be suggested that the smaller ANGII-induced rise in MAP after lithium is an artefact related to the unequal baseline values. However, this result is of interest irrespective of whether one expects ANGII infusion to increase blood pressure *by* or *to* a certain level, as the pressor response reported if lithium had been administered without a control experiment would have been substantially smaller than the true value. The uniformly low baseline plasma angiotensin II levels suggest that endogenous RAAS activation did not cause the rise in baseline blood pressure, and the mechanism of the increase is unexplained. Lithium affects other vasoactive hormone systems such as the kallikrein-kinin cascade and renal eicosanoids (Gross et al, 1990), and the effect of longer term lithium administration on hormonal responses, and the influence of sodium balance on these actions, has not been studied.

Pretreatment with lithium 750mg had a statistically significant effect on the fall in fractional sodium

excretion during ANGII infusion, but the difference between the responses after lithium and placebo was numerically much smaller than the effect of lithium on the natriuretic response to gludopa and atrial natriuretic peptide previously reported (Jeffrey et al, 1988; Freestone et al, 1990). This may stem partly from the use of pharmacological doses of dopamine (as gludopa) and ANP in those studies compared with the 'physiological' doses of ANGII infused in the present investigation, but may also reflect the fact that several distinct mechanisms exist by which ANGII can influence renal sodium handling. The changes in lithium clearance and the derived indices of tubular sodium handling during ANGII infusion under these particular experimental conditions were comparable after 750mg and 250mg doses of lithium, emphasising that the effect of lithium 750mg on the renal tubular response to ANGII was slight in comparison to the major changes in sodium reabsorption occurring during ANGII infusion. It is nonetheless undesirable to measure lithium clearance after a dose of lithium (750mg) which affects baseline sodium balance, and which may modify haemodynamic and antinatriuretic responses to ANGII. The lack of effect of a 250mg dose of lithium on the kidney is in accord with other studies (Shirley et al, 1991; Girbes et al, 1990; Strazzullo et al, 1988) and confirms the sense of using the lowest possible dose of lithium rather than the 'conventional'

dose of 500-600mg.

In conclusion, pretreatment with lithium in a dose of 750mg produces increases in sodium excretion and plasma aldosterone which suggest that baseline sodium balance is altered, and produces significant but numerically small changes in the measured responses to angiotensin II infusion. No interference with the response to ANGII infusion is apparent after a 250mg dose of lithium, making this a more suitable marker dose for the analysis of tubular sodium handling. The actions of lithium on the kidney may be different in normal man and in disease states; the assessment of these possible differential effects is essential before using lithium in studies of pathophysiological mechanisms.

CHAPTER SIX.

RENAL RESPONSES TO EXOGENOUS ANGIOTENSIN II IN TYPE 1 (INSULIN-DEPENDENT) DIABETES.

6.1 INTRODUCTION.

In experimental models of diabetes mellitus, an increase in intraglomerular pressure may initiate the development of glomerular sclerosis and proteinuria (Hostetter et al, 1982; Zatz et al, 1985). Treatment with angiotensin converting enzyme inhibitors (ACEI) prevents glomerulopathy in these animal models (Zatz et al, 1986), and may be protective in some human diabetic patients (Parving et al, 1989a), but it remains unclear whether these actions of ACEI indicate a role for an abnormal intrarenal vasoconstrictor response to endogenous angiotensin II (ANGII) in the pathogenesis of nephropathy.

Cross-sectional measures of plasma concentrations of RAAS components in diabetic subjects have provided variable results (Christlieb et al, 1976; DeChatel et al, 1977; Burden and Thurston, 1979; Ferriss et al, 1985). Plasma ANGII concentrations are suppressed in diabetic patients without complications (Feldt-Rasmussen et al, 1987), although plasma renin activity and converting enzyme levels are normal. A fall in plasma ANGII levels is the normal physiological response to sodium retention (Hollenberg et al, 1972), and the increased systemic pressor response to exogenous ANGII infusion in uncomplicated human Type 1 diabetes (Weidmann et al, 1979; Drury et al, 1984) could simply reflect the increase in total body sodium content in these patients

(Weidmann et al, 1985; Feldt-Rasmussen et al, 1987), but the absence of a concurrent fall in plasma renin activity is unusual.

The reduced density of ANGII receptors on glomerular mesangial cells in diabetic rats (Ballermann et al, 1984; Wilkes, 1987) and on platelets from patients with uncomplicated Type 1 diabetes (Connell et al, 1986) is consistent with the blunted renal haemodynamic response to exogenous ANGII recently reported in human diabetes (Fioretto et al, 1991). In normal man however sodium loading increases angiotensin II receptor density, without affecting receptor affinity (Moore et al, 1984). Because of the dichotomy that appears to exist between renal and systemic vascular reactivity to ANGII in human diabetes, the renal response to infused ANGII was examined in Type 1 diabetic patients and in non-diabetic controls after a period of controlled high dietary sodium intake.

6.1.1 Protocol.

Nine Type 1 diabetic patients (mean duration 18 (range 6-29) months) and nine matched non-diabetic controls were studied (Table 6.1). Seven of the diabetic patients were treated with a conventional insulin regime, and two were using a 'basal/bolus' regime ('Novopen', Novo-Nordisk). The normal diet was supplemented with sodium chloride 200 mmol/day ('Slow Sodium') for 5 days, and a 24h urine collection was completed on the fifth day

Table 6.1: Clinical details of diabetic subjects.

	Control	Diabetic
N	9	9
Age(years)	29(6)	28(5)
Body mass index ($kg.m^{-2}$)	24(2.3)	25(3.3)
Duration of diabetes(months)	-	18(6)
HbA_1 (%)	-	7.5(1.4)
Insulin dose ($U.kg^{-1}day^{-1}$)	-	0.53(0.21)
Urinary albumin excretion ($mg.24h^{-1}$)	10.2 (3.9-16.7)	16.6* (11.9-29.4)

Mean(SD), or median(range). *, P<0.05 vs control group. Normal range for HbA_1, 5.0-8.0 %.

of the sodium loading phase. Diabetic subjects were admitted to hospital at 1800h on the day before the study. Their normal evening insulin dose was omitted, and intravenous infusions of D-glucose 50 g/l (80 ml/h) and unmodified human insulin (Actrapid, Novo, Bagsvaerd, Denmark, 0.5 U/ml) were started with the evening meal. The rate of insulin infusion was adjusted manually to maintain a blood glucose concentration of around 6.5 mmol/l overnight and throughout the acute study. Both groups fasted from 2200h, and followed the clearance protocol from 0700h onwards. Renal responses to infusion of ANGII at 0.5 and 1.0 $ng.kg^{-1}min^{-1}$ were studied.

6.2 RESULTS.

6.2.1 Systemic and hormonal responses (Tables 6.2, 6.3).

There was no change in heart rate in either group during ANGII infusion, but small increases in systolic (diabetic P=0.023, control P=0.035), diastolic (diabetic P=0.014, control P=0.001) and mean arterial blood pressure (diabetic P=0.004, control P=0.002) occurred in both groups during ANGII infusion (Table 6.2), which did not differ between the two groups (diastolic blood pressure; MANOVA test of interaction between group and change in blood pressure with time, P=0.10). Blood glucose was adequately controlled in the diabetic subjects, plasma glucose being on average only 2 mmol/l higher than corresponding values in the control group (Table 6.2). No subject had glycosuria at any time. The overnight insulin infusion rate in the diabetic group was 1.25(0.10) U/h, which was not greater than the infusion rate before or during ANGII infusion (1.03(0.13) U/h and 0.97(0.12) U/h respectively). Plasma free insulin concentrations were low in both groups at baseline, and were essentially unchanged during ANGII (Table 6.2).

Plasma renin activity and plasma aldosterone concentrations were low at baseline, and did not change during ANGII infusion. However, plasma renin activity was significantly higher in the diabetic group throughout the clearance study (P<0.02, Table 6.3). Plasma ANGII

Table 6.2: Blood pressure, heart rate, plasma glucose and plasma free insulin concentrations during angiotensin II infusion in nine diabetic and nine control subjects.

		Baseline	ANGII ($ng.kg^{-1}min^{-1}$)	
			0.5	1.0
SBP	D	128.5(3.4)	129.8(3.1)	131.2(4.2)[a]
(mmHg)	C	126.9(2.0)	126.8(3.0)	129.8(3.5)[a]
DBP	D	76.4(3.2)	78.1(3.2)	80.3(3.5)[a]
(mmHg)	C	74.2(2.2)	73.8(2.5)	77.1(2.1)[a]
MAP	D	93.8(3.0)	95.3(3.0)	97.2(3.5)[a]
(mmHg)	C	91.8(2.0)	91.5(2.6)	94.7(2.4)[a]
Heart rate	D	65(5.0)	64(4.5)	62(4.0)
(bpm)	C	59(1.5)	56(1.4)	56(2.0)
Plasma glucose	D	6.9(0.4)[b]	6.8(0.5)[b]	6.2(0.6)[b]
(mmol/l)	C	5.1(0.3)	4.7(0.3)	4.2(0.3)
Plasma free insulin	D	8.0 (4.8-18)	8.4 (4.8-20)	4.8[a] (4.8-14)
(mu/l)	C	6.8 (4.8-23)	4.8 (4.8-18)	4.8 (4.8-6.2)

Mean(sem) or median(range). D, diabetic; C, control.
[a], $P<0.05$ vs baseline; [b], $P<0.02$ vs corresponding control.

Table 6.3: Plasma renin activity (PRA), plasma aldosterone (ALDO), and plasma angiotensin II (ANGII) during angiotensin II infusion in nine diabetic and nine control subjects.

		B/line1	B/line2	ANGII(ng.kg^{-1}min^{-1})	
				0.5	1.0
PRA (pmol ANGI.h^{-1} ml^{-1})	D	300[a] (30-720)	410[a] (80-1450)	240[a] (40-550)	300[a] (140-840)
	C	130 (10-290)	110 (20-220)	70 (10-140)	110 (20-300)
ALDO	D	60(20)	60(20)	80(20)	90(30)
(nmol/l)	C	90(30)	80(30)	80(20)	80(10)
ANGII	D	2.6(0.5)	2.8(0.5)	6.9(1.1)[b]	13.0(3.0)[b]
(pmol/l)	C	2.7(0.5)	2.6(0.6)	8.0(1.0)[b]	10.3(0.8)[c]

Mean(sem) or median(range). [a], P<0.02 vs control; [b], P<0.01; [c], P<0.001 vs baseline.

concentrations were suppressed at baseline in both groups, and comparable increments in plasma ANGII levels were produced during ANGII infusion (Table 6.3). Plasma potassium concentration was similar in both groups at baseline (diabetic 3.7(0.10), control 3.8(0.07) mmol/l), and did not change during ANGII, and urinary potassium excretion was similarly unaffected (data not shown). Urine flow rate fell during ANGII infusion in both diabetic and control subjects (diabetic 10.9(1.4) to 7.6(0.8) $mlmin^{-1}$, $P<0.05$; control 9.6(1.3) to 5.6(1.2) $mlmin^{-1}$, $P<0.05$). Venous haematocrit was unchanged during ANGII infusion.

6.2.2 Renal haemodynamics.

GFR was similar in both groups at baseline and fell significantly ($P<0.05$) and to a similar extent in both groups during ANGII infusion (Figure 6.1a). Effective renal plasma flow fell in a dose-dependent manner during ANGII, again to a similar extent in the two groups (diabetic 694(46) to 521(21) $ml.min^{-1}1.73m^{-2}$, $P<0.005$; control 665(41) to 498(30) $ml.min^{-1}1.73m^{-2}$, $P<0.02$), returning to the baseline values during the recovery period (Figure 6.1b). Filtration fraction (GFR/ERPF) increased significantly during ANGII infusion in both groups (Figure 6.1c).

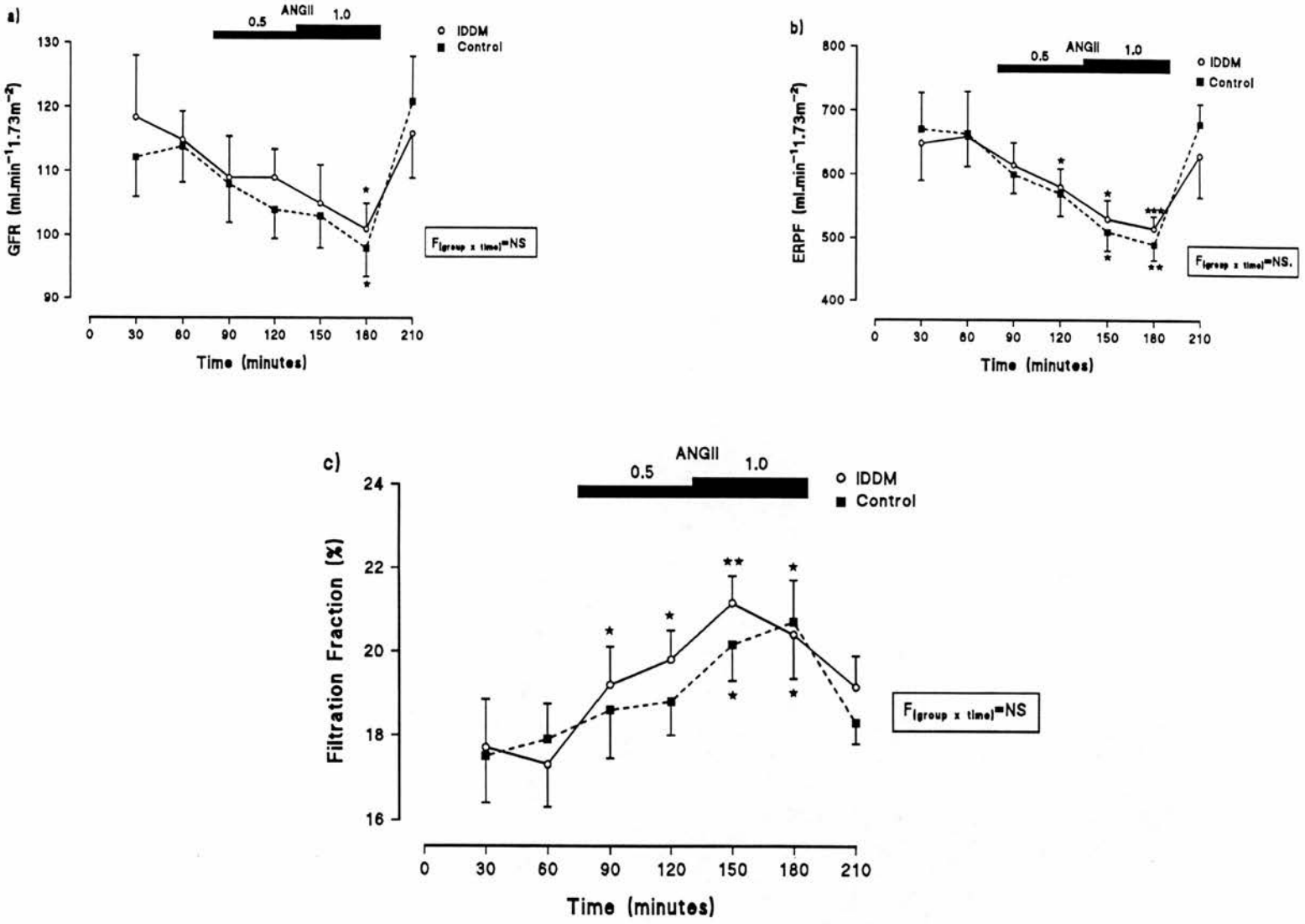

Figure 6.1: a) glomerular filtration rate, b) effective renal plasma flow and c) filtration fraction in nine diabetic and nine control subjects during ANGII infusion. Mean(sem). ⋆ P<0.05, ⋆⋆ P<0.02, ⋆⋆⋆ P<0.005 vs baseline.

6.2.3 Sodium excretion.

Total urinary sodium excretion and urine volume were similar in both groups in the 24 h period before the study (diabetic 335(21) mmol, 2.76(0.28)l; control 348(26) mmol, 2.52(0.33)l). The urinary excretion rate of sodium (UNaV) was lower in the diabetic subjects during the first control period ($P<0.05$, Figure 6.2a), a difference which persisted during the substantial antinatriuresis induced by ANGII infusion. Fractional sodium excretion (FENa) (Figure 6.2b) showed the same pattern. The decrements in absolute and fractional urinary sodium excretion during ANGII were not significantly different between the groups (absolute excretion; diabetic 103(24), control 195(42) μmol/min, $F_{(group\ x\ time)}=3.14$, $P=0.10$: fractional excretion; diabetic 0.58(0.10)%, control 1.09(.26)%, $F_{(group\ x\ time)}=3.56$, $P=0.08$). When the antinatriuretic response to ANGII was expressed as a percentage of the baseline result to compensate for the differing baseline sodium excretion rates, there was no difference in the tubular response to ANGII infusion between the two groups (% fall from baseline of UNaV; diabetic 30(7)%, control 38(6)%; % fall from baseline of FENa; diabetic 24(6)%, control 31(6)).

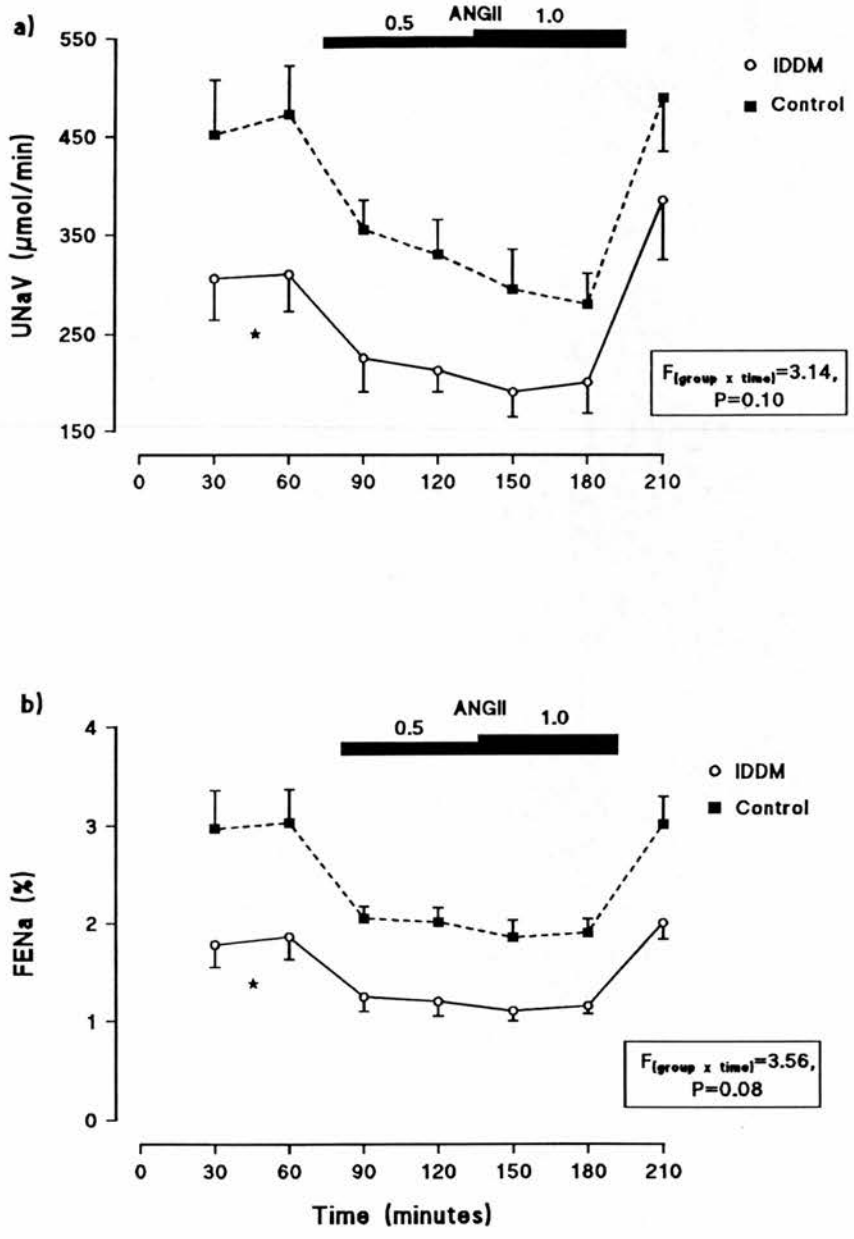

Figure 6.2: a) absolute and b) fractional sodium excretion during ANGII infusion in nine diabetic patients and nine control subjects. Mean(sem). ⋆, P<0.05 vs control group.

6.3 DISCUSSION.

Infusion of angiotensin II to levels within the physiological range produced similar changes in renal haemodynamics in both diabetic and control subjects as were reported in Chapter 4. The finding that renal vascular reactivity to ANGII in these diabetic patients with good chronic metabolic control did not differ from a matched control group extends the results of previous studies which measured the concentrations of RAAS components without examining dynamic renal responses. The renal tubular reabsorption of sodium was abnormal at baseline, but the antinatriuretic response to ANGII in the diabetic group did not differ from normal controls. However, since only whole kidney tubular responses were assessed it is not possible to comment on whether ANGII-mediated reabsorption of sodium in the proximal tubule was altered as a primary contributor to the increased fractional reabsorption of sodium seen in these patients at baseline.

Chronic glomerular hyperfiltration in Type 1 diabetic patients may increase the risk of diabetic nephropathy, and is at least partially ameliorated by improved glycaemic control (Christiansen et al, 1982). ANGII receptor density and affinity vary with the duration of hyperglycaemia and with the intensity of treatment of experimental diabetes (Ballermann et al, 1984; Wilkes, 1987), and RAAS activity is affected by

glycaemic control in human diabetes (Sullivan et al, 1980; Jenkins et al, 1990). Only one of the diabetic patients in this study had a glycated haemoglobin level >9%, and this uniformly good glycaemic control precludes any comment on whether any correlation exists between renal vascular reactivity to ANGII and the quality of glycaemic control. These patients are at low risk of diabetic nephropathy, and additional studies of the renal response to ANGII in patients with poorly controlled diabetes who have an increased long-term risk of kidney disease are needed to clarify whether ANGII has a primary role in the development of microvascular disease.

Urinary sodium excretion during the 24 hours before the study was similar in both groups, but was reduced in the diabetic group during the acute study. The volume of saline infused during the equilibration phase was too small (350 ml, 52 mmol of sodium) to suggest that the blunting of the acute natriuretic response to saline infusion seen in Type 1 diabetes was relevant (Roland et al, 1986), while the similar urine flow rates in both groups exclude any `washout` effects, and the day/night sodium excretion pattern is normal in uncomplicated Type 1 diabetes (Bell et al, 1987).

The period of high sodium intake before the study allowed infusion of low doses of ANGII which elicited only a minimal systemic pressor response and did not appreciably affect aldosterone secretion, and also

ensured that both groups had similar baseline plasma concentrations of ANGII so that the renal haemodynamic response to ANGII could be compared (Hollenberg et al, 1972). A criticism of this approach is that the responsiveness of the tubuloglomerular feedback loop is reduced by sodium loading and augmented by a rise in intrarenal ANGII (Wright and Briggs, 1979), so that sodium loading may change the feedback relationship between the tubular handling of sodium and intrarenal haemodynamics.

Acute insulin administration increases renal tubular reabsorption of sodium in normal man (DeFronzo et al, 1975; Skott et al, 1989a), and a correlation has been found between plasma free insulin concentrations and indirect indices of tubular sodium reabsorption in Type 2 diabetic patients (Mbanya et al, 1989). Chronic (24h) low dose insulin infusion in normal subjects causes a small reduction in urinary sodium excretion, from 242 to 221 umol/min (Trevisan et al, 1990). In the present study the overnight infusion rate of insulin received by the diabetic subjects was similar to the infusion rate during the clearance study, and to the patient's normal insulin dosage, and produced plasma free insulin levels which were certainly not higher (and were probably much lower) than those previously described (Trevisan et al, 1990). The antinatriuretic potential of exogenous insulin is retained in human Type 2 diabetes (Skott et

al, 1991; Gans et al, 1991b) but the effect of sodium balance on the renal actions of insulin, and the extent to which overnight hyperinsulinaemia may have contributed to the large difference in sodium excretion at baseline between these Type 1 diabetic patients and control subjects remains to be established.

Plasma ANGII levels were suppressed to the same extent at baseline in both groups, but plasma renin activity was higher in the diabetic group throughout the study. This is in keeping with some recent reports (Liebermann et al, 1991; O'Donnell et al, 1990), although PRA has previously been reported as normal in uncomplicated diabetic patients in sodium balance (Christlieb et al, 1976; DeChatel et al, 1977; Burden and Thurston, 1979; Feldt-Rasmussen et al, 1987). Plasma prorenin is also normal in most patients with diabetes of short duration (Feldt-Rasmussen et al, 1987), and artefactual cryoactivation of prorenin to active renin can not account for the difference between the groups since all the renin assays were performed on one occasion.

Incomplete suppression of renin release can not be attributed to retention of a smaller proportion of the supplementary dietary sodium load in the diabetic subjects because fractional sodium excretion was reduced at baseline and because osmotic diuresis with increased distal tubular sodium losses secondary to glycosuria did

not occur in any of the patients. One speculative explanation of the differences in plasma renin activity after sodium loading is that diabetic subjects may regain sodium balance more slowly after a change in dietary intake than normal subjects. The reduced fractional sodium excretion in the diabetic patients after sodium supplementation may in fact indicate that there was a greater increase in total body sodium in the diabetic group after sodium loading, although current methods for measuring total exchangeable body sodium in humans are not sufficiently sensitive to detect small differences. However, if this were so the normal renal response to exogenous ANG II would represent an attenuated response when compared to a normal subject in balance on the same dietary sodium intake.

In summary, the renal vascular response to exogenous angiotensin II was normal in these well controlled short duration Type 1 diabetic patients during euglycaemia. However, vascular reactivity to angiotensin II must always be considered in relation to the prevailing state of sodium balance, and altered renal tubular reabsorption of sodium was apparent even at this early stage of diabetes. This abnormal renal sodium handling was examined in more detail in Chapters 7 and 8.

CHAPTER SEVEN.

EFFECT OF LITHIUM ON RESPONSES TO ANGIOTENSIN II IN TYPE 1 DIABETES.

7.1 INTRODUCTION.

A parallel has been drawn between elevation of single nephron glomerular filtration rate and plasma flow in moderately hyperglycaemic rats (Hostetter et al, 1981a) and renal haemodynamics in a proportion of patients with Type 1 diabetes. Because increased renal plasma flow during hyperglycaemia is not a uniform finding in Type 1 diabetic patients with increased glomerular filtration rate (Ditzel and Junker, 1972; Stalder and Schmid, 1959; Jenkins et al, 1990), increased intraglomerular capillary pressure may be another precursor to the later development of nephropathy. Renal tubular sodium handling may modulate these haemodynamic factors in several different ways. The whole kidney sodium handling data in Chapter 6 gives no insight into the mechanism(s) of sodium retention in early diabetes, and more detail is needed concerning the reabsorption of sodium at specific tubular sites, and the humoral determinants acting at these sites .

Abnormal renal sodium handling and sodium retention may affect renal haemodynamics indirectly through effects on vascular reactivity and systemic blood pressure (Weidmann et al, 1985), or directly via intrarenal factors such as a change in sensitivity of the tubuloglomerular feedback mechanism (Ushiogi and Haberle, 1991). A correlation has been reported between increased absolute and fractional proximal tubular sodium

reabsorption, measured by lithium clearance, and glomerular filtration rate in Type 1 diabetes (Brochner-Mortensen et al, 1984; Skott et al, 1989b; Nosadini et al, 1989; Hannedouche et al, 1990a; Trevisan et al, 1990). This correlation suggests that glomerulotubular balance is appropriately adjusted to the increased glomerular filtration rate in diabetes in order to maintain a normal distal tubular sodium delivery, regardless of whether or not this occurs at a normal total body sodium content. There is a statistical problem in assigning physiological importance to this finding however, because the correlation of FE_{Li} (C_{Li}/GFR) with GFR will always be significant. The problems in using lithium clearance as a marker of tubular sodium handling discussed in Chapter 5 apply equally to its use in diabetes, notably doubt over the site(s) of tubular reabsorption of lithium, the effects of osmotic diuresis due to glycosuria (Skott et al, 1987), and the pharmacological interactions between lithium and intrarenal hormone systems demonstrated in normal man. The potential for interaction between lithium and the RAAS in Type 1 diabetes has not been explored, and in this study the effect of lithium on baseline renal function and the renal response to ANGII infusion was examined in a group of stable Type 1 diabetic patients, using the same 750mg dose of lithium as in the studies in Chapter 5.

7.1.1 Protocol.

Fifteen normotensive Type 1 diabetic patients (clinical details in Table 7.1) were studied on two occasions at least two weeks apart. Ten patients were treated with a twice daily insulin regime, while the other five were using a 'basal/bolus' regime. Their normal diet was supplemented with sodium chloride 100mmol/day ('Slow Sodium') for seven days before each study to allow comparison of the data with the experiments in Chapter 5. The subjects collected all urine for 24 hours before each study into 'Day' and 'Night' bottles, which were used respectively before and after 2100h when lithium carbonate 750mg (Li750) or placebo control (Pl) was taken in a single blind randomized order. After fasting from 2200h, they omitted the morning insulin dose, and attended the clinical laboratory at 0830h. An infusion of unmodified human insulin (Actrapid 0.2 Uml^{-1}, Novo, Bagsvaerd, Denmark) was started, and the infusion rate adjusted to establish and maintain a whole blood glucose concentration (Reflolux-S, Boehringer Mannheim, Germany) of 4-7 $mmoll^{-1}$. The absence of glycosuria (urine glucose <5mmol/l) was confirmed in all urine samples during the acute study (Diastix, Ames, England). Renal responses to infusion of ANGII at 1.25 and 2.5 $ng.kg^{-1}min^{-1}$ were studied. The patients were then fed and given an appropriate dose of insulin before leaving the hospital.

Table 7.1: Clinical details of diabetic patients.

N	15
Age	29.5 (5.5) years
Duration	7.1 (3.2) years
BMI	24.7 (2.9) kg/m^2
Insulin dose	0.72 (0.19) $U.kg^{-1}24h^{-1}$
HbA_1(%)	9.5 (1.3) (Normal range 5-8%)
Albumin:Creatinine Ratio (mg/mmol)	1.4 (0.4) (Normal <3.5)

Mean(SD).

7.2 RESULTS.

7.2.1 Systemic responses to angiotensin II infusion (Table 7.2, Table 7.3).

Whole blood glucose on arrival at the laboratory was 14.8(1.1) and 14.6(1.3) mmol/l after placebo and lithium pretreatment respectively. The blood glucose concentration and the insulin infusion rate were similar at all times throughout both studies, falling slightly as the study progressed. During ANGII infusion at 2.5 $ng.kg^{-1}min^{-1}$ one patient felt slightly nauseated and another noticed a sensation of mental alertness. Both these side effects occurred after placebo pretreatment.

Plasma ANGII concentrations were similar at baseline

on both days, and comparable elevations in plasma ANGII concentration occurred during ANGII infusion on each day. Baseline PRA was also low on each study day, and fell significantly during ANGII infusion, neither baseline PRA nor the fall in PRA during ANGII being affected by lithium. The fall in PRA during ANGII infusion was similar to the fall which occurred in the control subjects in Chapter 5 (Table 5.1); this supports a previous report suggesting that the intrarenal short feedback loop is intact in diabetes (Trujillo et al, 1989). Plasma ALDO levels rose during ANGII on each study day (all $P<0.005$); baseline ALDO and the increment in plasma ALDO during ANGII infusion were unaffected by lithium pretreatment.

Heart rate was similar on all three days, and did not change during ANGII (data not shown). Baseline mean arterial pressure, and the pressor response to ANGII were unchanged after lithium pretreatment (Figure 7.1).

7.2.2 Renal haemodynamics.

Baseline GFR did not change after lithium (Figure 7.2a), but baseline ERPF was significantly increased after Li750 (Figure 7.2b). This renal vasodilatation produced a fall in the filtration fraction (Figure 7.2c). Pretreatment with lithium did not alter the changes in ERPF ($F_{(group\ x\ time)}=1.55$, P=NS), GFR ($F_{(group\ x\ time)}=1.91$, $P=0.09$), or filtration fraction during ANGII

Table 7.2: Whole blood glucose (GLUC) and insulin infusion rate (INS) before and during angiotensin II infusion in fifteen diabetic subjects.

				ANGII infusion.		
Time(mins):		A	0	60	120	180
GLUC (mmol/l)	Pl	14.8 (1.1)	7.2 (0.5)	5.5 (0.3)	4.9 (0.3)	4.7 (0.2)
	Li250 (n=7)	11.9 (1.6)	6.2 (0.8)	6.2 (0.7)	5.8 (0.6)	5.2 (0.4)
	Li750	14.6 (1.3)	7.7 (0.5)	5.7 (0.4)	5.4 (0.4)	5.0 (0.4)
INS (U/h)	Pl	3.8 (0.6)	1.2 (0.3)	0.7 (0.2)	0.4 (0.15)	0.4 (0.15)
	Li250 (n=7)	5.1 (1.2)	0.7 (0.4)	0.7 (0.4)	0.6 (0.3)	0.2 (0.1)
	Li750	5.3 (0.8)	1.5 (0.4)	1.1 (0.3)	0.7 (0.2)	0.3 (0.1)

Mean(sem). A, arrival at laboratory; 0-60 mins, baseline; 60-180 mins, ANGII infusion.

Table 7.3: Plasma renin activity (PRA), plasma aldosterone (ALDO), and plasma angiotensin II (ANGII) during angiotensin II infusion in fifteen diabetic subjects.

		B/line	ANGII(ng.kg^{-1}min^{-1})	
			1.25	2.5
PRA (pmol AI. ml^{-1}h^{-1})	C	330(220-460)	-	190(120-260)[a]
	Li250	540(320-890)	-	270(140-365)
	Li750	520(260-670)	-	195(90-260)[a]
ALDO (pmol/l)	C	199(23)	350(35)[a]	458(43)[b]
	Li250	195(35)	374(44)[b]	422(33)[b]
	Li750	230(26)	408(33)[a]	480(46)[b]
ANGII (pg/ml)	C	9.8(1.2)	24.3(2.6)	47.4(3.1)
	Li250	11.8(0.8)	22.6(2.0)	40.2(2.6)
	Li750	11.8(1.4)	25.8(2.6)	44.2(4.9)

Mean(sem). [a], $P<0.05$; [b], $P<0.005$ vs baseline.

Technical note: plasma ANGII concentrations at baseline and during ANGII infusion are substantially higher than the levels reported in the control subjects in Chapter 5 (Table 5.1). The intraassay c.v. for this series of (triplicated) assays was acceptable at 9%, but the quality control samples indicated that the ANGII standard used had probably deteriorated with time. This shifts the standard curve to the right of its true position, hence the spuriously high results from experimental samples. Therefore, the ANGII data is useful in showing that a comparable stimulus was applied during each study in this diabetic group, but no valid comparisons can be made with the normal subjects in Chapter 5.

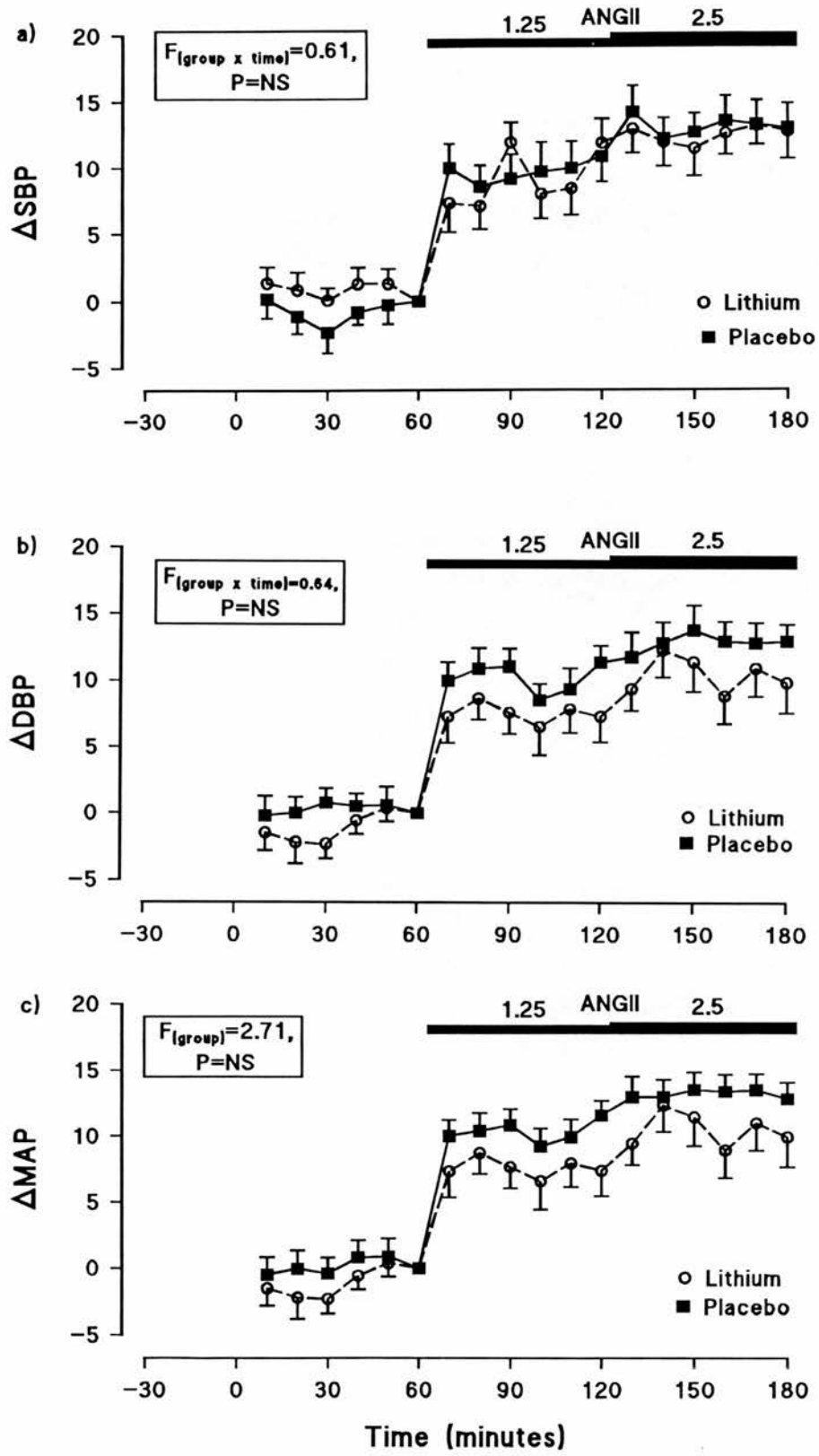

Figure 7.1: Change in a) systolic, b) diastolic, and c) mean arterial pressure during ANGII infusion in 15 diabetic patients after lithium or placebo. Mean(sem).

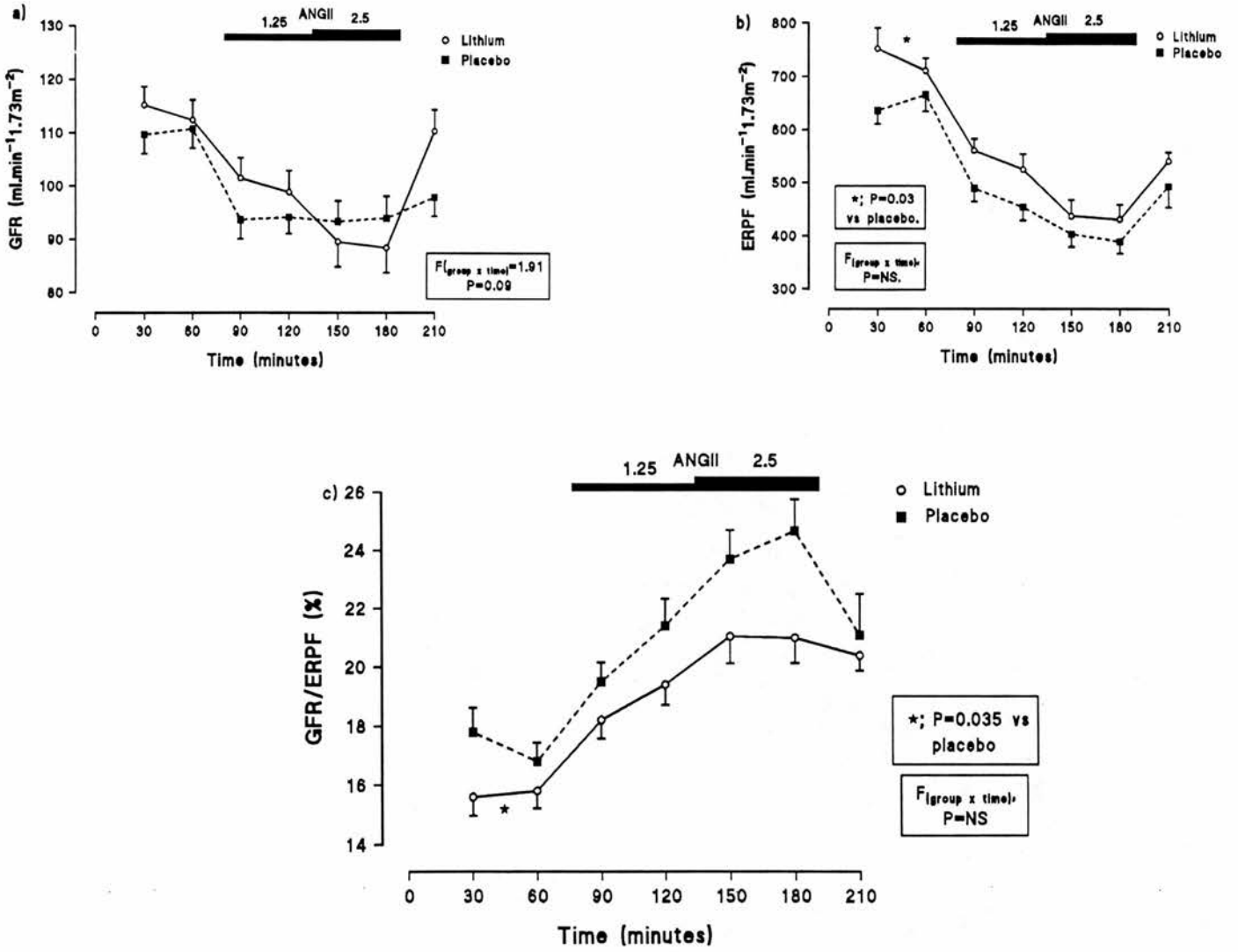

Figure 7.2: a) glomerular filtration rate, b) effective renal plasma flow, and c) filtration fraction in 15 diabetic patients during ANGII infusion after lithium 750mg or placebo.

infusion. The absence of effect of any lithium on renal haemodynamic responsiveness to ANGII was confirmed by the similar increase in effective renal vascular resistance during ANGII on both days (Pl 5260(440), Li750 5300(630) dyne.sec.cm^{-5}).

7.2.3 Sodium and water excretion.

24h sodium excretion was comparable before each study (Pl; Day 178(14) mmol, Night 125(12) mmol, Total 303(13) mmol: Li750; Day 166(15) mmol, Night 142(12) mmol, Total 308(13) mmol). Absolute and fractional urinary excretion of sodium were similar at baseline after both placebo and Li750, and the fall in both parameters during ANGII was unaffected by lithium (Figure 7.3).

Urine flow rate was similar at baseline and during ANGII infusion after Li750 and Pl (Li750 15.7(0.6) to 7.0(0.7) ml/min; Pl 15.6(0.7) to 6.8(0.9) ml/min). The fall in free water clearance (C_{H2O}) during ANGII infusion was also unaffected by lithium (Li750 10.6(0.5) to 4.6(0.6) ml/min; Pl 10.7(0.5) to 4.3(0.8) ml/min). Despite the fall in C_{H2O}, urinary osmolality (U_{Osm}) rose only slightly above the baseline value during ANGII infusion on both days. This is discussed in detail in Chapter 8 (Figure 8.5).

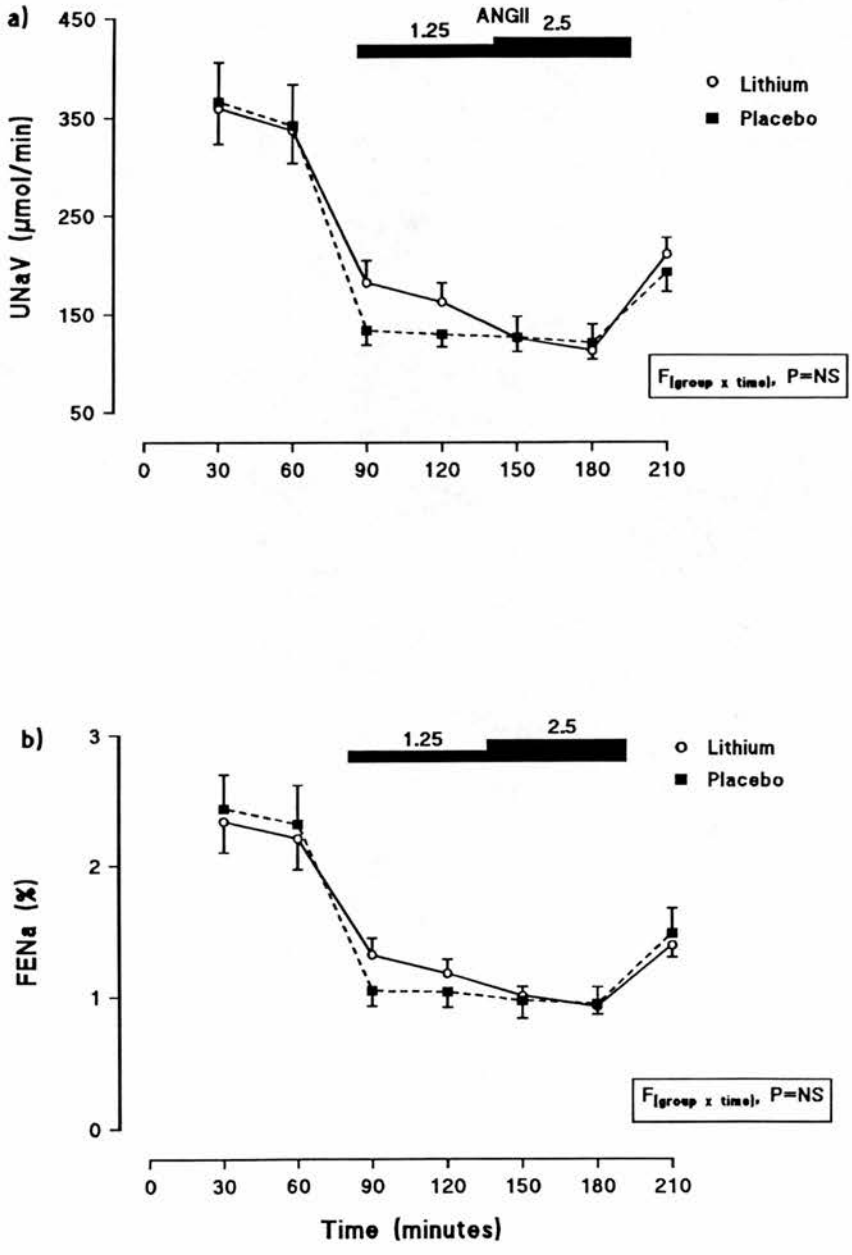

Figure 7.3: a) absolute and b) fractional urinary sodium excretion during ANGII infusion in 15 diabetic patients after placebo and lithium 750mg. Mean(sem).

7.3 PROBLEMS WITH COMPARISONS BETWEEN DIABETIC AND CONTROL GROUPS AFTER LITHIUM PRETREATMENT.

The effects of pretreatment with lithium 750mg on baseline renal haemodynamics and the response to ANGII infusion are different to the actions of lithium described in normal man in Chapter 5. This has implications for comparisons of haemodynamic data obtained in the two groups after lithium pretreatment. These will be discussed before examining the validity of lithium as a tubular marker in diabetes in more detail.

7.3.1. Effective renal plasma flow.

After placebo pretreatment baseline renal plasma flow and renal vascular reactivity to ANGII were identical in the diabetic group and in the normal subjects, data from whom were reported in Chapter 5 (Control group) (Figure 7.4a). This confirms in a larger group of subjects the result reported in Chapter 6. However, comparison of diabetic and control groups after lithium pretreatment indicated (misleadingly) that baseline ERPF was elevated in the diabetic patients (P=0.014)(Figure 7.4b), and that the fall in ERPF during ANGII infusion was greater in the diabetic patients than in the control group ($F_{(group \times time)}=2.66$, P=0.018).

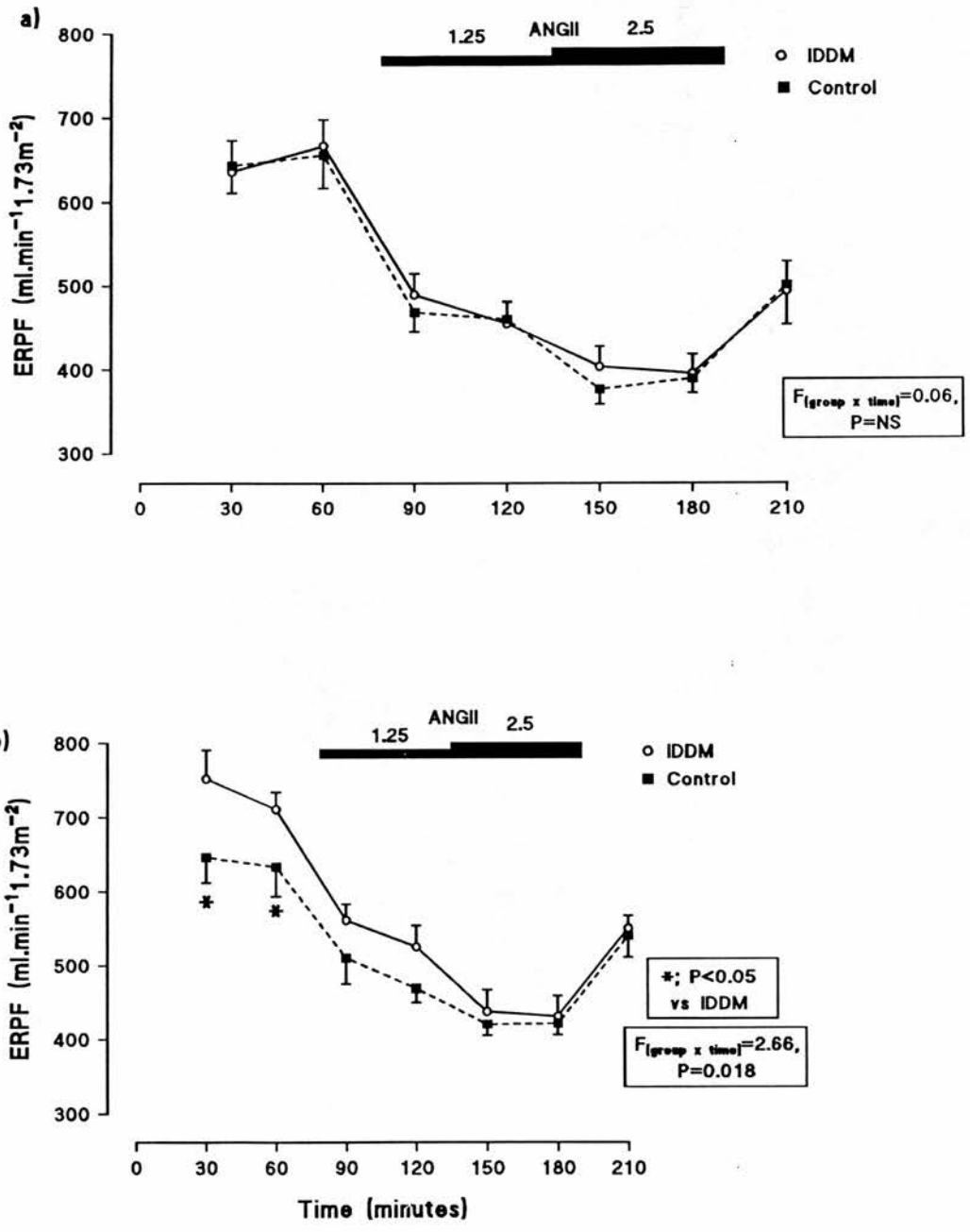

Figure 7.4: Effective renal plasma flow in 15 diabetic and 10 control subjects during ANGII infusion after pretreatment with a) placebo and b) lithium 750mg. Mean(sem).

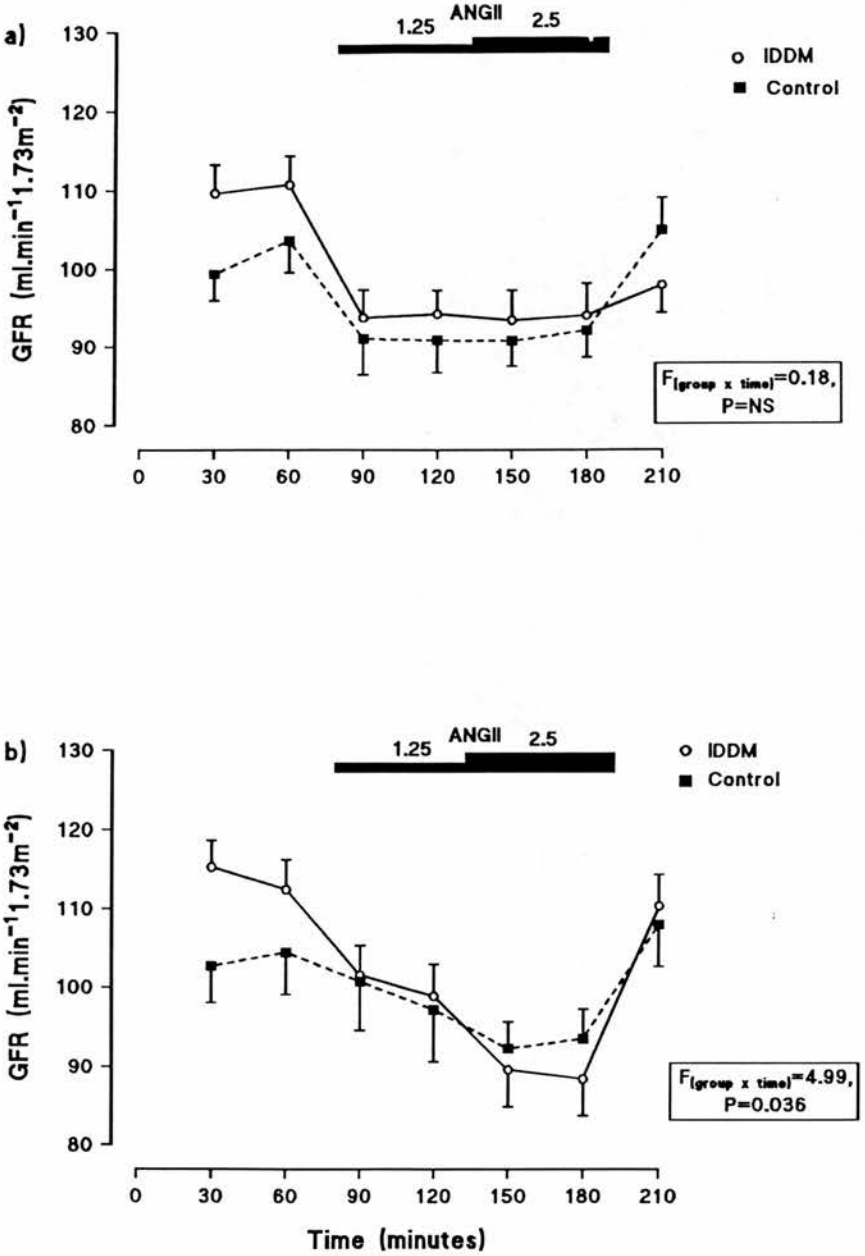

Figure 7.5: Glomerular filtration rate in 15 diabetic and 10 control subjects during ANGII infusion after pretreatment with a) placebo and b) lithuim 750mg. Mean(sem).

7.3.2. Glomerular filtration rate.

On the placebo day baseline GFR was slightly but but not significantly higher in the diabetic group (P=0.096). There was no difference between diabetic and control subjects either for the absolute data ($F_{(group)}$=1.11, P=0.303) or for the fall in GFR during ANGII ($F_{(group \times time)}$=1.54, P=0.227) (Figure 7.5a). After lithium, although the difference in baseline GFR between the two groups was the same as after placebo (P=0.076), the fall in GFR during ANGII infusion was greater in the diabetic patients ($F_{(group \times time)}$=4.99, P=0.036) (Figure 7.5b).

7.3.3. Effective renal vascular resistance.

After placebo pretreatment the baseline ERVR was higher in the diabetic group, due entirely to the slightly higher blood pressure in the diabetic patients (P<0.05) (Figure 7.6a). This difference was not apparent after lithium because of the renal vasodilatation which occurred in the diabetic group. Furthermore, the rise in ERVR during ANGII was similar in both groups after placebo, but because of the reduced rise in ERVR in the control group there was an accentuation of ANGII-induced renal vasoconstriction in the diabetic subjects after lithium (P=0.06)(Figure 7.6b).

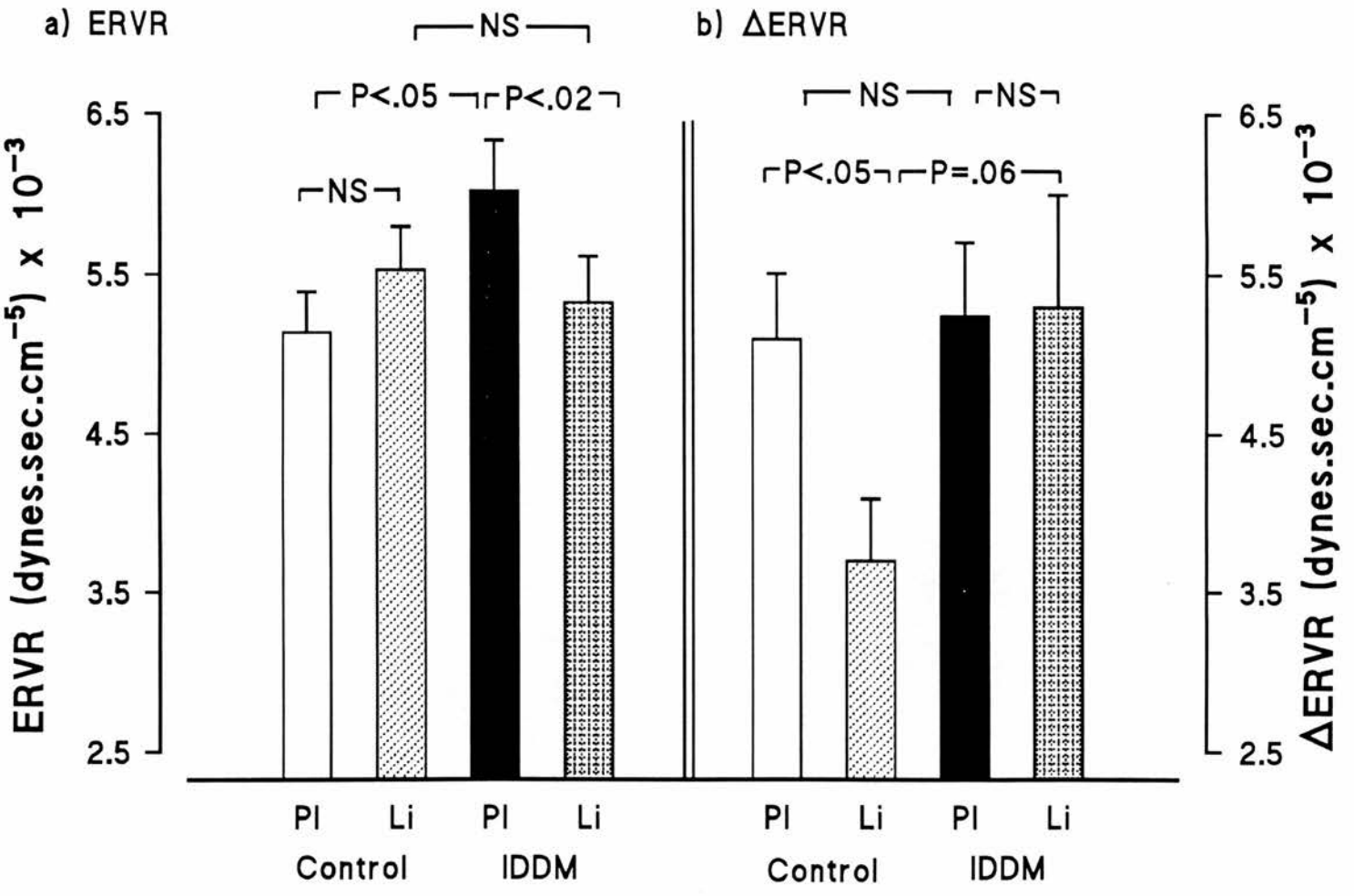

Figure 7.6: a) baseline and b) increment in effective renal vascular resistance (ERVR) during ANGII infusion in 15 diabetic patients (IDDM) and ten normal controls after placebo (Pl) or lithium 750mg (Li). Mean(sem). See text 7.3.3.

7.3.4. Systemic blood pressure.

After placebo pretreatment mean arterial pressure was significantly higher in this diabetic group than in age and BMI-matched controls at baseline (mean 98.5(2.1) mmHg vs 89.5(1.9) mmHg, $F_{(group)}=8.55$, P=0.007) and throughout ANGII infusion ($F_{(group)}=10.83$, P=0.003), despite the careful selection of lean subjects without any evidence of diabetic complications whose blood pressures were within the normal range for their age. Because of the consistent rise in MAP after lithium pretreatment in the control group, the difference in baseline MAP between the groups was smaller after lithium (mean 99.1(2.0) vs 93.1(1.9) mmHg, $F_{(group)}=4.49$, P=0.04). The rise in blood pressure during ANGII infusion was reduced after lithium in the controls (Chapter 5.2.2), but was unaffected in the diabetic patients, so that although the rise in mean arterial pressure was similar in both groups after placebo ($F_{(group\ x\ time)}=0.52$, P=NS) (Figure 7.7a), the pressor response to ANGII appeared to be accentuated in the diabetic group after lithium 750mg ($F_{(group\ x\ time)}=6.90$, P=0.014) (Figure 7.7b).

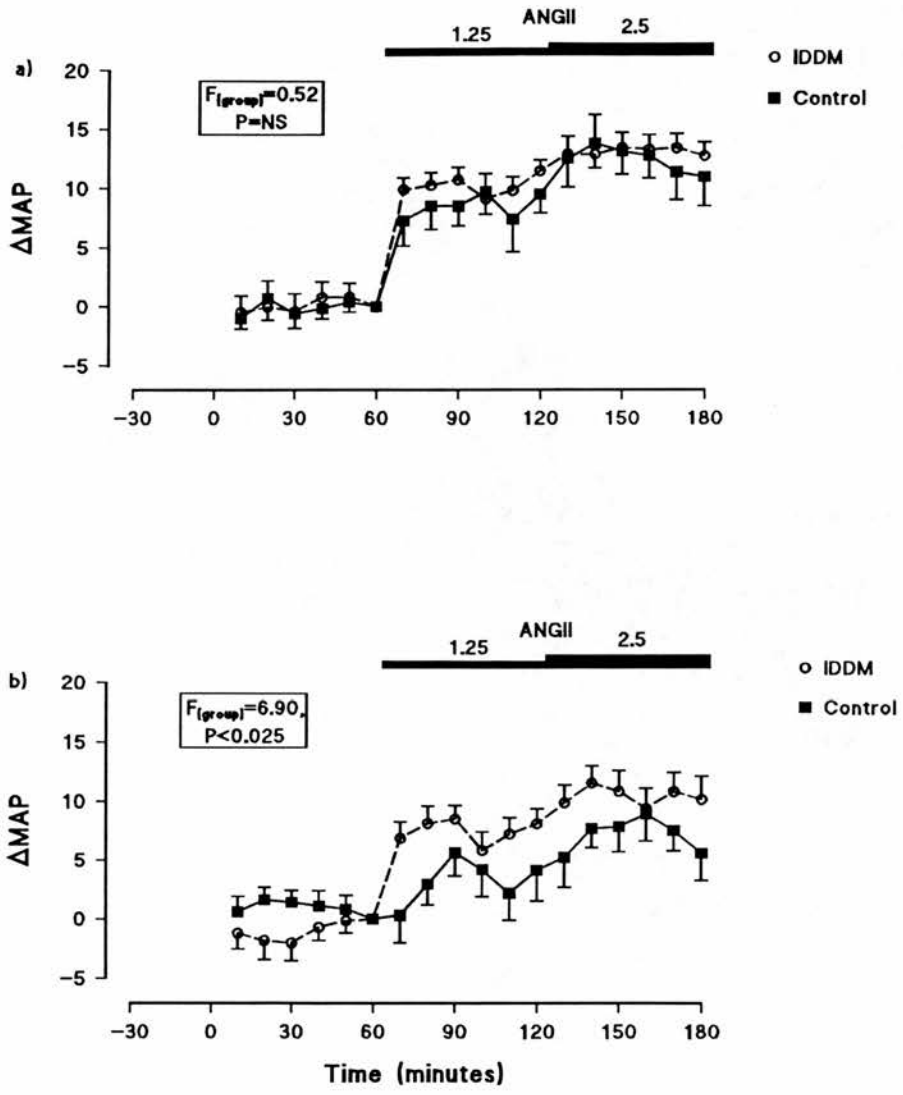

Figure 7.7: Change in mean arterial pressure (ΔMAP) during ANGII infusion in 15 diabetic patients and 10 normal subjects after a) placebo and b) lithium 750mg. Mean(sem).

7.4 VALIDITY OF LITHIUM CLEARANCE AS A TUBULAR MARKER IN DIABETES.

The differing actions of lithium in diabetic and control groups compromise comparisons of renal and systemic haemodynamics. This may affect the validity of lithium clearance as a tubular marker in diabetes in at least two ways. Firstly, changes in renal haemodynamics could influence tubular sodium reabsorption by changing peritubular physical forces or by altering the balance of cortical and medullary blood flow. Secondly, calculation of indices of proximal tubular sodium handling from lithium clearance data involves the glomerular filtration rate, which may change to a different extent during ANGII infusion after lithium. As part of the interpretation of the lithium clearance data in the diabetic subjects it was therefore necessary to consider how the effects of lithium 750mg on renal haemodynamic reactivity in diabetic and control groups might affect lithium clearance and associated derived data. Measurement of endogenous lithium in samples from the placebo studies would have been the ideal way to approach this problem, but this requires highly specialised atomic absorption spectrophotometry equipment. A compromise was therefore reached by examining seven randomly selected diabetic patients on a third occasion using the same protocol after pretreatment with a dose of lithium (250mg) which it was anticipated would not affect renal haemodynamics.

7.4.1 Results.

Baseline UNaV and FENa in these seven patients were lower after lithium 250mg than after either placebo or Li750 (FENa; Pl 2.37(0.26)%, Li750 2.53(0.42)%, Li250 1.99(0.23)%). Inspection of individual 24 hour sodium excretions after Li250 suggested that this difference was probably caused by two of the subjects not having taken their sodium supplement as requested (24h excretion of sodium; 290, 339, 296, 309, 279, 168*, 175* mmol). The antinatriuretic response during ANGII infusion was similar on all three days (FENa; Pl 2.37(0.26) to 0.95(0.13)%, Li750 2.53(0.42) to 1.06(0.10)%, Li750 1.99(0.23) to 0.81(0.05)%). ERPF was increased at baseline after Li750, as in the complete group of fifteen subjects, but baseline ERPF after Li250 did not differ from placebo (Pl 665(51), Li250 695(52), Li750 778(53) $ml.min^{-1}1.73m^{-2}$; Pl vs Li750, $P<0.05$). The fall in ERPF during ANGII infusion did not differ on the three study days.

Tables 7.4 and 7.5 summarise sodium and lithium clearances and the derived estimates of tubular reabsorption of sodium for the diabetic patients after pretreatment with both doses of lithium. There are some minor differences between the results for the two doses, which can be expected by chance alone in a small group of subjects, but the main effects of ANGII on tubular sodium handling are similar after both doses of lithium. In

Table 7.4: Sodium (C_{Na}) and lithium (C_{Li}) clearances, and derived expressions for fractional tubular reabsorption of sodium during angiotensin II infusion in diabetic patients after lithium pretreatment (Li750 data shown as N=15 and N=7).

			ANGII ($ng.kg^{-1}min^{-1}$)		
		B	1.25	2.5	R
C_{Na} (ml/min)	Li750 (N=15)	2.4(0.2)	1.3(0.2)	0.9(0.1)[a]	1.5(0.1)
	Li750 (N=7)	2.8(0.4)	1.5(0.3)	0.9(0.1)[a]	1.5(0.2)
	Li250 (N=7)	2.6(0.4)	1.5(0.2)	1.0(0.1)[a]	1.6(0.2)
C_{Li} (ml/min)	Li750 (N=15)	28.5(1.6)	19.6(1.4)	17.5(1.5)[a]	23.5(1.7)
	Li750 (N=7)	32.3(2.1)	22.3(2.2)	19.3(2.1)[a]	25.9(2.8)
	Li250 (N=7)	32.9(2.1)	25.8(2.2)	21.5(0.9)[a]	31.6(1.7)
FE_{Li} (%)	Li750 (N=15)	25.1(1.6)	19.5(1.1)	19.9(1.5)[b]	21.3(1.4)
	Li750 (N=7)	28.7(1.9)	22.2(1.3)	22.8(1.7)[b]	22.7(1.6)
	Li250 (N=7)	29.8(1.9)	25.3(1.2)	22.4(0.9)[b]	27.3(1.9)
C_{Na}/C_{Li} (%)	Li750 (N=15)	9.4(0.9)	6.6(0.6)	5.3(0.5)[b]	7.1(0.8)
	Li750 (N=7)	9.0(1.0)	6.5(0.9)	4.8(0.5)[b]	6.2(0.4)
	Li250 (N=7)	8.6(1.1)	5.8(0.8)	4.8(0.6)[b]	5.2(0.5)

Mean(sem). B, baseline, R, recovery period.

FE_{Li}, fractional proximal sodium rejection.

C_{Na}/C_{Li}, fractional distal sodium rejection.

[a], $P<0.001$; [b], $P<0.01$ vs baseline. The change during ANGII infusion was not different after either dose of lithium for any of the parameters.

Table 7.5: Derived expressions for absolute tubular reabsorption of sodium during angiotensin II infusion in diabetic patients after lithium pretreatment (Li750 data shown as N=15 and N=7).

		ANGII ($ng.kg^{-1}min^{-1}$)			
		B	1.25	2.5	R
APR_{Na} (mmol/min)	Li750 (N=15)	11.8(0.5)	11.1(0.5)	9.9(0.6)[a]	12.0(0.5)
	Li750 (N=7)	11.2(0.7)	10.8(0.9)	9.2(1.1)[a]	12.2(0.9)
	Li250 (N=7)	11.1(1.0)	10.5(0.7)	10.5(0.7)[c]	12.0(1.1)
ADR_{Na} (mmol/min)	Li750 (N=15)	3.3(0.2)	2.5(0.2)	2.3(0.3)[b]	3.0(0.2)
	Li750 (N=7)	4.2(0.3)	3.3(0.3)	2.9(0.1)[b]	4.2(0.1)
	Li250 (N=7)	4.2(0.3)	3.3(0.3)	2.8(0.1)[b]	4.2(0.2)

Mean(sem). B, baseline, R, recovery period.

APR_{Na}, absolute proximal sodium reabsorption.

ADR_{Na}, absolute distal sodium reabsorption.

[a], $P<0.05$; [b], $P<0.005$ vs baseline.

[c], $MANOVA_{(group \times time)}$ interaction, $P<0.01$ vs Li750.

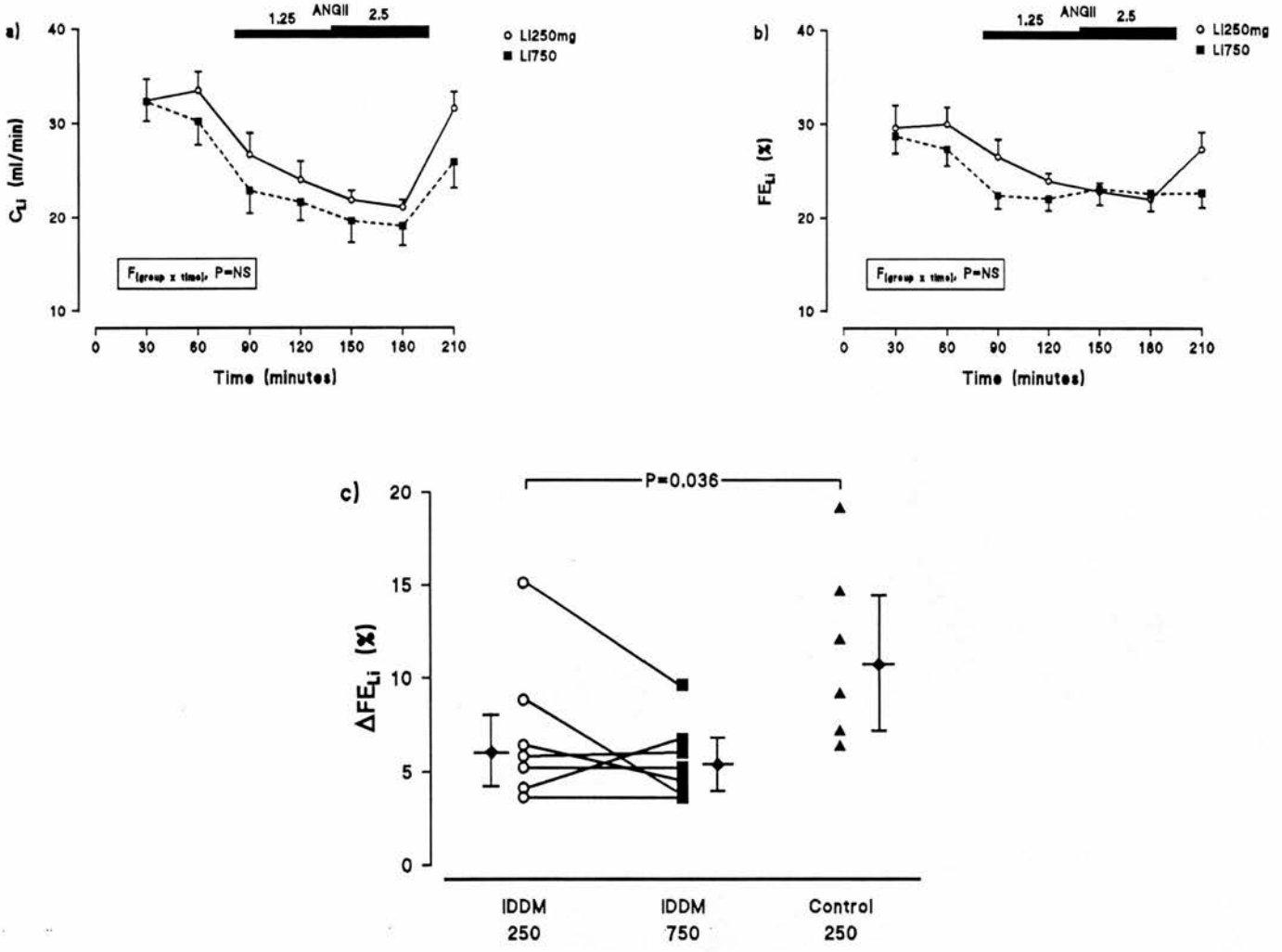

Figure 7.8: a) absolute and b) fractional lithium clearance in seven diabetic patients during ANGII infusion after lithium 250mg and 750mg. c) individual falls in FE_{LI} (median ±1 quartile) in seven diabetics and six matched controls.

particular, baseline absolute and fractional lithium clearances were similar after a 750mg and a 250mg dose of lithium (Figure 7.8a,b), and the fall in FELi during AII was not different on the two days; the data was comparable for individual subjects as well as the whole group (Figure 7.8c). The similar change in fractional proximal reabsorption of sodium on the two days confirms that the greater fall in *absolute* proximal reabsorption of sodium (APR_{Na}) during ANGII after Li750mg is an artefact caused by the larger reduction in GFR and filtered sodium load. It should be noted in passing that after the 250mg dose of lithium the change in FELi during ANGII infusion was significantly lower in the diabetic patients than in the six non-diabetic control subjects studied after lithium 250mg (data taken from Chapter 5).

7.5 DISCUSSION.

A number of studies have now used the lithium clearance method while analysing renal haemodynamics and sodium handling in diabetic patients. The errors due to 'post-proximal' reabsorption of lithium and the effect of osmotic diuresis on lithium clearance can be minimised by studying patients when salt replete and euglycaemic, but the potential for interaction between lithium and intrarenal hormone systems has not previously been studied in diabetes. Lithium 750mg caused dose-dependent

renal vasodilatation, but did not modify the falls in ERPF or GFR or the whole kidney tubular antinatriuretic response to exogenous ANGII. Systemic blood pressure and the pressor response to ANGII were also unaffected by lithium. The effects of lithium in diabetes proved sufficiently different from its actions in normal man to make comparisons of renal and systemic vascular reactivity data in diabetic and control groups misleading after lithium pretreatment. Despite this, baseline lithium clearance and fractional lithium excretion proved to be similar after both 'inert' and 'active' doses of lithium, as were the changes in lithium clearance during ANGII infusion, both on a group basis and within individual patients.

Lithium *in vitro* did not interfere with the assay system for PAH, and the reduced renal vascular resistance after lithium pretreatment is a real phenomenon. The reduction in filtration fraction after lithium pretreatment in the diabetic subjects could be regarded as indicating a fall in intraglomerular hydraulic pressure caused by efferent arteriolar vasodilatation, just as the increased filtration fraction seen in the diabetic compared to the control group after placebo pretreatment (IDDM 17.2(0.7)%, Control 15.3(0.4)%; P=0.043) might be construed as supporting the existence of intraglomerular hypertension due to relative efferent arteriolar vasoconstriction. However, the limitations of

filtration fraction as an index of intraglomerular pressure have already been mentioned (Smith, 1951; Carmines et al, 1987).

The renal haemodynamic response to ANGII in this group of diabetic subjects was not different from that in a matched control group, confirming the main conclusion of Chapter 6 in a larger group of subjects with a broader range of metabolic control. This result differs from a recent study of normotensive diabetic subjects (Fioretto et al, 1991) which reported a blunted fall in glomerular filtration rate and renal plasma flow during ANGII infusion. The abnormality was reversed by pretreatment for one week with indomethacin, and this was taken to indicate that renal vascular hyporeactivity to ANGII was due to antagonism of the renal vasoconstrictor effect of ANGII by excessive vasodilator prostaglandins. This hypothesis could not be substantiated because the acute changes in prostaglandin synthesis during ANGII infusion were not reported.

A *post hoc* power analysis of the data in Chapters 5 and 7.3 shows that the present studies had an 80% chance (at the 5% significance level) of detecting a difference in reactivity to ANGII 2.5 $ng.kg^{-1}min^{-1}$ of 10 ml/min for glomerular filtration rate and 90 ml/min for renal plasma flow between diabetic and control groups. The power to detect the difference in reactivity between diabetic and control subjects reported by Fioretto et al. (ΔGFR 15

ml/min, Δ ERPF 120 ml/min) was 98% for GFR and 96% for ERPF. It is therefore very unlikely that such large differences would have been overlooked if present, and reasons for the discordance between the two sets of data must be sought.

The inclusion of female subjects and the use of a supraphysiological dose of ANGII (4 $ng.kg^{-1}min^{-1}$) in the Italian study are unlikely to be major factors. The Italian study was performed after blood glucose had been stabilised overnight using an artificial pancreas, but the diabetic subjects in Chapter 6 had an unchanged haemodynamic response to ANGII when studied under similar conditions. Stabilisation of blood glucose for only 1-1.5 hours before an acute clearance study can be criticised on the basis of the changes in renal plasma flow described after acute changes in blood glucose (Christiansen et al, 1981b; Wiseman et al, 1987). However, these changes were small, and other studies have found no evidence that significant changes in renal haemodynamics occur during acute changes in blood glucose (Mogensen 1971c; Jenkins et al, 1989); furthermore, in the present studies blood glucose levels were comparable both before and throughout each study, as were the doses of insulin used to achieve near-normoglycaemia.

It is potentially important that the Italian patients were studied while on a lower sodium intake than the present subjects. The week of indomethacin

pretreatment may itself also have changed total body sodium and hence vascular reactivity to ANGII within the diabetic group. No data on renal sodium handling before or during ANGII infusion was given in the paper, so that the possibility that the disparate results are a result of differences in sodium balance between (and perhaps within) the two study populations due to differences in the experimental protocols can not be addressed.

In conclusion, despite the problems in interpreting haemodynamic responses after pretreatment with lithium 750mg, lithium clearance measured after this higher dose does appear reasonably reliable as an estimate of tubular sodium handling. The analysis of factors affecting lithium clearance therefore has internal consistency within a diabetic group. The validity of comparisons between diabetic and control groups will be considered in the next chapter.

CHAPTER EIGHT.

RENAL TUBULAR SODIUM HANDLING AND TUBULAR RESPONSES TO ANGIOTENSIN II IN TYPE 1 DIABETES.

8.1 INTRODUCTION.

Some of the problems associated with the use of lithium in diabetic patients were discussed in Chapter 7. The lithium clearance method does however have some validity as a tubular marker if interpreted cautiously. The results in this Chapter are based on analysis of the lithium clearance data (Table 8.1, 8.2) from the fifteen diabetic patients and ten control subjects who were pretreated with lithium 750mg. These tables also include the same parameters for the diabetic and control subjects who were pretreated with lithium 250mg, to show that comparable conclusions regarding tubular sodium handling in diabetes are drawn after both doses of lithium, provided that artefacts related to variations in the filtered load of sodium are recognized. This caveat is exemplified by the data for absolute proximal reabsorption rate of sodium (APR_{Na}) (Table 8.2), which fell to a greater extent in the diabetic than the control group after Li750 mainly due to the greater fall in the filtered sodium load resulting from the artefactually large fall in GFR.

The data on renal sodium reabsorption are followed by further results showing differences in renal water handling between diabetic and control groups.

Table 8.1: Sodium (C_{Na}) and lithium (C_{Li}) clearances, and derived expressions for fractional tubular reabsorption of sodium during ANGII infusion in diabetic (D) and control (C) patients after lithium 250mg (250) and 750mg (750).

		B/Line	ANGII(ng.kg^{-1}min^{-1})		R
			1.25	2.5	
C_{Na} (ml/min)	D750 (N=15)	2.4(0.2)	1.3(0.2)	0.9(0.1)[a]	1.5(0.1)
	C750 (N=10)	2.8(0.2)	1.6(0.2)[c]	1.2(0.1)[a]	2.0(0.2)
	D250 (N=7)	2.6(0.4)	1.5(0.2)	1.0(0.1)[a]	1.6(0.2)
	C250 (N=6)	2.4(0.2)	1.3(0.2)	1.0(0.1)[a]	1.8(0.3)
C_{Li} (ml/min)	D750 (N=15)	28.5(1.6)	19.6(1.4)	17.5(1.5)[a,d]	23.5(1.7)
	C750 (N=10)	31.5(2.1)	20.7(2.0)	17.8(1.7)[a]	25.8(2.5)
	D250 (N=7)	32.9(2.1)	25.8(2.2)	21.5(0.9)[a,d]	31.6(1.7)
	C250 (N=6)	29.4(2.0)	16.5(0.9)	14.9(0.9)[a]	20.3(1.2)
FE_{Li} (%)	D750 (N=15)	25.1(1.6)[g]	19.5(1.1)	19.9(1.5)[a,e]	21.3(1.4)
	C750 (N=10)	31.5(2.1)	21.8(2.6)	19.9(2.5)[a]	25.4(3.0)
	D250 (N=7)	29.8(1.9)	25.3(1.2)	22.4(0.9)[a,e]	27.3(1.9)
	C250 (N=6)	31.0(2.0)	20.3(2.4)	19.7(1.6)[a]	23.4(1.6)
C_{Na}/C_{Li} (%)	D750 (N=15)	9.4(0.9)	6.6(0.6)	5.3(0.5)[b,f]	7.1(0.8)
	C750 (N=10)	9.2(0.9)	8.8(1.3)	7.0(0.8)[c]	8.2(1.0)
	D250 (N=7)	8.6(1.1)	5.8(0.8)	4.8(0.6)[b,e]	5.2(0.5)
	C250 (N=6)	8.2(0.5)	8.1(1.1)	6.7(0.8)[c]	8.6(1.0)

Mean(sem). R, recovery period. FE_{Li}, fractional proximal sodium rejection; C_{Na}/C_{Li}, fractional distal sodium rejection.

a, P<0.001; b, P<0.01; c, P<0.05 vs baseline.
MANOVA(group x time) interactions vs Control: d, P=NS;
e, P<0.05; f, P<0.01.
g, P<0.05 vs C750.

Table 8.2: Derived expressions for absolute tubular reabsorption of sodium during ANGII infusion in diabetic (D) and control (C) patients after lithium 250mg (250) and 750mg (750).

		B/line	ANGII(ng.kg^{-1}min^{-1})		R
			1.25	2.5	
APR_{Na} (mmol/min)	D750 (N=15)	11.8(0.5)[a]	11.1(0.5)	9.9(0.6)[b,c]	12.0(0.5)
	C750 (N=10)	9.9(0.7)	10.8(0.8)	10.4(0.7)	12.0(0.5)
	D250 (N=7)	11.1(1.0)	10.5(0.7)	10.5(0.7)	12.0(1.1)
	C250 (N=6)	10.3(0.5)	10.6(0.3)	10.1(0.2)	10.5(0.5)
ADR_{Na} (mmol/min)	D750 (N=15)	3.3(0.2)	2.5(0.2)	2.3(0.3)[d]	3.0(0.2)
	C750 (N=10)	3.8(0.2)	2.7(0.2)	2.3(0.2)[d]	3.3(0.4)
	D250 (N=7)	4.2(0.3)	3.3(0.3)	2.8(0.1)[d]	4.2(0.2)
	C250 (N=6)	3.7(0.3)	2.1(0.2)	1.9(0.2)[d]	2.6(0.2)

Mean(sem). R, recovery period. APR_{Na} and ADR_{Na}; absolute proximal and distal reabsorption of sodium.

[a], P<0.05 vs C750.

[b], P<0.05 vs baseline.

[c], MANOVA(group x time) interaction vs C750, P=0.006.

[d], P<0.01 vs baseline.

8.2 RESULTS.

The serum lithium concentration twelve hours after the 750mg dose was 0.25(0.01)mM in the diabetic group and 0.29(0.02)mM in the control subjects.

8.2.1 Proximal tubular sodium handling.

Baseline absolute lithium clearance (C_{Li}) was not significantly reduced in the diabetic group (IDDM 28.5(1.6), Control 31.5(1.7) ml/min), and the fall in C_{Li} during ANGII infusion was similar in both groups (Figure 8.1a). However, because of the small difference in GFR between the diabetic and control groups, baseline fractional lithium excretion (FE_{Li}), representing the proportion of the filtered sodium load rejected by the proximal tubule, was reduced in the diabetic group ($P<0.05$), while the fall in FE_{Li} during ANGII infusion, representing a proximal tubular antinatriuretic response to ANGII, was blunted in the diabetic group (Figure 8.1b), the result already mentioned in Figure 7.8c.

Baseline absolute proximal reabsorption of sodium (APR_{Na}) was increased in the diabetic group ($P<0.05$). APR_{Na} did not change during ANGII infusion in the control group, and the apparent reduction in APR_{Na} in the diabetic patients during ANGII (Table 8.2) was as already discussed due mainly to artefactual changes in the filtered load of sodium.

The FE_{Li} data from the diabetic group is free of the

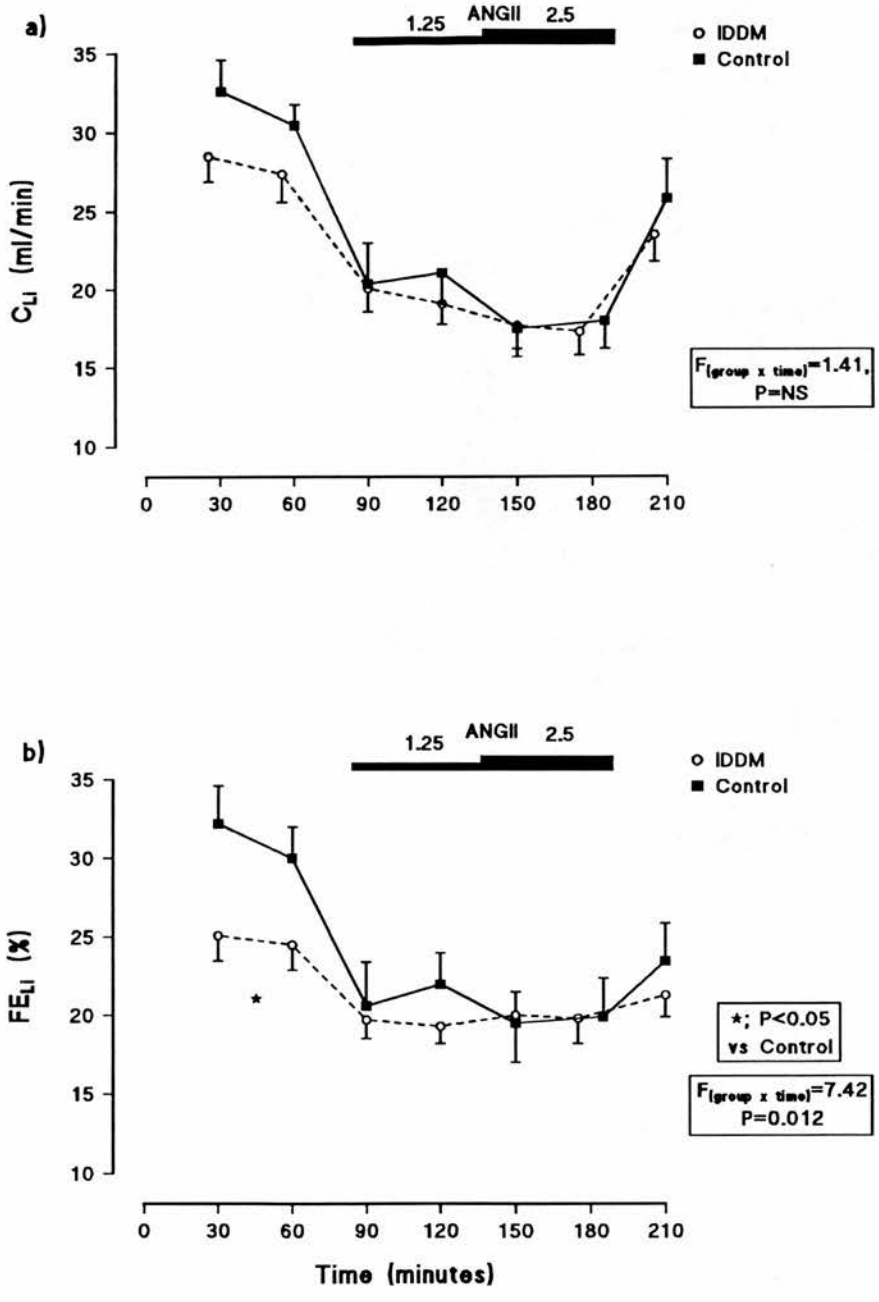

Figure 8.1: a) absolute and b) fractional lithium clearance in 15 diabetic and 10 control subjects during ANGII infusion after lithium 750mg. Mean(sem).

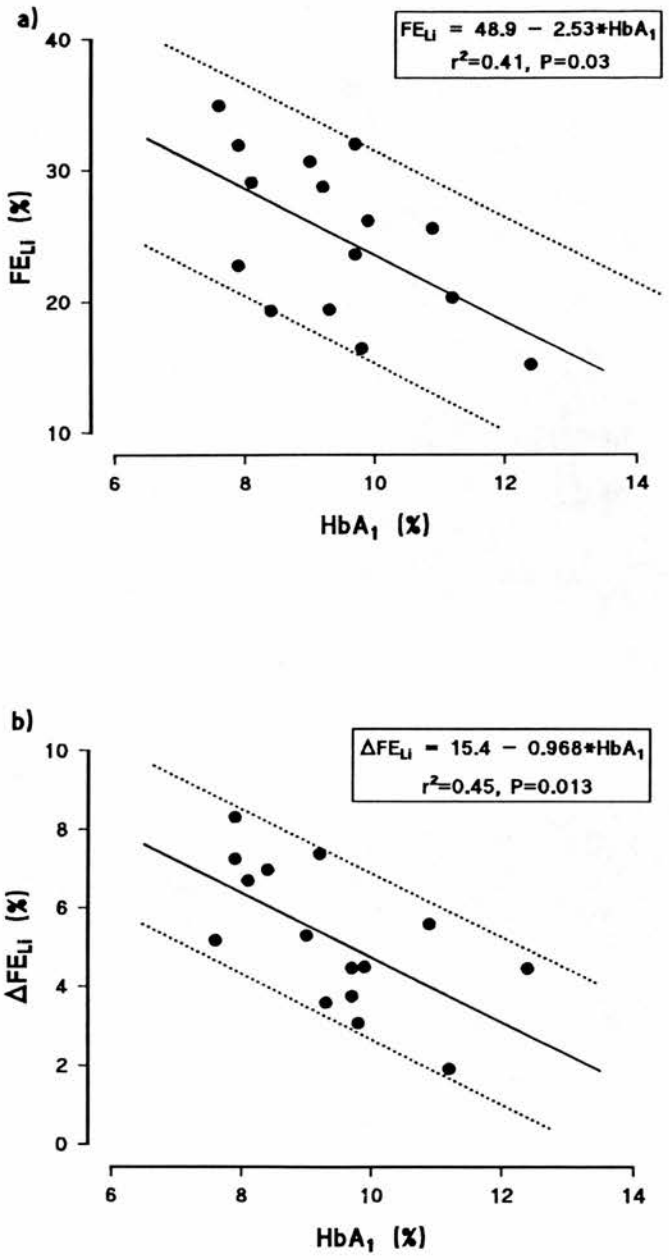

Figure 8.2: Correlation of HbA_1 with a) baseline and b) fall in fractional lithium excretion during ANGII infusion in 15 diabetic patients after lithium 750mg. Dotted lines indicate ±2SD from regression line.

problems associated with the estimation of absolute sodium reabsorption rate, and was examined by a stepwise linear regression procedure using glycated haemoglobin, fractional sodium excretion and duration of diabetes as possible predictors. The final regression equation shows that the only independent correlation is between baseline FE_{Li} and the level of chronic glycaemic control (Figure 8.2a). A similar inverse correlation was found between the *fall* in FE_{Li} during ANGII infusion and HbA_1 (Figure 8.2b). FE_{Li} is therefore increasingly reduced as the quality of chronic blood sugar control worsens, implying an increase in fractional proximal tubular sodium reabsorption, and the change in FE_{Li}, a surrogate measure of proximal antinatriuretic reactivity to exogenous ANGII, is increasingly blunted.

8.2.2. Distal tubular sodium handling.

The ratio C_{Na}/C_{Li}, representing fractional distal excretion of sodium, was similar in both groups at baseline (Figure 8.3a). Fractional distal excretion fell in both groups during ANGII infusion, but the size of the fall was significantly greater in the diabetic group ($P=0.02$). Baseline absolute distal sodium reabsorption (ADR_{Na}) was not different in the two groups (Figure 8.3b), falling substantially and to a similar extent during ANGII in both diabetic and control patients.

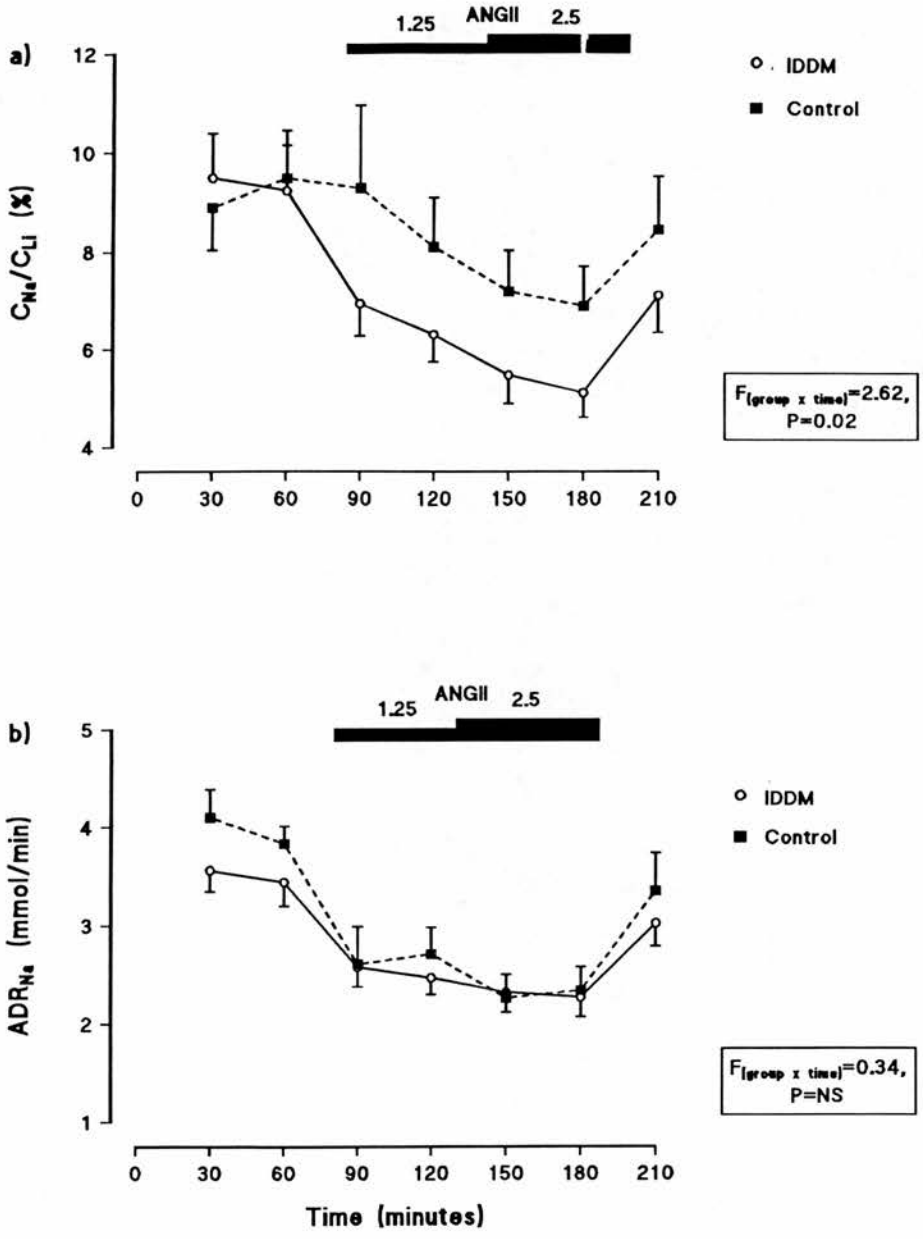

Figure 8.3: a) fractional, and b) absolute distal tubular reabsorption of sodium during ANGII infusion in 15 diabetic and 10 control subjects after lithium 750mg. Mean(sem).

8.2.3 Renal concentrating capacity and water handling.

During the clearance studies stable positive water balance was always maintained, resulting in a moderate but not maximal diuresis at baseline. The minimal increase in urinary osmolality (U_{Osm}) during ANGII infusion seen in the majority of the diabetic group contrasts with the rise in U_{Osm} seen in every control subject. These findings were noted after both placebo and lithium pretreatment (Figure 8.4a,b), and occurred despite similar falls in urine flow rate and free water clearance (C_{H2O}) in the two groups on both days (ΔV: IDDM, Pl 8.8(0.8), Li750 8.7(0.8) ml/min; Control, Pl 7.9(1.0), Li750 8.8(1.1) ml/min: ΔC_{H2O}: IDDM, Pl 6.4(0.6), Li750 6.1(0.7) ml/min; Control, Pl 6.4(0.8), Li750 6.7(1.0) ml/min). The individual changes in U_{Osm} emphasise how concentrating capacity was impaired in the diabetic group during ANGII infusion (Figure 8.5a). When the diabetic group was divided by the lower limit of the control range into normal 'responders' (R) and abnormal 'non- responders' (NR), glycaemic control in R was better than in NR (Figure 8.5b, 8.5c). Urine flow rate and free water clearance were similar at baseline in 'responding' and 'non-responding' subjects (V; R 16.1(1.2), NR 15.5(0.7) ml/min: C_{H2O}; R 11.4(0.7), NR 10.3(0.7) ml/min); the abnormal response to ANGII infusion was not therefore due to any differences in the initial degree of water loading.

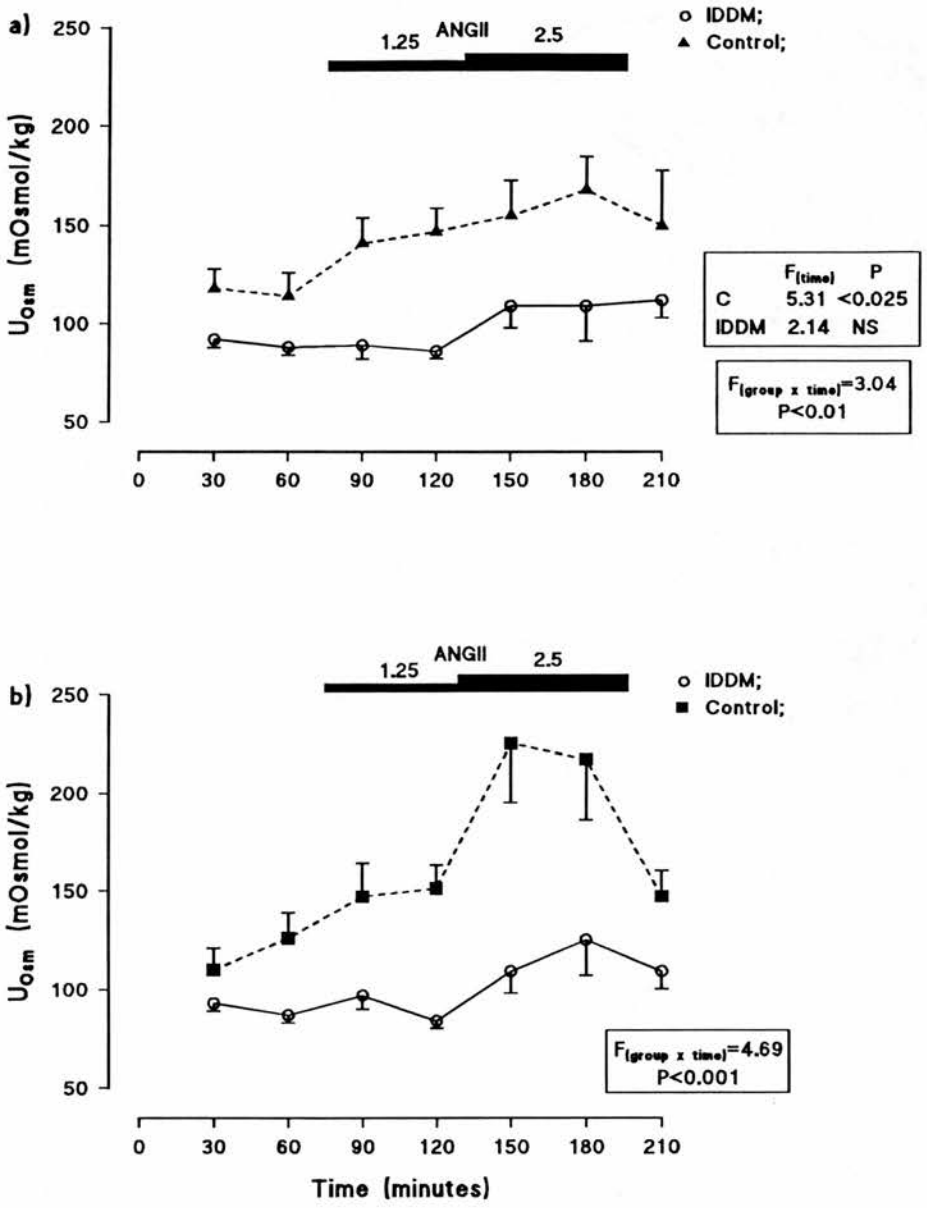

Figure 8.4: Urinary osmolality in 15 diabetic and 10 control subjects during ANG II infusion after a) placebo and b) lithium 750mg. Impaired concentrating ability is evident in the diabetic group on both days.

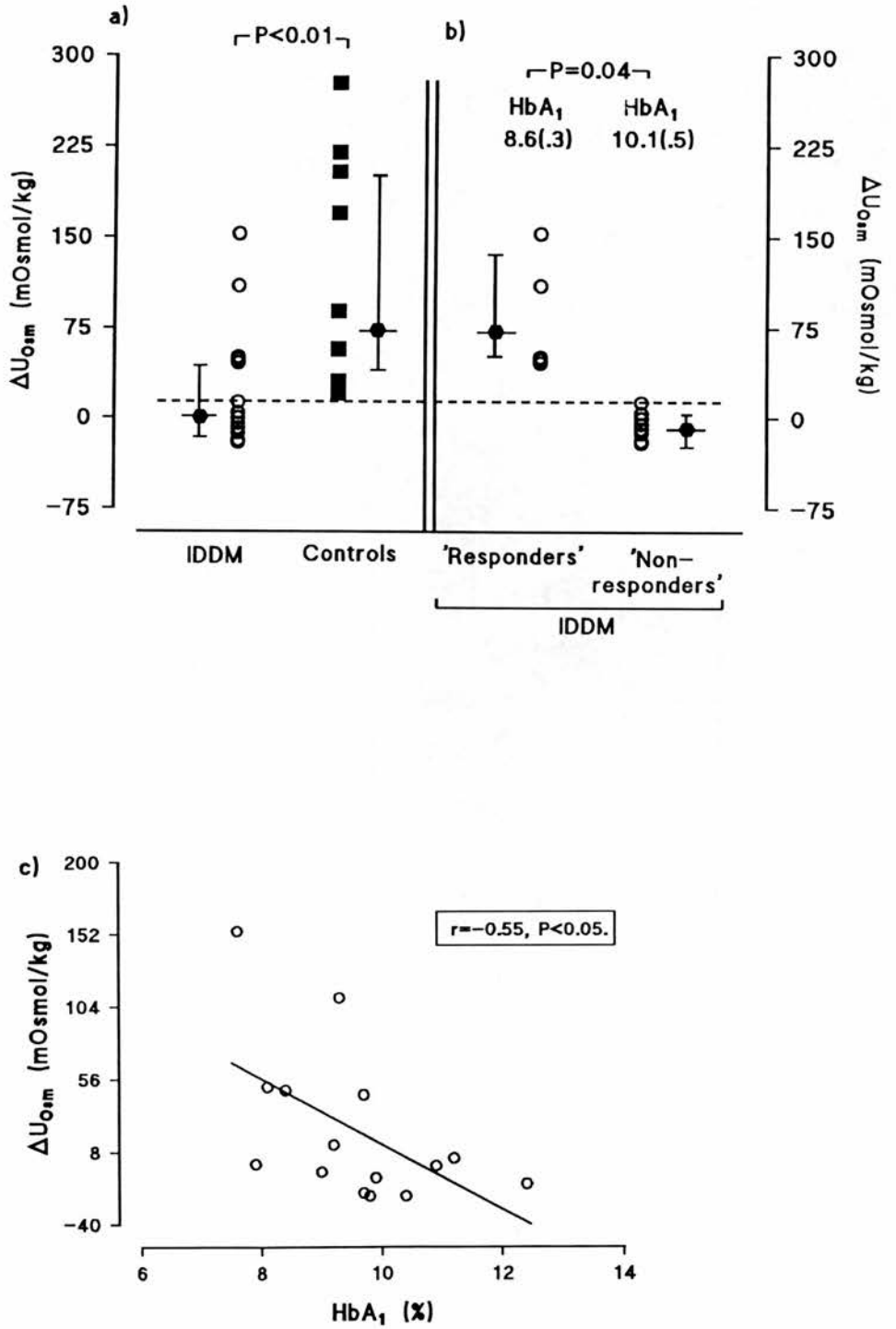

Figure 8.5: a) change in urinary osmolality during ANGII infusion in 15 diabetic and 10 control subjects. b) the diabetic group is divided according to ΔOsm (see p.205). c) confirms the result shown in b).

8.3 DISCUSSION.

Abnormalities of renal tubular sodium and water handling were apparent in this diabetic group. Importantly, the severity of both these abnormalities was correlated with the quality of chronic glycaemic control, implying that they were an acquired functional effect of the diabetic state.

The finding of an abnormal urinary concentrating response in Type 1 diabetes during ANGII infusion is not unprecedented; reduced concentrating ability has been described during infusion of the vasopressin analogue ddAVP in uncomplicated IDDM (Thompson et al, 1990), a defect which also correlated with the level of chronic glycaemic control. Infusion of ANGII in water replete normal humans stimulates the release of endogenous vasopressin to a very limited extent even at infusion rates 6-fold higher than those used in the present study (Phillips et al, 1985). The substantial urinary concentrating effect of ANGII infusion seen in these normal men was therefore independent of vasopressin release, making it difficult to propose an abnormality of systemic vasopressin release as the cause of the impaired concentrating response in Type 1 diabetes. The effect is probably due to a direct medullary action of ANGII, possibly related to reduced proximal tubular delivery of sodium and water.

The impaired concentrating ability in response to

two separate agonists now demonstrated in Type 1 diabetes seems most compatible with an intrarenal defect, but the present data can not determine the mechanism involved. Possibilities include partial washout of the medullary concentrating gradient following chronic intermittent glycosuria, or changes in epithelial transport function due to non-enzymatic glycation.

The positive correlation found in this study between fractional lithium clearance and glycaemic control, as judged by HbA_1, has not been described in previous investigations. This may be due to the confounding effect of the parallel variation of lithium clearance with dietary sodium intake (Atherton et al, 1987). Sodium loading before the study presumably facilitated an increase in proximal tubular sodium rejection towards a physiological maximum at baseline, and this may have allowed the underlying influence of chronic glycaemic control on tubular function to become more evident. The correlation of glycaemic control with fractional but not with absolute lithium clearance further suggests that an abnormality of glomerular filtration rate was of underlying importance in the expression of the effect.

Since GFR was slightly higher in the diabetic than in the control group, the increase in baseline proximal tubular reabsorption of sodium in the diabetic group can be interpreted as an appropriate response serving to maintain glomerulo-tubular balance, as already suggested

(Brochner-Mortensen et al, 1984; Skott et al, 1989b). It has been proposed that this is supported by sodium-glucose cotransport in the proximal tubule (Hannedouche, 1990a). In the present subjects studied at near-euglycaemia the potential for increased glucose-linked sodium transport may be too small to detect. Furthermore, lithium is not transported by the sodium-glucose cotransporter *in vitro* (Hopfer et al, 1973), and lithium clearance data may therefore be intrinsically unsuitable for detecting such an effect even if present.

The reduction in the fractional proximal anti-natriuretic response to ANGII infusion suggests an alternative mechanism by which glomerulotubular balance may have been preserved. Elevated levels of peptide hormones usually produce reciprocal down-regulation of tissue receptor density. The combination of enhanced baseline sodium reabsorption and blunted reactivity to an exogenous antinatriuretic agonist is characteristic of pre-activation of the endogenous agonist. It is therefore reasonable to speculate that elevation of intrarenal ANGII could be the mediator of enhanced fractional proximal sodium reabsorption in Type 1 diabetes. The correlation between tubular sodium reabsorption and HbA_1 would also be not inconsistent with the positive correlation between glycaemic control and plasma ANGII levels (Sullivan et al, 1980; O'Hare et al, 1982). However, the complexity of the receptor and

post-receptor events involved in the proximal tubular actions of ANGII and the dissociation between plasma and intrarenal ANGII levels (Mendelsohn, 1979) makes this postulate difficult to test directly.

Glomerulotubular balance is a substrate-driven homeostatic process which will not independently induce an increment in proximal tubular reabsorption exceeding the increase in the filtered load. Additional factors must be invoked if tubular sodium retention is to lead to expansion of total body sodium content in Type 1 diabetes. It is in this role that sodium-glucose cotransport is potentially more important; glucose-linked sodium hyperreabsorption during mild or moderate hyperglycaemia could be superimposed on the 'appropriate' excess reabsorption needed to preserve glomerulotubular balance, producing overall sodium retention. It is therefore especially interesting that ANGII has been shown to enhance the activity of this transporter (Garvin, 1990). The antinatriuretic effect of insulin may also be relevant, either independently or by modulating the activity of other systems.

There are several explanations for the fact that the baseline tubular sodium handling data in these studies gave no statistically significant evidence that overall proximal nor distal tubular sodium handling were inappropriately increased in the diabetic group under these particular experimental conditions. First, the

methodological limitations of clearance studies and the relative insensitivity of the lithium clearance method have been discussed at length. Second, there may be no additional abnormality of sodium handling at this stage of Type 1 diabetes; two studies have failed to find any significant increase in total exchangeable body sodium content in carefully selected uncomplicated Type 1 diabetic patients (Hommel et al, 1990; Catalano et al, 1991). Third, the experimental protocol may have modified the antinatriuretic defect, ie by reducing proximal sodium-glucose cotransport during euglycaemia. Finally, the kidney escapes from unphysiological antinatriuretic stimuli by activating opposing natriuretic systems, often with only minor changes in the plasma or urine levels of effector components; this is the rationale for studying the response to an intervention which disturbs the baseline situation when attempting to identify abnormalities in intrarenal sodium-retaining mechanisms.

The fraction of fluid and sodium delivered from the proximal tubule to the distal segments, fractional distal delivery, was reduced in the diabetic group, but the calculated absolute distal delivery was not significantly reduced. The increased fall in fractional distal excretion of sodium during ANGII infusion in the diabetic group suggests that distal tubular sensitivity to ANGII is increased, but the data can not establish if this is a

direct effect of ANGII. The increment in plasma aldosterone stimulated by ANGII did not differ in diabetic and control groups. Superimposed insulin-mediated distal tubular sodium retention is also unlikely to be a direct cause because baseline fractional distal excretion of sodium was not reduced in IDDM at the beginning of the study when the insulin infusion rate was highest, and the insulin infusion rate was falling during the ANGII infusion. An interaction between insulin and ANGII can not be excluded, and this will be discussed further in Chapter 9.

The discussion above views increased tubular sodium reabsorption in early diabetes as a secondary response to changes in glomerular haemodynamics, but a reduced proportional delivery of sodium to the macula densa in diabetes is an attractive mechanism by which deactivation of the tubuloglomerular feedback system could primarily elicit preglomerular vasodilatation and sustain an increased glomerular filtration rate (Blantz et al, 1982). The fall in glomerular filtration rate following the increase in distal sodium delivery (measured by lithium clearance after a 750mg loading dose) during acetazolamide infusion in normal man has been used to support this argument (Hannedouche et al, 1991). However, it seems dubious to ascribe the effect of this experimental intervention on glomerular filtration rate to a TGF response when the data on which this conclusion

is based is derived from equations which themselves include GFR as a dependent variable. The correlation coefficients quoted between glomerular filtration rate and proximal tubular rejection are very high (r=0.94-0.98) for an *in vivo* human study, suggesting that a mathematical artefact is present. The haemodynamic effects of lithium in diabetes also make it doubtful whether lithium clearance data can be used to analyse TGF in this way.

In conclusion, the data in this chapter shows that defects of tubular sodium and water handling are evident in uncomplicated diabetic patients. The severity of these abnormalities correlates with the level of chronic metabolic control. The alteration in proximal tubular sodium handling suggests that the kidney in early diabetes is responding appropriately, at least to some extent, to the abnormal environment in which it operates. The data does not exclude the presence of additional abnormalities of tubular sodium reabsorption, but it is clear that any hypothesis which attempts to unify the abnormalities of glomerular and tubular function in diabetes must acknowledge the central importance of the level of metabolic control as a factor modulating the expression of these renal functional abnormalities.

CHAPTER NINE.

THE EFFECTS OF INSULIN ON RENAL FUNCTION IN NORMAL MAN.

9.1 INTRODUCTION.

The interactions described between insulin and other hormone systems now extend far beyond those involved in carbohydrate and intermediate metabolism. A positive association has recently been found in epidemiological studies between fasting and post-prandial plasma free insulin levels and blood pressure in Type 2 diabetes mellitus (Modan et al, 1985; Zavaroni et al, 1987), and in subjects with normal glucose tolerance with or without hypertension (Ferranini et al, 1987; Fournier et al, 1986). This has stimulated interest in the importance of insulin as a vasoactive hormone, but the association between hyperinsulinaemia and increased blood pressure can not by itself distinguish genetic co-segregation from a direct pathophysiological relationship.

The acute cardiovascular effects of insulin include an increased heart rate in response to postural changes in uncomplicated Type 1 diabetic patients (Page et al, 1976a), vasodilatation in patients with diabetic autonomic neuropathy (Page et al, 1976b), and a weak systemic pressor effect at pharmacological levels in normal man (Gans et al, 1991a). These effects are tissue and species-specific; insulin attenuates vasoconstrictor responses to ANGII and noradrenaline (NE) in animal smooth muscle preparations *ex vivo* (Yagi et al, 1988), whereas ANGII-mediated contraction of glomerular mesangial cells is insulin-dependent (Kreisberg, 1982).

Insulin at physiological concentrations does not modify either the pressor response to acute ANGII infusion in normal humans (Vierhapper et al, 1983), or that induced during chronic ANGII infusion in the dog (Hall et al, 1990), although an interaction between pharmacological hyperinsulinaemia and the pressor response to ANGII infusion has been reported in the dog mediated through facilitation of sympathetic activation (Rocchini et al, 1990).

Acute hyperinsulinaemia reduces renal sodium excretion in normal man (Miller and Bogdonoff, 1954; DeFronzo et al, 1975; Skott et al, 1989a; Gans et al, 1991a) and in NIDDM (Skott et al, 1991). This provides a direct mechanism by which insulin might influence blood pressure, although in animal models the antinatriuretic effect appears to be transient (Hall et al, 1990). The reduction in sodium excretion when insulin is infused intrarenally (DeFronzo et al, 1976) or into the isolated perfused kidney (Cohen et al, 1989) indicates a direct effect on the kidney. However, systemic insulin infusion increases sympathetic outflow (Rowe et al, 1981), plasma renin activity and plasma ANGII levels (Trovati et al, 1989). Activation of these systems intrarenally may augment the sodium-retaining action of insulin. Clearly an intrarenal interaction between insulin and ANGII in the control of sodium handling deserves consideration.

The only difference between the experimental

protocols used in diabetic and control subjects in previous chapters was the small amount of insulin infused in the diabetic patients to maintain euglycaemia. The first objective of this study was therefore to determine how the known effect of insulin on renal sodium handling might affect the interpretation of the lithium clearance data in Chapters 7 and 8. Secondly, evidence was sought for an intrarenal interaction between insulin and the renin-angiotensin system by examining the effect of acute hyperinsulinaemia on renal sodium handling in normal humans after blockade of intrarenal ANGII generation with an angiotensin converting enzyme inhibitor (ACEI).

9.2 PROTOCOL.

Seven normal volunteers (mean age 32(SD 4) years), weight 76kg (SD 4, range 70 to 83kg), and body mass index 23.9(2.4) kg/m^2) were studied. All had normal glucose tolerance (two hour post-prandial blood glucose <6mM, mean HbA_1 5.8(0.2)% (normal range 5-8%)) and normal blood pressure (<140/90 mmHg, diastolic phase V). Enquiry was made about a family history of diabetes (none) and essential hypertension (one subject).

Each subject was studied three times in a randomised order, the studies being at least two weeks apart. One study served as a time control day (C); before the other two studies the subjects were treated for one week, double blind, either with a placebo preparation (Pl) or

with the angiotensin converting enzyme inhibitor perindopril 4mg o.d. (Servier Laboratories Ltd, Slough) (ACEI), a final dose of placebo or ACEI being taken on the morning of the acute study. Dietary advice was given with the aim of achieving a daily intake of 150-200 mmol sodium and 80-100 mmol potassium for one week before each study. Urine was collected for 24h before each study. At 2100h in the evening the subjects took 250mg of lithium carbonate, the dose which was shown to have no demonstrable pharmacological effects on the kidney in the studies reported in Chapter 5.

Renal haemodynamic parameters were measured using the clearance protocol already described. On the two days when insulin was infused a third cannula was inserted in a retrograde fashion into a dorsal hand vein for measurement of glucose concentrations in arterialized whole blood (oxygen saturation >98%); arterialization of blood was achieved by heating the right hand to $55^{o}C$ (Abumrad et al, 1981). After four baseline clearance periods, euglycaemic hyperinsulinaemia was established by starting an infusion of unmodified soluble human insulin (Actrapid, Novo-Nordisk, Denmark). The insulin was diluted (0.33 U/ml) into 100ml 0.15M sodium chloride containing 2ml of the subject's blood to prevent adsorption of the insulin onto plastic; an initial infusion rate of 120 $mU.m^{-2}min^{-1}$ was reduced every minute to reach the maintenance infusion rate of 40 $mU.m^{-2}min^{-1}$

after 10 minutes as previously described in detail (DeFronzo et al, 1979). The pre-infusion fasting blood glucose level was then maintained by infusing D-glucose 200g/l (1.15 mol/l) at a rate adjusted according to measurements of whole blood glucose made every 5 minutes at the bedside with a reflectance glucometer (Reflolux-S, Boehringer). Samples were collected every 30 minutes for later confirmation of the values obtained for whole blood glucose by a glucose oxidase method. Hyperinsulinaemia was maintained for two hours while four further clearance periods were completed. Subjects were fed after the insulin infusion had been stopped to avert the tendency to develop rebound hypoglycaemia. Insulin vehicle was infused alone during the time control study, the 20% D-glucose solution being omitted to avoid stimulating release of endogenous insulin.

Blood pressure was measured by an automated sphygmomanometer every 20 minutes, and the mean of all 'baseline' and 'clamp' results on different study days compared by paired t-test. When analyzing the data, a significant overall interaction in the data sets from all three study days was confirmed by three way analysis of variance before pairs of data sets were compared by two way MANOVA to produce the statistics quoted in the Figures.

9.3 RESULTS.

9.3.1 Dextrose utilisation during insulin infusion.

One subject noticed transient dizziness after starting perindopril, but continued taking the drug. No other side effects occurred. Basal fasting plasma free insulin levels were not affected by ACEI, and comparable increments in plasma free insulin levels were achieved during the two euglycaemic clamps in each subject (Table 9.1). Baseline whole blood glucose concentration was similar on each day, and remained stable throughout both insulin infusions (Table 9.1 and Figure 9.1). The dextrose infusion rate needed to maintain euglycaemia during the first hour of hyperinsulinaemia was higher after ACEI in five of the seven subjects, but was the same by the end of the second hour of insulin infusion on both days (Figure 9.1). The cumulative dextrose requirement during hyperinsulinaemia was not different on the two days (Pl 719(91) mg/kg, ACEI 824(87) mg/kg).

9.3.2 General.

Pretreatment with the ACEI produced a marked rise in plasma renin activity (Table 9.1) and a substantial fall in plasma converting enzyme activity in every subject (C 41(7) u/l, Pl 42(7) u/l, ACEI 5(1) u/l; C/Pl vs ACEI both $P<0.01$). PRA rose during hyperinsulinaemia compared to time alone; the insulin-induced rise in PRA was greater

Table 9.1: Plasma renin activity (PRA), plasma aldosterone (ALDO), whole blood glucose (GLUC) and plasma free insulin levels (INS) during euglycaemic hyperinsulinaemia (0-120 min) in seven normal subjects.

		Baseline	Insulin infusion (minutes).			
		0	30	60	90	120
PRA	C	860(180)	-	910(230)	-	530(140)
(pmol AI	Pl	680(180)	-	880(150)	-	880(240)[a]
$.ml^{-1}h^{-1}$	ACEI	2300(260)[c]	-	4080(740)	-	3190(590)[b]
ALDO	C	234(55)	-	157(30)	-	172(26)
(pmol/l)	Pl	197(39)	-	140(27)	-	128(22)
	ACEI	148(30)	-	129(26)	-	115(17)
GLUC (mmol/l)	Pl	4.30 (0.15)	4.48 (0.19)	4.34 (0.15)	4.62 (0.13)	4.44 (0.14)
	ACEI	4.24 (0.24)	4.33 (0.28)	4.36 (0.22)	4.30 (0.26)	4.50 (0.21)
INS*	C	3.8(0.7)	-	5.2(0.7)	-	4.2(1.0)
(mU/l)	Pl	5.2(0.5)	-	69.9(5.5)	-	70.2(5.3)
	ACEI	6.7(2.1)	-	73.6(6.8)	-	79.0(3.4)

Mean(sem). C, Time control; Pl, Placebo; ACEI, Perindopril. [a], $MANOVA_{(group\ x\ time\ vs\ C)}$ $P<0.01$; [b], $MANOVA_{(group\ x\ time\ vs\ Pl)}$ $P<0.01$, (% change in PRA from baseline, Pl 32(9)%, ACEI 50(18)%, P=NS). [c], $P<0.005$ vs C and Pl. *, normal range (fasting) 2.8-13.5 mU/l.

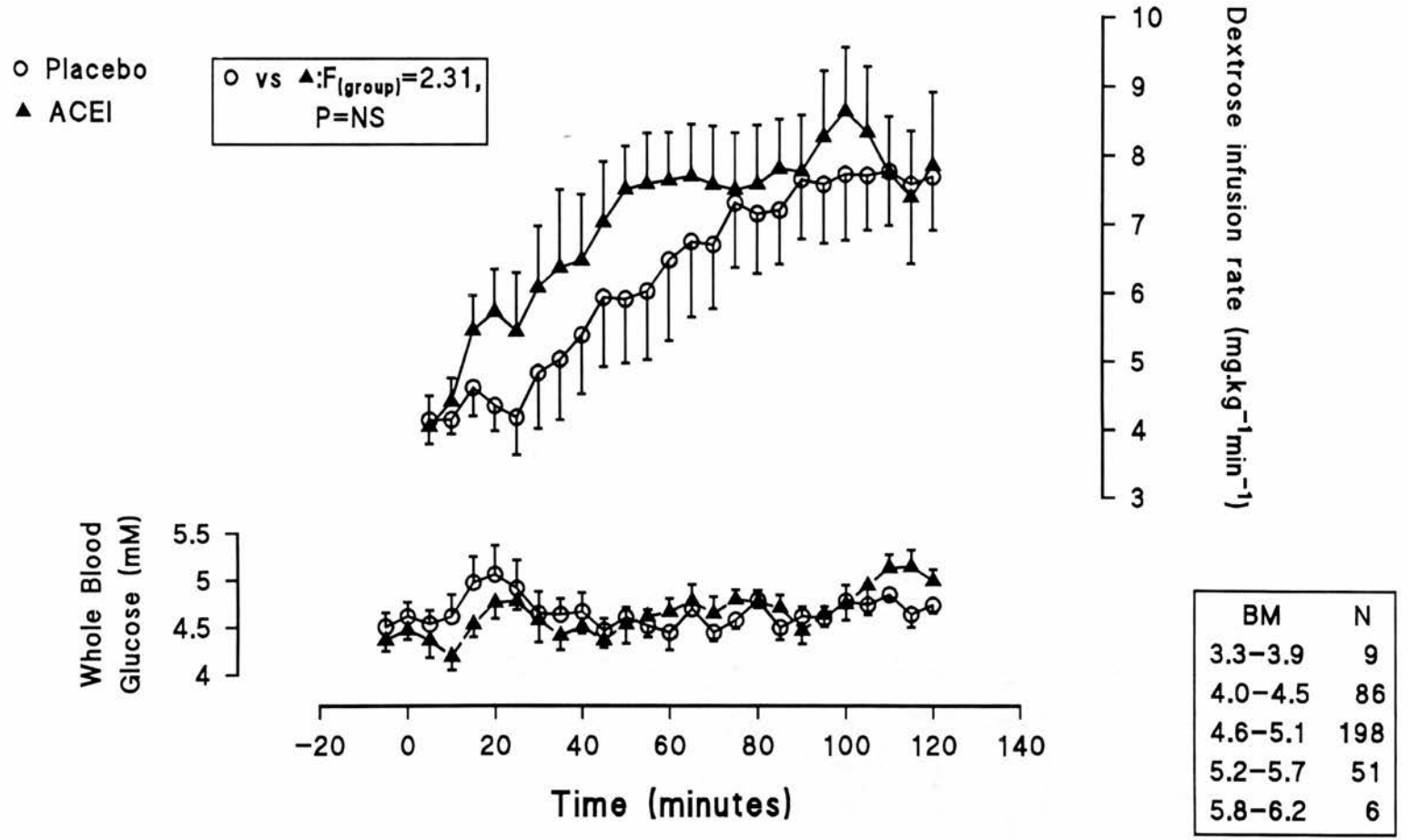

Figure 9.1: Whole blood glucose and dextrose infusion rate during euglycaemic hyperinsulinaemia (0−120min) in seven normal subjects. Mean(sem). The box shows the distribution of blood glucose values for both clamp studies combined.

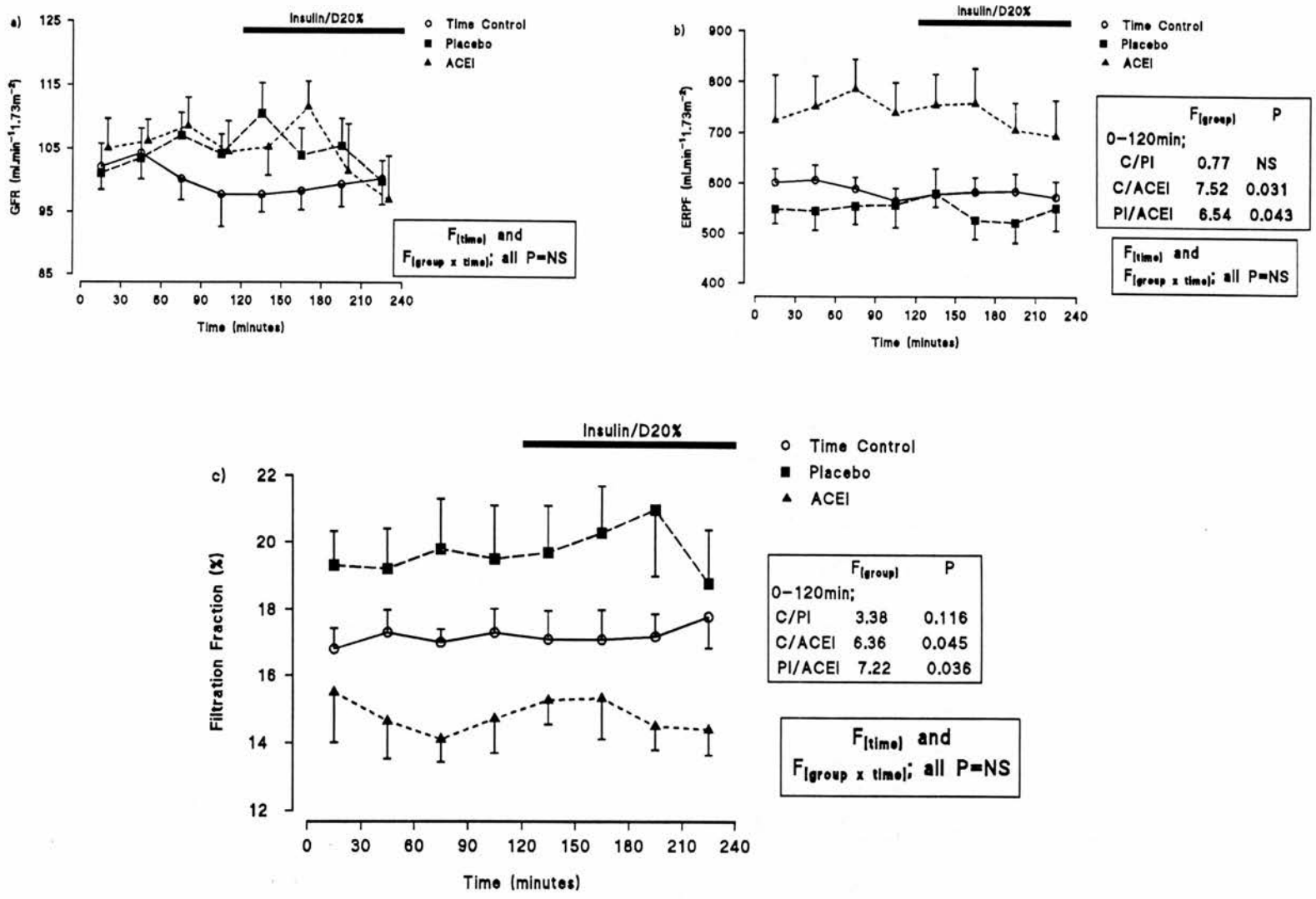

Figure 9.2: a) glomerular filtration rate, b) effective renal plasma flow, and c) filtration fraction in seven normal subjects during euglycaemic hyperinsulinaemia. Mean(sem). 0–120min refers to baseline.

in absolute terms after ACEI than placebo, but the percentage increases from the baseline value were not different. Baseline plasma aldosterone was not different between the three studies, and no significant change in aldosterone occurred during either insulin infusion. Mean arterial pressure was reduced after ACEI (Pl 96.0(2.9) mmHg, ACEI 89.0(2.1) mmHg, ΔMAP=7.1(1.2) mmHg, $P<0.005$). There was no change in blood pressure during hyperinsulinaemia on either day (Pl, 96.0(2.9) to 97.5(3.2) mmHg; ACEI, 89.0(2.1) to 89.2(2.2) mmHg). Heart rate did not change during insulin infusion (data not shown). Venous haematocrit fell during both insulin infusions (C 41(1.6) to 40.7(1.4)%; Pl 39.3(0.6) to 37.4(0.8)%, $P<0.05$; ACEI 40.2(0.9) to 38.7(0.8)%, $P<0.05$).

9.3.3 Renal haemodynamics.

Glomerular filtration rate was similar at baseline on all three days, and did not change during hyperinsulinaemia (Figure 9.2a). Effective renal plasma flow was significantly increased after ACEI in comparison to C and Pl, but did not change with time alone or during hyperinsulinaemia (Figure 9.2b). Baseline filtration fraction (GFR/ERPF) was reduced after ACEI (Figure 9.2c).

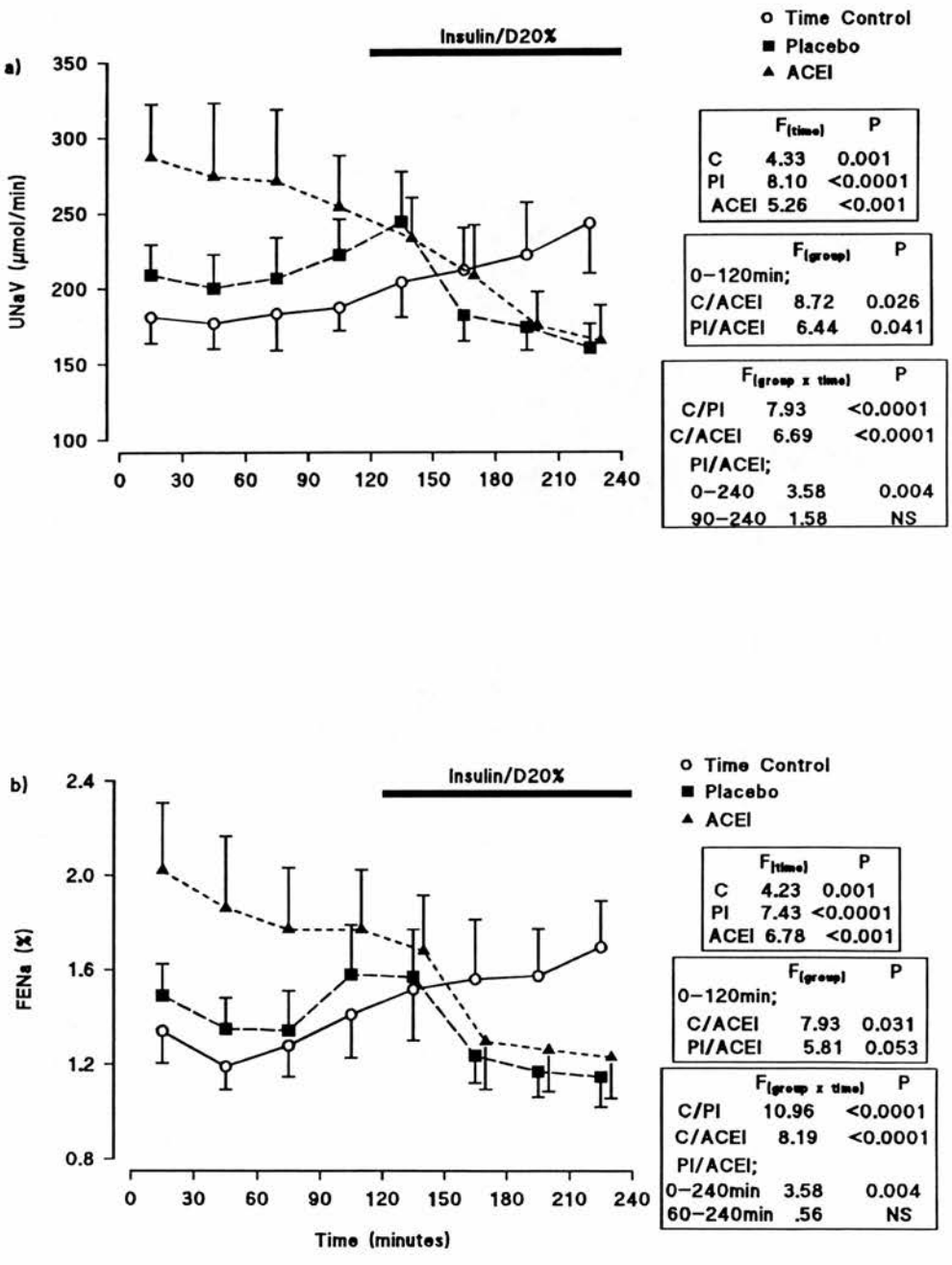

Figure 9.3: a) absolute and b) fractional urinary sodium excretion in seven normal subjects during euglycaemic hyperinsulinaemia. Mean(sem).

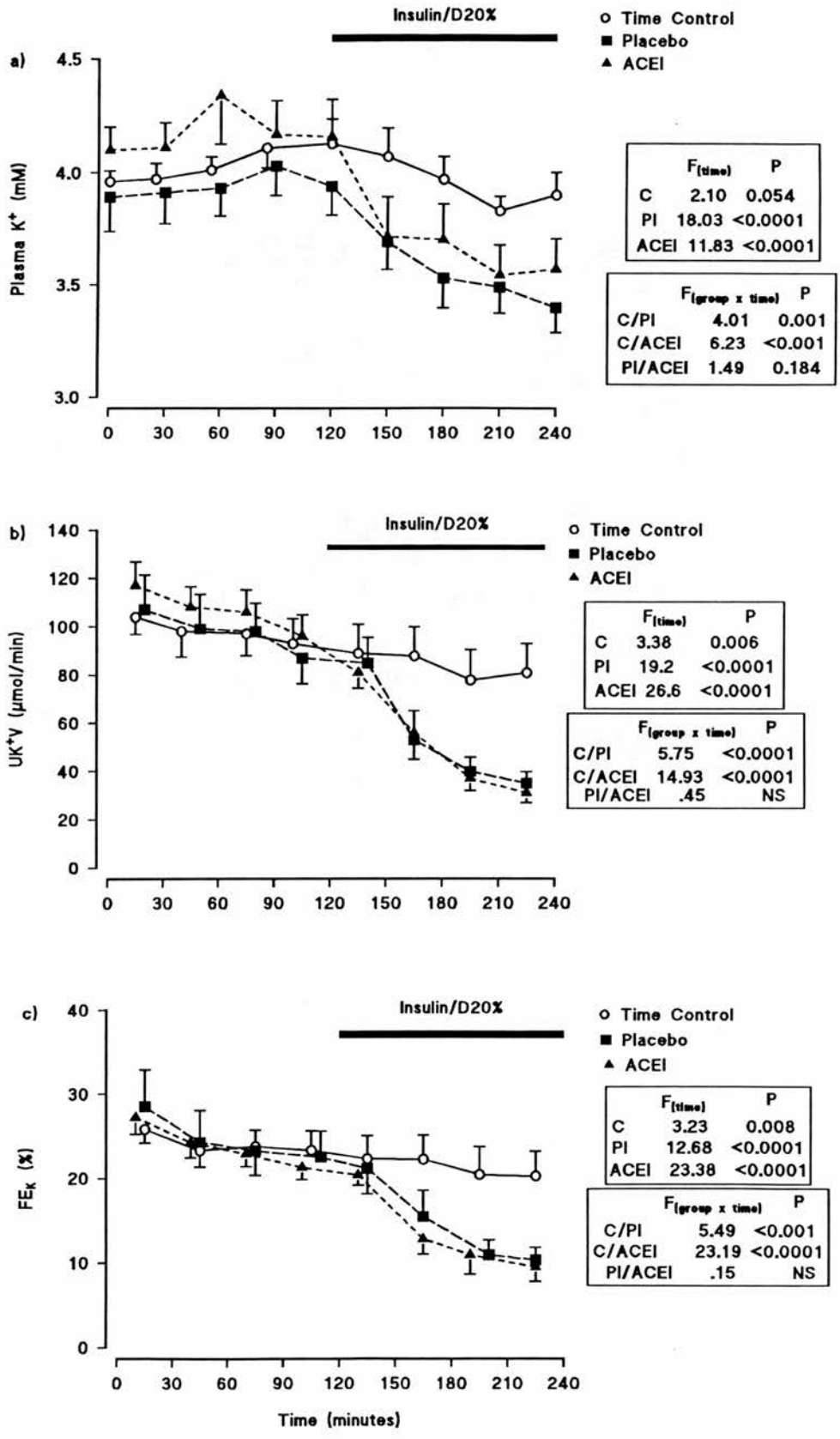

Figure 9.4: a) plasma potassium, and b) absolute, c) fractional urinary potassium excretion in seven normal subjects during hyperinsulinaemia. Mean(sem).

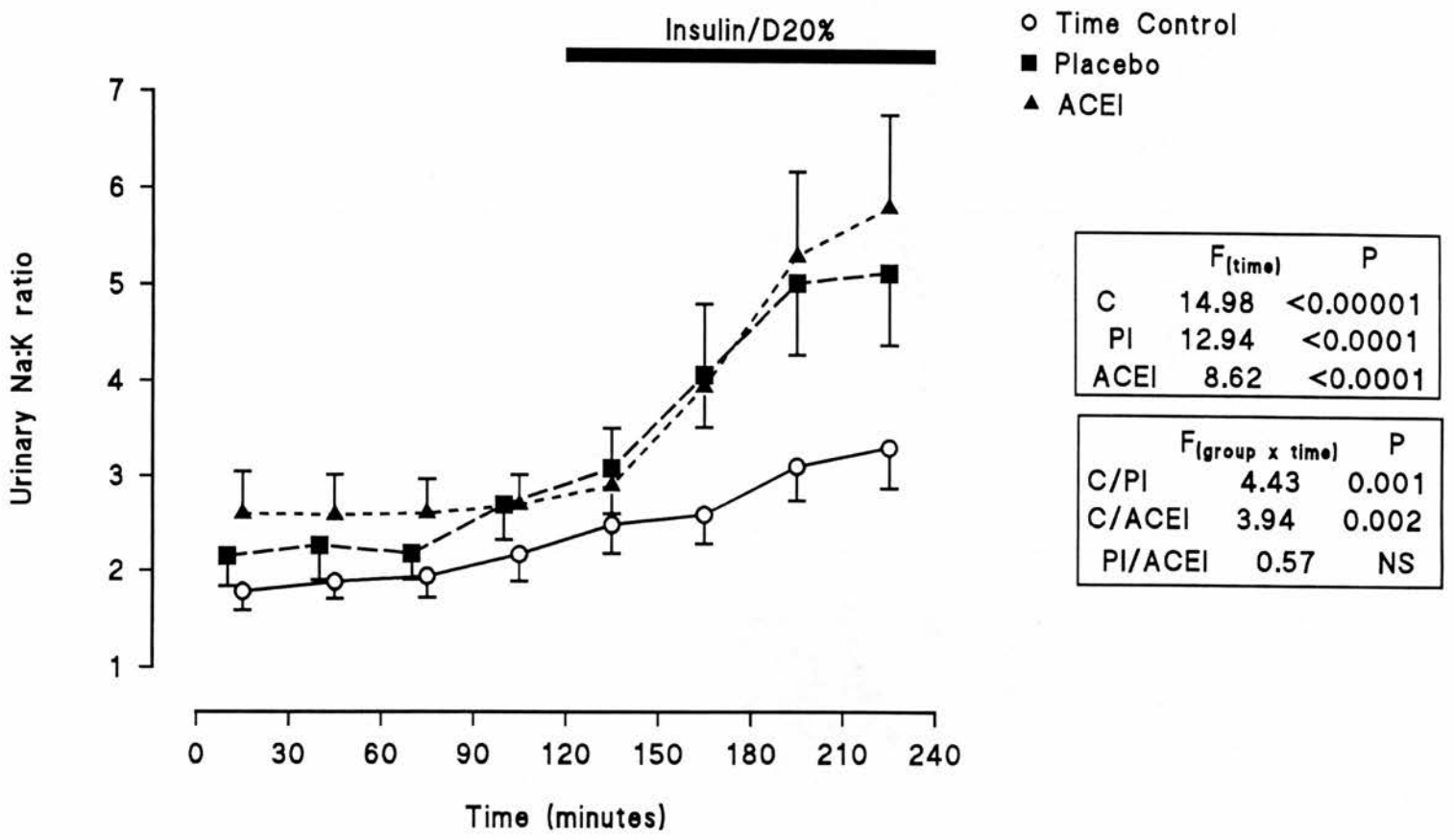

Figure 9.5: Urinary sodium:potassium ratio in seven normal subjects during euglycaemic hyperinsulinaemia. Mean(sem).

9.3.3 Electrolyte and water excretion.

24h potassium excretion was similar before each study (C 80(7), Pl 74(12), ACEI 79(7) mmol). Sodium excretion in the 24h before the study was greater after ACEI (C 176(16), Pl 217(28), ACEI 257(23) mmol; C vs ACEI, $P<0.05$). Plasma sodium was similar on all three days, and did not change during insulin infusion. The urinary excretion rate of sodium rose steadily during the time control study (Figure 9.3a) due to the infusion of about 650ml (90 mmol) of 0.15M sodium chloride as vehicle for Inutest and PAH. After placebo pretreatment a rising sodium excretion rate during the baseline phase was followed by a fall in urinary sodium excretion during hyperinsulinaemia (C vs Pl, $F_{(group \times time)}=7.93$, $P<0.0001$). Baseline sodium excretion was higher after ACEI than on either of the other two days (C vs ACEI, $F_{(group)}=8.72$, $P=0.026$), but antinatriuresis was still apparent during insulin infusion (C vs ACEI, $F_{(group \times time)}=6.69$, $P<0.0001$). There was a significant interaction between Pl and ACEI when all eight time points were analyzed ($F_{(group \times time)}=3.58$, $P=0.004$); however, this result was caused by the higher sodium excretion rate during the early baseline periods after ACEI because analysis of clearance periods 3 to 8 (the second hour of baseline and the period of insulin infusion) showed that the antinatriuretic potential of insulin was unaffected by ACEI ($F_{(group \times time)}=1.58$,

P=NS). The results for fractional sodium excretion showed the same pattern as the absolute sodium excretion data (Figure 9.3b).

Baseline plasma potassium was the same on each day, and a fall in plasma potassium concentration occurred during both insulin infusions which was not affected by ACEI pretreatment (Figure 9.4a). Baseline urinary potassium excretion (UKV) (Figure 9.4b) was similar on all three days. The fall in UKV during hyperinsulinaemia after Pl and ACEI exceeded the small fall with time alone (C vs Pl, $F_{(group \times time)}=5.75$, $P<0.001$; C vs ACEI, $F_{(group \times time)}=14.93$, $P<0.0001$); the antikaliuretic effect of insulin did not differ after ACEI (Pl vs ACEI, $F_{(group \times time)}=0.45$, P=NS). The same conclusion emerged when changes in fractional potassium excretion were analyzed (Figure 9.4c) to take account of the reduced filtered load of potassium resulting from the fall in plasma potassium during hyperinsulinaemia. The urinary $Na^+:K^+$ ratio (Figure 9.5) increased to a greater extent during hyperinsulinaemia than with time alone (C vs Pl, $F_{(group \times time)}=4.43$, $P=0.001$; C vs ACEI, $F_{(group \times time)}=3.94$, $P=0.002$) with no interaction attributable to ACEI. Urine flow rate (Figure 9.6a) and free water clearance (Figure 9.6b) fell with time on all three days, but urine osmolality did not change (data not shown).

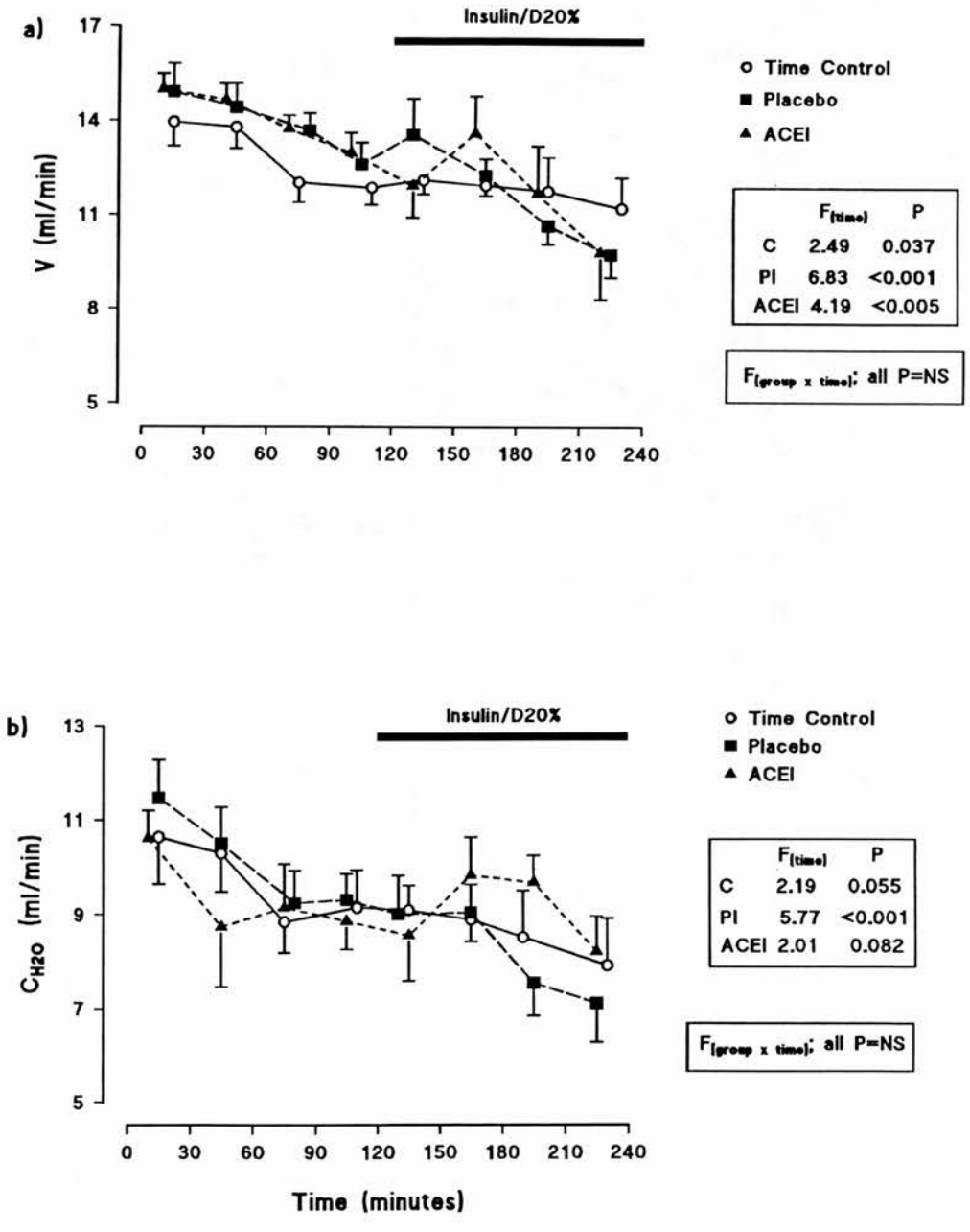

Figure 9.6: a) urine flow rate and b) free water clearance in seven normal subjects during euglycaemic hyperinsulinaemia. Mean(sem).

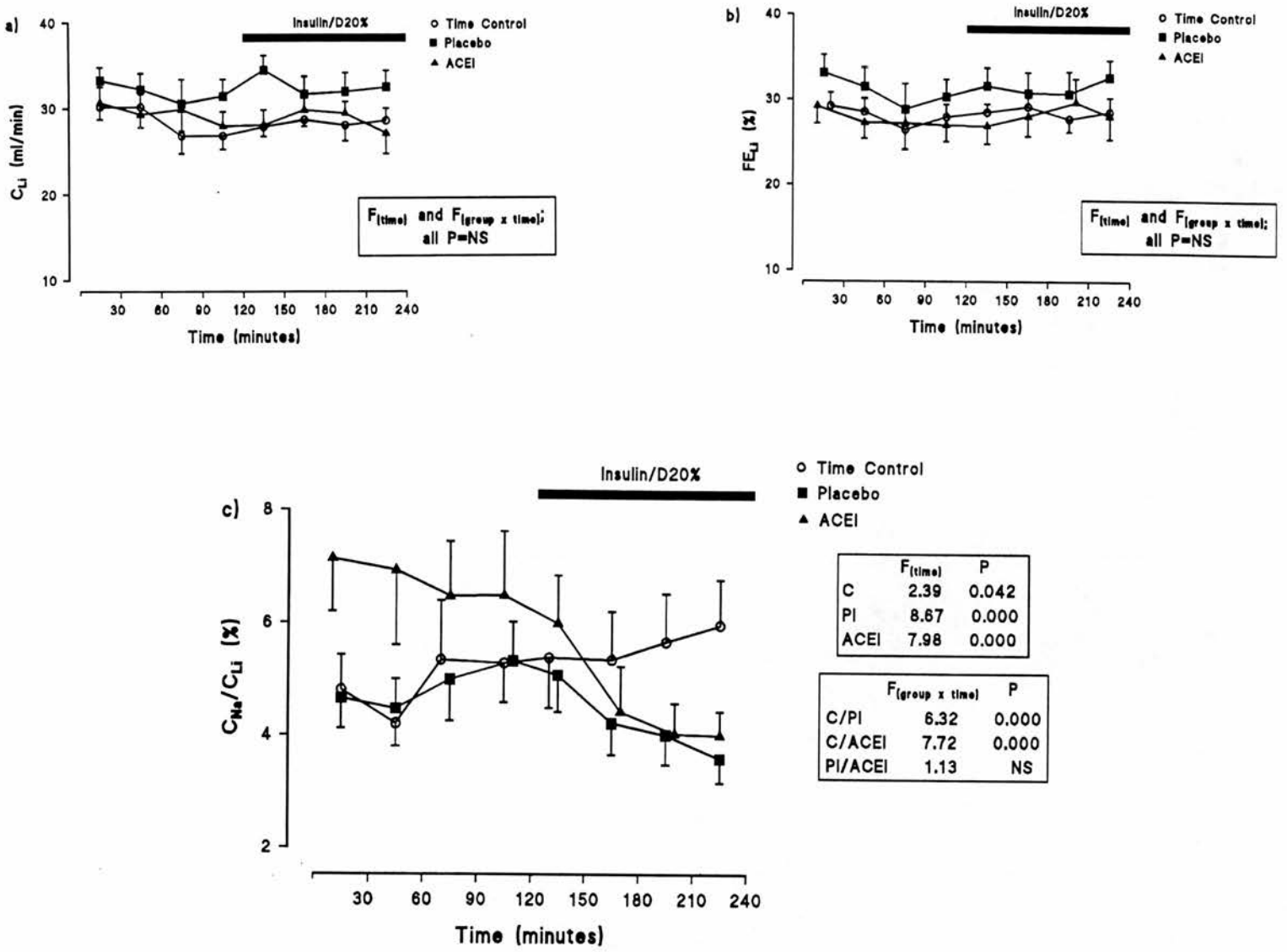

Figure 9.7: a) absolute and b) fractional lithium excretion, and c) fractional distal sodium rejection in seven normal subjects during euglycaemic hyperinsulinaemia. Mean(sem).

9.3.4 Lithium clearance data.

The serum lithium concentration at the beginning of the study was similar on all three days (C 0.104(0.01), Pl 0.107(0.01) , ACEI 0.117(0.005) mmol/l). There was no change in either absolute or fractional lithium clearance with time alone or during insulin infusion (Figure 9.7a,b). The ratio C_{Na}/C_{Li} (Figure 9.7c), representing fractional distal sodium excretion, fell during hyperinsulinaemia; the response of C_{Na}/C_{Li} to insulin infusion was not different after ACEI.

9.4 DISCUSSION.

This study confirms that an acute increase in plasma free insulin to levels within the pathophysiological human range reduces renal excretion of sodium and potassium. The time control day, which allowed for the circadian variation in sodium excretion and the natriuretic effect of the isotonic fluid infused during the study, showed that the antinatriuretic potential of insulin is greater than would otherwise have been concluded. The dose of insulin infused was low enough to avoid the systemic haemodynamic effects previously reported during higher infusion rates (Gans et al, 1991a). The lack of a measurable effect of hyperinsulinaemia on glomerular haemodynamics confirms a recent report (Vierhapper et al, 1991), and suggests that the antinatriuretic and antikaliuretic effects of insulin

involve changes primarily in the renal tubular handling of these ions. However, increased sodium reabsorption which is independent of sodium-glucose co-transport has been demonstrated following glucose exposure in the isolated perfused kidney (Frega et al, 1977). Glucose was deliberately not infused during the time control experiment to avoid stimulating endogenous release of insulin. Consequently, sodium retention due to increased utilization of glucose rather than to insulin infusion *per se* can not be completely excluded.

Insulin increases reabsorption of tubular fluid in isolated perfused rabbit proximal tubules (Baum, 1987), and stimulates Na^+-H^+ exchange in proximal tubule brush border membrane vesicles (Fine et al, 1985). There is however disagreement in human studies as to whether the antinatriuretic effect of insulin seen in human studies occurs in a distal (DeFronzo et al, 1975; Skott et al, 1989a) or a proximal tubular segment (Trevisan et al, 1990). In the present investigation the absence of a change in lithium clearance during hyperinsulinaemia, and the fall in the fractional distal rejection of sodium inferred from the change in the ratio C_{Na}/C_{Li} both suggest that the antinatriuretic action of insulin occurred in a segment distal to the proximal tubule. This interpretation is consistent with the reduction in fractional urinary potassium excretion during hyperinsulinaemia, since changes in urinary potassium

excretion are modulated predominantly in distal tubular segments. It can not be determined whether insulin was acting in the loop of Henle or the distal tubule, which is the tubular segment with the highest density of insulin receptors (Rabkin et al, 1984), but the absence of a rise in plasma aldosterone, the occurrence of antikaliuresis, and the rising urinary $Na^+:K^+$ ratio all indicate that insulin was acting directly and not through stimulation of aldosterone dependent Na^+-K^+-ATPase as previously suggested (Katz, 1982).

The potential relevance of insulin-stimulated release of renin and increased ANGII generation to the sodium retaining effect of insulin was examined by restudying the subjects in a new steady state after pretreatment for one week with an inhibitor of angiotensin converting enzyme. Plasma ANGII levels were not measured, but the *in vitro* effectiveness of the ACEI was mirrored *in vivo* by the increased renal plasma flow and the decreased filtration fraction which together are consistent with intrarenal vasodilatation following reduction of endogenous ANGII.

Because the antinatriuretic effect of euglycaemic hyperinsulinaemia persisted after ACEI pretreatment, increased intrarenal ANGII generation probably plays little if any part in mediating the renal response to insulin. Interpretation of this part of the study is complicated by the increase in baseline sodium excretion

after ACEI. Sodium intake before each study could not be rigidly controlled in an outpatient setting, making it uncertain whether the increased 24h sodium excretion after ACEI was due to a natriuretic effect of the drug following renal vasodilatation, an increase in dietary sodium intake, or a combination of these factors. It could be argued that the antinatriuresis during hyperinsulinaemia after ACEI was a purely time-related phenomenon occurring in subjects who were relatively sodium depleted in comparison to the placebo study by the natriuretic effect of the ACEI. This is unlikely because effective converting enzyme inhibition was present throughout the acute study at a time when the subjects were receiving an infusion of sodium, so that insulin still caused antinatriuresis despite the presence of these two natriuretic influences. Quantitative comparison of the antinatriuretic potential of insulin with and without prior ACEI must nonetheless be cautious, and a time control study after ACE inhibition would be desirable to address this point more fully. However, despite the differing baseline sodium excretion rates, the data strongly suggests that the antinatriuretic potential of insulin is not affected by a period of ACE inhibition.

How do these results bear upon the studies described in previous chapters? The study was planned bearing in mind the much greater antinatriuretic potential of ANGII

compared to insulin, which would have resulted in a very low power to detect modulation of the renal effects of ANGII infusion by insulin. The design used was intended to test first whether insulin infusion affected lithium clearance, and second whether the antinatriuretic effect of insulin infusion was mediated or modulated by intrarenal ANGII generation. The insulin infusion rate (about 4.5 u/h) and the plasma free insulin levels in these normal subjects during insulin infusion far exceed those measured in the diabetic patients reported in Chapter 6. Plasma insulin levels are not available for the studies described in Chapters 7 and 8, but the usual insulin dosage of this group of patients and the insulin infusion rate used to maintain stable glycaemia during the acute study were both similar to those of the diabetic patients described in Chapter 6. It is therefore likely that plasma insulin concentrations in all the studies involving diabetic patients were much lower than the levels produced in these normal men.

There is no *a priori* reason to expect the site of action of insulin within the nephron to be qualitatively different in diabetic subjects, and it is reassuring with regard to Chapter 8 that insulin infusion does not affect lithium clearance, as the correlation between fractional lithium excretion and glycaemic control could not have been biased by differing plasma free insulin levels in the diabetic subjects. However, because the

antinatriuretic effect of insulin apparently involved a distal tubular segment, the ratio C_{Na}/C_{Li} is more susceptible to interference and should be interpreted correspondingly cautiously; the experiment as designed can not determine whether insulin was in any way responsible for the accentuated fractional distal antinatriuretic effect of ANGII seen in the diabetic group. One unexplained feature of the studies in both Chapters 6 and 7 is that despite comparable levels of sodium intake, as judged by 24h sodium excretion, baseline fractional sodium excretion during the acute study was always lower in the diabetic patients than the controls, particularly after overnight insulin infusion. It is tempting to speculate that insulin-mediated antinatriuresis at a distal tubular site in the diabetic patients might have contributed to this phenomenon. This argument is compatible with the finding that the antinatriuretic potential of insulin is preserved in Type 2 diabetic patients (Skott et al, 1991; Gans et al, 1991b), but the effect of insulin on sodium handling in Type 1 diabetes has not hitherto been reported.

The euglycaemic hyperinsulinaemic clamp technique was used primarily to look at the effects of insulin on the kidney, and a two hour insulin infusion is not long enough to be absolutely confident about interpreting insulin-mediated glucose disposal as an index of insulin sensitivity. However, since the dextrose infusion rate

at the end of the second hour of insulin infusion appeared to be close to or at a plateau, the comparable final dextrose requirement after both placebo and ACEI in the face of identical plasma free insulin levels suggests that pretreatment with perindopril for one week had not appreciably altered peripheral insulin sensitivity. Chronic converting enzyme inhibition with enalapril also has little or no effect on insulin sensitivity in normal man (Gans et al, 1991c) or in uncomplicated Type 1 diabetes (Seefeldt et al, 1990). It is not surprising that ACEI has no effect on insulin-mediated glucose disposal in nonobese normotensive young men in whom insulin sensitivity is likely to already be high, and this finding is not inconsistent with the potentially beneficial effects of converting enzyme inhibition on insulin-mediated glucose disposal into muscle in insulin-resistant states such as hypertension, heart failure, and obesity (DeFronzo and Ferranini, 1991).

In conclusion, this study shows that acute hyperinsulinaemia has a significant antinatriuretic and antikaliuretic effect in normal man, manifested predominantly at a tubular segment(s) distal to the proximal tubule. The antinatriuretic effect of insulin is not modified by pretreatment with an angiotensin converting enzyme inhibitor, implying that insulin-dependent sodium retention is not mediated by intrarenal angiotensin II generation.

CHAPTER TEN.

FINAL DISCUSSION AND CONCLUSIONS.

Coordinated interactions between many physical and humoral systems allow the kidney to maintain sodium homeostasis by modulating renal haemodynamics and tubular sodium handling in response to changing environmental stresses. In defining the causes of abnormal renal function, the assumption that plasma or urinary activities of humoral mediators reflect the balance between vasoconstrictor/antinatriuretic stimuli and vasodilator/natriuretic forces has several limitations. Dynamic measures of renal response are potentially much more informative. For most systems the definition of normality includes some consideration of the prevailing state of sodium balance; this is particularly true of the actions of the renin-angiotensin system examined in this work.

The aim of the studies was to examine the renal response to exogenous angiotensin II in Type 1 diabetes under conditions of controlled sodium intake, in order to resolve some of the conflicting evidence from previous studies in animal models and human patients. As well as the conceptual deficiencies of this experimental approach already mentioned, the technical limitations of the methods used for analyzing renal function *in vivo* must also be acknowledged, especially in out-patient studies when the precise state of whole body sodium balance is unknown, interindividual variation is often large, and extraneous influences may not be identifiable or

controllable. Measurement of total exchangeable body sodium might have helped in interpreting some of the results, but was not attempted because of the large technical errors involved in estimating this parameter in small groups of subjects who were being studied after a change in sodium balance without the benefit of metabolic ward facilities. Furthermore, since the results obtained in a clearance study represent the final outcome of numerous events in compartmentalised regions of the kidney, the pathophysiological significance of any abnormality should be interpreted cautiously. Exogenous angiotensin II for instance has access to pre- and postglomerular arterioles and to proximal and distal tubular epithelia from the luminal and the basolateral surface, and the present data can not add to the debate on the possible changes in tubuloglomerular feedback sensitivity in diabetes. Another problem with reactivity studies is that following an experimental intervention the compensatory adjustments in other mechanisms may modify the expression of changes in the index system, particularly if physiological manipulations leading to subtle changes are examined.

Increased systemic pressor reactivity to exogenous ANGII in diabetes has been regarded as sodium-dependent, as vascular reactivity is restored to control levels by diuretic treatment (Weidmann et al, 1979). In the present studies an increased systemic pressor response to

ANGII occurred only as an artefact following lithium pretreatment, the small difference in response between diabetic and control groups after placebo treatment not being statistically significant. This is not necessarily inconsistent with the previous evidence, because the current studies were performed after a period of high sodium intake. A comparison of the dose response curves for blood pressure in the present work with published data (Drury et al, 1984) obtained using very comparable infusion rates of ANGII in subjects taking a normal sodium intake is shown in Figure 10.1. This figure suggests that the different conclusion of the two sets of data lies in the control studies. The pressor response to ANGII is increased in the present control studies compared to the normal sample studied by Drury et al., indicating normal modulation of pressor reactivity to ANGII with increasing sodium intake. In contrast, the pressor response in the diabetic patients appears relatively fixed irrespective of the differing sodium intakes of the two study samples. It appears that systemic pressor reactivity is not modulated normally by sodium intake in diabetic subjects. The present data is incomplete because of the absence of measurements during both normal and low sodium intake to confirm this postulate, but failure of sodium restriction to modulate pressor responsiveness to ANGII has recently been demonstrated in Type 2 diabetic patients with and without

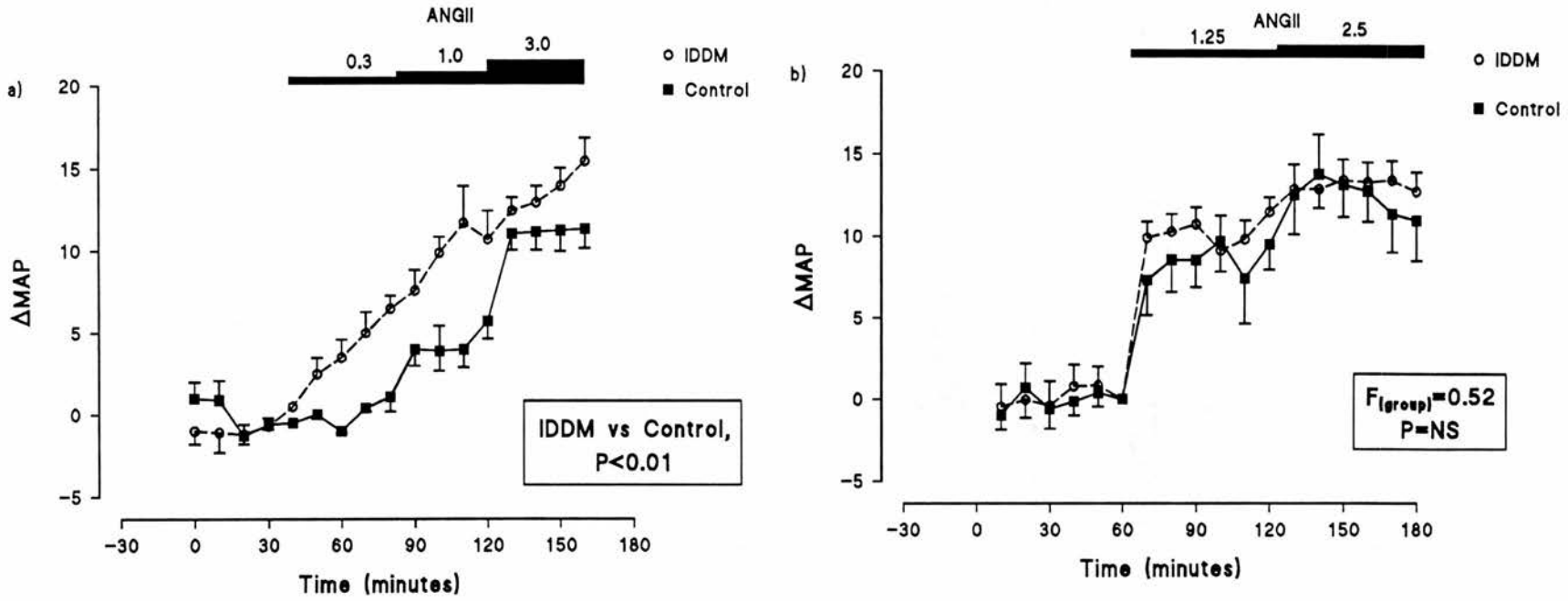

Figure 10.1: a) change in mean arterial pressure during ANGII infusion in 6 diabetic and 6 control subjects while on normal sodium intake (mean 160 mmol/day). Reproduced with permission of the Publisher from Drury et al, 1984.

b) change in MAP during ANGII infusion in 15 diabetic and 10 control subjects after high sodium intake (mean 300 mmol/day), (Figure 7.7a).

The different populations and slightly different infusion rates of ANGII preclude a direct comparison of a) and b), but the pressor response of diabetic patients appears relatively unaffected by high sodium intake. In the control subjects responsiveness is increased after sodium loading. See pp.242–3 for discussion.

hypertension (Tuck et al, 1990).

A rise in blood pressure (and renal perfusion pressure) is a central factor in permitting renal escape from various antinatriuretic stimuli, including chronic ANGII infusion. This has been used to support the existence of a primary renal defect in some hypertensive states, and may be a contributory factor to the intractable sodium retention in conditions where blood pressure is inappropriately low such as severe cardiac failure, cirrhosis and nephrotic syndrome. The selection criteria for the diabetic patients described in Chapter 7 included a blood pressure within the normal range for their age and sex, but a small increase in mean arterial pressure was nevertheless seen in comparison to a matched control group. This increase has been noted previously (Hommel et al, 1989). The higher blood pressure was not directly caused by sodium loading, as mean arterial pressure measured by the same semi-automated method while the patients and control subjects were taking their normal diet was not different, and remained higher in the diabetic group (IDDM 95.3(2.2) mmHg, control 88.6(2.1) mmHg; $P<0.05$). An increased blood pressure could be interpreted as a normal pressure-natriuresis response to an increase in total exchangeable sodium secondary to defective renal sodium handling, and is compatible with the correlation which exists between total exchangeable sodium and systolic or mean arterial pressure (Ferriss et

al, 1985; Feldt-Rasmussen et al, 1987; Hommel et al, 1990). However, this can not be tested because total exchangeable sodium was not measured. Additionally, several conflicting lines of evidence must also be considered. First, the fall in blood pressure which accompanies dietary sodium restriction in a proportion of Type 2 diabetic patients does not correlate with the rise in blood pressure during sodium loading in the same subjects (Tuck et al, 1990), indicating that different mechanisms may operate within the same patient depending on sodium intake. Second, large scale epidemiological evidence shows that the distribution of blood pressure in uncomplicated diabetic patients does not differ from the normal population (Norgaard et al, 1990), an increase in blood pressure only following the development of incipient nephropathy. Thirdly, total exchangeable sodium increases at a time when blood pressure is falling when glycaemic control is improved (O'Hare et al, 1982). Sodium retention may thus account for changes in cardiovascular responsiveness in diabetes, but is not the only factor modulating the final blood pressure.

There are also inconsistencies in existing data concerning the effect of changing glycaemic control on body sodium content. O'Hare et al (1982) studied a highly selected group of Type 2 diabetic patients who were being admitted to hospital for stabilisation of poorly controlled (mean HbA1 12.4%) but non-ketotic

diabetes. After a period of improved glycaemic control plasma volume and total exchangeable sodium increased above control levels, and there was a fall in plasma angiotensin II concentrations. A similar increase in extracellular fluid volume during short-term improved control occurs in normoalbuminuric Type 1 diabetes (Mathiesen et al, 1989). In contrast however, in Type 1 diabetic patients plasma atrial natriuretic peptide levels were higher and plasma renin activity lower in patients with poor glycaemic control (HbA1 >9%) (Bell et al, 1989), suggesting that extracellular volume expansion worsened with deteriorating glycaemic control. These reports vary in the populations studied and in the range of glycaemic control examined, only three subjects in the last study having a HbA1 greater than 11%. The greatest difference however is that the first two studies improved diabetic control by increasing exogenous insulin doses or by stimulating endogenous insulin production using oral sulphonylureas, and the results may simply reflect an acute antinatriuretic effect of insulin. Taken together the three sets of data are not inconsistent with a progressive expansion of extracellular volume as glycaemic control worsens up to an HbA_1 of 10%-11%, when sodium-glucose cotransport theoretically is at a maximum, followed by a reduction in total body sodium towards control levels if a marked deterioration in diabetic control (which is usually caused by insulinopenia)

produces glycosuric diuresis and distal tubular sodium losses.

Systemic factors may also promote sodium retention in some diabetic patients. Poor metabolic control, hypertension, and the presence of microangiopathy all independently increase the transcapillary escape rate of albumin in diabetic patients (O'Hare et al, 1983). In early diabetes improved metabolic control for one week normalises this microvascular leakage (Parving et al, 1976). This functional capillary defect may be caused by intracapillary hypertension, which is also aggravated by poor glycaemic control (Sandeman et al, 1991). Enhanced capillary permeability theoretically promotes movement of sodium and water from the intravascular to the interstitial space, leading to relative plasma volume depletion. Renal sodium retention is then an appropriate compensatory response restoring plasma volume to a normal level, the end result being plasma oncotic dilution and an increase in interstitial volume. There is no positive evidence that this sequence of events occurs in uncomplicated diabetes because plasma oncotic pressure is not reduced. However, plasma protein dilution and reduced intravascular oncotic pressure have been found in diabetic patients with established nephropathy and generalized oedema (Hommel et al, 1990). In these patients combination of a functional leak (capillary hypertension) with structural damage (microangiopathy)

may make the effect more relevant. A novel intrarenal mechanism has also been proposed (Pinter and Atkins, 1991), by which increased peritubular protein leakage may increase renal interstitial pressure, contributing to interstitial fibrosis (Thomsen et al, 1989) and glomerular injury.

The work in this thesis confirms that proximal renal tubular reabsorption of sodium is increased from an early stage of Type 1 diabetes. The abnormality is a functional effect of the diabetic state, directly related to the level of chronic diabetic control, and is not a secondary response to structural renal damage. The data are also consistent with the hypothesis that relative activation of the intrarenal renin-angiotensin system may play some part in causing this sodium retention. There is no direct evidence that the increase in renal sodium reabsorption during these experiments was not an appropriate response which maintained glomerulotubular balance in the face of a small increase in glomerular filtration rate, albeit still within the normal range. Because the majority of the diabetic patients had a normal glomerular filtration rate and renal plasma flow, the corollary is that abnormal renal sodium handling is unlikely to directly determine the development of glomerular hyperfiltration. However, the methods available in humans may not be sufficiently sensitive to detect small 'inappropriate' increases in tubular sodium

reabsorption due to for instance enhanced sodium-glucose cotransport or to hyperinsulinaemia, and the potential importance of these processes should not be discounted.

It is relevant at this stage to briefly reiterate the effects of glucose-mediated sodium retention at the cellular level. The induction of insulin-dependent diabetes in the rat is rapidly followed by an increase in the intracellular sodium content ($[Na]_i$) of proximal tubular cells, and a proportionate increase in Na^+-K^+-ATPase (Kumar et al, 1988). Furthermore, the tubules of chronically diabetic animals are adapted so as to maintain this higher $[Na]_i$ even in the presence of a normal extracellular glucose concentration. Thus changes in the intracellular electrolyte environment provide another pathway by which the diabetic state may influence protein kinase C activation and second messenger pathways to alter cellular reactivity, the regulation of cell pH, and the control of cellular hypertrophy.

The development of clinical nephropathy in Type 1 diabetes is a multifactorial process determined by the renal response to genetic and environmental factors. In the presence of as yet unidentified inherited influences (Seaquist et al, 1989), it is naive to automatically ascribe pathogenetic importance to individual functional abnormalities in early diabetes, unless this is also justified by evidence from long-term prospective studies. The abnormality in renal sodium handling in the present

studies correlated with the quality of chronic glycaemic control, but it is clearly illogical to suggest that the correlation between poor glycaemic control and the long-term risk of nephropathy *de facto* makes abnormal proximal renal sodium handling a risk factor for the development of diabetic nephropathy.

It is also important to recognise that the determinants and consequences of renal sodium retention are unlikely to be the same in different sub-groups of diabetic patients. The practical consequences of sodium retention in Type 1 diabetes are greatest in those patients who develop incipient or overt nephropathy, in whom the pathophysiological effects of microangiopathy and a falling nephron mass are superimposed on the functional defects already discussed. In these patients the secondary abnormalities which follow expansion of the extracellular sodium space, such as enhanced vascular reactivity and changes in intracellular electrolytes, will perpetuate the vicious cycle of oedema formation and volume-dependent hypertension which accelerates nephron loss and the progression to end stage renal failure. Therapeutic strategies which modify or abrogate the pathophysiological effects of sodium retention in this situation (Dodson et al, 1989) may therefore be of value in the management of diabetic hypertension and established chronic renal disease.

BIBLIOGRAPHY.

Abumrad NN, Rabin D, Diamond MP, Lacy WW. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics. *Metabolism* 1981;**30**:936-940.

Aiken JW, Vane JR. Intrarenal prostaglandin release attenuates the renal vasoconstrictor activity of angiotensin. *J. Pharmacol. Exp. Ther.* 1973;**184**:678-687.

Al-Barazanji KA, Balment RJ. The renal and vascular effects of central angiotensin II and atrial natriuretic factor in the anaesthetized rat. *J. Physiol.* 1990;**423**:485-493.

Ames RP, Borkowski AJ, Sicinski AM, Laragh JH. Prolonged infusions of angiotensin II and norepinephrine and blood pressure, electrolyte balance, and aldosterone and cortisol secretion in normal man and in cirrhosis with ascites. *J. Clin. Invest.* 1965;**44**:1171-86.

Anderson RJ, Berl T, McDonald KM, Schrier RW. Prostaglandins: effects on blood pressure, renal blood flow, sodium and water excretion. *Kidney Int.* 1976;**10**:205-215.

Anderson S, Rennke HG, Brenner BM. Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. *J. Clin. Invest.* 1986;**77**:1993-2000.

Anderson S, Brenner BM. Progressive renal disease: A disorder of adaptation. *Q. J. Med.* 1989;**70**:185-189.

Anton AH, Sayre DF. The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material. *J. Pharmacol. Exp. Ther.* 1964;**145**:326-336.

Arndt JO, Brambring P, Hindrof K, Rohnelt M. The afferent impulse traffic from atrial A-type receptors in cats. *Pfluegers Arch.* 1971;**326**:300-315.

Atchley DW, Loeb RF, Richards DW, Benedict EM, Driscoll ME. On diabetic acidosis. *J. Clin. Invest.* 1933;**12**:297-326.

Atherton JC, Green R, Hughes S, McFall V, Sharples JA, Solomon LR, Wilson L: Lithium clearance in man: effects of dietary salt intake, acute changes in extracellular fluid volume, amiloride and frusemide. *Clin. Sci.* 1987;**73**:645-651.

Atkins EL, Pearce JW. Mechanisms of the renal response to plasma volume expansion. *Can. J. Biochem. Physiol.* 1959;**37**:91-102.

August JT, Nelson DH, Thorn GW. Response of normal subjects to large amounts of aldosterone. *J. Clin. Invest.* 1958;**37**:1549-1555.

Bank N, Klose R, Aynedjian HS, Nguyen D, Sablay LB. Evidence against increased glomerular pressure initiating diabetic nephropathy. *Kidney Int.* 1987;**31**:898-905.

Bank N, Lahorra MAG, Aynedjian HS, Schlondorff D. Vasoregulatory hormones and the hyperfiltration of diabetes. *Am. J. Physiol.* 1988;**254**:F202-F209.

Ball SG, Lee MR. The effect of carbidopa administration on urinary dopamine excretion in man. Is dopamine an intrarenal natriuretic hormone? *Br. J. Clin. Pharmacol.* 1977;**4**:115-119.

Ball SG, Oates NS, Lee MR. Urinary dopamine in man and rat: effects of inorganic salts on dopamine excretion. *Clin. Sci. Mol. Med.* 1978;**55**:167-173.

Ballermann BJ, Skorecki KL, Brenner BM. Reduced glomerular angiotension II receptor density in early untreated diabetes mellitus in the rat. *Am. J. Physiol.* 1984;**247**:F110-F116.

Barajas L. Innervation of the renal cortex. *Fed. Proc.* 1978;**37**:1192-1201.

Barany FR. Abnormal vascular reaction in diabetes mellitus; clinical physiological study. *Acta Med. Scand.* 1955;**152**(Suppl.304):1-129.

Barnett R, Scharschmidt L, Ko Y-H, Schlondorff D. Comparison of glomerular and mesangial prostaglandin synthesis and glomerular contraction in two rat models of diabetes mellitus. *Diabetes* 1987;**36**:1468-1475.

Barraclough MA. Dose-dependent opposite effects of angiotensin on renal sodium excretion. *Lancet* 1965;**2**:987-988.

Bartoli E, Satta A, Faedda R, Olmeo NA, Soggia G, Branca GF. A furosemide test in the functional evaluation of the human nephron in vivo. *J. Clin. Pharmacol.* 1983;**23**:56-64.

Bartter FC, Mills IH, Biglieri EG, Delea C. Studies on the control and physiologic actions of aldosterone. *Recent Prog. Horm. Res.* 1959;**15**:311-335.

Baum M. Insulin stimulates volume absorption in the rabbit proximal convoluted tubule. *J. Clin. Invest.* 1987;**79**:1104-1109.

Bell PD, Thomas C, Williams RH, Navar LG. Filtration rate and stop-flow pressure feedback responses to nephron perfusion in the dog. *Am. J. Physiol.* 1978;**234**:F154-F165.

Bell PD, Navar LG. Stop-flow pressure feedback responses during reduced renal vascular resistance in the dog. *Am. J. Physiol.* 1979;**237**:F204-F209.

Bell GM, Reid W, Ewing DJ, Cumming AD, Watson ML, Doig A, Clarke BF. Abnormal diurnal urinary sodium and water excretion in diabetic autonomic neuropathy. *Clin. Sci.* 1987;**73**:259-265.

Bell GM, Bernstein RK, Laragh JH, Atlas SA, James GD, Pecker MS, Sealey JE. Increased plasma atrial natriuretic factor and reduced plasma renin in patients with poorly controlled diabetes mellitus. *Clin. Sci.* 1989;**77**:177-182.

Bello-Reuss E, Trevino DL, Gottschalk CW. Effect of renal sympathetic nerve stimulation on proximal sodium and water reabsorption. *J. Clin. Invest.* 1976;**57**:1104-1107.

Bellucci A, Wilkes BM. Mechanism of sodium modulation of glomerular angiotensin receptors in the rat. *J. Clin. Invest.* 1984;**74**:1593-1600.

Beretta-Piccoli C, Weidmann P, de Chatel R, Ziegler W, Gl≟ck Z, Keusch G. Plasma catecholamines and renin in diabetes mellitus. Relationships with posture, age, sodium, and blood pressure. *Klin. Wochenschr.* 1979;**57**:681-691.

Beretta-Piccoli C, Weidmann P, Keusch G. Responsiveness of plasma renin and aldosterone in diabetes mellitus. *Kidney Int.* 1981a;**20**:259-266.

Beretta-Piccoli C, Weidmann P. Exaggerated pressor responsiveness to norepinephrine in nonazotemic diabetes mellitus. *Am. J. Med.* 1981b;**71**:829-835.

Beretta-Piccoli C, Weidmann P. Body sodium-blood volume state in nonazotaemic diabetes mellitus. *Miner. Electrolyte Metab.* 1982a;**7**:36-47.

Beretta-Piccoli C, Weidmann P. Total plasma clearance of infused norepinephrine in non-azotemic diabetes mellitus. *Klin. Wochenschr.* 1982b;**60**:555-560.

Berglund J, Lins P-E, Adamson U, Lins L-E. Microalbuminuria in long-term insulin-dependent diabetes mellitus. Prevalence and clinical characteristics in a normotensive population. *Acta Med. Scand.* 1987;**222**:333-338.

Berk BC, Vekshtein V, Gordon HM, Tsuda T. Angiotensin II-stimulated protein synthesis in cultured vascular smooth muscle cells. *Hypertension* 1989;**13**:305-314.

Berkman J, Rifkin H. Unilateral nodular diabetic glomerulosclerosis (Kimmelstiel-Wilson): report of a case. *Metabolism* 1973;22:715-722.

Best JB, Coghlan JP, Bett JH, Cran EJ, Scoggins BA. Circulating angiotensin-II and aldosterone levels during dietary sodium restriction. *Lancet* 1971;**2**:1353-1359.

Bianchetti MG, Weidmann P, Beretta-Piccoli C, Rupp U, Boehringer K, Link L, Ferrier CF. Disturbed noradrenergic blood pressure control in normotensive members of hypertensive families. *Br. Heart J.* 1984;**51**:306-311.

Bianchi G, Fox U, Di Francesco GF, Giovanetti AM, Pagetti D. Blood pressure changes produced by kidney cross-transplantation between spontaneously hypertensive rats and normotensive rats. *Clin. Sci. Mol. Med.* 1978;**47**:435-448.

Bjorck S, Nyberg G, Mulec H, Granerus G, Herlitz H, Aurell M. Beneficial effects of angiotensin converting enzyme inhibition on renal function in patients with diabetic nephropathy. *Br. Med. J.* 1986;**293**:471-474.

Blaine EH, Davis JO, Witty RT. Renin release after hemorrhage and after suprarenal aortic constriction in dogs without sodium delivery to the macula densa. *Circ. Res.* 1970;**27**:1081-1090.

Blantz RC, Konnen KS, Tucker BJ. Angiotensin II effects upon the glomerular microcirculation and ultrafiltration coefficient of the rat. *J. Clin. Invest.* 1976;**57**:419-434.

Blantz RC, Peterson OW, Gushwa L, Tucker BJ. Effect of modest hyperglycaemia on tubuloglomerular feedback activity. *Kidney Int.* 1982;**22**(Suppl.12):S206-S212.

Blumenthal HT, Goldenberg S, Berns AW. Pathology and pathogenesis of the disseminated angiopathy of diabetes mellitus: on the nature and treatment of diabetes. In *Excerpta Med.*, eds. BS Leibel and GA Wrenshall, Amsterdam, 1965. Int. Congr. Ser. no. 84, pp.397-408.

Boer WH, Joles JA, Koomans HA, Doorhout Mees EJ. Decreased lithium clearance due to distal tubular lithium reabsorption in sodium-depleted dogs. *Renal Physiol.* 1987;**10**:65-68.

Boer WH, Koomans HA, Dorhout Mees EJ. Renal lithium handling during water loading and subsequent d-DAVP-induced anti-diuresis. *Eur. J. Clin. Invest.* 1988a;**18**:273-278.

Boer WH, Koomans HA, Dorhout Mees EJ, Gaillard CA, Rabelink AJ. Lithium clearance during variations in sodium intake in man: Effects of sodium restriction and amiloride. *Eur. J. Clin. Invest.* 1988b;**18**:279-283.

Boer WH, Koomans HA, Dorhout Mees EJ. Lithium clearance in healthy humans suggesting lithium reabsorption beyond the proximal tubules. *Kidney Int.* 1990;**37**(Suppl.28):S39-S44.

Borch-Johnsen K, Andersen PK, Deckert T. The effect of proteinuria on relative mortality in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1985;**28**:590-596.

Bourgoignie JJ, Hwang KH, Ipakchi E, Bricker NS. The presence of a natriuretic factor in urine of patients with chronic uraemia. *J. Clin. Invest.* 1974;**53**:1559-1567.

Bourgoignie JJ, Gavellas G, Martinez E, Pardo V. Glomerular function and morphology after renal mass reduction in dogs. *J. Lab. Clin. Med.* 1987;**109**:380-388.

Bovee KC, Kronfeld DS, Ramberg CS, Goldscmidt M. Long-term measurement of renal function in partially nephrectomized dogs fed 56, 27 or 19% protein. *Invest. Urol.* 1979;**16**:378-384.

Bras G. Age-associated kidney lesions in the rat. *J. Infect. Dis.* 1969;**120**:131-135.

Brenner BM, Falchuk KH, Keimowitz RI, Berliner RW. The relationship between peritubular capillary protein concentration and fluid reabsorption by the renal proximal tubule. *J. Clin. Invest.* 1969;**48**:1519-1531.

Brenner BM, Humes HD. Mechanics of glomerular ultrafiltration. *New Engl. J. Med.* 1977;**297**:148-154.

Brenner BM, Meyer TW, Hostetter TH. Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury

in the pathogenesis of glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *New Engl. J. Med.* 1982;**307**:652-659.

Brenner BM, Nephron adaptation to renal injury or ablation. *Am. J. Physiol.* 1985;**249**:F324-F337.

Bridges RJ, Cragoe Jr EJ, Frizzell RA, Benos DJ. Inhibition of colonic Na^+ transport by amiloride analogues. *Am. J. Physiol.* 1989;**256**:C67-C74.

Brier M, Aronoff G, Luft F. Sodium kinetics in normotensive white and black males. *J. Am. Soc. Nephrol.* 1991;**2**:487.

Brochner-Mortensen J, Stockel M, Sorensen PJ, Nielsen AH, Ditzel J. Proximal glomerulo-tubular balance in patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1984;**27**:189-192.

Brown D, Sorchser EJ, Ausiello DA, Benos DJ. Immunocytochemical localization of Na^+ channels in rat kidney medulla. *Am. J. Physiol.* 1989;**256**:F366-F369.

Brown GP, Douglas JG. Angiotensin II binding sites on isolated rat renal brush border membranes. *Endocrinology* 1982;**111**:1830-1836.

Brown GP, Douglas JG. Angiotensin II binding sites in the rat and primate isolated renal tubular basolateral membranes. *Endocrinology* 1983;**112**:2007-2014.

Brown MJ, Allison DJ. Renal conversion of plasma dopa to urine dopamine. *Br. J. Clin. Pharm.* 1981;**12**:251-253.

Bruun NE, Skott P, Lonborg-Jensen H, Giese J. Unchanged lithium clearance during acute amiloride treatment in sodium-depleted man. *Scand. J. Lab. Clin. Invest.* 1989;**49**:259-263.

Buckalew VM, Nelson DB. Natriuretic and sodium transport inhibitory activity in plasma of volume expanded dogs. *Kidney Int.* 1974;**5**:12-22.

Burden AC, Thurston H. Plasma renin activity in diabetes mellitus. *Clin. Sci.* 1979;**56**:255-259.

Burg M, Patlak C, Green N, Villey D. Organic solutes in fluid absorption by renal proximal convoluted tubules. *Am. J. Physiol.* 1976;**231**:627-637.

Burnett JC Jr, Knox FG. Renal interstitial pressure and sodium excretion during renal vein constriction. *Am J. Physiol.* 1980;**238**:F279-F282.

Cambier P. Application de la theorie de Rehberg a l'etude clinique des affections renales et du diabete. *Annales de Medecine* 1934;**35**:273-299.

Carey RM. Evidence for a splanchnic sodium input monitor regulating renal sodium excretion in man. Lack of dependence upon aldosterone. *Circ. Res.* 1978;**43**:19-23.

Carey RM. Angiotensin as a paracrine substance in the kidney. In *Cell to Cell Communication in Endocrinology*. eds. F Piva, CW Bardin, G Forti, M Motta. Raven Press, New York, 1988. pp.257-266.

Carmines PK, Perry MD, Hazelrig JB, Navar LG: Effects of preglomerular and postglomerular vascular resistance alterations on filtration fraction. *Kidney Int.* 1987;**31**(Suppl20): S229-S232.

Casson IF, Lee MR, Brownjohn AM, Parsons FM, Davison AM, Will EJ, Clayden AD. Failure of renal dopamine response to salt loading in chronic renal disease. *Br. Med. J.* 1983;**286**:503-506.

Catalano C, Winocour PH, Thomas TH, Rodham D, Wilkinson R. Erythrocyte sodium-lithium counter-transport, exchangeable sodium, and T_mPO_4 in normoalbuminuric Type 1 diabetes. *Diabetologia* 1991;**34**(Suppl.2):A68.

Chan YL. Cellular mechanisms of renal tubular transport of L-Dopa and its derivatives in the rat: microperfusion studies. *J. Pharm. Exp. Ther.* 1976;**199**:17-24.

Chan YL. The role of norepinephrine in the regulation of fluid absorption in the rat proximal tubule. *J. Pharmacol. Exp. Ther.* 1980;**215**:65-70.

Chavers BM, Bilous RW Ellis EN, Steffes MW, Mauer SM. Glomerular lesions and urinary albumin excretion in type 1 diabetes without overt proteinuria. *New. Engl. J. Med.* 1989;**320**:966-970.

Chinn RH, Dusterdieck G. The response of blood pressure to infusion of angiotensin II: relation to plasma concentrations of renin and angiotensin II. *Clin. Sci.* 1972;**42**:489-504.

Christensen NJ. Plasma catecholamines in long-term diabetics with and without neuropathy and in hypophysectomized subjects. *J. Clin. Invest.* 1972;**51**:779-787.

Christensen NJ. Catecholamines and diabetes mellitus. *Diabetologia* 1979;**16**:211-224.

Christiansen JS, Gammelgaard J, Frandsen M, Parving H-H. Increased kidney size, glomerular filtration rate, and renal plasma flow in short-term insulin-dependent diabetics. *Diabetologia* 1981a;**20**:451-456.

Christiansen JS, Frandsen M, Parving H-H. Effect of intravenous glucose infusion on renal function in normal man and in insulin-dependent diabetics. *Diabetologia* 1981b;**21**:368-373.

Christiansen JS, Frandsen M, Parving H-H. Effect of intravenous insulin infusion on kidney function in insulin-dependent diabetes mellitus. *Diabetologia* 1981c;**20**:199-204.

Christiansen JS, Gammelgaard J, Tronier B, Svensen PA, Parving H-H. Kidney function and size in diabetics before and during initial insulin treatment. *Kidney Int.* 1982;**21**:683-688.

Christiansen JS, Feldt-Rasmussen B, Parving H-H. Short-term inhibition of prostaglandin synthesis has no effect on the elevated glomerular filtration rate of early insulin-dependent diabetes. *Diabetic Med.* 1985;**2**:17-20.

Christlieb AR, Janka H-U, Kraus B, Gleason RE, Icasas-Cabral EA, Aiello LM, Cabral BV, Solano A. Vascular reactivity to angiotensin II and to norepinephrine in diabetic subjects. *Diabetes* 1976;**25**:268-274.

Cohen AJ, McCarthy DM, Stoff JS. Direct haemodynamic effect of insulin in the isolated perfused kidney. *Am. J. Physiol.* 1989;**257**:F580-F585.

Cohen D, Dodds R, Viberti GC. Effect of protein restriction in insulin dependent diabetics at risk of nephropathy. *Br. Med. J.* 1987;**294**:795-798.

Connell JMC, Ding Y-A, Fisher BM, Frier BM, Semple PF. Reduced number of angiotensin II receptors on platelets in insulin-dependent diabetes. *Clin. Sci.* 1986;**71**:217-220.

Connell JMC, Tonolo G, Davies DL et al. Dopamine affects angiotensin II-induced steroidogenesis by altering clearance of the peptide in man. *J. Endocrinol.* 1987;**113**:139-146.

Coruzzi P, Biggi A, Musiari L. Ravanetti C, Vescovi PP, Novarini A. Dopamine blockade and natriuresis during water immersion in normal man. *Clin. Sci.* 1986;**70**:523-526.

Cottier C, Matter L, Weidmann P, Shaw S, Gn╠dinger MP. Renal response to low-dose infusion of atrial natriuretic peptide in normal man. *Kidney Int.* 1988;**34**(Suppl.25);S72-S78.

Cotunnius D. *De Ischiade Nervosa.* Vienna 1770.

Cowie CC, Port FK, Wolfe RA, Savage PJ, Moll PP, Hawthorne VM. Disparities in incidence of diabetic end-stage renal disease according to race and type of diabetes. *New Engl. J. Med.* 1989;**321**:1074-1079.

Crane RK. The gradient hypothesis and other models of carrier-mediated active transport. *Rev. Physiol. Biochem. Pharmacol.* 1977;**78**:99-159.

Critchley JAJH, Cooper ES, Boye GL, Johnston NR, Samson RR, Lee MR. Failure of negroes to increase urine dopamine following an oral sodium load. *Br. J. Clin. Pharmacol.* 1987;**23**:113P-114P.

Cruickshanks KJ, Orchard TJ, Becker DJ. The cardiovascular risk profile of adolescents with insulin-dependent diabetes mellitus. *Diabetes Care* 1985;**8**:118-124.

Cupples WA, Marsh DJ. Autoregulation of blood flow in renal medulla of the rat: no role for angiotensin II. *Can. J. Physiol. Pharmacol.* 1988;**66**:833-836.

Dalton RN, Wiseman MJ, Turner C, Viberti G. Measurement of urinary para-aminohippuric acid in glycosuric diabetics. *Kidney Int.* 1988;**34**:117-120.

Daly JJ, Roe JW, Horrocks P. A comparison of sodium excretion following the infusion of saline into systemic and portal veins in the dog: Evidence for a hepatic role in the control of sodium excretion. *Clin. Sci.* 1967;**33**:481-487.

Davis JO, Freeman RH. Mechanisms regulating renin release. *Physiol. Rev.* 1976;**56**:1-56.

Dawborn JK. Applications of Heyrovsky's inulin method to automatic analysis. *Clin. Chim. Acta* 1965;**12**:63-66.

de Chatel R, Weidmann P, Flammer J, Ziegler WH, Beretta-Piccoli C, Vetter W, Reubi FC. Sodium, renin, aldosterone, catecholamines and blood pressure in diabetes mellitus. *Kidney Int.* 1977;**12**:412-421.

de Chatel R, Toth M, Barna I. Exchangeable body sodium: its relationship with blood pressure and atrial natriuretic factor in patients with diabetes mellitus.

J. Hypertens. 1986;**4**(Suppl.6):526-528.

Deckert T, Poulsen JE, Larsen M. Prognosis of diabetics with diabetes onset before the age of thirty-one. I. Survival, causes of death, and complications. *Diabetologia* 1978;**14**:363-370.

Deckert T, Anderson AR, Christiansen JS, Andersen JA. Course of diabetic nephropathy. Factors related to development. *Acta Endocrinol.* 1981a;**97**(Suppl.242):14-15.

Deckert T, Poulsen JE. Diabetic nephropathy: Fault or destiny? *Diabetologia* 1981b;**21**:178-183.

DeFronzo RA, Cooke CR, Andres R, Faloona GR, Davis PJ. The effects of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. *J. Clin. Invest.* 1975;**55**:845-855.

DeFronzo RA, Goldberg M, Agus ZS. The effects of glucose and insulin on renal electrolyte transport. *J. Clin. Invest.* 1976;**58**:83-90.

DeFronzo RA, Sherwin RS, Dillingham M, Hendler R, Tamborlane WV, Felig P. Influence of basal insulin and glucagon secretion on potassium and sodium metabolism. *J. Clin. Invest.* 1978;**61**:472-479.

DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin resistance and secretion. *Am. J. Physiol.* 1979;**237**:E214-E223.

DeFronzo RA. The effect of insulin on renal sodium metabolism. *Diabetologia* 1981;**21**:165-171.

DeFronzo RA, Ferrannini E. Insulin resistance. *Diabetes Care* 1991;**14**:173-194.

DeLeiva A, Christlieb AR, Melby JC, Graham CA, Day RP, Luetscher JA, Zager PG. Big renin and biosynthetic defect of aldosterone in diabetes mellitus. *New Engl. J. Med.* 1976;**295**:639-643.

Dev B, Drescher C, Schnermann J. Resetting of tubulo-glomerular feedback sensitivity by dietary salt intake. *Pfluegers Arch.* 1974;**346**:263-277.

DeWardener HE, Mills IH, Clapham WF, Hayter CJ. Studies on the efferent mechanism of the sodium diuresis which follows the administration of intravenous saline in the dog. *Clin. Sci.* 1961;**21**:249-258.

DeWardener HE. The control of sodium excretion. *Am. J. Physiol.* 1978;**235**:F163-F173.

DeWardener HE, MacGregor GA. The relation of a circulating sodium transport inhibitor (the natriuretic hormone?) to hypertension. *Medicine (Baltimore)* 1983;**62**:310-326.

DiBona GF, Sawin LL. Renal nerves in renal adaptation to dietary sodium restriction. *Am. J. Physiol.* 1983;**245**:F322-F328.

Ditzel J, Schwartz M. Abnormally increased glomerular filtration rate in short-term insulin-treated diabetic subjects. *Diabetes* 1967;**16**:264-267.

Ditzel J, Junker K. Abnormal glomerular filtration rate, renal plasma flow, and protein excretion in recent and short-term diabetics. *Br. Med. J.* 1972;**2**:13-19.

Dodson PM, Beevers M, Hallworth R, Webberley MJ, Fletcher RF, Taylor KG. Sodium restriction and blood pressure in hypertensive type II diabetics: Randomised blind controlled and crossover studies of moderate sodium restriction and supplementation. *Br. Med. J.* 1989;**298**:227-230.

Douglas JG, Romero M, Hopfer U: Signaling mechanisms coupled to the angiotensin receptor of proximal tubular epithelium. *Kidney Int.* 1990;**38**(Suppl 30):S43-S47.

Drake CR, Carey RM. Dopamine modulates sodium-dependent aldosterone responses to angiotensin II in humans. *Hypertension* 1984;**6**(Suppl.I):I.119-I.123.

Drury PL, Smith GM, Ferriss JB. Increased vasopressor responsiveness to angiotensin II in uncomplicated type 1 (insulin-dependent) diabetes. *Diabetologia* 1984;**27**:174-179.

Drury PL, Watkins PJ, Viberti GC, Walker JD. Diabetic nephropathy. *Br. Med. Bull.* 1989;**45**:127-147.

Dunham PB, Senyk O. Lithium efflux through the Na/K pump in human erythrocytes. *Proc. Natl. Acad. Sci.* 1977;**74**:3099-3103.

Dunn MJ. Renal prostaglandins: Influences on excretion of sodium and water, the renin-angiotensin system, renal blood flow, and hypertension. In *Contemporary Issues in Nephrology,* Vol 4., eds. B.M. Brenner and J.H. Stein. Churchill Livingstone, New York, 1979, pp.89-114.

Dunn BR, Ichikawa I, Pfeffer JM, Troy JL, Brenner BM. Renal and systemic hemodynamic effects of synthetic atrial natriuretic peptide in the anaesthetized rat. *Circ. Res.* 1986;**59**:237-246.

Edwards RM. Segmental effects of norepinephrine and angiotensin II on isolated renal microvessels. *Am. J. Physiol.* 1983:**244**:F526-F534.

Ejarque P, Marble A, Tuller EF. Proteins, lipoproteins and protein-bound carbohydrates in the serums of diabetic patients. *Am. J. Med.* 1959;**27**:221-230.

Ellis EN, Steffes MW, Goetz FC, Sutherland DER, Mauer SM. Glomerular filtration surface in type 1 diabetes mellitus. *Kidney Int.* 1986;**29**:889-894.

Epstein FH, Post RS, McDowell M. The effect of an arteriovenous fistula on renal haemodynamics and electrolyte excretion. *J. Clin. Invest.* 1953;**32**:233-241.

Epstein M, Bricker NS, Bourgoignie JJ. Presence of a natriuretic factor in urine of normal men undergoing water immersion. *Kidney Int.* 1978;**13**:152-158.

Esmatjes E, Fernandez MR, Halperin I, Camps J, Gaya J, Arroyo V, Rivera F, Figuerola D. Renal haemodynamic abnormalities in patients with short term insulin-dependent diabetes mellitus: Role of renal prostaglandins. *J. Clin. Endocrinol. Metab.* 1985;**60**:1231-1236.

Evanoff GV, Thompson CS, Brown J Weinmann EJ. The effect of dietary protein restriction on the progression of diabetic nephropathy: A 12-month follow-up. *Arch. Intern. Med.* 1987;**147**:492-495.

Fadem SZ, Hernandez-Llamas G, Patak RV, Rosenblatt SG, Lifschitz MD, Stein JH. Studies on the mechanism of sodium excretion during drug-induced vasodilatation in the dog. *J. Clin. Invest.* 1982;**69**:604-610.

Fahr T. Ueber Glomerulosklerose. *Virchows Archiv. fur Pathologische Anatomie und Physiologie und fur klinische Medizin* 1942;**309**:16-33.

Feldt-Rasmussen B, Mathiesen ER, Deckert T. Effects of two years of strict metabolic control on progression of incipient nephropathy in insulin-dependent diabetes. *Lancet* 1986;**2**:1300-1304.

Feldt-Rasmussen B, Mathiesen ER, Deckert T, Giese J, Christensen NJ, Bent-Hausen L, Nielsen MD. Central role for sodium in the pathogenesis of blood pressure changes independent of angiotensin II, aldosterone and catecholamines in type 1 diabetes mellitus. *Diabetologia* 1987;**30**:610-617.

Fernandez-Cruz A Jr, Noth RH, Lassman MN, Hollis JB, Mulrow PJ. Low plasma renin activity in normotensive patients with diabetes mellitus: relationship to neuropathy. *Hypertension* 1981;**3**:87-92.

Ferranini AM, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, Pedrinelli R, Brandi L, Bevilacqua S. Insulin resistance in essential hypertension. *New Engl. J. Med.* 1987;**317**:350-357.

Ferriss JB, Sullivan PA, Gonggrijp H, Cole M, O'Sullivan DJ. Plasma angiotensin II and aldosterone in unselected diabetic patients. *Clin. Endocrinol.* 1982;**17**:261-269.

Ferriss JB, O'Hare JA, Kelleher CCM, Sullivan PA, Cole MM, Ross HF, O'Sullivan DJ. Diabetic control and the renin-angiotensin system, catecholamines, and blood pressure. *Hypertension* 1985;**7**(Suppl. II):II.58-II.63.

Fidelman ML, Watlington CO. Insulin and aldosterone interaction on NA^+ and K^+ transport in cultured kidney cells (A6). *Endocrinology* 1984;**115**:1171-1178.

Fine LG, Badie-Dezfooly B, Lowe AG, Hamzeh A, Wells J, Salehmoghaddam S. Stimulation of Na^+/H^+ antiport is an early event in hypertrophy of renal proximal tubular cells. *Proc. Natl. Acad. Sci. USA* 1985;**82**:1736-1740.

Fine LG. How little kidney tissue is enough? *N. Engl. J. Med.* 1991;**325**:1097-1099.

Fioretto P, Sambatoro M, Cipollina MG, Duner E, Giorato C, Morocutti A et al. Impaired response to angiotensin II in Type 1 (insulin-dependent) diabetes mellitus. Role of prostaglandins and sodium-lithium countertransport activity. *Diabetologia* 1991;**34**:595-603.

Flamenbaum W, Gagnon J, Ramwell P. Bradykinin-induced renal haemodynamic alterations: renin and prostaglandin relationships. *Am. J. Physiol.* 1979;**237**:F433-F440.

Fogo A, Kawamura T, Ikoma M, Kon V, Ichikawa I. Role for the mesangium in glomerular neutrophil entrapment. *Kidney Int.* 1990;**37**:551.

Folkow B. The haemodynamic consequences of adaptive structural changes of the resistance vessels in hypertension. *Clin. Sci.* 1971;**41**:1-12.

Fournier AM, Gadia MT, Kubrusly DB, Skyler JS, Sosenko JM. Blood pressure, insulin and glycaemia in nondiabetic subjects. *Am. J. Med.* 1986;**80**:861-864.

Francis MJO, Ellis JP, Hockaday TDR. Skin collagen in diabetes mellitus in relation to treatment. *Proc. Royal. Soc. Med.* 1974;**67**:35-36.

Freestone S, Jeffrey RF, Bonner CV, Lee MR: Effect of lithium on the renal actions of ∝-human atrial natriuretic peptide in normal man. *Clin. Sci.* 1990;**78**:371-375.

Frega NS, Weinberg JM, Ross BD, Leaf A. Stimulation of sodium transport by glucose in the perfused rat kidney. *Am. J. Physiol.* 1977;**233**:F235-240.

Fries JU, Sandstrom D, Meyer TW, Rennke HG. Glomerular hypertrophy and epithelial cell injury are determinants of progressive glomerulosclerosis in the rat. *Kidney Int.* 1988;**33**:374.

Gans ROB, Toorn L, Bilo HJG, Nauta JJP, Heine RJ, Donker AJM. Renal and cardiovascular effects of exogenous insulin in healthy volunteers. *Clin. Sci.* 1991a;**80**:219-225.

Gans ROB, Bilo HJG, Heine RJ, Donker AJM. Hyperinsulinaemia: an unlikely mechanism for hypertension associated with non-insulin dependent diabetes mellitus (NIDDM). *J. Am. Soc. Nephrol.* 1991b;**2**:490.

Gans ROB, Bilo HJG, Nauta JJP, Popp-Snijders C, Heine RJ, Donker AJM. The effect of angiotensin-1 converting enzyme inhibition on insulin action in healthy volunteers. *Eur. J. Clin. Invest.* 1991c;**21**:527-533.

Garvin JL. Angiotensin stimulates glucose and fluid reabsorption by rat proximal straight tubules. *J. Am. Soc. Nephrol.* 1990;**1**:272-277.

Gassee JP, Du Bois R, Staroukine M, Lambert PP. Determination of glomerular intracapillary and transcapillary pressure gradients from sieving data: 3. The effects of angiotensin II. *Pflugers Arch.* 1976;**367**:15-24.

Gauer OH, Henry JP, Sieker HO, Wendt WE. The effect of negative pressure breathing on urine flow. *J. Clin. Invest.* 1954;**33**:287-296.

Gauer OH, Henry JP, Behn C. The regulation of extracellular fluid volume. *Ann. Rev. Physiol.* 1970;**32**:547-595.

Gauer OH, Henry JP. Neurohumoral control of plasma volume. *Int. Rev. Physiol.* 1976;**9**:145-190.

Giebisch G, Klose RM, Windhager EE. Micropuncture study of hypertonic sodium chloride loading in the rat. *Am. J. Physiol.* 1964;**206**:687-693.

Gilmore JP, Zucker IH. Contribution of vagal pathways to the renal responses to head-out immersion in the nonhuman primate. *Circ. Res.* 1978;**42**:263-267.

Gilmore JP, Cornish KG, Rogers SD, Joyner WL. Direct evidence for myogenic autoregulation of the renal microcirculation in the hamster. *Circ. Res.* 1980;**47**:226-230.

Girbes ARJ, Smit AJ, Meijer S, Reitsma WD: Lack of effect of lithium on the renal response to DA_1-dopamine receptor stimulation by fenoldopam in normal man. *Br. J. Clin. Pharmacol.* 1990;**29**:413-415.

Goetz KL, Hermreck AS, Slick GL, Starke HS. Atrial receptors and renal function in conscious dogs. *Am. J. Physiol.* 1970;**219**:1417-1423.

Goldblatt H, Lynch J, Hanzal RF, Summerville WW. Studies on experimental hypertension; production of persistent elevation of systolic blood pressure by means of renal ischaemia. *J. Exp. Med.* 1934;**59**:347-379.

Goldberg LI. Dopamine receptors and hypertension: physiologic and pharmacological implications. *Am. J. Med.* 1984;**77**(Suppl.4A):37-44.

Gordon MB, Moore TJ, Dluhy RG, Williams GH. Dopaminergic modulation of aldosterone responsiveness to angiotensin II with changes in sodium intake. *J. Clin. Endocrinol. Metab.* 1983;**56**:340-345.

Gotsche O, Gundersen HJG, Osterby R. Renal hypertrophy and glomerular basement membrane accumulation in early experimental diabetes - effect of islet transplantation. *Diabetes* 1981;**30**;481-485.

Gottschalk CW, Moss NG, Colindres RE. Neural control of renal function in health and disease. In, *The Kidney: Physiology and Pathophysiology*, eds. DW Seldin and G. Giebisch. Raven Press, New York, 1985. pp.581-611.

Green R, Windhager EE, Giebisch G. Protein oncotic pressure effects on proximal tubular movement in the rat. *Am. J. Physiol.* 1974;**226**:265-276.

Grez MM. The influence of antibodies against bradykinin on isotonic saline diuresis in the rat. *Pfluegers Arch.* 1974;**350**:231-239.

Grez MM, Schaechtelin G, Bonner G. Relationship between the renal kallikrein activity and the urinary excretion of kallikrein in rats. *Experimentia* 1982;**38**:941-943.

Gross F. Effects of aldosterone on blood pressure, water, and electrolytes. In *Handbook of Experimental Pharmacology. Angiotensin*, eds. IH Page and FM Bumpus. Springer-Verlag, Berlin, 1974. pp.369-399.

Gross P, Reinhard C, Grez MM, Fink E, Sauer H. Lithium treatment reduces the renal kallikrein excretion rate. *Kidney Int.* 1990;**38**:124-128.

Guyton AC, Scanlon LJ, Armstrong GG. Effect of pressoreceptor reflex and Cushing reflex on urinary output. *Fed. Proc.* 1952;**11**:61A.

Haak T, Jungmann E, Rosak C, Schwab N, Fassbinder W, Althoff P-H, Schoffling K. Evidence for decreased efficacy of human atrial natriuretic peptide in patients with Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1986;**29**:544A-545A.

Haber E, Koerner T, Page LB, Kliman B, Purnode A. Application of a radioimmunoassay for angiotensin I to the physiologic measurement of plasma renin activity in normal human subjects. *J. Clin. Endocrinol. Metab.* 1969;**29**:1349-55.

Haberle DA, Davis JM. Resetting of tubuloglomerular feedback: evidence for a humoral factor in tubular fluid. *Am. J. Physiol.* 1984;**246**:F495-F500.

Hall JE, Granger JP, Smith MJ, Premen AJ. Role of renal haemodynamics and arterial pressure in aldosterone "escape". *Hypertension* 1984a;**6**(Suppl.I):I.183-I.192.

Hall JE, Granger JP, Hester RL, Coleman TG, Smith MJ, Cross RB. Mechanisms of escape from sodium retention during angiotensin II hypertension. *Am. J. Physiol.* 1984b;**246**:F627-F634.

Hall JE: Control of sodium excretion by angiotensin II: Intrarenal mechanisms and blood pressure regulation. *Am. J. Physiol.* 1986;**250**:R960-R972.

Hall JE, Granger JP. Renal hemodynamics and arterial pressure during chronic intrarenal adenosine infusion in conscious dogs. *Am. J. Physiol.* 1986b;**250**:F32-F39.

Hall JE, Coleman TG, Mizelle HL, Smith MJ. Chronic hyperinsulinaemia and blood pressure regulation. *Am. J. Physiol.* 1990;**258**:F722-F731.

Hamlyn JM, Blaustein MP. Sodium chloride, extracellular fluid volume, and blood pressure regulation. *Am. J. Physiol.* 1986;**251**:F563-F575.

Hannedouche TP, Delgado AG, Gnionsahe DA, Boitard C, Lacour B, Grunfeld J-P. Renal haemodynamics and segmental tubular reabsorption in early type 1 diabetes. *Kidney Int.* 1990a;**37**:1126-1133.

Hannedouche T, Natov S, Ikeni A, Schmitt F, Lacour B, Grunfeld J-P. Does lithium affect renal sodium handling and renal haemodynamics in normal man? *Nephrol. Dial. Transplant.* 1990b;**5**:1007-1012.

Hannedouche T, Lazaro M, Delgado AG, Boitard C, Lacour B, Grunfeld J-P. Feedback-mediated reduction in glomerular filtration during acetazolamide infusion in insulin-dependent diabetic patients. *Clin. Sci.* 1991;**81**:457-464.

Harris PJ, Young JA. Dose-dependent stimulation and inhibition of proximal tubular sodium reabsorption by angiotensin II in the rat kidney. *Pfluegers Arch.* 1977;**367**:295-301.

Harris RC, Brenner BM, Seifert JL. Sodium-hydrogen exchange and glucose transport in renal microvillus membrane vesicles from rats with diabetes mellitus. *J. Clin. Invest.* 1986;**77**:724-733.

Harvey JN, Casson IF, Clayden AD, Cope GF, Perkins CM, Lee MR. A paradoxical fall in urine dopamine output when patients with essential hypertension are given added dietary salt. *Clin. Sci.* 1984;**67**:83-88.

Harvey JN, Jaffa AA, Margolius HS, Mayfield RK. Renal kallikrein and hemodynamic abnormalities of diabetic kidney. *Diabetes* 1990;**39**:299-304.

Harvey RB, Brothers AJ. Renal extraction of para-aminohippurate and creatinine measured by continuous in vivo sampling of arterial and renal vein blood. *Ann. N. Y. Acad. Sci.* 1962;**102**:46-54.

Hasslacher Ch., Stech W, Wahl P, Ritz E. Blood pressure and metabolic control as risk factors for nephropathy in Type 1 (insulin-dependent) diabetes. *Diabetologia* 1985;**28**:6-11.

Hayslett JP, Kashgarian M. A micropuncture study of the renal handling of lithium. *Pfluegers Arch.* 1979;**380**:159-163.

Hecht B, Kashgarian M, Forrest JN Jr, Hayslett JP. Micropuncture study on the effects of lithium on proximal and distal tubule function in the rat kidney. *Pfluegers Arch.* 1978;**377**:69-74.

Henrich WL, Anderson RJ, Bernes AS, McDonald KN, Paulsen PJ, Berl T, Schrier RW. The role of renal nerves and prostaglandins in control of renal haemodynamics and plasma renin activity during hypotensive hemorrhage in the dog. *J. Clin. Invest.* 1978a;**61**:744-750.

Henrich WL, Berl T, McDonald KM, Anderson RJ, Schrier RW. Angiotensin II, renal nerves, and prostaglandins in renal haemodynamics during hemorrhage. *Am. J. Physiol.* 1978b;**325**:F46-F51.

Hirose K, Tsuchida H, Osterby R, Gundersen HJG. A strong correlation between glomerular filtration rate and filtration surface in diabetic kidney hyperfunction. *J. Lab. Invest.* 1980;**43**:434-437.

Holzgreve H, Schrier RW. Variation of proximal tubular reabsorptive capacity by volume expansion and aortic constriction during constancy of peritubular capillary plasma concentration in rat kidney. *Pfluegers Arch.* 1975;**356**:73-86.

Hollenberg NK, Chenitz WR, Adams DF, Williams GH. Reciprocal influence of salt intake on adrenal glomerulosa and renal vascular responses to angiotensin II in normal man. *J. Clin. Invest.* 1972;**54**:34-42.

Hollenberg NK. Set point for sodium homeostasis: surfeit, deficit, and their implications. *Kidney Int.* 1980;**17**:423-429.

Hollenberg NK. Intrarenal and systemic actions of the renin-angiotensin system. Implications for renal excretory function and sodium homeostasis. *Contrib. Nephrol.* 1984:**43**:102-113.

Holstein-Rathlou N-H. Lithium transport across biological membranes. *Kidney Int.* 1990;**37**(Suppl28): S4-S9.

Homma T, Hoover RL, Ichikawa I, Harris RC. Angiotensin II (AII) induces hypertrophy and stimulates collagen production in cultured rat glomerular mesangial cell (MC). *Clin. Res.* 1990;**38**:358A.

Hommel E, Mathiesen ER, Giese J, Nielsen MD, Schutten HJ, Parving H-H. On the pathogenesis of arterial blood pressure elevation early in the course of diabetic nephropathy. *Scand. J. Lab. Clin. Invest.*

1989;**49**:537-544.

Hommel E, Mathiesen ER, Aukland, Parving H-H. Pathophysiological aspects of edema formation in diabetic nephropathy. *Kidney Int.* 1990;**38**:1187-1192.

Hopfer U, Nelson K, Perrotto J, Isselbacher KJ. Glucose transport in isolated brush border membrane from rat small intestine. *J. Biol. Chem.* 1973;**248**:25-32.

Hostetter TH, Troy JC, Brenner BM. Glomerular haemodynamics in experimental diabetes mellitus. *Kidney Int.* 1981a;**19**:410-415.

Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *Am. J. Physiol.* 1981b;**241**:F85-F93.

Hostetter TH, Rennke HG, Brenner BM. The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. *Am. J. Med.* 1982;**72**:375-380.

Hulet WH, Smith HH. Postural natriuresis and urine osmotic concentration in hydropenic subjects. *Am. J. Med.* 1961;**30**:8-25.

Ichikawa I. Direct analysis of the effector mechanism of the tubuloglomerular feedback system. *Am. J. Physiol.* 1982;**243**:F447-F455.

Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. *Br. Med. J.* 1988;**297**:319-328.

Ito S, Carretero OA. Macula densa control of glomerular haemodynamics. *Kidney Int.* 1991;**39**(Suppl.32):S83-S85.

Itskovitz HD, Gilberg N. Renal function and patterns of catecholamine excretion. *Kidney Int.* 1981;**19**:168A.

Jaffa AA, Miller DH, Bailey GS, Chao J, Margolius HS, Mayfield RK. Abnormal regulation of renal kallikrein in experimental diabetes. Effects of insulin on prokallikrein synthesis and activation. *J. Clin. Invest.* 1987;**80**:1651-1659.

Jeffrey RF, MacDonald TM, Rutter M Freestone S, Brown J, Samson RR, Lee MR. The effect of intravenous frusemide on urine dopamine in normal volunteers: studies with indomethacin and carbidopa. *Clin. Sci.* 1987;**73**:151-157.

Jeffrey RF, MacDonald TM, Brown J, Rae PWH, Lee MR. The effect of lithium on the renal response to the dopamine prodrug gludopa in normal man. *Br. J. Clin. Pharmacol.* 1988;**25**:725-732.

Jeffrey RF, MacDonald TM, Freestone S, Lee MR. The effect of carbidopa and lithium on the systemic and renal response to acute intravenous saline loading in normal man. *Nephrol. Dial. Transplant.* 1989;**4**:271-277.

Jenkins DAS, Cowan P, Watson ML, Clarke BF. Hyperglycaemia does not inhibit the renal haemodynamic response to angiotensin-converting enzyme inhibition in insulin-dependent diabetes mellitus. *Nephrol. Dial. Transplant.* 1989;**4**:436A.

Jenkins DAS, Cowan P, Collier A, Watson ML, Clarke BF. Blood glucose control determines the renal haemodynamic response to angiotensin converting enzyme inhibition in Type 1 diabetes. *Diabetic Med.* 1990;**7**:252-257.

Jensen JS, Mathiesen ER, Norgaard K, Hommel E, Borch-Jensen K, Funder J, Brahn J, Parving H-H, Deckert T. Increased blood pressure and erythrocyte sodium/lithium countertransport activity are not inherited in diabetic nephropathy. *Diabetologia* 1990;**33**:619-624.

Jensen PK, Christiansen JS, Steven K, Parving H-H. Renal function in streptozotocin-diabetic rats. *Diabetologia* 1981;**21**:409-414.

Jensen PK, Christiansen JS, Steven K, Parving H-H. Strict metabolic control and renal function in the streptozotocin diabetic rat. *Kidney Int.* 1987;**31**:47-51.

Johnson MD, Malvin RL. Stimulation of renal sodium reabsorption by angiotensin II. *Am. J. Physiol.* 1977;**232**:F298-F306.

Johnstone EC, Owens DGC, Lambert MT, Crow TJ, Frith CD, Done DJ: Combination tricyclic antidepressant and lithium maintenance medication in unipolar and bipolar depressed patients. *J. Affective Disorders* 1990;**20**:225-233.

Joint Working Party. Renal failure in diabetics in the UK: deficient provision of care in 1985. *Diabetic Med.* 1988;**5**:79-84.

Jorgensen PL. Sodium and potassium ion pump in kidney tubules. *Physiol. Rev.* 1980;**60**:864-917.

Kaas Ibsen K, Rotne H, Hongaard P. Blood pressure in children with diabetes mellitus. *Acta Pediatr. Scand.*

1983;**72**:191-196.

Katz AI. Renal Na-K-ATPase: its role in tubular sodium and potassium transport. *Am. J. Physiol.* 1982;**242**:F207-F219.

Kaufman JS, Hamburger RJ, Flamenbaum W. Tubuloglomerular feedback: effect of dietary NaCl intake. *Am. J. Physiol.* 1976;**231**:1744-1749.

Kaufman JS, Hamburger RJ, Flamenbaum W. Tubuloglomerular feedback response after hypotensive hemorrhage. *Renal Physiol.* 1982;**5**:173-181.

Keeler R. Natriuresis after unilateral stimulation of carotid receptors in unanaesthetized rats. *Am. J. Physiol.* 1974;**226**:507-511.

Keen H, Viberti GC. Genesis and evolution of diabetic nephropathy. *J. Clin. Path.* 1981;**34**:1261-1266.

Kimmelstiel P, Wilson C. Intercapillary lesions in glomeruli of kidney. *Am. J. Path.* 1936;**12**:83-98.

Kirchner KA. Lithium as a marker for proximal tubular delivery during low salt intake and diuretic infusion. *Am. J. Physiol.* 1987;**253**:F188-F196.

Knepper M, Burg M. Organization of nephron function. *Am. J. Physiol.* 1983;**244**:F579-F589.

Knowles HC Jr. Magnitude of the renal failure problem in diabetic patients. *Kidney Int.* 1974;**6**(Suppl.1):S2-S7.

Knox FG, Davis BB, Berliner RW. Effect of chronic cardiac denervation on renal response to saline infusion. *Am. J. Physiol.* 1967;**213**:174-178.

Knox FG, Mertz JI, Burnett JC, Haramati A. Role of hydrostatic and oncotic pressures in renal sodium reabsorption. *Circ. Res.* 1983;**52**:491-500.

Kreisberg JI. Insulin requirement for contraction of cultured rat glomerular mesangial cells in response to angiotensin II: Possible role for insulin in modulating glomerular hemodynamics. *Proc. Natl. Acad. Sci. USA* 1982;**79**:4190-4192.

Kreisberg JI, Patel PY. The effects of insulin, glucose and diabetes on prostaglandin production by rat kidney glomeruli and cultured glomerular mesangial cells. *Prostaglandins Leukot. Med.* 1983;**11**:431-442.

Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR. The changing natural history of diabetic nephropathy in Type 1 diabetes. *Am. J. Med.* 1985;**78**:785-794.

Krolewski AS, Canessa M, Warram JH, Laffel LMB, Christlieb AR, Knowler WC, Rand LI. Predisposition to hypertension and susceptibility to renal disease in insulin-dependent diabetes mellitus. *New Engl. J. Med.* 1988;**318**:140-145.

Kroustrup JP, Gundersen HJG, Osterby R. Glomerular size and structure in diabetes mellitus. *Diabetologia* 1977;**13**:207-210.

Kuchel O, Buu NT, Unger T. Dopamine-sodium relationship: is dopamine a part of the endogenous natriuretic system ? *Contrib. Nephrol.* 1978;**13**:27-36.

Kumar AM, Gupta RK, Spitzer A. Intracellular sodium in proximal tubules of diabetic rats. Role of glucose. *Kidney Int.* 1988;**33**:792-797.

Laragh JH, Atlas SA. Atrial natriuretic hormone: A regulator of blood pressure and volume homeostasis. *Kidney Int.* 1988;**34**(Suppl.25):S64-S71.

Laragh JH, Sealey JE. The renin-angiotensin-aldosterone hormonal system and regulation of sodium, potassium and blood pressure homeostasis. In *Handbook of Physiology,* vol.8, *Renal Physiology*, eds. Orloff J and Berliner RW, 1974. American Physiological Society, Washington, D.C., pp.831-908.

Lebel M, Schalekamp MA, Beevers et al. Sodium and the renin-angiotensin system in essential hypertension and mineralocorticoid excess. *Lancet* 1974;**2**:308-309.

Lee MR. Dopamine, the kidney and essential hypertension: studies with gludopa. *Clin. Exp. Hypertens.* 1987;**9**:977-986.

Lervang H-H, Jensen S, Brochner-Mortensen J, Ditzel J. Early glomerular hyperfiltration and the development of late nephropathy in Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1988;**31**:723-729.

Levens NR, Peach MJ, Carey RM. Role of the intrarenal renin-angiotensin system in the control of renal function. *Circ. Res.* 1981;**48**:157-167.

Levy M, Levinsky NG. Proximal reabsorption and intrarenal pressure during colloid infusions in the dog. *Am. J. Physiol.* 1972;**220**:415-421.

Liebermann JS, Parra L, Newton L, Scandling JD, Loon N, Myers BD. Atrial natriuretic peptide and response to changing plasma volume in diabetic nephropathy. *Diabetes* 1991;**40**:893-901.

Lindheimer MD, Lalone RC, Levinsky NG. Evidence that an acute increase in glomerular filtration has little effect on sodium excretion in the dog unless extracellular volume is expanded. *J. Clin. Invest.* 1967;**46**:256-265.

Liu F-Y, Cogan MG. Angiotensin II stimulates early proximal bicarbonate absorption in the rat by decreasing cyclic adenosine monophosphate. *J. Clin. Invest.* 1989;**84**:83-92.

Lundbaek K. Diabetic angiopathy: A specific vascular disease. *Lancet* 1954;**1**:377-379.

Lynn KL, Frendin TJ, Walker RJ, Bailey RR, Swainson CP. Renal disease in diabetics - which patients have diabetic nephropathy and what is their outcome? *Aust. NZ J. Med.* 1988;**18**:764-767.

MacDonald TM, Craig K, Watson ML. Frusemide, ACE inhibition, renal dopamine and prostaglandins: acute interactions in normal man. *Br. J. Clin. Pharm.* 1989:**28**:683-694.

Maguire GA, Price CP. A continuous monitoring spectrophotometric method for the measurement of angiotensin-converting enzyme in human serum. *Ann. Clin. Biochem.* 1985;**22**:204-210.

Mahnensmith RL, Aronson PS. The plasma membrane sodium-hydrogen exchanger and its role in physiological and pathophysiological processes. *Circ. Res.* 1985;**56**:773-788.

Mangili R, Bending JJ, Scott G, Li LK, Gupta A, Viberti GC. Increased sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 1988;**318**:146-150.

Mann GV, Gardner C, Root HF. Clinical manifestations of intercapillary glomerulosclerosis in diabetes mellitus. *Am. J. Med.* 1949;**7**:3-14.

Mann JFE, Murtz H, Sis J, Usadel KH, Hasslacher C, Ritz E. Specific binding of angiotensin II and atrial natriuretic factor in non-nephropathic type 1 diabetes mellitus. *Nephrol. Dial. Transplant.* 1989;**4**:530-534.

Mansell PI, Bisson D, O'Hare JP. Sodium excretion during water immersion is not impaired by physiological or

pharmacological hyperinsulinaemia. *Diabetic Med.* 1990;**7**(Suppl.2):30A.

Margolius HS, Horwitz D, Geller RG, Alexander RW, Gill JR, Pisano JJ, Keiser HR. Urinary kallikrein excretion in normal man. Relationships to sodium intake and sodium-retaining steroids. *Circ. Res.* 1974;**35**:812-819.

Margolius HS, Chao J, Kaizu T. The effects of aldosterone and spironolactone on renal kallikrein. *Clin. Sci. Mol. Med.* 1976;**51**(Suppl.3):279s-282s.

Marre M, Chatellier G, Leblanc H, Guyenne TT, Menard J, Passa P. Prevention of diabetic nephropathy with enalapril in normotensive diabetics with microalbuminuria. *Br. Med. J.* 1988;**297**:1092-1095.

Mathiesen ER, Gall M-A, Hommel E, Skott P, Parving H-H. Effects of short-term strict metabolic control on kidney function and extracellular fluid volume in incipient diabetic nephropathy. *Diabetic Med.* 1989;**6**:595-600.

Mathiesen ER, Hommel E, Giese J, Parving H-H. Efficacy of captopril in postponing nephropathy in normotensive insulin dependent diabetic patients with microalbuminuria. *Br. Med. J.* 1991;**303**:81-87.

Mathiesen ER, Ronn B, Jensen T, Storm B, Deckert T. Relationship between blood pressure and urinary albumin excretion in development of microalbuminuria. *Diabetes* 1990;**39**:245-249.

Mauer SM, Steffes MW, Ellis EN, Sutherland DER, Brown DM, Goetz FC. Structural-functional relationships in diabetic nephropathy. *J. Clin. Invest.* 1984;**74**:1143-1155.

Mbanya J-C, Thomas TH, Taylor R, Alberti KGMM, Wilkinson R. Increased proximal tubular sodium reabsorption in hypertensive patients with Type 2 diabetes. *Diabetic Med.* 1989;**6**:614-620.

McCance RA, Lawrence RD. Secretion of urine in diabetic coma. *Q. J. Med.* 1935;**4**:53-79.

McCance RA. Experimental sodium deficiency in man. *Proc. Roy. Soc.* 1936;**119**:245-268.

McClanachan M, Sowers JR, Beck FWJ, Mohanty PK, McKenzie T. Dopaminergic regulation of natriuretic response to acute volume expansion in dogs. *Clin. Sci.* 1985;**68**:263-269.

McDonald RH, Goldberg LI, McNay JL, Tuttle EP. Effects of dopamine in man: augmentation of sodium excretion,

glomerular filtration rate, and renal plasma flow. *J. Clin. Invest.* 1964;**43**:1116-23.

McNally PG, Burden AC, Swift PGF, Walls J, Hearnshaw JR. The prevalence and risk factors associated with the onset of diabetic nephropathy in juvenile-onset (insulin-dependent) diabetics diagnosed under the age of 17 years in Leicestershire 1930-1985. *Q. J. Med.* 1990;**76**:831-844.

Meltzer JI, Sealey J. Malignant hypertension during chronic maintenance lithium therapy: a new syndrome and cause of a false positive captopril test. *Am. J. Hypertension* 1990;**3**:73A.

Mendelsohn FAO. Evidence for the local occurrence of angiotensin II in rat kidney, and its modulation by dietary sodium intake and converting enzyme blockade. *Clin. Sci. Mol. Med.* 1979;**57**:173-179.

Messent J, Jones S, Wiseman M, Viberti GC. Glomerular hyperfiltration and albuminuria - a 8 year prospective study. *Diabetologia* 1991;**34**(Suppl.2):A1.

Michels LD, Davidman M, Keane WF. Determinants of glomerular filtration and plasma flow in experimental diabetic rats. *J. Lab. Clin. Med.* 1981;**98**:869-885.

Miller JH, Bogdonoff MD. Antidiuresis associated with administration of insulin. *J. Appl. Physiol.* 1954;**6**:509-512.

Mitchell KD, Navar LG. Superficial nephron responses to peritubular capillary infusions of angiotensin I and II. *Am. J. Physiol.* 1987;**252**:F818-F824.

Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, Shitrit A, Fuchs Z. Hyperinsulinaemia: a link between hypertension, obesity and glucose tolerance. *J. Clin. Invest.* 1985;**75**:809-817.

Mogensen CE. Glomerular filtration rate and renal plasma flow in short-term and long-term juvenile diabetes mellitus. *Scand. J. Clin. Lab. Invest.* 1971a;**28**:91-100.

Mogensen CE. Maximum tubular reabsorption capacity for glucose and renal haemodynamics during rapid hypertonic glucose infusion in normal and diabetic subjects. *Scand. J. Lab. Clin. Invest.* 1971b;**28**:101-109.

Mogensen CE. Glomerular filtration rate and renal plasma flow in normal and diabetic man during elevation of blood sugar levels. *Scand. J. Lab. Clin. Invest.*

1971c;**28**:177-182.

Mogensen CE, Andersen MJF. Increased kidney size and glomerular filtration rate in early juvenile diabetes. *Diabetes* 1973;**22**:706-712.

Mogensen CE. Renal function changes in diabetes. *Diabetes* 1976a;**25**:872-879.

Mogensen CE. Progression of nephropathy in long-term diabetics with proteinuria and effect of initial anti-hypertensive treatment. *Scand. J. Lab. Clin. Invest.* 1976b;**36**:383-388.

Mogensen CE, Christensen NJ, Gundersen HJG. The acute effect of insulin on renal haemodynamics and protein excretion in diabetics. *Diabetologia* 1978;**15**:153-157.

Mogensen CE. Long-term antihypertensive treatment inhibiting progression of diabetic nephropathy. *Br. Med. J.* 1982;**285**:685-688.

Mogensen CE. Early glomerular hyperfiltration in insulin-dependent diabetics and late nephropathy. *Scand. J. Lab. Clin. Invest.* 1986;**46**:201-206.

Mogensen CE. Natural history of renal function abnormalities in human diabetes mellitus: from microalbuminuria to incipient and overt nephropathy. In *The kidney in diabetes mellitus*. eds. BM Brenner, JH Stein. Churchill Livingstone, 1989, pp.19-49.

Moore LC, Yarimizu S, Schubert G, Weber PC, Schnermann J. Dynamics of tubuloglomerular feedback adaptation to acute and chronic changes in body fluid volume. *Pfluegers Arch.* 1980;**387**:39-45.

Moore LC, Mason J. Perturbation analysis of tubuloglomerular feedback in hydropenic and hemorrhaged rats. *Am. J. Physiol.* 1983;**245**:F554-F563.

Moore RD. Effects of insulin upon ion transport. *Biochim. Biophys. Acta* 1983;**737**:1-49.

Moore TJ, Taylor T, Williams GH. Human platelet angiotensin II receptors: regulation by the circulating angiotensin level. *J. Clin. Endocrinol. Metab.* 1984;**58**:778-782.

Morsing P, Persson AEG. Tubuloglomerular feedback in obstructive uropathy. *Kidney Int.* 1991;**39**(Suppl.32): S110-S114.

Morton JJ, Webb DJ. Measurement of plasma angiotensin II.

Clin. Sci. 1985;**68**:483-4.

Moss AJ. Blood pressure in children with diabetes mellitus. *Pediatrics.* 1962;**130**:932-936.

Mujais SK, Kaufmann S, Katz AI. Angiotensin II binding sites in individual segments of the rat nephron. *J. Clin. Invest.* 1986;**77**:315-318.

Muller-Suur R, Ulfendahl HR, Persson AEG. Evidence for tubuloglomerular feedback in juxtamedullary nephrons of young rats. *Am. J. Physiol.* 1983;**244**:F425-F431.

Murphy DL, Goodwin FK, Bunney WE: Aldosterone and sodium response to lithium administration in man. *Lancet* 1969;**2**:458-461.

Muther RS, Potter DM, Bennett WM. Aspirin-induced depression of glomerular filtration rate in normal humans: role of sodium balance. *Ann. Intern. Med.* 1981;**94**:317-321.

Myers BD, Deen WM, Brenner BM. Effects of norepinephrine and angiotensin II on the determinants of glomerular ultrafiltration and proximal tubular fluid reabsorption in the rat. *Circ. Res.* 1975;**37**:101-110.

Nasjletti A, Malik KU. Relationships between the kallikrein-kinin and prostaglandin system. *Life Sci.* 1979;**25**:99-110.

Nakagawa S, Nakayama H, Sasaki T, Yoshino K, Yu YY Shinozaki K, Aoki S, Mashimo K. A simple method for the determination of serum free insulin levels in insulin-treated patients. *Diabetes* 1973;**22**:590-600.

Nashat FS, Tappin JW, Wilcox CS. The renal blood flow and the glomerular filtration rate of anaesthetized dogs during acute changes in plasma sodium concentration. *J. Physiol.* 1976;**256**:731-745.

Navar LG, Ploth DW, Bell PD. Distal tubular feedback control of renal hemodynamics and autoregulation. *Ann. Rev. Physiol.* 1980;**42**:557-571.

Norgaard K, Feldt-Rasmussen B, Borch-Jensen K, Saelan H, Deckert T. Prevalence of hypertension in Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1990;**33**:407-410.

Nosadini R, Trevisan R, Fioretto P, et al. Kidney hemodynamics after ketone body and amino acid infusion in normal and IDDM subjects. *Diabetes* 1989;**38**:75-83.

Novick AC, Gephardt G, Guz B, Steinmuller D, Tubbs RR. Long-term follow-up after partial removal of a solitary kidney. *New Engl. J. Med.* 1991;**325**:1058-1062.

Nyberg G, Granerus G, Aurell M. Renal extraction ratios for ^{51}Cr-EDTA, PAH, and glucose in early insulin-dependent diabetic patients. *Kidney Int.* 1982;**21**:706-708.

Obika LFO, Fitzgerald EM, Gleason SD, Zucker A, Schneider EG. Lack of evidence for gastrointestinal control of sodium excretion in unanaesthetized rabbits. *Am. J. Physiol.* 1981;**240**:F94-F100.

O'Donnell MJ, Lawson N, Barnett AH. Activity of the unstimulated renin-angiotensin-aldosterone system in Type 1 diabetic patients with and without proteinuria. *Diabetic Med.* 1989;**6**:422-425.

O'Donnell MP, Kasiske BL, Daniels FX, Keane WF. Effects of nephron loss on glomerular hemodynamics and morphology in diabetic rats. *Diabetes* 1986;**35**:1011-1015.

O'Hare JA, Ferriss JB, Twomey BM, Gonggrijp H, O'Sullivan DJ. Changes in blood pressure, body fluids, circulating ngiotensin II and aldosterone with improved diabetic control. *Clin. Sci.* 1982;**63**:415s-418s.

O'Hare JA, Ferriss JB, Twomey B, O'Sullivan DJ. Poor metabolic control, hypertension, and microangiopathy independently increase the transcapillary escape rate of albumin in diabetes. *Diabetologia* 1983;**25**:260-263.

O'Hare JA, Ferriss JB, Brady D, Twomey B, O'Sullivan DJ. Exchangeable sodium and renin in hypertensive diabetic patients with and without nephropathy. *Hypertension* 1985;**7**(Suppl.II):II.43-II.48.

O'Hare JP, Roland JM, Walters G, Corrall RJM. Impaired sodium excretion in response to volume expansion induced by water immersion in insulin dependent diabetes mellitus. *Clin. Sci.* 1986;**71**:403-409.

O'Hare JP, Anderson JV, Millar ND, Bloom SR, Corrall RJM. The relationship of the renin-angiotensin-aldosterone system to atrial natriuretic peptide and the natriuresis of volume expansion in diabetics with and without proteinuria. *Postgrad. Med. J.* 1988;**64**(Suppl.3):35-38.

Omvik P, Tarazi RC, Bravo EL. Regulation of sodium balance in hypertension. *Hypertension* 1980;**2**:515-523.

Orstavvik TB, Inagami T. Localisation of kallikrein in the rat kidney and its anatomical relationship to renin.

J. Histochem. Cytochem. 1982;**30**:385-390.

Ortola FV, Ballermann BJ, Anderson S, Mendez RE, Brenner BM. Elevated plasma atrial natriuretic peptide levels in diabetic rats: potential mediator of hyperfiltration. *J. Clin. Invest.* 1987;**80**:670-674.

Osgood RW, Reineck HJ, Stein JH. Further studies on segmental sodium transport in the rat kidney during expansion of the extracellular fluid volume. *J. Clin. Invest.* 1978;**62**:311-320.

Osswald H, Nabakowski G, Hermes H. Adenosine as a possible mediator of metabolic control of glomerular filtration rate. *Int. J. Biochem.* 1980;**12**:263-267.

Osswald H, Hermes HH, Nabakowski G. Role of adenosine in signal transmission of tubuloglomerular feedback. *Kidney Int.* 1982;**22**(Suppl.12):S136-S142.

Osterby R, Parving H-H, Nyberg G, Hommel E, Jorgensen HE, Lokkegaard H, Svalander C. A strong correlation between glomerular filtration rate and filtration surface in diabetic nephropathy. *Diabetologia* 1988;**31**:265-270.

Page M McB, Smith RBW, Watkins PJ. Cardiovascular effects of insulin. *Br. Med. J.* 1976a;**1**:430-432.

Page M McB, Watkins PJ. Provocation of postural hypotension by insulin in diabetic autonomic neuropathy. *Diabetes* 1976b;**25**:90-95.

Paintal AS. Vagal sensory receptors and their reflex effects. *Physiol. Rev.* 1973;**53**:159-227.

Parving H-H. Increased metabolic turnover rate and transcapillary escape rate of albumin in long-term juvenile diabetics. *Scand. J. Clin. Lab. Invest.* 1975;**35**:59-66.

Parving H-H, Noer I, Deckert T, et al. The effect of metabolic regulation on microvascular permeability to small and large molecules in short-term juvenile diabetics. *Diabetologia* 1976;**12**:161-166.

Parving H-H, Andersen AR, Smidt UM, Oxenboll B, Edsberg B, Christiansen JS. Diabetic nephropathy and arterial hypertension. *Diabetologia* 1983a;**24**:10-12.

Parving H-H, Andersen AR, Smidt UM, Svendsen PA. Early aggressive antihypertensive treatment reduces rate of decline in kidney function in diabetic nephropathy. *Lancet* 1983b;**1**:1175-1179.

Parving H-H, Viberti GC, Keen H, Christiansen JS, Lassen NA. Hemodynamic factors in the genesis of diabetic microangiopathy. *Metabolism* 1983c;**32**:943-949.

Parving H-H, Andersen AR, Smidt UM, Hommel E, Mathiesen ER, Svendsen PA. Effect of antihypertensive treatment on kidney function in diabetic nephropathy. *Br. Med. J.* 1987;**294**:1443-1447.

Parving H-H, Hommel E, Smidt UM. Protection of kidney function and decrease in albuminuria by captopril in insulin dependent diabetics with nephropathy. *Br. Med. J.* 1988;**297**:1086-1091.

Parving H-H, Hommel E, Nielsen MD, Giese J. Effect of captopril on blood pressure and kidney function in normotensive insulin dependent diabetics with nephropathy. *Br. Med. J.* 1989a;**299**:533-536.

Parving H-H, Hommel E. Prognosis in diabetic nephropathy. *Br. Med. J.* 1989b;**299**:230-233.

Passo SS, Thornborough JR, Rothballer AB. Natriuresis following fourth ventricle perfusion with high-sodium artificial CSF. *Can. J. Physiol. Pharmacol.* 1975;**53**:363-367.

Patrick AW, Hepburn DA, Craig K, Thomson I, Swainson CP, Frier BM. The effects of acute insulin-induced hypoglycaemia on renal function in normal human subjects. *Diabetic Med.* 1989;**6**:703-708.

Persson AEG, Muller-Suur R, Selen G. Capillary oncotic pressure as a modifier for tubuloglomerular feedback. *Am. J. Physiol.* 1979a;**236**:F97-F102.

Persson AEG, Schnermann J, Wright FS. Modification of feedback influence on glomerular filtration rate by acute isotonic extracellular volume expansion. *Pfluegers Arch.* 1979b;**381**:99-105.

Persson AEG, Boberg U, Hahne B, Muller-Suur R, Norlen B-J, Selen G. Interstitial pressure as a modulator of tubuloglomerular feedback control. *Kidney Int.* 1982;**22**(Suppl.12):S122-S128.

Philipp T, Distler A, Cordes U. Sympathetic nervous system and blood-pressure control in essential hypertension. *Lancet* 1978;**2**:959-963.

Phillips PA, Rolls BJ, Ledingham JGG, Morton JJ, Forsling ML. Angiotensin II-induced thirst and vasopressin release in man. *Clin. Sci.* 1985;**68**:669-674.

Pinter GG, Atkins JL. Role of postglomerular microvessels in pathophysiology of diabetic nephropathy. *Diabetes* 1991;**40**:791-795.

Pirart J. Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. *Diabetes Care* 1978;**1**:168-188.

Ploth DW, Schnermann J, Dahlheim H, Hermle M, Schmidmeier E. Autoregulation and tubuloglomerular feedback in normotensive and hypertensive rats. *Kidney Int.* 1977;**12**:253-267.

Ploth DW, Rudulph J, Thomas C, Navar LG. Renal and tubuloglomerular feedback responses to plasma expansion in the rat. *Am. J. Physiol.* 1978;**235**:F156-F162.

Ploth DW, Rudulph J, Lagrange R, Navar LG. Tubuloglomerular feedback and single nephron function after converting enzyme inhibition in the rat. *J. Clin. Invest.* 1979;**64**:1325-1335.

Ploth DW, Roy RN. Renal and tubuloglomerular feedback effects of (Sar^1,Ala^8)angiotensin II in the rat. *Am. J. Physiol.* 1982;**242**:F149-F157.

Pollock CA, Lawrence JR, Field MJ. Tubuloglomerular feedback in renal hypertrophy. *Kidney Int.* 1991;**39**(Suupl.32):S106-S109.

Premen AJ. Potential mechanisms mediating postprandial renal hyperemia and hyperfiltration. *FASEB J.* 1988;**2**:131-137.

Prosnitz EH, Zambraski EJ, DiBona GF. Mechanism of intrarenal blood flow redistribution after carotid artery occlusion. *Am. J. Physiol.* 1977;**232**:F167-F172.

Prosnitz EH, DiBona GF. Effect of decreased renal sympathetic nerve activity on renal tubular sodium reabsorption. *Am. J. Physiol.* 1978;**235**:F557-F563.

Puig JG, Mateos Anton F, Grande C et al. Relation of kidney size to kidney function in early insulin-dependent diabetes. *Diabetologia* 1981;**21**:363-367.

Rabelink TJ, Koomans HA, Boer WH, Dorhout Mees EJ, van Rijn HJM. Indomethacin increases renal lithium reabsorption in man. *Nephrol. Dial. Transplant.* 1989;**4**:27-31.

Rabkin R, Ryan MP, Duckworth WC. The renal metabolism of insulin. *Diabetologia* 1984;**27**:351-357.

Read A, Tibi L, Smith AF. Assessment of a simple electrophoretic method for measuring HbA_1. *Clin. Chim. Acta* 1980;**108**:487-491.

Reid W, Ewing DJ, Lightman SL, Williams TDM, Eadington DW, Clarke BF. The pressor effect of arginine vasopressin in diabetic autonomic neuropathy. *Diabet. Nutrit. Metab.* 1991;**4**:29-34.

Reineck HJ, Stein JH, Seldin DW. In *The Kidney: Physiology and Pathophysiology,* eds. DW Seldin and G Giebisch. Raven Press, New York, 1985. pp.1150-1152.

Relman AS, Schwartz WB. The effect of DOCA on electrolyte balance in normal man and its relation to sodium chloride intake. *Yale J. Biol. Med.* 1952;**24**:540-558.

Risdon RA, Sloper JC, DeWardener HE. Relationship between renal function and histological changes found in renal-biopsy specimens from patients with persistent glomerular nephritis. *Lancet* 1968;**2**:363-366.

Rocchini AP, Moorehead C, DeRemer S, Goodfriend TL, Ball DL. Hyperinsulinaemia and the aldosterone and pressor responses to angiotensin II. *Hypertension* 1990;**15**:861-866.

Roland JM, O'Hare JP, Walters G, Corrall RJM. Sodium retention in response to saline infusion in uncomplicated diabetes mellitus. *Diabetes Res.* 1986;**3**:213-215.

Rollo J. *Cases of the Diabetes Mellitus,* ed 2. London, Dilly, 1798.

Roos JC, Koomans HA, Dorhout Mees EJ, Delawi IMK. Renal sodium handling in normal humans subjected to low, normal, and extremely high sodium supplies. *Am. J. Physiol.* 1985;**249**:F941-F947.

Rosenbaum JD, Papper S, Aschley MM. Variations in renal excretion of sodium independent of changes in adrenal cortical hormone doseage in patients with Addison's disease. *J. Clin. Endocrinol. Metab.* 1959;**15**:1459-1467.

Rowe JW, Young JB, Minaker KL, Stevens AL, Pallotta J, Landsberg L. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes* 1981;**30**:219-225.

Rudberg S, Persson B, Dahlquist G. Increased glomerular filtration rate predicts diabetic nephropathy - results from an 8 year prospective study. *Diabetologia* 1991;**34**(Suppl.2):A1.

Saito I, Takeshita E, Saruta T, Nagano S, Sekihara T. Urinary dopamine excretion in normotensive subjects with or without family history of hypertension. *J. Hypertension* 1986;**4**:57-60.

Samani NJ. New developments in renin and hypertension. *Br. Med. J.* 1991;**302**:981-982.

Sandeman DD, Shore AC, Tooke JE. Nailfold capillary pressure falls with improved glycaemic control in Type 1 diabetic subjects. *Diabetic Med.* 1991;**8**(Suppl.1):38A.

Saudek CD, Boulter PR, Knopp RH, Arky RA. Sodium retention accompanying insulin treatment of diabetes mellitus. *Diabetes* 1974;**23**:240-246.

Sawicki PT, Heinemann L, Rave K, Hohmann A, Berger M. Atrial natriuretic factor in various stages of diabetic nephropathy. *J. Diabet. Complicat.* 1988;**2**:207-209.

Schalekamp MA, Lebel M, Beevers et al. Body-fluid volume in low-renin hypertension. *Lancet* 1974;**2**:310-311.

Schambelan M, Blake S, Sraer J, Bens M, Nivez MP, Wahbe F. Increased prostaglandin production by glomeruli isolated from rats with streptozotocin-induced diabetes. *J. Clin. Invest.* 1985;**75**:404-412.

Schimamura T, Morrison AB. A progressive glomerulosclerosis occurring in partial five-sixths nephrectomy. *Am. J. Pathol.* 1975;**79**:95-106.

Schnermann J, Hermle M, Schmidmeier F, Dahlheim H. Impaired potency for feedback regulation of glomerular filtration rate in DOCA escaped rats. *Pfluegers Arch.* 1975;**358**:325-338.

Schnermann J, Ploth DW, Hermle M. Activation of tubulo-glomerular feedback by chloride transport. *Pfluegers Arch.* 1976;**362**:229-240.

Schnermann J, Osswald H, Hermle M. Inhibitory effect of methylxanthines on feedback control of glomerular filtration rate in the rat kidney. *Pfluegers Arch.* 1977a;**369**:39-48.

Schnermann J, Stowe NT, Yarimizu S, Magnusson M. Feedback regulation of glomerular filtration rate in isolated kidneys. *Pfluegers Arch.* 1977b;**368**(Suppl.):R14.

Schnermann J, Schubert G, Hermle M, Herbst R, Stowe NT, Yarimizu S, Weber PC. The effect of inhibition of prostaglandin synthesis on tubuloglomerular feedback in

the rat kidney. *Pfluegers Arch.* 1979;**379**:269-279.

Schnermann J, Briggs J, Wright FS. Feedback-mediated reduction of glomerular filtration rate during infusion of hypertonic saline. *Kidney Int.* 1981;**20**:462-468.

Schnermann J, Briggs JP, Weber PC. Tubuloglomerular feedback, prostaglandins, and angiotensin in the autoregulation of glomerular filtration rate. *Kidney Int.* 1984;**25**:53-64.

Schnermann J, Briggs J. Function of the juxtaglomerular apparatus: Local control of glomerular haemodynamics. In *The Kidney: Physiology and Pathology*, eds. DW Seldin and G Giebisch. New York, Raven Press, 1985, pp.669-697.

Schuster VL, Kokko JP, Jacobson HR. Angiotensin II directly stimulates sodium transport in rabbit proximal convoluted tubules. *J. Clin. Invest.* 1984;**73**:507-515.

Scicli AG, Carretaro OA. Renal kallikrein-kinin system. *Kidney Int.* 1986;**29**:120-130.

Scroop GC, Lowe RD. Efferent pathways of the cardiovascular resonse to vertebral artery infusions of angiotensin in the dog. *Clin. Sci.* 1969;**37**:605-619.

Sealey JE, Atlas SA, Laragh JH, Oza NB, Ryan JW. Human urinary kallikrein converts inactive to active renin and is a possible physiological activator of renin. *Nature* 1978;**275**:144-145.

Seaquist ER, Goetz FC, Rich S, Barbosa J. Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. *New Engl. J. Med.* 1989;**320**:1161-1165.

Seefeldt T, Orskov L, Mengel A, Rasmussen O, Pedersen MM, Moller N, Christiansen JS, Schmitz O. Lack of effects of angiotensin-converting enzyme (ACE)-inhibitors on glucose metabolism in Type 1 diabetes. *Diabetic Med.* 1990;**7**:700-704.

Seidelin PH, McMurray JJ, Struthers AD. Mechanisms of the antinatriuretic action of physiological doses of angiotensin II in man. *Clin. Sci.* 1989;**76**:653-8.

Seney FD, Salmond R. Tubuloglomerular feedback in diabetic rats. *Kidney Int.* 1991;**33**:412.

Shimizu K, Kurosawa T, Maeda T, Yoshitoshi Y. Free water excretion and washout of renal medullary urea by prostaglandin E_1. *Jpn Heart J.* 1969;**10**:437-455.

Shirley DG, Walter SJ, Thomsen K. A comparison of micropuncture and lithium clearance methods in the assessment of renal tubular function in rats with diabetes insipidus. *Pfluegers Arch.* 1983;**399**:266-270.

Shirley DG, Singer DRJ, Sagnella GA, et al. Effect of a single test dose of lithium carbonate on sodium and potassium excretion in man. *Clin. Sci.* 1991;**81**:59-63.

Singer DRJ, Shirley DG, Markandu ND, Miller MA, Buckley MG, Sugden AL, Sagnella GA, MacGregor GA. How important are suppression of aldosterone and stimulation of atrial natriuretic peptide secretion in the natriuretic response to an acute sodium load in man? *Clin. Sci.* 1991;**80**:293-299.

Singhal PC, Santiago A, Satriano J, Hays RM, Schlondorff D. Effects of vasoactive agents on uptake of immunoglobulin G complexes by mesangial cells. *Am. J. Physiol.* 1990;**258**:F589-F596.

Skott P, Bruun NE, Giese J, Holstein-Rathlou N-H, Leyssac PP. What does lithium clearance measure during osmotic diuresis ? *Clin. Sci.* 1987;**73**:126-127.

Skott P, Hother-Nielsen O, Bruun NE, Giese J, Nielsen MD, Beck-Nielsen H, Parving H-H. Effects of insulin on kidney function and sodium excretion in healthy subjects. *Diabetologia* 1989a;**32**:694-699.

Skott P, Mathiesen ER, Hommel E, Gall M-A, Bruun NE, Parving H-H. The increased proximal tubular reabsorption of sodium and water is maintained in long-term insulin-dependent diabetics with early nephropathy. *Scand. J. Clin. Lab. Invest.* 1989b;**49**:419-425.

Skott P, Vaag A, Bruun NE, Hother-Nielsen O, Gall M-A, Beck-Nielsen H, Parving H-H. Effect of insulin on renal sodium handling in hyperinsulinaemic Type 2 (non-insulin-dependent) diabetic patients with peripheral insulin resistance. *Diabetologia* 1991;**34**:275-281.

Smith HW. *The Kidney. Structure and Function in Health and Disease*. Oxford University Press, 1951.

Snedecor GW, Watson WG. *Statistical Methods,* 7th edn, 1980. Ames, Iowa State University Press.

Soeldner JS, Stone D. Critical variables in the radioimmunoassay of serum insulin using the double antibody technic. *Diabetes* 1965;**14**:771-779.

Solez K, Fox JA, Miller M, Heptinstall RH. Effects of indomethacin on renal inner medullary plasma flow.

Prostaglandins 1974;**7**:91-98.

Spencer K, Price CP. Kinetic immunoturbimetry: the estimation of albumin. *Clin. Chim. Acta* 1979;**95**:263-276.

Spiro RG. Search for a biochemical basis of diabetic microangiopathy. *Diabetologia* 1976;**12**:1-14.

Stalder G, Schmid R. Severe functional disorders of glomerular capillaries and renal hemodynamics in treated diabetes mellitus during childhood. *Ann. Paediatr.* 1959;**193**:129-138.

Starling EH. The fluids of the body. In: The Herter Lectures, p.106. 1909, W.T. Keener & Co., Chicago.

Steffes MW, Osterby R, Chavers B, Mauer SM. Mesangial expansion as a central mechanism for loss of kidney function in diabetic patients. *Diabetes* 1989;**38**:1077-1081.

Stein JH, Osgood RW, Kunau RT Jr. Direct measurement of papillary collecting duct sodium transport in the rat. *J. Clin. Invest.* 1976;**58**:767-773.

Steinhausen M, Endlich K, Wiegman DL. Glomerular blood flow. *Kidney Int.* 1990;**38**:769-784.

Stewart PM, Grieve J, Nairn IM, Padfield PL, Edwards CRW. Lithium inhibits the action of fludrocortisone on the kidney. *Clin. Endocrinol.* 1987;**27**:63-68.

Stewart PM, Atherden SM, Whalley L, Edwards CRW, Padfield PL. Lithium carbonate - a competitive aldosterone antagonist? *Br. J. Psychiatry* 1988;**153**:205-207.

Stokes JB, Kokko JP. Inhibition of sodium transport by prostaglandin E_2 across the isolated, perfused rabbit collecting tubule. *J. Clin. Invest.* 1977;**59**:1099-1104.

Stokes JB. Effect of prostaglandin E_2 on chloride transport across the rabbit thick ascending limb of Henle. *J. Clin. Invest.* 1979;**64**:495-502.

Stowe N, Schnermann J, Hermle M. Feedback regulation of nephron filtration rate during pharmacologic interference with the renin-angiotensin and adrenergic systems in rats. *Kidney Int.* 1979;**15**:473-486.

Strauss MB, Lamdin E, Smith WP, Bleifer DJ. Surfeit and deficit of sodium: A kinetic concept of sodium excretion. *Arch. Int. Med.* 1958;**102**:527-536.

Strazzullo P, Iacoviello L, Iacone R, Giorgione N. Use of fractional lithium clearance in clinical and epidemiological investigations: a methodological assessment. *Clin. Sci.* 1988;**74**:651-657.

Sullivan PA, Gonggrijp H, Crowley MJ, Ferriss JB, O'Sullivan DJ. Plasma angiotensin II and the control of diabetes mellitus. *Clin. Endocrinol.* 1980;**13**:387-392.

Tan SY, Noth R, Mulrow PJ. Direct non-chromatographic radioimmunoassay of aldosterone: validation of a commercially available kit and observations on age-related changes in concentration in plasma. *Clin. Chem.* 1978;**24**:1531-1533.

Tarn AC, Drury PL. Blod pressure in children, adolescents and adults with type 1 (insulin-dependent) diabetes. *Diabetologia* 1986;**29**:275-281.

Thames MD, Miller BD, Abboud FM. Baroreflex regulation of renal nerve activity during volume expansion. *Am. J. Physiol.* 1982;**243**:H810-H814.

Thompson CJ, Baylis PH, Frier BM. Renal resistance to vasopressin in Type 1 (insulin-dependent) diabetes. *Diabetic Med.* 1991;**7**(Suppl.2):6A.

Thompson JMA, Dickinson CJ. The relation between the excretion of sodium and water and the perfusion pressure in the isolated, blood-perfused, rabbit kidney, with special reference to changes occurring in clip-hypertension. *Clin. Sci. Mol. Med.* 1976;**50**:223-236.

Thomsen AC. *The Kidney in Diabetes Mellitus*. Thesis, 1965. Copenhagen: Munksgaard.

Thomsen K, Schou M. Renal lithium clearance in man. *Am. J. Physiol.* 1968;**215**:823-827.

Thomsen K, Schou M, Steiness I, Hansen HE. Lithium as an indicator of proximal sodium reabsorption. *Pflugers Arch.* 1969;**308**:180-184.

Thomsen K. The renal handling of lithium: Relation between lithium clearance, sodium clearance and urine flow in rats with diabetes insipidus. *Acta Pharmacol. Toxicol.* 1977;**40**:491-496.

Thomsen K, Holstein-Rathlou N-H, Leyssac PP. Comparison of three measures of proximal tubular reabsorption: lithium clearance, occlusion time, and micropuncture. *Am. J. Physiol.* 1981;**241**:F348-F355.

Thomsen K. Lithium clearance: a new method for determining proximal and distal tubular reabsorption of sodium and water. *Nephron* 1984;**37**:217-223.

Thomsen K, Leyssac PP: Acute effects of various diuretics on lithium clearance. Studies in rats on medium and low sodium diet. *Renal Physiol.* 1986;**9**:1-8.

Thomsen K. Lithium clearance as a measure of sodium and water delivery from the proximal tubules. *Kidney Int.* 1990;**37**(Suppl.28):S10-S16.

Thomsen OF, Andersen AR, Christiansen JS, Deckert T. Renal changes in long-term type 1 (insulin-dependent) diabetic patients with and without clinical nephropathy: a light microscopic morphometric study of autopsy material. *Diabetologia* 1989;**26**:361-365.

Thornborough JR, Passo SS, Rothballer AB. Receptors in cerebral circulation affecting sodium excretion in the cat. *Am. J. Physiol.* 1973;**225**:138-141.

Thurau K, Schnermann J. Die Natriumkonzentration an den Macula densa Zellen als regulierender Faktor fur das Glomerulumfiltrat (Mikropunktionsversuche). *Klin. Wochenschr.* 1965;**43**:410-413.

Thurau KWC, Dahlheim H, Gr≟ner A, Mason J, Granger P. Activation of renin in the single juxtaglomerular apparatus by sodium chloride in the tubular fluid at the macula densa. *Circ. Res.* 1972;**31**(Suppl.II): II.182-II.186.

Tigerstedt R, Bergman PG. Niere und Kreislauf. *Skandin. Archiv. f. Physiol.* 1898;**viii**:223-271.

Tobian L, Tomboulian A, Janecek J. The effect of high perfusion pressure on the granulation of juxtaglomerular cells in an isolated kidney. *J. Clin. Invest.* 1959;**38**:605-610.

Trevisan R, Fioretto P, Semplicini A, Opocher G, Mantero F, Rocco S, Remuzzi G et al. Role of insulin and atrial natriuretic peptide in sodium retention in insulin-treated IDDM patients during isotonic volume expansion. *Diabetes* 1990;**39**:289-298.

Trost BN, Weidmann P. Effects of calcium antagonists on glucose homeostasis and serum lipids in non-diabetic and diabetic subjects: a review. *J. Hypertens.* 1987;**5**(Suppl.4):S81-S104.

Trovati M, Massucco P, Anfossi G et al. Insulin influences the renin-angiotensin-aldosterone system in

humans. *Metab. Clin. Exp.* 1989;**38**:501-503.

Trujillo A, Eggena P, Barrett J, Tuck M. Renin regulation in type II diabetes mellitus: influence of dietary sodium. *Hypertension* 1989;**13**:200-205.

Tuck M, Corry D, Trujillo A. Salt-sensitive blood pressure and exaggerated vascular reactivity in the hypertension of diabetes mellitus. *Am. J. Med.* 1990;**68**:210-216.

Tucker BJ, Collins RC, Ziegler MG, Blantz RC. Disassociation between glomerular hyperfiltration and extracellular volume in diabetic rats. *Kidney Int.* 1991;**39**:1176-1183.

Ullrich KJ. Renal tubular mechanisms of organic solute transport. *Kidney Int.* 1976;**9**:134-148

Ushiogi Y, Haberle DA. Hyperreactivity of tubuloglomerular feedback in chronically salt-loaded spontaneous hypertensive rats. *Kidney Int.* 1991;**39**(Suppl.32):S142-S147.

Vander AJ, Geelhoed GW. Inhibition of renin secretion by angiotensin II. *Proc. Soc. Exp. Biol. Med.* 1965;**120**:399-403.

Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet* 1982;**1**:1430-1432.

Viberti GC, Wiseman M, Mackintosh D, Jarrett RJ, Keen H. Microalbuminuria and marginal blood pressure changes in insulin-dependent diabetes. *Diabetic Nephropathy* 1985;**4**:32-33.

Viberti GC, Keen H, Wiseman MJ. Raised arterial pressure in parents of proteinuric insulin dependent diabetics. *Br. Med. J.* 1987;**295**:515-517.

Vierhapper H, Waldhausl W, Nowotny P. The effect of insulin on the rise in blood pressure and plasma aldosterone after angiotensin II in normal man. *Clin. Sci.* 1983;**64**:383-386.

Vierhapper H, Gasic S, Roden M, Waldhausl W. Increase in skeletal muscle blood flow but not in renal blood flow during euglycaemic hyperinsulinaemia in man. *Diabetologia* 1991;**34**(Suppl.2):A147.

Walker AM, Bott PA, Oliver J, MacDowell MC. The collection and analysis of fluid from single nephrons of

the mammalian kidney. *Am. J. Physiol.* 1941;**134**:580-595.

Walker JD, Tariq T, Viberti GC. Sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes and nephropathy and their parents. *Br. Med. J.* 1990;**301**:635-638.

Watnick TJ, Jenkins RA, Rackoff P, Baumgarten A, Bia MJ. Microalbuminuria and hypertension in long-term renal donors. *Transplantation* 1988;**45**:59-65.

Waugh WH. Angiotensin II: Local renal effects of physiological increments in concentration. *Can. J. Physiol. Pharmacol.* 1972;**50**:711-716.

Weder AB. Red-cell lithium-sodium countertransport and renal lithium clearance in hypertension. *New Engl. J. Med.* 1986;**314**:198-201.

Weidmann P, Reinhart R, Maxwell MH, Massry SG, Lupu AN, Coburn JW, Kleeman CR. Syndrome of hyporeninemic hypoaldosteronism and hyperkalemia in renal disease. *J. Clin. Endocrinol. Metab.* 1973;**36**:965-977.

Weidmann P, Beretta-Piccoli C, Keusch G, Gluck Z, Mujagic M, Meier A, Ziegler WH. Sodium-volume factor, cardiovascular reactivity and hypotensive mechanisms of diuretic therapy in hypertension associated with diabetes mellitus. *Am. J. Med.* 1979;**67**:779-784.

Weidmann P, Beretta-Piccoli C, Trost BN. Pressor factors and responsiveness in hypertension accompanying diabetes mellitus. *Hypertension* 1985;**7**(Suppl.II):II.33-II.42.

Wennergren G, Henriksson B-A, Weiss L-G, Oberg B. Effects of stimulation of nonmedullated cardiac afferents on renal water and sodium excretion. *Acta Physiol. Scand.* 1976;**97**:261-263.

Wilkes B. Reduced glomerular angiotensin II receptor density in diabetes mellitus in the rat: time course and mechanism. *Endocrinol.* 1987;**120**:1291-1298.

Wiseman MJ, Viberti GC, Keen H. Threshold effect of plasma glucose in the glomerular hyperfiltration of diabetes. *Nephron* 1984;**38**:257-260.

Wiseman MJ, Mangili R, Alberetto M, Keen H, Viberti GC. Glomerular response mechanisms to glycaemic changes in insulin-dependent diabetics *Kidney Int.* 1987;**31**:1012-1018

Woods LL, Mizelle HL, Hall JE. Control of renal hemodynamics in hyperglycaemia: possible role of

tubuloglomerular feedback. *Am. J. Physiol.* 1987;**252**:F65-F73.

Worth DP, Harvey JN, Brown J, Lee MR. Gamma-L-glutamyl-L-dopa is a dopamine pro-drug, relatively specific for the kidney in normal subjects. *Clin. Sci.* 1985;**69**:207-214.

Wright FS, Schnermann J. Interference with feedback control of glomerular filtration rate by furosemide, triflocin, and cyanide. *J. Clin. Invest.* 1974;**53**:1695-1708.

Wright FS, Briggs JP. Feedback control of glomerular blood flow, pressure, and filtration rate. *Physiol. Rev.* 1979;**59**:958-1006.

Xiong W, Chao L, Chao J. Renal kallikrein mRNA localization by in situ hybridization. *Kidney Int.* 1989;**35**:1324-1329.

Yagi S, Takata S, Kiyokawa H, Yamamoto M, Noto Y, Ikeda T, Hattori N. Effects of insulin on vasoconstrictive responses to norepinephrine and angiotensin II in rabbit femoral artery and vein. *Diabetes* 1988;**37**:1064-1067.

Yoshida Y, Fogo A, Ichikawa I. Glomerular hemodynamic changes vs. hypertrophy in experimental glomerular sclerosis. *Kidney Int.* 1989;**35**:654-660.

Yoshimoto M, Awazu M, Ichikawa I, Hoover R. Effect of vasoactive agents on myosin light-chain phosphorylation in rat mesangial cells. *J. Am. Soc. Nephrol.* 1990;**1**:736.

Young DK, Marsh DJ. Pulse wave propagation in rat renal tubules: Implications for GFR autoregulation. *Am. J. Physiol.* 1981;**240**:F446-F458.

Zambraski EJ, DiBona GF. Angiotensin II in anti-natriuresis of low-level renal nerve stimulation. *Am. J. Physiol.* 1976;**231**:1105-1110.

Zatz R, Meyer TW, Rennke HG, Brenner BM. Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. *Proc. Natl. Acad. Sci. USA* 1985;**82**:5963-5967.

Zatz R, Dunn RB, Meyer TW, Anderson S, Rennke HG, Brenner BM. Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. *J. Clin. Invest.* 1986;**77**:1925-1930.

Zavaroni I, Dall'Aglio E, Bonora E, Alpi O, Passeri M, Reaven GM. Evidence that multiple risk factors for coronary artery disease exist in persons with abnormal glucose tolerance. *Am. J. Med.* 1987;**83**:609-612.

Publications arising from this work.

1. Oral carbidopa does not affect the renal response to angiotensin II infusion in normal man.
Eadington DW, Swainson CP, Lee MR.
Clinical Science 1991;**80**:149-154.

2. Renal responses to angiotensin II infusion in early Type 1 (insulin-dependent) diabetes.
Eadington DW, Swainson CP, Frier BM, Semple PF.
Diabetic Medicine 1991;**8**:524-531.

3. Effect of lithium on renal and systemic responses to angiotensin II infusion in normal man.
Eadington DW, Freestone S, Waugh C, Swainson CP, Lee MR.
Clinical Science (1992, in press).

Submitted:

4. The antinatriuretic effect of insulin is preserved after angiotensin-I converting enzyme inhibition in normal man.
Eadington DW, Hepburn DA, Swainson CP.

Presentations to scientific societies.

5. Lithium invalidates comparisons between systemic pressor responses to angiotensin II (ANGII) in diabetic and normal man.
Eadington DW, Frier BM, Swainson CP.
Clinical Science 1991;**81**:9P.
Medical Research Society, Cambridge, July 1991.

6. Effect of lithium on renal and systemic responses to angiotensin II infusion in Type 1 diabetes.
Eadington DW, Frier BM, Swainson CP.
Diabetologia 1991;**34**(Suppl.2):A137.
European Association for the Study of Diabetes, Dublin, September 1991.

7. Is lithium suitable as a marker of tubular sodium handling in Type 1 diabetes?
Eadington DW, Frier BM, Swainson CP.
Scottish Renal Association, Glasgow, October 1991.

8. Reduced effect of angiotensin II (ANGII) on lithium clearance in Type 1 diabetes (IDDM): correlation with glycaemic control.
Eadington DW, Frier BM, Swainson CP.
J. Amer. Soc. Nephrol. 1991;**2**:488.
American Society of Nephrology, Baltimore, November 1991.