A THESIS

ENTITLED

PROSTAGLANDINS, RENAL FUNCTION AND THE DEVELOPMENT OF HYPERTENSION

BY

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Submitted for the degree of Doctor of Medicine in The University of Edinburgh

University of Edinburgh March 1986

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To my wife Penny and children Fiona and James for their encouragement and moral support.

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UNIVERSITY OF EDINBURGH

ABSTRACT OF THESIS (Regulation 7.9)

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Degree M.D. *Date* 12th JUNE, 1985

Title of Thesis PROSTAGLANDINS, RENAL FUNCTION AND THE DEVELOPMENT OF HYPERTENSION

No. of words in the main text of Thesis 30,000

Renal function is a key factor in the long term control of blood pressure. The kidney has a large capacity to synthesise a number of prostaglandins, particularly PGE_2 and PGI_2. These compounds are systemic vasodilators and when infused into the kidney cause renal vasodilatation, renin release and an increase in sodium excretion. By virtue of these potential actions they may, therefore, have an important role in the control of renal function and ultimately in the long term control of blood pressure. In this thesis the results of a number of studies in both animals and man are described which aim to clarify the importance of prostaglandins as determinants of renal function. Both radioimmunoassay and gas chromatography - mass spectrometric methods for measuring prostaglandins and their metabolites were developed.

These methods were then employed to determine the effect of changes in sodium balance on renal PGE synthesis in conscious dogs during the development of one clip-two kidney hypertension. Sodium balance was manipulated during the early phase of this process by the controlled removal of salt and water using haemofiltration. The interrelationship between prostaglandins and the renin-angiotensin system was explored in a series of experiments; firstly by measuring changes in systemic PGI_2 synthesis in conscious dogs during infusion of angiotensin II and secondly by measuring changes in renal haemodynamics, sodium excretion and PGE excretion in response to infusion of different doses of angiotensin II before and after the development of one clip-two kidney hypertension. In view of the importance of renal interstitial cells as a source of prostaglandins, changes in their morphology was also studied in normotensive and hypertensive dogs.

The second part of the thesis concentrates on studies in man. The effect of rapid expansion of the extracellular fluid volume on renal PGE and systemic PGI_2 synthesis was examined. Systemic PGI_2 synthesis was measured during varying levels of activity of the renin-angiotensin system, induced by changes in dietary sodium intake and also by comparing synthesis before and after removal of an aldosterone secreting adenoma from patients.

The results support the hypothesis that renal PGE synthesis is important in maintaining renal blood flow in the presence of vasoconstrictor stimuli such as angiotensin II. The role of changes in renal PGE synthesis during the development of renal hypertension is less clear but the results are consistent with the hypothesis that PGE may serve as an intrarenal natriuretic agent. Systemic PGI_2 synthesis does not appear to be of importance in modulating the systemic vasoconstrictor effects of angiotensin II. The observation of relative increases in its synthesis during period of high sodium intake, immediately after a rapid intravenous infusion of sodium chloride and in the presence of hyperaldosteronism are all consistent with the concept that changes in vascular filling pressure may be modulated by changes in systemic PGI_2 synthesis.

Use this side only

DECLARATION

The work in this thesis was performed by myself (unless otherwise stated) in the Departments of Pharmacology and of Medicine, in the University of Edinburgh and in the Department of Clinical Pharmacology, Vanderbilt University, Nashville TN, between 1977 and 1983.

Abbreviations

cms	centimetres
d^4	deuterium
d^o	proton
EDTA	ethylenediamine tetra acetic acid
fg	fentogram
g	gram
Hg	mercury
Hz	hertz
h	hour
^3H	tritium
i.v.	intravenous
i.u.	international unit
kf	ultrafiltration coefficient
kgs	kilograms
l	litre
m^2	square metre
M	molar
mgs	milligrams
mins	minutes
ml	millilitre
mm	millimetre
mmols	millimoles
mOsm	milliosmoles
ng	nanogram
nm	nanometre
nmol	nanomol

P and II	hydraulic and oncotic pressure
pg	picogram
pG	prostaglandin
%	percentage
SNGFR	single nephron glomerular filtration rate
Tc	technetium
ugms	microgram
umol	micromole
w/v	weight per unit volume

INTRODUCTION

1. GENERAL CONSIDERATION

In experimental animal models and in man the transition from normotension to hypertension is a complex process characterised by, amongst other things, changes in cardiac output, total peripheral resistance and renal function. The relative importance of these these variables in the transition remains controversial. One theory that attempts to integrate all the known factors, and may also account for some that are still unknown, is that of whole body autoregulation. First proposed by Ledingham and Cohen (1964) and expanded upon by Guyton, Coleman, Cowley, Scheel, Manning and Norman, (1972), the theory proposes that the rise in blood pressure that occurs during the development of hypertension is mediated entirely through a rise in cardiac output. This is believed to trigger a myogenic response in the resistance vessels producing an 'autoregulatory' rise in total peripheral resistance which eventually leads to a restoration of normal cardiac output. An additional feature of the theory which has been emphasised by others (Kaplan 1979), is the pre-eminent role of the kidney in this process. Blood pressure cannot be persistently elevated over a long period of time without the occurrence of some adaptation of renal function. Any acute elevation in blood pressure will result in sustained natriuresis, followed by a decrease in extracellular fluid volume

and cardiac output, and a restoration of normotension. The proposed adaptation of the kidney in established hypertension involves resetting of the natriuretic response to increased blood pressure such that diminished natriuresis occurs and the hypertension persists.

This is envisaged to be an 'infinite gain' long term effect, and therefore assigns a critical role to the kidney in any theory accounting for long term blood pressure elevation.

The theory naturally arouses much controversy, perhaps fueled by its heavy reliance on theoretical aspects of control systems, and the expression of these concepts in terms of engineering principles. Supportive experimental evidence for the occurrence of long term autoregulaton is limited and has been criticised as inadequate (Korner 1980). The pre-eminent role assigned to the kidney for the long term control of blood pressure has more general support and has led to much work aimed at determining the mechanism controlling renal sodium excretion.

2. CONTROL OF SODIUM EXCRETION

There are numerous postulated mechanisms for the control of sodium excretion but this introduction particularly explores those mechanisms in which prostaglandins and the renin angiotensin system may participate directly. The mechanisms may best be divided into those primarily operating through changes in vascular tone, and those directly affecting tubular

function.

Renal blood flow and glomerular filtration rate are the basic physical determinants of sodium excretion. The glomerular filtration rate of a single nephron (SNGFR) maybe expressed as:-

$$SNGFR = K_f \ (P-II)$$

K_f = ultrafiltration coefficient

$$K_f = KxS$$

K = effective hydraulic permeability of capillary wall

S = total surface area for filtration

P - II = net driving force for filtration

ie. the difference between hydraulic and oncotic presssures.

The hydraulic pressure difference across the glomerulus is determined by afferent and efferent arteriolar tone. Thus increased renal blood flow, if accompanied by no change in either afferent or efferent arteriolar tone, will not change glomerular filtration rate. Renal blood flow and efferent arteriole tone are together the important determinants of peritubular capillary hydraulic pressure. Under most circumstances there is a large drop in hydraulic pressure between the glomerular capillary circulation and the peritubular capillaries at the end of the efferent arteriole. Peritubular capillary oncotic pressure usually exceeds the hydraulic pressure and there is therefore a net force for fluid reabsorption from the adjacent proximal tubule. Increased renal blood flow without change in efferent arteriolar tone may increase peritubular

hydraulic pressure, thereby reducing the drive for fluid reabsorption from the proximal tubule. On the other hand, unchanged or increased renal blood flow with an increase in efferent arteriole tone will result in increased hydraulic driving force for glomerular filtration. Changes in physical structure of the glomerulus may also change the ultrafiltraton coefficient and thus result in changes in glomerular filtration rate.

Activity of the renin angiotensin system is an important determinant of glomerular afferent and particularly efferent arteriolar tone (Myers, Deen and Brenner 1975; Brenner 1980). However there also appears to be an effect of angiotensin II on mesangial cell tone, perhaps via interference with transmembrane calcium transport (Ichikawa, Miele and Brenner 1979). Such an effect could well result in dramatic changes in glomerular capillary surface area available for ultrafiltration and therefore in Kf. Despite the renal vasodilator activity of prostaglandin E_2, D_2 and I_2 (Friesinger, Oelz, Sweetman, Nies and Data 1978; Jones, Watson and Ungar 1981) they only have a marginal effect on glomerular filtration rate when infused into the kidney, since under these conditions they produce equal effects on both afferent and efferent vessels, or a dominant effect on the efferent vessel (Baylis, Deen, Myers and Brenner 1976). However prostaglandins are synthesised by mesangial cells, glomerular epithelial cells and afferent and efferent arterioles (see later)

and are therefore well placed to modulate locally glomerular filtration rate, renal blood flow and therefore proximal tubular sodium reabsorption.

Distribution of blood flow within the kidney varies under different conditions. Evidence is accummulating that nephrons in outer and inner cortex vary considerably in their capacity to excrete sodium and water, partly by virtue of different filtration rates and length of tubules in nephrons from different sites (Horster and Thurau 1968). In the rat expansion of extracellular fluid volume results in greater delivery of sodium out of juxtamedullary nephrons, suggesting that these nephrons differ significantly from those in the outer cortex in their response to volume expansion (Stein, Osgood and Kunau 1976). There is evidence in favour of prostaglandins having a role in increasing blood flow preferentially towards these inner cortical nephrons (Kirschenbaum, White, Stein and Ferris 1974; Chang, Splawinski, Oates and Nies, 1975), thus providing an alternative mechanism by which prostalgandins may promote sodium excretion.

Direct effects of hormones on tubular sodium reabsorption seems an obvious means of controlling sodium excretion. Definitive evidence on involvement of particular hormones has not been easy to obtain since direct micropuncture methods are the only useful techniques. The role of aldosterone as a determinant of distal tubular sodium transport is most clearly established (Schwartz and Burg 1978). Angiotensin II

also probably has a direct effect on proximal tubular sodium transport. The effect observed on the isolated proximal tubules is dose dependent but at concentrations liable to occur in the kidney it is most likely to inhibit tubular sodium reabsorption (Harris and Young 1977; Schuster, Kokko and Jacobson, 1984). Direct effects of prostaglandins on tubular sodium transport have also been reported (see later).

Many factors other than prostaglandins and the renin angiotensin system have been invoked as determinants of renal haemodynamics and sodium excretion and therefore by implication in controlling systemic blood pressure. However there is also an entirely separate mechanism by which the kidney, independent of its capacity to excrete sodium, may lower blood pressure.

3. ANTIHYPERTENSIVE FUNCTION OF THE KIDNEY

Goldblatt first reported in 1934 that partial occlusion of one renal artery with the opposite kidney remaining intact, led to modest elevation of blood pressure (Goldblatt, Lynch, Hanzal and Summerville 1934) (1 clip - 2 kidney model of hypertension). Later it was demonstrated that much more severe hypertension followed if one renal artery was partially occluded and the contralateral kidney was removed (Goldblatt 1938). The contralateral kidney was apparently exerting antihypertensive activity. At first sight it might be assumed that this was a result of impaired renal excretory function after removal of the

contralateral kidney. In 1949 it was clearly established that the antihypertensive effect is independent of renal excretory function, but likely to be due to a blood born secretion from the kidney (Grollman, Muirhead, Vanatta 1949). Production of the factor is localised in the renal medulla (Lee, Covino, Takman and Smith 1965), indeed its effect can be mimicked in hypertensive rabbits by injection of cultured medullary interstitial cells (Muirhead, Brooks, Pitcock and Stephenson, 1972). In view of the known capacity of interstitial cells to synthesise prostaglandins it was thought that these might be the responsible hypotensive agents (Muirhead, Germain, Leach, Pitcock, Stephenson, Brooks, Brosius, Daniels and Hermain 1972). PGA, although subsequently shown to be an artifact, appeared at one time to be a particularly likely candidate (Lee, Covino, Takman and Smith 1965). It now seems most likely that the antihypertensive compound is a glyceryl ether derivative (Muirhead, Byers, Desiderio, Brooks and Brosius 1981). The interstitial cells are intimately associated with vasa rectae, collecting ducts and loops of Henle and are therefore well placed to influence tubular functions. Although prostaglandins do not function as circulating antihypertensive agents, several of them are potent vasodilators and may also have direct effects on renal tubular function (see later). Once synthesised in these cells they are well placed to exert effects on sodium and water excretion, and therefore on blood

pressure.

Many studies have commented on the presence of dark osmiophilic granules in the interstitial cells (Osvaldo and Latta 1966), and it has been demonstrated that the number of such granules per interstitial cell is altered under a variety of different conditions of sodium balance and blood pressure (Nissen 1968; Tobian, Ishii and Duke 1969; Pitcock, Brown, Byers, Brooks and Brosius 1981). The relationship of these granules to prostaglandin synthesis by the cells is unclear. Part of this thesis presents results of a study in dogs that aims to clarify this point, and relate it to the possible antihypertensive role of these cells.

The 1 clip - 2 kidney model of hypertension in the dog, described by Goldblatt in 1934, has proven to be a useful experimental model for investigating both the mechanisms by which the kidney may increase blood pressure, and the mechanism by which it serves to ameliorate increases in blood pressure.

The mechanism responsible for sustained elevation of blood pressure in this model is complex. The cause of the initial rise in pressure after partial occlusion of a renal artery is increased release of renin from the clamped kidney. The increased renin stimulates formation of angiotensin II. The rise in plasma concentration of angiotensin II is sufficient to account entirely for the rise in blood pressure (Caravaggi, Bianchi, Brown, Lever, Morton, Powell-Jackson, Robertson and Semple 1976; Freeman, Davis,

Watkins and Lohmeir 1977). Within a few days of partial constriction of the renal artery, plasma renin activity returns towards normal, but the blood pressure remains elevated ie. established hypertension develops (Bianchi, Baldoli, Lucca and Barbin, 1972; Watkins, Davis, Hanson, Lohmeir and Freeman, 1976; Lupu, Maxwell and Kaufman 1977; Maxwell, Lupu, Viskoper, Aravena and Waks 1977).

During the renin dependent phase of 1 clip - 2 kidney hypertension,there is a transient modest retention of water (Bianchi, Baldoli, Lucca and Barbin 1972) and probably sodium (Watkins, Davis, Hanson, Lohmeir and Freeman 1976; Maxwell, Lupu, Viskoper, Aravena and Waks 1977). Although subsequent excretion of the retained salt and water appears to be temporarily related to the decline in plasma renin activity, evidence for the importance of this retained salt and water in the progression to established hypertension in such a model is lacking. In the 1 clip - 1 kidney model of hypertension (partial occlusion of one renal artery in the absence of the contralateral kidney) the situation is more clear. Although salt and water is retained in the period immediately after induction of this type of hypertension (Bianchi, Tenconi and Lucca 1970), this is neither a prerequisite for the development of, nor essential for the maintenance of the hypertension (Fray, Johnson and Barger 1977).

Previous studies of 1 clip - 2 kidney hypertensive dogs suggested that increased PGE production by the

untouched kidney several days after the partial occlusion of the contralateral renal artery mediated the excretion of salt and water that had been retained over the first four days (Dighe, Smith, Ungar and Whelpdale 1978). One of the studies in this thesis aimed to further test this hypothesis and at the same time provide further information on the role of retained sodium and water in mediating transition to established hypertension.

4. MECHANISMS OF PROSTANOID SYNTHESIS

4.1 Phospholipase

Most of the prostanoids that are at present thought to have important roles in regulation of vascular tone and renal function are derived from arachidonic acid (C20:4). Substantial amounts of arachidonic acid in the body are esterified to either triglycerides or cholesterol esters, particularly for example in the renal medulla (Nissen and Bojesen 1969; Comai, Farber and Paulsrud, 1975) but in most tissues the major pool of arachidonic acid is in phospholipids (Morgan, Tinker and Hananhan 1963; Miller, Kaiser, Baver, Scheiber and Hohenegger 1976). Synthesis of prostanoids requires initial release of the fatty acid from the phospholipid (Lands and Samuelson 1968). Phospholipase A_2, the enzyme most commonly responsible for this process (Flower and Blackwell, 1976) is highly specific, both with regard to the position on the phospholipid of the fatty acid hydrolyzed (2 position only) (Isakson, Raz and Needleman, 1976) and the length of its carbon chain.

The metabolic pool of arachidonate available for prostanoid synthesis also does not appear to be a single entity; changes in fatty acid availability, for example by manipulation of dietary intake (Vergroesen, Dedecker, Tenhoor and Hornstra 1980) rapidly affect prostanoid synthesis, whilst at the same time causing little change in the fatty acid composition of membrane phospholipids. There are a large number of factors that potentially exert control on phospholipase A activity, including vasoactive peptide hormones such as bradykinin and angiotensin II (Limas and Limas 1979). Whilst the availability of free arachidonic acid is certainly a major determinant of the rates of prostanoid synthesis, the rate of re-acylaton of arachidnoic acid into phospholipids, by limiting the amount of free arachidonate available, may also be important.

4.2 Fatty acid oxygenation

Arachidonic acid is further metabolised to a large range of prostanoid products after incorporation of molecular oxygen by one of at least three different mechanisms (oxygenase reactions). The monoxygenase (e.g. lipoxygenase) and dioxygenase (e.g. cyclo-oxygenase) reactions are considered by the far most important, but a variety of hydroxylated derivatives of arachidonic acid can also be synthesised by a mixed function oxidase system. Examples of some of the reaction products of each system are shown in Fig 1.

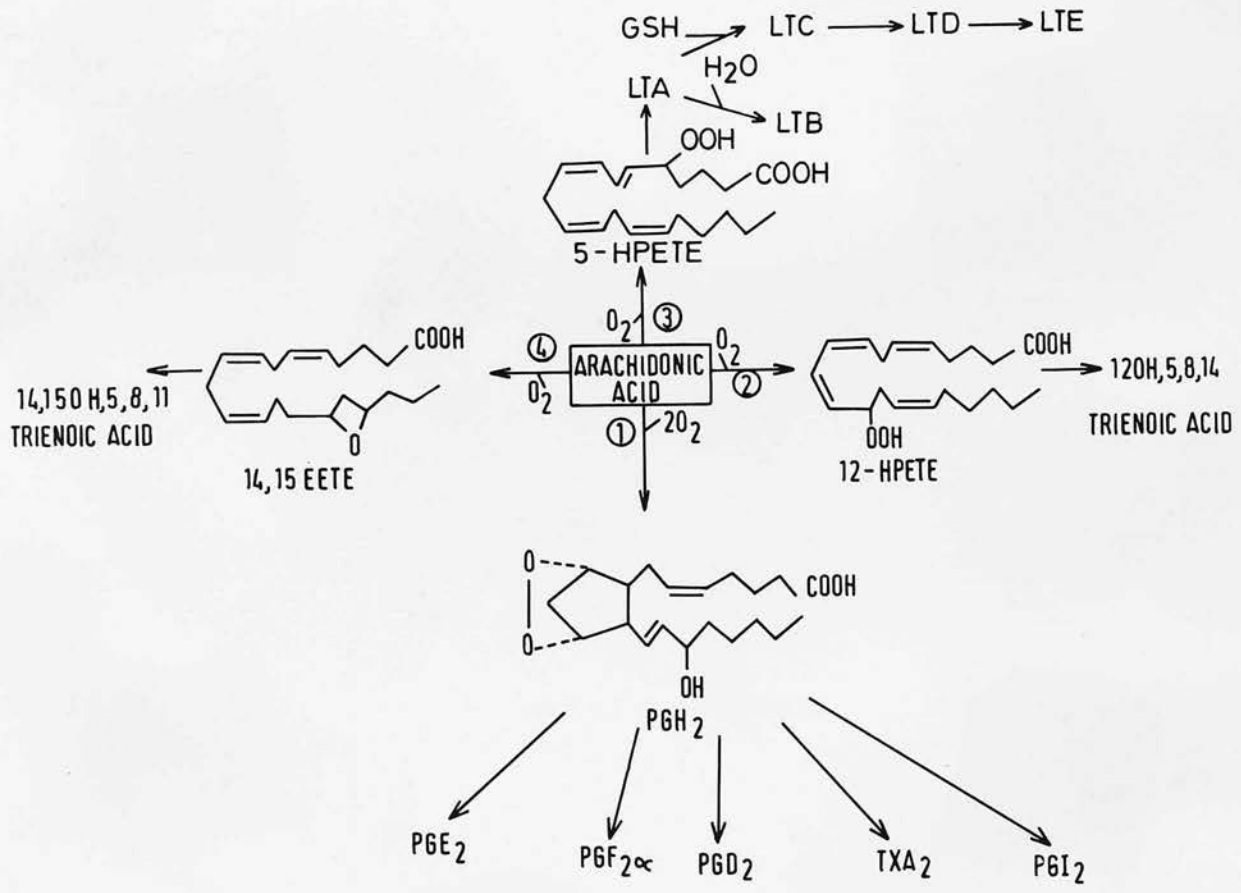

Fig 1. Oxygenation reactions for metabolism for arachidonic acid 1 = Cyclo-oxygenase; 2 = 12- lipoxygenase; 3 = 5 lipoxygenase; 4 = Cytochrome P450 mixed function oxidase. HPETE = hydroperoxy eicosatetraenoic acid; EETE = epoxy eicosatrienoic acid LT = leukotriene.

4.2.1. Cyclo-oxygenase

The products of the arachidonic acid cyclo-oxygenase reaction have been extensively documented. Incorporation of two atoms of molecular oxygen leads to formation of an unstable endoperoxide intermediate which is further metabolised to a variety of products. Until 1976, PGE_2, PGF_2 and PGD_2 were regarded as the prostaglandins of most biological significance, but the discovery of thromboxane A_2 and PGI_2 has modified this view. Indeed on the basis of biological activity there are reasonable grounds to implicate all of these metabolites as potential modulators of blood pressure, and/or renal function.

Whilst arachidonate is the major substrate for cyclo-oxygenase, other fatty acids such as dihomo γ linolenic acid (20:3) and eicosapentaenoic acid (C20:5) are also tightly bound to the enzyme although less rapidly metabolised to 1 and 3 series prostanoids respectively (Needleman, Raz, Minkes, Ferrendelli and Sprecher 1979). Phospholipase also releases C20:3 and C20:5 from phospholipids. The relative quantities of these different fatty acids released in response to a given stimulus and therefore competing with arachidonic acid for binding to the cyclo-oxygenase enzyme, may serve as an important factor determining the relative amounts of products formed (Siess, Roth, Scherer, Kurzman, Bohlig and Weber 1980).

4.2.2. Lipoxygenase

A single atom of molecular oxygen is inserted into the arachidonate molecule by a lipoxygenase system (Borgeat, Hamberg and Samuelsson 1976). This occurs across CIS double bonds leading to formation of chemically unstable epoxide intermediates across the C5-6, C11-12, and C14-15 double bonds. These epoxides are further metabolised, predominantly to the 5 series leukotrienes (Murphy, Hammarstrom and Samuelsson 1979), 12 and 15 hydroxyeicosatetraenoic acids and 15 series leukotrienes (Maas, Brash and Oates 1982).

4.2.3 Mixed function oxidase

Incorporation of molecular oxygen via a cytrochrome P450 dependent mixed function oxidase system also leads to formation of a range of unstable epoxide intermediates which are further metabolised to a variety of hydroxy fatty acids (Oliw, Lawson, Brash and Oates 1981; Capdevila, Marnett, Chacos, Prough and Estabrook 1982).

The biological significance of the lipoxygenase and mixed function oxidase products remains uncertain. Their mere existence serves to confuse the picture since, although under normal circumstances they may have little function, they may become important when the metabolic pathways are perturbed. For example whilst endoperoxides are unstable, they have significant biological activity (Feigen, Chapnick, Fleming and Kadowitz 1978). Inhibition of their metabolism to, for example, thromboxane A_2, may well lead to their

accumulation and to occupation of any available thromboxane receptors (Jones and Wilson 1980).

Likewise inhibition of cyclo-oxygenase by non-steroidal anti-inflammatory drugs, may cause accummulation of arachidonic acid, and so increase its metabolism by adjacent metabolic pathways such as the lipoxygenase or cytochrome P450 systems. These possibilities must always be kept in mind when assessing the evidence for the importance of these compounds in biological systems, where selective inhibitors are the only investigation technique utilised.

Only a limited number of these fatty acid oxygenation products are likely to have a significant role in the control of blood pressure and renal function. This thesis particuarly concentrates on the role of PGE_2 and PGI_2. The importance attached to the function of these two prostanoids is based on the description of their synthesis by tissues and organs that are most involved in the control of renal function and blood pressure.

5. LOCALISATION OF SYNTHESIS OF PGE_2 AND PGI_2 IN KIDNEY AND VASCULATURE

PGE_2 and PGI_2 are both synthesised by vascular endothelium (Terragno, Crowshaw, Terragno and McGiff 1975; Moncada, Higgs and Vane 1977) and isolated endothelial cells in culture (Gimbrone and Alexander 1975; Remuzzi, Mecca, Livio, De Gaetano, Bonati, Pearson and Gordon 1980; Whorton, Young, Data, Barchowsky and Kent 1982). The distribution of the sites of synthesis

of PGE_2 and PGI_2 within the kidney is complex. The renal medulla has a massive capacity to synthesise PGE_2 (Daniels, Hinman, Leach and Muirhead 1967; Larsson and Anggard 1973; Frolich, Sweetman, Carr and Oates 1975) which is primarily localised in the interstitial cells (Muirhead, Germain, Leach Pitcock, Stephenson, Brooks, Brosius, Daniels and Kinman 1971; Zusman and Keiser 1977). Many factors modulate interstitial cell synthesis of PGE_2 including angiotensin II, bradykinin, vasopressin and potassium concentration and osmolarity of the surrounding fluid (Zusman and Keiser 1977). Indeed the concept of single hormones or factors stimulating PGE synthesis is unduly simple. For example, aldosterone, although not itself stimulating prostaglandin synthesis, alters the stimulation of PGE_2 synthesis by vasopressin, probably by stimulatng phospholipase A_2 synthesis, thus giving rise to the potential for extremely complex multifactorial control systems (Zusman and Keiser 1977). The renal cortex also synthesisessmall amounts of PGE_2 (Frolich, Sweetman, Carr and Oates 1975) which may be important functionally (see later). Initial reports suggested that PGI_2 in contrast to PGE_2 was primarily synthesised in the renal cortex (Remuzzi, Cavenaghi, Mecca, Donati and De Gaetano 1978; Whorton, Smigel, Oates and Frolich 1978) with comparatively little being formed in the renal medulla (Whorton, Smigel, Oates and Frolich 1978). More recent studies however suggest that PGI_2 is also synthesised in

significant quantities by renal medullary tissue (Okahara, Imanishi and Yamamoto 1983).

Most investigators agree that prostanoids are likely to exert their physiological effects at or near their site of synthesis. Studies of prostanoid synthesis in whole tissue homogenates of complex organs such as the kidney therefore provide limited information. Determination of specific cellular sites of prostaglandin synthesis within the kidney is difficult, particularly because of changes in prostanoid synthesis that occur during the tissue separation and fractionation procedures. Isolated glomeruli studied in this fashion have been shown to synthesise a range of arachidonate-derivedproducts particularly PGE_2 but also PGI_2 (Folkert and Schlondorff 1979; Hassid, Konieczkowski and Dunn 1979; Sraer, Sraer, Chansel, Russo-Marie, Koutnetzova and Ardaillou 1979), some thromboxane A_2 (Hassid and Dunn 1979; Petrulis, Aikawa and Dunn 1981) and to a lesser extent ill-defined lipoxygenase products (Lianos and Dunn 1983; Petrulis, Aikawa and Dunn 1981). Isolated segments of renal vasculature have also been shown to synthesise prostaglandins (Terragno, Terragno and McGiff 1978). An alternative approach has been to culture cells derived from important structures in the renal cortex and medulla. Cell culture studies of renal medullary cells have already been described but more recent studies have also been undertaken using cell lines derived from renal cortical tissues. Mesangial cells derived from

glomeruli appear primarily to synthesise PGE_2, whilst glomerular epithelial cells synthesise PGI_2 (Kreisberg, Karnovsky and Levine 1982). This observation may be of considerable functional importance. Mesangial cells are contractile and by this means probably alter the surface area of the glomerulus available for filtration, thereby altering glomerular hydraulic conductivity. Recent observations suggest that angiotensin II stimulates PGE_2 release from these mesangial cells (Sharschmidt and Dunn 1983) which may then modulate the effects of angiotensin II on mesangial cell tone. Juxtaglomerular cells which synthesise renin have also been grown in cell culture (Rightsel, Okamura, Inagami, Pitcock, Takii, Brooks, Brown and Muirhead 1982). Both these cells and mesangial cells are similar to smooth muscle derived cells, and like mesangial cells, the juxtaglomerular cells also preferentially appear to synthesise PGE_2, in addition to much smaller amounts of PGI_2 (Watson, Inagami and Branch unpublished observations).

An alternative method of identifying sites of prostaglandin synthesis is by using histochemical techniques to localise prostaglandin endoperoxide synthetase, since prostaglandins are not themselves stored in cells. This evidence suggests that there is likely to be active synthesis of prostaglandins in the collecting ducts of the renal medulla (Janszen and Nugteren 1971; Bohman 1977; Smith and Wilkin 1977) and in the vascular tissue and to a lesser extent in the glomeruli of the renal cortex (Smith and Bell 1978).

6. PROSTANOID METABOLISM

Investigation of the metabolism of prostanoids has relied heavily on the mass spectrometric analysis of urinary metabolites. The typical approach employed is to inject intravenously a mixture of tritiated and protonated compound, and make collections of urine subsequently voided. Radioactive peaks are separated by high performance liquid chromatography and the identity of each peak determined by measuring its full mass spectrum (Brash, Jackson, Saggesse, Lawson and Fitzgerald 1983). Identity of the metabolites then permits deductions as to the major routes of metabolism.

Most prostanoids are metabolised by similar pathways. The most important enzymes are prostaglandin 15 - hydroxy dehydrogenase and prostaglandin 13-14 reductase. Both lung and kidney contain large amounts of these enzymes (Anggard, Larsson and Samuelsson 1971), thus PGE_2 neither acts as a circulating hormone, nor appears in the urine unchanged after an intravenous injection (Hamberg and Samuelsson, 1971). As with other fatty acids, prostanoids are also susceptible to beta oxidation (Hamberg and Samuelson 1972), occurring primarily in the liver and kidneys. Further metabolism may also occur by both alpha and Omega oxidation (Roberts, Sweetman and Oates 1981). The metabolism of PGI_2 is a little more complex than PGE_2. PGI_2 itself is an unstable compound that spontaneously hydrolyses to 6 keto PGF_{1a} in aqueous solution (Cho and Allan 1978). Whilst both PGI_2 and 6 keto PGF_{1a} are substrates for the

PG 15 hydroxy dehydrogenase, PGI_2 in particular has poor affinity for the receptor governing uptake into the lung alveolar cells, where most of the enzyme is situated (McGuire and Sun 1978). Much of the further metabolism of these two compounds therefore occurs in liver and kidney, which, as with PGE_2, includes in addition to 15 hydroxy dehydrogenation, alpha, beta and omega oxidation (Rosenkranz, Fischer, Weimer and Frolich 1980; Brash, Jackson, Saggesse, Lawson and Fitzgerald 1983). Fig 2 illustrates some of the metabolites of PGI_2 most prevalent in the urine.

Metabolism of PGE_2 and PGI_2 also occurs at the 9 position on the carbon chain. Prostaglandin 9 ketoreductase, an NADH dependent enzyme present in both liver and kidney, promotes the conversion of PGE_2 to PGF_{2a} (Leslie and Levine 1973; Lee and Levine 1974; Mooreand Hoult 1978). In rabbits activity of this enzyme system in the kidney has been related to the state of sodium balance (Weber, Larsson and Scherer 1977). Shunting of PGE_2 metabolism through this pathway may provide a further means of controlling the local concentration of PGE_2. 6 keto PGF_{1a} may be metabolised by a similar NADPH dependent enzyme, 9 hydroxy dehydrogenase to 6 keto PGE_1 (Wong, McGiff, Cagan, Malik and Sun 1979). This compound has potent activity as both a vasodilator and renin secretagogue (Jackson, Herzer, Zimmermann, Branch, Oates and Gerkins 1981) and this reaction could therefore be of considerable significance. However, although there is potential for

6 keto PGE_1 synthesis, it remains to be proved whether biologically significant quantities of this compound are acutally synthesised in vivo (Jackson and Goodman 1981).

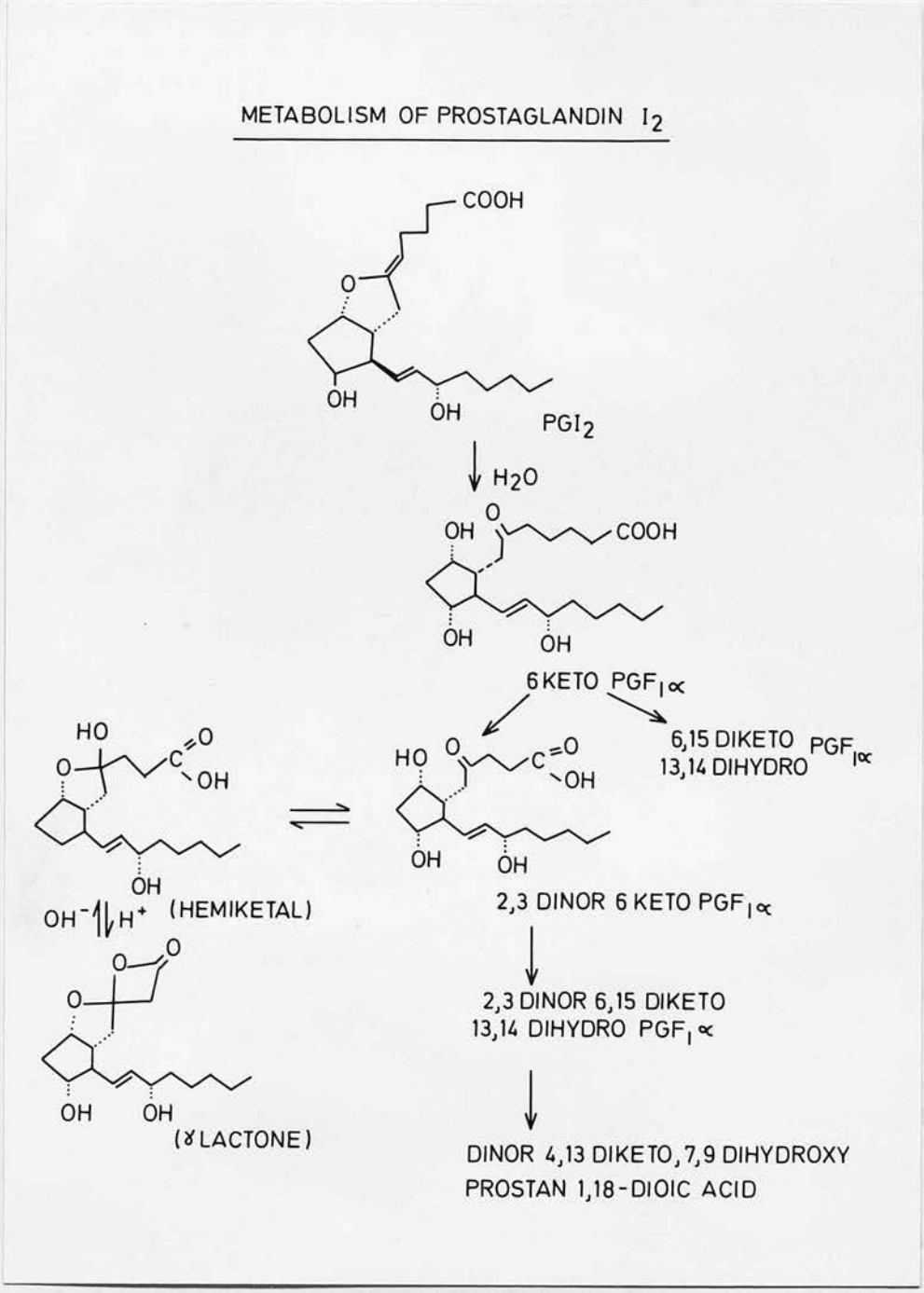

Fig 2. Metabolic path for metabolism of PGI_2. Enzymes implicated are 15 hydroxy dehydrogenase, 13, 14 reductase, Beta and Omega oxidation. The hemiketal form of dinor 6 keto $PGF_{1\alpha}$ is in equilibrium with the open form. Formation of the complete lactone is promoted under acid conditions.

7. RENAL EFFECTS OF PGE_2 AND PGI_2

The simplest approach to assessing the effect of PGE_2 and PGI_2 on renal function is by infusing the compounds directly into the renal artery of anaesthetised animals and observing the changes in renal haemodynamics and excretory function. Both PGE_2 and PGI_2 are potent renal vasodilators (Jackson, Heideman, Branch and Gerkins 1982), increments in renal arterial plasma PGE_2 concentration of as low as 25 pg/ml causing significant increases in renal blood flow (Jones, Watson and Ungar 1981). Infusion of low doses of both compounds also promotes natriuresis and diuresis (Martinez-Maldonado, Tsaparas, Eknoyan and Suki 1972; Bolger, Eisner, Ramwell and Slotkoff 1978). Infusion of PGI_2 into the kidney significantly increases renin release (Bolger, Eisner, Ramwell and Slotkoff 1978; Gerber, Keller and Nies 1979). Most supporting evidence implicating prostaglandins in the control of renin release in vivo is based on studies using prostaglandin synthetase inhibitors (Frolich, Hollifield, Dormois, Frolich, Seyberth, Michelakis and Oates 1976; Speckart, Zia, Zipser and Horton 1977). Such studies have implicated prostaglandins as mediators of renin release as a result of baroreceptor and macula densa stimulation (Data, Gerber, Crump, Frolich, Hollifield and Nies 1978; Blackshear, Spielman, Knox and Romero 1979; Gerber, Nies and Olsen 1981). Release as a result of sympathetic nervous activity is more likely to be a direct effect that does not involve prostaglandins (Berl, Henrich,

Erikson and Schrier 1979; Vandongen, Tunney, Mahoney and Barden 1981). An important specific role for PGI_2 in this process is further supported by its demonstrated ability to release renin from incubated slices of renal cortex, and also to enhance release from beta blocked, indomethacin treated, non-filtering kidneys (Whorton, Misono, Hollifield, Frolich, Inagami and Oates 1977; Seymour 1979).

As already indicated the major action of prostaglandins is likely to depend on their site of synthesis, since they are unlikely to function as circulating hormones. Infusion of prostaglandins into the whole kidney therefore provides only limited information on their renal actions. The use of prostaglandin synthesis inhibitors provides an alternative method of investigation.

In conscious animals inhibition of prostaglandin synthesis has comparatively minor effects on renal function - inducing no real change in renal haemodynamics and if anything a mild natriuresis (Swain, Heyndricky, Boettcher and Vatner 1975; Kirschenbaum and Stein 1976). In anaesthetised traumatised animals the situation is rather different with several studies documenting a reduction in renal blood flow and diminuition of sodium and water excretion after inhibition of prostaglandin synthesis (Aiken and Vane 1973; Terragno, Terragno and McGiff 1977; Beilin and Bhattacharya 1977; Henrich, Anderson, Berns, McDonald, Paulsen, Berl and Schrier 1978). Indeed a period of

sodium restriction is sufficient to sensitise the renal vasculature to the effects of prostaglandin inhibition (Blasingham and Nasjletti 1980). The mechanism of stimulation of prostaglandin synthesis under these stressful conditions is unclear, but is probably a response to associated neuro-humeral events. It is known that the renal vasocontrictors angiotensin II and noradrenaline, both of which are released during sodium restriction (Oliver, Pinto, Sciacca and Cannon 1980) stimulate release of PGE_2 from the kidney (McGiff, Crowshaw, Terragno and Lonigro 1970; McGiff, Crowshaw, Terragno, Malik and Lonigro 1972; Rowe and Nasjletti 1983). The renal vasoconstrictor properties of these compounds are enhanced by inhibition of prostaglandin synthesis, implying a role for prostaglandins as modulators of vascular tone, at least under these conditions, in the kidney (Aiken and Vane 1973). Furthermore renal nerve stimulation has been clearly documented to stimulate renal prostaglandin synthesis, particularly PGE_2 (Davis and Horton 1972) and the effects of renal nerve stimulation are also enhanced by inhibition of prostaglandin synthesis (Malik and McGiff 1975; Henrich, Anderson, Bern, McDonald, Paulsen, Berl and Schrier 1978: Susic and Malik 1981).

All of these findings can best be summarised by concluding that under a variety of stressful conditions, particularly when the kidney is influenced by vasoconstrictor activity, prostaglandins become important in maintaining renal blood flow. Having

generalised on this point it is still by no means clear which particular prostaglandins are most important in this respect; this question is addressed in some detail in some of the studies to be described.

Involvement of prostaglandins in control of sodium balance is still more controversial. PGE_2 and PGI_2 cause natriuresis when infused into the kidney, but renal blood flow increases at the same time. It is therefore difficult to be certain whether the natriuresis is the result of renal vasodilation or direct inhibition of tubular sodium transport. Micropuncture studies indicate that the latter is a distinct possibility (Stokes and Kokko 1977; Stokes 1979). However, it has been reported (Weber, Larsson and Scherer 1977) that a high sodium intake in rabbits stimulates conversion of PGE_2 to PGF_{2a} via a prostaglandin 9 ketoreductase. The resulting reduction in PGE_2 during sodium loading argues against a direct role for PGE_2 in promoting sodium excretion. Perhaps the decrease in PGE_2 levels represents a means by which renal renin release is supressed in response to a high sodium intake, in view of the known ability of PGE_2 to stimulate renin release. Other studies in rabbits, dogs and man have demonstrated that a low sodium intake increases renal synthesis of PGE_2, as measured by synthesis in incubated sections of kidney (Stahl, Attallah, Bloch and Lee 1979) and excretion in both urine (Rathaus,Podjarny, Weiss, Ravid, Bauminger and Bernheim 1981) and renal venous blood (Oliver, Pinto,

Sciacca and Cannon 1980). Whilst this may reflect a direct effect of the changes in extracellular fluid volume, it is more likely to be due to increased adrenergic nervous stimulation and/or renin angiotensin activity, supporting the possibility that vasodilator prostaglandins modulate the pressor effects of such systems. Whilst this is an attractive hypothesis, there is also evidence to suggest that pressor activity of these systems may be influenced by other factors. For example the increase in endogenous concentration of angiotensin II during sodium depletion maybe associated with increased receptor occupancy (Thurston and Laragh 1975) and alteration of receptor sensitivity (Strewler, Hinrichs, Guido and Hollenberg 1972) and numbers (Aguilera and Catt 1981), all factors which might decrease the apparent pressor activity of angiotensin II. Any role under physiological conditions for prostaglandins in this process remains unproven.

Although it is well established that infusion of PGE_2 and PGI_2 into the renal artery induces natriuresis, their role as endogenous intra-renal natriuretic substance is less clear. Previous studies on human subjects have suggested that the increased rate of excretion of sodium and water by the kidney which follows expansion of the volume of extracellular fluid is associated with increased production of renal prostaglandins (Papanicoloau, Safar, Hornych, Fontaliran, Weiss, Bariety and Milliez 1975; Epstein, Lifschitz, Hoffman and Stein 1979). In keeping with

these observations, most workers have found that in human subjects inhibitors of prostaglandin synthetase cause sodium retention (Donker, Arisz, Brentyens, Van Der Hem and Hollemans 1976; Berg 1977). By contrast, pre-treatment of normal human subjects with indomethacin has been shown to enhance the rate of sodium excretion after an intravenous infusion of sodium chloride solution, suggesting that under certain conditions prostaglandins may have anti-natriuretic effects (Mountokalakis, Karambasis, Mayopoulou-Symvoulidou and Merikas 1978), and this concept receives some support from the studies of Kirschenbaum and Stein (1976), who observed a natriuretic effect of meclofenamate in conscious dogs undergoing water diuresis. Such discrepant results are not easy to explain and have been further explored in this thesis.

PGE_2 in particular also appears to have a separate effect on renal function by modulatng the anti-diuretic actions of vasopressin on the collecting ducts. Initial studies indicated that PGE_1 opposed the action of vasopressin on the isolated toad bladder (Orloff, Handler and Bergstrom 1965) and collecting duct preparations (Grantham and Orloff 1968). In water diuresing whole animals it was then shown that inhibition of prostaglandin synthesis enhanced the anti-diuretic effect of vasopressin (Lum, Aisenbrey, Dunn, Berl, Schrier and McDonald 1977). Subsequent studies in both the Brattelboro rats (Walker, Whorton, Smigel, France and Frolich 1978) and in patients with diabetes

insipidus (Fichman, Zia and Zipser 1980) have provided supporting evidence for this possible role of prostaglandins.

The apparently contrasting actions of prostaglandins under different conditions emphasise that their actions on the kidney are likely to depend on the exact site of synthesis. For example increased synthesis of prostaglandins in the mesangial cells of the glomerulus, the afferent arterioles or collecting duct cells must certainly have very different effects on renal function.

8. PGE_2, PGI_2 AND SYSTEMIC VASCULATURE

Initial studies with prostaglandins raised the possibility, first with PGE_2, then with PGA_2 and finally with PGI_2 that they acted as circulating vasodepressor hormones. It subsequently became clear approximately 70% of PGE_1 in venous blood was metabolised on single passage through the lungs (Golub, Zia, Matsuno and Horton 1975) and that PGA_2 in plasma was an artifact as a result both of its conversion from PGE_2 during extraction and insufficiently specific radioimmunoassay (Frolich Sweetman, Carr, Hollifield and Oates 1975). PGI_2 escapes metabolism on the passage through the lungs (Dusting, Moncada and Vane 1978) and, based on the apparent plasma concentration of its stable hydrolysis product, 6 keto PGF_{1a}, was thought to circulate in significant concentrations and thus possibly act as a circulating hypotensive agent (Hensby,

Fitzgerald, Friedman, Lewis and Dollery 1979). Subsequent improvements in methodology have now clearly established that the plasma concentration of 6 keto PGF_{1a} is extremely low (Christ-Hazelhof and Nugteren 1981; Blair, Barrow, Waddell, Lewis and Dollery 1982; Siess and Dray 1982), and certainly the concentration of PGI_2 in the systemic circulation is well below that required for a hypotensive effect.

PGI_2 and PGE_2 may however exert vasodilator actions at or near their site of synthesis. Both compounds are synthesised by arteries (Blumberg, Denny, Marshal and Needleman 1977; Pace-Asciak, Carrara, Rangaraj and Nicolaou 1978) and veins (Hamilton, Rosza and Hutton 1981). Increased synthesis by arterioles or even venous capacitance vessels might well produce sufficient local vasodilation to lower blood pressure. In support of this hypothesis is some evidence that vasculature from spontaneously hypertensive rats has an increased capacity to synthesise PGI_2 compared with normotensive controls (Pace-Asciak, Carrara, Rangaraj and Nicolaou 1978).

It is particularly attractive to spectulate that vasodilator prostaglandins may be released from the systemic vasculature in response to infusion of vasoconstrictor compounds such as angiotensin II. In a number of in vitro preparations of isolated vascular strips, or perfused vascular beds, angiotensin II has been reported to release both PGE_2 and PGI_2 (McGiff, Crowshaw, Terragno and Lonigro 1970; Aiken and Vane

1973; Shebuski and Aiken 1980; Dusting, Mullins and Doyle 1980). On the other hand angiotensin II failed to stimulate PGI_2 release from vascular endothelial cells in culture (Whorton, Young, Data, Barchowsky and Kent 1982). In all instances however the doses of angiotensin II used were well in excess of those likely to occur in vivo. If prostaglandins significantly modulate the pressor activity of angiotensin II in man it might be expected that the pressor response to infused angiotensin II would be increased in the presence of prostaglandin synthetase inhibitors. Several studies in man support this hypothesis (Negus, Tannen and Dunn 1976; Vierhapper, Waldhausl and Nowotny 1981) but the evidence is unconvincing. Renal release of renin is inhibited by inhibition of prostaglandin synthesis (see previously) and the circulating concentration of angiotensin II falls. The change in concentration of endogenous angiotensin II alters the basis on which the dose response curve is constructed, such that the pressor response to infusion of angiotensin II is apparently increased, and the spurious assumption made that this a consequence of the loss of some vasodilator activity. Part of the present thesis further addresses this particular question.

1. PREPARATION OF ANIMAL MODEL

1.1 Introduction

The protocol employed for the initial studies demanded that the animals remain healthy with patent arterial and venous catheters for at least 25 days. Despite repeated attempts using numerous different methods of inserting catheters into arteries this proved possible on only rare occasions (2 out of 25 animals). The procedures described below were therefore employed (formation of carotid arterial loops) with a subsequent dramatic improvement in the success rate achieved. Integral to this success was the employment of inhalation anaesthesia during the surgical procedures and a consistent post-operative antibiotic policy. In the studies where catheter patency was required for shorter periods (10 days approximately), a satisfactory success rate was achieved with chronic indwelling catheters.

1.2 Animals

Fox hound bitches were used in studies in 2.1 and 2.3 (weight 16.8 -26.2 kgs) and mongrol bitches in study 2.2 (weight 17-24kgs).

1.3 Surgical Procedures

1.3.1 Anaesthesia

After pre-medication with chlorpromazine (50 mgs iv) anaesthesia was induced by intravenous thiopentone, an endotracheal tube was inserted and a satisfactory depth

of anaesthesia subsequently maintained by administration of a mixture of oxygen/nitrous oxide/halothane.

1.3.2. Formation of carotid arterial loop

The common carotid artery was exposed by a ventral mid line incision (15cms long) in the neck and mobilised for approximately 12 cms distal to the carotid bifurcation. The superior thyroid artery was tied and cut. A second slightly shorter incision through the skin in the neck was made approximately 7 cms lateral to the first. The carotid artery was then drawn into the flap of skin formed and the edges opposed and fixed with interrupted nylon sutures, so as to place the artery in a tunnel of skin. The lateral edges of each incision were then drawn together underneath the loop and again fixed with interrupted nylon sutures. It was thus possible to place at least two fingers between the tunnel of skin containing the artery and the skin of the rest of the neck. After the operation the neck was protected by a light bandage. Twenty-four hours later this was changed to a plaster of paris collar around the neck to protect the loop from trauma. This was changed weekly for three weeks, the sutures being removed after two weeks. Healing was complete within one month, the plaster was removed and no further protection was required thereafter.

1.3.3 Positioning of renal artery clamp and venous catheters

A laparotomy was performed via a right paramedian incision and the right renal artery exposed through the

posterior peritoneum. A metal externally adjustable screw clamp was introduced through a stab wound in the subcostal angle and placed on the right renal artery. The clamps used were the same as those described previously (Dighe, Smith, Ungar and Whelpdale 1978) and were held in position around the artery by a metal clip. The silastic tube leading to the clamp was buried subcutaneously.

Two silastic catheters (Esco Ltd 1.0 mm internal diameter, 2.0 mm external diameter, 70 cm long) were introduced at the back of the neck, and with the assistance of a metal rod led down the back into the abdomen. One catheter was inserted into the left renal vein via the left ovarian vein and the other into the inferior vena cava, usually via the right ovarian vein. Catheters were terminated by metal luer fittings, one way metal taps and metal obturators. They were flushed on at least every third day with sodium chloride solution (150 mmols/l) after removal of the dead space, and filled with heparinised saline (1000 iu/ml).

When arterial catheters were used they were also introduced at the back of the neck and lead down to the groin. They were then inserted into the femoral artery via a small incision. This was closed with thin nylon sutures, and the catheter was also fixed to the external surface of the vessel by thin nylon sutures.

Post-operative analgesia was maintained with intramuscular cyclimorph for 36 hours. Sutures were removed after 10 days.

1.3.4 Placement of flow probes on a renal artery.

With the animals anaesthetised in the usual fashion the left renal artery was exposed by a flank incision. An electromagnetic flow probe (Statham 3 or 4 mm diameter) was clipped around the vessel and the electrical connecting cable was exteriorised via a stab incision in the flank. The leads were protected by, and carefully sutured to a close fitting nylon jacket which the animals wore at all times after the procedure.

1.3.5 Production of hypertension

Hypertension was induced by partial occlusion of one renal artery by the screw clamp previously positioned in the animal. On the day of clamping a control blood pressure was first measured and the animals were then lightly anaesthetised with thiopentone. The tube from the clamp was retrieved through a smallflank incision and the screw driver inserted. The screw was turned to complete occlusion and then backed off by one and half turns (approx 0.8 mm). The blood pressure was monitored during the clamping procedure and in most cases there was an immediate increase in mean pressure of 10-15 mmHg, which returned to control values over the ensuing 5 minutes.

1.4 Diets

The animals were given two separate diets depending on the period in the study (study 2.1 only). During the high sodium period they received a diet containing an estimated 60 mmols of sodium and 40 mmols of potassium per day. During the low sodium period the diet

contained less than 10 mmols of sodium per day and potassium capsules (KC1) were given to maintain a daily potassium intake of 40 mmols. Duplicate diets were analysed for sodium and potassium content and these estimates were confirmed.

1.5 Recording of blood pressure, heart rate and renal blood flow

The dogs were introduced to the recording room one week after the initial operation and trained to sit on a table where the blood pressure would subsequently be recorded. The arterial loops were cannulated with a 19 guage butterfly needle (Abbot ltd) with prior local anaesthetic (2% W/V lignocaine). The first blood pressure recording was obtained three weeks after formation of the loop. Further recordings were then obtained during the control period after laparotomy and placement of clamp and catheters. The pressure was obtained from the arterial catheter (Consolidated Electro-dynamics L223 or Statham P36 pressure transducers) and mean blood pressure was continuously recorded during each session for at least one hour, while pulsatile pressure, from which heart rate was derived was recorded for 5 minutes at 15 minute intervals.

The electrical scale of the pressure recording system was calibrated at approximately three monthly intervals with a mercury manometer system. Day to day calibration of the system was then made with reference to this electrical scale. The frequency response of the

entire pressure recording system was flat within 5% up to 25 Hz.

In studies in which renal blood flow was measured (study 2.2) the animals were kept upright in a harness system. The flow probe was connected to a meter (Statham) and the system calibrated with reference to an electrical zero. The signal for mean renal blood flow was then continuously recorded on a Hewlett Packard chart recorder. The electrical zero position of the meter was confirmed at the end of the study by intravenous injection of 2 ugms of angiotensin II, which transiently reduce renal blood flow to zero.

1.6. Urine collection procedures

Achievement of satisfactory sodium and potassium balance was confirmed by collection of urine excreted over a 24 hour period. During this time the animals remained in their kennels, apart from a short episode of supervised excerise. A false base was inserted into the kennel so that all urine voided was collected in a stainless steel tray.

In two of the studies (2.2 and 2.3), catheters were inserted into the bladder to permit continuous collection of urine for periods of up to six hours. Catheters were either inserted after induction of light anaesthesia with intravenous thiopentone, (study 2.3) or the conscious animal was catheterised (study 2.2). The urine was allowed to continuously drain into a flask during a collection period, and at the end of the period complete bladder empyting was ensured by manual

palpation and injection of air.

2. MEASUREMENT OF RENAL PLASMA FLOW, GLOMERULAR FILTRATION RATE, SAMPLE COLLECTION AND ANALYTICAL METHODS

2.1 Introduction

In one of the studies (study 2.2) renal blood flow was monitored directly from an indwelling electromagnetic flow probe placed around a renal artery. In all other studies renal plasma flow was derived indirectly from the clearance of p-amino hippurate (PAH). Glomerular filtration rate was always measured by a clearance technique.

2.2 Clearance techniques

For most studies PAH and inulin clearances were measured simultaneously. A loading intravenous dose of each compound was first given followed by a continuous intravenous infusion of a suitable dilution of the compounds in dextrose solution (50g/l) so as to maintain plasma levels of approximately 3 and 20 mg/100mls of PAH and inulin respectively. The loading dose of each compound was varied according to body weight, based on assumed distribution throughout the extracellular fluid volume.

Urine collected during timed periods was analysed for PAH and inulin. Similarly blood samples were always collected at the beginning and end of each period and analysed in the same way. A mean value was then determined from the two plasma results, which was then used for calculation of clearances.

In study 2.2 glomerular filtration rate was calculated from the clearance of ^{99}Tc diethylene triaminepentaacetic acid (DTPA). The method is very similar to that for inulin, except that radioactivity of the samples is determined, rather than chemical assay of a compound.

2.3 Sample collection and techniques of analysis

2.3.1 Sample collection

Blood samples were collected into tubes at 4°C containing either lithium heparin, EDTA (for analysis of plasma renin activity and aldosterone, study 2) or pepstatin and nonapeptide converting enzyme inhibitor (angiotensin II). All samples were kept on ice, centrifuged at 2000g for 20 minutes soon after collection, and the plasma was separated and stored at -20°C.

Urine samples were collected at room temperature, their volume measured and then separated into aliquots which were stored at -20°C or -70°C (prostaglandin samples).

2.3.2 Routine laboratory analysis

Plasma and urine, sodium and potassium were measured by flame photometry (Corning 430). A freezing point depression method was used for osmolality (Advanced Inst Inc), and a standard autoanalyser technique for urea and creatinine (Technicon autoanalyser). Inulin was measured by a modification of Heyrovsky's technique for automatic analysis (Technicon antoanalyser) (Dawborn 1965). In brief the inulin was hydrolysed to fructose

by heating with hydrochloric acid. After removal of protein by dialysis the colour was developed by incubation with concentrated hydrochloric acid and 3-indolylacetic acid and its intensity measured in a spectrophotometer recording absorbance at 520 nm.

PAH was also measured on an autoanalyser system (Technicon autoanalyser) (Harvey and Brothers 1962). The compound was first removed from urine and plasma by dialysis and subsequently diazotised with nitrite. The excess nitrite was destroyed by sulphamate and the colour produced by developing with N(1-Naphthyl) ethylene diamine dihydrochloride was monitored at 550 nm.

Radioactivity of DTPA was measured in 1 ml of urine and plasma with an automated gamma counter (Packard Inst Co).

2.3.3 Radioimmunoassays

2.3.3.1 Plasma renin activity, angiotensin II and aldosterone.

The method for measurment of plasma renin activity is well described (Haber, Koerner, Page, Kilman and Purnode 1969) and based on the generation of angiotensin I during incubation of the samples at 37°C. Commercially available antibody and radioactive angiotensin I (C.I.S.) were used throughout. In the initial studies a commercial assay kit was used (C.I.S. Ltd), but later a modified system was used (Roulston, Wathen, Sanger and Muir 1983) which gave results similar to those with the commercial kit, but less susceptible to interference by

plasma being kept too warm during sample collection.

In study 2.2 aldosterone was measured by a commercial system (C.I.S. Ltd) that did not require extraction of the plasma samples (McKenzie and Clements 1974). In other studies aldosterone was first extracted from plasma prior to assay. Angiotensin II was measured on unextracted plasma samples using the method in routine use in the Department of Endocrinology, Vanderbilt University (Dr R J Workman).

2.3.3.2 Prostaglandin E_2 (Plasma)

The method was based on that described by Dray et al (Dray, Charbonnel and MacLouf 1975) and was a further modification of that developed by Dr K K Dighe in the Department of Pharmacology, Edinburgh University. In brief, 1 ml of plasma was extracted, after acidification to pH 3, with ethyl acetate (x3). The organic fractions were pooled, dried and then subjected to silicic acid chromatography (Unisil). The fraction containing PGE_2 was dried and the residue resuspended in phosphate buffer. The PGE content was quantified by a radioimmunoassay system using specific rabbit anti-PGE antibody (Institut Pasteur, Paris), and $3_H PGE_2$ (Amersham Chemicals). A double antibody method was used to separate the antibody bound from free tracer (Dighe, Emslie, Henderson, Simon and Rutherford 1975). Since recovery of PGE from plasma was less than 100%, the recovery was determined from the mean recovery of 3_H PGE_2 added to a standard plasma, and extracted in parallel with the unknown. Mean recovery of PGE_2 in

these samples was 44+2%. The appropriate correction factor could then be used when calculating the final concentration of PGE in the unknown samples.

2.3.3.3 Prostaglandin E_2 (urine)

The method is a further modification of that described above for plasma. A small quantity of 3_H PGE_2 was first added to 5-10 ml urine to permit subsequent calculation of recovery of extracted PGE_2. After acidification (pH3.0) the urine was passed through a C18 Sep-Pak (Waters Associates). The column was washed with water followed by 20% Ethanol:80% water and the retained prostaglandin was then eluted with ethyl acetate. The eluate was taken to dryness and the residue resuspended in phosphate buffer. The 3_H PGE_2 content of part of this fraction was determined to permit calculation of recovery, and aliquots of the remainder were subjected to radioimmunoassay by the same system described above. Recovery of extracted PGE_2 was 82+5% by this method.

2.3.4 Mass Spectrometry

2.3.4.1 Introduction

Gas chromatography in combination with a mass spectrometer (GC-MS) has proved to be a useful technique for quantitative analysis of prostaglandins. Before a final analysis on such a system most biological samples have to be substantially purified by organic extractions and chromatography. The size of the sample required for extraction is determined by the sensitivity of detection of the compound in the mass spectrometer. The most widely used method of inducing molecular fragmentation

within the mass spectrometer is by electron impact ionisation. Whilst this usually provides a large range of molecular fragments, any one of which can be monitored with the instrument in single ion mode, the sensitivity of detection of any one of these ions is limited, since each constitutes only a small proportion of the total ion current. Ionisation techniques that limit molecular fragmentation permit selection of ions for monitoring that constitute a much larger proportion of the total ion current, with corresponding gains in the sensitivity of detection. Chemical ionisation is a useful means of inducing such ionisation and has been widely used in the analysis of prostaglandins (Suzuki, Morita, Kawamura,Murota, Nishizawa, Miyatake, Nagase, Ohno and Shimizu 1980). An alternative approach has been to use chemical ionisation techniques, but to measure the negatively charged ions formed in the ion source, negative ion chemical ionisation mass spectrometry (NICI) (Dougherty 1981). The great sensitivity of this method for measuring certain prostaglandins has already been reported (Blair, Barrow, Waddell, Lewis and Dollery 1982). The method to be described was specifically developed for analysis of a urinary metabolite of PGI_2, 2,3 dinor 6 keto PGF_{1a}.

2.3.4.2 Preparation of standard fatty acid derivatives.

In order to optimise the working conditions of the mass spectrometer in NICI mode, electrophilic fatty acid derivatives were synthesized. The pentafluorobenzyl ester of stearic acid was synthesized by extractive

alkylation (Rosenfeld, Ting and Phatak 1981). 100 ul of a 25% (W/V) solution of tetrabutyl ammonium in methanol was added to 900 ul of 0.1 M solution of sodium carbonate. Stearic acid was added (0.5 mg in 1 ml dichloromethane) and reacted for 2 hours at 25°C. 10 ul of a solution of pentafluorobenzyl bromide (60 ul in 200 ul of acetonitrile) was added. After 10 minutes the upper aqueous phase was removed. The fatty acid pentafluorobenzyl ester remained in the organic phase.

2.3.4.3 Separation of 2,3 dinor 6 keto PGF_{1a} from urine and its derivatisation.

The procedure was a modification of that described by Falardeau et al (Falardeau, Oates and Brash 1981) and relies on separation of 2,3 dinor 6 keto PGF_{1a} from other carboxylic acids by utilising its ability to form a complete lactone under acid conditions thereby manipulating its solubility in polar and non-polar organic solvents (Fig 2).

Urine (5-10 mls) was spiked with 10 ng of d^4 dinor 6 keto PGF_{1a}. After initial alkalinisation to pH 10 the mixture was acidified to pH2 with concentrated hydrochloric acid. This was poured onto a 5 or 10 ml capacity Clin-Elut column and the prostaglandin was eluted with two volumes of dichloromethane into sialinized glass tubes. The eluate was extracted 3 times with 5 or 10 ml of pH 8 borate buffer (0.05 M sodium borate) and the aqueous phase was discarded. After evaporation of dichloromethane with nitrogen the sample was taken up in 50 ul pyridine and mixed with 1

ml of borate buffer. After standing 10 mins this was extracted twice with 10 ml ethyl acetate and the organic phase was discarded. The aqueous phase was acidified to pH2 with concentrated hydrochloric acid and extracted with 4 ml dichloromethane. The aqueous phase was discarded and the dichloromethane was evaporated under nitrogen. The residue was then redissolved in 20 ul pyridine and the methoxime derivative formed by addition of an excess of methoxyamine hydrochloride in the presence of triethylamine, followed by incubation for 1 hour at 60°C. Pyridine was evaporated with nitrogen and the pentafluorbenzyl ester formed by addition of 15 ul of pentafluorobenzyl bromide (50 ul in 200 ul acetonitrile), 10 ul of diisopropylethylamine (60 ul in 250 ul acetonitrile) and 20 ul of acetonitrile, followed by incubation at 50°C for 20 minutes. After evaporation under nitrogen, the sample was resuspended in 20 ul acetonitrile and applied to a thin layer chromatography plate (Whatman LK6DF linear K Silica gel, 250 um thick with preadsorbent zone), and developed in a solvent system of ethyl acetate:methanol (98:2). Dinor 6 keto PGF_{1a} had an Rf of approximately 0.15 in this system, but its exact position on the plate was determined by running a similarly derivatised standard in parallel and subsequently staining this part of the plate with a colour reagent (calcium sulphate 5 g, sulphuric acid (con) 20 ml and water 100 ml). The region of interest was scrapped from the plate and after addition of 250 ul of water (containing 0.1% acetic acid) the prostaglandin

was extracted into a 5 fold excess of ethyl acetate. The organic phase was separated, taken to dryness and derivatised with N,N-bis-(trimethyl silyl) trifluoroacetamide (BSTFA) to form the methoxime trimethyl silyl ether derivative.

2.3.4.4 Gas chromatography - mass sepctrometry system

A Hewlett Packard HP 5982 mass spectrometer was modified for use in the negative ion mode (Dr B J Sweetman and Mr W Levine) (Fig 3). The reagent gas used was the same as the carrier gas for the gas chromatography system (3 foot SP 2100 column) and could therefore be led directly into the ion source of the mass spectrometer via a jet separator. Estimates of ion source pressure were derived from the pressure in the manifold. Methane was routinely used as the carrier gas, which with a column temperature of 260^{o}C gave a retention time of 4.7 minutes for dinor 6 keto PGF_{1a} (pentafluorobenzyl ester, methoxime trimethyl silyl ether derivative).

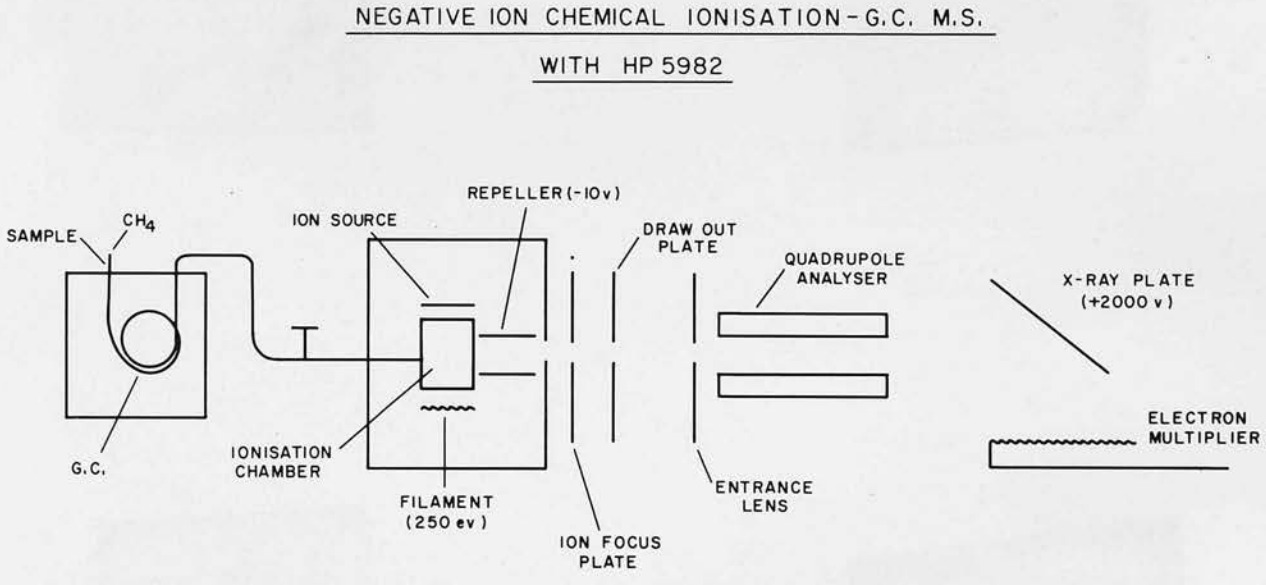

Fig 3. Schematic representation of HP5982 mass spectrometer modified for negative ion chemical ionisation. Chemical ionisation is induced in the ion source in a methane atmostphere. Repeller, ion focus and draw out plates and the entrance lens are all held at a negative charge to repel the ions into the quadripole analyser. The positively charged x-ray plate draws out the ions which are detected on the electron multiplier.

2.3.4.5. Quantitation by GC-MS.

Derivatised standards containing constant amounts of d^4 dinor 6 keto PGF_{1a}, and variable quantities of the protonated form were prepared by the method above. A standard calibration curve could then be constructed by measuring peak sizes of d^o and d^4 compounds (586 and 590 m/z respectively) in the different samples. Amounts of d^o dinor 6 keto PGF_{1a} in particular biological samples could then be calculated by reference to the standard curve. The d^o blank obtained using pure d^4 standard was in the region of 0.5%.

2.4 Calculation of results.

2.4.1 Clearance

Clearance (C) of all compounds (n) was calculated from the following formula

$$C_n = \frac{U_n V}{P_n}$$

where U_n = concentration (or activity in the case of osmoles) in urine

V = urine flow rate

P_n = concentration or activity of the compound in plasma

In this thesis n is restricted to inulin, PAH, creatinine and osmoles.

2.4.2 Expression and analysis of results

All results have been expressed as mean ± standard error of the mean. Comparisons between samples have been made with students two tailed t test for paired or unpaired samples. On occasion non-parametric analysis with Wilcoxon sign rank test was used. The null hypothesis for differences between the samples was rejected when $P < 0.05$.

RESULTS

1. ANALYTICAL METHODS FOR PROSTAGLANDINS

1.1 Validation of radioimmunoassay for PGE

Precision of the analytical system for urinary PGE was determined by extracting different volumes of the same urine sample and determining the amount of PGE present by analysing an appropriate volume of eluate from the Sep-Pak. Fig 4 demonstrates that there was linearity between the volume of urine extracted and the PGE detected over an appropriate working range.

Addition of known varying amounts of PGE to a fixed volume of urine allowed assessment of the acurracy of the system. There was again reasonable linearity between the amount of PGE added and the amount detected by radioimmunoassay (Fig 5).

Many of the studies involved the assessment of effective renal plasma flow by PAH clearance. PAH is an organic acid which may only be partially removed from urine by the Sep Pak system, its potential for interfering with the assay system was therefore assessed. Known varying amounts of PAH were added to a fixed volume of urine. Samples were extracted and analysed in the usual fashion. Although very high concentrations of PAH may have interfered with the assay (fig 6), this was not a significant factor in the effective working range.

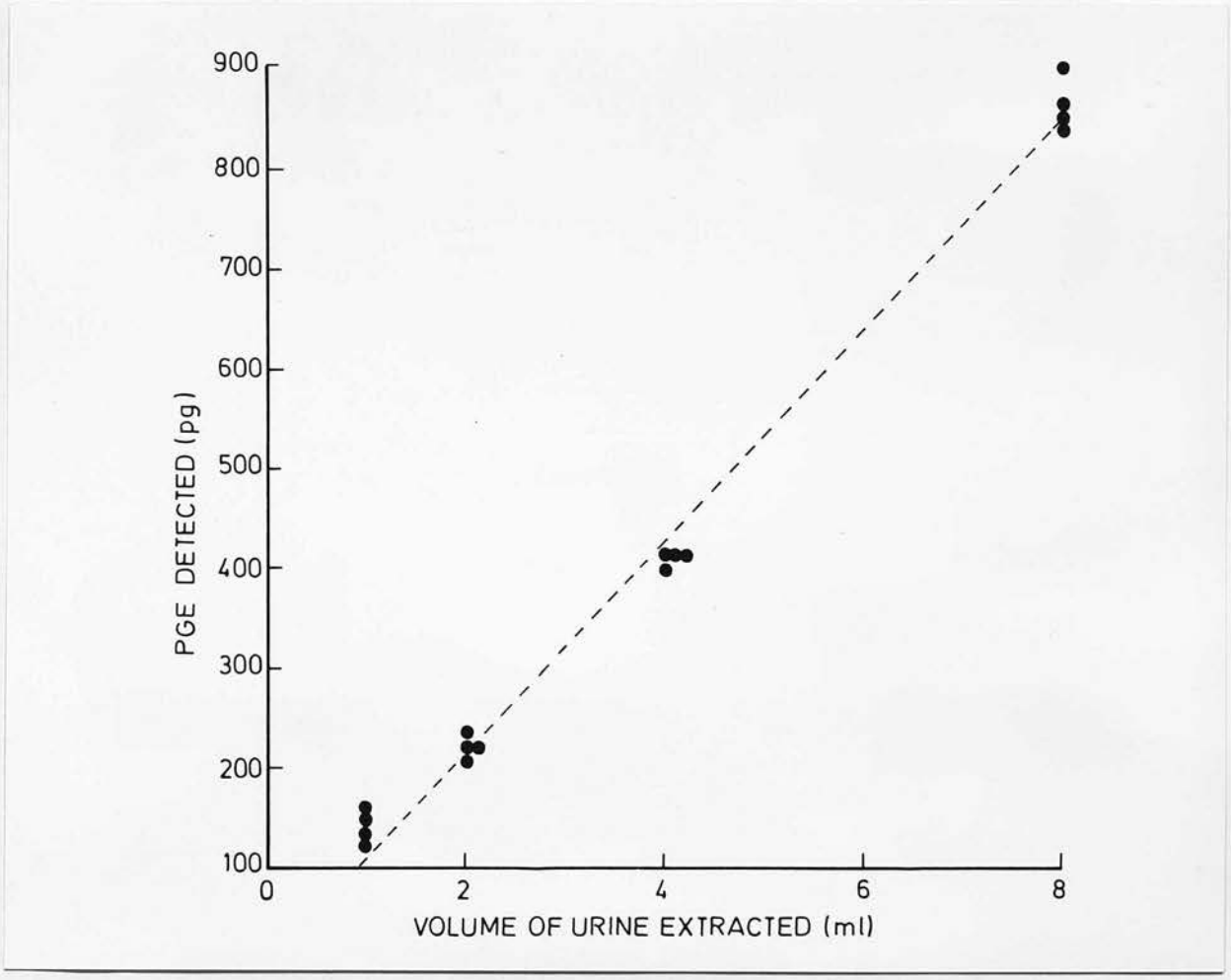

Fig 4. Effect of the volume of urine extracted on the amount of PGE detected by radioimmunoassay. Broken line indicates expected values if detection is linear.

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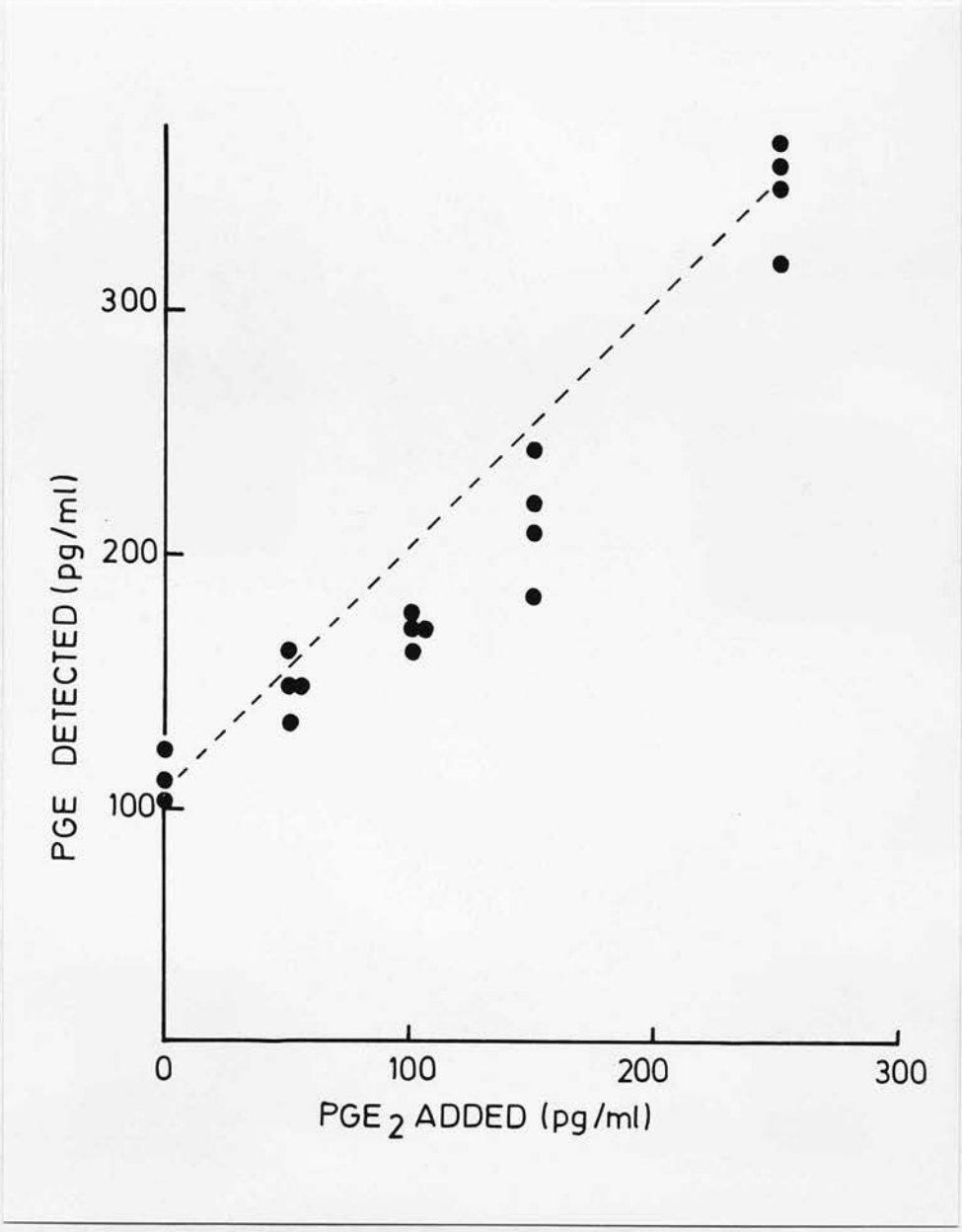

Fig 5. Effect of addition of varying quantities of PGE_2 (pg/ml urine) to a fixed volume of the same urine sample on the amount of PGE detected by radioimmunoassay. Broken line indicates expected results if detection is linear.

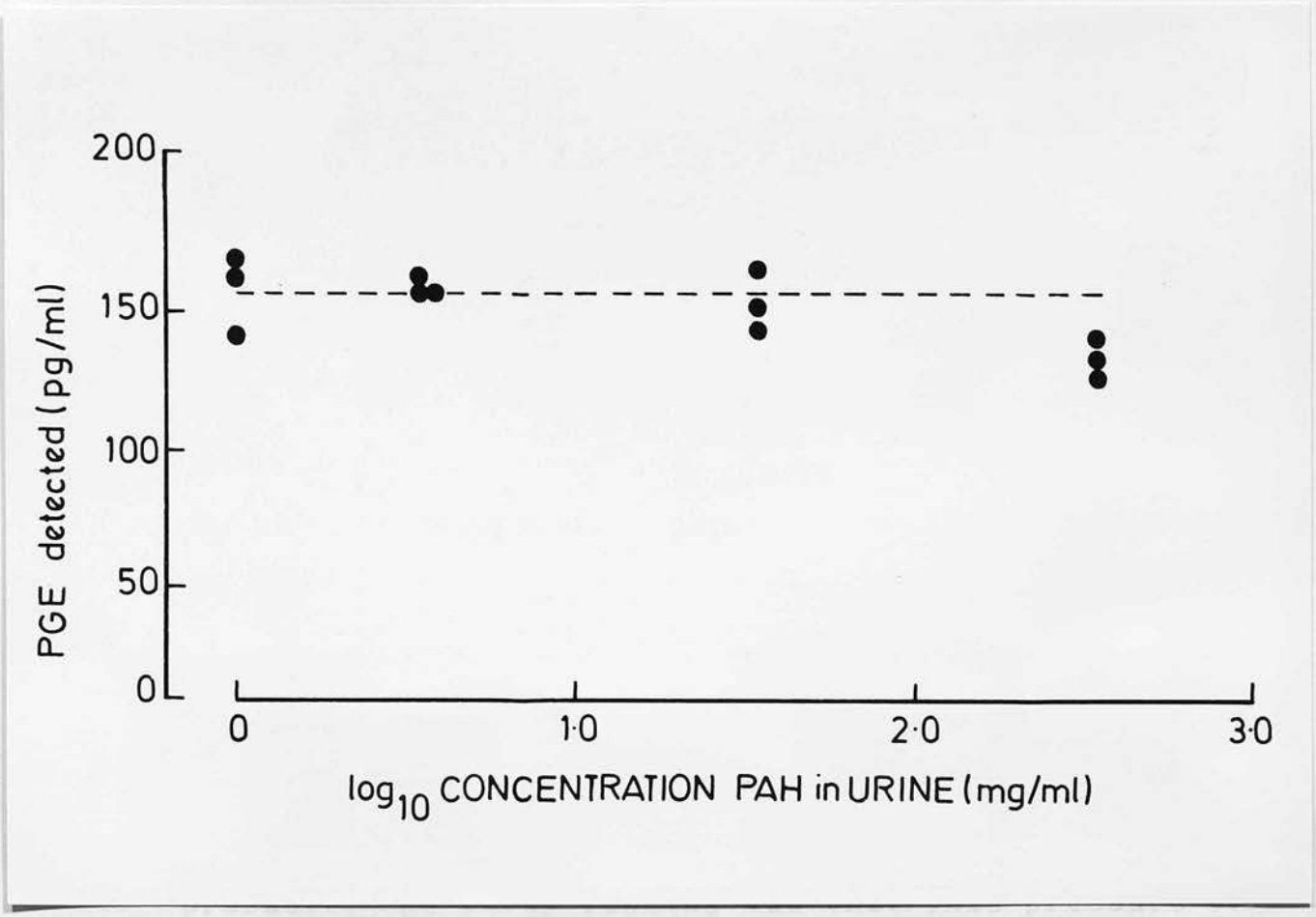

Fig 6. Effect of increasing concentration of PAH (3.5 mg/ml to 350 mg/ml) on the amount of PGE detected in the same sample of urine. Results expressed as $\log_{10}$ of concentration of PAH. Broken line indicates expected values if PAH does not interfere with the assay.

1.2 Optimisation of gas chromatography mass spectrometry system for dinor 6 keto PGF_{1a}

1.2.1 Reagent gas and sensitivity of detection.

Although satisfactory negative ion chemical ionisation spectra of fatty acid and prostaglandin pentafluorobenzyl ester methoxime trimethylsilyl ether derivatives were obtained with both ammonia and argon as reagent gasses, the greatest sensitivity was obtained with methane. In this reagent gas the pentafluorobenzyl ester of prostaglandins tested, and stearate, ionised with at least 50% of the total ion current being accounted for by the M -pentafluorobenzyl fragment (Fig 7). This contrasts with the fragmentation pattern when the same molecule is ionised by the more usual electron impact method (Fig 8). Many more ion fragments are produced and the proportion of the total ion current in any one fragment is comparatively small. The operating conditions of the instrument were then optimised using stearate pentafluorobenzyl ester and monitoring M/Z (mass/energy) in single ion monitoring mode. Although ion source pressure was not monitored directly, this was reflected in changes in flight tube manifold pressure. An early finding was that this pressure of reagent gas critically determined ionisation efficiency Increased pressure in the ion source was produced by increasing the methane flow through the gas chromatogram column. Increasing methane flow through the column however, also shortens the retention time of any compound on the gas chromatogram. To standardise the

effects of changes in ion source pressure the temperature of the gas chromatography column was varied to maintain a constant compound retention time for any particular gas flow. With this system it was possible to demonstrate in excess of 100 fold changes in sensitivity of detection over a manifold pressure range of 2.8 - 14 x 10^{-6} torr (Fig 9). Under optimal operating conditions it was possible to detect, with a signal to noise ratio of better than 3-1, as little as 25 fg of stearate and approximately 350 fg of 6 keto PGF_{1a} placed on column (Fig 10).

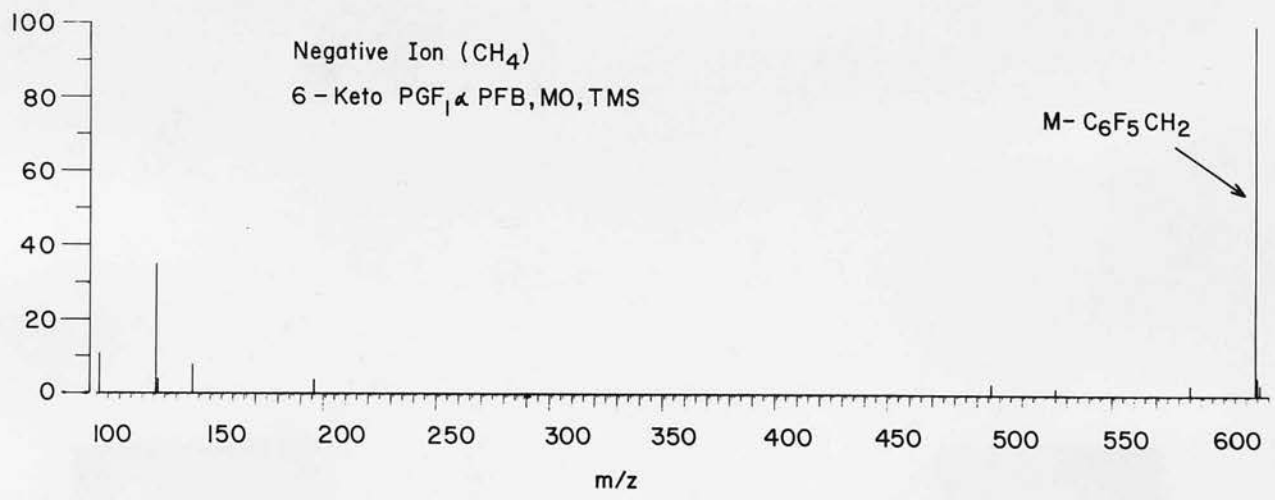

Fig 7. Negative ion spectrum of 6 keto PGF_{1a} pentaflurobenzyl ester methoxime trimethyl silyl ether. Loss of pentaflurobenzyl group leaves the molecular fragment (614 m/z) by far the most abundant ion. HP 5982 mass spectrometer.

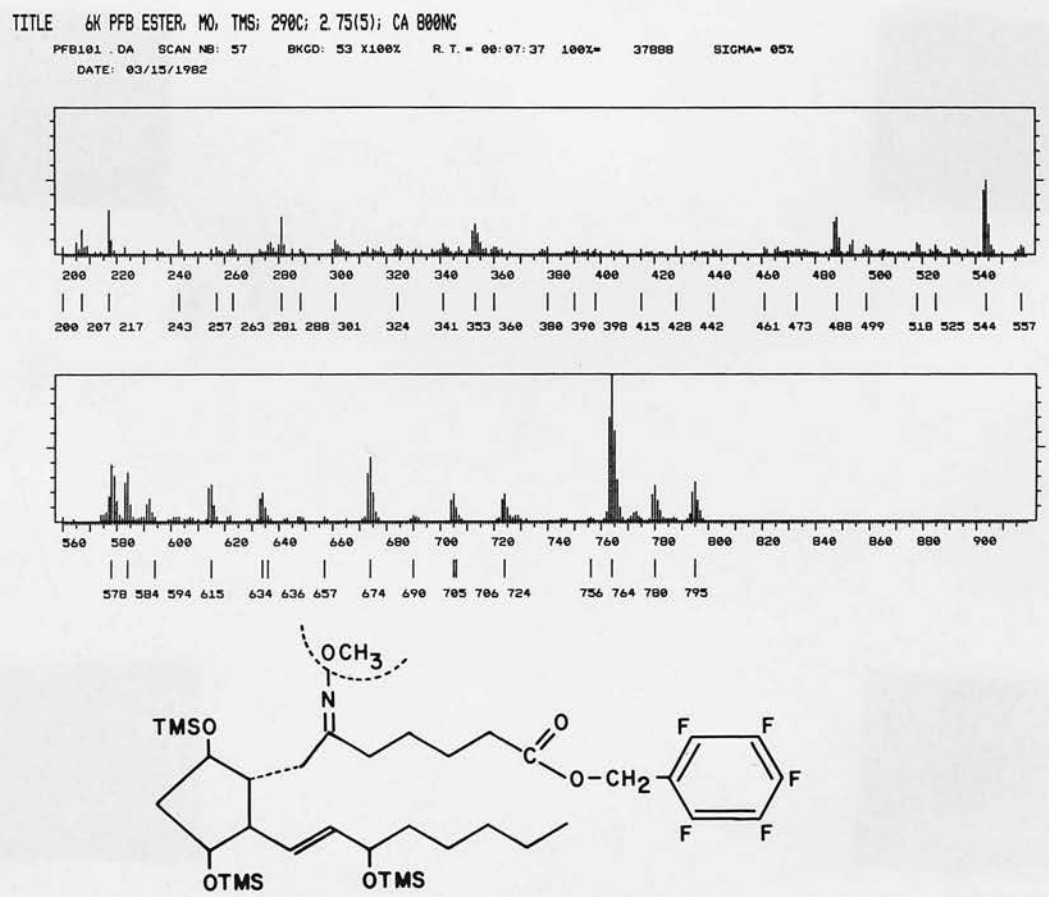

Fig 8. Electron impact ionisation spectra of 6 keto PGF_{1a} pentaflurobenzyl ester methoxime trimethyl silyl ether. Relatively abundant ions are M-31 (764) - loss of OCH_3 and M-121 (674) - loss of OCH_3 and OTMS. M-PFB is not a significant ion.

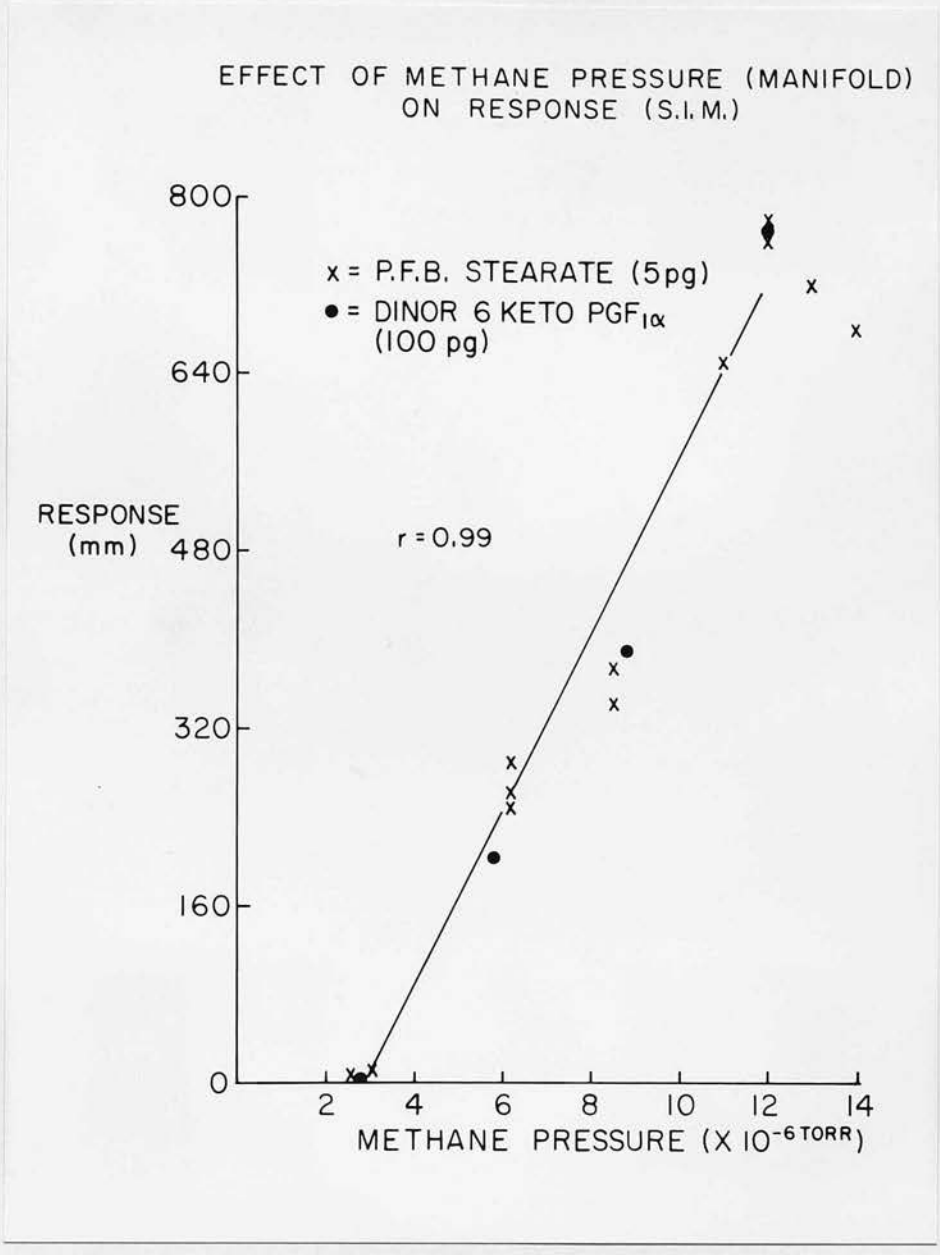

Fig 9. Dependency of sensitivity of detection of 6 keto PGF_{1a} PFBMOTMS on methane pressure in the ion source manifold. Gas chromatography operating temperature was manipulated to ensure a retention time of approx 2 mins.

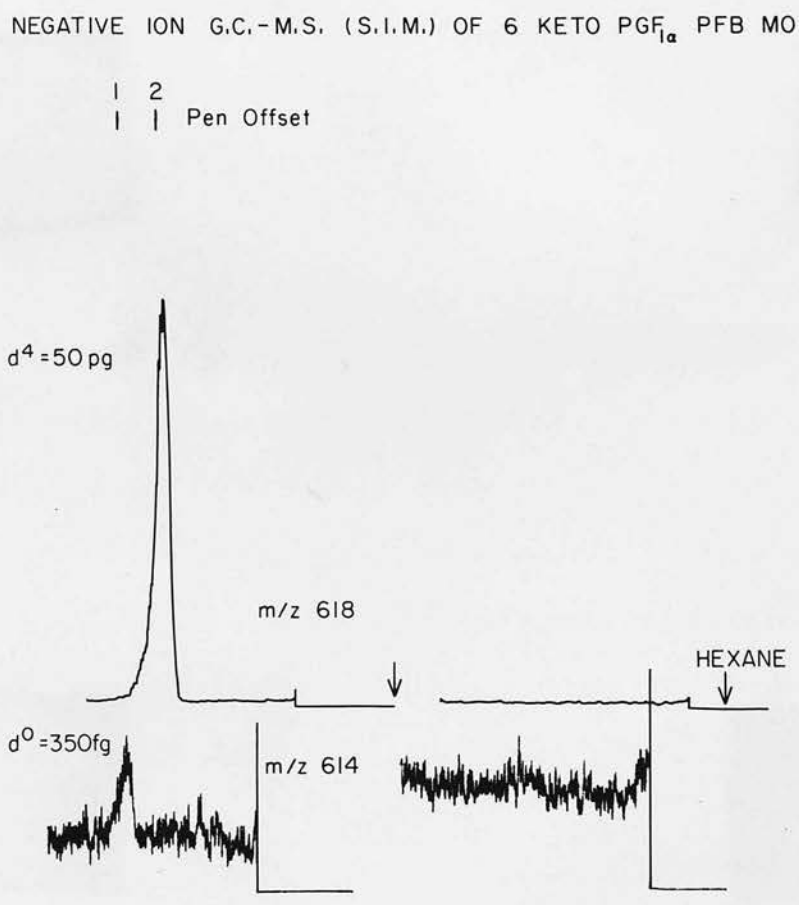

Fig 10. Maximum sensitivity of detection of 6 keto PGF_{1a} PFBMOTMS. 50 pg d^4 derivative applied to the column, known d^0 component 7:1000. Hexane blank for base line comparison. Retention time at 260°C. Machine in selective ion monitoring mode.

1.2.2 Application to urinary and plasma 2,3 dinor 6 keto PGF_{1a} estimation.

The sensitivity of detection of the compound was such that prostaglandin extracted from 0.5 - 2 mls of urine was sufficient to provide an excellent signal in the d^{o} channel, the typical concentration in urine being 100-200 pg/ml. Precision of the assay was determined by extracting different volumes of the same urine sample (Fig 11). Accuracy was determined by extracting fixed volumes of the same urine samples, spiked with increasing amounts of protonated standard (Fig 12). In each case there was linearity between the amount detected and the amount extracted.

Plasma samples were extracted in the same way as urine samples. Since the concentration in plasma was lower than that in urine the system was operated at high sensitivity so as to provide appropriate detection limits.

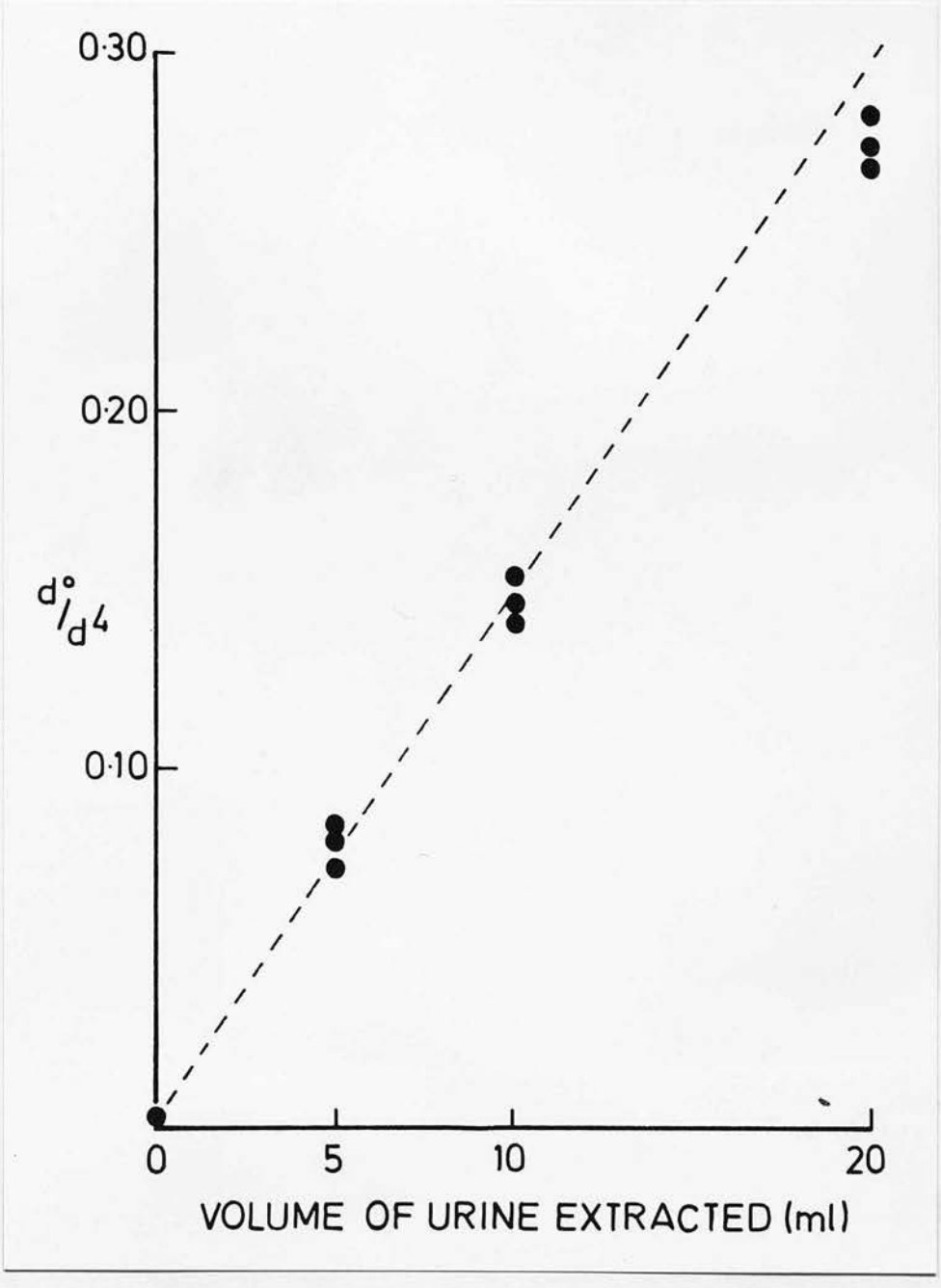

Fig 11. Peak ratio d^o/d^4 of dinor 6 keto PGF_{1a} as function of volume of urine extracted. d^o blank equivalent to 5:1000 of d^4 standards. Broken line indicates expected values if detection is linear.

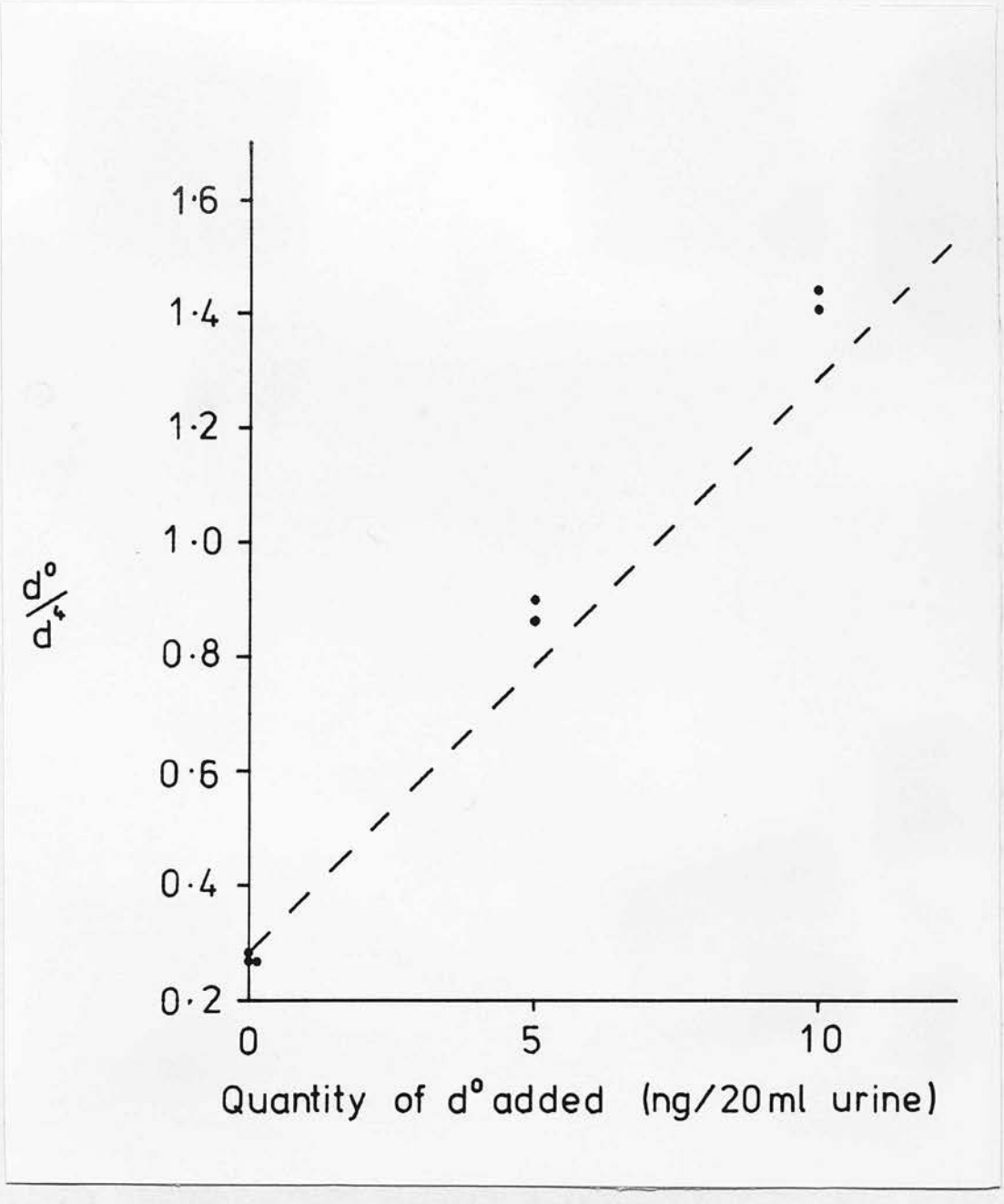

Fig 12. Peak ratio d^0/d^4 on addition of varying amounts of protonated dinor 6 keto PGF_{1a} to a fixed volume of urine (20 ml).
Broken line indicates expected values if detection is linear.

2. ANIMAL STUDIES

Four different studies are described in this section:-

2.1 Extracellular fluid volume expansion and the development of one clip two kidney hypertension.

2.2 Systemic angiotensin II infusion and PGI_2 release.

2.3 Systemic and renal vascular sensitivity to angiotensin II in normotensive and hypertensive dogs.

2.4 Interstitial cell morphology and the development of one clip-two kidney hypertension.

2.1 Extracellular fluid volume expansion and the development of one clip two kidney hypertension.

The potential role of sodium and water retained in the early period after partial occlusion of a renal artery in the development of established hypertension in conscious dogs has been discussed in the introduction. To explore further the role the sodium and water that accummulates during the two days after the partial occlusion of one renal arteryit was removed by haemodialysis. Subsequent retention of sodium and water was prevented for seven days by feeding a low sodium diet. Plasma levels of renin, aldosterone and PGE were monitored at intervals and the development of hypertension was observed.

2.1.1 Protocol and Special Methods

The protocol for the timing of blood pressure recordings, induction of hypertension, dialysis and dietary restrictions are illustrated in Fig 13. Haemodialysis was undertaken on day 3, 48 hours after

partial occlusion of a renal artery, with an EX 20 $1m^2$ coil (Extracorporeal), paediatric connecting lines (Travenol) and a roller pump. The arterial blood supply was taken from the carotid artery loop and venous return was via a 17 gauge Medicut inserted into a foreleg vein. Ultrafiltraton was achieved with a blood flow of approximately 120 mls/min and the pressure in the venous return circuit was maintained at approximately 200 mmHg. The ultrafiltrate from the coil was collected in a measuring cylinder. Before dialysis the dogs were heparinised with 5000 i.u. injected intravenously and at the end of the dialysis anticoagulation was reversed with an equivalent quantity of protamine. Each dialysis lasted for approximately 2 hours during which time the animals remained in the sitting position. The length of each dialysis and the rate of fluid removal was determined by the quantity of fluid to be removed. Fluid gain and loss from day to day and before and after dialysis were estimated from changes in body weight. The animals were weighed at the same time each day on an electronic weighing balance with an electronic integrator which gave results that were accurate to within $\pm$ 1 gm.

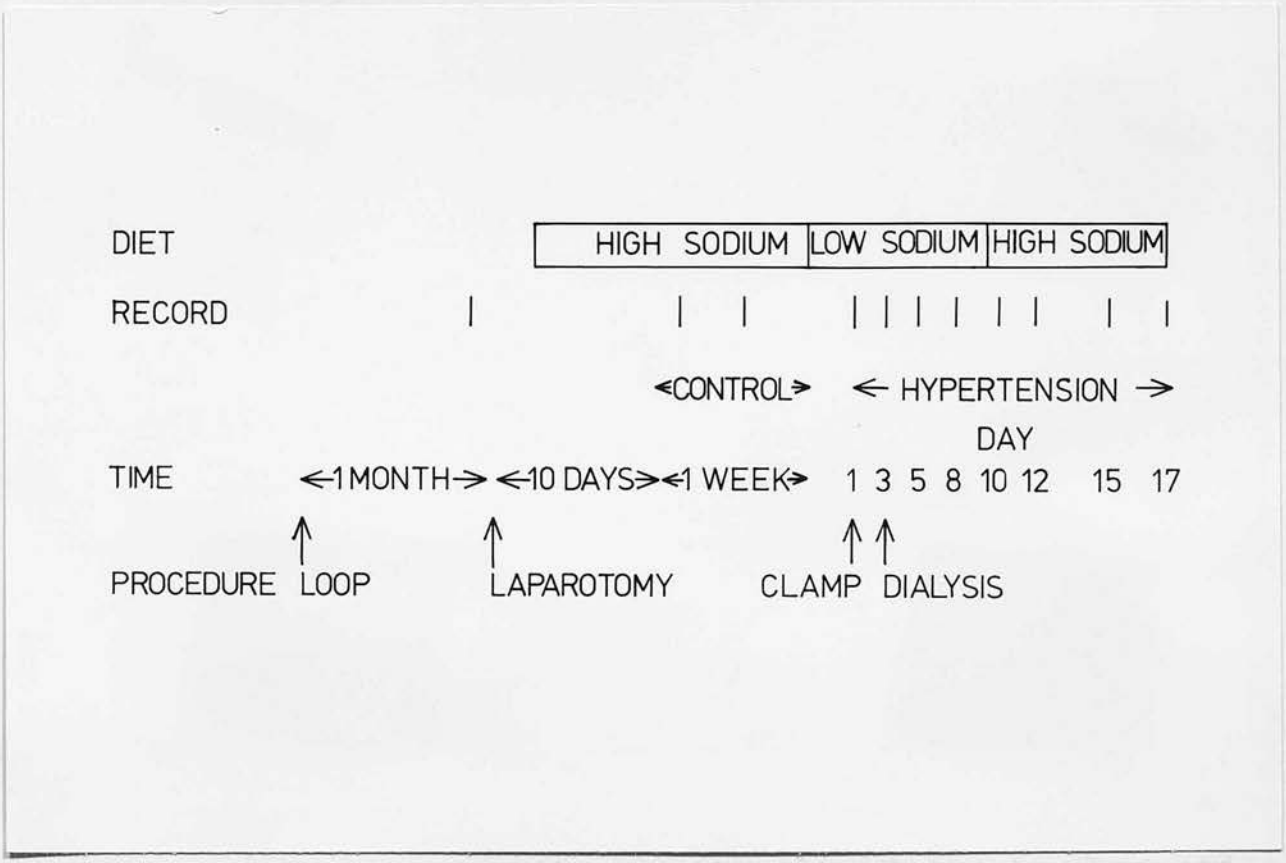

Fig 13. Protocol for procedures and recordings. The control period started 10 days after laparotomy for placing renal artery clamp and catheters. Each recording period lasts about one hour.

2.1.2 Results

2.1.2.1 Body weight and packed cell volume (PCV)

The weight of the animals (n=6) increased by a mean of 0.49 kg between the clamping of the renal artery on day 1 and day 3 (22.40 ± 1.3 - 22.89 + 1.4 kg, $p<0.01$). There was also a significant increase in weight between days 10 and 13 (21.35 ± 1.1 - 22.07 ± 1.2 kg $p<0.02$). There were substantial long-term changes in body weight particularly during the low sodium period, which were almost certainly a result of changes in nitrogen balance. The large short term changes in body weight described are likely to be due to changes in fluid balance, the increase in weight between days 1 and 3 representing a mean accumulation of 76 mmols of sodium. PCV decreased significantly between days 1 and 3 (38.3 ± 1.4 - 33.9 ± 1.4%, $p<0.005$).

2.1.2.2 Plasma creatinine and electrolytes

There was no significant change in plasma concentration of sodium (mean control value 146 ± 1.6 mmols/l) or potassium (mean control value 3.9 ± 0.2 mmols/l) throughout the course of the study. There was in particular no decrease in plasma sodium concentration between days 1 and 3 indicating that the increase in weight was due to retention of both salt and water. The concentration of sodium in the ultrafiltrate produced during dialysis was identical with the plasma sodium concentration of the animal on each occasion. There was no evidence of deterioration in renal function at any stage, with plasma creatinine concentration never

increasing above 100 umol/l in any animal.

2.1.2.3 Blood pressure and heart rate.

Mean blood pressure increased by 24 mmHg within 2 days of partial occlusion of a renal artery (91.8 $\pm$ 0.9 - 116.5 $\pm$ 2.7 mmHg, $p<0.001$) (Fig 14). Dialysis on day 3 was not followed by a significant change in blood pressure until day 10, indicating that the acute accummulation of fluid after partial renal artery occlusion was not necessary for the maintenance of hypertension. The decrease of 8 mmHg in blood pressure during the period of sodium restriction between days 3 and 10 ($p<0.05$) was followed by a small but significant increase (6 mm Hg) after the restoration of sodium intake between days 10 and 17 ($p< 0.01$). The results of blood pressure estimations on day 19 are not shown, since one of the animals had dropped out of the series, however, the pressure at day 19 in the remaining animals was not significantly different from the pressure on day 10.

The heart rate decreased significantly between 1 and 3 (94.0 $\pm$ 3.8 - 70.0 $\pm$ 3.7 beats/min, $p < 0.005$) but progressively increased during the low sodium period to 95 $\pm$ 12 beats/min on day 10 ($p < 0.05$) (Fig 14). Institution of a normal sodium intake on day 10 resulted in a small decrease in heart rate, which on day 17 was not significantly different from the control value. The product of heart rate and pulse pressure maybe taken as an approximate index of cardiac output. The results from this study (Fig 14), suggest that there was an

initial fall in cardiac output between days 1 and 3 with a subsequent progressive increase between days 3 and 10. Between days 10 and 17 there was a return to the values which were very similar to those obtained during the control period.

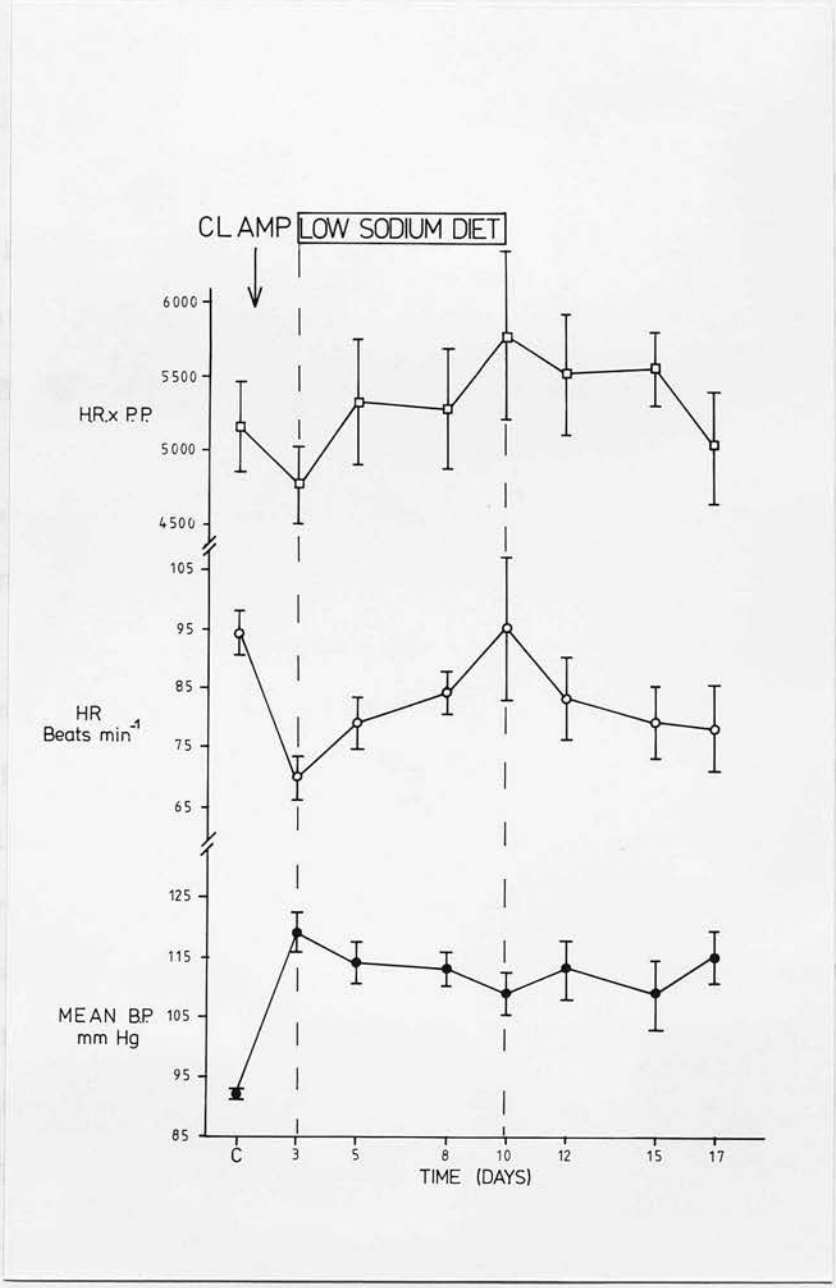

Fig 14. Product of heartrate and pulse pressure (HRxPP), heartrate (HR) and blood pressure (BP) before and after the induction of hypertension.

2.1.2.4 Endocrine Changes

Partial occlusion of one renal artery resulted in a significant increase in arterial plasma renin activity (3.4 ± 0.3 - 10.5 ± 1.5 ng/h/ml, $P < 0.005$), which remained high during the period of low sodium intake. There was then a gradual decrease in the concentration, although by day 17 it was still significantly higher (5.7 ± 1.7 ng/ml) than the control level ($P < 0.05$) (Fig 15). Plasma renin activity in renal venous plasma from the untouched kidney paralleled levels in arterial blood during the study, suggesting that almost all of the renin was released from the clamped kidney.

The time course of the change in arterial plasma concentration of aldosterone was similar to that of renin (Fig 15), although the level of aldosterone continued to rise until day 5, whereas plasma renin activity reached its maximum on day 3. As with renin, the aldosterone level remained significantly elevated at day 17 compared with control value ($P < 0.05$). Despite the changes in sodium balance and arterial plasma renin activity, there were no significant changes in the arterial and renal venous plasma concentration of PGE throughout the course of the study (Fig 15).

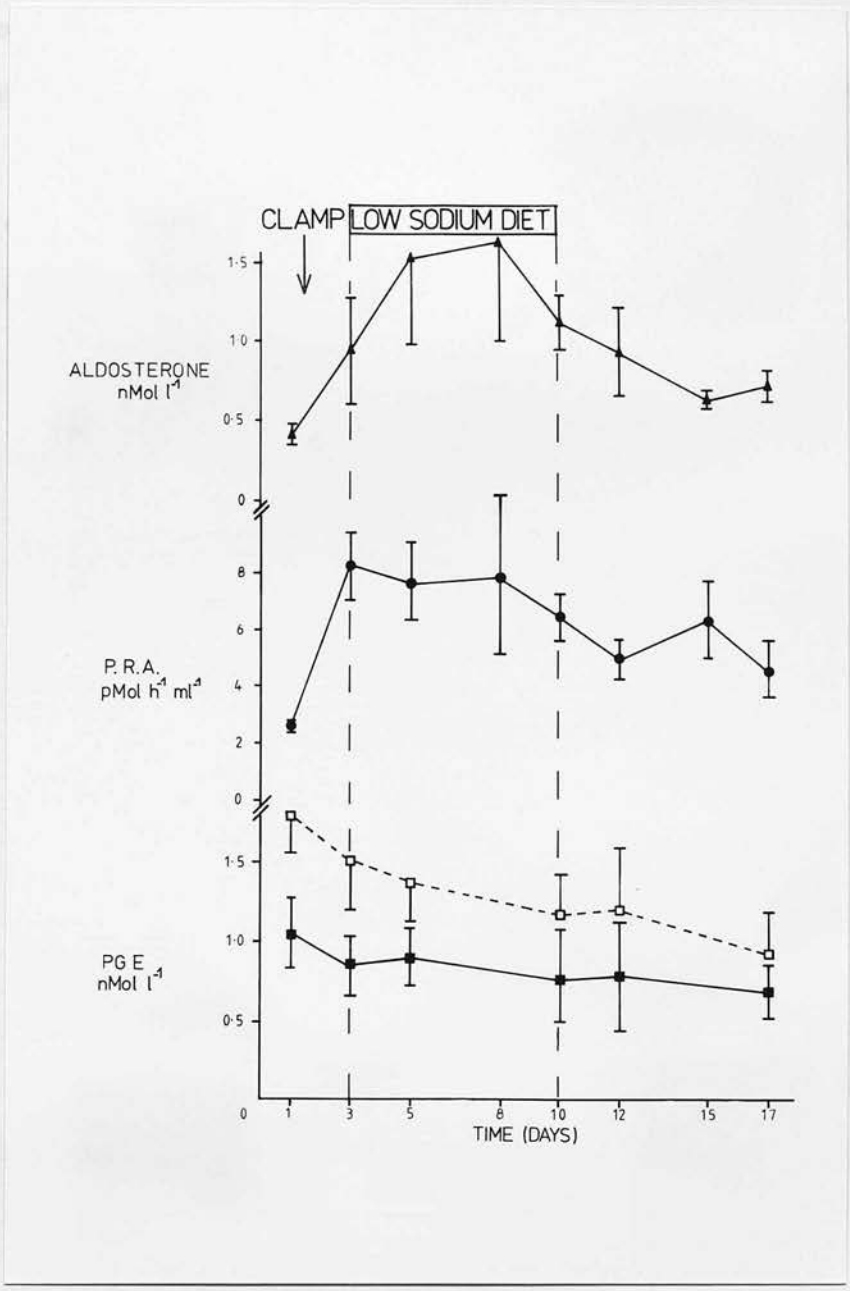

Fig 15. Plasma aldosterone, plasma renin activity, (PRA) and plasma PGE before and after induction of hypertension.
■——■ = Arterial □---□ = Renal venous

2.1.2.5 Urinary Output

The results of 24 hour urine volume, sodium and potassium excretion and creatinine clearances are shown in Table 1. The results show that the animals were in similar sodium balance during both the control period and postclamp period, while taking the same high sodium diet. During the low sodium diet there was avid sodium retention with a mean urinary excretion of only 8.6 mmols/24 hrs.

TABLE 1. PARAMETERS OF RENAL FUNCTION DURING 24-H URINE COLLECTION PERIODS

Diet...	High Sodium		Low Sodium
Time...	Control (n=5)	Postclamp (n=11)	Postclamp (n=6)
V (ml/24 h)	583±51	628±78	523±53
$U_{Na}V$ (mmol/24 h)	55±7	48±18	9±3.5
U_kV (mmol/24 h)	44±2	47±4	38±3
CrCl (ml/min)	44±10	65±16	42±10

Urine flow rate (V), sodium excretion ($U_{Na}V$), potassium excretion (U_kV) and creatinine clearance (CrCl) derived from urine collections during periods of high- and low-sodium intake before (control) and after induction of hypertension (postclamp). n=Number of collection periods.

2.2 Systemic angiotensin II infusion and PGI_2 release

Evidence that the vasodilator prostaglandin PGI_2 modulates the vasoconstrictor activity of angiotensin II is mainly derived from studies in vitro and in anaesthetised animals. However the extent to which PGI_2 modulates the systemic vasoconstrictor activity of angiotensin II in conscious animals has not been established. The objective of this study was to determine whether infusion of angiotensin II into conscious dogs in a dose sufficient to cause a modest increase in systemic blood pressure and renal vascular resistance also resulted in increased release of PGI_2 into the systemic circulation, as assessed by measurement of plasma and urinary levels of its stable metabolite dinor 6 keto PGF_{1a}.

2.2.1 Protocol and Special Methods

On the day of the study the urinary bladder was catheterised without anaesthesia. An initial intravenous infusion of 500 ml of dextrose solution (50 g/l) was given over 30 minutes via a catheter in the inferior vena cava, followed by continuous infusion of dextrose solution (50 g/l) at 3 ml/min and sodium chloride solution (150 mmol/l) at 0.6 ml/min using a Harvard infusion pump. After a 60 min control period either the sodium chloride infusion was continued (vehicle) or a freshly prepared solution containing angiotensin II amide (Hypertensin, Ciba) in sodium chloride solution was substituted. The concentration of angiotensin II solution was adjusted for each dog such

that the final rate of infusion was 15 ng/min/kg body weight. After a further 180 min sodium chloride solution was substituted for the angiotensin II and the infusion continued for a further 60 min recovery period.

To permit calculation of glomerular filtration rate ^{99}Tc DTPA in sodium chloride solution was also continuously infused via the same catheter at a rate of 0.18 ml/min (Harvard pump), after an appropriate loading dose. After an initial period of 90 min, 30 min urine collections were made throughout the study. Blood samples were also collected every 30 min for estimation of DTPA activity, while samples for estimation of plasma renin activity, angiotensin II, aldosterone, dinor 6 keto PGF_{1a} and haematocrit were collected every hour. Each of the six animals underwent two studies during which either angiotensin II or vehicle was infused.

2.2.2. Results

2.2.2.1 Renal and systemic haemodynamics

There were no significant differences in systemic blood pressure, glomerular filtration rate and renal blood flow between the two groups during the control period. On starting infusion of angiotensin II there was a prompt increase in systemic blood pressure and decrease in renal blood flow and glomerular filtration rate which was sustained throughout the angiotensin II infusion (Fig 16). There was no change in these parameters during vehicle infusion. All parameters returned to near control values after completion of the angiotensin II infusion.

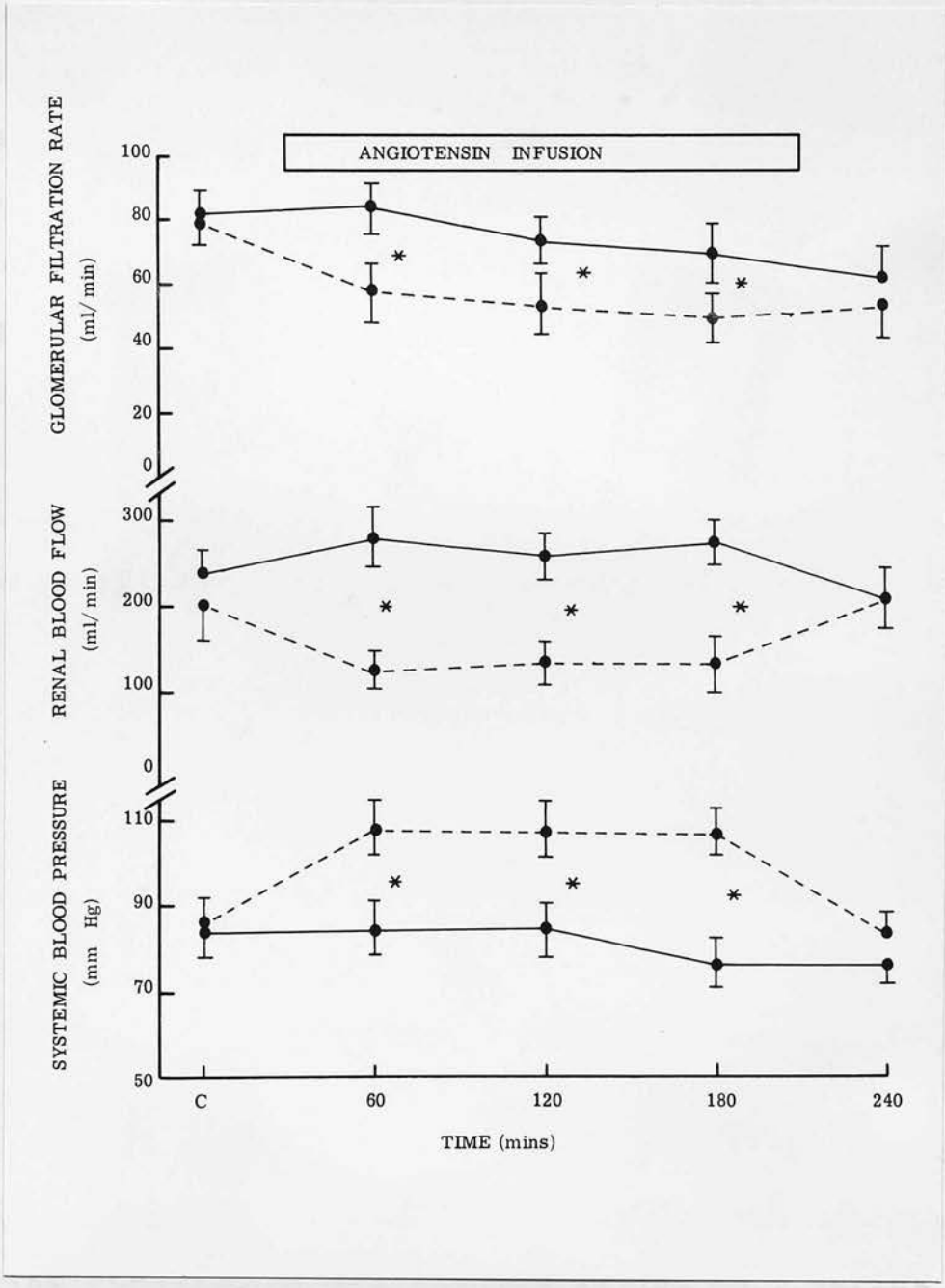

Fig 16. Changes in glomerular filtration rate, renal blood flow and systemic blood pressure as a result of angiotensin II infusion (15 ng/min/kg).
* = P<0.05 for difference from saline control
---- = angiotensin II infusion

——— = saline infusion

2.2.2.2 Humoral factors

Plasma angiotensin II increased by 3-fold within one hour of starting the infusion, ($p < 0.01$) (Fig 17), remained at the same level during the second hour, and then increased further during the third hour before returning to control values. Plasma renin activity was significantly decreased throughout infusion of angiotensin II. Plasma aldosterone also increased during the angiotensin II infusion. In contrast to other parameters, the level of aldosterone progressively increased during the course of the infusion and then returned to control values within one hour of the end of the infusion. The rate of urinary excretion of dinor 6 keto PGF_{1a} was unchanged during infusion of angiotensin II (Fig 18). Since there were significant changes in glomerular filtration rate during the angiotensin II infusion, the results have also been expressed as a fraction of the glomerular filtration rate. There was still no significant difference in excretion rate during the angiotensin II infusion. The plasma concentration of dinor 6 keto PGF_{1a} was measured in 4 of the animals during infusion of angiotensin II and in 5 during the vehicle infusion (Fig 19). There was no change during infusion of either angiotensin II or vehicle although in one animal the plasma concentration was substantially higher than the others before infusion of vehicle. The urinary excretion rate of dinor 6 keto PGF_{1a} was also high in this animal, which in part explained the apparently higher excretion rate in the vehicle infusion group.

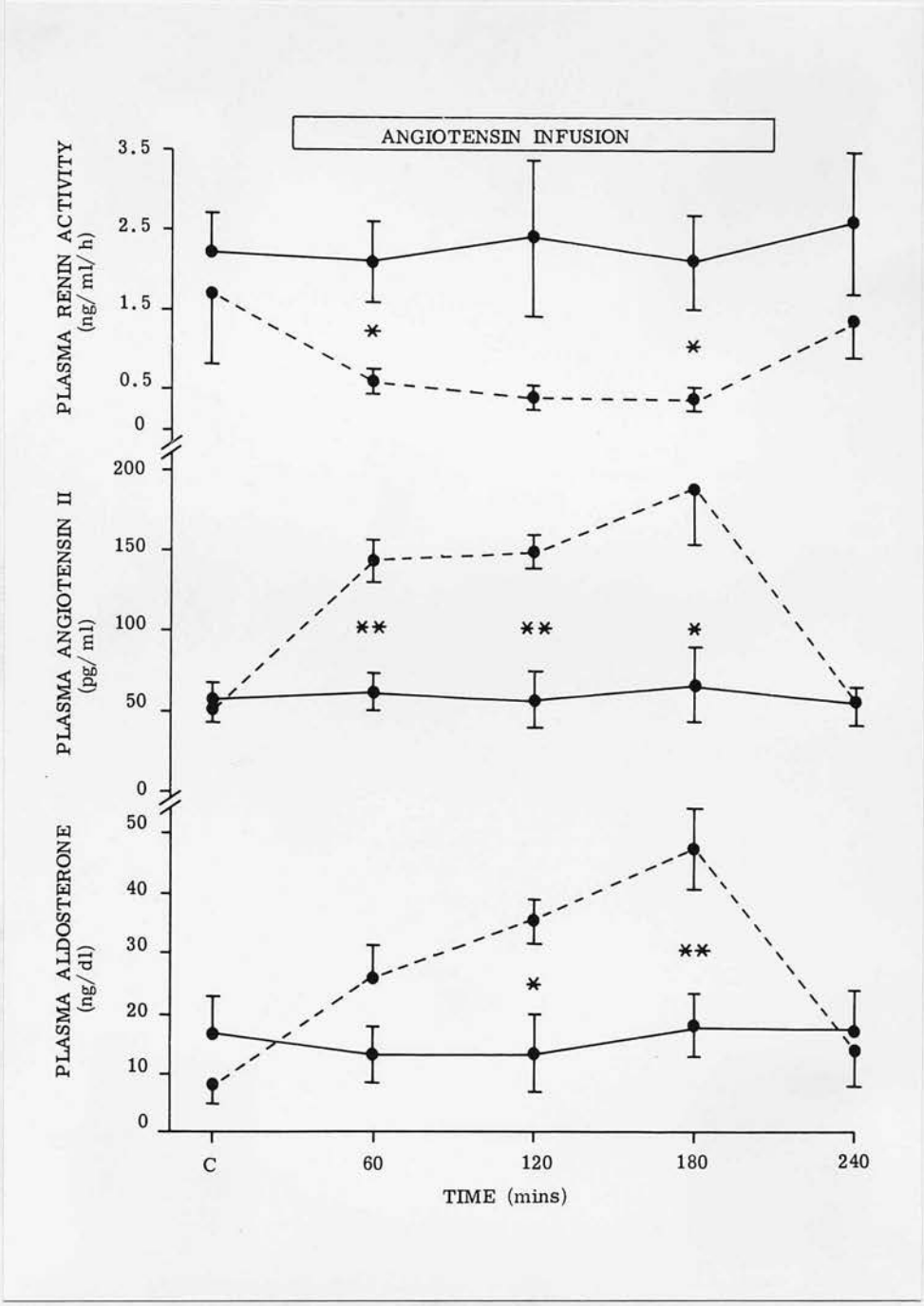

Fig 17. Changes in plasma renin activity, angiotensin II and aldosterone as a result of angiotensin II infusion (15ng/min/kg)
* = $P<0.05$
** = $P<0.01$ for difference from saline control
---- = angiotensin II infusion

——— = saline infusion

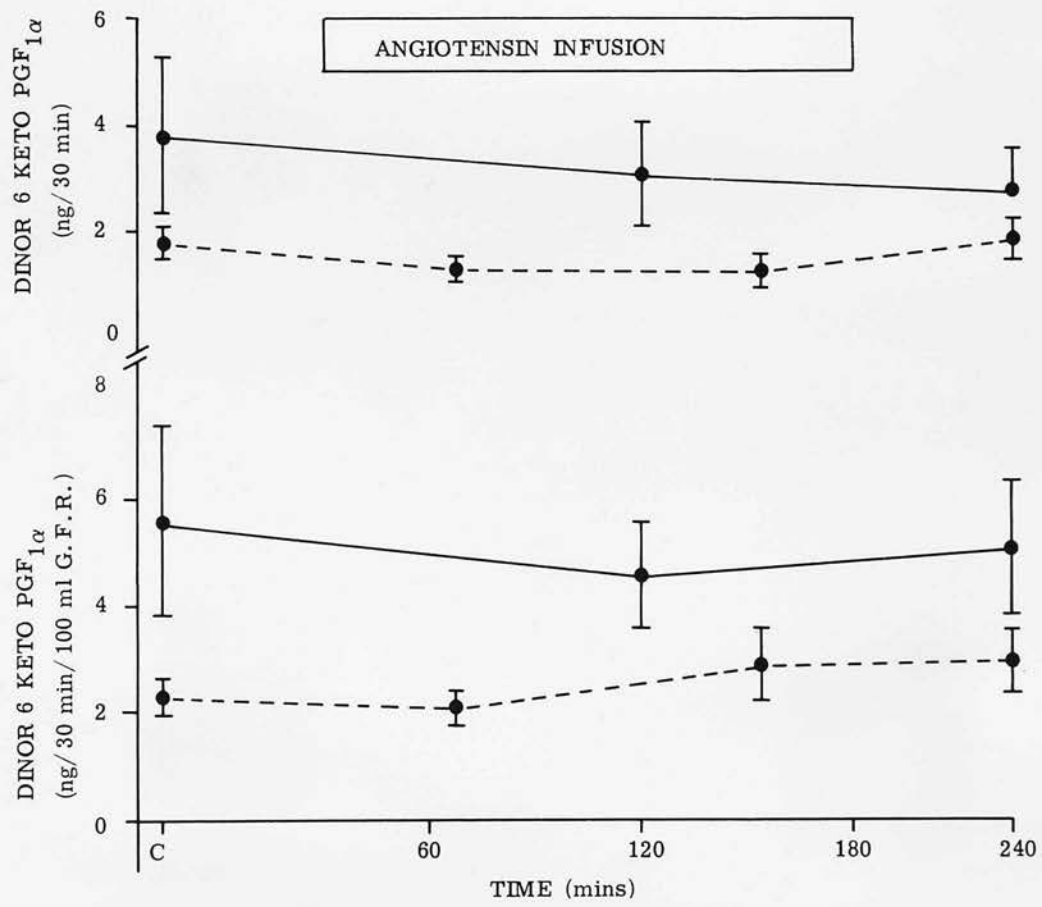

Fig 18. Changes in rate of urinary excretion of dinor 6 keto PGF_{1a} also expressed as a function of glomerular filtration rate, as a result of angiotensin II infusion (15ng/min/kg).
---- = angiotensin II infusion

—— = saline infusion

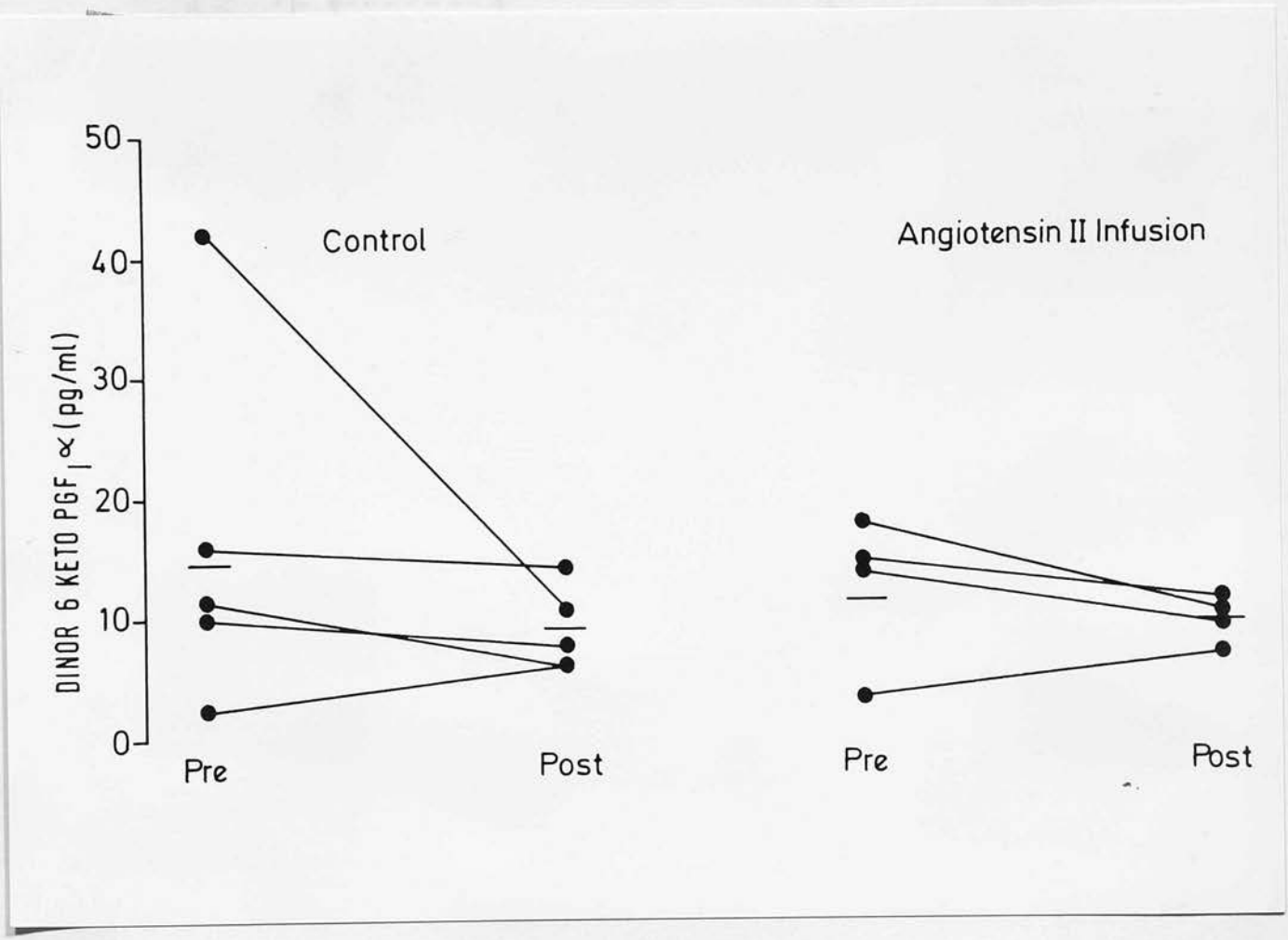

Fig 19. Plasma concentration of dinor 6 keto PGF_{1a} before and 30 minutes after infusion of angiotensin II (15 ng/min/kg) for 180 minutes. Horizontal bar indicates mean value.

2.2.2.3 Renal excretory function

There were no significant differences in urinary excretion of water, sodium or potassium between the dogs receiving angiotensin II and the vehicle (Fig 20).

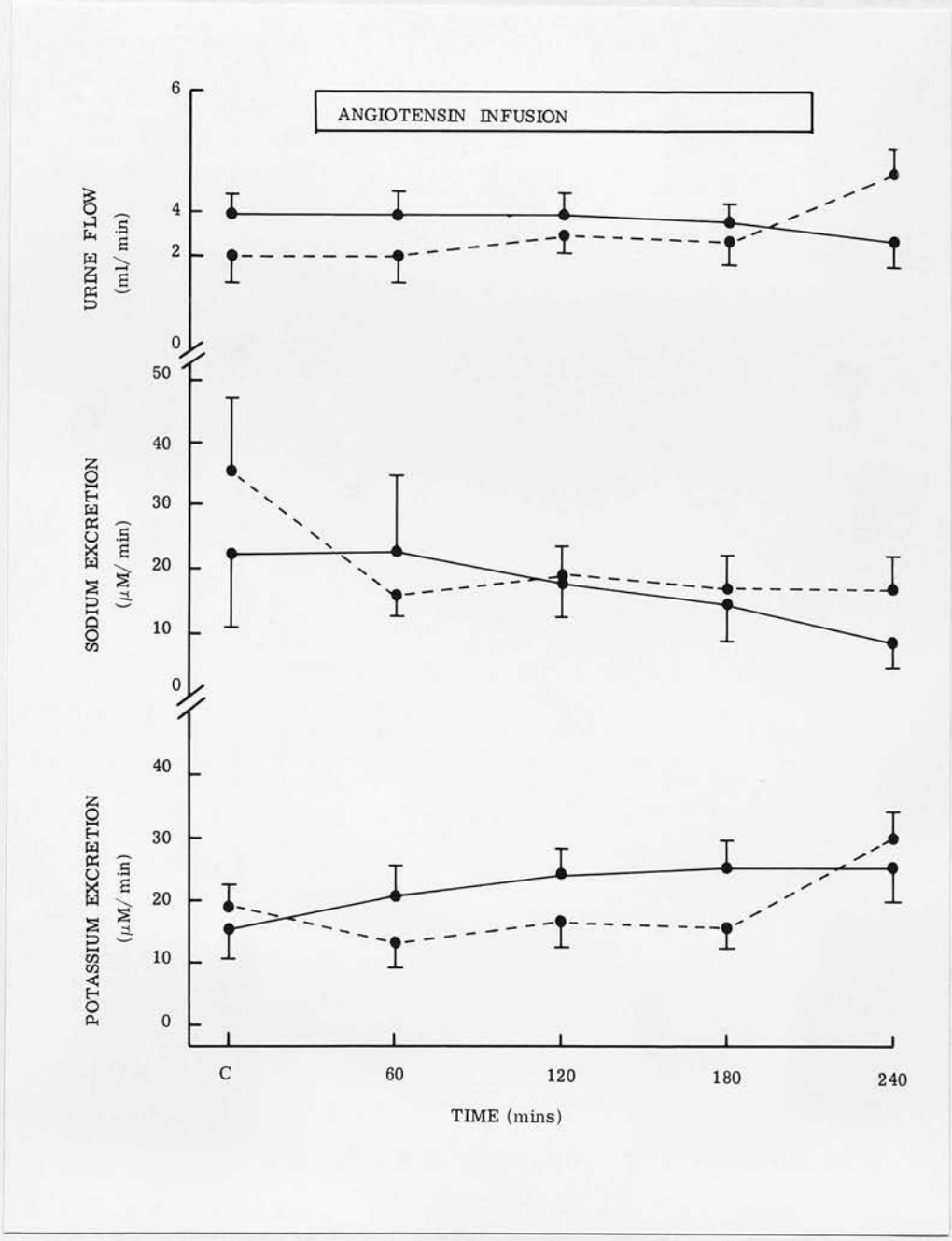

Fig 20. Changes in urine flow, sodium excretion, and potassium excretion as a result of angiotensin II infusion (15 ng/min/kg).
---- = angiotensin II infusion
——— = saline infusion

2.3 Systemic and renal vascular sensitivity to angiotensin II in normotensive and hypertensive dogs.

The results of the previous study suggest that in normotensive sodium replete dogs, systemic synthesis of PGI_2 is not significantly altered by infusion of physiological quantities of angiotensin II. The same may not be true for renal PGI_2, and moreover, the synthesis of other vasodilator prostaglandins such as PGE_2 maybe stimulated by angiotensin II. As indicated in the introduction, other factors than prostaglandins may also modify the pressor response to angiotensin II, for example, there maybe changes in vascular sensitivity to angiotensin II.

Some of these aspects of the actions of angiotensin II were further explored by comparing the systemic and renal effects of infusions of angiotensin II in conscious dogs before and 14 days after the development of one clip two kidney hypertension. Thirteen animals were used, 7 of which were studied when hypertensive.

2.3.1 Protocol and Special Methods

A catheter was inserted into the bladder of each animal on the morning of the study, as described in the general methods section. A water diuresis was initiated by an intravenous infusion of 300 ml dextrose solution (50g/l) over 40 minutes and then maintained by infusion of dextrose solution (50g/l) containing PAH and inulin, via a Watson Marlow MHRE pump at 4 ml/min for the remainder of the study. Sodium chloride solution (150 mmol/l) was infused intravenously at 0.5 ml/min via a

Harvard infusion pump. After a 90 minute equilibration period urine was collected for 2 successive 20 min control periods. Angiotensin II solution was then substituted for the sodium chloride solution and infused at 0.5 ml/min to give a final infusion rate of 5 ng/min/kg body weight of angiotensin II. Urine was collected for 20 minutes. After a 20 minute recovery period, during which time sodium chloride solution was again substituted for angiotensin II, the same protocol was repeated with infusions of 10 and 15 ng/min/kg body weight of angiotensin II, each infusion being preceeded by a 20 minute control collection. Clearance results were calculated for each urine collection period. Blood pressure was monitored continuously throughout, and arterial blood samples were collected at the beginning and end of each urine collection.

2.3. Results

2.3.2.1 Normotension vs hypertension

Blood pressure was sigificantly increased 14 days after partial occlusion of one renal artery (106.6$\pm$ 3.2 - 133.5 $\pm$ 4.5 mmHg, $p < 0.01$, n=7) and plasma renin activity was also significantly different in the two groups (3.0 $\pm$ 0.2 vs 5.1 $\pm$ 0.8 ng/ml/h, $p<0.01$, n=7). Urine flow, sodium excretion, effective renal plasma flow and glomerular filtration rate were all similar during the control period before and after induction of hypertension (Tables 2 and 3). The rate of urinary excretion of PGE was, however, significantly higher in the hypertensive compared with the normotensive animals (82 $\pm$ 224 df=17 vs. 255 $\pm$ 66 pg/min, df=12, $p < 0.02$).

TABLE 2. ANGIOTENSIN II (ANG II) INFUSION AND RENAL FUNCTION

	Normotensive				
Infusion	V (ml/min)	$U_{Na}V$ (μM/min)	C_{PAH} (ml/min)	C_{Inulin} (ml/min)	BP (mm Hg)
Control (n=9)	5.4±0.5	32.3±5.5	282.0±19.0	96.9±4.7	106.6±3.2
ANG II (5 ng/min/Kg)	4.1±0.6**	24.8±3.6*	209.0±18.0**	83.3±7.3	116.2±3.6***
Control (n=9)	5.6±0.7	46.8±7.9	273.0±27.0	99.8±5.2	106.8±2.8
ANG II (10 ng/min/Kg)	3.1±0.3**	22.3±3.5**	205.0±23.0**	78.3±6.5**	126.7±2.4***
Control (n=8)	6.4±0.4	73.7±20.5	279.0±33.0	100.3±4.1	105.6±1.8
ANG II (15 ng/min/kg)	3.0±0.2***	29.1±6.5*	200.0+32.0***	85.8±5.9	130.2±2.4***

V = Urine volume, $U_{Na}V$ = urinary sodium excretion, C_{PAH} = PAH clearance, C_{Inulin} = inulin clearance, PAH = para aminohippuric acid, BP = mean blood pressure. *P<0.05, **P<0.01, ***P<0.001.

TABLE 3. (ANG II) INFUSION AND RENAL FUNCTION

	Hypertensive				
Infusion	V (ml/min)	$U_{Na}V$ (μM/min)	C_{PAH} (ml/min)	C_{Inulin} (ml/min)	BP (mm Hg)
Control (n=7)	5.7±0.5	55.4±11.4	289.0±26.0	102.1±4.8	133.5±4.5
ANG II (5 ng/min/Kg)	5.7±0.6	66.7±14.5	306.0±32.0	113.2±12.8	136.8±4.0
Control (n=7)	6.0±0.4	38.6±4.8	289.0±28.0	96.1+5.5	131.9±4.6
ANG II (10 ng/min/Kg)	5.2±0.8	79.2±16.1	265.0±36.0	99.6±11.3	150.4±5.0***
Control (n=7)	5.2±0.7	64.3±20.7	277.0±27.0	95.0±7.5	131.3±2.6
ANG II (15 ng/min/Kg)	5.6±0.5	119.9±26.9*	264.0±27.0	103.8±9.1	153.1±1.8***

V = Urine volume, $U_{Na}V$ = urinary sodium excretion, C_{PAH} = PAH clearance, C_{Inulin} = inulin clearance, PAH = para aminohippuric acid, BP = mean blood pressure. *P<0.05, **P<0.01, ***P<0.001.

2.3.2.2 Angiotensin infusion in normotensive groups

There were dose related increases in blood pressure during infusion of all three doses of angiotensin II (Table 2). At the same time there were dose related decreases in urine flow, sodium excretion and effective renal plasma flow. Glomerular filtration rate was only significantly decreased during infusion of the two larger doses of angiotensin II.

2.3.2.3 Angiotensin infusion in hypertensive group

As in the normotensive group there were dose related increases in blood pressure with infusion of angiotensin II (Table 3), but neither effective renal plasma flow nor glomerular filtration rate were significantly altered. Urine flow also was not significantly changed (Table 3) but during the highest infusion rate of angiotensin II there was a significant increase in sodium excretion ($p<0.05$).

2.3.2.4 Comparison between angiotensin infusion in normotensive and hypertensive groups.

The differences have been summarised in Figs 21-23. Increments in systemic blood pressure in response to angiotensin II were not significantly different in the two groups (Fig 21). The change in effective renal plasma flow during the 5ng/min/kg body weight infusion rate was significantly different with a decrease occurring in the normotensive group and small increase in the hypertensive group (Fig 22). The decrease in glomerular filtration rate during the 10 ng/min/kg body weight infusion was also greater in the normotensive

group as was the change in urine flow during the 15 ng/min/kg infusions. The changes in sodium excretion were significantly different during both 10 and 15 ng/min/kg infusions, a substantial decrease occurring in the normtensive group and increase in the hypertensive group (Fig 23).

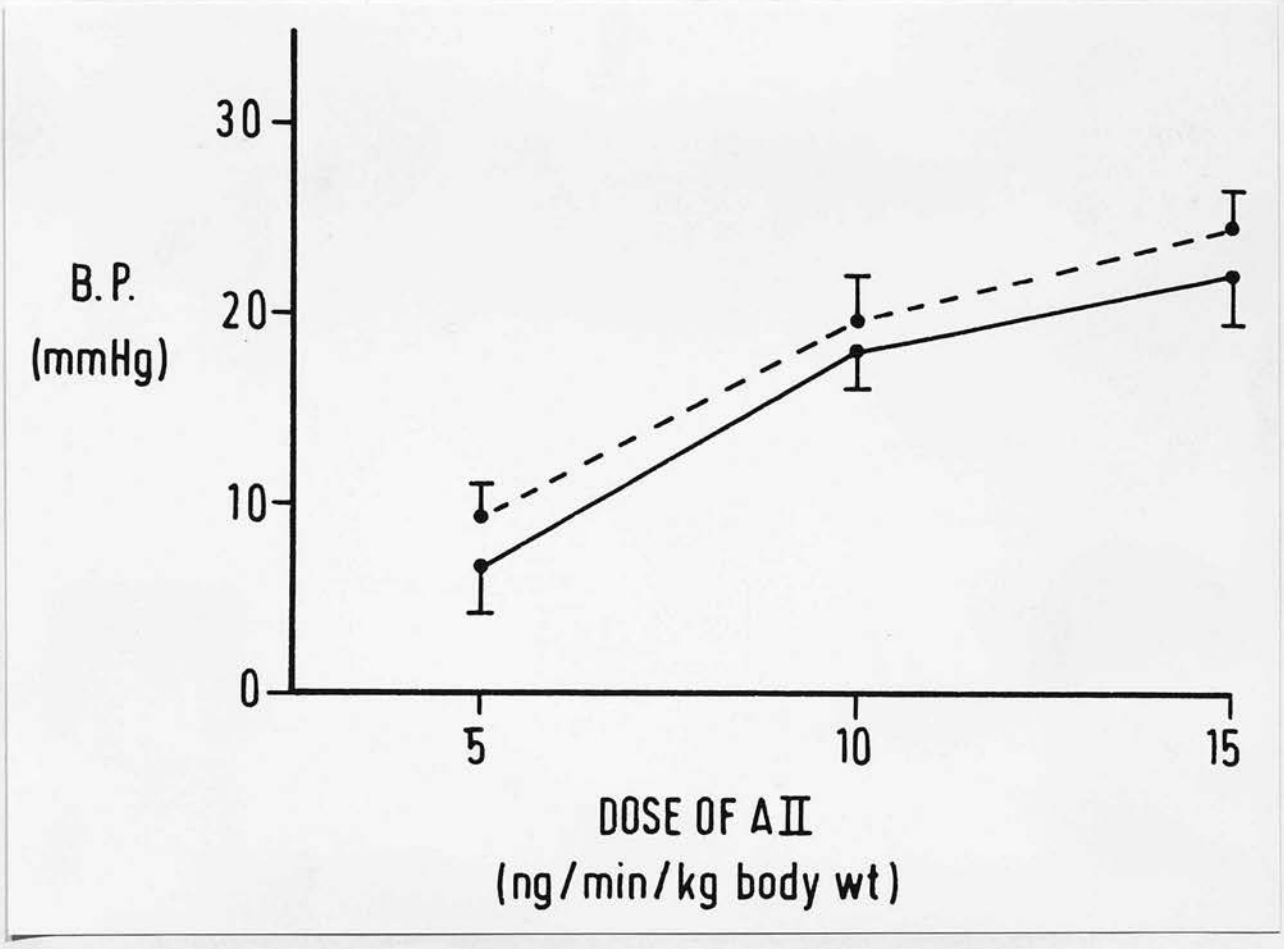

Fig 21. Increment in mean blood pressure (BP) during infusion of different doses of angiotensin II. Results represent values during each preceeding control period subtracted from those during infusion period.
--- = Normotensive

—— = Hypertensive

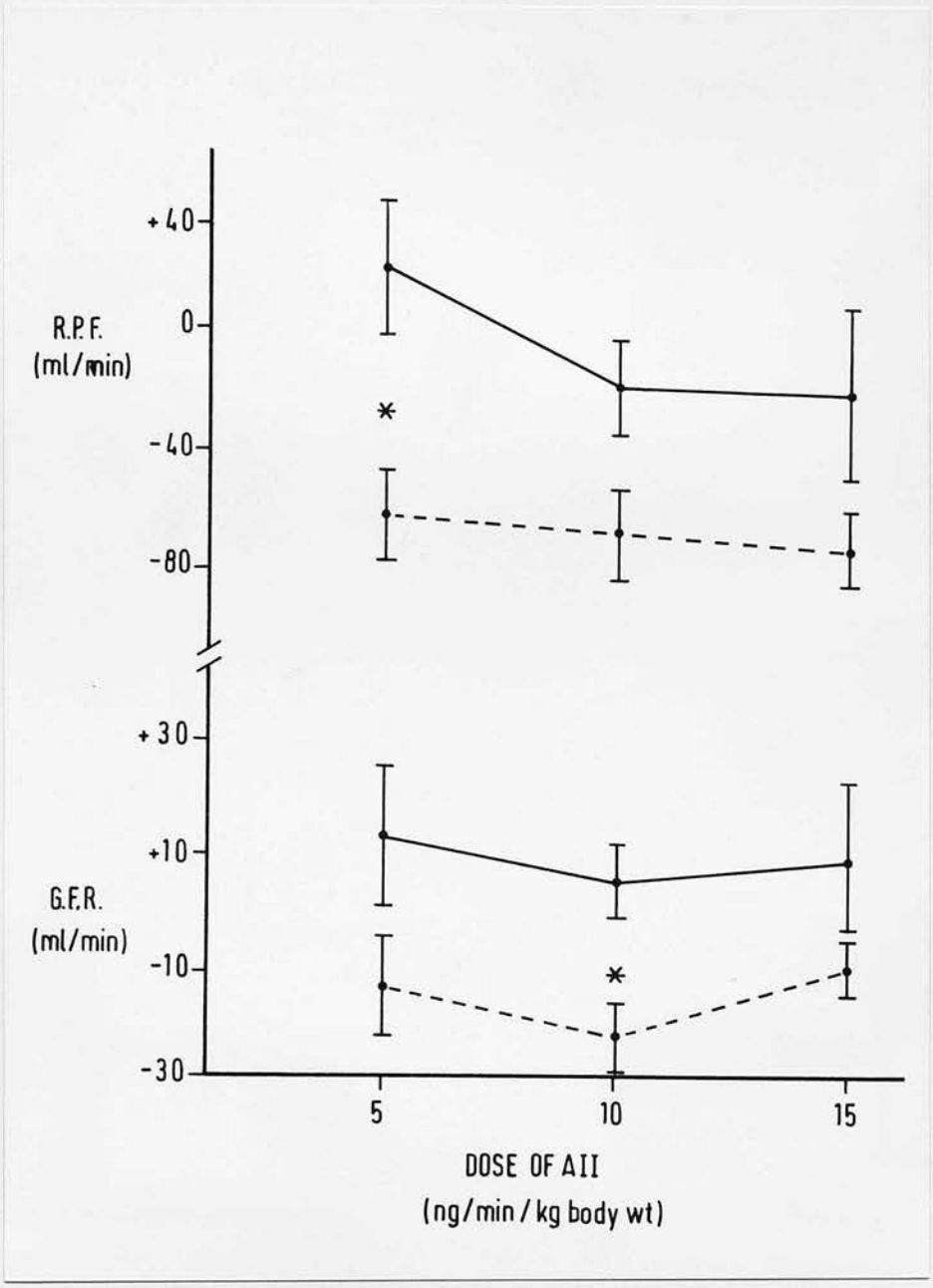

Fig 22. Increment or decrement in effective renal plasma flow (RPF) andglomerular filtration rate (GFR) during infusion of different doses of angiotensin II. Results represents values during each preceeding control period subtracted from those during infusion period.
---- = Normotensive

——— = Hypertensive

* = P<0.05

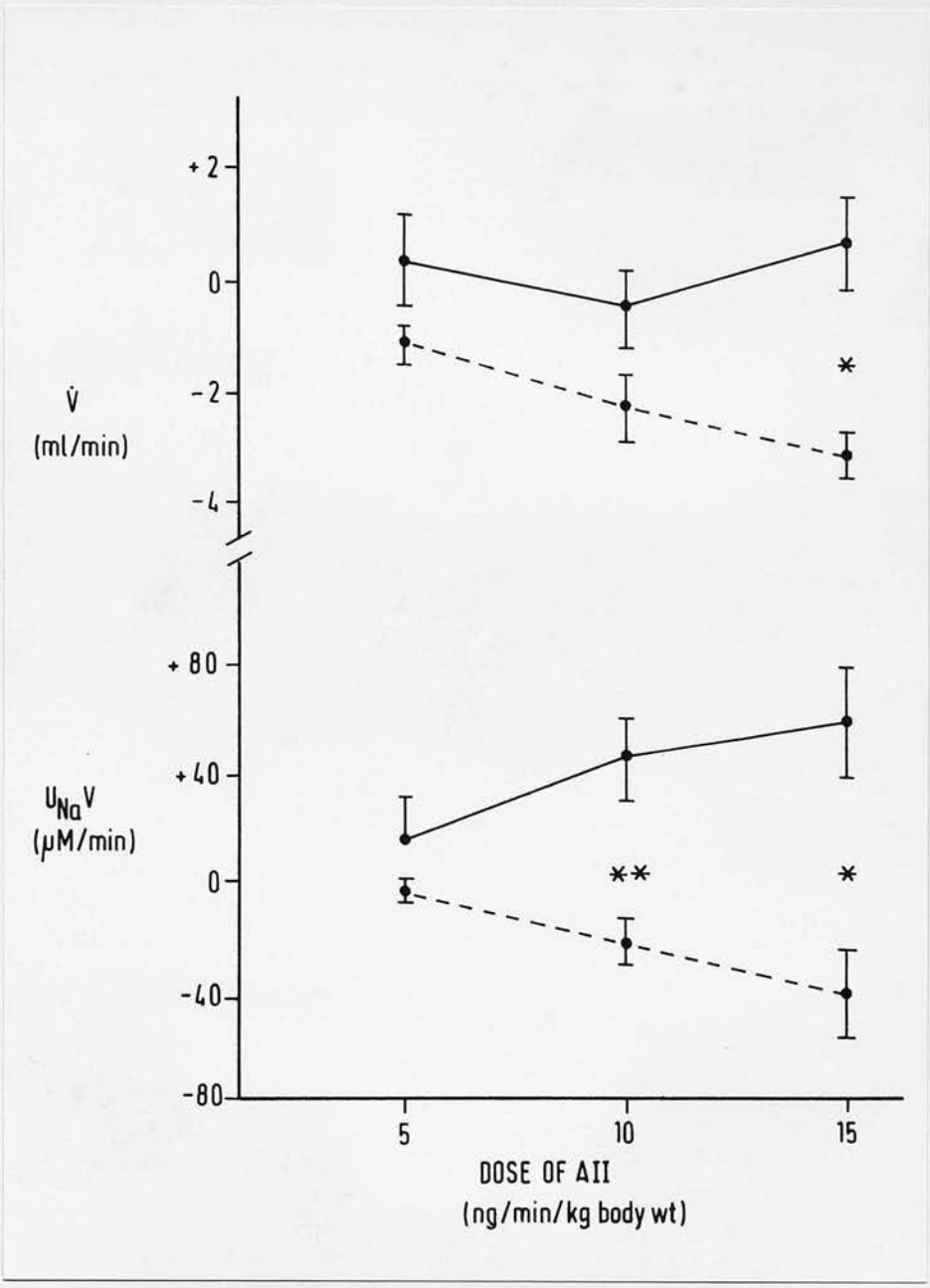

Fig 23. Increment or decrement in urine flow (V) and urinary sodium excretion ($U_{Na}V$) during infusion of doses of angiotensin II. Results represent values during each preceeding control period subtracted from those during infusion period.
--- = Normotensive

——— = Hypertensive
* = P<0.05
** = P<0.01

Although PGE excretion was significantly higher in the hypertensive group, further increases occurred during infusion of angiotensin II in both groups. The presence of haematuria in some animals limited the number of analyses which could be performed, and therefore clear cut dose dependent relationships could not be established for the increment in PGE excretion during angiotensin II infusion (Table 4). However the mean rate of excretion during angiotensin II infusions was significantly larger than the mean rate during the control periods in both normotensive (82 ± 24 - 115 ± 29 pg/min, $df=17$, $p<0.05$) and hypertensive groups (255 ± 66 to 341 ± 60 pg/min $df=12$, $p<0.02$).

TABLE 4. INCREMENT IN URINARY PGE EXCRETION ($U_{PGE}V$) DURING ANGIOTENSIN II (ANG II) INFUSION

Dose of ANG II (ng/min/Kg)	$U_{PGE}V$ (Infuse-Control) (pg/min)	
	Normotensive (n=7)	Hypertensive (n=5)
5	73.0±25.0	38.0±19.0
10	20.0±34.0	131.0±93.0
15	19.0±24.0	174.0±79.0

$U_{PGE}V$ = Urinary prostaglandin E excretion

2.4 Interstitial cell morphology and the development of one clip - two kidney hypertension

It is attractive to speculate that prostaglandins exert a modulatory role on vasoconstrictor systems in the kidney. Interstitial cells in the renal medulla certainly synthesise prostaglandins, in addition to other anti-hypertensive compounds. The dark osmiophilic granules in the interstitial cells appear to have an important function in this secretory role of the cells. The next study aimed to clarify the electron microscopic appearance of these granules and observe changes in their numbers in dogs with one clip - two kidney hypertension in an effort to correlate any changes with associated changes in renal prostaglandin synthesis.

2.4.1 Protocol and Special Methods

Sixteen female fox hounds (weight 16.0 to 27.0 kg) were used. The animals were divided into two groups. The hypertensive group consisted of 8 dogs that underwent surgery and subsequent partial clamping of a renal artery. The control group consisted of two dogs (Nos 5 and 13) that were surgically prepared as described, but in whom the clamp was not tightened and a further 6 dogs that did not undergo surgery.

The kidneys from the two groups of animals were allocated into 1 of 3 categories. Kidneys from animals in the control group were designated normals. The kidneys from the hypertensive animals were divided into two further categories. The kidneys with a partially clamped renal artery were designated 'clamped'

kidneys and the opposite unclamped kidneys in the same animals were designated 'untouched'. All kidneys were weighed, halved coronally, and tissue blocks were dissected from the cortex and outer and inner medulla. The blocks were fixed in 3% glutaraldehyde (PH 7.4, 625 mOsm/l) for 24 hours at 20°C, postfixed in 3% osmium tetroxide for 2 hours at 4°C and then embedded in Araldite. Initial screening of specially treated 1.5 um thick sections with light microscopy permitted areas in which all tubules were cut transversely to be chosen. 500 A° sections from this area were floated onto 400 mesh copper grids, stained with uranyl acetate and lead citrate, and examined on an AEI Corinth 275 electron microscpe (AEI Scientific Inst, San Diego, Calif).

All granule counts were undertaken by a single observer (Ms J. Taylor) who was unaware of the origin of a particular section at the time of counting. The sections on the grid were scanned at low power, and the largest intact section chosen for counting. Each square of the copper mesh grid containing part of the section was viewed at 6000 x magnification. The number of granules in each complete interstitial cell was recorded and a minimum of 70 interstitial cells per medulla examined.

2.4.2 Results

2.4.2.1 Blood pressure monitoring

As a result of technical problems with the arterial catheters, blood pressure recordings were only obtained from 6 of the animals in the hypertensive group. In 5

of these animals the mean blood pressure increased from 101 ± 2 mm Hg during the control period to 119 ± 5 mmHg ($p<0.02$), an increase of 17 ± 4 mmHg at the time of termination. The time course of the development of hypertension was different in each animal. Some animals showed a rapid initial rise in pressure with a subsequent small fall after some days, whereas other showed a gradual increase between the time of clamping and termination. The rise in blood pressure for the group was the same on the third day as at termination.

In one dog (No 14) the renal artery was overclamped, and there was no change in blood pressure (95 ± 1 mmHg before clamping and 97 ± 2 mmHg after clamping). The two dogs in the control group in whom the clamp was not tightened had a mean blood pressure of 110 ± 2 mmHg (No 5) and 99 ± 3 mmHg (No 13) at termination.

Renal function assessed by estimation of plasma creatinine was normal in all 16 animals.

2.4.2.2 Kidneys

The kidneys were obtained a mean of 12 ± 1.7 days after clamping. The mean weight of the clamped kidneys was 47 ± 5 gms which was significantly less than the mean weight of the untouched kidneys (81 ± 5 gms, $p<0.005$). Normal kidneys had a mean weight of 64 ± 3 gms, which was significantly greater than that of the clamped kidneys ($p<0.05$) but did not differ significantly from the weight of the untouched kidneys.

2.4.2.3 Light microscopy

Sections of renal cortex and medulla were initially

scanned by light microscopy. There were no gross morphological abnormalities in any of the kidneys. The impression was that there were increased numbers of cells in clamped kidneys compared with normal and untouched kidneys, but little difference between untouched and normal. Quantification of the numbers of interstitial cells in the medullae of the three groups could not be attempted by light microscopy for two main reasons. First, difficulties in accurate cell identification by this method are considerable. Second, the distribution of interstitial cells throughout the medulla may not be uniform and accurate identification of the original location of a tissue block after processing is difficult; this would make it impossible to be sure that comparable areas in different kidneys were being counted.

2.4.2.4 Electron microscopy

The medullary interstitial cell is a stellate cell, often ill-defined with one or more cytoplasmic processes, and frequently closely applied to adjacent capillaries or tubules (Fig 24). The cytoplasm of the cell has a characteristic lacey appearance due to dilated rough and smooth endoplasmic reticulum. The osmiophilic granules are located mainly in the perinuclear region or clustered in the cytoplasm. The dark type have a granular appearance, are membrane bound, and are smaller than the light granules (Fig 25, 26). The light granule is a large homogeneous structure that is not bound by a membrane and is less electron

dense than the dark granule. Both types of granule were observed apparently being extruded from cells (Fig 25, 26), suggesting that the granule contents may not be released within the interstitial cells. There appeared to be an inverse relationship between the presence of cisternae and the prevalence of granules within the cell. Collagen fibres were often present around the cells, and in certain sections intracellular fibrillar material was seen, virtually always in cytoplasmic processes, extracellular fibres being closely related to the overlying plasma membrane (Fig 27). The amount of collagen present in the sections was surprising, particularly when compared with the amount present around interstitial cells from other species. Its presence was, however, a consistent feature in sections both from the normal and hypertensive animals.

Fig 24. Clamped kidney: inner medulla. Interstitial cell containing numerous dark granules and mitochondria. The cell is close to an adjacent basement membrane (arrow) of a tubule, and the surrounding interstitial space shows abundant collagen (x8000).

Fig 25. Untouched kidney: inner medulla. Three light granules and one dark granule lie near the plasma membrane of this interstitial cell. One light granule is in the process of being extruded from the cell (arrow). The cytoplasm around these granules contains dilated rough endoplasmic recticulum (x20,000).

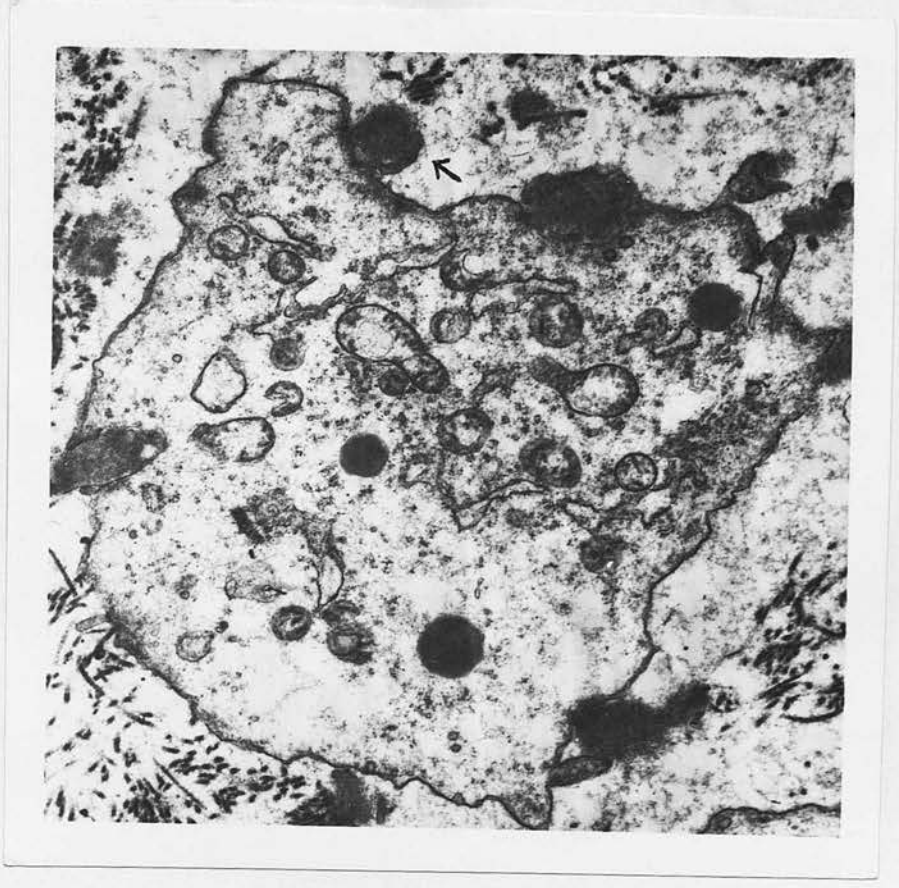

Fig 26. Clamped kidney: inner medulla. In this cross section of an interstitial cell process three dark granules are present in the cytoplasm. Several dark granules are seen apparently in the process of extrusion (arrow) (x20,000).

Fig 27. Normal kidney: inner medulla. In this interstitial cell process intracellular fibrils can be seen lying parallel to the plasma membrane, and extracellular collagen fibres are apparently attached to the membrane and at right angles to it. Clumps of these fibres also lie free in the interstitial space (x12,000).

2.4.2.5 Quantification

Numbers of cells could not be accurately counted on electron microscope sections, because of the high magnification involved and the impossibility of ensuring that sections from comparable sites in all groups of kidneys were being assessed.

The total number of granules per interstitial cell was different in the three groups (Fig 28). The number of dark granules per cell in clamped kidneys (4.69 $\pm$0.50) was significantly greater than in the normal kidneys (0.63 $\pm$0.12, $p<0.01$), whereas the count in the untouched kidneys (0.14 $\pm$ 0.04) was significantly less than in both the contralateral clamped kidneys and in normal kidneys ($p<0.01$). The proportion of light and dark granules in interstitial cells from kidneys in each group are shown in Fig 29. The proportion of dark granules (95.3%) in the clamped kidneys was significantly greater than in the untouched kidneys (31.1%, $p<0.001$), whereas the proportion of dark granules in normal kidneys (79.5%) was significantly higher than that in the untouched kidneys and lower than that in the clamped kidneys ($p<0.001$ in each case).

There were fewer light granules per cell in the clamped kidneys (0.23 $\pm$ 0.05) than in the untouched kidneys (0.44 $\pm$ 0.15), but the difference was not significant. There was, however, a significantly larger number of light granules per cell in the untouched kidneys than in normal kidneys (0.16 $\pm$ 0.04, $p<0.01$). There was no correlation between the number of granules

per cell in either kidney and the extent of blood pressure elevation in individual dogs.

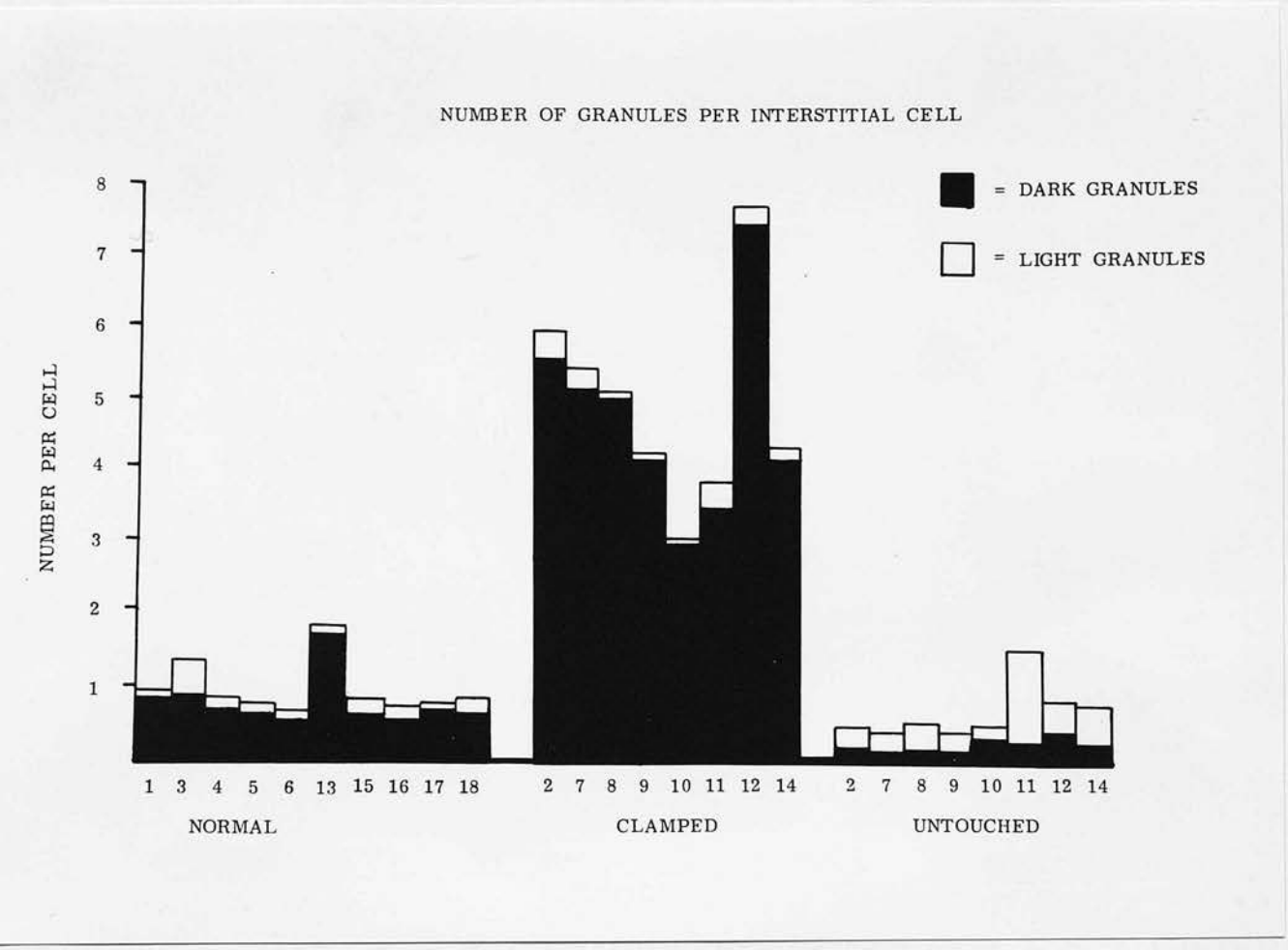

Fig 28. Number of granules per interstitial cell in sections from normal, clamped and untouched kidneys.

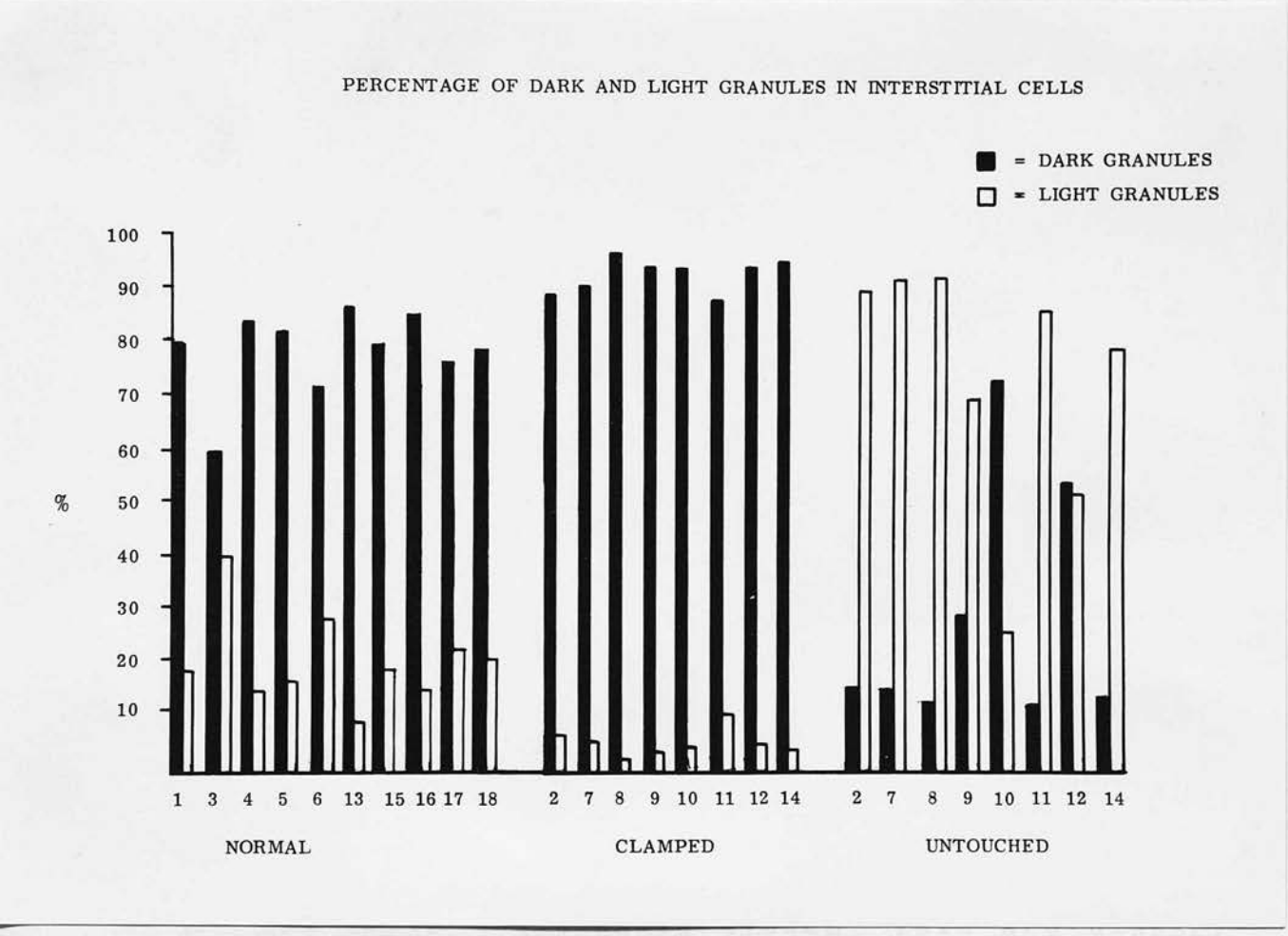

Fig 29. Relative proportions of dark and light granules ininterstitial cells in sections from normal, clamped and untouched kidneys.

3. STUDIES IN MAN

The following studies are described in this section:-

3.1 The renin angiotensin system, PGI_2 and dietary sodium intake.

3.2 Prostaglandins and the renal response to isotonic sodium chloride infusion.

3.3 Systemic PGI_2 synthesis and the surgical correction of hypertension in Conn's syndrome.

The extrapolation of experimental results obtained in vitro to derive explanations for physiological and pathological events in man is an attractive scientific exercise. Proof of the validity of such hypotheses is always difficult to obtain. The many possible interactions between prostaglandins, sodium balance and the state of the renin angiotensin system provides a typical example of such a situation. The next series of studies were designed to provide information to help define these interactions more clearly in normotensive and hypertensive man. All of these investigations were carried out with the informed consent of the individuals involved and the protocols employed had been approved by the Ethical Review Committees of the respective institutions.

3.1 The renin angiotensin system, PGI_2 and dietary sodium intake.

The renin angiotensin system is activated in normal man during periods of dietary sodium restriction. If PGI_2 was implicated in this process, either by increased

renal synthesis promoting renin release, or by increased vascular PGI_2 synthesis in response to increased circulating concentrations of angiotensin II, then an increase in excretion of its major urinary metabolite dinor 6 keto PGF_{1a}, might be expected.

3.1.1 Protocol and Special Methods

Ten healthy male volunteers (age 26-32 years) were included in this study at Vanderbilt University. They progressed through the dietary periods in random order. Three different diets were used with a sodium content of 10 mmol per day (low), 150 mmol per day (normal), and 300 mmol per day (high). The potassium content of each diet was constant (60 mmol per day). Each subject took a particular diet for a preliminary four day period and continued to take it for a further three days, during which time 24 hour collections of urine were made. The subjects remained fully ambulant during this period, but on the final day of the study they lay recumbant for two hours. Blood pressure was then checked and blood drawn for measurement of plasma renin activity, aldosterone and angiotensin II. A two-week washout period intervened between the diets.

3.1.2 Results

The rate of urinary excretion of sodium and potassium derived from the 24 hour urine collections corresponded to the predicted dietary intake (Table 5). The blood pressure of volunteers was less than 130/90 mmHg supine at entry to the study and did not significantly differ at the end of the three dietary regimes. Both plasma

TABLE 5. URINARY ELECTROLYTES AND MEAN ARTERIAL PRESSURE DURING VARIED DIETARY SODIUM INTAKE

Predicted dietary Na^+ (mM/24 h)	10	150	300
Actual urinary Na^+ (mM/24 h)	7.4±2.4	133±15	239±44
Actual urinary K^+ (mM/24 h)	57±7	72±9	60±9
Mean arterial pressure (mm Hg)	110±3.5	108±5.4	108±6.7

Urinary Na^+ excretion significantly differed between diets by analysis of variance ($P<0.01$). The variation in urinary K^+ excretion and mean arterial pressure did not attain statistical significance.

renin activity and plasma angiotensin II concentrations were significantly increased by low sodium intake ($p<0.01$) but there was no significant difference between the levels on normal and high sodium intake. The excretion of 2, 3 dinor 6 keto PGF_{1a} was highest on the normal sodium intake, although the difference between the levels on normal and high sodium intake did not attain significance (Fig 30). By contrast, excretion during sodium restriction was significantly lower ($P < 0.01$) at a time when both plasma renin activity and angiotensin II were substantially increased.

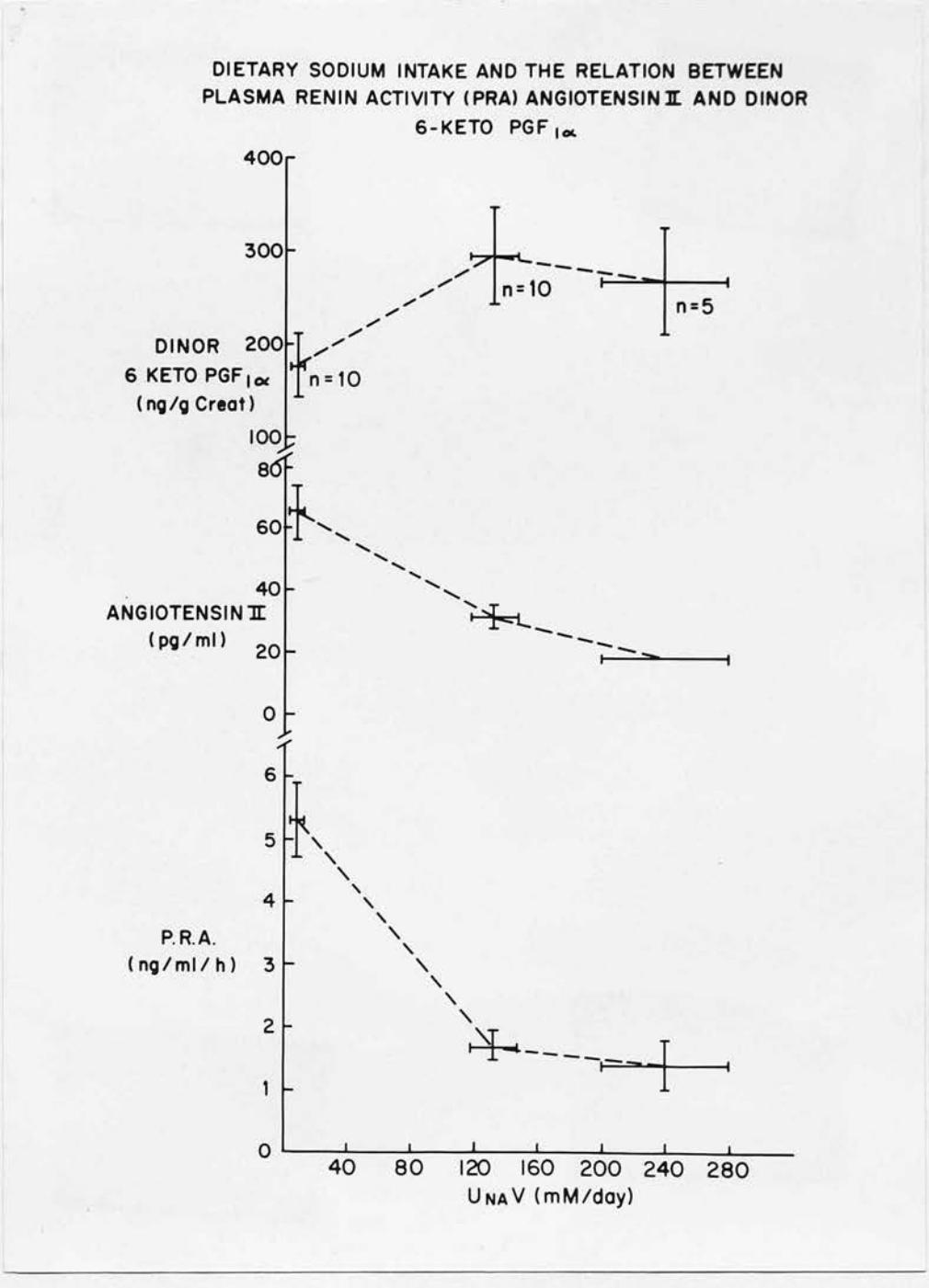

Fig 30. Changes in urinary excretion of dinor 6 keto PGF_{1a}, plasma angiotensin II and plasma renin activity (PRA) as a function of 24 hour urinary sodium excretion in normal subjects on three different dietary sodium intakes. Dinor 6 ketoPGF_{1a} and sodium were measured in the same 24 hour urine specimens.

3.2 Prostaglandins and the renal response to isotonic sodium chloride infusion

Restriction of dietary sodium intake as well as activating the renin angiotensin system, also leads to a contraction of the extracellular fluid volume. An alternative approach to examining the interrelationship between prostaglandins and sodium excretion is to observe the effects of expansion of the extracellular fluid volume. In the next study extracellular volume was acutely expanded by rapid intravenous infusion of isotonic sodium chloride solution in normal man. Changes in renal excretion of PGE and dinor 6 keto PGF_{1a} were monitored, and the effects of inhibition of prostaglandin synthesis with indomethacin observed.

3.2.1. Protocol and Special Methods

Studies were carried out on ten normal human male subjects, mean age 30 years. All subjects ate an unrestricted diet and were requested to refrain from sexual intercourse for at least 24 hours before the study. Urine was collected for the 24 hours up to 2200 hours on the evening before the study. Subjects then fasted and were requested not to smoke. At 0800 hours the next morning a catheter was inserted into a vein in the antecubital fossa of each arm, a sample of blood was obtained, and an intravenous infusion of dextrose solution containing PAH and inulin was started at a rate of 1 ml/min, after suitable loading doses of each compound. Between 1000 hours and 1100 hours an intravenous infusion of sodium chloride solution (150

mmol/l) was given at a rate of 50 ml/min (3 litres in total) and the subjects remained supine until 1300 hours except for rising to pass urine. Blood and urine samples were collected at timed intervals of approximately 30 minutes throughout the study. Blood pressure was measured with the subject recumbant, before each blood sample was taken. Additional blood samples for the measurement of plasma renin activity were collected immediately before and after infusion of sodium chloride and again two hours later at the end of the study. In the studies with indomethacin (50 mg) 140 umol of the drug was taken by mouth at 2200 hours on the evening before the study and a similar dose at 0830 hours on the day of the infusion.

The results of each study were accummulated into three separate periods. Period 1 was a 1 hour control period preceeding sodium chloride infusion. Period II was from 1000-1130 hours, including 1 hour during the sodium chloride infusion and the next half hour. Period III was the final one and half hours of the study. Prostaglandins were measured in the last urine collected from each subject during each of the three periods. Mean blood pressure was calculated as diastolic pressure + (pulse pressure $\div$ 3) and the results given are the mean of at least three separate estimations on each individual during each period.

3.2.2 Results

3.2.2.1 Sodium chloride infusion without indomethacin pre-treatment.

3.2.2.1.1 Systemic blood pressure

Mean blood pressure was not altered significantly by the intravenous infusion of sodium chloride solution (Table 6).

TABLE 6. CHANGES IN RENAL FUNCTION, MEAN BLOOD PRESSURE AND PLASMA RENIN ACTIVITY DURING DIFFERENT PERIODS IN STUDIES IN WHICH SODIUM CHLORIDE SOLUTION WAS INFUSED WITH (+) AND WITHOUT (-) INDOMETHACIN (IND) PRETREATMENT

Period	1			2			3		
Measurement	(-)IND	(+)IND	P	(-)IND	(+)IND	P	(-)IND	(+)IND	P
Renal plasma flow (ml/min)	882±84	700±79	<0.05	921±88	741±49	<0.05	813±72	684±39	<0.025
Glomerular filtration rate (ml/min)	109±10	113±3	N.S.	115±7	125±8	N.S.	105±4	118±10.2	N.S.
Mean blood pressure (mmHg)	88.6±2.2	89.3±1.8	N.S.	90.7±2.0	92.4±3.4	N.S.	88.1±2.6	89.0±2.0	N.S.
Plasma renin activity (pmol $h^{-1}ml^{-1}$)	1.17±0.17	1.03±0.29	N.S.	0.66±0.08	0.65±0.15	N.S.	0.61±0.10	0.57±0.11	N.S.

P values represent the statistical differences between results obtained with and without indomethacin in separate periods by Student's test. N.S. - not significant.

3.2.2.1.2 Renal function

Effective renal plasma flow decreased significantly between period II (921 ± 88 ml/min) and period III (813 ± 72 ml/min, $p<0.01$) to a value not significantly different from period 1 (Table 6).

Glomerular filtration rate did not change significantly throughout the study period (Table 6). There was great individual variability in the maximum rate of urine flow achieved during period II (1.0 - 12.4 ml/min;), and this was associated with a similar wide variation in the rate of sodium excretion. Two of the subjects showed no change in the rate of sodium excretion during period II whereas others showed very large increments (Fig 31). The percentage of the infused solution which was excreted within two hours after the end of the infusion of sodium chloride varied between 17 and 100% for different indiviudals. Free water clearance increased significantly during period II ($p<0.05$) but had returned to values below control by period III (Fig 31).

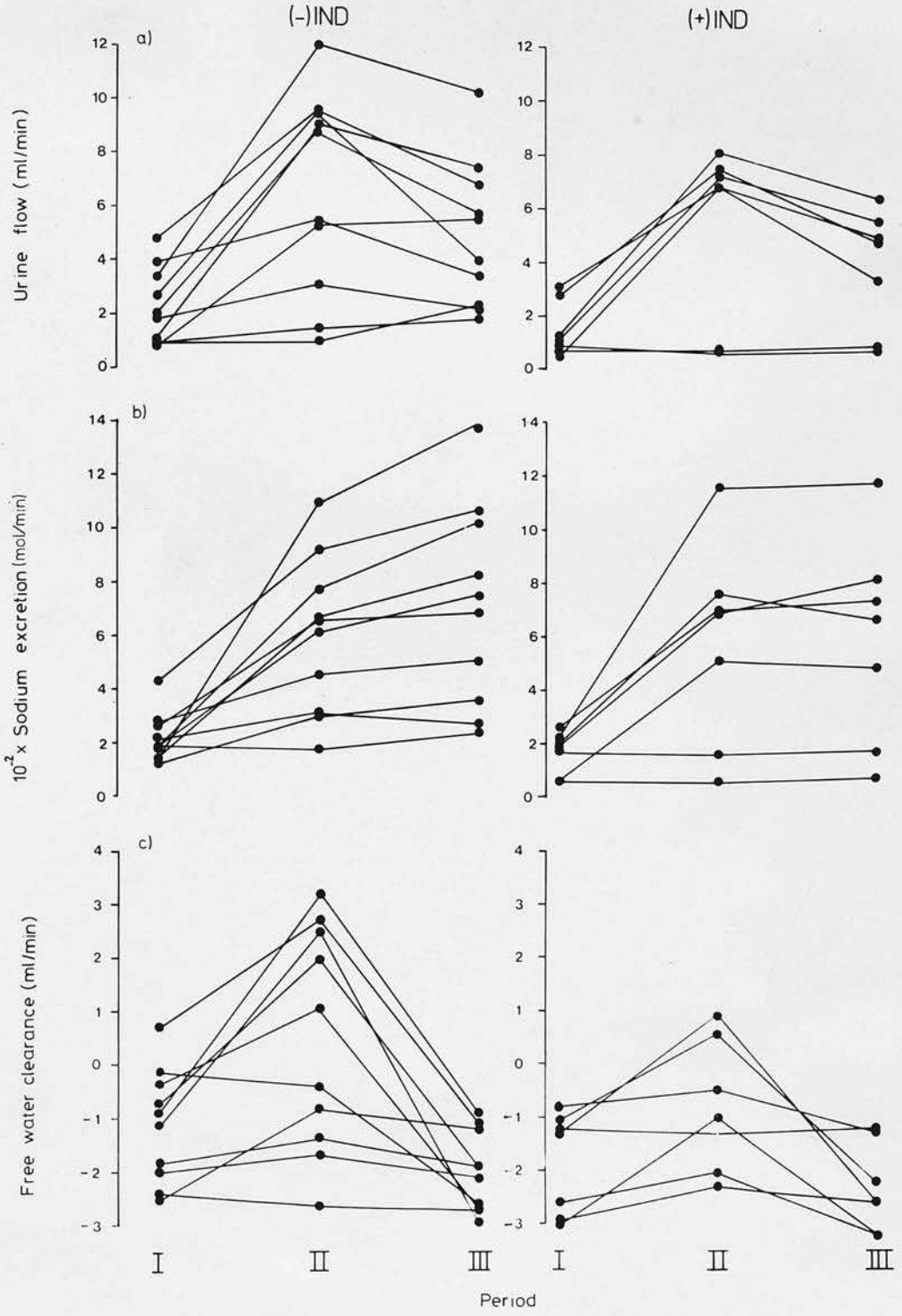

Fig 31. Values in individual subjects for urine flow, sodium excretion and free water clearance before (I), immediately after (II) and 90 minutes after (III) infusion of three litres of sodium chloride solution. Each column of results refers to pre-treatment with (+IND) or without (-IND) indomethacin.

3.2.2.1.3 Plasma renin and urinary prostaglandins

Plasma renin activity decreased significantly between periods I and II ($p<0.01$) and remained suppressed at the end of period III (Fig 32). Excretion of PGE during the day before the study (1.17 $\pm$ 0.21 nmol/24) showed no correlation with either the sodium content of the urine during the same period (166 $\pm$ 15 mmol/24) ($r=0.10$, $p>0.05$) or with the subsequent rate of excretion of infused sodium chloride solution, as indicated by the percentage of the infused sodium chloride solution that had been excreted by the end of period III ($r=0.54$, $p>0.05$). There was no relationship between the rate of excretion of the infused sodium chloride solution and the excretion of sodium over the the 24 hours before the study (Fig 33). The rate of excretion of PGE decreased significantly between the end of period 1 and the end of period III (Fig 34) ($p<0.05$). Urinary PGE excretion decreased during period II, the concentration of PGE in the urine being below the reliable limit of detection in all samples.

Excretion of dinor 6 keto PGF_{1a} was higher after infusion of sodium chloride (period II) than during the control period 1, although the difference narrowly failed to achieve significance. Excretion during period III was significantly lower than that during period II ($p<0.05$, Fig 32). The rate of excretion of dinor 6 keto PGF_{1a} did not correlate with glomerular filtration, urine flow or sodium excretion although there was a weak correlation with plasma renin activity ($r=0.48$, $p<0.02$).

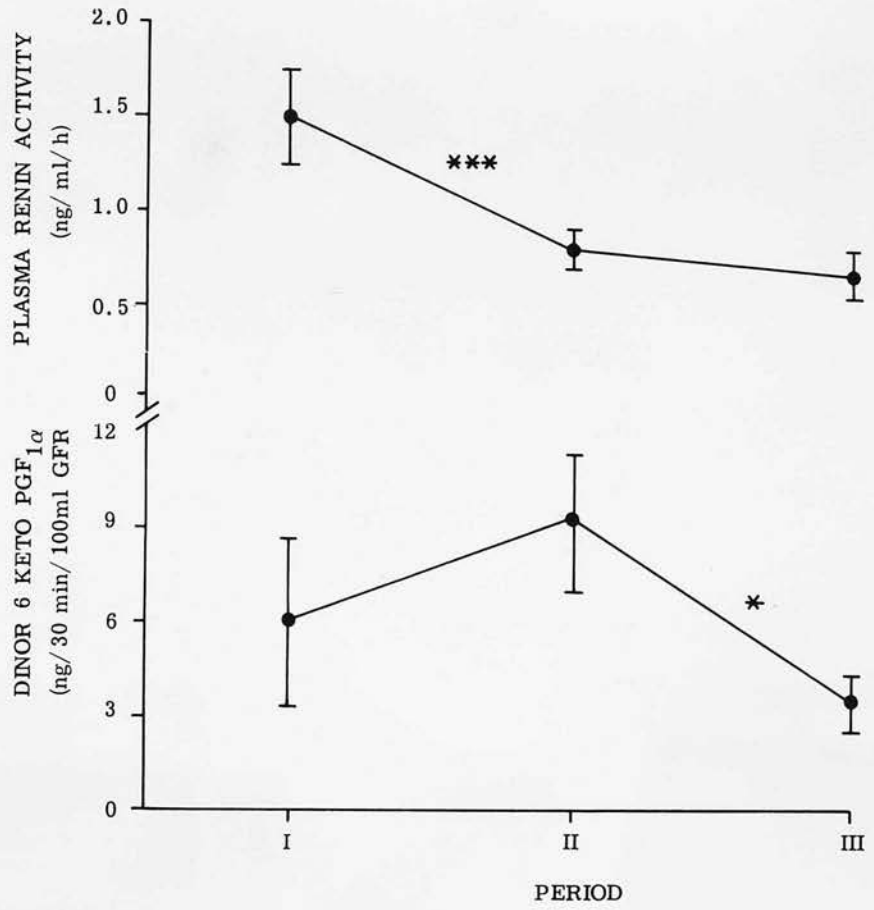

Fig 32. Plasma renin activity and urinary excretion of dinor 6 keto PGF_{1a} (expressed as a function of glomerular filtration rate) before and immediately after sodium chloride infusion (periods I and II) and again 90 minutes later (period III).
* = $p<0.05$
*** = $P<0.01$

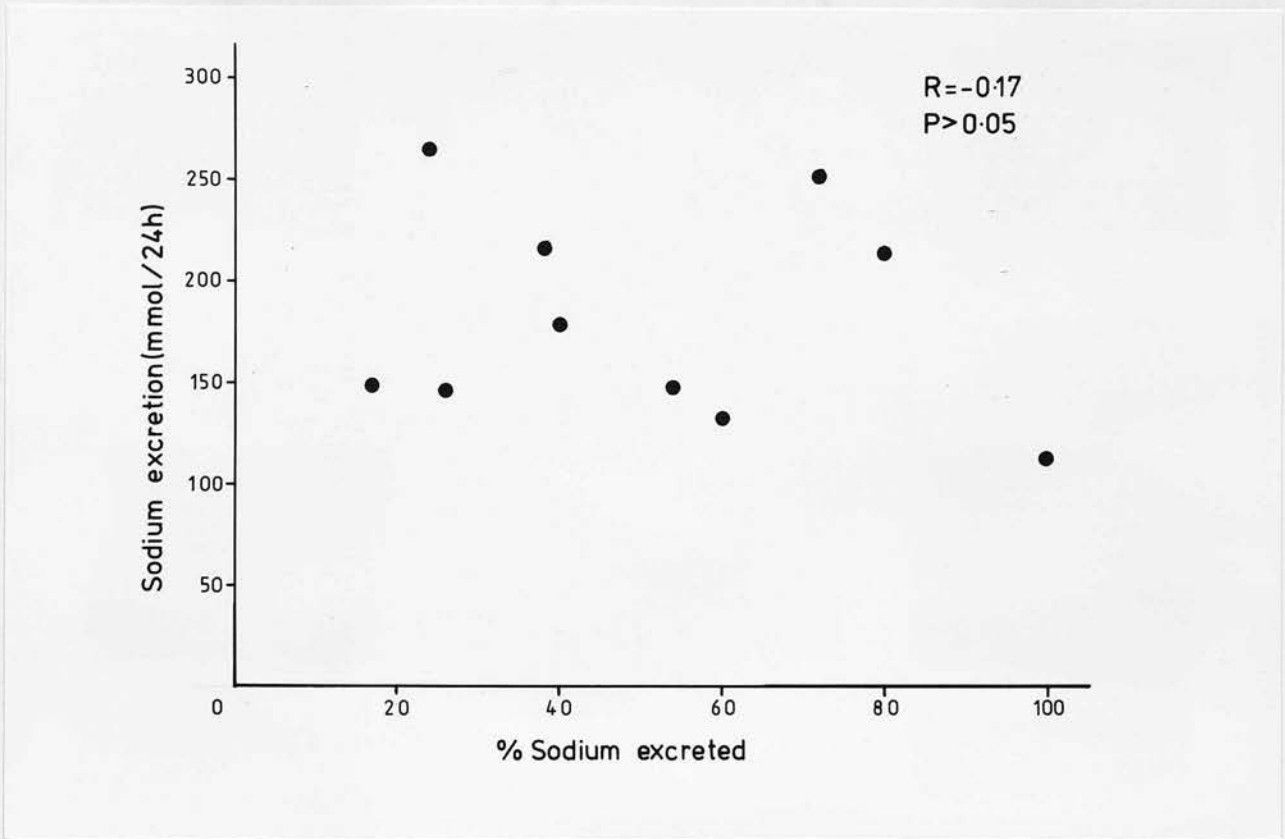

Fig 33. Percentage of the infused sodium load that was excreted within 120 minutes of completing the sodium chloride infusion, expressed as a function of urinary sodium excretion over the 24 hours before the start of the study.

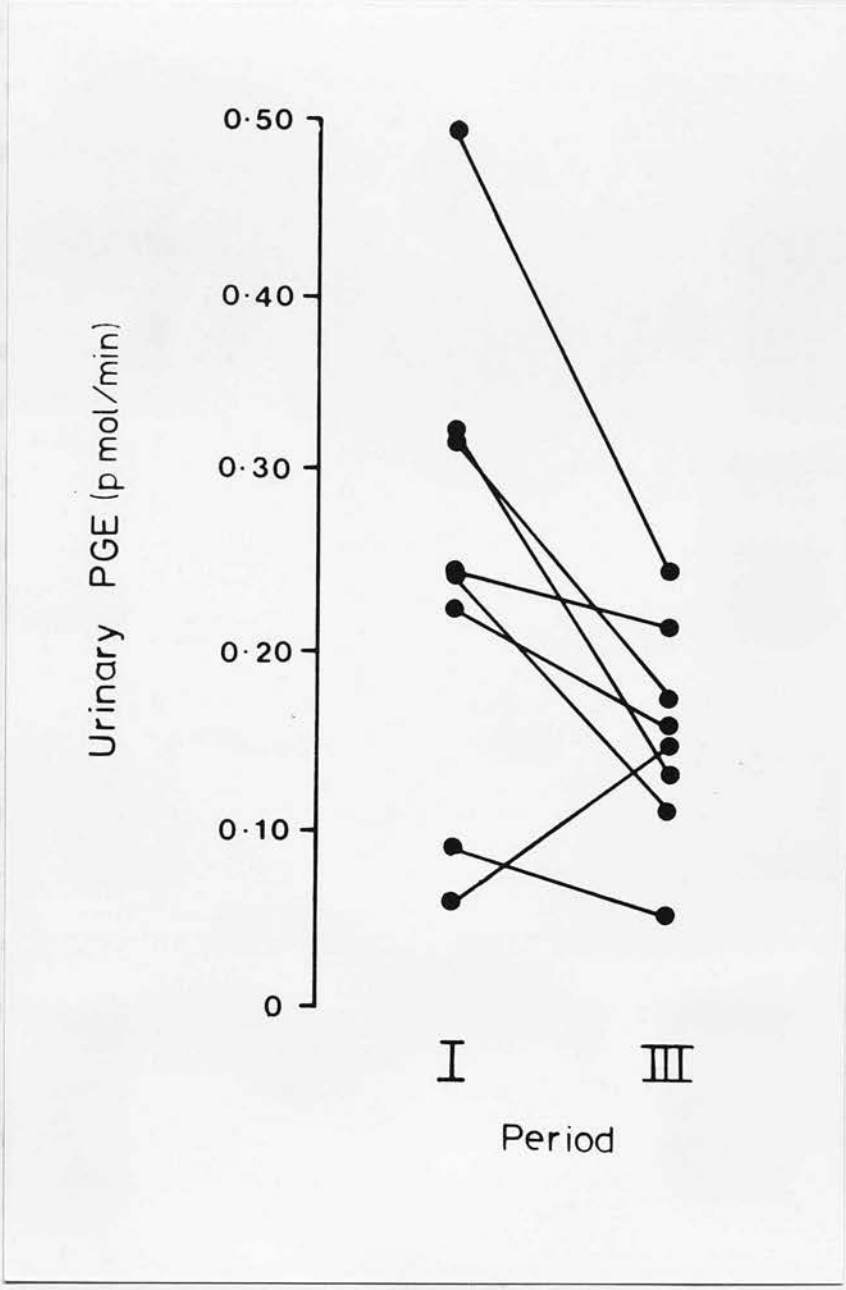

Fig 34. Rate of urinary PGE excretion over 30 mins before sodium chloride infusion (I) and during a 30 min collection starting90 mins after completion of the sodium chloride infusion (III).

3.2.2.2 Sodium chloride infusion with indomethacin pre-treatment

3.2.2.2.1 Systemic blood pressure

There was no significant change in mean blood pressure during the infusion of sodium chloride solution, the blood pressure being the same as in the control experiments (Table 6).

3.2.2.2.2 Renal function

As in the control studies, effective renal plasma flow decreased significantly between period II (741 $\pm$ 49 ml/min and period III (684 $\pm$ 39ml/min, $p<0.02$). Effective renal plasma flow was significantly lower during each period after indomethacin compared with the values obtained in the control experiment (Table 6).

Glomerular filtration rate was again unaltered by infusion of sodium chloride and the value during each period was not significantly different in the studies with and without indomethacin (Table 6). The patterns of increased urine flow were similar, but the actual rates of urine flow were significantly lower during the first two periods $p<0.05$) with indomethacin pre-treatment (Fig 35). In most subjects there was a substantial increase in sodium excretion between periods 1 and II (Fig 31), however, the further increment in sodium excretion between periods II and III was significantly less with indomethacin ($p<0.02$, Fig 31). Free water clearance was again significantly increased ($p<0.05$, Fig 31) but the results did not differ significantly from those obtained without indomethacin

pre-treatment.

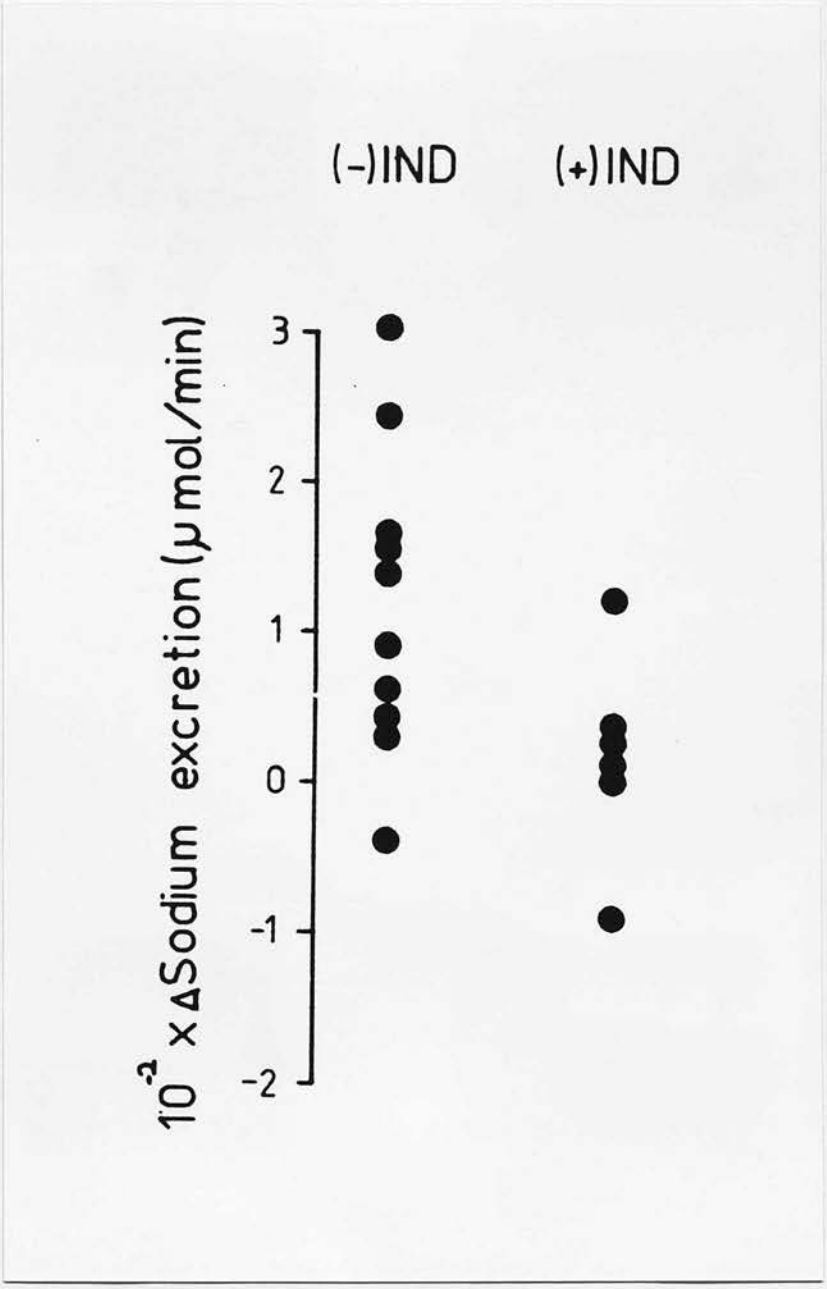

Fig 35. Difference in rate of urinary sodium excretion during period II and period III in normal subjects with (+IND) and without (-IND) pre-treatment with indomethacin.

3.2.2.2.3 Plasma renin activity and urinary PGE

There was again a significant decrease in plasma renin activity between periods I and II ($p<0.05$, Table 6) although the values were not significantly different with and without indomethacin. The concentration of PGE in the urine was below the reliable limit of detection in all but one subject.

3.3 Systemic PGI_2 synthesis and the surgical correction of hypertension in Conn's Syndrome.

The potent vasodilator activity of PGI_2 suggests an important potential role as an antihypertensive factor, particularly if sufficient quantities are synthesised to affect peripheral vascular resistance. This possibility was examined in a small group of patients with hypertension due to primary hyperaldosteronism. By studying them before and after removal of an adrenal adenoma it was also possible to further examine the effect of changes in activity of the renin angiotensin system on systemic PGI_2 synthesis.

3.3.1 Protocol and Special Methods

Five women with primary hyperaldosteronism were studied (age 35-66: mean 49 years). In th pre-operative period each patient was maintained on a dietary sodium intake of 100 mmoles/day and blood pressure was monitored every four hours. After at least three days, 24 hour urine collections were made and blood was drawn for measurement of plasma renin activity and aldosterone after overnight bedrest (supine), and again after standing or walking for two hours (upright). In each patient an adrenal adenoma was removed at a subsequent operation. Three weeks later the patients were again given the same diet and further recordings of blood pressure and collections of blood and urine samples were performed.

3.3.2 Results

Plasma aldosterone was increased in all patients pre-operatively and in every instance was lower within three weeks of removal of the adenoma (Fig 36). Similarly, all patients had a low plasma potassium pre-operatively which was corrected by surgery. Removal of the adenoma provoked a substantial increase in activity of the renin angiotensin system as shown by the increase in plasma renin activity, yet systolic and diastolic blood pressure fell significantly. After operation, excretion of dinor 6 keto PGF_{1a} decreased in all of thepatients from the pre-operative level (Fig 37). Creatinine clearance was not significantly modified by removal of the adenomas (88.6 $\pm$ 7.0 vs 74.6 $\pm$ 11.1 ml/min, P=ns).

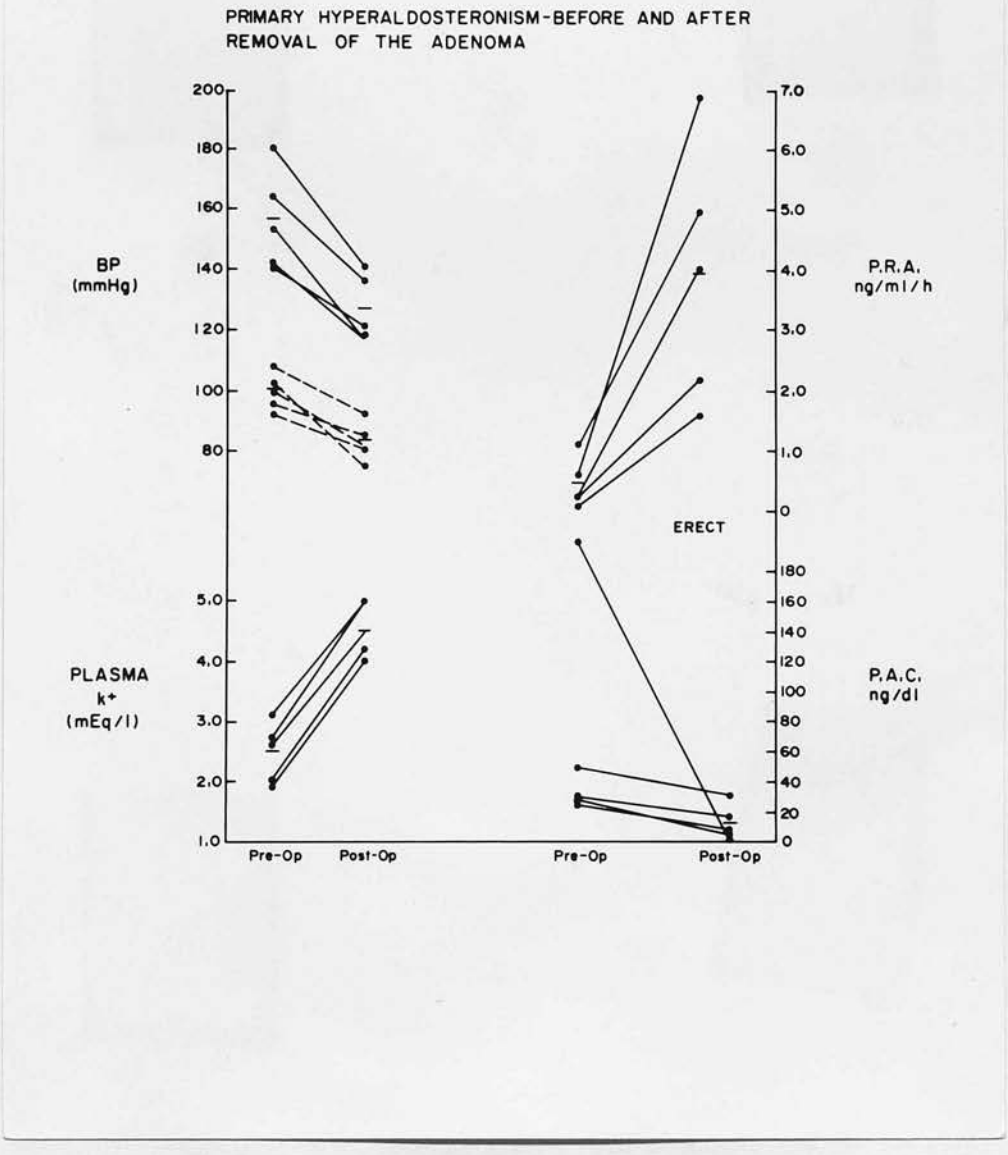

Fig 36. Systolic (——) and diastolic (---) blood pressure, plasma potassium, plasma renin activity (PRA) and plasma aldosterone concentration (PAC) before and three weeks after removal of an aldosterone secreting adenoma. Horizontal bars represent mean values for the group.

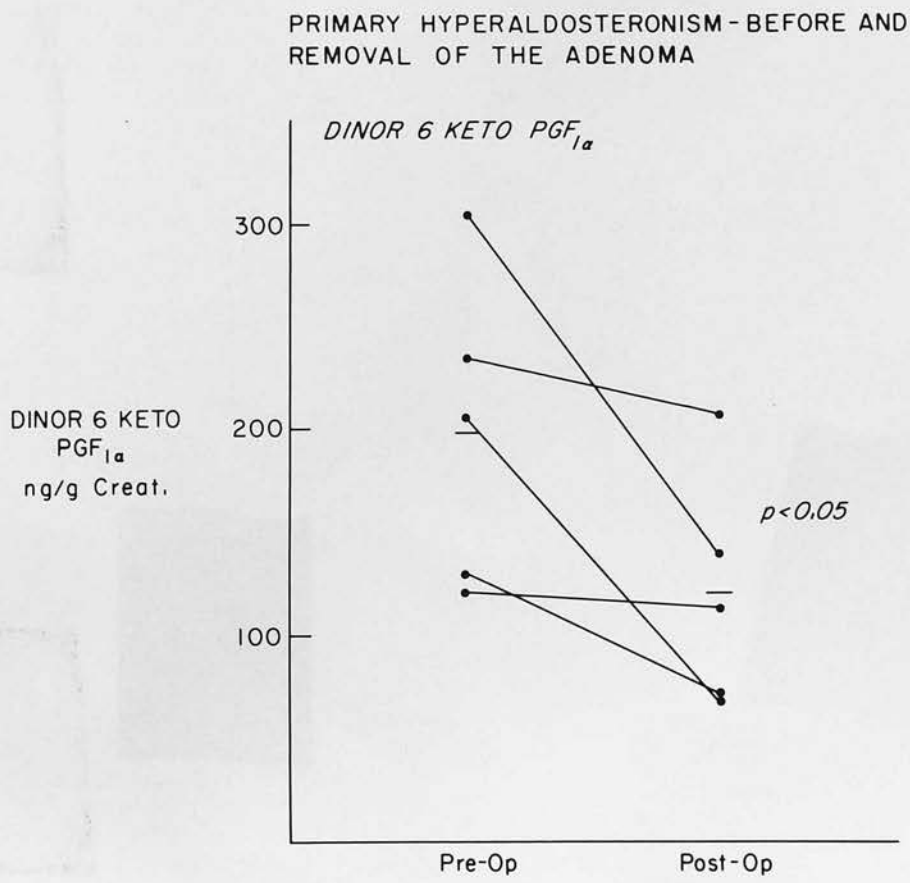

Fig 37. Dinor 6 keto PGF_{1a} excretion in 24 hour urine collections made before and again 3 weeks after removal of an aldosterone secreting adenoma. Horizontal bars represent mean values for the group.

DISCUSSION

1. Methodology

The development of reliable methods for quantitative analysis of prostaglandins in biological fluids has been a difficult and controversial area of research. The technique of mass spectrometry has been of major importance in this research. Its initial application was in the studies of Bergstrom and colleagues on the structure of prostaglandins (Bergstrom, Ryhage, Samuelsson and Sjovall 1963); subsequently it was developed for quantitative analysis (Morton 1972). Indeed the technique has been the yard stick by which the validity of other methods of analysis have been assessed. Various factors have limited its widespread use. The instrumentation is expensive, complex and requires the services of skilled maintenance and operating staff. Internal standards are required to permit corrections to be made for losses of compound during extraction from biological fluids. By far the most satisfactory standards are derivatives of the molecule under study in which 3 or 4 of the protons have been replaced by deuterium atoms. Such molecules are difficult and expensive to synthesise and are thus limited in availability. Finally the sensitivity of the electron impact method of mass spectrometry is such that large amounts of compound have to be extracted from biological fluids to provide sufficient for analysis. Contamination of the sample to be analysed is a major

problem and time consuming methods of extracting and purifying prostaglandins from the biological samples have to be employed. When correctly applied the method does however provide a precise means of quantitative analysis.

Development of negative ion chemical ionisation mass spectrometry has lead to significant improvements in the sensitivity of detection of the system. Chemical ionisation using positive ion detection has been used for some time (Suzuki, Morita, Kawamura, Murota, Nishizawa, Miyatake, Nagase, Ohno and Shimizu 1980) but only recently have the advantages of the negative ion technique been realised (Dougherty 1981). The technqiue is not only an order of magnitude more sensitive than the electron impact method in most instruments, but the ion to be monitored is also usually of higher molecular weight, therefore reducing contamination by interfering signals from low molecular weight ions. Employing this method, analysis of one of the metabolites of prostaglandin I_2, as described in the methods section, has been particularly successful. The volume of biological sample which has to be extracted has been substantially reduced, eg. 5 mls of urine is often sufficient. Moreover exploitation of the chemical properties of 2, 3 dinor 6 keto PGF_{1a}, ie. its ability to form a complete lactone under acid conditions has meant that a series of organic extractions followed by thin layer chromatography provides a sample that can be applied directly to the gas chromatography column

attached to the mass spectrometry system. This has meant that precise analyses can be quickly obtained on a comparatively large number of samples.

Until the development of this methodology, radioimmunoassay provided the only reliable means of achieving such results. Indeed this is still by and large the case for most compounds that do not lend themselves to such easy extraction from biological fluids as 2,3 dinor 6 keto PGF_{1a}. Radioimmunoassay relies on the availablity of specific antibodies to the compound of interest. The only antibody used for the current studies is both specific and sensitive for PGE_1 and PGE_2 (Dray, Charbonel and Maclouf 1975). PGE_2 is the dominant renal prostaglandin but wherever immunoassay measurements have been employed the more general term PGE has been used. Prior extraction of the prostaglandin from biological fluid is required, but the C18 microcolumns described have proved satisfactory. The demonstration of linearity with respect to the amount of PGE detected after extraction of differing volumes of urine, and also after addition of different amounts of PGE_2 suggests that the assay is reliable.

Whilst mass spectrometric analysis of PGE_2 in urine is possible (Frolich, Wilson, Sweetman, Smigel, Nies, Carr, Watson and Oates 1975), a number of time consuming purification steps must be employed, including high performance liquid chromatography. This severely limits the number of samples that can be analysed, and overall provides little advantage over a reliably validated

radioimmunoassay system.

A number of different methods have been used for assessing renal synthesis of the two different prostaglandins. Measurement of the concentration of PGE in renal venous plasma provides a useful measure of renal PGE synthesis (Beckman and Zehr 1975). The rate of renal PGE synthesis assessed by this method is different from that calculated from measurement of PGE in urine but various manipulations that interfere with renal PGE synthesis cause parallel changes in the rates of production derived from both urine and renal venous plasma (Dunn, Liard and Dray 1978). The advantages of measuring concentrations of PGE in renal venous plasma is that the results are not subject to some of the difficulties of interpretation that occur in assessing changes in urinary excretion rates (see later). The disadvantage is that renal plasma flow should be measured if production rates are to be calculated. Moreover release of PGE_2 by platelets during the passage of blood along the renal venous catheter may result in appearance of spuriously high levels of PGE in the plasma. Measurement of PGE_2 in urine has a number of attractions, in particular it does appear to reflect renal PGE_2 synthesis (Frolich, Wilson, Sweetman, Smigel, Nies, Carr, Watson and Oates 1975). A number of factors however complicate interpretation of the significance of changes in urinary PGE excretion. Urine flow is undoubtedly an important determinant of PGE excretion (Kirschenbaum and Serros 1980, Kaye, Zipser, Hahn, Zia

and Horton 1980, Kaye, Zipser, Wright, Rosenblatt and Lifschitz 1981), although there is not always a proportionate relationship between the two parameters (Bowden, Ware, Demets and Keiser 1977). Urinary pH may also influence PGE excretion rates since it is a weak acid with a pk of approximately 6.5 (Haylor Lote and Thewles 1984), however in most of the studies described there are unlikely to be major changes in urinary pH. Any contamination of urine with blood or semen will also invalidate the results (Patrono, Wenmalm,Ciabattoni, Nowak, Pugliese and Cinotti 1979) as will delays in storage at low temperature.

Interpretation of the significance of changes in excretion of dinor 6 keto PGF_{1a} is less prone to errors. The compound is stable in urine and its excretion is independent of urine flow rate. Its limitation however is that it only permits interpretation of systemic changes in PGI_2 synthesis, rather than selective renal changes (Fitzgerald, Brash, Falardeau and Oates 1981). There is also the theoretical possibility that changes in liver and renal blood flow may significantly alter rates of beta oxidation, thereby interfering with formation of the metabolite. There is at present no good evidence to support such a hypothesis.

Despite these reservations and limitations, useful conclusions on the role of both prostaglandin E_2 and I_2 in controlling sodium excretion, renal and systemic vascular resistance can be made from the various studies

described.

These studies explore a variety of possible roles for prostalgandins E_2 and I_2 in both modulating renal sodium excretion and renal and systemic vascular responses to a variety of vasoconstrictor stimuli. Particular attention has been directed at assessing the relationship between the prostaglandins and the renin angiotensin system. At the same time the models used also permit some more general conclusions to be drawn about the inter-relation between sodium balance and the state of the cardiovascular system.

2. The development of renal hypertension

The first study examined factors important in the development of the established phase of one clip two kidney hypertension. Accumulation of fluid during the early phase of increased blood pressure in experimental renal hypertension has been previously documented (Bianchi, Baldoli, Lucca and Barbin 1972; Watkins, Davis, Hanson,Lohmeir and Freeman 1976; Rocchini and Barger 1979), although it seems to be less pronounced during the development of one clip two kidney hypertension compared with one clip one kidney hypertension (Bianchi, Tenconi and Lucca 1970; Liard, Cowley McCaa, McCaa and Guyton 1974). The rapid increase in body weight of the animals in the present studies 1-3 days after clamping was almost entirely due to fluid retention although formal metabolic balance studies were not undertaken. The plasma sodium

concentration remained constant during the increase in weight, indicating that sodium and water were retained in proportion to maintain isotonicity and were therefore evenly distributed between the interstitial space and the plasma. If it is assumed that extracellular fluid volume is equal to 20% of the body weight at the end of the control period, this implies that the estimated extracellular fluid increased by 11% between days 1 and 3. The plasma volume increased by 13%, as calculated from the fall in PCV during this period suggesting that even distribution of the accumulated fluid between plasma and interstitial space had indeed occurred.

The rise in plasma renin acitvity during the same period is comparable with the rise in other studies of one clip two kidney hypertension (Bianchi, Baldoli, Lucca and Barbin 1972, Watkins, Davis, Hanson, Lohmeir and Freeman 1976; Maxwell, Lupu, Viskoper, Aravena and Waks 1977) and is compatible with the hypothesis that the early phase of hypertension in this model is angiotensin dependent (Caravaggi, Bianchi, Brown, Lever, Morton, Powell-Jackson, Robertson and Semple 1976). Under normal circumstances the development of the established phase (Phase 2) of renal hypertension (Brown, Cuesta, Davies, Lever, Morton, Padfield, Robertson, Trust, Bianchi and Schalekamp 1976) is associated with a decrease in plasma renin activity to normal or only slightly elevated levels (Bianchi, Baldoli, Lucca and Barbin 1972). At this stage the persistent elevation of blood pressure is unlikely to be

accounted for by the immediate vasoconstrictor actions of angiotensin II (see later) and there is only a small decrease in blood pressure after inhibition of the actions of angiotensin II (Watkins, Davis, Hanson, Lohmeir and Freeman 1976; Masaki, Ferrario, Bumpus, Bravo and Khosla 1977). Sodium restriction in both normotensive dogs and in those with established hypertension alters the pattern completely. Although the level of blood pressure does not change there is an increase in plasma renin activity and the blood pressure becomes dependent on the immediate vasoconstrictor actions of angiotensin II, as shown by the decrease in blood pressure immediately after infusion of angiotensin II antagonists (Watkins, Davis, Hanson, Lohmeir and Freeman 1976; Liang, Gavras and Hood 1978). Sodium restriction at the time of partial occlusion of a renal artery does not prevent the development of hypertension, at least in the one clip, one kidney model (Brown, Davis, Olichney and Johnston 1966; Conway 1968), although very large increases in plasma renin activity may occur (Fray, Johnson and Barger 1977) presumably reflecting changes in smooth muscle or receptor sensitivity to angiotensin II (Brunner, Chang, Wallace, Sealey and Laragh 1972; Strewler, Hinrichs, Guiod and Hollenberg 1972). In the present study plasma renin activity and aldosterone remained at a constant level between days 3 and 8 during the period of low sodium intake, while there was only a small decrease in mean blood pressure. The factors responsible for the

transition from the early renin dependent phase (phase I) to phase 2 of renal hypertension are unclear at present. It has been suggested that a transient period of sodium retention in the early phase of renal hypertension in the dog is important in mediating the fall in plasma renin activity and the development of the long acting vasoconstrictor action of angiotensin II (Brown, Cuesta, Davis, Lever, Morton, Padfield, Robertson, Trust, Bianchi and Schalekamp 1976). The data obtained from our study are consistent with this hypothesis in that plasma renin activity only decreased on restoration of a high sodium intake.

It has been postulated by Ledingham and Cohen (1964) that the fluid accummulation in the early period after partial occlusion of a renal artery causes an increase in cardiac output and subsequent maintenance of hypertension as a result of 'whole-body autoregulation'. The accummulated fluid is, however, clearly not required for the initial elevation of blood pressure, since hypertension develops in the one kidney animal despite a very low dietary intake of sodium (Brown, Davis, Olichney and Johnson 1966; Conway 1968) and as shown, in the experiments reported here acute removal of the retained fluid does not result in an immediate decrease in blood pressure.

The factors inducing the fluid retention have not been clearly defined. There was a significant increase in plasma aldosterone by day 3. Although this is presumably secondary to the increase in concentration of

angiotensin II in the plasma, the rise appears to occur more slowly than the increases in plasma renin activity that were measured. A sodium retaining action of angiotensin II acting within the kidney may also be important (Lohmeir, Cowley, Trippodo, Hall and Guyton 1977).

The effect of renal artery occlusion on cardiac output remains controversial (Ferrario and Page 1978). Bianchi et al (1972) were able to demonstrate an increase in cardiac output during the early phase of renal hypertension, whereas Korner, Anderson, Johnson, Angus and Fletcher (1978) suggested that the changes in cardiac output in the early phase of hypertension play no role in the development of hypertension. Changes in heart rate have also been variously reported (Bianchi, Baldoli, Lucca and Barbin 1972; Masaki, Ferrario, Bumpus, Bravo and Khosla 1977) but in the present study there was a significant fall in heart rate between days 1 and 3 which is presumably a result of a reflex response initiated by stimulation of the baroreceptors after the rise in systemic blood pressure (Liard, Cowley, McCaa, McCaa and Guyton 1974). The subsequent increase in heart rate during the low dietary sodium period probably reflects adaptation of the baroreceptor reflexes to the elevated blood pressure. Although no direct measurements of cardiac output were made, the product of heart rate and pulse pressure provides an approximate index of cardiac output. These results would suggest that there is a fall in cardiac output

during the early period after induction of hypertension with the increase in blood pressure being entirely accounted for by an increase in peripheral resistance.

3. Prostaglandin E and sodium excretion in renal hypertension

Acute infusion of PGE_2 into the kidney has a natriuretic action (Vander 1968), whereas long term infusions of PGE_2 into the kidneys of conscious animals result in an early natriuresis followed by a significant rise in blood pressure over several days as a result of increased renin release from the kidney (Hockel and Cowley 1980). Conversely, sodium restriction increases renal production of PGE in the rabbit (Stahl, Attallah, Bloch and Lee 1979). In a previous study on one clip two kidney hypertension in conscious dogs, results suggested that there is an increase in the concentration of PGE in renal venous blood from the intact kidney after the early phase of hypertension, associated with the spontaneous excretion of retained sodium and water (Dighe, Smith, Ungar and Whelpdale 1978). In the study described here the accumulated salt and water was removed by dialysison day 3. The failure of the concentration of PGE in renal venous blood to increase 5 days after induction of hypertension (Dighe, Smith, Ungar and Whelpdale 1978) implies that the PGE released after the early phase of hypertension by the untouched kidney, is involved in sodium excretion, and would be consistent with the suggestion that PGE produced within

the kidney exerts a natriuretic action, and that its release from the intact kidney is triggered by the retention of sodium and water.

4. Factors determining vascular sensitivity to angiotensin II

As already discussed, vascular sensitivity to angiotensin II maybe markedly altered by changes in sodium balance. The mechanism of the apparent tachyphalaxis to infused angiotensin II, for example under conditions of sodium depletion, is controversial (Reid and Laragh 1965), but release of vasodilator prostalgandins may be important. Studies in anaesthetised animals have demonstrated that the renal vasoconstriction caused by infusion of angiotensin II into the kidney is partially opposed by the release of vasodilator prostaglandins (McGiff, Crowshaw, Terragno and Lonigro 1970; Aiken and Vane 1973). Two more recent studies particularly implicate PGI_2 as the vasodilator released by angiotensin II in both the renal and mesenteric vasculature (Shebuski and Aiken 1980; Dusting, Mullins and Doyle 1980). Care must be taken in extending such observations to conscious unstressed animals in which circulating concentrations of angiotensin II are very low. In both studies very large doses of angiotensin II were infused and the surgical trauma inherent in such studies stimulates renal prostaglandin synthesis (Terragno, Terragno and McGiff 1977). Evidence that vasodilator prostaglandins

modulate the vasoconstrictor activity of angiotensin II in the systemic circulation is even less clear. Aiken and Vane were unable to demonstrate any effect of indomethacin on angiotensin II induced vasoconstriction in the isolated hind limb of dogs (Aiken and Vane 1973). However, inhibitors of prostaglandin synthetase have been reported to increase the pressor response to infused angiotensin II in man (Negus, Tannen and Dunn 1976; Vierhapper, Waldhaus and Nowotny 1981). In experiments described here the role of PGI_2 as a systemic modulator of angiotensin II activity was investigated by infusion of low doses of angiotensin II into conscious unstressed animals and changes in plasma concentration and urinary excretion of a major metabolite of PGI_2 were monitored. The latter approach in particular provides an accurate measure of the rate of entry of PGI_2 into the circulation (Fitzgerald, Brash, Falardeau and Oates 1981) which in man is decreased by aspirin therapy (Fitzgerald, Oates, Hawiger, Maas, Roberts, Lawson and Brash 1983) and increased by stressful procedures such as cardiac catheterisation and angiography (Roy, Knapp, Robertson and Fitzgerald 1983).

In the present study, despite infusion of a dose of angiotensin II sufficient to cause a mean increase in blood pressure of 24 mmHg and decrease in renal blood of 31% for three hours, there was no change in excretion of the metabolite of PGI_2 nor a change in its concentration in plasma. Moreover, it is highly

unlikely that changes in glomerular filtration rate altered excretion of the metabolite since there was also no change in excretion rate when expressed as a fraction of glomerular filtration rate. It is possible that infusion of angiotensin II for only three hours might cause only a small, but still significant increase in PGI_2 synthesis which would not be reflected by changes in urinary excretion of dinor 6 keto PGF_{1a}. The lack of change in plasma concentration of dinor 6 keto PGF_{1a} before and after angiotensin II makes this a less likely explanation. Another possibility is that PGI_2 released in response to angiotensin II in the vasculature is metabolised differently than PGI_2 formed in the systemic circulation (Brash, Jackson,Saggesse, Lawson and Fitzgerald 1983), in which case dinor 6 keto PGF_{1a} might not be the most appropriate metabolite to measure. Recently it has been shown that dinor 6 keto PGF_{1a} consistutes only a small proportion of total PGI_2 metabolites in plasma (Taylor, Shebuski and Sun 1983). However after systemic infusion of PGI_2, the size and time course of the increase in plasma concentration of dinor 6 keto PGF_{1a} was similar to that for another metabolite of PGI_2, dinor 6,15 diketo PGF_{1a}, suggesting that both compounds provide suitable measures of entry of PGI_2 into the circulation. Indeed this confirms the findings of Fitzgerald et al (1981) that urinary dinor 6 keto PGF_{1a} and dinor 6,15 diketo PGF_{1a} equally reflect changes in entry of PGI_2 into the systemic circulation.

The observation that angiotensin II increases 6 keto PGF_{1a} and dinor 6 keto PGF_{1a} in plasma is therefore in contrast with the presently described finding of no change in plasma and urinary of dinor 6 keto PGF_{1a} (Machleidt, Forstermann, Anhut and Hertting 1981). The increases were transient however and had returned to control within one hour of the infusion. The simplest explanation for these discrepancies is that angiotensin II does stimulate an immediate increase in PGI_2 synthesis (within 2 minutes) but that this is not sustained; this small transient increase not being detected by the later urine and plasma measurements used in the present study. The real point at issue however is whether PGI_2 antagonises the systemic vasoconstrictor activity of angiotensin II. Whilst this may be the case in the early period after angiotensin II infusion the absence of change in urine and plasma dinor 6 keto PGF_{1a} suggests that this is not a significant factor within 30 minutes of any increase in angiotensin II concentration. The results also do not exclude the possible stimulation by angiotensin II of PGI_2 synthesis within the renal vasculature. Such PGI_2 synthesis maybe measured better by monitoring urinary excretion of 6 keto PGF_{1a}, the stable hydrolysis product of PGI_2 (Rosenkranz, Kitajima and Frolich 1981; Patrono , Pugliese, Ciabattoni, Patrignani, Maseri, Chierchia, Peskar, Cinotti, Simonetti and Pierucci 1982; Wilson, Loadholt, Privitera and Halushka 1982) rather than the dinor derivative produced by systemic metabolism of PGI_2.

Despite the substantial effects of this dose of angiotensin II on systemic and renal haemodynamics, there was no apparent stimulation of systemic PGI_2 synthesis. Acute elevations of angiotensin II in sodium replete subjects, for example during changes in posture (Brown, Davis, Lever, McPherson and Robertson 1966) are therefore unlikely to be modulated by increased systemic PGI_2 synthesis. Although angiotensin II may stimulate the synthesis of some other prostaglandins, the apparent resistance to the vasopressor effects of angiotensin II may be best accounted for by changes in position of the dose response curve for angiotensin II. If dose response curves are constructed for the effects of angiotensin II on blood pressure as a function of the plasma concentration of angiotensin II, under certain circumstances then changes in response may be entirely accounted for by changes in position of the dose response curve, and the associated release of a vasodilator need not be postulated (Bean, Brown, Casals-Stenzel, Fraser, Lever, Millar, Morton, Petch, Riegger, Robertson and Tree 1979). Such an explanation might also explain the apparently increased pressor response of normal subjects to angiotensin II during treatment with indomethacin (Negus, Tannen and Dunn 1976; Speckart, Zia, Zipser and Horton 1977; Vierhapper, Waldhaus and Nowotny 1981). In two of these studies (Speckart, Zipser and Horton 1977; Vierhapper, Waldhaus and Nowotny 1981) plasma renin activity decreased while the subjects were on indomethacin, implying that basal

synthesis of angiotensin II was also decreased. The resulting decreased receptor occupancy by endogenous angiotensin II would then result in an increased response to the infused compund.

There is therefore little support for the concept that release of PGI_2 from the systemic vasculature modulates the pressor effects of angiotensin II during sodium replete conditions. Are there are any conditions under which systemic vascular sensitivity to angiotensin II cannot be accounted for by normal dose response relationships? One possible condition when this might be the case is during the established phase of one clip two kidney hypertension. Although the plasma concentration of angiotensin II increases shortly after partial occlusion of a renal artery (Caravaggi, Bianchi, Brown, Lever, Morton, Powell-Jackson, Robertson and Semple 1976; Maxwell, Lupu, Viskoper, Aravena and Waks 1977) it returns towards control within 14 days, despite persistence of the hypertension (Watkins, Davis, Hanson, Lohmeir and Freeman 1976; Freeman, Davis, Watkins and Lohmeir 1977, Lupu, Maxwell and Kaufman 1977). Unless there is a substantial increase in the vascular sensitivity to angiotensin II, it is unlikely to be directly causing the persisting hypertension. The observed similarity of the dose response effect of infused angiotensin II on blood pressure, in both the normotensive and hypertensive groups in the present studies, confirms that pressor sensitivity to angiotensin II is not increased in the established phase

of two kidney one clip hypertension. Circulating levels of angiotensin II are therefore likely to be insufficient to maintain blood pressure by their direct vasconstrictor activity.

5. Factors determining renal sensitivity to angiotensin II

The response of the kidneys to infused angiotensin II was rather different after induction of hypertension. The dose related decreases in urine flow, sodium excretion, effective renal plasma flow and glomerular filtration rate in the normotensive group are similar to the changes described in other studies in dogs (Waugh 1972). In the hypertensive group, despite similar increases in blood pressure, urine flow decreased to a much smaller extent during infusion of all three doses of angiotensin II, indeed during infusion of the highest dose there was a small increase in flow. Sodium excretion also increased rather than decreased during all three infusions in the hypertensive group, and there was not the same dose related decrease in either renal plasma flow or glomerular filtration rate, as in the normotensive groups. Renal clearance studies could only be performed on urine obtained from both kidneys. This is not a limitation in the normotensive group but in the hypertensive group the two kidneys will be very different, thus complicating any explanation of the observed effects. Divided renal function studies by Deforrest, Davis, Freeman, Watkins and Stevens (1975) in this model of hypertension suggest that after two weeks

the unclamped kidney contributes the greater proportion of total renal function, particularly in regard to urine flow, sodium excretion and glomerular filtration rate. The major part of the changes in observed renal function in response to angiotensin II are therefore likely to be occurring in the unclamped kidney.

The decreased response to angiotensin II in the hypertensive group could in theory have three components. Firstly, there may be receptor desensitisation to angiotensin II. Secondly there may be indirect tachyphalaxis to angiotensin II, due for example to metabolic smooth muscle or neural adaptation. Thirdly it may be some consequence of clamping, other than the raised level of angiotensin II. Explanations of possible receptor desensitisation to angiotensin II in the hypertensive group can be derived from previous studies of normal animals under varying conditions of sodium balance. Resistance to the effects of infused angiotensin II in sodium depleted rats when the plasma concentration of endogenous angiotensin II is increased, may be the result of increased receptor occupancy by endogenous angiotensin II (Thurston and Laragh 1975; Cowley and Lohmeir 1977),or alternatively there may be a decrease in the actual number of receptors or their affinity for angiotensin II (Strewler, Hinrichs, Guiod and Hollenberg 1972; Aguilera and Catt 1981). Previous studies in rat suggest that renin is depleted in the untouched kidney of the one

clip, two kidney model (Brunner, Desaulles, Regoli and Gross 1962; Huang, Ploth, Bell, Work and Navar 1981). Intra-renal angiotensin II levels may not however be depressed to the same extent (Mendelsohn 1980). Moreover captopril increased renal blood flow in the untouched kidney of hypertensive dogs (Zimmerman, Mommsen and Kraft 1980) and rats (Huang, Ploth, Bell, Work and Navar 1981), suggesting that angiotensin II levels maybe higher than predicted. If this was indeed the case then the relative resistence to the vasoconstrictor effects of angiotensin II could be caused by any one of these potential changes in receptor function.

The angiotensin II induced natriuresis might reflect a direct tubular action of angiotensin II, since its effect on tubular sodium reabsorption is dose dependent (Harris and Young 1977). It is well known that increases in systemic blood pressure can cause a pressure natriuresis (Guyton, Coleman, Cowley, Scheel, Manning and Norman 1972). The increased pressure as a result of angiotensin II infusion in the hypertensive animals might then increase renal perfusion pressure to a level sufficient to induce a natriuresis, even if there was no difference between normotensive and hypertensive kidneys. This is unlikely since the level of blood pressure in the normotensive animals during infusion of 15 ng/min/kg body weight angiotensin II was similar to the pressure in hypertensive animals during infusion of 5 ng/min/kg body weight and yet very

different effects were observed on renal function.

Persisting hypertension might impair renal autoregulation such that a rise in perfusion pressure, as a result of angiotensin II infusion, will cause a larger increase in renal blood flow. The decrease in tubular sodium reabsorption as a result of the increase in renal blood flow might then be sufficient to exceed other antinatriuretic effects of angiotensin II and result in an overall increase in sodium excretion.

An entirely different explanation would be that release of vasodilator compounds such as prostaglandins is increased in the hypertensive state and that they partially antagonise the effects of angiotensin II in the kidney. Infusion of angiotensin II into the kidney of anaesthetised animals (McGiff 1970) and into the systemic circulation of conscious man (Frolich, Wilson, Sweetman, Smigel, Nies, Carr, Watson and Oates 1975) results in increased synthesis of PGE_2. Its local release therefore may modulate the vasoconstrictor action of angiotensin II. Indeed angiotensin II is a more potent renal vasoconstrictor if infused into the kidneys of anaesthetised dogs when prostaglandin synthesis has been inhibited (Aiken and Vane 1973). Although renal PGE release probably increases transiently after partial occlusion of one renal artery (Dighe, Smith, Ungar and Whelpdale 1978) it returns to control values within 8 days. Inhibition of prostaglandin synthesis at this time does not result in significant changes in blood flow of the untouched

kidney (Zimmerman 1978). However, in the present study, there was a significant increase in renal PGE release as reflected in urinary PGE excretion, within 14 days of the development of hypertension. It is possible that this enhanced renal PGE synthesis occurs in the clipped kidney, but the histological studies described in this thesis suggest that the untouched kidney maybe more active than the clipped kidney in this respect. Enhanced PGE synthesis within this kidney would provide an explanation for the decreased sensitivity to angiotensin II and explain the results obtained. The renal synthesis of prostaglandins other than PGE_2, and thromboxanes, may also be altered in this model of hypertension, and therefore use of prostaglandin synthetase inhibitors may not provide a useful means of defining the particular role of PGE_2. Future availability of selective antagonists of PGE_2 is likely to provide a more specific approach. Some studies have however demonstrated exacerbation of hypertension by long term treatment with prostaglandin synthetase inhibitors supporting the possibility of a general or renal vasodilator role for some prostaglandins (Pugsley, Beilin and Peto 1975; Romero and Strong 1977).

The ultimate explanation for the altered renal response to angiotensin II in the hypertensive state is probably a combination of effects. Whilst changes in receptor numbers, occupancy or affinity maybe important in mediating the decreased renal sensitivity to angiotensin II, increased systemic blood pressure and

enhanced release of vasodilator prostaglandins may also have a role.

6. The renomedullary interstitial cells

The morphological studies of renal medullary interstitial cell granules provide an alternative approach for assessing possible roles for the kidney in modulating blood pressure. The physiological significance of the granules in these cells is unclear, but their anatomical localisation suggests that they are well placed to have an important role in the regulation of nephron function.

They appear able to synthesise collagen and ground substances, (Osvaldo and Latta 1966) which will directly influence the osmotic environment of the medullary papilla. As discussed previously, the cells also have the capacity to produce large quantities of prostaglandin (Muirhead, Germain, Leach, Pitcock, Stephenson, Brooks, Brosius, Daniels and Kinman 1972). Many studies have commented on the presence of dark osmiophilic granules in the interstitial cells, and it has been demonstrated that the number of such granules per interstitial cell is altered under a variety of different conditions. Fluid deprivation or dietary salt restriction causes a decrease in the numbers of dark granules in the interstitial cells (Nissen 1968a; Nissen 1968b; Osvaldo and Latta 1969). In both spontaneously hypertensive and deoxycorticosterone acetate treated salt loaded hypertensive rats, the granule count per

intersitial cell is decreased compared with the count in cells from normal rat kidneys (Muehrcke, Mandal, Epstein and Volini 1969; Tobian, Ishii and Duke 1969; Mandal, Frolich, Chrysant, Pfeffer, Yunice and Nordquist 1974). In rats with hypertension induced by partial occlusion of one renal artery, the granule count is also decreased in both kidneys, although more so in the untouched kidney (Ishii and Tobian 1969). In contrast the present data from studies on dog kidneys show a significant decrease in the total number of granules per interstitial cell in the untouched kidney, but in kidneys with a partially occluded renal artery a very marked increase in interstitial cell granularity compared with normal and untouched kidneys. An inverse relationship between the numbers of dark granules and the degree of hypertension has been claimed (Tobian, Ischii and Duke 1969; Ischii and Tobian 1969) whereas the results of this study and those of other workers (Muehrcke, Mandal, Epstein and Volini 1969) do not provide support for such a correlation. Species differences may partly explain these differences, but it is also possible that the changes in granule count per interstitial cell are not a true reflection of the total number of granules in the medulla. Variations in the numbers of interstitial cells under different conditions may be as important as the changes in granule counts per cell. The overall number of interstitial cells cannot be quantitified by the present techniques.

In the dog kidney the light and dark granules in the

interstitial cells appeared to be quite distinct morphological entities. The large proportion of dark granules in the clamped kidneys (95.3%) is in direct contrast to the small proportion in the untouched kidneys (31.1%). The actual number of light granules per cell in the untouched kidneys is larger than in both the clamped kidneys and normal. It is impossible to determine from this study whether there is any structural relationship between light and dark granules. The inverse change in number of dark and light granules in the clamped and untouched kidneys might suggest that dark granules transform into light granules, becoming larger in the process, and that this process is enhanced in the untouched kidneys. The secretory function of the interstitial cell is controversial (Mandal, Frolich, Chrysant, Pfeffer, Yunice and Nordquist 1974; Bohman and Jensen 1976); however, both types of granule have been observed when being extruded from the cell, suggesting that the contents of each are secreted separately. The electron microscopic appearances would suggest that the lipid content of the dark granule is greater than that of the light granule.

Increased granularity of interstitial cells in hydronephrotic kidneys (Comai, Farber and Paulsrud 1975) is accompanied by an increased capacity for prostaglandin synthesis in response to a variety of stimuli (Nishikawa, Morrison and Needleman 1977). The lipid content of interstitial cells mainly consists of triglyceride, containing a high proportion of

arachidonic acid (Comai, Farber and Paulsrud 1975; Nissen and Bojesen 1969). Although this may represent a store of prostaglandin precursor (Comai, Prose, Farber and Paulsrud 1974; Vogt 1978) fatty acid precursors of prostaglandin synthesis are thought to be released from phospholipids (Vogt 1978) and not triglycerides.

It is therefore unclear whether the granule count bears any direct relation to prostaglandin synthesis. However the presence of large numbers of light granules in the untouched kidney may relate to an increased capapcity for prostaglandin synthesis under certain circumstances.

To summarise, there is little evidence from direct infusion studies of angiotensin II to support the concept that prostaglandins (particularly PGE_2 and PGI_2) modulate its systemic vasoconstrictor activity. The results of the morphological study of interstitial cell granules are consistent with the possibility that a lipid material may modulate the renal vasoconstrictor response to angiotensin II particularly in the untouched kidney. Indeed the angiotensin II infusion studies suggest that PGE_2 is a potential candidiate, and that its release in response to angiotensin II is enchanced under stressful conditions, such as after the development of hypertension. To examine this question further and at the same time provide more information on the relationship between prostaglandin synthesis, sodium balance and renin release a number of studies were

undertaken in man.

7. Prostaglandins, sodium and renin release in man

Prostaglandins have been implicated as an integral component in the mechanism of renin release resulting from stimulation of both renal baroreceptors (Data, Gerber, Crump, Frolich, Hollifield and Nies 1978) and the macula densa (Gerber, Nies and Olsen 1981). Studies in the isolated perfused hog kidney indicate that PGI_2 stimulates the release of both active and inactive renin (Ohde, Ogilhara, Nakamaru, Higaki, Gotoh, Masuo, Ohtsuka, Saeki and Kumahara 1982). This suggests that PGI_2 maybe the endogenous prostanoid responsible for renin release. This hypothesis is supported by the observation that renal artery constriction in the B-adrenergically blocked dog induces concomitant increases in both renin and renal secretion of 6 keto PGF_{1a}, the hydrolysis product of PGI_2 (Jackson, Gerkens, Brash and Branch 1982). Thus, sodium deprivation, which increases renin by a prostaglandin mediated mechanism (Frolich, Hollifield, Michelakis, Vesper, Wilson, Shand, Seyberth, Frolich and Oates 1979) might be associated with an increase in PGI_2 biosynthesis. During the study on sodium deprivation in normal volunteers described in this thesis, there was a substantial increase in plasma renin activity and yet there was at the same time a reduction in urinary excretion of dinor 6 keto PGF_{1a}. These results do not support the hypothesis linking PGI_2 synthesis and renin release. Furthermore

the levels of plasma renin activity increased in the patients with Conn's syndrome after removal of the aldosterone secreting adenoma and yet the urinary excretion of dinor 6 keto PGF_{1a} again moved in the opposite direction and decreased.

The direct infusion of angiotensin II in animals failed to increase PGI_2 synthesis. Stimulation of endogenous angiotensin II synthesis both by dietary restriction of sodium in normal subjects, and by removal of the adenoma in patients with Conn's syndrome, provided an alternative means of addressing the same questions. In each instance the urinary excretion of dinor 6 keto PGF_{1a} moved in the direction opposite to that expected if angiotensin II was indeed a major determinant of PGI_2 synthesis. A possibility that cannot completely be excluded is that subtle changes in renal function may have altered dinor 6 keto PGF_{1a} excretion. Although metabolite concentrations were corrected for creatinine excretion, renal function was not measured directly in the normal subjects, however, changes in creatinine clearance post-operatively were not marked, and the slight decrement in the glomerular filtration rate bore no relationship to the decrement in urinary dinor 6 keto PGF_{1a} excretion. Metabolite excretion is unlikely to be altered by changes in serum potassium resulting from removal of the adenomas, as urinary dinor 6 keto PGF_{1a} excretion was normal in patients with Bartter's syndrome and remain unaltered during administration of a potassium load (Watson, Gill,

Branch, Oates and Brash 1983).

An alternative explanation for the changes in urinary dinor 6 keto PGF_{1a} excretion is that they are directly reflective of alterations in extracellular fluid volume status. Removal of aldosterone secreting tumours and keeping normal subjects on a low sodium intake are both manoeuvers that are associated with contraction of the extracellular fluid volume (Perera and Blood 1946; Biglieri and Forsham 1961). Under these conditions it is possible that changes in endogenous PGI_2 synthesis reflect alterations in vascular tone secondary to those in extracellular fluid volume.

There are two comparatively simple methods of effectively expanding extracellular fluid volume; either by intravenous infusion of isotonic sodium chloride solution, or by immersing subjects up to the neck in a water bath. Both methods provide a means of assessing the role of prostaglandins in mediating the response to effective extracellular fluid volume expansion. It has been reported that during water immersion urinary excretion of PGE increases in parallel with that of sodium in both sodium loaded and sodium depleted subjects. However pretreatment with indomethacin inhibited the increase in sodium excretion only in sodium depleted subjects, despite a decrease in PGE in both groups (Epstein, Lifschitz, Hoffman and Stein 1979). Renal plasma flow was not measured in these studies. In a previous study in which normal subjects received an infusion of sodium chloride solution, a

significant decrease in urinary PGE excretion was observed after the infusion (Kramer, Prior, Stinnesbeck, Backer, Eden and Dusing 1980). It was also found that indomethacin given before and during the infusion of sodium chloride inhibited the increase in renal plasma flow.

The results of the study reported here show that effective renal plasma flow was significantly decreased by indomethacin throughout, providing further evidence for the importance of prostaglandins in modulating renal vascular tone, at least under these conditions (Stein and Fadem 1980). However the acute increase in effective renal plasma flow observed during the sodium chloride infusion was not affected by prostaglandin inhibition. In addition to the observed effects of indomethacin on effective renal plasma flow there was also, in contrast with the study by Kramer et al (Kramer, Prior, Stinnesbeck, Backer, Eden and Dusing 1980), a small inhibitory effect on the increment in sodium excretion that occurred in the period following completion of the sodium chloride infusion.

The different results obtained in these studies may have arisen as a result of either the different techniques used to induce natriuresis, or the different doses of indomethacin employed. In some studies larger doses of indomethacin were given than in the present study, but even then complete inhibition of prostaglandin synthesis, as indicated by absence of PGE in the urine, was never achieved (Epstein, Liftschitz,

Hoffman and Stein 1979; Kramer, Prior, Stinnesbeck, Backer, Eden and Dusing 1980).

Decreased urinary PGE excretion during the sodium chloride infusion suggests that PGE is not directly involved in mediating the increase in sodium excretion and indeed may be exerting an antinatriuretic effect. It is very unlikely however that PGE within the urinary space has any direct effect on sodium excretion since micropuncture studies indicate that PGE_2 must act on the serosal surface of the tubule if it is to influence sodium excretion (Fine and Trizna 1977; Stokes and Kokko 1977). In view of the multiplicity of sites of PGE synthesis in the kidney it is therefore possible that urinary PGE is not a good measure of PGE that is exerting an effect on sodium excretion within the kidney. Chronic increases in sodium intake increased the rate of conversion of PGE_2 to PGF_{2a} via PGE_2 9 ketoreductase (Weber,Larsson and Scherer 1977). The effect of acute infusion of sodium chloride on this enzyme system has not been examined but such changes might account for the observed decrease in urinary PGE excretion.

The apparent increase in urinary PGE excretion during water immersion remains difficult to reconcile with the results of the sodium chloride studies. These immersion studies have however been repeated recently and the results suggest that, at least in sodium replete subjects, there is no change in urinary PGE excretion (O'Hare, Roland, Watson, Dalton, Brimble, Chapman,

Bhoola and Corrall 1985).

Indomethacin has many effects other than inhibition of prostaglandin synthesis (Flower 1974). Indeed, as has already been pointed out, inhibition of prostaglandin synthetase, even when specific, may affect flux through the lipoxygenase pathway. None the less, in the absence of more specific compounds it does permit some conclusions to be drawn about the relative role of prostaglandins. Indomethacin treatment before the sodium chloride infusion caused a small inhibition in the subsequent rate of sodium excretion and a decrease in renal plasma flow. The inhibition of sodium excretion may be a result of inhibition of a direct effect of prostaglandins on tubular sodium reabsorption, but it is more likely to be a consequence of the decrease in renal plasma flow, which may result in changes in physical forces acting across the proximal tubule (Earley and Friedler 1965). Alternatively, it could be a result of an intrarenal redistribution of blood flow that has been noted previously in animals after prostaglandin inhibition (Kirshenbaum, White, Stein and Ferris 1974; Beilin and Bhattacharya 1977).

Intravenous infusion of large volumes of sodium chloride solution will cause an early transient expansion of the vascular volume, after which the sodium chloride solution will diffuse into the interstitial space. Urinary excretion of dinor 6 keto PGF_{1a} transiently increased during the sodium chloride infusion suggesting that systemic PGI_2 synthesis was

also increased. The source of the increased PGI_2 synthesis can only be speculated, but studies of the portal vasculature during portal hypertension indicate that venous distension may be sufficient to stimulate PGI_2 synthesis (Hamilton, Rosza and Hutton 1981; Hamilton, Phing, Hutton, Dandona and Hobbs 1982). This may be a purely passive phenomenon of little functional significance, or alternatively it may be an important mechanism dilating capacitance vessels to accomodate changes in vascular filling pressures. It is even possible that the increased PGI_2 synthesis may have a role in causing the dilatation of the renal vasculature and the observed increase in effective renal plasma flow.

8. Conclusions

Investigation of the role of prostaglandins in controlling the systemic vasculature and renal function has not proven to be easy. Prostaglandins primarily function as local hormones. The presently available methods of analysis provide comparatively poor means of measuring local changes in prostaglandins, particularly with respect to kidneys. The difficulties are compounded by the multiple potential actions of prostaglandins, depending on their site of synthesis within the kidney. The use of inhibitors of their synthesis is an attractive experimental tool but is limited by the multiple effects of the presently used non-steroidal anti-inflammatory drugs. Despite these

limitations, work in the last decade has greatly improved our knowledge of the role of prostaglandins in blood pressure control and renal function. The further refinement of analytical techniques combined with the availablility of more specific inhibitors and selective antagonists will lead to further improvements in our understanding over the next decade.

LIST OF PUBLICATIONS DESCRIBING RESULTS IN THIS THESIS

TAYLOR J.D., WATSON M.L., THOMSON D. AND UNGAR A. RENAL INTERSTITIAL GRNULARITY IN DOGS WITH RENAL HYPERTENSION. JOURNAL OF LABORATORY AND CLINICAL MEDICINE, 98,78-88,1981.

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ACKNOWLEDGEMENTS

The work described was begun during tenure of a Medical Research Council Training Research Fellowship in the Departments of Medicine and of Pharmacology in the University of Edinburgh. It was subsequently continued in Edinburgh during tenure of a lectureship in the Department of Medicine, and completed whilst a Fogarty International Research Fellow at Vanderbilt University, Nashville, Tennessee.

All the projects have been collaborative and I am indebted to the help and encouragement of numerous co-workers. I am particularly grateful to Dr Alex Ungar in the Department of Pharmacology, Edinburgh for helping to initiate the animal experiments and provide stimulating discussions of many of the investigations. Miss Jill Taylor undertook all of the histological studies and the counting of granules in the interstitial cells. Dr Anne Lambie was a continual source of knowledge, help and encouragement in relation to all of the work in this thesis. I am most grateful to Professor John Oates in the Department of Clinical Pharmacology in Nashville for being so generous in giving me access to the facilities and expertise in his excellent department, and for Dr Alan Brash for his many hours of painful tuition in the art of mass spectrometry and science of chemistry! Dr John Gill of National Insitutes of Health, USA was a most helpful collaborator in the study of Conn's syndrome. I am most indebted of all to Professor J S

Robson for his continued substantial support of my work over the last 10 years. He must have felt on numerous occasions that he had bought "a pig in a poke". Such unfailing support is a prerequisite for the conduct of any substantial scientific investigations. Massive technical assistance has been provided by MrP. Whelpdale, Ms A. Thom, Ms J. M. McCormick, Ms B. Sanger and the technicians in the Medical Renal Unit laboratories. The conscious animal studies would have been impossible without the dedicated care of Ms Jean Hunter.

I am indebited to Ms W Dunnett who has typed the manuscript and Ms A Macnicol and Ms K Craig who have toiled for hours over the references. There are many others from whom I have sought and obtained advice and assistance and I am grateful to all of them.

The work was supported by funds provided by :

Medical Renal Unit Research Fund (Edinburgh)

Scottish Hospitals Endowment Research Trust

Medical Research Council (UK)

British Heart Foundation

National Institute of Health (USA).

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