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ATRIAL NATRIURETIC FACTOR AND CHRONIC RENAL DISEASE.

M.D. Thesis 1989.

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ABSTRACT.

Atrial natriuretic factor (ANF) is secreted by the heart in response to increased atrial stretch, and acts as a hormone which protects against blood volume expansion by enhancing renal sodium excretion. Cyclic guanosine monophosphate is an intracellular 'second-messenger' of ANF.

Peripheral venous (99-126) ANF was measured by radioimmunoassay after plasma extraction. In patients with chronic renal failure who did not require dialysis, the mean ANF was raised two-fold compared to healthy subjects of a similar age, and a positive correlation with arterial blood pressure suggested a dependence of pressure on blood volume. The highest levels of ANF were found in patients who required haemodialysis, and ANF fell both after dialysis and after successful renal transplantation. Intravenous infusions of (99-126) ANF into normals, patients with the nephrotic syndrome and a separate group with chronic renal impairment produced rises of plasma cGMP, urine cGMP and renal sodium excretion. In addition, ANF enhanced albuminuria in these patients.

In chronic renal disease high plasma ANF may reflect blood volume expansion and decreased renal clearance. ANF may help to maintain sodium balance, but ANF-induced albuminuria may harm long term renal function.

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LIST OF ABBREVIATIONS.

ANF: Atrial Natriuretic Factor.

cGMP: Cyclic Guanosine Monophosphate.

CRF: Chronic Renal Failure.

EDTA: Ethylene-diamine-tetra-acetic Acid.

ERPF: Effective Renal Plasma Flow.

GFR: Glomerular Filtration Rate.

MBP: Mean Blood Pressure.

PAH: Para-aminohippurate.

PRA: Plasma Renin Activity.

RIA: Radioimmunoassay.

SNGFR: Single Nephron Glomerular Filtration Rate.

TFA: Trifluoroacetic acid.

PART I.

CHAPTER 1.

THE OUTLINE OF THE THESIS.

Introduction.

The discovery that the heart is an endocrine organ has led to an explosion of information (de Bold et al, 1981; de Bold et al, 1988). The first phase of work, from 1981 to 1984, led to the sequencing of the major circulating form of Atrial Natriuretic Factor (ANF) and its precursors within cardiac atrial myocytes (de Bold et al, 1988). At the same time, the pharmacological effects of ANF on isolated tissues and the whole animal were studied, and it was found that large doses of either atrial extracts or synthetic ANF had the following effects:

a) Atrial peptides relaxed arterial strips which had been precontracted with vasoconstrictors such as angiotensin II and noradrenaline (Winquist et al, 1984).

b) ANF caused natriuresis (de Bold et al, 1981; Maack et al, 1984). Both the vascular and renal effects were dose-dependent and were used as a bioassay of ANF before ANF radioimmunoassay (RIA) became available.

c) Large doses of ANF inhibited the release of both renin from the kidney (Maack et al, 1984) and

aldosterone from the zona glomerulosa of the adrenal gland (Matsuoka et al, 1985).

d) Evidence accumulated that cyclic guanosine monophosphate (cGMP) might act as an intracellular 'second-messenger' for ANF (Hamet et al, 1984; Winquist et al, 1984).

e) In the whole organism, arterial pressure fell after large doses of ANF (de Bold et al, 1981). This was thought to be due to either plasma volume depletion, vasodilatation or inhibition of the renin-aldosterone axis, or to a combination of these three.

From 1985 polyclonal RIAs were used to measure plasma ANF in healthy humans both in the basal state and also after stimulation by blood volume expansion (Sagnella et al, 1985; Shenker et al, 1985; Anderson et al, 1986a).

In addition, the natriuretic and hypotensive effects documented in animal experiments, were confirmed when pharmacological amounts of ANF were injected intravenously into normal man (Richards et al, 1985; Gerzer et al, 1985). From 1985 to 1986, reports appeared stating that plasma ANF was raised in disease states associated with blood volume expansion, including congestive cardiac failure (Raine et al, 1986) and severe renal failure (Rascher et al, 1985).

Aims of the Thesis.

This was the background when the current project of 'ANF and Chronic Renal Disease' was planned. The subject was thought worthy of attention because kidney diseases are often accompanied by disorders of sodium balance. An increase of total body sodium and extracellular volume is often found in chronic renal failure (CRF) before the start of dialysis, and may contribute to the genesis of hypertension in some patients (Weidmann, 1984). In general, sodium retention is not progressive because daily urinary sodium excretion is maintained to match dietary intake, until renal failure is very severe, by an increased fractional excretion of the ion (Slatopolsky et al, 1968). In contrast, progressive sodium retention and marked peripheral oedema are found in patients with the nephrotic syndrome even in the presence of a normal glomerular filtration rate (GFR) (Geers et al, 1984).

The thesis is divided into four parts. Part I (Chapters 1-7) describes the principles of RIA and the methods used to measure plasma ANF, cGMP and other hormones. The measurement of ANF involves blood collection, plasma extraction and RIA and these steps will be described in detail (Chapters 2 and 3).

Part II (Chapters 8-13) starts with a historical review of the discovery of ANF and the relation of its

secretion with blood volume. Cyclic GMP is a second messenger of ANF, and I discuss whether extracellular levels of this nucleotide might give a guide to the tissue effects of ANF. The rest of Part II investigates plasma levels of ANF in health, both in the basal state and after volume expansion with intravenous saline. These levels are compared with values in patients with chronic renal failure, both before and after the initiation of dialysis. The relation of ANF with arterial blood pressure is discussed, and the effects of renal transplantation on plasma ANF will be followed.

Part III (Chapters 14-20) reviews the action of ANF on kidney fuction in healthy animals and humans, and then decribes my experiments which followed the renal and endocrine effects of intravenous ANF infusions into healthy subjects, patients with chronic renal failure and also those who suffer from the nephrotic syndrome. Low doses of ANF were used in an attempt to make the results of physiological rather than pharmacological relevance. In Chapter 20 I summarise these results, and the possible biological significance of ANF in chronic renal disease is discussed.

Finally, Part IV comprises the references (Chapter 21) and the raw data derived from my experiments (Chapter 22).

CHAPTER 2.

THE BACKGROUND TO THE RADIOIMMUNOASSAY OF ATRIAL NATRIURETIC FACTOR

Introduction.

The basis of radioimmunoassay (RIA) is a competitive inhibition of the binding of a radioactively labellel hormone to a limited concentration of antibody by unlabelled hormone contained in standards or unknown samples (Yalow, 1985). After these components have reacted, the bound and unbound antibody are separated, and the unknown's concentration derived by comparing the radioactivity of the sample with that of the standards.

Antibodies.

Most hormone antibodies are raised by immunisation of rabbits, sheep, goats or guinea pigs. Hormones of large molecular weight are administered as an emulsion with Freund's adjuvant, but smaller molecules may need conjugation with bovine serum albumin or glutaraldehyde in order to stimulate the immune reaction. Animals are bled and reimmunised at intervals until a suitable antibody is produced.

The antiserum produced in this fashion will be a polyclonal mixture of antibodies of different

immunoglobulin classes with varied affinities for the hormone. However, at a high dilution only the antibody with the highest affinity will take part in the RIA, and therefore the mixture will behave approximately as a single reagent. Monoclonal antibodies, which react to different domains of the hormone, may be used in immunoradiometric assays. However, these are not generally used for RIA of ANF and will not be discussed.

Labelling of Hormone.

Radioactivity can be added to the hormone by introducing ^{3}H or ^{125}I atoms. The latter have been used to 'label' ANF. In general, radioiodine can be substituted on a tyrosine or histidine residue, and this is achieved by gentle oxidation with chloramine-T. The radiolabelled hormone is purified by 'desalting' to remove any free iodine, and by chromatography to ensure a homogeneous result. Although a larger number of ^{125}I groups per molecule would increase the radioactivity and allow shorter counting times, this results in accelerated radiation damage and diminished immunoreactivity of the molecule.

Radioimmunoassay Kinetics.

The details of the RIA, involving the binding of

antibody and hormone, will vary. For example, the speed and completeness of this reaction are determined by the pH and temperature. In order to maximise the sensitivity of the assay, ANF RIAs often use prolonged incubations (up to days) at low temperatures ($4^{o}C$).

Separation Techniques.

At the end of the reaction, the bound and unbound label must be separated. In classic immunological reactions the antigen/antibody complex spontaneously precipitated but in RIA the concentrations used are too low for this to occur. Organic solvents can precipitate the complexes, the antigen or antibody can be adsorbed onto solid phase material, or a second antibody is added to the mixture to make the complex insoluble.

Validation of the Radioimmunoassay.

Radioimmunoassay does not test the biological activity of the substance, but simply depends on the interaction of chemical components by the laws of mass action. A "necessary condition for ensuring a properly validated RIA is the superimposability of a dilution curve of the unknown sample along a dilution curve of standards" (Yalow, 1985).

However, this alone is not sufficient proof that plasma does not contain a biologically alien substance that

cross-reacts in the RIA. Indeed, hormones may circulate in a variety of forms, and this is especially so in renal impairment when non-biologically active fragments may be present in high concentration (eg. parathyroid hormone). This problem will be discussed in detail later in this chapter with reference to circulating forms of ANF in renal failure.

Blood Collection Technique for ANF.

The site of blood sampling for ANF is important because the peptide is cleared from the plasma not only by the kidney but also by peripheral vascular tissues. Therefore, arterial values are higher than venous levels in the same limb (Espiner and Nicholls, 1987). Secondly, ANF immunoreactivity is labile in untreated blood, probably due to breakdown by proteases, and the addition of enzyme inhibitors can make a significant difference to the measured ANF value (Tan et al, 1987).

ANF Antibody and Radiolabel.

The major circulating form of the carboxyterminus of the (1-126) ANF propeptide in healthy humans and rats is (99-126) ANF (Schwartz et al, 1985; Yandle et al 1986a). Polyclonal antibodies have been raised against both human and rat ANF (Sagnella et al, 1985; Sugawara et al, 1985; Shenker et al, 1985; Richards et al, 1987). Most were raised against (99-126) ANF but

shorter fragments have also been used for innoculation (Epstein et al, 1986).

Polyclonal antibodies raised against (99-126) ANF may vary in specificity. For example, the antibody used in this thesis (Peninsula Laboratories, RAS 8798) recognises the carboxy-terminal, while other antibodies bind with the the central ring structure of ANF (Ogawa et al, 1987). Therefore caution is required when comparing plasma ANF measured by different antibodies.

In general, (99-126) ANF has been used for the label, and ^{125}I has been introduced by the chloramine-T method. After the incubation of antibody and label, most published RIAs have separated bound and free fractions using either charcoal to bind free hormone, or a second antibody to precipitate the antibody/hormone complex.

The Importance of Plasma ANF Ex traction.

Unless ANF is extracted from plasma, spuriously high levels of the peptide are measured in RIA (Gutkowska et al, 1987a; Yandle et al 1986a; Richards et al 1987), and the correlation between direct and extracted assays is poor, especially at healthy physiological concentrations (Sugawara et al, 1985). Richards et al (1987) found that the 'interference' in the direct RIA

was proportional to the number of platelets in the sample. Platelets possess ANF receptors, and in the direct RIA this ANF might be released into the incubation mixture.

Most published reports have used octadecylsilica (ODS), often in the form of commercially available columns (Sep-Pak), to extract ANF by reverse phase chromatography. ODS is pre-treated with an acid solution and the ANF eluted with either acetonitrile or an alcohol (ethanol or methanol). Acidification of plasma before passing it through the ODS column has been reported to improve recovery (Cappuccio et al, 1987). Usually the fraction containing ANF has been dried with a stream of nitrogen and/or freeze dried before reconstitution for the RIA. The mean recovery of both radiolabelled and synthetic ANF (Table 2.1) has been as low as 57% (Shenker et al, 1985) or as high as 100% (Tan et al, 1987).

In order to validate the assay, serial dilutions of extracts from healthy plasma should parallel the ANF standard curve. After extraction of healthy plasma with ODS, Richards et al (1987) identified only one peak on high performance liquid chromatography which corresponded to (99-126) ANF and reacted with the Peninsula RAS 8798 antibody.

Table 2.1.

Review of ANF radioimmunossays.

Author (Year)	ANF (pmol/l)	Enzyme inhibitors	Extraction Efficiency (%)
Sugawara (1985)	12.2±2.3	EDTA+Aprotinin	70-80
Anderson (1986a)	3.4±0.8	EDTA+Aprotinin	79±2
Epstein (1986)	1.5-20.0	EDTA	79±9
Gutkowska (1987)	3.9±0.4	EDTA+Pepstatin	77±2
Yandle (1986)	14.1±1.0	EDTA+Aprotinin	87
Weidmann (1986a and b)	18.8±3.9	EDTA+Aprotinin	60-68
Richards (1987)	6.6±4.0	EDTA+Aprotinin	>80
Tan (1987)	9.4±0.5	EDTA	94-108

Values are given as mean±SEM or ranges as quoted in the original publications.

Healthy Plasma Values of ANF.

Table 2.1 shows published healthy peripheral venous plasma ANF levels. The variation of the ranges and mean values of plasma ANF might be explained by differences in RIA and extraction techniques, in subject age, in dietary sodium , the posture of the subject and use of enzyme inhibitors during blood collection.

Circulating ANF in Renal Failure.

In renal failure, the secretion and disposal of peptide hormones may be altered, and the plasma levels and molecular forms of these substances can differ from normal. Numerous groups have investigated the nature of circulating ANF in end-stage renal failure.

Anderson et al (1986b) extracted uraemic plasma with octadecylsilica, and then performed fractionation on gel-permeation columns using Sephadex G-50 Superfine. The elution position, as determined by RIA for (99-126) ANF, demonstrated that "the majority of ANP (ANF) immunoreactivity eluted in a position similar to synthetic human-ANP". Walker et al (1987) extracted uraemic plasma from a single anephric subject with ODS and used high performance liquid chromatography to confirm that most ANF-like radioimmunoreactivity, as determined by the Peninsula ANF antiserum used in this

thesis, corresponded to (99-126) ANF. In contrast, another group found more than one peak of immunoreactive ANF in uraemic plasma; however, this method used extraction with Vycor glass beads together with an antibody directed to the ring structure of the (99-126) molecule (Ogawa et al, 1987).

More recently, Winters et al (1988) have identified high concentrations of N-terminal fragments of the (1-126) ANF propeptide in normal plasma and in patients with renal failure. The ratio of these fragments to immunoreactive (99-126) ANF was higher in the patients, an observation confirmed by Itoh et al (1988a). The later group have performed detailed analysis of plasma from healthy subjects and patients with severe renal failure using gel permeation chromatography. They found that in health a 3 kilodalton fragment, corresponding to (99-126) ANF, is the only species which is immunoreactive with antibodies directed to the carboxy-terminus of (99-126) ANF. In addition, there is one species of 10 kilodaltons, corresponding to (1-98) ANF, which reacts with antibodies raised against (1-25) ANF. Itoh found that (99-126) and (1-98) ANF were also the predominant circulating species in uraemic plasma. However, in some patients small amounts of two molecules larger than (99-126) ANF were identified which reacted with antibodies raised against the carboxy-terminus of (99-126) ANF. On the basis of their

molecular weights, these were thought to possibly represent the (1-126) ANF propeptide and the antiparallel dimer of (99-126) ANF.

Currently, only (99-126) ANF has been shown to have biological activity in physiological concentrations in health (Anderson et al, 1987; Richards et al, 1988) although pharmacological doses of the anti-parallel dimer can cause a natriuresis in normals (Itoh et al, 1988b) and fragments derived from the (1-98) region of ANF can vasodilate aorta in vitro (Vesely et al, 1987). This thesis only considers the role of (99-126) ANF in health and renal diisease. However, it is possible that other molecules derived from the ANF propetide may in future be shown to have biological activity at concentrations measured in the plasma of both normal subjects and patients.

Summary of Chapter 2.

1. The principles of radioimmunoassay were discussed, and the importance of plasma collection technique and extraction in the ANF assay was emphasised.

2. The major circulating carboxy-terminal fragment of the ANF propeptide is (99-126) ANF in health and renal failure. However, in renal failure ANF antisera may cross-react with lesser amounts of other molecules derived from the ANF propeptide and thus the measured 'ANF' may overestimate true (99-126) ANF values.

CHAPTER 3.

THE MEASUREMENT OF PLASMA ANF DEVELOPED FOR THE STUDIES REPORTED IN THIS THESIS.

Introduction.

The method used for the assay of ANF was investigated in detail in order to obtain reliable rules for blood collection, plasma extraction and radioimmunoassay. The first part of the chapter describes the protocol which was finally developed, and the second part, the experiments used to devise it.

Blood Collection.

Materials.

1. Trasylol (Aprotinin, Bayer).
2. Plastic tubes containing K_2EDTA (Ethylene-diamine-tetra-acetic acid) (Sarsted 27.326).

Method.

5-10 ml venous blood was added to 2000 KIU/ml of Trasylol in a cooled plastic tube containing 15mg EDTA. This was mixed and cooled rapidly on ice. The sample was spun at 540 x G for 10 minutes and the supernatant plasma removed into a polypropylene container. The plasma was extracted on the same day.

Plasma Extraction.

Materials.

1. Octadecylsilica (ODS, Sepralyte C18, particle size 40 µm, preparative grade, Analytichem International).
2. Polypropylene columns (Bio-rad 731-1550).
3. Methanol (BDH AnalaR).
4. Trifluoroacetic acid (TFA) (BDH AnalaR).
5. Oxygen-free Nitrogen (BOC Industrial Gases).
6. Freeze drier (Edwards Modulyo).

Method.

0.25 g of ODS was placed in the column, by pouring the packing material through a small funnel onto the floor of the column. An even surface was ensured by gentle tapping of the side of the tube. 4 ml of methanol was passed through the column under suction, followed by 4 mls of 0.5% TFA. Then, 0.5 to 3mls of the plasma sample was passed through, followed by 4 mls of 40% methanol in 0.5% TFA. The fraction containing the ANF was eluted with 4 ml of 90% methanol in 0.5% TFA.

The 4 ml containing the final extract was blown down under a stream of nitrogen until only the aqueous phase remained. This portion was freeze-dried overnight, and the lyophilised material was stored in this form for up to three months before the ANF radioimmunoassay.

Radioimmunoassay of ANF.

Materials.

1. (99-126) ANF (Bachem UK) for standard curve. The peptide was dissolved in small volumes of acetic acid and stored in 0.1 ml aliquots of millimolar concentration. At three-monthly intervals 10 sets of standards were made up, by dilution in phosphate buffer.

2. Alpha-human atrial natriuretic peptide antiserum (rabbit) (Peninsula Laboratories RAS 8798). Specificity: 100% crossreactivity to (99-126) ANF, 100% to (103-126) ANF, 5% to (103-125) ANF. The polyclonal antibody was directed to the carboxy-terminus of the ANF molecule.

3. Tracer: ^{125}I-(99-126) ANF (Amersham International IM.187). Purity >90% On arrival the label had the acivity of 5 μCi. It is used within eight weeks of iodination.

4. ANF assay phosphate buffer: pH 7.4, NaH_2PO_4 19mM, Na_2HPO_4 81mM, NaCl 50mM, Triton X-100 0.1%, Bovine serum albumin 0.1%, NaN_3 0.01%.

5. Dextran-charcoal mixture: 0.5 g dextran (Sigma D1390) in 100ml phosphate pH 7.4 buffer, to which was added 2.5 g charcoal (Sigma C5260). The mixture was continuously stirred and kept between 0-4^{o}C by surrounding the beaker with ice water.

6. Polystyrene tubes (RT 30, Sterilin).

7. Gamma counter (Nuclear Enterprises 1600).

8. Apple computer with Nuclear Enterprise programme for plotting of standard curve and calculation of unknowns.

Method.

The freeze dried sample was reconstituted in 1ml of assay buffer, and 0.3 ml aliquots were assayed in duplicate. They were placed in polystyrene tubes and 50 µl of ANF antiserum was added at a final titre of 1:40000. Six duplicate sets of ANF standards were also set up from 12.18 fmol/tube at doubling dilutions to 0.38 fmol/tube. In addition, duplicate tubes for non-specific binding (using phosphate buffer instead of antiserum) and zero-standards were prepared. At least two different quality controls were included in each assay. The tubes were then incubated for 24 hours at $4^{o}C$.

After this, 50 µl of ^{125}I-ANF was added to each tube. When the label had been freshly prepared and diluted in phosphate buffer, radioactivity was 7000 counts per minute. The tubes were further incubated for 24 hours. Finally, 0.3 ml of ice-cold dextran/charcoal solution was added, and after centrifugation for 20 minutes at 850 x G at $4^{o}C$, the supernatant was carefully aspirated and the bound radioactivity counted for 300 seconds in the gamma counter.

The standard curve was plotted on a semi-log scale with the radioactive counts, expressed as the ratio of bound

counts versus the zero binding, on the vertical axis and the logarithmic transformed concentration of ANF per tube on the horizontal axis. The concentration of peptide in the original plasma was calculated by dividing the unknown sample concentration (fmol/tube) by the volume of plasma used for the extraction and by 0.3 (because only 0.3 ml of the one ml of reconstituted extract was added to each assay tube). No correction was made for extraction losses.

Stability of ANF in Venesected Blood.

Tan et al (1987) reported that the use of different enzyme inhibitors affected measured plasma ANF. Therefore, I studied use use of a variety of collection techniques. 100 ml of venous blood was collected from a healthy subject and was added to either lithium heparin (Sterilin), EDTA only (Sterilin), or EDTA and Trasylol as previously described. The plasma was either centrifuged and then separated within 10 minutes of venesection, or left at room temperature for 30 or 120 minutes before separation. All plasma was frozen once at -80^{o}C, except the samples that were given two freeze-thaw cycles, and ANF was extracted on the same day. Results of measured ANF immunoreactivity are shown in Table 3.1. The preparation which produced the highest ANF immunoreactivity contained both EDTA and Trasylol and was separated and frozen as soon as possible. Collection into lithium heparin tubes

resulted in significantly lower immunoreactive ANF ($p<0.01$, unpaired Student's t-test). Moreover, when EDTA and Trasylol were used together, neither delayed plasma separation nor repeated freezing significantly reduced immunoreactive plasma ANF. Subsequently blood was collected into EDTA and Trasylol, and plasma was separated within 15 minutes of venesection, although these results (Table 3.1) suggested that blood left unseparated for up to 120 minutes would give similar results.

Table 3.1.

The stability of ANF in venesected blood.

How blood was treated	n	ANF (pmol/l) mean±SD	% of best method
Lithium Heparin	8	2.1±0.5	36
EDTA	8	4.2±0.4	72
EDTA and Trasylol	8	5.9±0.5	100
EDTA and Trasylol (left at room temperature for 30 minutes).	6	5.1±0.3	88
EDTA and Trasylol (left at room temperature for 120 minutes)	8	5.2±0.6	89
EDTA and Trasylol (two freeze-thaw cycles)	8	5.6±0.6	96

Stability of ANF in Frozen Plasma.

There is little information regarding the stability of ANF in stored plasma, so this was investigated as follows. Forearm venous blood was drawn and placed into plastic tubes containing EDTA and Trasylol, as previously described, and the plasma was separated and stored in four different ways:

A. ANF was extracted from plasma on the day of venesection and measured in the RIA.

B. ANF was extracted on the day of venesection and the freeze-dried extract stored at -80^{o}C for three months and then reconstituted in phosphate buffer and measured in the RIA.

C. Unprocessed plasma was stored at -80^{o}C for one month and then extracted and assayed.

D. As for C, but stored for three months.

Table 3.2 shows that ANF immunoreactivity was stable up to three months when stored as the freeze-dried extract. However, losses of immunoreactive peptide occurred when neat plasma was kept for this period in the same freezer. Thereafter, all samples were extracted on the same day as venesection and stored for up to, but no longer than, three months before RIA.

Table 3.2.

Effect of freezer storage on immunoreactive ANF.

Method of Storage (see text for key)	n	ANF (pmol/l) (mean±SD)	% versus method A
A	8	5.9±0.5	100
B	8	6.1±1.0	104
C	7	4.9±1.6	83
D	6	3.7±0.4	63

Comparison of ANF Standard Curve Using Phosphate Buffer and 'ANF-free' Plasma as Solvents.

One ml of fresh plasma was collected into EDTA and Trasylol as previously described and gently mixed by hand at room temperature for 5 minutes with 1ml of the phosphate buffer containing the dextran and charcoal mixture, and after centrifugation at 850 x G for 10 minutes the treated plasma was then extracted in the usual fashion. When 5 similarly prepared extracts were measured in the ANF RIA, no immunoreactive peptide was detected. Therefore, plasma treated in this fashion was considered 'ANF-free' and was used as a solvent for an ANF standard curve, which was compared to a simultaneously performed curve with standards dissolved

in phosphate buffer.

The standard curve which used 'ANF-free' plasma as a solvent was shallower than the curve prepared in phosphate buffer (not shown), and the zero binding of the former was only 40% of the latter method. Therefore, plasma devoid of ANF immunoreactivity when measured in the extracted assay, contained a component which displaced ^{125}I-ANF when added directly to the RIA. The result agreed with previous reports which found that unextracted plasma contained a component which interfered with the ANF RIA (Chapter 2).

Recovery of Radioactive ANF from Plasma.

The method for plasma extraction used in this thesis was based on the protocol of reverse phase chromatography described by Sagnella et al (1985), except that the final wash of the ODS column was performed with methanol rather than ethanol.

Labelled ANF recovery was studied in 20 separate plasma samples (10 healthy subjects, and 10 with renal failure). One ml of plasma was mixed with 50 ul of ^{125}I-ANF (approx 3 fmol, 10000 counts per minute). The plasma was extracted by the routine method and radioactivity in the final wash was counted. The mean$\pm$SD recovery of label was 79.5$\pm$2.1%, and was not different between the patients and healthy plasmas.

In order to determine the concentration of methanol which would give the optimum recovery of radiolabelled plasma, the standard extraction was modified so that the final wash was performed with a range of methanol concentrations, using 0.5% TFA as the solvent.

Table 3.3.

The effect of methanol concentration on ^{125}I-ANF recovery.

(mean of two healthy plasmas)

Methanol Concentration (%)	% Recovery
40	0.5
50	9.0
60	52.5
70	71.1
85	84.7
90	83.6
95	69.5
100	48.9

Table 3.3 shows that the maximum recovery lay between 85-90% methanol. Thereafter, the 90% concentration was used for extractions.

Two further modifications were studied. Firstly, the volume of 90% methanol in the final wash was varied in

order to see whether recovery of spiked plasma was improved: and these results are shown in Table 3.4.

Table 3.4.

Effect of methanol volume on ^{125}I-ANF recovery.

Methanol (ml)	% Recovery
One	65.2
Two	73.3
Four	79.4
Eight	75.5

There was no advantage in percent recovery by increasing the volume of the final wash above 4 ml. Finally, I attempted to maximise the recovery of ANF by varying the volume of ODS in the column (Table 3.5), but the yield was not improved by increasing the volume of packing material beyond the standard 0.25 g.

Table 3.5.

Effect of ODS mass on ^{125}I-ANF recovery.

ODS (g)	% Recovery
0.125	72.3
0.25	75.3
0.375	74.9
0.5	77.6

Recovery of Immunoreactive ANF from Plasma.

Experiments were performed in order to measure the recovery rates of unlabelled (99-126) ANF. Firstly, 4 fmol of the peptide (Bachem, UK) was added to 1 ml plasma samples (3 from healthy subjects, 3 from renal failure patients), collected in the usual fashion. Secondly, different amounts of ANF were added to the same plasma sample of two subjects (both healthy). The plasma were then extracted and immunoreactive ANF measured. Results are shown in Table 3.6.

The mean$\pm$SD recovery of these 16 tests was 78.5$\pm$9.5%, and this value was close to the mean recovery of labelled peptide (79.5%) although the standard deviation was larger (9.5 versus 2.1%), possibly due to the variation added by the ANF RIA. There was no difference of percent recovery of immunoreactive ANF over a wide range of added ANF concentrations (1-32 pmol/l), and recovery from normal and patient plasma was similar, in agreement with the recovery of labelled ANF.

On account of this relatively constant ANF recovery, it was decided that there was little point in performing a test of extraction efficiency for each individual unknown plasma. Therefore, ANF results in the thesis are not corrected for the approximate 20% loss in the extraction process. The recovery rate of the current

protocol compares favourably with reports from other authors who use methods similar to my protocol (Table 2.1).

Table 3.6.

The recovery of immunoreactive ANF.

(* = renal failure plasma, otherwise healthy plasma)

Endogenous ANF (pmol/l)	ANF added (pmol/l)	ANF measured (pmol/l)	Recovery (%)
7.9	4.0	10.9	75
9.1	4.0	11.8	68
*15.7	4.0	19.6	98
4.5	4.0	8.3	96
*14.3	4.0	17.5	80
*19.6	4.0	22.8	80
1.7	1.0	2.4	70
1.7	2.0	3.4	85
1.7	4.1	4.4	67
1.7	8.1	7.8	75
1.7	16.2	13.3	70
1.7	32.4	23.8	69
3.2	2.0	4.7	75
3.2	4.0	6.2	75
3.2	8.1	10.2	87
3.2	16.2	13.9	86

Effect of Sample Volume on Recovery of ANF.

Firstly, ^{125}I-ANF was added to freshly obtained healthy plasma to give approximately 10000 counts per minute/ml. The routine extraction was followed with 0.5, 1.0, 2.0 and 3.0 ml of plasma.

Table 3.7.

Effect of sample volume on ^{125}I-ANF recovery.

(mean of two healthy plasmas)

Volume of plasma (ml)	Recovered Radioactivity (% of original total)
0.5	83.6
1.0	81.5
2.0	81.5
3.0	84.1

Next, the recovery of endogenous ANF was determined in plasma from a healthy adult and also in patient with renal failure (Table 3.8). The derived plasma concentration of immunoreactive ANF was consistent using sample volumes between 0.2 to 3.0 ml. Therefore, taking into account the range of standards, the method could measure unknown plasma ANF concentrations between 0.4 and 200 pmol/l, which encompassed the physiological and pathological range (Chapters 10-12).

Table 3.8.

Effect of sample volume on immunoreactive ANF.

	Volume of plasma (ml)	Plasma ANF measured by RIA (pmol/l)
Healthy	0.5	4.9
	1.0	4.9
	2.0	4.9
	3.0	4.6
Patient	0.2	14.7
	0.4	16.4
	0.8	16.2
	1.6	15.4

Parallelism of Extracts in the ANF Radioimmunoassay.

In order to show that the immunoreactive ANF produced by extraction 'resembled' synthetic (99-126) ANF, serial dilutions, in phosphate buffer, were made of:

1) An extract of synthetic ANF (Bachem UK) added to healthy human plasma to give a concentration of 162 pmol/l.

2) An extract of endogenous plasma ANF (43 pmol/l) from a patient with chronic renal failure.

Serial dilutions of both extracts were parallel to the ANF standard curve (Figure 3.1 A and B).

Figure 3.1 (following page).

A. Parallelism of ANF standard curve with serial dilutions of extract of synthetic ANF added to healthy plasma.

B. Parallelism of ANF standard curve with serial dilutions of extract of plasma of a patient with chronic renal failure.

Key: solid line represents ANF standard curve and the dotted line represents the plasma extract.

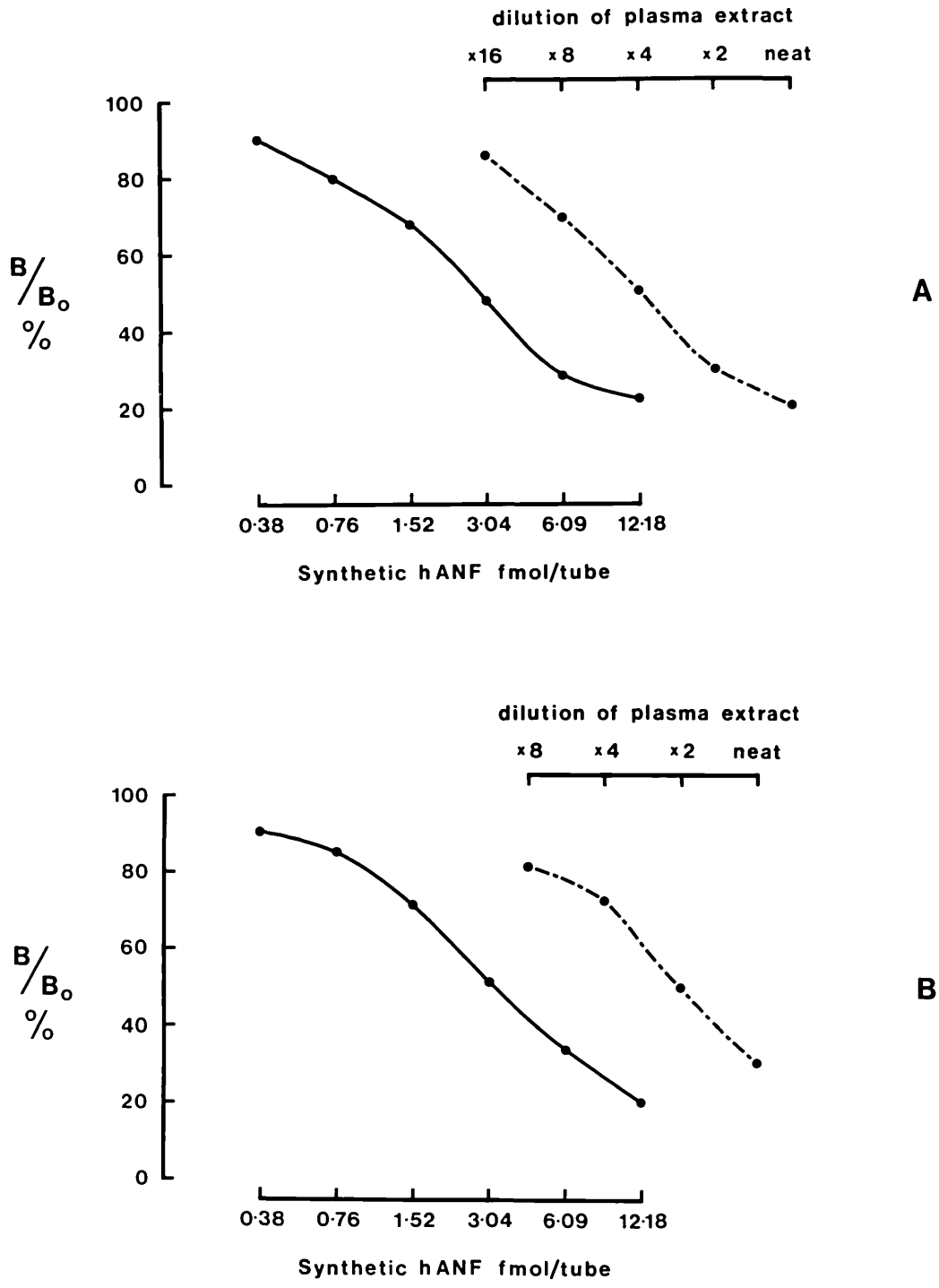


Figure 3 1.A and B.

3) Finally, serial dilutions were made of synthetic (99-126) ANF (Bissendorf GmbH Peptide). This preparation, which was used in the ANF infusion studies (Chapters 17-19), also showed parallelism with the standard curve (not shown).

ANF Radioimmunoassay Kinetics.

The ANF RIA developed in this thesis used a delayed addition of radiolabel, and followed a protocol described by Peninsula Laboratories. However, Amersham International suggested mixing all components simultaneously, and then incubating for four days.

When standard curves were set up using these two methods, a difference in slope was apparent so that the 50% displacement of tracer was 7.0 fmol/tube and 12.0 fmol/tube by the 'Peninsula' and 'Amersham' methods respectively. Thereafter, the method of delayed addition of label was used becuase it gave a greater sensitivity when used with the Peninsula 8798 antiserum.

Next, in order to ascertain the optimum delay between the addition of ^{125}I-ANF and the separation of bound and free components, the RIA was terminated at times between 4-72 hours after addition of label. The results (Table 3.9) showed that large increases of zero binding

occurred if incubation was continued up to 24 hours, although there was little gained by prolonging the incubation beyond this time.

Table 3.9.

Effect of incubation time on zero-binding.

Hours	Zero Binding (% Total Count)	Non Specific Binding (% Total Count)
4	10.4	3.4
8	17.8	3.2
24	36.0	2.5
48	40.6	4.5
72	41.0	4.0

The ANF Standard Curve and its Precision Profile.

The characteristics of the standard curve were studied in order to obtain a precision profile. Figure 3.2 shows a curve representing the mean$\pm$SD of ten standards at each concentration performed within the same assay. The lowest standard (0.38 fmol/tube) could be distinguished from zero binding ($p<0.01$, Student's unpaired t-test). The coefficient of variation at 0.38 fmol/tube was 3.2%, at 1.52 fmol/tube was 2.9% and at

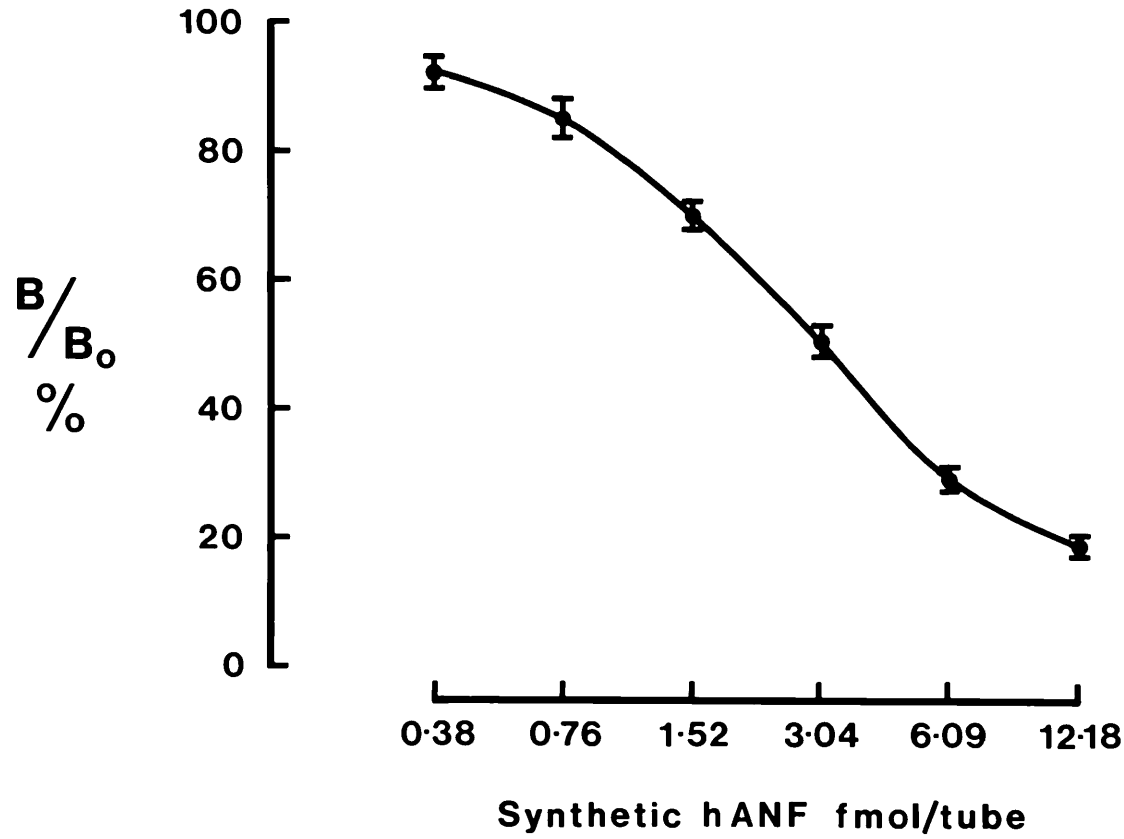


Figure 3.2.

The ANF standard curve.

Each point is the mean±SD of ten standards. B/B_o is the ratio of the standard binding versus zero-binding.

12.18 fmol/tube was 6.4%.

In 16 individual assays performed between 1986-1988, the mean (range) of absolute total counts was 6900 (3800-9500) per minute. Zero binding was 36 (25-48)% of the total count, and the non-specific binding was 3 (1-5)% of the total count.

Intra- and Interassay Variation.

The intra-assay variations at different concentrations of the standard curve are discussed above. Next, variation of plasma extracts was investigated. Every three months pooled plasma was used to make batches of extracts (Quality Controls A-D, Table 3.10). These were stored at -80^{o}C, and after reconstitution in phosphate buffer, at least two extracts of different concentrations were used in each ANF assay. Note that unextracted plasma was not a suitable material with which to assess inter-assay variation because ANF immunoreactivity had been shown to fall with prolonged freezer storage (Table 3.2).

Table 3.10 shows the coefficient of variation of different samples ranging between 0.4 and 5.5 fmol/tube. If any one RIA produced 'quality control' results that deviated widely from the expected variation, then the whole assay was repeated. In addition, in each RIA, at least five freshly extracted

healthy plasmas were included, and any deviation of the measured ANF from the established normal range (Chapter 10) signalled that the assay may have been flawed.

Table 3.10.

Quality controls of the ANF radioimmunoassay.

Name of Quality Control	n	ANF (fmol/tube) (mean±SD)	Coefficient of variation (%)
A. Intra-assay	10	1.4±0.2	12
Inter-assay	8	1.4±0.1	9
B. Intra-assay	10	5.5±0.7	12
Inter-assay	6	5.3±0.8	14
C. Inter-assay	12	4.1±0.2	6
D. Inter-assay	7	0.4±0.05	13

These results show that the coefficient of variation over the range of the standard curve was satisfactory.

Healthy Plasma Levels of ANF.

Using the current scheme of blood collection, ANF extraction and RIA, immunoreactive ANF in healthy plasma ranged between 1.5 and 18.1 pmol/l (Chapter 10), comparable with ranges established by other groups (Table 2.1).

Summary of Chapter 3.

1. The methods developed for the blood collection, plasma extraction and radioimmunoassay of plasma ANF were described.

2. The whole procedure appeared robust because:

A. It minimised peptide degradation at the time of blood collection.

B. It allowed stable storage of freeze-dried extracts for up to three months.

C. ANF was extracted from venous plasma using octadecylsilica. The recovery rate was high and reproducible.

D. By extraction of different volumes of plasma, the assay was able to measure the physiological and pathological range plasma ANF values.

E. The method incorporated a radioimmunoassay with acceptable intra- and interassay coefficients of variation.

CHAPTER 4.

THE MEASUREMENT OF CYCLIC GUANOSINE MONOPHOSPHATE.

Introduction.

I am grateful to Dr.P.J.Wood (Southampton Hospital Endocrine Laboratory) for advice and guidance regarding the measurement of cGMP.

Sample Collection.

A) Urine. Cyclic GMP is stable in urine (Wood and Marks, 1978) and therefore no preservatives were used. Urine was collected and immediately frozen and stored in polypropylene tubes at -80^{o}C until RIA.

B) Plasma. In contrast, plasma is rich in phosphodiesterases and therefore I followed a collection procedure obtained from Amersham International. Venesected blood was placed in polypropylene tubes containing 1% volume of 0.5M EDTA to act as an inhibitor of nucleotide degradation. The sample was then centrifuged at 540 x G for 10 minutes at 4^{o}C and the plasma separated and frozen at -80^{o}C. It was stored in a freezer under similar conditions to the urine samples until deproteinisation.

Plasma Deproteinisation.

Materials and Methods.

1. 95% ethanol (BDH AnalR).
2. Oxygen-free nitrogen (BOC Industrial gases).
3. Freeze-drier (Edwards Modulyo).

Before addition to the RIA, plasma was deproteinised in order to eradicate non-specific matrix effects. The method followed has been modified from a protocol supplied by Amersham International. One ml of plasma was added to 2 ml of 95% ethanol and the tube mixed by shaking and vortexing. After standing for 10 minutes at room temperature, tubes were centrifuged for 15 minutes at at 540 x G at $4^{o}C$. The supernatant was decanted and the ethanol component blown away by a stream of oxygen-free nitrogen. The remaining aqueous phase was freeze dried overnight. The lyophilised samples were stored at -80^{o} until RIA, when they were reconstituted with 1ml of assay buffer.

The Cyclic GMP Radioimmunoassay.

Materials and Methods.

1. Polystyrene tubes (RT30, Sterilin).
2. Assay buffer: Tris 50 mM with 5 mM EDTA, pH 7.4.
3. Cyclic GMP antiserum was a gift from Dr.P.J.Wood (Southampton Hospital Endocrine Laboratory), but was also available from Cymbus Bioscience Ltd, Romsey, UK. It was raised in rabbits by immunisation against 2'-o-

succinyl cGMP conjugated to bovine serum albumin (BSA). The lyophilised antiserum was reconstituted in BSA solution (1 g/l, pH 7.0). Cross reactivity to other nucleotides, quoted by Cymbus Bioscience, were: succinyl cGMP, 100%; cGMP, 20%; succinyl cAMP, 0.05%; cAMP, 0.004%.

4. Cyclic GMP radiolabel was purchased from Amersham International, UK: guanosine 3',5'-cyclic phosphoric acid 2'-o-succinyl 3-^{125}I-iodotyrosine methyl ester. The lyophilised tracer was reconstituted in the assay buffer and was used within two months of iodination.

5. For the standard curve, cGMP was purchased from Sigma.

6. Second antibody: sheep anti-rabbit coupled to microsphere beads (Pharmacia).

7. NE 1600 gamma radiation counter

For RIA, the following protocol was followed in duplicate. 100 µl of antiserum (final dilution 1/20000) was placed in polypropylene tubes together with 50 µl of the unknowns or standards. For the standard curve cGMP was added to give a total of 0.018 to 1.250 pmol cGMP per assay tube. This standard curve allowed plasma concentrations of the nucleotide between 0.36 and 25 nmol/l to be measured: this encompassed physiological and pathological ranges (Chapters 12,13,17-19). In addition, non-specific binding tubes were set up with assay buffer substituted for cGMP antiserum.

Tubes were incubated at 4°C for 24 hours and then 50µl of label (10,000 counts per minute) was added and then incubated for a further 24 hours at 4°C. Bound and free label were separated by addition of 100 µl of the second antibody, and after 30 minutes, 4 ml of 0.1% Triton was added and the mixture was centrifuged for 15 minutes at 850 x G at 4°C. The supernatant was decanted and radioactivity of the precipitate, containing the first antibody bound with cGMP, was measured for 120 seconds.

Studies of Extraction Efficiency.

The recovery of cGMP during plasma deproteinisation was studied as follows.

A) ^{125}I-cGMP was added to human plasma and 1ml aliquots were deproteinised, freeze dried and reconstituted in buffer as described above. The mean±SD recovery of radioactivity was 89±8% (n=10).

B) Next, 10 pmol of unlabelled cGMP was added to six 1 ml aliquots of a single healthy subject's plasma. The mean±SD recovery was 11.2±0.36 pmol.

C) Finally, 5 pmol of unlabelled cGMP was added to 1 ml aliquots of plasma from four patients with chronic renal failure. In addition, samples of the same plasma were incubated overnight in a water bath at 37°C with

0.05 ml of phosphodiesterase solution (kindly provided by Dr.P.J.Wood, Southampton Hospital Endocrine Laboratory). The samples were deproteinised and cGMP measured in the RIA (Table 4.1). The addition of phosphodiesterase rendered immunoreactive cGMP undetectable and this demonstrated that deproteinised plasma did not interfere with the RIA. When cGMP was measured in the plasmas which were spiked with extra nucleotide, the recovery was >90% in all samples.

On account of the consistently high recovery rates of both radiolabelled and immunoreactive cGMP, no correction was made for possible losses from the deproteinisation.

Table 14.1.

The recovery of immunoreactive cGMP.

(results are mean of two plasmas)

Original Plasma	After Phosphodiesterase (nmol/l)	After adding 5 pmol/ml cGMP	Recovery (%)
7.36	<0.36	12.30	99
4.08	<0.36	8.78	94
6.05	<0.36	10.76	94
5.64	<0.36	10.54	98

Results of the cGMP Assay.

Intra-assay coefficient of variation (CV) was: 2% at 0.224 pmol/tube (n=6), and 5% at 0.108 pmol/tube (n=9). The interassay CV of a single quality control plasma was 11% (0.187 pmol/tube, n=6). The lowest standard, 0.018 pmol/tube, could be distinguished from zero binding with >95% confidence. A typical standard curve had a total count of 6330 counts per minute, a zero-binding of 43% of total, 50% displacement at approximately 0.156 pmol/tube and a non-specific binding of 4% of the total count.

Results for healthy plasma and urine cGMP values were in agreement with previously published ranges (Wood and Marks, 1978; Gerzer et al, 1985; Broadus et al, 1970). The mean$\pm$SEM plasma values of 14 healthy adults on a 150 mmol sodium diet was 3.1$\pm$0.3 nmol/l (range 1.6-5.4nmol/l). Their 24 hour urinary cGMP excretion was 356$\pm$42 nmol (range 43-510 nmol).

Summary of Chapter 4.

1. The measurement of plasma and urinary cGMP was described.

2. The method incorporated collection of blood into enzyme inhibitors, plasma detproteinisation and a sensitive RIA.

CHAPTER 5.

THE MEASUREMENT OF PLASMA RENIN ACTIVITY.

Introduction.

The protocol for measurement of plasma renin activity (PRA) was identical to that used by the Supra-Regional Assay Unit at the Cobbold Laboratories, Middlesex Hospital, London. Angiotensin I is generated in the presence of angiotensin converting enzyme inhibitors, followed by the RIA of angiotensin I, and this method was modified from the work of Menard and Catt (1972).

I am grateful to Dr.J.D.H.Slater and Mrs.N.N.Payne for generously providing both the method, the angiotensin I radiolabel and the antiserum.

Plasma Renin Activity in Renal Disease.

In patients with renal disease, renin precursors can circulate and under certain conditions can contribute to measured PRA. However, the current method which stores plasma at -80^{o}C and generates angiotensin at pH 5.5 should avoid this effect, which has been reported to occur with prolonged storage of plasma just below its freezing point and exposure to pH 3.3 (Hsueh et al, 1978).

Materials for Measurement of PRA.

1. Lithium heparin tubes (Sterilin) for blood collection.
2. Polystyrene tubes for RIA (RT 30 Sterilin).
3. Angiotensin converting enzyme inhibitor solution: 120 mg of 8-hydroxyquinolone hemisulphate, 660 mg EDTA dissolved in 10 ml distilled water.
4. pH 5.5 buffer: 57g sodium acetate, 4.6 ml concentrated acetic acid, 200 mg neomycin sulphate dissolved in 1 litre of distilled water.
5. pH 8.5 buffer: 12.5 G Tris hydroxymethyl-methylamine, 160 ml 0.1 M hydrochloric acid, 0.5 g bovine serum albumin (Miles Laboratories Ltd) dissolved in 1 litre of distilled water.
5. 37^{o} water bath (Grant Circulation type).
6. Angiotensin I antiserum, used at final dilution of 1/80000, which was raised by the Middlesex Hospital.
7. Angiotensin I, for the standard curve (National Institute for Biological Standards).
8. Angiotensin label: ^{125}I-ileu-5-angiotensin I (CIS International), used within two months of iodination.
9. Dextran-charcoal mixture: 2.5 g activated charcoal (Sigma C-5260) and 0.5 g dextran (molecular weight, 70300. Sigma D-1390) in 100 mls of pH 8.5 buffer. The mixture was continuously stirred and kept in a container of ice-cool water.
10. NE 1600 gamma radiation counter.

The Generation of Angiotensin I.

Five mls of venous blood was collected and placed into cooled tubes containing lithium heparin. The blood was spun at 540 x G for 15 minutes and the plasma separated and stored in plastic tubes at -80°C. On the day of the assay, two 500 µl aliquots were placed into polystyrene tubes and mixed with 50 µl of inhibitor solution and 450 ul of pH 5.5 buffer.

In order to measure the basal level of angiotensin I, one tube was incubated at 4°C for three hours. In the final calculation of PRA, this value was subtracted from the amount of angiotensin I measured in the same original plasma after incubation at 37°C.

In the 37°C water bath, tubes were arranged in a rack so that their liquid contents were submerged. This rack was moved too and fro along the bottom of the bath by a mechanical device at one traverse per second. In addition, the tubes were vortex mixed for ten seconds every 30 minutes. After three hours, the reaction was terminated by adding 0.2 ml of the mixture to 1.8 ml of the 0.1M Tris buffer, pH 8.5.

Radioimmunoassay of Angiotensin I.

RIA was performed in duplicate. To each 0.5 ml sample, having been processed as described above, was added 0.5 ml of antiserum and 1.0 ml of ^{125}I-angiotensin I

(5000 counts per minute), both dissolved in pH 8.5 buffer. A standard curve of angiotensin I was set up from 5.3 to 385 fmol/tube, and non-specific binding tubes included. The components were mixed and incubated at 4°C for 48 hours.

In order to separate the bound and free components, 0.3 ml of charcoal/dextran solution was added and the tubes were then spun at 850 x G at 4°C for 30 minutes. The supernatant containing the antigen/antibody complexes was decanted and its radioactivity counted for 300 seconds. A typical standard curve comprised a zero binding of approximately 33% of the total count, with a 50% displacement of label at 50 fmol/tube, and a non-specific binding of less than 2% of the zero binding.

After multiplication of the unknown value by the appropriate dilution factor, PRA was expressed as nmol of angiotensin I generated per hour per litre of plasma. The intra-assay coefficient of variation was (n=12) 9.8%, and the mean±SD PRA of this sample was 1.73±0.17 nmol/h/l. A 'quality control' plasma was used in each assay (2.1±0.21 nmol/h/l) and the intra-assay coefficient of variation was 10% (n=9).

The PRA of 14 healthy young adults who ate a 150 mmol sodium diet for three days the mean±SD, after sitting quietly for one hour between 0900 and 1000h, was (mean±SD) 1.02±0.36 nmol/h/l (range 0.45 to 1.74).

CHAPTER 6.

THE MEASUREMENT OF PLASMA ALDOSTERONE

Introduction.

In the presence of chronic renal failure, circulating glucuronide derivatives interfere with the RIA of plasma aldosterone. Therefore, plasma aldostserone was extracted before addition to the RIA (Jones et al, 1981).

The complete method follows the protocol used by the Middlesex Hospital Cobbold Laboratories Supra-Regional Assay Service, and I am grateful to Mrs.N.N.Payne for advice on this technique.

Materials.

1. Dichloromethane (BDH, Analar Grade).
2. 10 and 20 ml glass stoppered test tubes (Quickfit).
3. Oxygen-free nitrogen (BOC Industrial Gases).
4. Bovine serum albumin solution pH 7.4: 18g BSA (Miles Fr V), 0.9g NaCl (BDH Analar), 0.1G Neomycin sulphate (Upjohn), 0.1G NaN_3 (BDH Analar) made up in 100 ml distilled water.
5. Lithium heparin tubes for blood collection (Sterilin).
6. CIS SB-ALDO-2 radioimmunoassay kit, containing standards, label and antibody-coated tubes.

7. NE 1600 gamma counter.

Plasma Extraction.

Venous blood was collected into glass tubes containing Lithium Heparin as an anti-coagulant. After centrifugation at 540 x G at 4°C for 15 minutes, 0.5-1.0 ml of plasma was added to 8 ml dichloromethane in a stoppered glass test tube. After vortexing briefly, the tube was gently rolled for 30 minutes, with further brief vortexing at 10 and 20 minutes. The tube was then unstoppered and left for 5 minutes to allow phase separation, after which the aldosterone-containing dichloromethane fraction was carefully pipetted off into 10 ml glass tubes, and the extract was dried under a stream of nitrogen. The extract was then reconstituted by adding 1 ml of the BSA solution.

Preliminary experiments had shown that the extract was slow to dissolve, and therefore the solutions were rolled gently overnight at room temperature after which any residue, previously on the sides of the tube, had dissolved. Before addition to the RIA the solution was vortexed until it became homogenous.

Radioimmunoassay of Aldosterone.

A commercial RIA kit (CIS SB-ALDO-2) was used which incorporated an antibody-coated tube method. The

antibody was quoted to have the following cross-reactivities: 100% with aldosterone, 4.2% with 3 beta, 5 alpha-tetrahydroaldosterone, 0.3% with 3 alpha, 5 beta tetra hydroaldosterone, and less than 0.001% with corticosterone, deoxycorticosterone, cortisol and cortisone.

0.2 ml of plasma or aldosterone standard was mixed with 0.5 ml ^{125}I-aldosterone in the coated tube and incubated overnight at room temperature (18-25^{o}C). The liquid within each tube was then aspirated and the remaining radioactivity bound to the tube's interior was counted for 180 seconds. The standard curve ran from 69 to 2770 pmol/l. All samples from a project were measured in the same assay and the intra-assay variation at 277 pmol/l was 11%, and was 17% at 69 pmol/l.

Plasma Extraction Efficiency.

Ten 0.5 ml samples of plasma were selected at random from subjects with chronic renal failure and 0.1 ml ^{125}I-aldosterone label was added. The mixture was extracted and resuspended as described above. The mean$\pm$SD radioactivity in the extract, compared to 0.1 ml label in 0.5 ml BSA solution (2800 counts per minute) was 94$\pm$2%.

Next, a smaller series of 'cold' extraction experiments

were performed in which 277 pmol aldosterone was added to to the plasma of four patients with chronic renal failure, and recovery was 76-105% (Table 6.1). Finally, doubling dilutions of an extract of a patient's plasma were made from x1 to x16 and the series demonstrated parallelism with the aldosterone standard curve.

Table 6.1.

The recovery of immunoreactive aldosterone.

(277 pmol/l added to all plasmas)

Aldosterone in original sample (pmol/l)	Aldosterone in 'spiked' sample (pmol/l)	Efficiency of extraction (%)
1080	1360	101
840	1060	76
780	1030	91
470	760	105

Results of the Aldosterorne Assay.

The mean±SEM (range) of plasma aldosterone in 14 healthy adults, who had eaten a 150 mmol sodium diet for three days, with blood collected at 1000h after one hour of quiet sitting, was (mean±SD) 263±30 (range 125-460) pmol/l.

CHAPTER 7.

THE MEASUREMENT OF OTHER SUBSTANCES.

1. The Measurement of Plasma Total Protein.

Reagents.

Biuret Reagent, in each litre: 3.0g $CaSO_4.5H_2O$.
5.0g KI, 9.0g Na/K Tartarate, 1.4 ml AW7 detergent.

Blank Reagent, in each litre: 4g KI, 6.4g AW7 detergent.

Spectrophotometer: LKB Ultrospec 4050.

Method.

Biuret Total Protein Assay. The following were performed in duplicate. 0.1 ml of plasma was added to 0.9 ml of distilled water in a RT30 test tube, and 1.5 ml of the Biuret Reagent added. This was vortex mixed and incubated for 10 minutes in a water bath.

The absorbance was then read at 540 nm against a blank sample, and the amount of protein calculated in the unknown sample was calculated after comparison with a standard curve which incorporated bovine serum albumin The intraassay variation was <5% and all samples from one project were measured in a single assay.

2. The Measurement of Plasma Albumin.

Reagents.

Bromocresol Green Reagent: 0.8 ml (BDH) was added to

200 ml of citrate buffer pH 3.8 (BDH).
'Albumin Water': 0.2 ml of Brij 35 (BDH) added to 200 ml of deionised water.

Method.
The following scheme was performed in duplicate. 0.1 ml of plasma was added to 3 ml of 'albumin water'. Then 0.3 ml of this mixture was added to 3 ml of bromocresol green reagent and vortex mixed. The absorbances were read immediately at 615 nm. Standards and controls were obtained from the Department of Chemical Pathology at the Whittington Hospital. The intra-assay variation was <5%.

3. The Measurement of Urinary Albumin.

A commercial RIA kit was used to measure urinary albumin (Pharmacia Albumin RIA 100). This incorporated ^{125}I-albumin as the competing ligand, and the bound and free components were separated by the addition of a second antibody immunoadsorbent followed by centrifugation and decanting.

Urine was collected into plastic containers and kept at -80^{o}C until RIA. Urinary albumin is stable when frozen for up to two months (Pharmacia). For the normal subjects, urine could be used neat but in pathological samples with high albumin levels urine was diluted with deionised water before addition to the RIA.

The span of the standard curve was from 0.8-80.0 mg/l. If necessary, the lower detection limit could be increased to 0.4 mg/l by using twice the conventional sample volume, as recommended by Pharmacia: this allowed measurement of healthy albumin excretion rates during high urinary flow rates. All samples from one project were analysed in the same RIA and the intra-assay coefficient of variation was 5% at 8 mg/l but was 11% at the lowest standard of 0.8 mg/l.

4. The Measurement of Plasma and Urine Electrolytes and Creatinine.

For the measurement of plasma and urine sodium and potassium concentrations I used the Flame Photometer (Instumentation Laboratory, 543) in the Department of Chemical Pathology at the Whittington Hospital. Standards were incorporated between every few samples in each assay to check for drift and the machine was regularly serviced by the Department of Chemical Pathology who also used it for routine work. The coefficient of variation was 0.7%.

Creatinine was measured by the Alkaline Picrate method by the Biochemistry Laboratory, St.Paul's Hospital, London. The coefficient of variation was 3.9%.

5. The Measurement of Inulin and Para-aminohippurate.

During the ANF infusions (Chapters 17-19), inulin and para-aminohippurate (PAH) have been used to measure glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). The details of the infusion rates are given in the appropriate chapters, and the method of measurement is described below.

Materials.

These were BDH, Analar grade, unless otherwise stated.

1. Inulin was purchased from Laevosan-Gessellschaft as vials of 5g in 50ml of water.
2. PAH sodium was purchased from Merck Sharp and Dohme as vails of 2g in 10ml of water.
3. Lithium heparin tubes (Sterilin).
4. Cadmium sulphate solution composed of 17.3g $3CdSO_4 8H_2O$, 86g 1N H_2SO_4 made up to 500 ml with distilled water.
5. Solutions of 1.1N sodium hydroxide, 1.2N and concentrated (36%) hydrochloric acid, 0.15% resorcinol in absolute ethanol, 0.5% ammonium sulphamate and 0.1% $NaNO_2$.
6. Coupling reagent comprised 0.1% N-1-napthyl-ethylene diamine hydrochloride.
7. Water bath, 80^{o}C (Grant Circulation type).
8. LKB Ultrospec 4050 spectrophotometer.

For plasma sampling venous blood was collected from the

forearm contralateral to the inulin and PAH infusion at a time three minutes before the midpoint of the corresponding urine collection. Blood was placed in lithium heparin glass tubes and the plasma separated after centrifugation for 10 minutes. Plasma was stored at -80^{o}C until assay, which was usually within one week of the experiment.

Plasma was then processed in a glass tube by adding 1ml to 3ml of the cadmium sulphate solution and then adding 1ml of 1.1N NaOH. The tubes were vortex mixed and then left to stand at room temperature for 10 minutes. After centrifugation at 80 x G for ten minutes the supernatant, which was a five-fold dilution of the original plasma, was added to the assays. Urine was added to the assays after dilution in distilled water (1/200 for PAH and 1/50 for inulin).

The PAH Assay.

This was performed in duplicate. Urine samples had their absorbances read relative to a water blank (water plus reagents), and plasma samples were read relative to a plasma plus reagent blank. Two ml of the diluted urine or processed plasma sample was added to 0.5ml of 1.2N HCl and 0.25 ml 0.1% $NaNO_2$ solution and then mixed. After 4 minutes 0.25 ml of the 0.5% ammonium sulphamate solution was added, and after a further 4 minutes 0.2 ml of the coupling reagent was added. The whole was mixed and the absorbance read after 10

minutes at a wavelength of 540 nm. Plasma unknowns were compared against standards of 2 and 4 mg/l in order to ensure that the correct dose of PAH had been administered during the experiment. In normals, if plasma PAH exceeds 600 mg/l, tubular secretion becomes saturated. Therefore, in the current experiments, PAH was infused to keep plasma levels at approximately 20 mg/l. PAH clearance, or effective renal plasma flow, was calculated, after appropriate correction for plasma and urine dilution from the equation:

$$\frac{\text{(urinary PAH concentration) x (urine flow rate)}}{\text{(plasma PAH concentration)}}$$

The Inulin Assay.

One ml of the processed plasma or diluted urine sample was added to 1.5 ml of 0.15% resorcinol solution in a glass bottle. After mixing, 1 ml of 36% HCl was added. The bottles were stoppered and vortex mixed and then incubated in the water bath at 80°C for 40 minutes. After cooling for 20 minutes, the absorbances were read at 480 nm. After correction for water and plasma blanks, inulin clearance (GFR) was calculated as:

$$\frac{\text{(urinary inulin concentration) x (urine flow rate)}}{\text{(plasma inulin concentration)}}$$

PART II.

CHAPTER 8.

ANF AND THE CONTROL OF PLASMA VOLUME: A REVIEW.

Introduction.

In this chapter two concepts that were current before the discovery of ANF are reviewed, namely the cardiac atria as blood volume sensors and the natriuretic hormone. The remainder of the chapter describes the discovery of ANF, the control of its secretion and various conditions in which plasma levels are high.

Evidence that the Atria are Blood Volume Sensors.

Sodium ions comprise most of the osmotically active cation in the extracellular fluid, and therefore the long-term control of both the plasma and the interstitial fluid volumes depends on a balance between sodium intake and excretion (Raymond et al, 1985). In man, sodium ingestion is culturally, rather than biologically determined and the final adjustment of plasma volume depends on renal sodium excretion which itself must be regulated by a volume sensor.

In 1956 Henry described a diuresis which followed the inflation of a balloon in the left atrium of the anaesthetised dog and postulated that cardiac stretch receptors might control kidney function. This experiment was refined and the inflation of small

balloons at the junction of the venous system and the atria increased both urinary sodium and water excretion (Linden, 1979).

Further evidence for the atrial location of volume sensors derived from study of head-out water immersion during which hydrostatic pressure shifts blood into the thorax followed by a natriuresis and diuresis (Epstein, et al 1978). Similarly, when venous return to the heart was enhanced by assuming the supine or head-down position, a natriuresis occurred (Karnad et al, 1987). The observation that, in some species, the removal of the right atrial appendage caused a blunted natriuretic response to acute hypervolaemia provided further circumstantial evidence which suggested the atria were important in volume control (Goetz, 1988).

Even before the discovery of ANF (de Bold et al, 1981), enhanced atrial filling was known to be associated with suppression of antidiuretic hormone, plasma renin activity, aldosterone and a reduction of renal efferent sympathetic nerve activity (Share et al, 1969; Linden et al, 1979; Reinhardt et al, 1980). These responses were in part mediated by activity of stretch receptors connected to vagal afferent nerves, and cardiac denervation blunted the natriuresis in response to acute volume expansion in some (Goetz, 1988) but not other studies (de Wardener et al, 1961).

Other organs also monitor blood volume, including the central nervous system (Kalayonides et al, 1978), the arterial system (Proznitz et al, 1977) and the splanchnic area (Carey, 1978). The kidney itself can be included in this list because in the isolated organ, perfusion pressure determines sodium excretion (Selkurt, 1951). Manning and Guyton (1982) argued that enhanced sodium intake would increase blood volume and cardiac venous return: the subsequent increase of cardiac output would cause a small rise in arterial blood pressure which would cause a 'pressure natriuresis' with a return to sodium balance.

This plethora of volume sensors should not detract from the potential importance of the the cardiac atria in volume control. In fact, the existence of more than one sensor would be advantageous.

The Concept of a Natriuretic Hormone.

In 1961 de Wardener reported that volume expansion in dogs increased urinary sodium excretion despite the control of GFR (by aortic constriction) and the use of pharmacological doses of mineralocorticoids. The results were taken to indicate that "the saline diuresis is due to the action of a hormonal agent, other than aldosterone, on the tubules". Initially, the methodology was critised because volume expansion by saline caused haemodilution and a fall of plasma

protein which may have reduced proximal tubular sodium reabsorption (Spitzer and Windhager, 1970). However, refinements of de Wardener's original experiment, using blood equilibrated with the animal's circulation, supported his original conclusions.

Subsequently, circulating natriuretic substances were reported in other conditions associated with central blood volume expansion. These include primary aldosteronism (Sealey et al 1969), chronic renal failure (Bourgoignie et al, 1974), and head-out water immersion (Epstein et al, 1978).

De Wardener's original observations suggested that the the putative natriuretic hormone acted on the kidney tubule, and micropuncture studies confirmed that such partially purified agents inhibited sodium reabsorption in the proximal tubule (Weber et al, 1974) and in the collecting tubule (Fine et al, 1976). The nature of these agents proved to be elusive, but they were thought to be low molecular weight molecules devoid of amino-acids. They inhibited Na^+-K^+-ATPase and cross-reacted with antibodies to digoxin, and the hypothalamus was considered a likely source (de Wardener and MacGregor, 1982).

The Discovery of Atrial Natriuretic Factor.

The discovery of ANF (de Bold et al, 1981) was preceded by histological studies which suggested that the heart was an endocrine organ as well as a muscular pump. In 1956, Kisch reported that guinea-pig atria contained "endoplasmic bodies" and in 1964, Jamieson and Palade found large populations of "spherical, electron opaque granules" within the muscle fibres of mammalian atria. Although these granules stained in a similar fashion to hormone-containing organelles in other tissues, tests for catecholamines were negative. Their significance remained unknown until 1979 when de Bold noted that atrial granularity varied with salt balance, and this led him to inject atrial extracts into the circulation of other animals and to discover their natriuretic effects (de Bold et al, 1981 and 1988).

Purification of protein within these granules, followed by DNA cloning studies, quickly identified an amino-acid sequence that constituted the prepropeptide ANF (de Bold et al, 1988). It consisted of 151 aminoacids in man and 152 in the rat, and was coded for by three exons in the nuclear DNA. The initial 25 or 24 amino acids, in man and rat respectively, composed an N-terminal signal peptide, and the subsequent 126 amino acids (the propeptide) was designated 1-126 ANF. The latter molecule was found within heart granules

and although smaller molecular weight peptides derivatives were isolated, some may have been artefacts (Kangawa et al 1984).

During the secretion of the hormone, the propeptide is processed by tryptic digestion at position 98-99. The cellular site of this cleavage is debated: digestion may occur within the atrial cell or at the cell membrane during secretion (Trippodo et al, 1984). The 28 amino acid peptide, (99-126) ANF, was identified as the principal form released by both the isolated mammalian heart (Thibault et al, 1986) and by the healthy human heart (Yandle et al, 1987). Recently, peptides derived from (1-98) ANF have been identified in the blood stream (Winters et al, 1987; Itoh et al, 1988a). Their physiological significance is unclear, although two have been reported to vasodilate vascular strips (Vesely et al, 1987; Chapter 2).

The Control of ANF Secretion from the Heart.

Studies in healthy humans have confirmed that immunoreactive (99-126) ANF was released from the heart (Sato et al, 1986; Singer et al, 1987a) and that there was a step-up in concentration from the vena cava to the right atrium, with the highest concentrations measured at the mouth of the coronary sinus. Initially it was assumed that this material derived exclusively

from the atria, but Gutkowska (1987b) demonstrated that the isolated lung also secreted ANF, and Currie et al (1987) showed that ventricles from diseased hearts contained measurable quantities of ANF. However, the absolute contribution to the plasma pool of ANF by these non-atrial sources is unknown.

What controls the release of ANF ? Isolated strips of atrial muscle released ANF when put under tension (de Bold et al, 1988), and when atrial pressure was experimentally raised in isolated animal hearts immunoreactive and biologically active ANF was secreted (Dietz et al, 1984; Ledsome et al, 1985). The importance of atrial stretch rather than the pressure was emphasised by Edwards et al (1986) who studied a group animals with cardiac tamponade: when atrial pressure was experimentally increased no rise of plasma ANF occurred until the release of the tamponade and stretching of the atrial walls. In contrast, the rate of atrial muscle cell contraction did not alter ANF secretion (de Bold et al, 1988).

In healthy humans, there was a positive correlation between pulmonary artery pressures and plasma ANF (Sato et al, 1986), and after intravenous volume expansion rises of atrial pressure and ANF occurred (Sato et al, 1986; Anderson et al, 1986a). The increase in central blood volume after head-out water immersion caused a two to four-fold rise in plasma ANF (Epstein et al,

1986), and postural changes which enhanced cardiac venous return increased ANF two-fold (Solomon et al, 1987). Large increases of dietary sodium (eg from 10 to 300 mmol/day) caused small increases in plasma ANF (Shenker et al, 1985; Sagnella et al, 1985). Such a change of diet is accompanied by the retention of approximately two litres of fluid before a new steady state is established (Raymond et al, 1985), and the subsequent rise in plasma volume may be responsible for the rise of ANF.

The administration of mineralocorticoids to healthy subjects was associated with a rise in plasma ANF which may result from sodium retention and plasma volume expansion (Cappucio et al, 1987). Peripheral plasma ANF levels were high in congestive cardiac failure and correlated with intracardiac pressures (Raine et al, 1986; Richards et al, 1986). Similarly, values are high in primary aldosteronism (Espiner and Nicholls, 1987), end-stage renal failure (Rascher et al, 1985) and hyperthyroidism (Woolf and Moult, 1987a), all conditions assoiated with blood volume expansion.

The role of cardiac innervation in modulating ANF release has been investigated, but no firm conclusions have been reached. In rats, cardiac denervation blunted the ANF rise to volume expansion (Eskay et al, 1986), yet ANF was released by both isolated atrial

strips and anatomically and pharmacologically denervated hearts (de Bold, 1986; Dietz et al, 1984; Ledsome et al, 1984). In humans with cardiac transplants immunoreactive plasma ANF was detectable (Singer et al, 1986) and rose in response to blood volume expansion (Woolf et al, 1987b). In these patients only a remnant of the native right atrium remains, and most of the atrial tissue, derived from the donor, was not innervated. When isolated rat atrial tissue was exposed to muscarinic cholinergic agents or to adrenaline, ANF was released (Sonnerberg and Veress, 1984). However, the rise of plasma ANF after exposure to phenylephrine in the whole animal was proportional to elevated arterial and intracardiac pressures secondary to peripheral vasoconstriction (Katsube et al, 1985).

Summary of Chapter 8.

1. The discovery of ANF linked the concepts that the cardiac atria acted as blood volume sensors and that a natriuretic hormone circulated in states of volume expansion. However, other volume sensors exist apart from the atria, and there are circulating natriuretic substances other than ANF.

2. Plasma ANF is high in a variety of physiological and pathological conditions associated with blood volume expansion and enhanced atrial filling.

CHAPTER 9.

CYCLIC GUANOSINE MONOPHOSPHATE: A REVIEW

Introduction.

The identification of cGMP in rat urine (Ashman et al, 1963) long preceded an appreciation of its significance as a 'second-messenger' of ANF (Hamet et al, 1984). Before reviewing the relationship of ANF and cGMP, general aspects of the nucleotide will be described.

Cyclic GMP is present in cells, blood, CSF and urine. The production of cGMP from guanosine triphosphate is catalysed by guanylate cyclase which exists in a 'soluble' and 'particulate' form (Murad, 1986). After it is formed, cGMP is destroyed by phosphodiesterases in cells and in the extracellular fluid. A cGMP-dependent protein kinase has been identified and consists of one regulatory and one catalytic subunit and may be responsible for protein phosphorylation and further cellular effects (Rapoport, et al, 1982).

In healthy subjects plasma cGMP is 1-20 nmol/l, and is not protein-bound (Broadus et al, 1970; Wood and Marks, 1978). Broadus (1970) intravenously infused ^{3}H-cGMP into normal subjects and calculated a volume of distribution of 38% body weight. The plasma clearance was 855 ml/minute and the half-life 30 minutes. The kidney contributed only 15% to the clearance and all

urinary cGMP was accounted for by filtration of the nucleotide from the plasma. In contrast, a rat micropuncture study (Huang et al, 1986) showed that only two thirds of cGMP that passed into the glomerular filtrate reached the urine, suggesting that tubular reabsorption had occurred.

In thyrotoxicosis urinary cGMP excretion is increased (Tucci and Kopp, 1976). Alpha-adrenergic and angiotensin II administration both raise extracellular levels of cGMP (Rosman et al, 1976; Okada et al, 1982).

Cyclic GMP: a Second Messenger of ANF.

In 1984 Hamet demonstrated that ANF induced intra- and extracellular rises of cGMP. Since then, it has been found that ANF membrane receptors were often coupled to particulate guanylate cyclase, and that the binding of the peptide to vascular, renal and adrenal tissue resulted in rises of intracellular cGMP (Murad, 1986; Leitman and Murad, 1987). The renal action of ANF on cGMP will be reviewed in detail in Chapter 15.

This discovery has allowed new interpretation of previous observations. For example, the changes in cGMP with thyroid hyperfunction may result from the raised plasma ANF in thyrotoxicosis (Woolf and Moult, 1987a). Similarly, the rise in extracellular cGMP which followed angiotensin II and alpha-adrenergic

stimulation may be mediated by rises in plasma ANF: both of these stimuli are known to enhance cardiac secretion of ANF (Sanfield et al, 1986).

Intravenous administration of pharmacological doses of ANF increase plasma and urinary cGMP in man (Gerzer et al, 1985), the dog (Seymour et al, 1985) and the rat (Hamet et al, 1984). When ANF was infused into the brachial artery, forearm vascular resistance fell accompanied by a rise of ipsilateral venous cGMP (Fujita et al, 1987), supporting in vitro experiments which suggested that cGMP mediated ANF-induced vasodilatation (Murad et al, 1986). Moreover, plasma ANF and cGMP were both elevated in states of central blood volume expansion including intravenous saline infusion (Weil et al, 1985), congestive heart failure (Weil et al, 1987) and hyperthyroidism (Woolf and Moult, 1987a).

Are extracellular levels of cGMP a marker for the intracellular effects of ANF ? For this to be true a number of conditions must be met. Firstly, ANF binding to cells and its final action must be firmly associated with increases in cGMP. Although there is much to support this, there is also evidence that not all ANF receptors are linked to guanyl cyclase (Leitman and Murad, 1987; Takayanagi et al, 1987), and cGMP analogues did not invariably mimick the effects of the peptide (Elliot and Goodfriend, 1986).

Furthermore, it is an assumption that plasma cGMP accurately reflects levels of intracellular nucleotide, and the contribution of each organ with ANF receptors to the the plasma pool of cGMP is unknown. On the basis of size, it is likely that the majority of plasma cGMP derives from blood vessel arterial walls (Leitman and Murad, 1987). Therefore, in the whole animal, ANF-induced rises of plasma cGMP would not be organ specific. Chapters 17-19 further consider the use of urinary cGMP as a marker for the renal actions of ANF.

Lastly, local mediators (acetylcholine and endothelium-derived relaxant factor) and exogenous drugs (nitrates and nitroprusside) also stimulate cGMP production (Murad, 1986). Therefore, extracellular levels of the nucleotide are not solely the result of stimulation of particulate guanylate cyclase by ANF.

Summary to Chapter 9.

1. Cyclic GMP might act as an intracellular 'second-messenger' for ANF. In states of blood volume expansion, extracellular cGMP and plasma ANF are raised above healthy values.

2. Pharmacological doses of ANF administered to man and animals increase plamsa and urinary cGMP, and in the whole animal, these may act as a non-organ specific marker for the tissue effects of ANF.

CHAPTER 10.

PLASMA LEVELS OF ANF IN HEALTHY SUBJECTS AND THE EFFECTS OF INTRAVENOUS SALINE INFUSION

Introduction.

The aim was to establish a normal range of plasma ANF (Study A) and to follow the effect of acute blood volume expansion on plasma ANF in normals (Study B).

Subjects and Methods: Study A.

24 healthy volunteers, aged 24-72 years (10 female), drawn from hospital staff, ate their regular diet and performed a 24 hour urine collection. At 0900h on that morning, they were seated with legs dependent in a quiet room. After 30 minutes a single blood pressure reading was taken with a mercury sphygmomanometer (using Korotkoff V for diastolic reading) and then forearm venous blood was collected for ANF, plasma renin activity (PRA), sodium and creatinine measurement. Urine was analysed for sodium and total protein excretion. All had blood pressure <150/90 mmHg, mean blood pressure (MBP = diastolic plus one third of pulse pressure) <110 mmHg and <0.05 g of proteinuria per day. Creatinine clearances were not measured, but all had plasma creatinine <120 umol/l. Simple and partial correlations were sought with ANF as the dependent variable and age, MBP and 24 hour sodium

excretion as the independent variables.

Subjects and Methods: Study B.

This was approved by the Hospital Ethical Committee and 7 healthy subjects (one female), aged (mean±SEM) 34±2 years, gave informed consent. All had blood pressures of <150/90 mmHg and were within 20% of ideal body weight. They were studied twice, with and without saline infusion, in random order and at least two weeks apart. Dietary sodium was not controlled but the volunteers were asked to eat a similar diet for three days prior to each study. At 0900h they sat with legs dependent and remained in this position apart from rising briefly every half-hour in order to micturate. Between 0900h and 1000h they each drank 500 ml of water and thereafter drank 150 ml half-hourly. At 0900h a 'butterfly' cannula was inserted into a forearm vein. Between 1000h and 1100h 2 litres of isotonic (0.9%) saline was infused intravenously at a constant rate to give a total dose of 300 mmol sodium ions. The control study was identical except that no saline was infused. The one female volunteer took no oral contraception and active and placebo studies were performed in the follicular phase of the cycle. Plasma was sampled half-hourly for ANF, and urine volume and sodium was measured. In the active study group only, hourly plasma total protein, cGMP, aldosterone and PRA were measured.

Statistics.

Serial results were expressed as absolute values as mean±SEM. Plasma ANF and sodium excretion were initially examined by two way analysis of variance upon active-placebo pair differences. Only if significant ($p<0.05$) changes were found, Duncan's test for mutiple comparisons was used to determine the times at which the mean pair differences differered significantly from both basal pair differences (Geigy Scientific Tables, 1984, after Duncan, 1945).

Changes of plasma cGMP, PRA, aldosterone and plasma total protein, which were measured hourly in the active group only, were analysed by Student's t-test compared to the basal sample.

Results of Study A.

Raw data are given in the Data Appendix (Chapter 22), and Tables 10.1 and 10.2 show basal levels and correlations. The mean plasma level of ANF in the healthy group was 6.9±0.8 pmol/l. PRA, urinary sodium excretion and MBP are shown in Table 10.1.

Simple correlations (Table 10.2) revealed that ANF was related to age ($r= +0.66$ $p<0.01$) and MBP ($r= +0.52$ $p<0.01$). In addition, simple correlations showed that age was related to MBP ($r= +0.53$ $p<0.01$).

Table 10.1.

Basal ANF values of 24 healthy subjects.

Age (years)	Urine Sodium (mmol/day)	MBP (mmHg)	ANF (pmol/l)	PRA (nmol/l/h)
42±2	140±17	87±2	6.9±0.8	1.21±0.16

Results are mean±SEM.

Key: MBP = mean blood pressure, ANF = atrial natriuretic factor, PRA = plasma renin activity.

Table 10.2.

Correlations with ANF in health.

	Age	Urine Sodium	MBP	PRA
Simple	** +0.66	NS	** +0.52	NS
Partial	** +0.68	** 0.60	NS	NS

NS = $p>0.05$, ** = $p<0.01$.

Key as for Table 10.1.

Partial correlations (Table 10.2) showed that ANF was significantly related to age ($r = +0.68$ $p<0.01$) and daily urinary sodium excretion ($r = +0.60$ $p<0.01$). The appearance of sodium excretion as a significant partial correlate, which was not apparent on univarate analysis, may be explained by the tendency for elderly to have a lower sodium output (age versus urinary sodium $r = -0.58$ $p<0.01$).

Finally, multiple regression revealed that age and urinary sodium could explain 0.62 of the variation of plasma ANF ($r = 0.82$ $p<0.001$) but that the addition of PRA and MBP did not significantly help to account for further variation of the peptide.

Results of Study B.

The raw data are given in the Data Appendix (Chapter 22).

ANF.

Figure 10.1 demonstrates the serial changes of plasma ANF and urine sodium excretion after saline infusion. The basal plasma ANF in the active (3.0 ± 0.5 pmol/l) and placebo (4.2 ± 0.6 pmol/l) groups were similar. However, these values were lower than the group mean in Study A (6.9 ± 0.8 pmol/l). This might be explained by the relative youth of the volunteers in Study B.

Figure 10.1 (following page).

The effect of intravenous saline infusion on ANF.

The effects of two litre saline infusion administered intravenously on plasma ANF and urinary sodium excretion (Study B). Serial results are shown as mean±SEM. ** indicates a significant difference between means of saline and control group ($p<0.01$) at individual time points, compared to both basal differences, otherwise points are not significantly different ($p>0.05$).

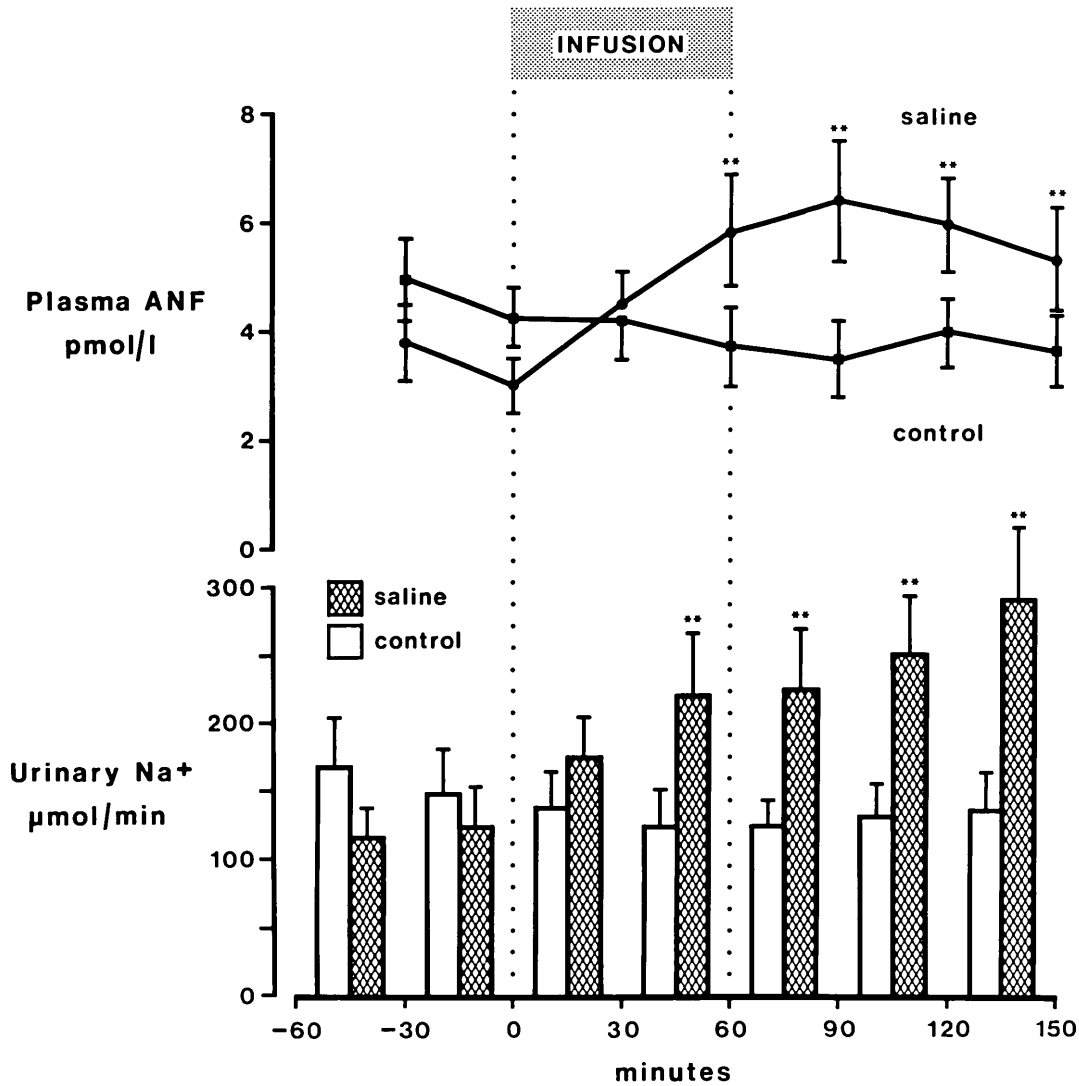


Figure 10.1.

The effect of intravenous saline infusion on ANF.

For key see previous page.

During saline infusion plasma ANF rose two-fold to reach a peak mean by 30 minutes after the end of the saline infusion (Figure 10.1). The mean values of the active group were significantly higher than controls from the end of the saline infusion up to 90 minutes after the end of the infusion (all $p<0.01$). ANF tended to fall in the final hour of the study.

Cyclic GMP.

Plasma cGMP in the active group rose from a basal value of 4.1 ± 0.9 nmol/l to a peak mean of 5.0 ± 0.9 nmol/l 15 minutes after the end of the saline infusion. This change was not significant.

Sodium Excretion.

Basal urinary sodium excretion rate was similar in the two groups (Figure 10.1). The difference between the natriuresis of the two groups became significant in the second half hour of the saline infusion ($p<0.01$). In the final hour, sodium excretion in the group infused with saline continued to rise at a time when the mean plasma ANF tended to fall. By the end of the experiment only a fraction of the total sodium load (<15%) had been excreted into the urine.

Plasma Renin Acivity and Aldosterone.

In the active group PRA became significantly suppressed (basal 0.99 ± 0.22 nmol/l/h to 0.36 ± 0.09 nmol/l/h at 30 minutes after the end of the infusion). During the same

period plasma aldosterone fell from 420±118 pmol/l to 208±45 pmol/l, and total protein decreased from 78±3 g/l to 70±2 g/l (all $p<0.05$). No significant change occurred in MBP.

Discussion.

The resting ANF plasma levels in studies A and B should be compared with values measured by other authors listed in Table 2.1. The mean value and range that I measured in study A was similar to the findings some authors (Epstein et al, 1986; Gutkowska et al, 1987; Anderson et al, 1986a; Richards et al, 1987; Sagnella et al, 1985). However, the levels in the study A and B were lower than those quoted by Weidmann et al (1986) and Yandle et al (1986a). This might be explained by differences in posture, enzyme inhibitors used during blood collection and in the ANF RIA. In addition, as discussed in the methods section, my own results were not corrected for the approximately 20% loss of ANF during extraction: other authors have not always clarified whether their own values have been corrected in this fashion.

The positive relation between age and plasma ANF found in the current group agreed with the findings of Sagnella et al (1986) and Montorsi et al (1987), and may be explained by decreased ANF clearance in the elderly (Ohashi et al, 1987). The significant partial

correlation of ANF and urinary sodium was in accord with observations that plasma ANF rises in normals on high sodium diets (Shenker et al, 1985; Sagnella et al, 1985).

The two-fold rise of plasma ANF after saline infusion (Figure 10.1) has also been recorded studies with a similar protocols (Sagnella et al, 1985; Singer et al, 1987a), but brisker saline challenges have resulted in a four-fold rise of basal values (Anderson et al, 1986a). Head-out water immersion, a procedure which causes central blood volume expansion of a similar order as a two litre saline infusion, produced comparable ANF rises to saline infusions (Epstein et al, 1986).

Does ANF contribute to the natriuresis after acute blood volume expansion ? In rats, both atrial appendectomy (Kobrin et al, 1985) and also the administration of antibodies raised against ANF (Stasch et al, 1988; Greenwald et al, 1988) blunted the natriuresis and fall in PRA and aldosterone after acute volume expansion. In contrast, in dogs (Goetz, 1988; Knapp et al, 1986) cardiac denervation abolished the natriuresis after expansion despite a rise of plasma ANF. Similar experiments can not be performed in humans, but the use of an ANF receptor blocking drug would help to clarify the role of ANF in this context.

In man, when the period of observation after a saline infusion was extended for 2-3 hours, ANF fell to basal values but natriuresis continued (Singer et al, 1987b). Therefore, other mechanisms must ensure the complete exretion of the acute sodium load. These could include a dilutional effect on plasma proteins, which would decrease renal proximal tubular reabsorption (Spitzer and Windhager, 1970), and the release of other unidentified natriuretic substances (de Wardener et al, 1961; Chapter 8).

Another means of determining the biological significance of small rises in ANF has been to record the effects of low dose intravenous infusions of the peptide. These experiments (Chapter 17; Espiner and Richards, 1989) suggest that physiological rises of plasma ANF do indeed cause natriuresis and suppression of the renin-aldosterone axis.

After volume expansion, over three quarters of an acute intravenous saline load enters the interstitial space, protecting against dangerous increases in blood volume (Manning and Guyton, 1982). Pharmacological doses of ANF increase the permeability of vascular walls, so the rise of ANF after volume expansion might contribute to movement of fluid out of the vascular compartment (Huxley at al, 1987). However, it is currently unknown whether a physiological rise of

plasma ANF could produce this effect.

The basal levels of plasma cGMP agrees with those obtained by other authors (Wood and Marks, 1978; Broadus el at, 1970). The non-significant rise of cGMP after saline infusion (Study B) could suggest that cGMP is not produced in response to a small rise of plasma ANF. Alternatively, the rise of intracellular nucleotide may have been too small to cause a significant increase of extracellular levels. In Chapters 17-19 in which I follow the effects of intravenous ANF infusions, the percent rise of plasma cGMP was always less than that of plasma ANF, confirming the pattern which I found after saline infusion. In contrast with my study, Weil et al (1985) documented a significant rise of plasma cGMP after a brisker saline infusion in healthy subjects.

Summary of Chapter 10.

1. The mean plasma ANF in 24 healthy subjects was 6.9±0.8 pmol/l and after intravenous infusion of two litres of isotonic saline, mean plasma ANF rose two-fold. Basal values and the response to volume expansion agreed with the results of other authors, supporting the use of the 'in-house' RIA in futher experiments.

2. Basal plasma levels of cGMP were similar to those measured by other authors, but rise after the saline infusion was not significant.

CHAPTER 11.

PLASMA ANF IN PATIENTS WITH CHRONIC RENAL FAILURE WHO DO NOT REQUIRE DIALYSIS.

Introduction.

Plasma (99-126) ANF values have been measured in patients with chronic renal failure (CRF) who did not require dialysis (Rascher et al, 1985; Hasegawa et al, 1986; Yamamoto et al, 1987; Shenker et al, 1987; Cannella et al, 1987; Predel et al, 1987). Some of these studies suffered from the following drawbacks.

1. Two groups used radioimmunoassay of unextracted plasma to measure ANF (Yamamoto et al, 1987; Cannella et al, 1987), a method which produces spuriously high ANF values (Richards et al, 1987; Chapter 2).

2. Some patients in two studies took diuretics and drugs used to treat hypertension (Hasegawa et al, 1986; Shenker et al, 1987). However, antihypertensive drugs alter plasma ANF: beta-adrenergic blockade increased ANF in hypertensives with normal and impaired renal function (Kingswood et al, 1987; Nakaora et al, 1987), and angiotensin converting enzyme inhibitors lowered ANF in normals and CRF patients (Mann et al, 1986; Kingswoood et al, 1987). Moreover, diuretic administration to volume expanded normal man caused large falls of plasma ANF (Kimura et al, 1986).

3. Plasma ANF rises with age in normals and patients with essential hypertension (Sagnella et al, 1986; Larochelle et al, 1987). If a similar relation exists in CRF, the conclusion of two studies are open to question. One study used healthy controls who were 30 years younger than the patients (Shenker et al, 1987) and another did not state the age of the controls (Predel et al, 1987).

4. In normals plasma ANF rises when changing from a low to high salt intake (Shenker et al, 1985) but neither the sodium intake nor urinary output has been quoted in previous CRF reports.

In this chapter I describe a group with CRF who took neither diuretics nor anti-hypertensive medication. ANF was measured by the extracted RIA and values were compared with those of healthy subjects of a similar age range (Chapter 10). The relation between ANF and creatinine clearance, arterial blood pressure, urinary sodium excretion, PRA and age was investigated.

Patients and Methods.

The study was approved by the Hospital Ethical Committee and patients gave informed consent. They numbered 36 (14 female) and were aged 19-67 years. They had a wide range of renal impairment but all had plasma

creatinine above 150 umol/l (creatinine clearance: range 4-67, mean 21 ml/min) but were not receiving dialysis. Primary diagnoses were: chronic glomerulonephritis (13), reflux nephropathy and chronic pyelonephritis (11), chronic urinary obstruction (3), adult polycystic disease (3), calculi (2), diabetic nephropathy (2), renal tuberculosis (1) and renal tubular acidosis (1). None took diuretics or antihypertensive drugs for at least one month prior to study. Patients with clinically significant valvular heart disease, malignant hypertension and acute illness were excluded.

Dietary sodium was not controlled but on the study day a 24 hour urine collection was made to measure sodium excretion. None had peripheral or pulmonary oedema and therefore it was assumed that sodium excretion approximated with intake.

Forearm venous blood was drawn between 0900-1200h, with the subject in the sitting position, for plasma ANF, plasma renin activity (PRA), sodium and creatinine. Creatinine clearance and fractional excretion of sodium (only measured in patient group) were calculated by standard formulae. Blood pressure was measured once by mercury sphygmomanometer directly before venesection and expressed as mean blood pressure (MBP: diastolic plus one third pulse pressure). The 24 healthy subjects described in Chapter 10 were used as a control group.

Statistics.

Results are given as mean±SEM. Comparisons between groups were made using unpaired Student's t tests. In order to study the levels of ANF in 'hypertensive' patients, they were divided into subgroups with MBP under 110 mmHg (n=19) and with higher pressures (n=17). This division was somewhat artificial because pressure formed a continuum within the whole group, but allowed comparison with results of another study which used the same definition (Yamamoto et al, 1987). Moreover, an identical division has been used by Weidmann (1984) in a major review of hypertension in renal disease.

In addition, simple Pearson and partial correlations were sought between plasma ANF and other variables. Fractional sodium excretion showed a skew distribution and was converted into $\log_{10}$ for statistical manipulations. Non-parametric Spearman Rank correlations were also performed but the results are not quoted because they closely resemble the parametric correlations.

Lastly, the 95% confidence intervals (CI) are given both for the comparison of means and the correlation coefficients (Gardner and Altman, 1986; Altman and Gardener, 1988). Results were considered significant when $p<0.05$.

Results.

Raw data are shown in the Data Appendix (Chapter 22).

The mean plasma ANF was significantly elevated ($p<0.01$) in the patients compared to controls (Figure 11.1 and Table 11.1). The difference between the means was 6.8 pmol/l (95% CI 2.3 to 11.1 pmol/l). In addition, in the CRF group as a whole, MBP was significantly higher ($p<0.01$) than in the controls (difference between means 20 mmHg; 95% CI 13 to 27 mmHg). Age, daily sodium excretion and PRA did not differ between these groups.

The sub-group of 17 patients with pressures $\geq$110 mmHg had a significantly higher ($p<0.05$) plasma ANF than 19 patients with lower pressures (difference between means of 7.4 pmol/l; 95% CI 0.8 to 13.9 pmol/l) (Figure 11.2). Both patient sub-groups had similar mean ages, creatinine clearances and urinary sodium excretions (Table 11.2). It was striking that the mean ANF in the 'normotensive' patients did not differ significantly from that of the healthy controls despite their impaired renal function.

Simple univarate analysis in the whole patient group (Table 11.3) showed significant positive relationships between plasma ANF and age ($r= +0.45$, $p<0.01$), MBP ($r= +0.42$, $p<0.01$) and fractional sodium excretion ($r= +0.34$, $p<0.05$). Significant negative correlations were found between ANF and PRA ($r= -0.38$, $p<0.05$) and

Table 11.1.

Basal plasma ANF in patients with CRF.

	Age (years)	Urinary Sodium (mmol/day)	MBP (mmHg)	ANF (pmol/l)	PRA (nmol/l/h)
Healthy Controls (n=24)	42 ±3	140 ±10	87 ±2	6.9 ±0.8	1.21 ±0.16
Patients (n=36)	43±3	119±8	107±2**	13.7±1.7**	1.17±0.29

** p <0.01 versus to healthy controls,
otherwise p > 0.05.

Key: MBP = mean blood pressure, ANF = atrial natriuretic factor, PRA = plasma renin activity.

Table 11.2.

Plasma ANF in normotensive and hypertensive CRF subgroups.

	Age (years)	Urinary Sodium (mmol/day)	Creatinine Clearance (ml/min)	ANF (pmol/l)
MBP<110 mmHg (n=19)	43±4	122±13	22±4	10.2±1.5
MBP>110 mmHg (n=17)	44±4	116±9	21±4	17.6±3.0 *

* $p<0.05$ between the two patient groups, otherwise $p>0.05$.

Table 11.3.

Correlations with plasma ANF in the whole CRF group.

	PRA	MBP	Urinary Sodium	Fe.Na	Age	Creatinine Clearance
Simple	* -0.38	** +0.42	NS	* +0.34	** +0.45	# -0.40
Partial	NS	# +0.41	NS	NS	* +0.35	NS

NS = $p>0.05$, * $p<0.05$, # $p=0.01$, ** $p<0.01$.



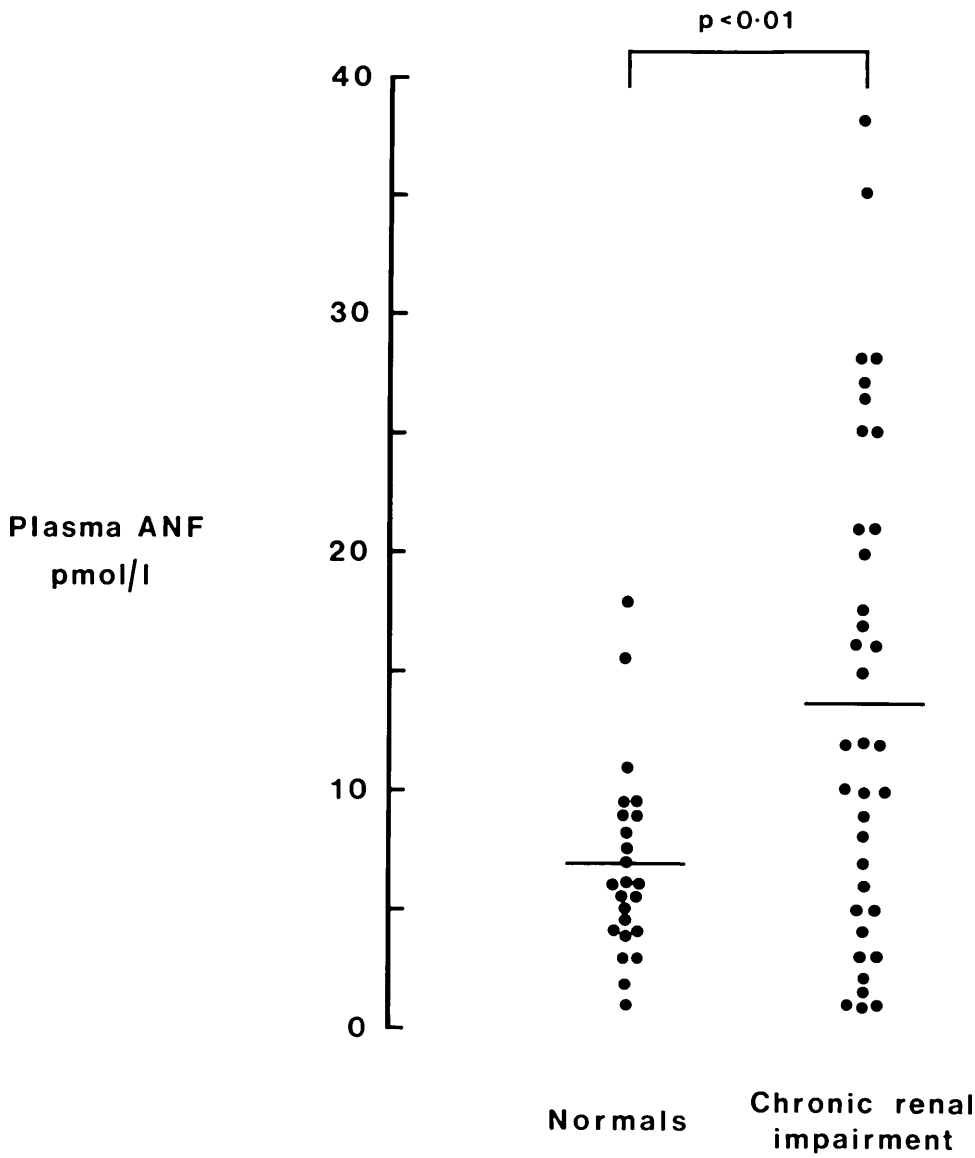


Figure 11.1.

Plasma ANF in patients with chronic renal failure.

The horizontal bars indicate mean values.

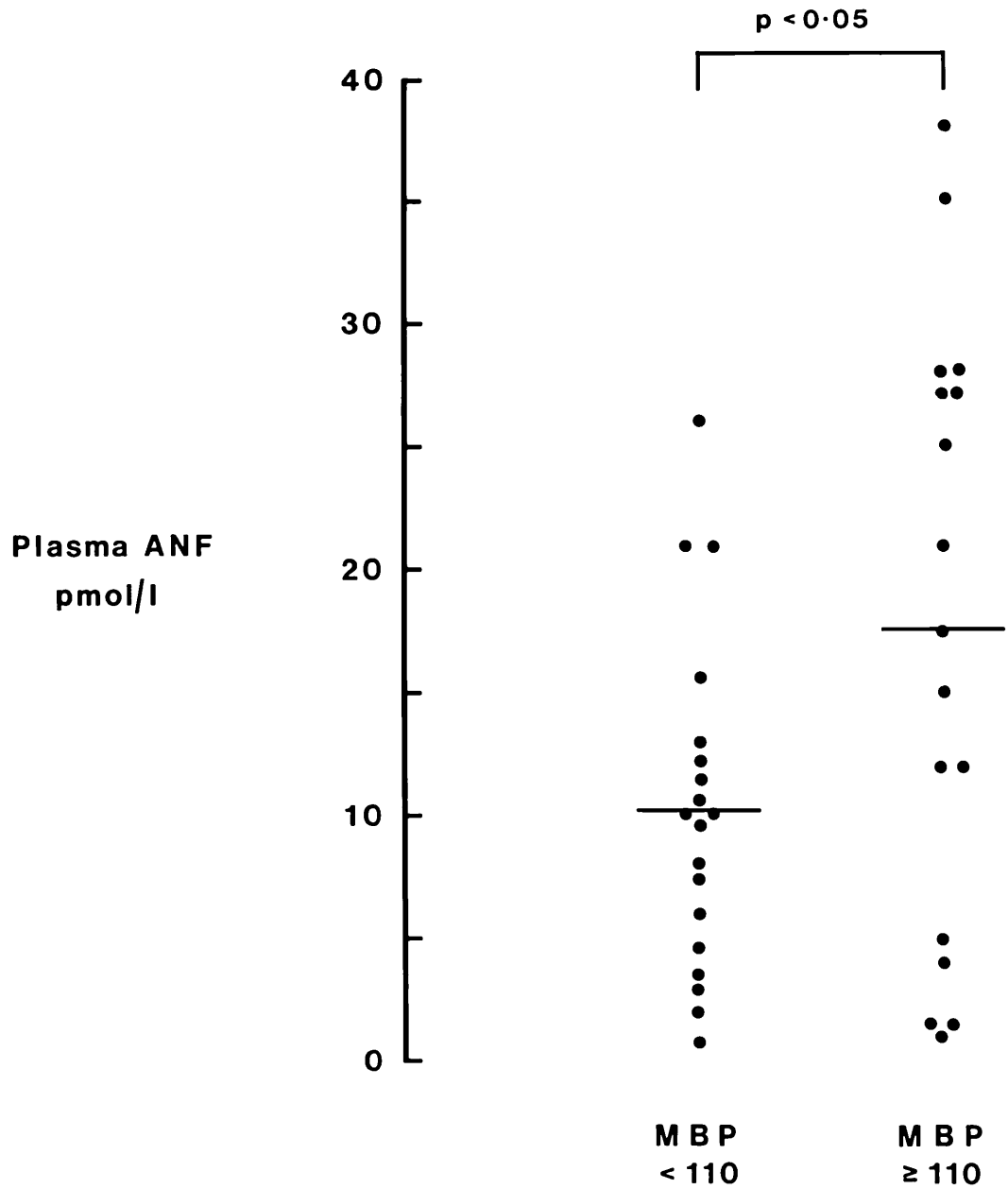


Figure 11.2.

Plasma ANF in chronic renal failure patients with a mean blood pressure <110 and ≥110 mmHg.

The horizontal bars indicate mean values.

creatinine clearance (r= -0.40 p=0.01). No significant relation existed between ANF and total urinary sodium excretion.

Partial correlations were sought with plasma ANF as the dependent variable and age, MBP etc as independent variables. ANF was related to MBP (r=+0.41, p=0.01; 95% CI +0.65 to +0.10) and to patient age (r= +0.35, p<0.05; 95% CI +0.61 to +0.02). The relationship of ANF with creatinine clearance, PRA and fractional sodium excretion was no longer significant after correcting for the effects of other variables.

Multiple regression analysis demonstrated that age and MBP could together account for 0.35 of the variation in plasma ANF (r=0.59, p<0.001) and if the contribution from creatinine clearance was added, 0.45 was accounted for (r=0.67, p<0.001). Adding the contributions of PRA, total or fractional urinary sodium did not significantly increase the variation of ANF which could be explained.

Discussion.

The immunoreactive ANF measured in these patients may be a slight, but unknown, overestimate of the true levels of (99-126) ANF. This is because fragments of the ANF propeptide circulate in the plasma of some patients with severe CRF, and may cross-react with

antisera directed to the carboxy-terminus of (99-126) ANF (Itoh et al, 1988a). However, in both health and in renal failure, the majority of material which reacts with antisera to (99-126) ANF does indeed correspond with the 28 amino acid peptide (Reviewed in chapter 2).

The mean plasma ANF of the CRF group was higher than that of the healthy adults, a conclusion shared by four other studies (Hasegawa et al, 1986; Yamamoto et al, 1987; Shenker et al, 1987; Predel et al, 1987). However, Rascher el al (1985) found normal ANF levels in children with CRF who did not require dialysis, possibly because of the small number of patients (n=6). In contrast to my study, Shenker et al (1987) found little overlap between ANF in patients and normals. However, if ANF values are age-related, Shenker's results may be explained by the youth of the healthy control group. Hasegawa et al (1986), using an extracted assay, reported values similar my own, but assays of unextracted plasma gave levels five to ten-fold higher (Yamamoto et al, 1987; Cannella et al, 1987).

The raised ANF is compatible with reports of a raised exchangeable body sodium in mild to moderate renal failure, and of an increased extracellular volume found in most patients with severe renal failure before dialysis is started (Weidmann, 1984).

I found a weak but significant simple and partial correlation between plasma ANF and MBP, confirming simple correlations found by other groups (Hasegawa et al, 1986; Yamamoto et al, 1987; Predel et al, 1987). Indeed, in the subgroup of patients with a MBP of <110 mmHg, ANF was not significantly higher than controls of a similar age, despite the presence of renal impairment. This agrees with the observation by Yamamoto et al (1987) that plasma ANF was not raised in patients with severe renal failure who were normotensive (MBP <110 mmHg) and had a normal cardiothoracic ratio.

The single blood pressure measurement used in my study may not give a precise idea of the severity of the hypertension. Knowledge of the duration of hypertension and ECG criteria of left venticular hypertrophy may have given more insight into the relation between ANF and blood pressure but that data was not available in the current analysis.

In mild essential hypertension most authors have found that plasma ANF is identical to (Larochelle et al, 1987; Genest et al, 1988) or marginally elevated compared to carefully age-matched normals (Montorsi et al, 1988). Atrial pressures are elevated in this disorder, and Genest et al (1988) suggested that the atria may be 'hyporesponsive', and the lack of ANF could contribute to the genesis of the hypertension.

In contrast, ANF was markedly raised in more severe essential hypertension (Sagnella et al, 1986).

Cangiano et al (1976) found an increased plasma volume in hypertensive CRF patients, and Tarazi et al (1970) described a positive relationship between plasma volume and blood pressure in a similar group. In normals ANF secretion is stimulated by blood volume expansion (Chapters 8 and 10) and in CRF the correlation of plasma ANF and arterial blood pressure may reflect a partial dependence of pressure on blood volume. Although Cannella et al (1987) found no relation between plasma ANF and blood volume in CRF, some patients took salt-restricted diets and their blood volumes, which fell in the normal range, may not have represented the untreated state.

If ANF were a true measure of volume expansion, then PRA should be suppressed. However, PRA in my CRF group was not low. This agrees with some other observations: for example, PRA was raised inappropriately for the level of plasma volume in hypertensive patients with CRF (Cangiano et al, 1976), and did not suppress completely after volume expansion (Warren and Ferris, 1970). The cause of this distorted relationship is not known, but the inappropriate interplay of the renin-angiotensin axis and body sodium correlates significantly with hypertension in CRF before the start

of dialysis (Davies et al, 1973; Weidmann, 1984).

An alternative explanation for the discrepancy between the raised plasma ANF and normal PRA is that the elimination of ANF was severely impaired in the patients, so that the levels were inappropriately high for the blood volume. The isolated kidney removes over 80% of ANF in one passage of perfusate (Weselcouch et al, 1985), and renal tissue contains potent enzymes capable of breaking down ANF (Raine et al, 1989). Despite this, nephrectomised and hepatectomised rats clear radiolabelled ANF as rapidly as normals (Murthy et al, 1986a), and in healthy man the clearance of plasma ANF exceeds 5 l/min (Gillies et al, 1987).

Therefore, even if the kidneys removed all ANF in one transit of plasma, they could account for only a fraction of the total clearance. In patients who required dialysis, and therefore had lower GFR that my current group, the half-life of immunoreactive ANF was prolonged by 30% (Tonolo et al, 1988).

In normal man ANF is cleared across many different vascular beds (Espiner and Nicholls, 1987): vascular strips take up plasma ANF which is destroyed by lysosomes (Hirata et al, 1985) or processsed into smaller peptides (Murthy et al, 1986b). While it is possible that clearance by these extrarenal sites is impaired in CRF, this theory is not currently proven.

In my patients there was no significant relation between ANF and creatinine clearance after the variation caused by age and MBP had been taken into account. Although there was a significant simple inverse correlation between ANF and creatinine clearance, the true relation between the two variables was confounded by the tendency for older patients to have lower clearances (age versus clearance, $r= -0.31$, $p<0.05$) and the tendency of plasma ANF to rise with age (Table 11.3). In contrast, other studies found a highly significant inverse relation between ANF and renal function, but only univarate analysis was used (Shenker et al, 1987; Predel et al, 1987; Cannella et al, 1987).

In uraemia sodium balance is maintained, until renal function is severely impaired, by an increased fractional excretion of the ion, and a circulating natriuretic substance has been postulated to explain the increased tubular rejection of sodium (Bourgoignie et al, 1974). In the current series there was a striking negative relation between creatinine clearance and fractional sodium excretion ($r = -0.88$, $p<0.01$) as would be expected from other studies (Slatopolsky et al, 1968). After correction for age and MBP, no significant relation was discerned between plasma ANF and either daily or fractional urinary sodium excretion, and therefore this study did not support a

natriuretic role for ANF in CRF. However, the sodium excretion was expressed as an integrated value over 24 hours, and comparison with a single morning ANF may not have been valid. Plasma aldosterone, the degree of tubular structural damage and natriuretic substances other than ANF might also be important in determing sodium excretion and could have confounded any relationship between ANF and sodium excretion (Berl et al, 1978; Uribarri et al, 1983; Fine et al, 1976).

The positive relation ANF with age found in the current study has been observed by other authors both in of healthy adults (Sagnella et al, 1986; Montorsi et al, 1988) and in essential hypertensives (Larochelle et al, 1987). The relation may reflect decreased clearance of ANF (Ohashi et al, 1987) or a tendency for atrial pressures to be higher in the elderly (Ehrsam et al, 1983).

By combining MBP, age and creatinine clearance only 45% of the variation in plasma ANF could be explained. The the rest of the variation may comprise (apart from experimental error) cardiac valvular disease and mild degrees of heart failure that were not apparent on clinical examination.

Summary of Chapter 11.

1. The mean plasma ANF was raised two-fold in a group of 36 patients with chronic renal failure, with creatinine clearances of 4-67 ml/min, compared to 24 healthy subjects of a similar age range and who excreted a similar quantity of urinary sodium per day.

2. In a subgroup of normotensive patients (mean blood pressure less than 110 mmHg), with a mean creatinine clearance of 22 ml/min, mean plasma ANF was slightly but not significantly higher than normal. The positive relation of ANF and mean blood pressure in the patients suggests a partial dependence of arterial pressure on blood volume.

3. There was a significant positive relation between age and ANF, as previously described in healthy subjects. No significant independent relation was found between plasma ANF and either creatinine clearance or urinary sodium excretion.

4. There was no single factor or combination of factors measured in this study which could explain the majority of the variation of plasma ANF.

CHAPTER 12.

PLASMA LEVELS OF ANF IN CHRONIC HAEMODIALYSIS PATIENTS.

Introduction.

Plasma levels of ANF have been measured in patients who require chronic dialysis, and the acute effects of haemodialysis, haemofiltration and isolated ultrafiltration have been followed (Wilkins et al, 1986; Eisenhauer et al, 1986; Zoccali et al, 1986; Anderson et al, 1986b; Walker et al, 1987).

The source of the blood for ANF measurement in these studies was not always stated, and one group used arterial blood from shunts and arterialised blood from fistulae (Anderson et al, 1986b). In healthy subjects and patients with renal failure, arterial ANF values can exceed venous values in the same limb (Sato et al, 1986; Ogawa et al, 1986) because ANF is cleared by peripheral vascular beds.

In this chapter I have measured peripheral venous ANF in similar patients, and compared these values with healthy subjects and patients with renal impairment who did not require dialysis (Chapters 10 and 11). In addition, the effects of acute haemodialysis on plasma ANF and cGMP were studied.

Study A.

11 patients aged 25-61 years (5 female) were studied. All had received regular twice-weekly haemodialysis for between 6 months and 5 years. None was acutely ill or had clinically significant valvular or ischaemic heart disease. None had required nephrectomy. Hypotensive drugs were stopped for one month before the study. Two days after the previous haemodialysis forearm venous blood was sampled between 0900-1200h with the subject sitting quietly. Before venesection, blood pressure was measured once by mercury sphygmomanometer.

Study B.

The acute effects of haemodialysis was studied in 8 patients who required regular dialysis, and had their own kidneys in situ. Three patients took regular atenolol and/or nifedipine. However, these drugs were omitted on the morning before the dialysis. Blood was drawn from a peripheral vein, from the arm opposite the arteriovenous fistula, immediately before dialysis began and again immediately after the end of dialysis. Arterial blood pressure was measured before and after dialysis by mercury sphygmomanometer.

Dialysis was performed for six hours using a Monitral S pump (BSM 22) and either a 1 m^2 hollow fibre (Discap

110) or 0.7 m^2 plate dialyser (Biospal 1800 S). The composition of the dialysate was: sodium 137 mmol/l, calcium 1.53 mmol/l, potassium 2.0 mmol/l, magnesium 0.85 mmol/l, chloride 104 mmol/l and acetate 40 mmol/l. Ultrafiltration was performed to attain 'dry weight' (further weight loss would cause cramps or symptomatic hypotension).

Statistics.

Results were expressed as median and range because the number of patients was small and ANF showed a skew distribution. For comparison between groups I used unpaired Wilcoxon Rank tests (haemodialysed patients versus normals and patients with renal impairment) and paired Mann Whitney tests (pre- versus post-dialysis). Correlations between ANF and other variables were sought using the non-parametric Spearman rank test (R_S). Results were considered significant when $p<0.05$. Raw data are given in Chapter 22.

Results of Study A.

Plasma ANF was significantly elevated in the dialysis group compared to both healthy subjects and patients with CRF who did not require dialysis (Figure 12.1), despite similar age ranges (Table 12.1). MBP was significantly raised in the dialysis group compared to normals but was not different to MBP of the patients

with less severe renal failure (Table 12.1).

Within the dialysis group plasma ANF was significantly correlated with age (R_s +0.82, $p<0.01$) and PRA (R_s +0.72, $p<0.01$) but the relation with MBP was was not significant (R_s +0.36, $p>0.05$).

Table 12.1.

Basal ANF in the dialysis, renal impairment and healthy groups.

	Age (years)	MBP (mmHg)	Plasma ANF (pmol/l)
Dialysed (n=11)	40 (25-63)	101 (95-114) **	28.8 (12.3-124.0) **/++
Renal Impairment (n=36)	46 (19-66)	109 (80-140)	12.0 (0.9-37.6)
Healthy Controls (n=24)	39 (24-72)	85 (70-106)	6.2 (1.5-18.1)

Note that all results are median (range) in order to allow comparisons with the dialysis group, whose data showed skew distributions. ** $p<0.01$ dialysed versus controls, ++ $p<0.01$ dialysed versus patients with renal impairment. Otherwise $p>0.05$.

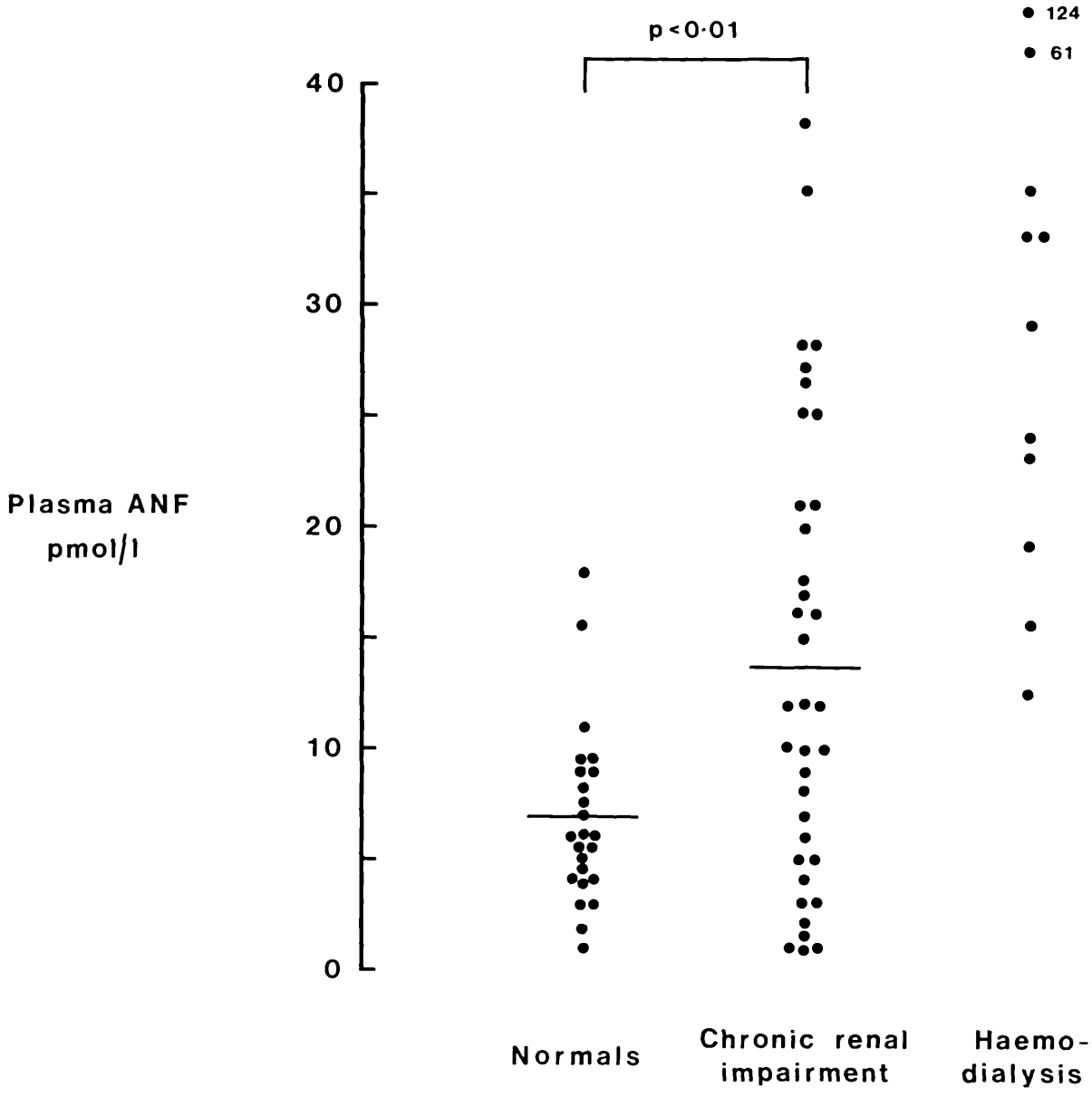


Figure 12.1.

Plasma ANF in normals, patients with chronic renal impairment and haemodialysis patients.

Table 12.2.

The effect of acute haemodialysis on plasma ANF.

	Before Dialysis (n=8)	After Dialysis (n=8)
ANF (pmol/l)	27.6 (18.8-69.5)	* 19.7 (13.3-51.9)
Cyclic GMP (nmol/l)	14.6 (9.0-21.1)	* 10.4 (7.1-16.8)
PRA (nmol/l/h)	0.94 (0.52-1.60)	* 1.16 (0.79-2.29)
MBP (mmHg)	100 (87-113)	103 (80-110)

* $p<0.05$ Post versus pre-dialysis values, otherwise $p>0.05$.

Healthy sitting ranges:

ANF 1.5-18.1 pmol/l (Chapter 10).

cGMP 1.2-7.7 nmol/l (Chapter 10 and 17).

PRA 0.46-4.08 nmol/l/h (Chapter 10).

Results of Study B.

Plasma ANF and cGMP values fell significantly ($p<0.05$), although both remained elevated compared to healthy control ranges (Table 12.2). The range of basal PRA was within the healthy range and levels of rose slightly but significantly ($p<0.05$) by the end of dialysis. No change was observed in MBP. All patients lost weight (range 0.2-4.7 kg).

Discussion.

Interdialysis immunoreactive plasma (99-126) ANF was high and fell during acute haemodialysis which was accompanied by weight loss. These results agree with reports of other authors (Walker et al, 1987; Anderson et al, 1986b). Is is of note that plasma ANF was still raised in some patients even after weight removal during dialysis.

Zoccali et al (1986) performed isolated ultrafiltration (fluid removal without dialysis) and found that the changes of plasma ANF correlated with reductions of cardiac atrial pressure. Eisenhauer et al (1986) reported that isovolaemic haemofiltration (removal of protein-free plasma with equivalent simultaneous crystalloid relacement) did not alter plasma ANF, despite immunoreactive peptide appearing in the haemofiltrate. Kingswood et al (1987) found significant

falls of interdialysis plasma ANF in 6 patients after two weeks of dialysis against a low bath sodium concentration, a manouevre which also reduced exchangeable sodium and arterial blood pressure. These results support the hypothesis that plasma ANF in these patients is sensitive to manipulations of sodium and blood volume status, and that plasma ANF is not simply determined by impaired renal clearance.

However, why do plasma levels of ANF do not always fall into the healthy adult range after dialysis or ultrafiltration which achieves 'dry-weight' ? In response, many patients with end-stage renal failure have ischaemic and hypertensive heart disease and their atria may not be 'healthy' even after fluid removal (Huting et al, 1988). For instance, such hearts may require higher filling pressures to maintain an adequate cardiac output.

Walker et al (1987) recorded a significant positive relation between ANF and blood pressure in patients who required regular dialysis. In contrast, I found no significant correlation between ANF and arterial pressure. In my group, although ANF fell after dialysis this was not accompanied by a fall in blood pressure. Moreover, Walker et al (1987) and Anderson et al (1986b) found no correlation between the changes of ANF and of arterial pressure during acute dialysis and ultrafiltration.

However, blood pressure in this context is not only dependent on volume status but is also influenced by the use of acetate versus bicarbonate in the dialysate, the integrity of the autonomic nervous system and by the ability to secrete renin in response to volume subtraction (Imai et al, 1981; Hsu et al, 1984).

Taken together, these observations suggest that the relation of blood volume, and hence ANF, with blood pressure in haemodialysis patients is likely to be complex. Dathan et al (1973) observed that the positive correlation between blood pressure and volume which was found in patients before commencing regular dialysis was lost once regular dialysis was started. Similarly, the relation of ANF with arterial pressure might also be lost, if the peptide level is an index of blood volume.

Inappropriate activity of the renin-angiotensin system may play a role in causing hypertension in end-stage renal failure (Weidemann, 1984; Warren and Ferris, 1970), and it was interesting to find normal levels of PRA in the dialysis group in the presence of raised ANF: indeed, I found an un-physiological positive correlation between ANF and PRA in the group (R_S= +0.72, $p<0.01$).

Plasma levels of cGMP were high before dialysis and

fell towards but not into the normal range after the procedure. Increased cGMP clearance by the dialyser may have contributed to this effect, or alternatively the fall could be explained by a diminished production of the nucleotide in response to a fall of ANF.

What effects could ANF have in patients with minimal residual renal function ? Tonolo et al (1988) infused a similar group with synthetic ANF and recorded a rise of plasma protein and haematocrit. Therefore, in the face of sodium retention, high plasma ANF might prevent dangerous rises of blood volume by increasing vascular hydraulic permeability (Huxley et al, 1987).

Summary of Chapter 12.

1. In 11 patients who required regular haemodialysis, immunoreactive peripheral venous ANF, measured on a day midway between twice-weekly dialysis, was high and the range of values showed little overlap with healthy controls.

3. In 8 patients acute haemodialysis, accompanied by a fall in weight, was associated with a fall of plasma ANF.

2. The high basal plasma cGMP values and their fall during haemodialysis, suggests that ANF may exert a tissue effect in these patients.

CHAPTER 13.

THE EFFECT OF RENAL TRANSPLANTATION ON PLASMA ANF.

Introduction.

As discussed Chapter 12, plasma ANF is high in patients who require regular haemodialysis, and values fall toward the healthy range after an acute dialysis accompanied by weight reduction. After successful renal transplantation rapid alterations in plasma volume may be expected. In this study I have measured plasma ANF in 4 subjects after cadaveric transplantation and investigated the relation with plasma cGMP, right atrial pressure, weight changes and urinary sodium excretion.

Patients and Methods.

Four patients (A,B,C and D), aged 25-45 years, gave informed consent for the study which was approved by the Hospital Ethical Committee. End-stage renal failure (primary diagnoses: A, malignant hypertension; B, pyelonephritis; C, glomerulonephritis; D, diabetic nephropathy) had been treated with haemodialysis for 1-5 years. None had clinically significant valvular nor ischaemic heart disease. Although three had received anti-hypertensive medication (beta-blockade and nifedipine), these drugs were not administered after

transplantation. They took prednisolone (0.5 mg/kg/day) and cyclosporine (14 mg/kg/day for the first week and 10mg/kg/day thereafter), and none developed cyclosporine toxicity.

The following measurements were made immediately before transplantation and daily thereafter, for 8 to 23 days: weight, plasma and urinary creatinine and sodium, plasma ANF and PRA. In addition, plasma aldosterone and cGMP were measured in patients A, B and C. All blood was sampled from a forearm vein between 0800-1000h, with the patient sitting. During the first 72 hours after transplantation daily right atrial pressures were measured through a cannula inserted through the jugular vein. On the first post-operative day intravenous fluids were administered, but the patients took oral fluids thereafter. No attempt was made to induce a 'forced-diuresis' by overtransfusion of intravenous fluids.

Statistics.

For each patient the correlation of plasma ANF with plasma cGMP, weight, mean blood pressure and total and fractional excretion of sodium was sought. Much of the data showed a skew distribution and the non-parametric Spearman rank correlation (R_s) was used. A relation was considered significant when $p<0.05$. Raw data are given

in the Data Appendix (Chapter 22). The daily levels of ANF, aldosterone, weight, creatinine clearance and fractional sodium excretion of patient B are shown in Figure 13.1. Figure 13.2 follows daily plasma ANF, aldosterone and cGMP in three successful transplants.

Results of Three Successful Transplants.

Transplant function in patients A,B and C was adequate and no further haemodialysis was required. They showed no clinical signs of fluid retention and diuretics were not used. Their creatinine clearances rose to 55-78 ml/min after 5-13 days.

ANF levels were high immediately before transplantation (range 15.6-36.1 pmol/l), then fell over the next two weeks after transplantation (Figures 13.1 and 13.2). By the end of the study ANF had fallen to within the healthy range (A: 3.8 pmol/l; B: 2.0 pmol/l; C: 17.2 pmol/l). In patient B, ANF fell steadily during a period when creatinine clearance changed from only 40-46 ml/min (Figure 13.1).

Their weight decreased (by 2-6 kg) after transplantation and there was a positive correlation of plasma ANF with weight which was statistically significant in two patients (A: R_S= +0.47, $p>0.05$; B: R_S= +0.78, $p<0.01$; C: R_S= +0.75, $p<0.01$).

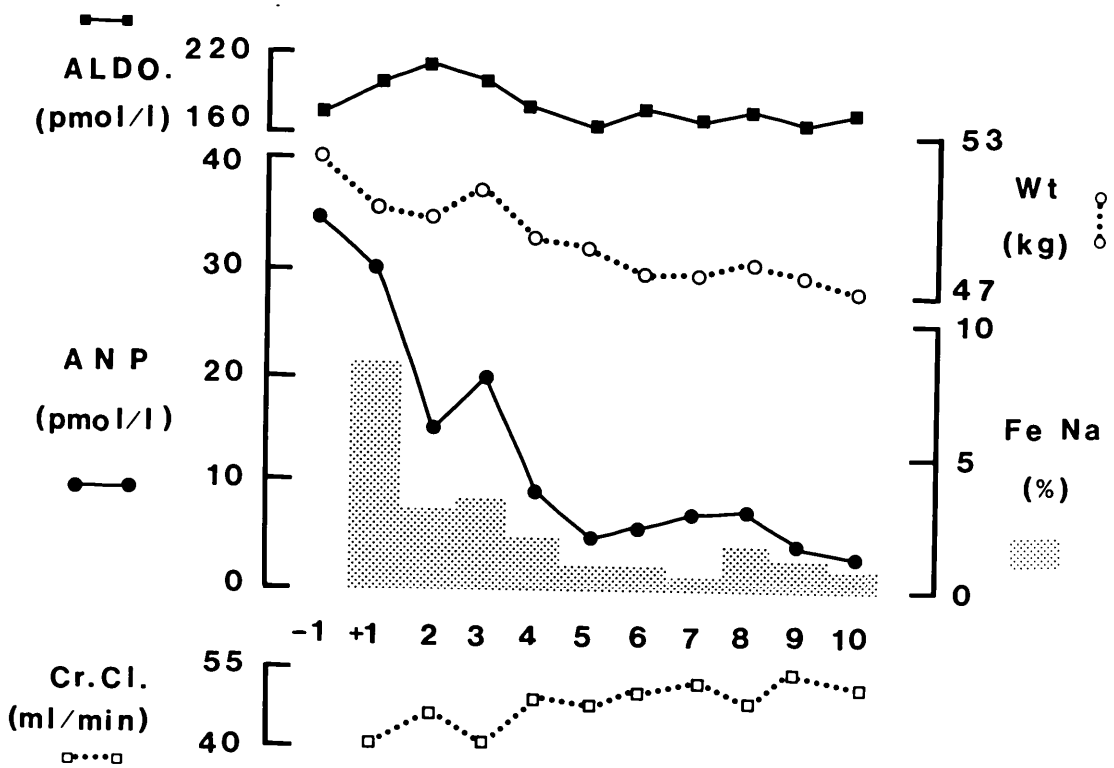


Figure 13.1.

Daily ANF levels in a successful renal transplant.

Key: -1 is the day before transplantation, +1 and onwards are the days after operation. ANP = atrial natriuretic factor, ALDO = plasma aldosterone, Cr.Cl = creatinine clearance, Wt = weight, FeNa = fractional sodium excretion.

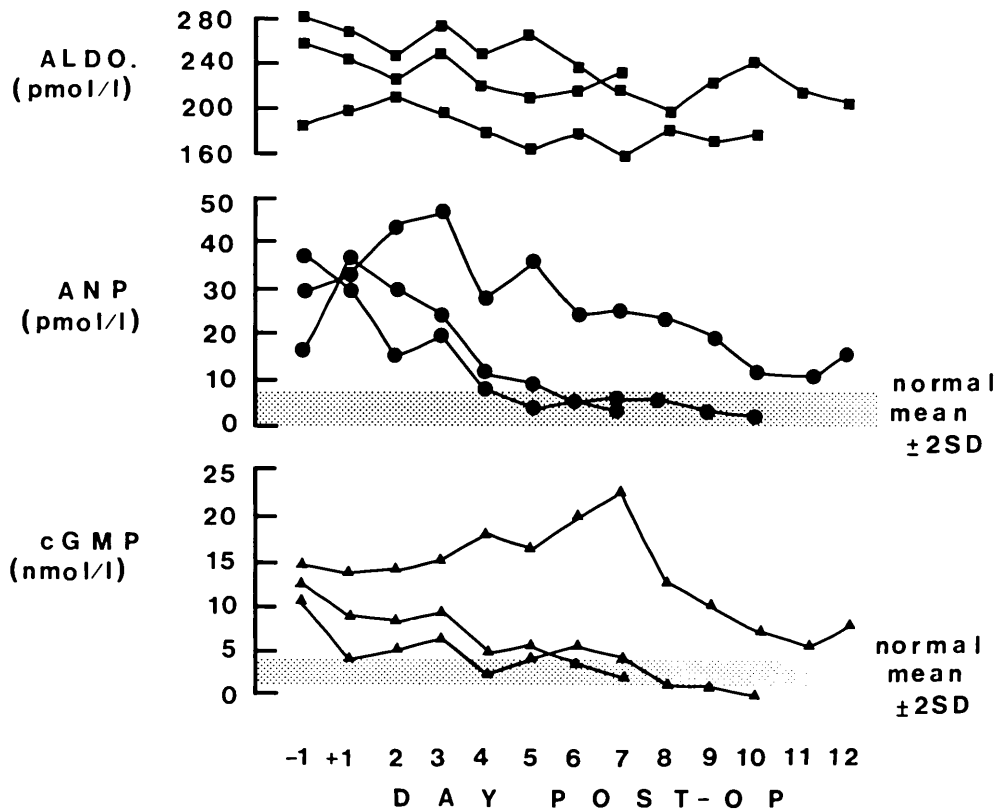


Figure 13.2.

Daily levels of plasma ANF, aldosterone and cGMP in three successful renal transplants.

Note that he shaded areas are the healthy mean±2SD at the time of the study (n=14).

Plasma cGMP levels were high immediately before transplantation (range 10.4-14.4 nmol/l) and tended to fall in parallel with plasma ANF. The correlations between ANF and cGMP in individual patients were: A: R_S= +0.81, p<0.01; B: R_S= +0.74, p<0.01; C: R_S= +0.61, p=0.01.

Fractional excretion of sodium was high on the first post-operative day (8.6-19.0%) and fell towards normal over the next two weeks (0.7-1.8% at end of study). There was a positive correlation between ANF and fractional excretion but this was statistically significant in only one patient (A: R_S= +0.47, p>0.05; B: R_S= +0.87, p<0.01; C: R_S= +0.38, p>0.05). The total sodium excretion per day held no definite pattern throughout the study and showed no significant relation to plasma ANF.

Aldosterone levels tended to fall during the study, and PRA remained in the low/normal range. Neither PRA nor aldosterone showed a significant relationship with ANF.

Results of the Unsuccessful Transplant.

In contrast, the creatinine clearance of patient D did not rise above 15 ml/min. Transplant biopsy showed acute vascular rejection but treatment with intravenous methylprednisolone did not improve renal function. In the final study week, a weight gain of 5.6kg occurred

and severe peripheral oedema appeared. ANF remained strikingly elevated (peak value 58 pmol/l), but fractional sodium excretion remained less than 1% and daily sodium excretion was only 10-20 mmol. However, high-dose oral frusemide initiated a natriuresis, increasing fractional sodium excretion to 5-8% and daily sodium excretion to 140-200 mmol.

In the group as a whole there was a significant positive correlation between plasma ANF drawn from the peripheral venous system and right atrial pressure (R_S= +0.78, $p<0.05$).

Discussion.

Pre-transplant plasma ANF levels were high, in agreement with previous reports of subjects requiring chronic haemodialysis (Chapter 12). In three patients, with no clinical evidence of rejection, the reduction of volume overload, reflected by daily weight changes, was associated with a fall in ANF levels. Moreover, the positive relation of plasma ANF with right atrial pressure agreed with previous observations made in healthy subjects (Sato et al, 1986) and in patients who required regular haemodialysis (Zocalli et al, 1986).

Although improved renal function may have contributed to the fall of ANF by increasing renal clearance, in patient B large changes in ANF occurred over a period

of four days when renal function was relatively stable (Figure 13.1), so that renal excretory function could not have been the sole determinant of the ANF fall.

The fall in plasma cGMP showed a close parallel with that of ANF. In normals 15% of the total clearance of cGMP from the plasma is attributable to renal excretion (Broadus et al, 1970) and thus improved kidney function may have played a part in the fall of plasma nucleotide. Alternatively, the fall in plasma ANF may have resulted in a decrease of cGMP production by vascular and renal tissues.

There was a striking fall in the fractional excretion of sodium following transplantation, but this was only significanlty correlated with ANF in one subject. Even though the high levels of ANF in the first few days after transplantation might have contributed to the high fractional sodium excretion in these patients (none of whom took diuretic drugs), there must be multiple factors responsible for the enhanced tubular rejection of sodium. These include mild tubular necrosis and the osmotic natriuresis resulting from the glomerular filtration of large amounts of urea.

A negative correlation between ANF and PRA has been recorded in normal man during ANF infusions (Richards et al, 1988). However, I found no such relationship in this study and the use of cyclosporine, which can

suppress PRA by an unknown mechanism, may have altered the expected relationship (Bantle et al, 1985).

In patient D, natriuresis was minimal, despite the highest levels of plasma ANF. Severe vascular rejection may have prevented the action of ANF, but the mechanism of this interference is not known.

One study has followed the effects of pharmacological doses of ANF (0.7 mg over 30 minutes) administered to oliguric renal transplants within two days of grafting (Bozkut et al, 1987). In all six patients, pulmonary and systemic arterial blood pressures fell, and in a few subjects urine flow rate increased. However, the physiological significance of these results is uncertain because plasma ANF levels, which were not measured, were likely to have been extremely high.

Summary of Chapter 13.

1. After succesful renal transplantation plasma ANF and cGMP fell rapidly towards the normal aldult range.

2. The fall in ANF may indicate the reduction of volume overload as reflected by a fall in patients' weight and the correlation of ANF with right atrial pressure.

3. During graft rejection plasma ANF remained very high, but natriuresis was minimal.

PART III.

CHAPTER 14.

GLOMERULAR FILTRATION RATE, PROTEINURIA AND NATRIURESIS: A REVIEW.

Introduction.

Part III of the thesis explores the effects of ANF on the kidney, with respect to natriuresis and proteinuria. This chapter introduces the basic mechanisms of glomerular filtration and urinary sodium excretion, and decribes the pathogenesis of glomerular proteinuria.

Glomerular Filtration Rate (GFR).

The mechanisms of filtration have been studied in the Munich-Wistar rat, an unusual strain which has surface glumeruli within reach of micropuncture (Brenner et al, 1985). Single nephron glomerular filtration rate (SNGFR) is expressed as:

$$SNGFR = K_f.PUF.$$

K_f is the ultrafiltration coefficient of the glomerular barrier, itself the product of k, the effective hydraulic permeability of the capillary wall, and S, the filtration surface area. In this animal, the permeability of the glomerular capillary is one hundred times greater than in other organs. PUF is the mean net

ultrafiltration pressure, being the sum of the opposing forces of glomerular capillary hydraulic pressure and plasma protein oncotic pressure.

In the glomerular capillary of this strain of rat filtration stops before the efferent glomerular capillary at the point of 'filtration pressure equilibrium'. A mathematical model predicts that, as glomerular plasma flow increases, this point becomes displaced further from the afferent arteriole because oncotic pressure increases less steeply along the length of the capillary. Consequently SNGFR increases in proportion to plasma flow until the point of equilibrium reaches the most distal end of the capillary filtration surface: further rises of plasma flow cause proportionately lesser increases of SNGFR. Although this theoretical dependence of ultrafiltration on plasma flow has been confirmed in some species of rat during plasma volume expansion, there is uncertainty whether the principle applies in other mammals.

The same model predicts that an isolated increase of mean glomerular hydrostatic pressure produces only a small rise of SNGFR because of a concomitant rise of oncotic pressure which limits the rise in PUF by displacing the point filtration pressure equilibrium towards the afferent end of the glomerular capillary.

In the isolated kidney, renal blood flow is autoregulated over a wide range of perfusion pressures, an ability abolished by muscle relaxants, such as papaverine. SNGFR is also preserved despite fluctuations of perfusion pressure, a process which may be mediated by angiotensin II generated within the kidney. Indeed, angiotensin converting enzyme inhibition destroys the ability of the nephron to autoregulate (Brenner et al, 1985).

In Chapters 15-21 I discuss the effects of ANF on GFR, but the concept of a hormonal control of glomerular haemodynamics is not new (Ardaillou et al, 1984). Glomerular cells bind angiotensin II at physiological concentration. Angiotensin II-induced efferent glomerular arteriole constriction would reduce plasma flow and increase hydrostatic pressure within the glomerulus; the same peptide causes mesangial cell contraction which may lead to a decrease of the area available for glomerular filtration and thus a reduced SNGRF. Other substances, including noradrenaline, parathyroid hormone, antidiuretic hormone, aswell as prostaglandin E_2 also cause mesangial cell contraction.

Finally, SNGFR falls when the delivery of fluid to the distal nephron rises. The precise mechanism of this 'tubulo-glomerular feedback' is not clear but its efficiency is reduced during blood volume expansion, allowing a natriuresis without depression of GFR

(Carmines et al, 1984).

How are the determinants of filtration rate altered in renal diseases? In rats with 5/6th nephrectomy, one model for the progression of CRF in humans, remnant nephrons are larger than normal and have higher plasma flows, elevated capillary hydraulic pressures and SNGFRs (Meyer et al, 1986). In rat nephrotoxic serum nephritis, which may be comparable to some forms of human proliferative glomerulonephritis, there is a heterogeneity of SNGFR, with increased glomerular capillary hydraulic pressures offset by reduced plasma flow and K_f. In contrast, in the puromycin-induced glomerular lesion, a model for human minimal change disease, SNGFR and K_f are both reduced but but there is no increase of glomerular hydrostatic pressure (El Nahas, 1988; Skorecki et al, 1985). Dissection of kidneys from humans with CRF reveals a heterogeneity of nephron size and degree of structural damage (Oliver, 1939), and the determinants of GFR are open to speculation.

The Production of Glomerular Proteinuria.

Small molecules such as inulin (molecular weight 5200) move freely across the healthy glomerular barrier, but the passage of larger molecules is impaired. The healthy barrier can be considered to have one type of

water filled cylindrical pore through which macromolecules move either by convection or diffusion. In healthy rats, the pore radius has been estimated as 50 Angstroms and the pores occupy 10% of the surface of the glomerular filter (Skorecki et al, 1985).

Dextrans are hexose polymers which are not reabsorbed by the renal tubule, and are ideal markers of glomerular permeability. The fractional clearance (100% x clearance/GFR) of uncharged dextrans the size of albumin (36 Angstroms) is 10%, but anionic dextran sulphate of a similar size has a fractional clearance of only 0.1%. This is caused by repulsion from negatively charged proteoglycans and heparan sulphate in the glomerular barrier, and explains why little albumin, which is also anionic, enters the glomerular filtrate.

In addition to the intrinsic properties of the glomerular membrane, the filtration of macromolecules is determined by glomerular haemodymanics (Skorecki et al, 1985). For example, isolated rises of renal plasma flow or glomerular hydraulic pressure are predicted to increase the total convective flux of macromolecules, although the percentage rise of SNGFR will be greater and the fractional clearances of those macromolecules may fall. Enhanced passage by diffusion would theoretically occur when the concentration of macromolecules within the glomerular circulation rises,

either as a result of a rise in peripheral plasma concentration, or secondary to a rise of filtration fraction (GFR/plasma flow).

The final effect may represent the sum of a number of haemodynamic alterations. For example, angiotensin II infusion into the healthy rat causes decreased glomerular plasma flow together with increased filtration fraction and glomerular hydraulic pressure. Together, these changes result in increased clearances of dextrans between 22 and 42 Angstroms (Bohrer er al, 1977).

Heavy proteinuria is the hall-mark of primary glomerular disease, but is also found as a result of secondary glomerular damage in the course of primary interstitial disorders such as reflux nephropathy (Williams et al, 1988). What causes the enhanced leak of proteins in renal disease ?

In adriamycin-induced nephrotoxicity in the rat, a pore size defect explains the proteinuria, but in puromycin-induced disease a charge defect predominates (Skorecki et al, 1985). In the rat remnant kidney the fractional clearances of neutral dextrans below 38 angstroms are unchanged but they are increased for larger molecules (Olson et al, 1982). The haemodynamic alterations in this model, which comprise increased capillary plasma flow and hydrostatic pressure, should theoretically

reduce fractional clearances, and therefore the existence of a size-selective defect has been postulated: fractional clearances for anionic dextrans were even more exaggerated, suggesting a coexisting charge defect.

Mechanisms of glomerular proteinuria in human renal disease are less well documented (Skorecki et al, 1985). In minimal change glomerulonephritis the fractional clearances of neutral dextrans are reduced, and loss of glomerular anionic charge may be important in the genesis of albuminuria. In diabetics with nephropathy, the fractional clearance of macromolecules with radii less than 70 Angstroms falls as renal disease progresses. At the same time, the clearance of larger dextrans increases, and the appearance of a second, larger type of pore has been postulated in order to explain this phenomenon. Clearly no single mechanism is likely to explain the proteinuria that results from glomerular disease, and structural, electrostatic and haemodynamic factors must all be considered to be important.

The Determinants of Natriuresis.

Effects of GFR.

In healthy humans, about 20,000 mmol of sodium ion appears in the daily glomerular filtrate but less than

2% is excreted in the urine. Although small changes of GFR could have major effects on natriuresis if tubular reabsorbtion was unchanged, changes in GFR are generally matched by a proportionate increase in sodium reabsorption by the tubules. This phenomenon, known as 'glomerular-tubular balance', can be partly explained by considering Starling forces around the proximal tubule (Raymond et al, 1985). In this segment sodium is actively transported from the lumen into the peritubular space, and would tend to leak back into the tubule were it not for the physical forces which draw fluid into nearby capillaries.

For example, suppose that GFR rose in the presence of a constant plasma flow: the increased filtration fraction would result in increased oncotic pressure and decreased hydraulic pressure in the peritubular capillaries, both of which would tend to enhance absolute proximal reabsorption. In this fashion the effect of fluctuations of GFR on final sodium excretion is minimised.

If alterations of GFR have little effect on urinary sodium, what of the effects of dietary sodium on GFR ? In health, changes in dietary sodium are not accompanied by major changes of GFR, even though sodium excretion rises or falls to match dietary input (Raymond et al, 1985). In humans with chronic renal impairment an appropriate urinary response to altered

sodium intake tends to be maintained despite a reduced GFR: fractional sodium excretion is enhanced compared to normal for any level of dietary sodium (Slatopolsky et al, 1968). Similarly, in animals with remnant kidneys, adaptations to sodium challenges are reported to occur by tubular adjustments of reabsorption of the ion rather than by alterations of GFR (Wilson and Sonnenberg, 1979).

In summary, the potential effect on sodium excretion by an acute change of GFR is minimised by 'glomerulo-tubular balance'. Renal tubular adaptation, rather than GFR changes, appear to be an important response to alterations of sodium input in health and in renal disease.

Effects of Peritubular Forces.

We must return to peritubular physical forces to explain the 'pressure-natriuresis' in which increased renal perfusion pressure caused a rise of sodium excretion (Selkurt, 1951): an increase of peritubular hydrostatic pressure would decrease fluid clearance into the bloodstream. Similarly, a fall of peritubular plasma oncotic pressure may contribute to the natriuresis that follows an intravenous saline load (Spitzer and Windhager, 1970). In renal disorders, the importance of peritubular physical factors is little studied, but in nephrotic syndrome, proximal tubular

fluid reabsorption is decreased, probably as a result of the low plasma oncotic pressure (Grausz et al, 1972; Ichikawa et al, 1983).

Effects of Solute in the Medullary Interstitium.

The natriuresis after volume expansion is enhanced by water loading, an observation explained by postulating a dissipatation of high solute concentrations in the medulla. This solute draws water from the descending loop of Henle, thus raising sodium concentration within the tubule and permitting the passive movement of sodium out of thin ascending loop of Henle (Raymond et al, 1985).

Effects of Renal Innervation.

In 1859 it was reported that interruption of the greater splanchnic nerve resulted in a diuresis. Recent studies have shown that a low level of stimulation of renal sympathic nerves enhances sodium reabsorption in the proximal tubule. However, if these nerves are stimulated with greater intensity, renin release also contributes to sodium retention. Decreased renal nerve activity, mediated by atrial and arterial stretch receptors, may contribute to natriuresis after acute blood volume loading (Goetz, 1988).

Effects of Aldosterone.

Aldosterone enhances sodium reabsorption in the cortical collecting tubule, and is important in sodium

balance. In health, plasma levels vary inversely with sodium balance, and the lack of aldosterone in Addisons disease results in severe salt wasting (Raymond et al, 1985). In patients with CRF plasma levels are often high, correlating with plasma potassium levels, and the small increase in sodium excretion that follows spironolactone suggests that aldosterone affects sodium balance (Berl et al, 1978).

Effects of Angiotensin II.

1) It stimulates the secretion of aldosterone.

2) It is a vasoconstrictor, and at high concentrations causes arterial hypertension and a 'pressure-natriuresis': this effect may explain the natriuresis sometimes found in malignant hypertension, a disorder accompanied by high circulating angiotensin II.

3) It increases fluid reabsorption in the proximal tubule by causing a rise of filtration fraction and also by a direct action (Harris et al, 1987).

Effects of Circulating Natriuretic Sustances.

These have been reviewed in Chapter 9.

Summary of Chapter 14.

As an aid to the interprtation of the effects of ANF on the kidney in health and renal disease, the major determinants of glomerular filtration rate, glomerular proteinuria and natriuresis were reviewed.

CHAPTER 15.

THE RENAL EFFECTS OF ANF:

A REVIEW OF ANIMAL EXPERIMENTS

Introduction.

Animal experiments have contributed to our knowledge of ANF-induced natriuresis and of cGMP as a 'second-messenger' to ANF. However, we must be cautious about the relevance of these studies to human physiology because some used atrial extracts, which contain a variety of ANFs as well as other unknown substances, and when synthetic ANF was used, the dose was often pharmacological. Having said this, ANF does bind to specific renal receptors and also has biological renal effects both in vitro and in vivo at physiological concentrations (Napier el al, 1984; Zeidel et al, 1986; Kurtz et al, 1986; Zimmerman et al, 1987).

Effects of ANF on the Glomerulus.

The mechanism of ANF-induced natriuresis is debated. The original report using whole rats (de Bold et al, 1981) suggested that it occurred with a constant GFR. However, in 1984, Maack et al reported that ANF induced rises of GFR, sodium, calcium and phosphate excretion in whole animals. Camargo et al (1984) found that atrial extract decreased the renal vascular

resistance of isolated perfused kidneys, but only after vessels were pre-constricted with angiotensin II: at the same time, GFR doubled. Verapamil blunted both the natriuresis and rise of GFR, suggesting that they were linked. Cogan (1986) came to a similar conclusion after finding that aortic constriction abolished the ANF-induced rise of GFR and also blunted the natriuresis.

Ichikawa et al (1985) found, in rat micropuncture studies, that ANF increased SNGFR, by raising mean glomerular capillary pressure, a consequence of efferent arteriolar constriction and afferent dilatation. Marin-Grez et al (1986) confirmed these effects on vessel diameter by visualisation of isolated dog glomeruli. Fried et al (1986) perfused isolated glomeruli with high dose ANF and found a rise SNGFR, increased glomerular hydrostatic capillary pressure and decreased plasma flow. Moreover, the calculated permeability of the glomerular barrier increased, an effect consistent with mesangial relaxation.

In fact, mesangial cells have ANF receptors and show a reduction in the density of ANF binding sites upon exposure to high ANF levels: such a 'down-regulation' suggests a physiological role for ANF binding (Ballerman et al, 1985), and raises the possibility that ANF might affect GFR and glomerular permeability by an action on these cell.

These studies suggested that, after high doses of ANF, sodium excretion followed a rise in GFR. In contrast, other authors reported that lower doses of ANF increased sodium excretion without a detectable rise in GFR (Yukimura et al, 1984; Zimmerman et al, 1987). In Zimmerman's study a physiological rise of plasma ANF of 10 pmol/l increased sodium excretion. Although the latter studies could have missed a small rise of GFR due to experimental error, they throw doubt on the theory that increased GFR is essential for ANF-induced natriuresis.

Effects of ANF on the Tubule.

Sonnerberg proposed a primary tubular action of ANF after he found that atrial extracts (Sonnenberg et al, 1982) cuased natriuresis despite a constant GFR: both atrial and ventricular extracts had the non-specific effect of increasing delivery out of the proximal tubule, but only in the animals treated with atrial extract did sodium reabsorption fail to rise in the medullary collecting duct. Briggs et al (1982) performed a similar study and found no change in GFR, or reabsorption in the proximal tubule or loop of Henle, despite a natriuresis, again supporting a distal tubular effect. These conclusions were later confirmed by Sonnenberg et al (1986) using synthetic ANF.

ANF has no direct effect on the proximal tubule. Baum and Toto (1986) found no change in the transepithelial potential difference of rabbit proximal tubules upon exposure to ANF, nor did ANF alter PAH secretion by the straight segment (Ando et al, 1987) or affect metabolism in this part of the nephron (Vinay et al, 1987). This accords with the lack of ANF receptors in this segment (deLean et al, 1985). However, Harris et al (1987) found that ANF had an indirect effect in this region by inhibiting angiotensin II-induced sodium reabsorption.

In the loop of Henle there is also little evidence of a direct action (Vinay et al, 1987), despite a low density of ANF receptors (de Lean et al, 1985). Zeidel et al (1986) found that ANF at physiological concentration (10 pmol/l) inhibited oxygen consumption in rabbit inner medullary collecting duct and Cantiello et al (1986) recorded that ANF prevented amiloride-sensitive sodium uptake in a cell line which resembles tubular epithelium. In contrast, another group found no effect on the collecting duct of the dog (Vinay et al, 1987).

Indirect effects of ANF on tubular sodium reabsorption may also be important. In 1983 Borenstein reported that atrial extracts increased medullary blood flow: this might decrease passive sodium reabsorption in the thin loop of Henle by dissipating the high urea

concentration of the medullary interstitium. Alternatively, increased hydraulic pressure in the vasa recta, would favour the direct entry of sodium-rich fluid from the medullary interstitium into the collecting duct (Ballerman and Brenner, 1987). However, Torikai (1986) found an ANF-induced natriuresis in rats with destroyed papillae, and moreover, other agents which increase medullary blood flow, such as acetylcholine, cause a lesser natriuresis than ANF. Therefore, the 'medullary washout' effect can not explain the full potency of the peptide (Borenstein et al, 1983).

Effects of ANF on the Renin-Aldosterone System.

ANF inhibits renin release from mammalian kidney slices (Henrich et al, 1986), and ANF infusion into whole animals reduced PRA (Zimmerman et al, 1987; Maack et el, 1984). In contrast, Opgenorth (1986) could not detect ANF-induced suppression of renin secretion in the non-filtering kidney and suggested that the fall in secretion found in other studies was mediated by an increased delivery of sodium to the macula densa. However, this is difficult to reconcile with the direct inhibitory effect on renin release upon exposure of juxtaglomerular cells to low concentrations of ANF (Kurtz et al, 1986). ANF directly inhibits the secretion of aldosterone by the adrenal zona

glomerulosa (Matsuoka et al, 1985), possibly by blocking the conversion of cholesterol to pregnenolone (Elliot and Goodfriend, 1986), and Maack et al (1984) documented falls in plasma aldosterone in dogs infused with atrial extracts.

Relationship of ANF and Cyclic GMP in the Kidney.

In 1984 Hamet recorded rises of plasma and urinary cGMP during ANF-induced natriuresis. Glomerular mesangial cells (Ballerman et al, 1985) and epithelial cells (Ardaillou et al, 1986) produce cGMP when exposed to ANF, and a cGMP-dependent protein kinase is present in the renal mesangial and vascular smooth muscle cells (Joyce et al, 1986). Micropuncture studies in rats, performed during intrarenal infusion of pharmacological doses of ANF, suggested that rises of cGMP in the glomerular filtrate derived from the glomerulus (Huang et al, 1986). The same group showed that the intrarenal administration of a stable cGMP analogue mimicked the rise in GFR produced by ANF.

When the proximal tubule was exposed to ANF no increases occur in cGMP, in accord with its lack of ANF binding sites (de Lean et al, 1985; Vinay et al, 1987). In collecting tubule cells ANF stimulates cGMP production (Ishikawa et al, 1985) and in LLC-PK_1 cells, which resemble renal tubular elements, both ANF and cGMP analogues prevent sodium uptake by an amiloride-

sensitive conductive pathway (Cantiello et al, 1986).

ANF inhibits aldosterone secretion and increases adrenal cGMP production (Matsuoka et al, 1985), and in cultured rat juxta-glomerular cells ANF was found to inhibit renin release and to enhance cGMP production: both effects were diminished by methylene blue, an inhibitor of guanylate cyclase (Kurtz et al, 1986).

Is urinary cGMP a good guide to the renal effects of ANF? Plasma cyclic GMP isfreely filtered by the glomerulus, and in normals the filtered load accounts for all urinary cGMP (Broadus et al, 1970). The difficultly of interpretation of urinary nucleotide is illustrated by Seymour et al (1985) who infused ANF into one renal artery of the intact dog. A rise in cGMP was measured in the systemic arterial blood and in the urine from both the infused and contralateral kidney. However, only the kidney directly infused with ANF produced a natriuresis. Therefore, because cGMP is produced in extrarenal sites and is filtered by the glomerulus, in the whole animal urinary levels do not indicate a unique renal action of ANF.

Physical Factors Modulate the Renal Action of ANF.

Hyperoncotic albumin infusion decreased the ANF-induced natriuresis despite a rise in GFR (Mendez et al, 1986), perhaps because the increase of peritubular osmotic pressure increased reabsorption in the proximal tubule

(Spitzer and Windhager, 1970), and infusion of angiotensin II, which increased systemic arterial pressure, enhanced the sodium excretion after ANF adminstration (Mendez et al, 1986): this may represent the additive effect of increased renal perfusion pressure and ANF on sodium excretion (Raine et al, 1989). The natriuresis after ANF is exaggereted in hypertensive rats (Pang et al, 1985), despite similar production of cGMP to healthy animals, and Reigger (1988) found a blunted urinary sodium excretion in dogs with cardiac failure despite normal ANF-induced rise in plasma cGMP. Thus, the natriuretic response to ANF was modulated by changes of renal perfusion pressure rather than alterations of cGMP production.

Summary of Chapter 15.

1. High dose ANF increases GFR but this effect does not accompany natriuresis induced by lower doses. The major site of renal tubular action may be the medullary collecting duct.

2. Natriuresis is associated with suppression of the renin-aldosterone axis.

3. Physical factors alter the ANF-induced natriuresis.

4. Renal ANF receptors are linked to cGMP production but urinary nucleotide is derived from plasma cGMP in addition to putative nephrogenous nucleotide.

CHAPTER 16.

THE RENAL EFFECTS OF PHARMACOLOGICAL DOSES OF ANF IN HEALTHY HUMANS: A REVIEW.

Introduction.

This chapter reviews the effects of high doses of ANF administered intravenously to healthy subjects. Whenever plasma ANF was measured during these experiments, levels greatly exceeded the healthy range, and therefore the results of these studies may not pertain to normal physiology.

Early Studies.

In 1985 Richards et al administered a 33 nmol intravenous bolus of (99-126) ANF to six healthy adults. In the next 30 minutes there was a fourfold increase of urinary sodium, and water, calcium, magnesium and phosphorus (but not potassium) excretion rose significantly. In the first five minutes there was a transient fall in arterial blood pressure, associated with cutaneous flushing, followed by a small but significant drop which lasted for two hours. No changes occurred in the renin-aldosterone system.

Tikkanen et al (1985) infused 40 nmol of (99-126) ANF over 30 minutes into three adults and observed a two-fold increase in urinary sodium and volume. There was

no effect on potassium excretion, the renin-alosterone axis, plasma anti-diuretic hormone, arterial blood pressure or pulse rate. Plasma ANF exceeded the healthy mean by a factor of 10-30, and was equal to values measured in severe heart failure.

In 1986 two groups demonstrated that the absolute sodium excretion after ANF was greater on a high than on a low-sodium diet (Weidmann et al, 1986a and b; Cuneo et al, 1986). Systemic arterial blood pressure fell and heart rate rose significantly only in Weidmann's study, perhaps explaining the rise in PRA. In contrast, Cuneo found that PRA fell significantly. Both groups recorded falls of plasma aldosterone.

The effects of high dose ANF infusions on GFR and renal plasma flow are not clear-cut. Transient rises of inulin and ^{51}Cr-EDTA clearances were recorded (Weidmann et al, 1986a and b; Cody et al, 1986), but urine volume rose markedly and the apparent effect on GFR might have been caused by a sudden wash-out of inulin or tracer from structures beyond the glomerulus. In these studies the effects on renal plasma flow (PAH clearance) were variable.

Renal Interaction with other Hormones.

Vierhapper et al (1988) found that the ANF induced-natriuresis was not altered by dDAVP administration despite a reduction in free water clearance, suggesting

a separate mechanism for ANF-induced sodium and water excretion. Physiological doses of angiotensin II cause renal sodium retention, an effect partially abolished by large doses of ANF (McMurray and Struthers, 1988a). Angiotensin-converting-enzyme blockade blunts the natriuretic effect of pharmacological doses of ANF (Wilkins et al, 1987), suggesting that basal angiotensin II must be present before ANF can cause maximal natriuresis.

Effects on Plasma Volume.

High dose ANF increased haematocrit and plasma total protein concentration to a degree greater than could be accounted for by renal fluid loss (Weidmann et al, 1986 a and b). This might be explained by increased capillary hydraulic conductivity that has been reported to occur in vitro after massive doses of ANF (Huxley et al, 1987).

Clearance of ANF.

The pharmacokinetics of ANF has been studied during steady-state infusions. The volume of distribution was 11 litres, and the plasma clearance rate of immunoreactive ANF during pharmacological infusions rates was 2 l/min (Yandle et al, 1986b), with values as high as 7 l/min documented with low dose infusions (Gillies et al, 1987). The use of a high or low sodium

diet does not change the metabolic clearance rate (Cuneo et al, 1986), and clearance was higher in the supine versus the sitting position (Gillies et al, 1987).

Effects on cGMP.

Finally, pharmacological doses of ANF increase both plasma and urinary cGMP (Gerzer et al, 1985).

Summary of Chapter 15.

1. Intravenous infusions of large doses of ANF into healthy man increase urinary sodium, water, phosphate, calcium and magnesium excretion.

2. Glomerular filtration rate may increase transiently but the effects on renal plasma flow are not constant.

3. The effects of high dose ANF on plasma renin varies: if arterial blood pressure is constant, PRA becomes suppressed. However, very high doses of ANF may produce falls in arterial pressure with subsequent rise of PRA.

4. Plasma aldosterone falls during prolonged ANF infusion, whatever the behaviour of PRA.

5. ANF clearance from the plasma of healthy subjects is rapid and exceeds renal plasma flow.

6. Pharmacological doses of ANF cause significant rises of extracellular cGMP.

CHAPTER 17.

THE RENAL EFFECTS OF LOW DOSE ANF INFUSION IN HEALTHY SUBJECTS.

Introduction.

Administration of small doses of ANF into healthy subjects causes a significant natriuresis (Anderson et al, 1987; Richards et al, 1988). In this chapter I decribe my own study which follows the renal and hormonal responses to a low dose ANF infusion in normals, during which plasma ANF was raised to the limit of the physiological range. In chapter 18 these results will be compared with the effects of ANF in patients with the nephrotic syndrome.

Subjects and Methods.

Seven healthy volunteers (1 female) gave consent to undergo the study which was approved by the Department of Health and the Hospital Ethical Committee. Their age (mean±SEM) was 29±2 years, height 176±3 cm, weight 76±4 kg, systolic blood pressure 106±6 mmHg, diastolic blood pressure 76±4 mmHg, urinary total protein excretion <0.10 g/day and urinary albumin excretion <35 µg/day. They took no drugs during the study and the woman did not use oral contraception.

They ate a diet containing 150 mmol sodium per day for

3 days as an outpatient and a 24 hour urine collection was made on the third day. On the fourth day, upon rising, each drank 500 ml of water and ate a light breakfast which did not include meat. At 0900h they were admitted into the investigation room which was kept at a comfortable, constant temperature. They drank 500ml of water and thereafter 200ml was taken each half-hour. They sat with legs dependent, rising briefly to pass urine each 30 minutes. At 0900h sodium aminohippurate (PAH: Merk, Sharp and Dohme) and inulin (Laevosan International) were infused, through a forearm vein, to achieve approximate plasma levels of 20 mg/l and 250 mg/l respectively and continued for 5 hours. After an hour for equilibration, collections of blood and urine were made half hourly. Urine was obtained by asking the volunteer to void completely.

Between 1100 and 1300h (99-126) ANF (Bissendorf Peptide) was infused by the same route at 2.5 pmol/kg/min dissolved in 100ml 0.9% saline. The collections between 1000 and 1100h served as a control period and the final hour, 1300-1400h, as a recovery period. The total vehicle sodium administered was 30mmol. Blood was drawn from the opposite antecubital vein, three minutes before the mid-point of each urine collection period (total 150ml). The experiment was performed twice, at least one week apart, in a single-blind fashion, using either ANF or vehicle alone.

Plasma was sampled half-hourly for ANF, PRA, cGMP, sodium, potassium, inulin, PAH, and total protein. Plasma aldosterone was measured hourly after the two basal samples. Urinary parameters measured were: volume, sodium, potassium, inulin, PAH, cGMP and albumin. A single observer measured blood pressure half-hourly using a standard mercury sphygmomanometer, just before the subject rose to micturate. The inulin and PAH clearances were taken to be equivalent to GFR and ERPF respectively. Fractional cGMP excretion equalled the cGMP clearance/GFR x 100%.

Percent changes in plasma volume were derived from measurement (using the cyanomethamoglobin prodedure and Coulter Counter) of haematocrit and haemoglobin on blood drawn immediately before the start and also the end of the ANF infusion (Tulassay et al, 1987) using the formula:

$$\% \text{ change} = 100 \times \frac{\text{initial Hb}}{\text{second Hb}} \times \frac{\text{1-second Hct}}{\text{1-initial Hct}} - 100$$

Statistics.

Serial results (mean$\pm$SEM) were examined by two way analysis of variance upon the active-placebo pair differences at each time point. If significant changes were found, then Duncan's test for multiple comparisons determined times at which the mean pair

differences differed significantly from both basal pair differences (Geigy Scientific Tables, 1984).

Results.

Basal values (mean±SEM) are shown in Table 17.1, and Figure 17.1 shows plasma ANF, cGMP, PRA, urinary sodium excretion and filtration fraction in active and placebo groups. The raw data are listed in Chapter 22.

Plasma ANF.

The mean basal ANF was similar on the placebo and active days. On the placebo day plasma ANF tended to fall from an initial value of 2.7±0.7 pmol/l at 0900h to a nadir of 1.4±0.0.3 pmol/l at 1100h, rising to 2.3±0.2 pmol/l by the end of the experiment. During ANF infusion, levels rose to a plateau by one hour, with a peak mean increment of 17.6±2.0 pmol/l, and then returned to baseline during the recovery hour. The plasma ANF in the active versus placebo study was significantly increased throughout the ANF infusion period ($p<0.01$ at all time points. Figure 17.1).

Plasma and Urinary cGMP.

Basal plasma cGMP was similar in both groups and during ANF infusion levels rose from 2.9±0.6 nmol/l to a peak of 10.4±1.8 nmol/l in the final period of the ANF infusion. Compared to pattern for plasma ANF, the cGMP rise showed an approximate 30 minute lag, with

significant differences between the active and placebo group from the second half-hour of the infusion, extending to the first time point of the recovery hour (all $p<0.01$. Fig 17.1).

The basal urinary cGMP excretion was similar in the active (217±28 pmol/min) and placebo groups (213±21 pmol/min), and rose significantly in the active group to a peak of 1111±234 pmol/min and fell in the recovery hour. On the active day the fractional excretion of cGMP increased slightly from a basal value of 56±7% to 75±7% during the final collection in the ANF infusion. Therefore, in all periods, cGMP filtered from the plasma accounted for the measured urinary cGMP.

Plasma Renin Activity and Aldosterone.

Basal PRA was similar in the active and placebo groups, and on the active day PRA fell from 0.96±0.13 nmol/h/l to a nadir of 0.50±0.06 nmol/h/l despite a calculated fall of plasma volume of 7±2%. On the placebo day, PRA fell slightly but not significantly, and plasma volume increased by 4±2% The difference in response between the two groups was significant by the second half hour of the ANF infusion period, and PRA in the active group remained significantly suppressed in the recovery hour (Figure 17.1).

Plasma aldosterone fell in the active group from a

Table 17.1.

Basal values in normals before ANF infusion.

	Active Day	Placebo Day
Plasma data on the second basal period		
ANF (pmol/l)	1.9±0.4	1.4±0.2
cGMP (nmol/l)	2.9±0.6	3.1±0.4
Sodium (mmol/l)	136±1	135±1
Potassium (mmol/l)	4.3±0.3	3.9±0.1
PRA (nmol/h/l)	0.96±0.13	0.98±0.14
Aldosterone (pmol/l)	258±67	223±32
Urine data on the second basal period		
Sodium (µmol/min)	163±31	165±22
Potassium (µmol/min)	97±11	92±13
Volume (ml/min)	12.9±1.4	12.1±1.6
cGMP (pmol/min)	217±28	213±21
GFR (ml/min/1.73 m^2)	130±5	124±5
ERPF (ml/min/1.73 m^2)	558±24	541±29
Filtration Fraction(%)	23.4±1.1	23.0±1.2
Albumin (µg/min)	6±2	8±2
Pulse (beats/min)	65±3	58±3
Mean BP (mmHg)	85±4	79±2

Data are mean±SEM. Note that GFR and ERPF have been corrected for body surface area.

Figure 17.1 (following page).

ANF infusion in healthy subjects.

Mean±SEM levels of plasma ANF, plasma cGMP, PRA, urinary sodium excretion and filtration fraction following the start of the ANF infusion.

Note that the third blood sample is taken only 15 minutes after starting the infusion. Data for sodium excretion are plotted at the midpoint of each half hour collection period. Solid lines represent healthy subjects receiving ANF, and short dashes the same volunteers receiving a placebo infusion.

The asterisks indicate the pair differences which differed significantly from both basal pair differences (Duncans test for multiple comparisons).
* $p<0.05$, ** $p<0.01$.

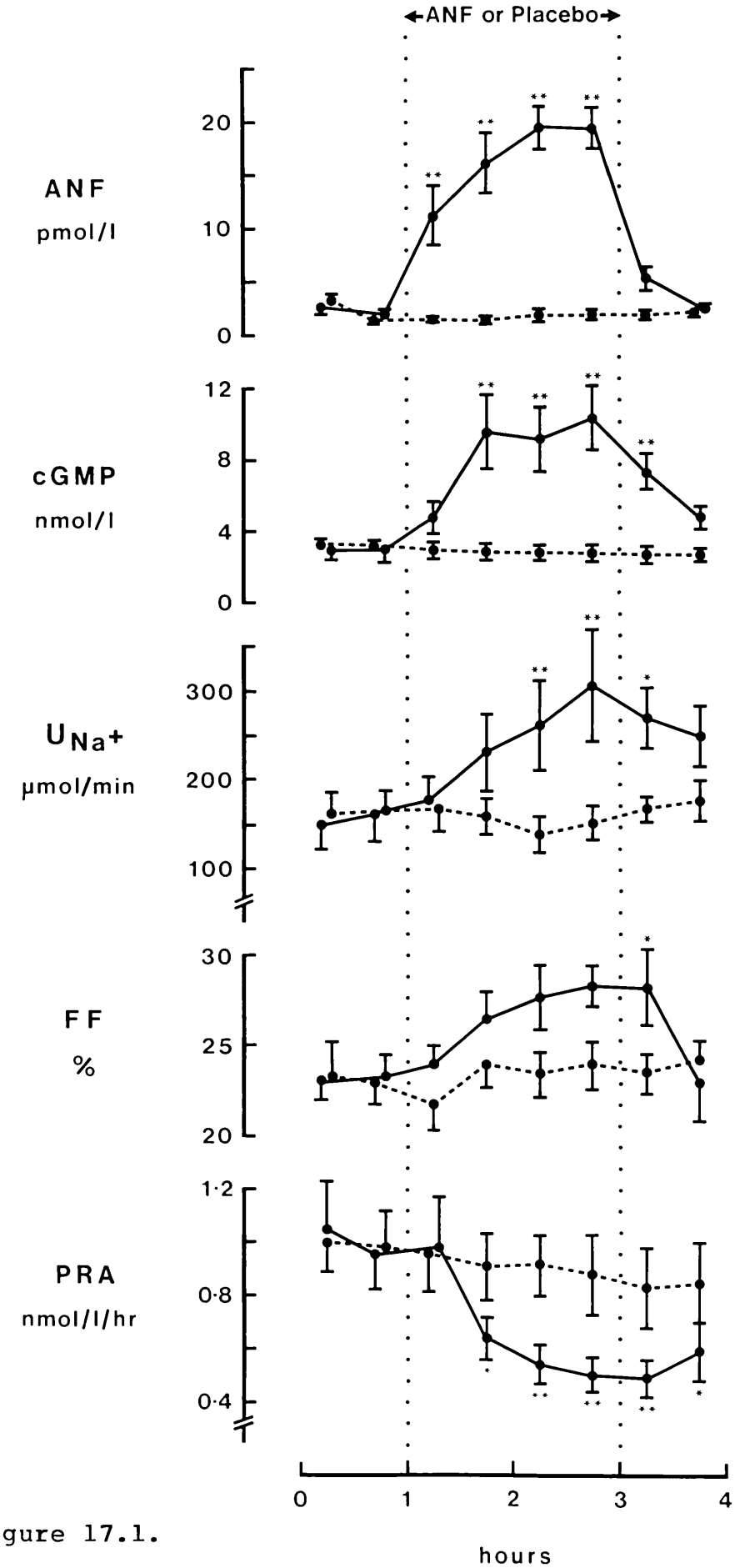

←ANF or Placebo→
ANF
pmol/l
20
10
0
cGMP
nmol/l
12
8
4
0
U_{Na^+}
µmol/min
300
200
100
FF
%
30
25
20
PRA
nmol/l/hr
1·2
0·8
0·4
0
1
2
3
4
hours

Figure 17.1.

basal value of 258±67 pmol/l to a nadir of 107±32 pmol/l, and remained suppressed during the recovery hour. The pattern of response of the active versus placebo group resembled that observed for PRA.

Plasma and Urine Electrolytes and Volume.

No change was seen in plasma concentrations of either sodium or potassium. Urine sodium excretion on the day before the study, indicated that the groups were in balance with the 150mmol diet (152±10 mmol and 162±9 mmol in the active and placebo groups respectively).

Basal sodium excretion was similar on the experimental day in both groups (Table 17.1), and ANF infusion caused a peak mean two-fold increase in sodium excretion. In the recovery hour sodium excretion returned towards placebo values. Compared to the placebo group, there was a significant difference between the means during the second hour of the ANF infusion period and in the first half hour of the recovery period (Figure 17.1). Basal urinary potassium excretion was similar in both groups and fell similary in active and placebo studies.

The basal urinary volumes were similar in each group but were significantly higher on the active compared to placebo day during the final half hour of the ANF infusion (12.4±1.3 versus 8.6±0.9 ml/min). During the recovery hour urinary volumes fell in the active group

to values similar to those on the placebo day.

Renal Haemodynamics.

The GFR did not change through the ANF infusion and there was no difference in the response on the active and placebo days. ERPF fell on the active day reaching a nadir of 19±3% below the basal by the end of the infusion. ANF infusion was associated with a steady increase in filtration fraction from 23.4±1.1% to a peak of 28.5±1.1% in the final half-hour of the peptide infusion, with a fall observed in the second half of the recovery hour: there was a significant difference between active and placebo groups in the period immediately after the end of the ANF infusion (Figure 17.1).

Plasma and Urinary Proteins.

In both groups plasma total protein was constant. Neither group as a whole showed a significant change in urinary albumin excretion rate, but in one subject albuminuria increased from 5.0 µg/min to a peak of 201 µg/min at the end of the ANF infusion, falling to basal values in the recovery period. This surprising result was checked, by repeating the albumin RIA, and found to be correct.

Blood Pressure.

Near the end of the ANF infusion, one subject experienced a transient scotoma, associated with a fall

of MBP of 11 mmHg. This symptom disappeared after ANF infusion was discontinued. Despite this individual reaction, there were no significant differences in blood pressure response between the two groups.

Discussion.

ANF and cGMP.

The basal ANF levels were similar to values in healthy young adults as reported by others who extracted plasma with octadecylsilica (Anderson et al, 1986a; Singer et al, 1987b). The mean rise of plasma ANF in the active group was similar to that attained during two other low dose infusions (Anderson et al, 1987; Richards et al, 1988) but greater than the peptide rise after intravenously infusing 2 litres of saline into normals (Chapter 10). However, it is similar to the greatest increase produced by more brisk saline challenges (Anderson et al, 1986a) and head-out water water immersion (Epstein et al, 1986). Therefore, the rise in plasma ANF was at the limit of the healthy physiological range.

The basal levels of plasma cGMP in this experiment are similar to those found by other groups (Wood and Marks, 1978; Gerzer et al, 1985). Gerzer et al (1985) reported rises of plasma and urinary cGMP in healthy subjects after a pharmacological intravenous bolus of ANF, and

the current study showed that extracellular nucleotide rose after physiological doses of ANF. In contrast to plasma ANF, plasma cGMP remained elevated in the first sample of the recovery period in the active versus placebo group. This might represent continued nucleotide production, but the effect is more easily explained by the plasma half-life of cGMP which, at 30 minutes (Broadus et al, 1970), is considerably longer than that of ANF (Yandle et al, 1986b).

The kidney possesses ANF receptors linked to guanylate cyclase, the enzyme responsible for production of cGMP (Chapter 15) and the question arises whether urinary cGMP levels indicate the renal response to ANF. The current results show that although there was a small rise in the fractional excretion of cGMP on the active day, the absolute value was always less than 100% of the GFR. Therefore, the rise in urinary cGMP could be accounted for by the nucleotide filtered from the plasma, which may in turn have its origin in non-renal tissue.

This conclusion does not account for possible reabsorption of the nucleotide by the renal tubule. Although Broadus (1970) found that the ratio of inulin to cGMP excretion was unity, another group found evidence for a small but measurable reabsorption of the nucleotide beyond the proximal tubule (Huang et al, 1986). Therefore, until more is known about the origin

of plasma cGMP and its renal tubular handling, caution should be applied when using urinary cGMP as a marker for the renal effects of ANF.

Renin and Aldosterone.

The fall of PRA accords with other low dose human studies (Anderson et al, 1987; Richards et al, 1988) and may be due to a direct effect of ANF on renin secretion (Kurtz et al, 1986) or to increased sodium delivery to the macula densa. In contrast, very high doses of ANF may stimulate renin secretion secondary to large falls in systemic blood pressure (Weidmann et al, 1986 a and b). In the last half of the recovery hour PRA remained suppressed while sodium excretion fell to placebo values, possibly suggesting that a fall in PRA is not of primary importance in causing the natriuresis.

A fall in aldosterone has also occurred in another human study which used a similar protocol (Richards et al, 1988). In contrast, Anderson et al (1987) who employed a slightly lower rate of ANF infusion (1.2 pmol/kg/min), found no significant change. A fall of aldosterone may have resulted from reduced stimulation by angiotensin II subsequent to the fall of PRA, or from direct inhibition of steroid production (Matsuoka et al, 1985).

Sodium Excretion.

The magnitude of the natriuresis together with the delayed rise in sodium excretion, resembles results of other low dose infusions (Anderson et al, 1987; Biollaz et al, 1987; Richards et al, 1988). In contrast, infusions of pharmacological doses of ANF produce larger rises in sodium excretion (Cody et al, 1986; Weidmann et al, 1986a and b; Gerzer et al, 1985).

In common with Anderson et al (1987), I found that sodium excretion remained elevated compared to placebos during the recovery period when plasma ANF had returned to basal values. The might result from continued cGMP production after the disappearance of high levels of ANF. I found no increase in potassium output compared to placebo values in agreement with other authors.

Although urinary volume did not increase during the ANF infusion, it was higher in the final hour of the infusion compared to the placebo group because urinary volume fell in the latter group.

Biollaz et al (1987) found that ANF infusion into water-loaded healthy subjects increased both the absolute and the fractional clearance of solute-free water. In that study antidiuretic hormone was suppressed before the ANF infusion began, and the rise of free-water excretion was explained by both an enhanced volume delivery from the proximal tubule and

a rise of free-water generation in the diluting segments of the tubule.

Glomerular Filtration and Renal Plasma Flow.

In healthy subjects pharmacological doses of ANF transiently elevate GFR and this could contribute to the natriuresis (Cody et al, 1986; Weidmann et al, 1986a and b). Both my study and others which also used physiological doses of ANF have not demonstrated significant rises in inulin or creatinine clearances (Anderson et al, 1987; Biollaz et al, 1987; Richards et al, 1988).

Although it is acknowledged that rises in GFR of <10% are difficult to document by conventional clearance techniques, the evidence supports a primary tubular action of ANF. Biollaz et al (1987), using the lithium clearance technique (Thomsen, 1984), suggested that an increased fractional delivery of sodium out of the proximal tubule, which then escaped reabsorption by the nephron after the diluting segment, was the cause of the natriuresis, rather than a rise of filtered load.

In my study, ANF infusion was associated with significant falls of ERPF, and the filtration fraction rose. Similar responses have been described by other authors (Richards et al, 1988; Biollaz et al, 1987). Although ANF was originally thought to act as a specific renal vasodilator (Wakitani et al 1985), other

studies showed that this effect only occurred when vessels were precontricted (Camargo et al, 1984). In animals, when renal vessels were relaxed, pharmacological doses of ANF reduced renal plasma flow and increase renal vascular resistance. At the same time, filtration fraction rose, because of preferential constriction of the efferent glomerular arteriole (Camargo et al, 1984; Fried et al, 1986; Marin-Grez et al, 1986; Ballerman and Brenner, 1987; Chapter 15). A similar mechanism may explain the rise in filtration fraction observed during low dose ANF infusion in healthy subjects.

ANF does not alter PAH secretion by the isolated proximal tubule (Ando et al, 1987), and therefore the fall of PAH clearance in the active group should represent a real fall in renal plasma flow. Alternatively, a redistribution of flow towards the renal medulla, which extracts PAH less efficiently, would have the same effect (Stein, 1973).

Arterial Blood Pressure.

In one subject the sudden symptomatic fall of blood pressure, may have been caused by an ANF-induced fall of plasma volume or arterial vasodilatation, and may have contributed to the fall of ERPF. However, arterial hypotension was not a constant feature of this or other studies which infused low dose ANF over a period of up to few hours (Anderson et al, 1987; Biollaz et

al, 1987; Richards et al, 1988). Janssen et al (1986) reported that a low salt diet exacerbated the hypotensive response to low dose ANF in patients with essential hypertension, but my healthy volunteers took a normal sodium intake.

Higher doses ANF apppear to produce hypotension on a more predictable basis (Weidmann et al, 1986a and b;Richards et al, 1985), and a recent review (Genest et al, 1988) suggested that during short infusion studies plasma ANF must rise above 83 pmol/l for hypotension to occur with regularity, and this exceeds the highest value attained by any subjects in the current study. Very recently, preliminary evidence has suggested that more prolonged low dose ANF infusions do indeed result in regular falls of blood pressure (Espiner and Richards, 1989).

Albumin Excretion.

There was no change in albuminuria in the group as a whole on either experimental day. However, in one subject albuminuria increased by over ten-fold. McMurray et al (1988b) recorded a significant rise of albuminuria after a pharmacological ANF infusion in normals, although Ishii et al (1988) found no increase of total proteinuria in a similar experiment. In my study, a small effect on albuminuria may have been missed because of the high coefficient of variation of

the albumin RIA when measuring dilute urine from healthy subjects (Chapter 7). The possible mechanisms of ANF-induced proteinuria will be discussed in Chapters 18 and 19.

Summary of Chapter 17.

1. Intravenous infusion of (99-126) ANF in healthy adults increased plasma immunoreactive peptide to the upper limit of the physiological range.

2. Plasma and urinary cGMP rose but filtered cGMP accounted for all measured urinary nucleotide.

3. Urinary sodium rose slowly to a peak of two-fold over placebo values, and significant suppression of the renin-aldosterone axis occurred.

4. Glomerular filtration rate was constant but ERPF fell significantly. Therefore, ANF caused a significant rise in filtration fraction.

5. No consistent effects of ANF were found on either arterial blood pressure or urinary albumin excretion.

CHAPTER 18.

THE RENAL EFFECTS OF ANF INFUSION IN THE NEPHROTIC SYNDROME.

Introduction.

This chapter examines the renal effects of ANF in the nephrotic syndrome, a disorder characterised by heavy proteinuria (>3.5g/day in adults), hypoalbuminaemia and peripheral oedema. Administration of small doses of ANF into healthy subjects causes a significant natriuresis (Chapter 17), and similar increases of plasma ANF occur when normals and nephrotic patients undergo head-out water immersion (Epstein et al, 1986; Peterson et al, 1988). The latter manoeuvre produces variable increases in sodium excretion in patients with the syndrome (Berlyne et al, 1981; Krishna et al, 1982; Peterson et al, 1988). In addition, Tulassay et al (1987) have recorded concomitant rises of plasma ANF and sodium excretion in nephrotic children who underwent blood volume expansion after intravenous albumin infusion. Therefore, in these patients who suffer from oedema, evidence suggests that a physiological rise in ANF is accociated with natriuresis.

Recent reports have shown a markedly attenuated natriuretic response to pharmacological doses of ANF in

rats with nephrotic syndrome induced by adriamycin and aminonucleoside (Perico et al, 1987; Hildebrandt and Banks, 1988) but a normal response in humans with the syndrome after a large intravenous bolus of the peptide (Rabelink et al, 1987). In the current study I followed the renal and hormonal response to a physiological ANF infusion in six nephrotic patients. These results were compared to those of the normal subjects described in Chapter 17.

Patients and Methods.

Six patients with nephrotic syndrome due to primary renal disease gave consent to undergo the study which was approved by the Department of Health and the Hospital Ethical Committee. None took regular medication, except one patient who stopped frusemide one week before study. However, three patients had received courses of steroids which were not effective at inducing remission and had been stopped at least two months prior to the study. All had clinical oedema. None had more than trace haematuria (Multistix, Ames), nor was their urine infected. The patient and the healthy control details are shown in Table 18.1. The two groups had similar mean age and sex distribution. No subject had a basal blood pressure over 150/90 mmHg, and although the patient group tended to have higher pressures, the difference was not significant.

The protocol for the ANF, PAH and inulin infusion together with dietary sodium control, water loading and blood and urine sampling were identical to the scheme decribed in detail in Chapter 17. Unfortunately the patients were unwilling to undergo a placebo study, and no extra patients could be found to perform a separate control group (nephrotics who take no medication are quite unusual !). Therefore, the nephrotic syndrome study comprised only of an active infusion group.

Statistics.

Serial results of the patient group (mean±SEM) were examined by two way analysis of variance. If this gave a significant result then Duncan's test test for multiple comparisons was used to determine times at which levels differed significantly from both basal observations. Total responses in the nephrotic group and the healthy subjects (Chapter 17) were compared by Student's t-tests on the incremental areas under the curves following the second basal sample.

Results.

Basal levels are given in Table 18.2, and the total response of nephrotics and normals in Table 18.3. The responses of plasma ANF, cGMP, renin activity, urinary sodium excretion and filtration fraction are shown in

Figure 18.1, and changes in albuminuria for the nephrotic group in Figure 18.2. The raw results are given in the Data Appendix (Chapter 22).

Plasma ANF.

The mean basal immunoreactive ANF in these water-loaded nephrotics (4.0±0.8 pmol/l) was higher than that of the normal subjects (1.9±0.4 pmol/l). During ANF infusion, levels rose to a plateau by one hour, with a peak increment in normals of 17.6±2.0 pmol/l and in nephrotics of 22.1±3.3 pmol/l. The incremental area was similar in both groups, and ANF returned to basal levels by the first sample in the recovery hour.

Plasma and Urinary cGMP.

Basal plasma cGMP was similar in both patients and normals and mean levels rose significantly versus basal levels in both groups by the second half hour of the ANF infusion. In both active groups, plasma cGMP remained elevated above basal values ($p<0.01$) in the first half of the recovery hour when plasma ANF had fallen to basal levels. On the day before the ANF infusion, 24 hour urinary cGMP was similar in normals and patients (Table 18.1), and during the ANF infusion in the nephrotic patients urinary cGMP rose from 168±102 pmol/min to a peak of 624±214 pmol/min. In the patients cGMP fractional clearance rose only slightly from a basal mean of 57±13% to a peak mean of 83±26% in the final collection period of the ANF infusion.

Table 18.1.

Nephrotic patient details.

Diagnosis Sex/Age (years)	Blood Pressure (mmHg)	Plasma Albumin (g/l)	Urinary Protein (g/24h)	Urinary Sodium (mmol/24h)	Urinary cGMP (nmol/24h)
FGS/M/35	108/70	30	4.7	147	808
MEMB/M/21	112/90	31	5.2	119	378
MP/M/35	136/91	18	10.1	64	408
MP/M/32	118/80	24	21.3	196	732
FGS/F/27	102/72	22	10.2	67	205
FGS/M/33	144/88	18	17.6	70	120
30±2	120±7/83±4	24±2	11.5±2.7	111±22	442±113
Healthy Subjects					
29±2	106±6/76±4	*45±4	*<0.05	152±10	330±62

FGS = focal glomerulosclerosis

MEMB = membranous glomerulonephritis

MP =membranoproliferative glomerulonephritis

The group data are given as mean±SEM. * = $p<0.01$ in normal group compared to nephrotics, otherwise $p>0.05$. Urinary excretions refer to the 24 hour collection before the study.

Table 18.2.

Basal values in nephrotic patients.

	Normal Subjects (n=7)	Nephrotics (n=6)
Plasma data on the second basal period		
ANF (pmol/l)	1.9±0.4	*4.0±0.8
cGMP (nmol/l)	2.9±0.6	3.4±0.4
Sodium (mmol/l)	136±1	*132±1
Potassium (mmol/l)	4.3±0.3	4.6±0.3
PRA (nmol/l/h)	0.96±0.13	1.07±0.10
Aldosterone (pmol/l)	258±67	429±165
Urine data on the second basal period		
Sodium (μmol/min)	163±31	108±26
Potassium (μmol/min)	97±11	91±20
Volume (ml/min)	12.9±1.4	*6.8±2.3
cGMP (pmol/min)	217±27	168±42
GFR (ml/min/1.73 m^2)	130±5	96±22
ERPF (ml/min/1.73 m^2)	558±24	447±125
Filtration Fraction(%)	23.4±1.1	23.8±2.7
Albumin (μg/min)	6±2	*4900±1400
Pulse (beats/min)	65±3	69±7
Mean BP (mmHg)	85±4	92±5

Key: *= $p<0.05$ on nephrotic basal levels versus active day in normal subjects. Results are given as mean±SEM.

Figure 18.1 (following page).

Effects of ANF in the nephrotic syndrome compared with the healthy response.

Mean±SEM increments of plasma ANF, plasma cGMP, plasma renin activity (PRA), urinary sodium excretion ($U_{Na}+$) and filtration fraction (FF) following the start of the ANF infusion. Note that the third blood sample is taken only 15 minutes after starting the infusion. Data for sodium excretion are plotted at the midpoint of each half hour collection period.

Solid lines represent patients with the nephrotic syndrome, long dashes normal subjects receiving ANF, and short dashes normals receiving placebo.

The asterisks indicate means which differed significantly from both basal samples in the same study group (Duncans test for multiple comparisons).
* $p<0.05$, ** $p<0.01$ otherwise $p>0.05$.

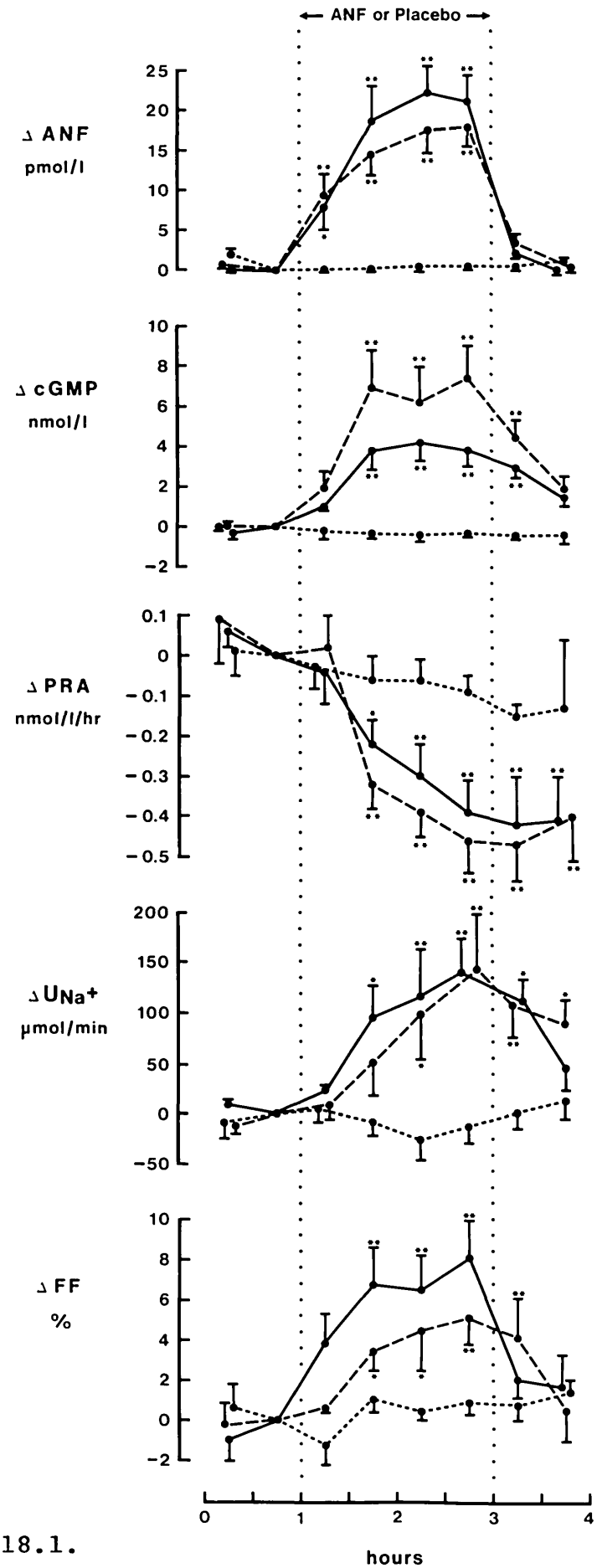

ANF or Placebo
Δ ANF
pmol/l
Δ cGMP
nmol/l
Δ PRA
nmol/l/hr
Δ UNa+
µmol/min
Δ FF
%
hours

Figure 18.1.

Table 18.3.

Comparison of the response of nephrotic patients with healthy subjects.

	Normals	Nephrotic
Plasma ANF (pmol.hr/l)	+31.5±3.8	+36.2±5.4
Plasma cGMP (nmol.hr/l)	+14.3±3.2	+8.9±0.9
GFR (l/1.73 m^2)	-1.20±0.84	+0.54±0.84
ERPF (l/1.73 m^2)	-14.58±2.88	-9.36±5.34
Filtration fraction (% hr)	+9.2±2.8	+14.2±3.2
Urinary Sodium (mmol)	+15.7±5.2	+15.6±3.7
Fractional Na^+excretion(%hr)	+1.51±0.45	+2.00±0.40
PRA (nmol/l)	-1.02±0.18	-0.91±0.24

The incremental areas are shown as mean±SEM after the second basal sample. Note that the values quoted are those in Table 18.2 multiplied by the dimension of time in hours. Thus, for example the data for urinary sodium excretion represents the amount of the ion excreted in excess of basal values during the three hour period.

There was no significant difference between the normal controls and the nephrotics in any category ($p>0.05$).

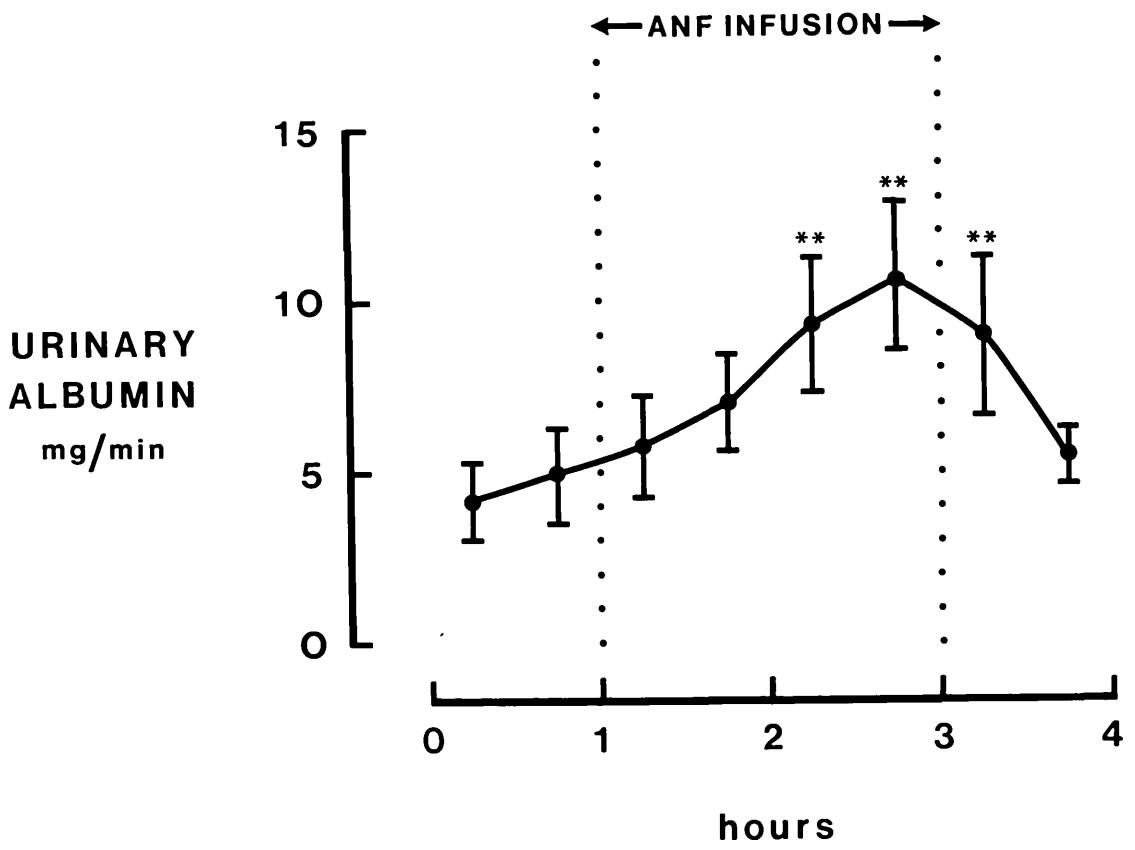


Figure 18.2.

Effect of ANF on urine albumin excretion in the nephrotic syndrome.

Data are mean±SEM, and the analysis as for Figure 18.1.
** = $p<0.01$ versus both basal values, otherwise $p>0.05$.

Plasma Renin Activity and Aldosterone.

Basal PRA was similar in normals and patients (Table 18.2) and in the patients it fell with a similar pattern and total response to the normal group (Figure 18.1, Table 18.3). In the patients as a whole the changes in plasma volume, calculated from changes in haemoglobin and haematocrit, were not significant and individual responses varied from -20 to +6%.

In nephrotic patients the basal mean plasma aldosterone was similar to the healthy range (Table 18.2). During ANF infusion aldosterone levels fell from 429±165 pmol/l to a nadir of 165±64 pmol/l and tended to rise during the recovery hour so that the final value was similar to the basal ($p<0.005$).

Plasma and Urinary Electrolytes.

In the patients plasma potassium concentration fell significanlty during the study, from a peak of 4.6±0.3 to 4.0±0.2 mmol/l. There was no change of plasma sodium concentration.

In the patients the mean daily excretion of sodium on the day before the experiment was not significantly different from the normals although three patients excreted less than 75 mmol/day, suggesting mild active sodium retention (Table 18.1).

In the patients and healthy subjects ANF infusion was

associated with a progressive rise in sodium excretion over the two hours (Figure 18.1), and the total response was similar in both groups (Table 18.3). In the recovery hour, the natriuresis abated more rapidly in the nephrotics than in the normals subjects. In both patients and the healthy subjects a similar rise was measured in fractional sodium excretion (Table 18.3). Urinary potassium excretion fell similarly in both groups during the course of the active infusion.

Urine Volume.

The lower basal urine flow in the nephrotic patients suggested a degree of water retention in this group which received identical water loading to the healthy subjects. In the patients there was no significant change in volume excretion during the study, although the mean flow rate tended to fall in the recovery hour.

Renal Haemodynamics.

In patients the mean GFR tended to rise during the ANF infusion and fall in the recovery period, but the change was not significant. Although their mean ERPF tended to fall, individual responses were variable and therefore the response of the whole group was also not significant (ERPF rose in one patient, but it fell in the rest). In both the healthy and nephrotic group ANF infusion was associated with similar significant increases in filtration fraction (Figure 18.1, Table 18.3).

Plasma and Urinary Proteins.

There was no significant change of plasma total protein and albumin in the patient group. Albumin comprised 80±8% of the total urinary protein excreted during the preceeding 24 hours (9.1±3.4 g albumin versus 11.5±2.7g total protein per day). During the ANF infusion albumin excretion rate rose two-fold, falling to baseline values during recovery (Figure 18.2), and there was also a significant rise in the fractional excretion of albumin (100% x albumin clearance/ GFR) from a basal value of 0.37±0.22% to a peak of 0.58±0.23% at the end of the ANF infusion and falling to 0.46±0.24 % during the recovery hour ($p<0.005$).

Blood Pressure.

Towards the end of the ANF infusion one patient experienced nausea associated with a fall of blood pressure of less than 10 mmHg. These symptoms disappeared rapidly after ANF infusion was discontinued. Despite this reaction, there were no significant change of blood pressure in the nephrotic group as a whole.

Discussion.

ANF and cGMP.

During the ANF infusion the response of plasma peptide was similar in patients and healthy subjects, and, as discussed in Chapter 17, the rise is probably

equivalent to the maximal increment in plasma peptide produced by physiological stimuli such as head-out water immersion. The mean basal level of ANF in the patients with the nephrotic syndrome was twice as high as the healthy subjects, who had a similar age and blood pressure. Therefore, my patients may fit into the subgroup of nephrotics who have an overfilled circulation (Meltzer et al, 1979). However, two patients had a subnormal GFR, which could have lowered ANF clearance (Chapter 11). Moreover, the nephrotic patients may have been artificially volume expanded because thay had low basal urine flow rates verus the healthy group, despite identical water loading.

Tulassay et al (1987) found a range of ANF values, from sub- to supranormal, in nephrotic children. In adults Pedersen et al (1988) described elevated plasma ANF in patients who had oedema resistant to diuretics, but Peterson et al (1988) found strikingly low levels in another group of patients with nephrotic syndrome. This lack of consistency may reflect the wide range of plasma volumes that have been reported in the syndrome (Meltzer et al, 1979).

The basal plasma cGMP was similar in normals and patients. The absolute rise of plasma nucleotide was the same in both groups, suggesting a similar tissue effect of ANF. The fall of plasma cGMP after the

peptide infusion in both groups was delayed compared to that of ANF: this may represent a slower plasma clearance of cGMP versus ANF, or the continued production of cGMP.

Plasma Renin Activity and Aldosterone.

In the nephrotic syndrome the renin-aldosterone axis may be activated, normal or suppressed depending on the primary disorder, and the stage of evolution of the syndrome (Meltzer et al, 1979; Geers et al, 1984; Dorhout Mees et al, 1984). My patients had a variety of histological lesions, although none had minimal change disease, and only one was studied at the onset of the syndrome, the others having been been nephrotic for up to three years. Although the plasma ANF in my patient group was elevated compared with the normals, PRA was not low. One interpretation would be that in the presence of volume expansion, renin secretion was inappropriately high. This could be resolved by studying the relation of plasma ANF, PRA and blood volume in a larger group of nephrotic patients.

The role of aldosterone in the genesis of sodium retention in the nephrotic syndrome is debated. Although high levels of the steroid were thought to be important in causing sodium retention, angiotensin-converting enzyme inhibitors do not regularly induce a natriuresis, and after steroid-induced remission in minimal change disease plasma aldosterone may rise

without associated sodium retention (Brown et al, 1982 a and b).

In the current study, as well as suppression by ANF, the small but significant fall of plasma potassium may have contributed to the fall in aldosterone levels. Therefore, there must be uncertainty regarding both the cause of the fall in aldosterone and its significance in the natriuresis.

Sodium Excretion.

The classical explanation for the sodium retention in the syndrome comprised the sequence of proteinuria, hypoalbuminaemia, loss of plasma volume and activation of the renin-aldosterone system. This theory has been challenged: firstly, not all nephrotics have low plasma volumes and elevated PRA (Meltzer et al, 1979; Geers et al, 1984), and during steroid-induced natriuresis blood volume was unchanged (Brown et al, 1985a). Secondly, in animal models, severe proteinuria is not always accompanied by sodium retention (Van Liew et al, 1985) and oedema formation can precede proteinuria (Kalant et al, 1962).

Other studies found that proximal tubular sodium reabsorption is depressed in the syndrome and that excessive sodium reabsorption occurred in the distal part of the nephron (Grausz et al, 1972; Ichikawa et al, 1983). At least part of the ANF-induced natriuresis

in normals man and animals is attributed to a distal renal tubular action (Sonnenberg et al, 1986; Biollaz et al, 1987; Chapter 15 and 17), and therefore it appeared possible that in the nephrotic syndrome, sodium retention might be caused or exacerbated by a resistance to that action of ANF.

In my patients the renal effects of ANF were not diminished, confirming observations by Rabelink et al (1988) who used a pharmacological bolus of ANF in six nephrotic subjects. However, despite the presence of oedema some of my nephrotics may have reached a steady-state of sodium intake and excretion. It remains to be determined whether physiological doses ANF would cause a natriuresis in the presence of extremely avid sodium retention that can occur earlier in the disease.

In contrast my results, Hildebrandt et al (1988) who used amino-nucleoside to induce the disorder in rats and Perico et al (1987), who employed adriamycin, reported a diminished natriuresis compared to normal after pharmacological doses of ANF. Keeler et al (1987) found a blunted natriuresis to blood volume expansion in nephrotic rats despite a normal rise of plasma ANF, and similar results were reported in nephrotic humans during head-out water immersion (Peterson et al, 1988). However, many of these protocols used aminals and patients with a reduced GFR, and this may have

modified the response. Indeed, in my own group the greatest and smallest absolute increases in sodium excretion occurred in the patients with the highest and lowest basal GFR respectively, although in the group as a whole there was no significant correlation between natriuresis and basal GFR.

Renal Haemodynamics.

Rabelink et al (1987) recorded an ANF-induced rise of GFR nephrotic patients. However, a phamacological dose was used and the results may not be comaprable to my more physiological study in which no significant change occurred in GFR. My study agrees with previous low dose infusions in healthy subjects (Chapter 17). In the current study, the tendency for the ERPF to fall, together with the small rise of GFR, produced a significant rise in filtration fraction. Similar changes in filtration fraction are found during low dose ANF infusions in healthy subjects (Chapter 17), and also occur during higher dose infusion into nephrotic patients (Zietse and Schalekamp, 1988).

Rises in filtration fraction are also found in animal studies and may be the result of efferent glomerular arteriolar constriction, and afferent dilatation together with an increase in glomerular permeability (Camargo et al, 1984; Fried et al, 1986). Although these observations could explain the rise in filtration fraction in the current study, caution should be

applied in comparing animal experiments which used massive ANF doses to human pathophysiology.

Albuminuria.

The enhanced absolute and fractional albumin excretion, found in the nephrotic group and one normal volunteer (Chapter 17), has also been reported when higher doses of ANF were given to healthy subjects (McMurray et al, 1988b) and to patients with the nephrotic syndrome (Zietse and Schalekamp, 1988). The explanation for this phenomenon is not known but the following possibilities can be considered.

a) An acute water load enhances albuminuria in normals, possibly by a tubular 'wash-out' effect (Viberti et al, 1982), but in my patient group there was no significant increase of urine volume during the ANF infusion.

b) Proteinuria may follow a circadian rhythm, the cause of which is not known (Koopman et al, 1987). Although my nephrotic study lacked a placebo group, the clear temporal association between the rise and fall of albuminuria and the beginning and end of the ANF infusion, makes a circadian rhythm a less likely explanation.

c) Albumin is partly reabsorbed by the tubules and it is theoretically possible that ANF might inhibit this process. However, the failure of McMurray et al (1988b)

to find a significant rise of B2-microglobulin, which is also reabsorbed, after pharmacological doses of ANF argues against this idea. This question could be resolved by studying the effects of ANF on the excretion of dextrans which are not reabsorbed.

d) The mechanism of ANF-induced albuminuria may have a haemodynamic basis, like angiotensin II-induced proteinuria (Bohrer et al, 1977). According to this model, a fall in renal glomerular plasma flow, associated with an increase in filtration fraction and concentration of protein within the glomerular capillary, would tend to increase the fractional and absolute clearance of macromolecules, despite an unchanged GFR. However, despite a fall of renal plasma flow and a rise of filtration fraction in my healthy subjects during ANF infusion, that group as a whole showed no change in albumin excretion. Therefore, haemodynamic factors alone probably do not account for the rise in albumin excretion.

e) In renal disease, alterations of both the charge of glomerular barrier and its size-selectivity contribute to the proteinuria (Skorecki et al, 1985). The area for glomerular filtration is partly determined by the state of contraction of mesangial cells (Brenner at al, 1985), and these cells bind ANF (Ballerman et al, 1985). If ANF could cause relaxation of contractile mesangial cells, then glomerular barrier permeability

and proteinuria might be enhanced. The disproportionate effect on albuminuria compared to inulin clearance could be explained by the appearance of a small proportion of larger channels that would make a large impact on percentage change in macromolecule clearance, but have little effect on total inulin clearance. However, at the time of writing, there is no direct evidence to support such a mechanism. Finally, ANF infusions into nephrotic patients enhanced the excretion of both neutral immumoglobulin G and anionic albumin, suggesting that ANF does not affect the electrical charge of the glomerular barrier (Zietse and Schalekamp, 1988).

Summary of Chapter 18.

1. Plasma ANF was higher in a group of water-loaded patients with the nephrotic syndrome compared with a healthy group of a similar age. Two patients had a low GFR and three had mild active sodium retention.

2. A low dose intravenous ANF infusion produced similar natriuresis, rise of plasma cGMP, suppression of plasma renin activity and rise of filtration fraction compared to the healthy group.

3. Absolute and fractional urinary albumin excretion increased significantly during the ANF infusion.

CHAPTER 19.

THE RENAL EFFECTS OF ANF INFUSION IN PATIENTS WITH CHRONIC RENAL FAILURE.

Introduction.

Previous studies have suggested that sodium balance is maintained in chronic renal failure (CRF) by an increase in fractional excretion of sodium (Slatopolsky et al, 1968; Danovitch et al, 1977), and that this effect might be mediated by an unidentified circulating natriuretic agent (Bourgoignie et al, 1974; Fine et al, 1976).

Plasma ANF values are often raised in patients with CRF who do not require dialysis, and this may be due to blood volume overload, decreased clearance of the peptide and coexisting cardiac disease (Chapter 11). However, the biological effects of these high levels are unknown. In order to study the role of ANF in this disorder I have followed the renal and hormonal effects of intravenous ANF infusion.

Patients and Methods.

After approval by the Department of Health and the Hospitals' Ethical Committees, 15 patients with CRF were recruited from nephrology clinics, and they gave informed consent. None had valvular heart disease, a

history of cardiac ischaemia, nephrotic syndrome nor peripheral oedema. In addition, none had a severe salt-wasting state as judged by the absence of postural hypotension and the lack of need of dietary sodium supplements. Finally, none had received immunosuppressive treatment within the previous year.

They were assigned alternately to have either an ANF or placebo infusion, and the experiment was performed in single blind fashion. The active group (n=8, 4 males), had diagnoses of glomerulonephritis (3), chronic pyelonephritis (3), nephrolithiasis (1) and adult polycystic disease (1). The placebo group (n=7, 3 males) had diagnoses of glomerulonephritis (3), pyelonephritis (3) and analgesic abuse (1). One patient in each group took their regular nifedipine on the study day, and one subject in each group stopped a thiazide and beta-blockade two days before the study. Dietary sodium was uncontrolled but the groups had similar urinary sodium excretion on the day before the experiment (ANF group, 127±21 mmol/day and placebo group, 145±23 mmol/day). The daily protein excretion of the active group was 1.27±0.30g, and that of the placebo group was 1.05±0.33g. Their plasma creatinines were 474±88 and 398±86 umol/l respectively. Individual details are given in Table 19.1.

On the the study day the experimental protocol was identical to that in healthy subjects (Chapter 17) with

regard to water loading, ANF infusion and blood and urine collections. After the loading dose, the rate of infusion of PAH and inulin was modified according to individual patient's renal function calculated from recent creatinine clearances performed as an outpatient. Inulin and PAH clearances were taken to equal the GFR and ERPF respectively. No correction was made for tubular extraction efficiency of PAH which was unknown in these patients. Fractional clearances of solutes were calculated by standard formulae: eg: fractional cGMP excretion = 100% X cGMP clearance/GFR. Finally, note that ERPF and serial ANF could not be measured in one active patient due to technical reasons.

Statistics.

Unless otherwise stated results are given as mean$\pm$SEM. Because of the wide variation between subjects in basal GFR, ERPF, sodium and albumin excretion, these responses were expressed as percentages of the values during the basal period immediately before the start of the ANF or placebo infusion. When the results were studied, it was apparent that the placebo group's absolute and fractional sodium fell during the experiment, and so the statistical method had to consider the response of both groups together. Therefore, firstly the total response of each group was

compared by applying Stutent's t-tests to the areas under the curves after the second basal sample. Only if a significant difference was found ($p<0.05$, Table 19.3), then Student's unpaired t-tests were applied between mean active and placebo values at each time point.

Results.

Basal values in the two groups were similar (Table 19.2). Total responses are shown in Table 19.3, and serial results of plasma ANF, plasma cGMP, urinary sodium, GFR and albuminuria are shown in Figures 19.1 and 19.2. The raw data are tabulated in the Data Appendix (Chapter 22).

Plasma ANF.

During the active infusion plasma immunoreactive (99-126) ANF rose fourfold, and rapidly returned to basal levels in the recovery hour. The total response was significantly greater than that of the placebo group, in which plasma ANF was constant. The mean ANF of the active group was significantly higher than placebos at all time points during the ANF infusion, and levels of the peptide had fallen to placebo levels by the first sample of the recovery hour.

Plasma and Urine cGMP.

On the active day plasma cGMP increased two-fold and

urinary cGMP rose from 140±30 pmol/min to 396±82 pmol/min. At corresponding periods the fractional excretion of the nucleotide did not change significantly (from 115±19 to 104±9% of GFR). During the placebo study no change occurred in plasma or urinary cGMP. Compared to the plasma ANF response, plasma cGMP showed a lag in rising and remained significantly eleveated compared to placebos in the first sample of the recovery hour (Figure 19.1).

Renin and Aldosterone.

Plasma aldosterone fell on the active day from a basal value of 1595±498 to a nadir of 826±134 pmol/l. Aldosterone was constant on the placebo day, but there was no significant difference between the groups. PRA showed a tendency to fall throughout the study in both groups, possibly due to the oral water loading.

Renal Haemodynamics.

On the active day mean GFR tended to increase, and in the recovery period fell rapidly to basal values. However, the individual absolute increments were variable, ranging from 2-20 ml/min. Although GFR in the placebos was unchanged through the experiment, the difference of incremental areas between the two groups failed to reach significance (p=0.077, Table 19.3). In both groups, ERPF was constant, and although the filtration fraction of the active group tended to rise,

there was no significant difference between the total response of each group (Table 19.3).

Urinary Electrolytes and Volume.

On the active day, urinary sodium response of the active group was significantly greater than that of the placebos ($p=0.001$, Table 19.3). In the active group natriuresis increased slowly to reach a peak of 168±20% of basal values in the final half-hour of the ANF infusion. By the end of the recovery hour the excretion rate had returned to basal values but was still significantly higher than that of the placebo group (Figure 19.1). In all of the patients in the placebo group there was a striking steady fall of sodium excretion rate to approximately 50% of basal values by the end of the study.

During the ANF infusion, fractional excretion of sodium tended to rise from 5.6±1.1% to 7.0±1.0% by the end of the infusion, and it then fell rapidly in the recovery hour to 5.4±1.3%. In contrast, fractional excretion fell steadily in the placebo group from 6.1±1.7% to 3.3±0.8% by the end of the study. However, because of some variation of individual responses, there was no significant difference between the groups as a whole.

Urine potassium excretion remained constant in both groups, and urine flow rate showed a parallel rise and fall with sodium excretion on the active day but

remained unchanged on the placebo day.

Urinary Albumin.

Note that the basal protein excretion on the day before the study showed that none of the patients had nephrotic-range proteinuria (Table 19.1).

On the active day urinary albumin increased in all patients. The individual absolute responses varied widely from approximately x2 to x10 of the basal levels. The active group mean rose to a peak of 280±110% of basal values during the second hour of the ANF infusion, and the total response was significantly greater in the active patients than in the placebo group ($p=0.018$, Table 19.3). Albumin excretion in the active group tended to fall in the recovery hour but excretion still exceeded the placebo group's response at these time points (Figure 19.2). This pattern resembled the natriuretic and plasma cGMP response to ANF. The fractional excretion of albumin also rose in the patients infused with ANF, but because of the tendency for GFR to increase, this rise was not significant.

Blood Pressure.

In both groups blood pressure, pulse, plasma electrolytes and plasma total protein were constant. No patient experienced any side-effects during either the ANF or placebo infusions.

Table 19.1.

Chronic renal failure patient details.

Diagnosis/Age/Sex	Plasma Creatinine (μmol/l)	Urine Sodium (mmol/day)	Urine Protein (mmol/day)
	Active Group		
GN/42/M	747	91	0.52
PY/30/F	651	130	0.58
PY/30/M	253	126	1.92
GN/33/F	615	150	2.23
PY/62/F	448	91	1.02
PCK/44/M	140	240	0.38
GN/47/M	191	150	0.98
LITH/30/F	738	41	2.56
	Placebo Group		
GN/44/M	198	96	1.01
AN/60/F	624	201	0.32
PY/41/F	172	120	0.45
GN/47/F	563	132	1.97
PY/27/M	704	152	0.78
GN/47/M	184	244	0.23
PY/18/F	341	70	2.54

Key: GN = chronic glomerulonephritis, PY = chronic pyelonephritis, PCK = polycystic disease, LITH = nephrolithiasis, AN = analgesic nephropathy. Urinary excretions refer to the day before the ANF infusion.

Table 19.2.

Basal values of chronic renal failure patients.

	Active Group (n=8)	Placebo Group (n=7)
Age (years)	40±4	41±5
Mean BP (mmHg)	96±4	97±7
Pulse (bpm)	64±6	62±2
	Plasma Indices	
Sodium (mmol/l)	136±1	134±1
Potassium (mmol/l)	4.5±0.2	4.5±0.3
Albumin (g/l)	40±2	41±1
ANF (pmol/l)	10.2±2.4	10.6±1.8
PRA (nmol/h/l)	1.24±0.33	1.36±0.59
Aldosterone (pmol/l)	1595±498	1207±425
Cyclic GMP (nmol/l)	7.0±1.4	6.4±1.3
	Urine Indices	
Cyclic GMP (pmol/min)	140±30	167±44
GFR (ml/min/1.73m^2)	13.5 (8.3-52.4)	23.3 (13.3-45.1)
ERPF(ml/min/1.73m^2)	*30 (20-176)	88 (23-157)
Volume(ml/min)	4.4±0.8	4.0±0.7
Sodium (µmol/min)	115 (44-408)	179 (77-224)
Sodium (% GFR)	5.62±1.06	6.13±1.66
Potassium (µmol/min)	51±5	47±8
Albumin (µg/min)	575 (70-1612)	385 (120-1610)
Albumin (%GFR)	0.054(0.003-0.406)	0.045(0.006-0.195)

Basal data are given as mean±SEM or median (range).

* Note the n=7 only on Active ANF and ERPF values.

Figure 19.1 (following page).

Effect of ANF infusion in chronic renal failure.

Mean±SEM values of plasma ANF, plasma cyclic GMP, inulin clearance (GFR) and urinary sodium excretion (UNa^+). Data for GFR and urinary excretions are plotted at the midpoint of each half hour period and are expressed as percentages of the second basal collection.

Solid lines represent the group infused with ANF and short dashes represent the separate placebo group who received vehicle only. The asterisks indicate a significant difference between means of each group at each time point, * $p<0.05$ and ** $p<0.01$.

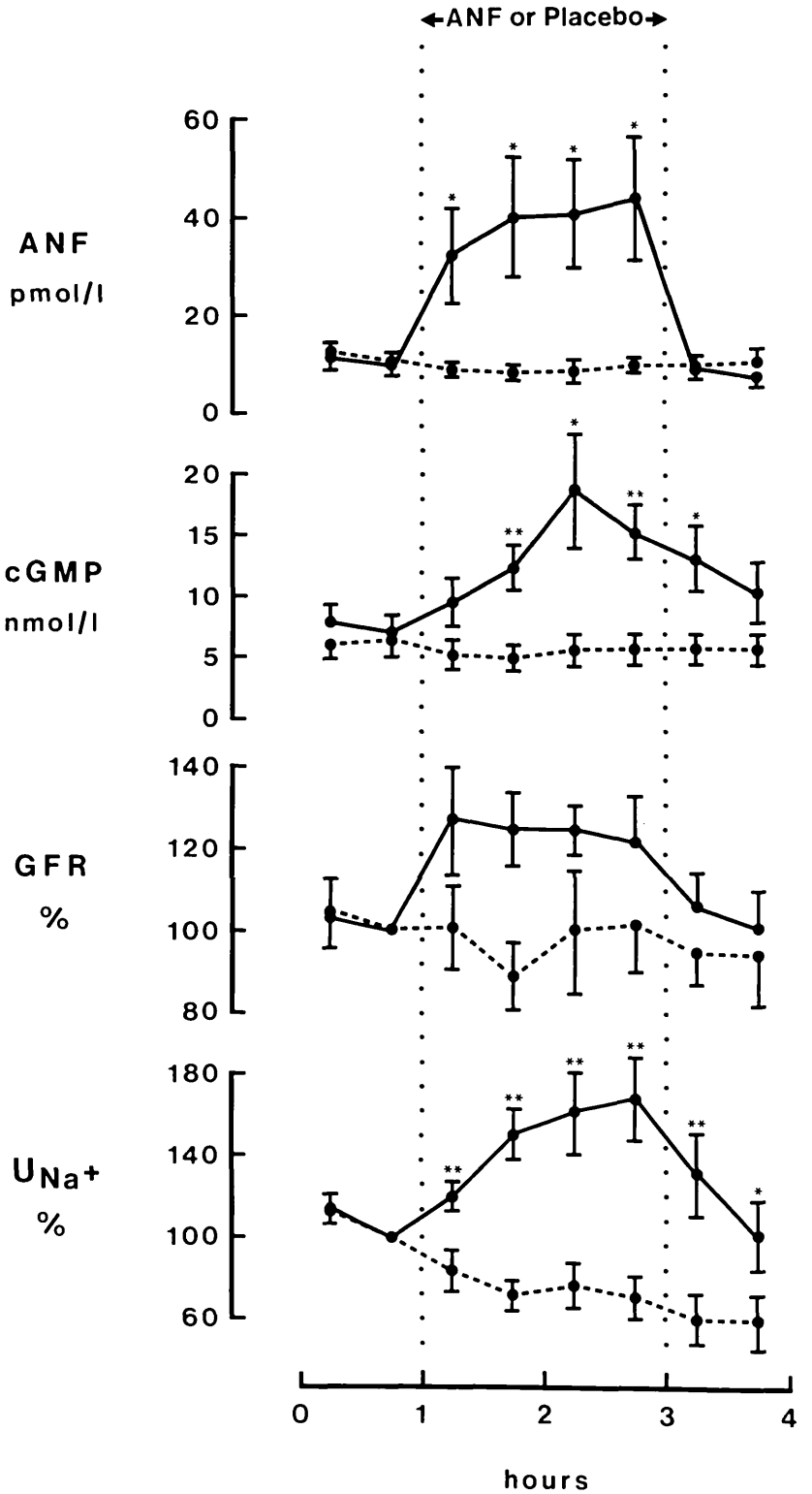


Figure 19.1.

Effects of ANF infusion in chronic renal failure.

For key see previous page.

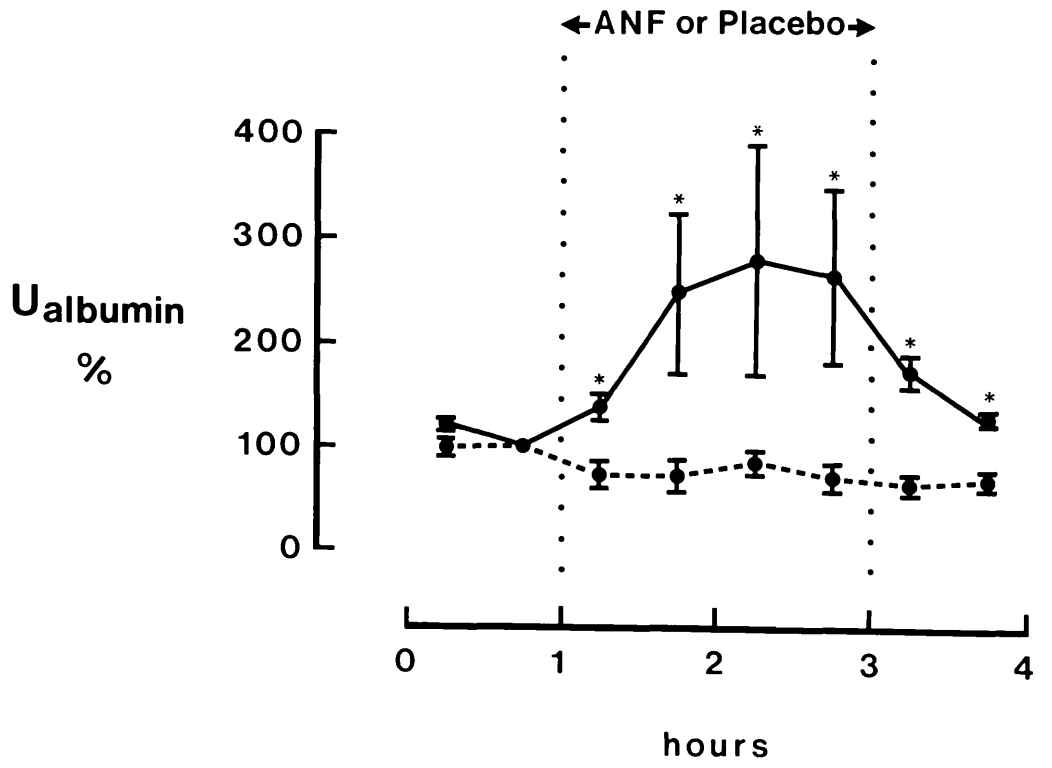


Figure 19.2.

Effect of ANF infusion on albumin excretion in chronic renal failure.

$U_{albumin}$ is the urinary albumin excretion expressed as percent of second basal sample, otherwise the key is as for Figure 19.1.

Table 19.3.

Effect of ANF in chronic renal failure: incremental areas after second basal sample.

	Active (n=8)	Placebo (n=7)	p
Plasma ANF (pmol.h/l)	+57.2±20.3	+2.9±2.7	0.013
Plasma cGMP (nmol.h/l)	+21.9±5.2	-2.3±2.5	0.001
Urine Sodium (%.h)	+121.0±4.0	-90.3±27.5	0.001
Urine Albumin (%.h)	+316±145	-4.9±1.7	0.018
GFR (%.h)	+71.8±31.9	-10.0±28.3	0.077
Filtration Fraction (%.h)	+5.8±5.2	-6.4±6.4	0.165

This Table shows the areas under the curve (mean±SEM) after the second basal sample. Units are the same as those in Table 19.2, multiplied by the time factor in hours. The response of each group is compared by Student's t-test.

Discussion.

ANF and cGMP.

The peak mean plasma value of ANF during the peptide infusion (44.0±12.4 pmol/l), was four times the basal value, and was just above the upper limit (37.6 pmol/l) of a range of basal plasma levels measured in 36 patients of a similar age range and degree of renal impairment (Chapter 11).

The rise of ANF caused a significant tissue effect as judged by the increases of plasma and urinary cGMP. At all time periods, in both the active and placebo groups, the ratio of cGMP to inulin clearance was approximately unity and therefore no nephrogenous nucleotide was detected, confirming previous observations in healthy subjects (Broadus et al, 1970; Chapter 17). Arterial walls are rich in ANF receptors (Leitman et al, 1987) and may have contributed the majority of both plasma and urinary cGMP. Therefore, urinary cGMP could not have been a marker for the specific renal effects of ANF in these CRF patients.

Sodium Excretion and Glomerular Filtration Rate.

The natriuresis during the ANF infusion contrasted with a fall which was measured in the placebo group. A similar fall has been recorded in another study, and patients with CRF excrete more sodium at night compared to daytime hours (Hillier et al, 1980).

Although early views suggested that patients with CRF must always waste sodium on low salt intakes (Coleman et al, 1966), other authors found that the kidneys of similar patients could adapt to both increases and decreases of dietary sodium, albeit more sluggishly than in healthy subjects (Slatopolsky et al, 1968; Danovitch et al, 1977).

It is possible that ANF may participate in this adaptation because in animals and humans with CRF plasma ANF rises when dietary salt is increased (Smith et al, 1986; Czekalski et al, 1988). The four-fold increase of plasma ANF in the current study is likely to represent a change of the same order as that induced by sodium loading: central blood volume expansion in normals cause a two to four-fold rise of plasma ANF. Moreover, patients with severe renal failure are reported to show an exaggerated ANF rise to acute intravenous saline infusion (Walker et al, 1987).

Bricker has argued that salt balance is maintained in patients with CRF by an increased tubular rejection of sodium (Slatopolsky et al, 1968), and animal studies have also emphasised the importance of tubular adaptations. Schultze et al (1972) found an increased proximal tubular rejection of sodium in uraemic animals, although more recent evidence has pointed to the distal tubule, specifically the collecting ducts,

as the final regulator of sodium excretion in renal impairment (Kahn et al, 1972; Wilson and Sonnerberg, 1979). Importantly, these studies did not document changes in whole animal or single nephron GFR which might theoretically have played a role when changing from a low to high sodium diet. Unfortunately, my current study did not differentiate between a glomerular and a tubular mechanism of natriuresis, because although both GFR and fractional sodium excretion tended to increase, neither change was significantly different from the placebo group.

GFR rises after the administration of pharmacological doses of ANF to rats with CRF (Cole et al, 1985; Sterzel et al, 1987), to healthy humans (Chapter 16), and to patients with CRF and gomerulonephritis (Suda et al, 1988; Ishii et al, 1988). Even though I may have 'missed' a significant rise of GFR because of experimental error, when low-dose ANF is administered to healthy subjects (Richards et al, 1988; Biollaz et al, 1987; Chapter 17) and to patients with nephrotic syndrome (Chapter 18; Zietse and Schalekamp, 1988) natriuresis occurs despite a constant GFR. Although the importance of a rise in GFR in the ANF-induced natriuresis is likely to remain uncertain because of the difficulty in measuring small changes of GFR with great accuracy, it should be noted that acute changes in GFR generally have minor effects on final sodium

excretion as long as 'glomerulo-tubular balance' is maintained (Chapter 14).

Within the current study group, there was no correlation between percent rise in sodium excretion and the basal GFR. However, the search for this type of correlation is not be justified because of the range of individual basal arterial blood pressures and a wide range of plasma ANF increments. Such factors may have determined the natriuresis in addition to GFR. In contrast, comparisons between the active and placebo groups as a whole was valid because each comprised patients who had a similar range of basal arterial pressures, plasma ANF and GFR (Table 19.2).

There are also problems when attempting to compare the natriuretic effect of ANF in patients with CRF with the natriuresis found in healthy subjects using a similar dose of ANF (Chapter 17). The peak mean ANF increment in the CRF study was 50% greater than the ANF rise in the normals, possibly reflecting decreased ANF clearance secondary to increased age (Ohashi et al, 1987) or decreased GFR (Weselcouch et al, 1985). Moreover, the CRF group had a higher basal blood pressure (MBP 96$\pm$4 mmHg) than the normals (85$\pm$4 mmHg), and this might have affected the subsequent natriuresis (Raine et al, 1989; Pang et al, 1985). Despite these confounding influences, the general time course for the rise and fall of plasma ANF, plasma cGMP and

natriuresis were similar in healthy subjects and patients with CRF. Therefore, there appeared to be no major resistance to ANF-induced natriuresis despite renal impairment and high ambient levels of ANF.

Urinary Albumin Excretion.

A rise in albuminuria has been recorded after high doses of ANF in both healthy subjects (McMurray et al, 1988b) and in patients with glomerulonephritis (Zietse and Schalekamp, 1988; Ishii et al 1987; Chapter 18). The current study shows that the effect occurs with low doses of the peptide in patients with renal impairment who have both primary glomerular and interstitial diseases.

The possible causes of this phenomenon have been discussed in the previous chapter, and include tubular effects such as a 'wash-out' of albumin (due to water loading) and decreased tubular uptake and the effect of circadian rhythms. In the current study both water loading and circadian rhythms are unlikely explanations for the albuminuria because no change was observed in the placebo group.

Although haemodynamic changes may affect glomerular proteinuria (Chapter 14), and may have contributed to the ANF-induced rise in albumin excretion in nephrotics (Chapter 18), no significant changes in GFR, ERPF or filtration fraction could be discerned in the current

study. This negative finding may reflect the difficulty of accurately measuring small changes of inulin and PAH clearances.

Direct measurement of glomerular haemodynamics is not possible in humans, but in future the study of ANF's effect on the urinary clearance of dextrans of different molecular sizes and charges might yield useful data about alterations of permeability of the glomerular filter.

Summary of Chapter 19.

1. Infusion of ANF into eight patients with CRF, who did not require dialysis, raised immunoreactive plasma (99-126) ANF four-fold, to the upper limit of a range of basal values previously recorded in a larger group of similar patients.

2. Significant rises, compared to a separate placebo group, were recorded in plasma cGMP and in urinary sodium excretion.

3. No significant change was observed in renal haemodynamics, although the GFR tended to rise.

4. During ANF infusion, urinary excretion of albumin rose in all patients. The mechanism of this effect requires further investigation.

CHAPTER 20.

CONCLUSIONS.

Introduction.

My studies have measured basal levels of plasma ANF in healthy subjects (Chapter 10), nephrotic patients (Chapter 18) and patients with CRF, both before (Chapters 11 and 19) and after (Chapters 12 and 13) the need for the start of haemodialysis. In addition I have infused low doses of ANF into normals, patients with the nephrotic syndrome and also patients with renal failure in order to study the renal effects of the peptide (Chapters 17 to 19). Conclusions regarding the biological significance of ANF make two assumptions.

1) Firstly, comparison of immunoreactive plasma ANF in renal disease and health assumes that the measured substances are the same. Although I have not performed detailed analysis of plasma extracts, I have argued that other investigators, who used silmilar extraction methods and antisera, have found that the majority of ANF immunoreactivity in renal failure plasma corresponds to a substance similar to (99-126) ANF, as identified by a number of techniques (Chapter 2).

2) Secondly, if the effects caused by the ANF infusions are to have physiological significance, then the attained plasma peptide levels must not be

excessively high. The rises of plasma ANF were thought to be similar to the maximum stimulation attained in health and renal disease by sodium loading and by head-out water immersion.

Plasma Levels of ANF in Renal Disease.

In patients with CRF who did not require dialysis (Chapter 11) mean plasma ANF was twice that of a group of healthy subjects who had a similar age and sodium excretion (Chapter 10), although there was a large overlap between the ranges. In the heamodialysis group ANF was strikingly elevated, with little overlap with the healthy basal range. Elevated plasma ANF might represent blood volume expansion, or decreased clearance of the peptide. Although, renal ANF clearance must fall in the presence of kidney disease, I have argued that this mechanism can account for only a small proportion of total ANF clearance (Chapter 11).

In nephrotic patients (Chapter 18) basal plasma ANF were slightly, but significantly elevated when compared to normals of the same age sex and blood pressure, suggesting an expansion of plasma volume. However, this result must not be extrapolated to all nephrotic patients because of the variety of blood volumes and degrees of renal impairment which can occur in the disorder.

ANF and Arterial Blood Pressure in Renal Disease.

In the non-dialysed group with renal impairment there was a weak but significant positive correlation between basal ANF and blood pressure (Chapter 11). In contrast, in the small group of dialysis patients there was no such relation (Chapter 12).

If ANF levels are a marker of blood volume, this suggests that before the initiation of dialysis blood volume is related to arterial blood pressure, but that this no longer is true after the initiation of dialysis. Although this theory agrees with the conclusions of some authors on the relation of blood volume with arterial pressure (Weidmann, 1984), it must be noted that others have found a significant positive correlation between basal ANF and arterial pressure in haemodialysis patients (Walker et al, 1987).

Although pharmacological doses of ANF regularly lower arterial blood pressure in normals (Genest et al, 1988), the two hour infusion which raised mean levels in the CRF patients to a peak of about 40 pmol/l failed to lower blood pressure (Chapter 19). This suggests that in renal impairment plasma ANF is a marker of volume expansion rather than a major determinant of blood pressure.

Having said this, it is possible that more prolonged infusions might have a hypotensive effect by enhancing

sodium excretion, and that a more accurate method of pressure recording, such as intra-arterial readings, would have detected small changes which not apparent from the sphygmomanometer readings. Indeed, preliminary reports suggest that prolonged infusion of low-dose ANF may cause hypotension in healthy subjects (Espiner and Richards, 1989).

ANF and Sodium Excretion in Renal Disease.

I found no correlation between basal plasma ANF and either daily total or fractional sodium excretion in the group with renal impairment (Chapter 11). Therefore, other hormonal systems (aldosterone and natriuretic substances other than ANF) together with the degree of renal tubular damage may also determine daily sodium excretion in this group.

However, when the effects of ANF infusion were studied in individual patients (Chapter 19), a significant natriuresis occurred. Although the results of that study could not be directly compared to the effect of ANF in normals (Chapter 17), because of differences of group age, blood pressure and ANF levels (basal and incremental), both the pattern and time course of natriuresis was similar in the two groups. It can be concluded that there is no major resistance to the natriuretic effects of ANF in these patients. In

contrast, during ANF infusion in subjects with congestive cardiac failure, a disorder also associated with volume overload and high basal plasma ANF, the natriuresis after ANF is markedly blunted, possibly due to diminished renal perfusion and high levels of renin and aldosterone (Cody et al, 1986).

Unfortunately, my study of ANF infusion in renal impairment could not answer the important question whether the natriuresis was mediated by a rise of GFR or by an increase of fractional sodium excretion (or by both of these). Fractional sodium excretion and GFR tended to rise during the ANF-induced natriuresis but neither response was significantly different in the active compared to the placebo group.

In the nephrotic patients (Chapter 18), the absolute ANF-induced natriuresis was the same as that in normals of a similar age, sex and arterial blood pressure. Therefore, resistance to ANF probably did not explain the oedema of these patients. However, none suffered from very avid active renal sodium retention, and it is possible that such a group might demonstrate a blunted natriuretic response to ANF infusion.

ANF and cGMP in Renal Disease.

Plasma cGMP was elevated in patients with renal failure both before and after the need for dialysis (Chapters

12,13 and 19). After renal transplantation plasma levels showed a parallel fall with ANF, reflecting either increased renal clearance or a lesser tissue effect of ANF.

During ANF infusions, plasma cGMP rose significantly and then fell in the recovery hour, indicating a significant tissue effect of ANF. The time course of response in normals, patients with nephrotic syndrome and renal impairment was similar.

Finally, the pattern of urinary response mirrored plasma nucleotide levels, and in all studies urinary cGMP could be accounted for by nucleotide calculated to have been filtered by the glomerulus. Until more is known of the intra-renal handling of cGMP, urinary nucleotide should not be used as a marker for the specific renal action of ANF.

The Effect of ANF on Albuminuria.

Apart from natriuresis and rise of cGMP, the most marked effect of the ANF infusions in the patients was the rise in albumin excretion. I could detect no consistent effect of ANF on albumin excretion in normals, but a small change might have been missed due to experimental error.

Micropucture studies of isolated nephrons from healthy animals show that pharmacological doses of ANF lead to

increases of glomerular capillary hydrostatic pressure, glomerular barrier permeability and filtration fraction (Chapter 15), changes that would encourage macromolecular passage across the glomerular barrier by convection and diffusion (Chapter 14). In humans it is not possible to make direct measurements of glomerular haemodynamics, but the ANF-induced rise of filtration fraction which I documented in healthy subjects and in nephrotic patients (Chapters 17 and 18) is at least consistent with the animal studies. In my nephrotic study, the rise of albuminuria during ANF, and the fall upon stopping the infusion, would also be consistent with a rapidly reversible enhanced passage of macromolecules across the glomerulus mediated by haemodynamic changes.

However, this can not be the whole explanation, because similar rises of filtration fraction occurred in the healthy group yet there was no consistent effect on albumin excretion. Moreover, I found a rise of albumin excretion in all patients with CRF yet no significant effects on GFR or ERPF (although small changes may have been missed because of experimantal error). My experiments suggest that, compared to healthy subjects, patients with renal disease may be more susceptible to ANF-induced albuminuria.

Clearly more work needs to be done to elucidate the

mechanism of this striking effect. In particular, an unknown quantity of albumin filtered by the glomerulus is reabsorbed by the tubule, and a study of the effect of ANF infusion on the clearances of dextrans (which are not reabsorbed) would eliminate this confounding factor.

ANF, Sodium Balance and Progression of Renal Disease.

Recently, there has been much investigation into non-immunological mechanisms which may cause the progression of CRF. Harmful factors may include elevated systemic arterial blood pressure, raised intraglomerular hydraulic pressure, high protein and/or phosphate diets and high levels of plasma lipids (Klahr et al, 1988; El Nahas, 1988; Meyer et al, 1986).

In chronic glomerulonephritis and pyelonephritis the degree of proteinuria is a marker for the rate of renal deterioration (Williams et al, 1988). In addition to indicating the severity of glomerular structural and functional changes, the enhanced passage of macromolecules across the glomerular barrier may cause further structural damage (Couser et al, 1975). In animals with partial renal ablation (Meyer et al, 1986) and in humans with a variety of primary renal diseases (Alvestrand et al, 1988) the control of arterial hypertension can reduce proteinuria and stabilise renal function, an effect which may be mediated by the

lowering of glomerular capillary pressure in surviving nephrons (Meyer et al, 1986).

How might ANF fit into this scheme ? In CRF sodium excretion tends to be impaired by the decrease of functioning renal tissue. Volume expansion would result in enhanced atrial filling and secretion of ANF from the heart: together with a contribution from impaired renal clearance of the peptide, plasma ANF would rise. High levels of the peptide would then enhance urinary sodium excretion and maintain a constant sodium excretion but at the expense of a chronically expanded blood volume.

To this extent, high levels of ANF would beneficial, but at the same time proteinuria would increase. I speculate that in some patients with renal failure the retention of sodium may lead to both arterial hypertension and a rise of plasma ANF. Both of these changes may alter glomerular haemodymanics and increase proteinuria to the detriment of long-term renal function.

This is an example of Bricker's "trade-off" hypothesis, in which adaptations to a decrease in GFR not only tend to maintain a healthy composition of body fluids but also have harmful effects (Bricker et al, 1981). For example, the rise of parathyroid hormone which occurs as CRF progresses tends to maintain a constant plasma

phosphorus and calcium concentration, but at the same time causes erosive bone disease.

The ANF hypothesis could be tested. For example, low sodium diets should retard the progression of renal failure, but effect would be difficult to distinguish from a simultaneous fall in arterial blood pressure which might also occur. Secondly, the rate of progression in individual patients should correlate with plasma ANF. Thirdly, the use of different classes of anti-hypertensive drugs which may either increase or decrease plasma ANF (Chapter 11) should have different effects on the progression of renal failure. Finally, an ANF receptor-blocking drug should produce beneficial decreases of proteinuria, although if ANF is truly an important natriuretic hormone in these patients, then the use of such a drug may also produce dangerous rises of blood volume.

Summary of Chapter 20.

1. High plasma levels of immunoreactive (99-126) ANF occur in some patients wich chronic renal disorders, and may represent a combination of blood volume expansion, decreased renal clearance of the peptide, and occult cardiac disease. However, a contribution by small quantities of other ANF propeptide fragments which cross-react with (99-126) antisera, can not be

ruled out.

2. Low dose ANF intravenous infusion causes a similar pattern of rise of extracellular cGMP in normals and patients with renal disease.

3. Low doses of ANF cause significant natriuresis in health, in chronic renal impairment and in the nephrotic syndrome.

4. Low dose ANF increased albumin excretion in patients with renal disease, but no significant effect was noted in the normal study group as a whole.

5. In chronic renal failure, before end-satge disease, ANF may help to maintain sodium balance at the expense of enhanced proteinuria.

PART IV.

CHAPTER 21.

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CHAPTER 22.

DATA APPENDIX.

DATA FROM CHAPTER 10 (STUDY A): ANF IN HEALTHY ADULTS.

ID	Age (yrs)	Daily Na+ Excretion (mmol)	Mean Blood Pressure (mmHg)	Plasma ANF (pmol/l)	Plasma Renin Activity (nmol/h/l)
1	29	148	79	3.1	1.69
2	33	216	93	8.8	0.57
3	42	110	85	2.9	1.56
4	27	172	85	7.5	0.58
5	42	115	73	3.9	1.33
6	37	100	85	3.9	0.78
7	31	100	100	5.5	1.92
8	37	120	87	4.0	0.96
9	40	288	92	9.5	0.93
10	27	172	70	9.4	0.78
11	40	190	77	6.6	0.58
12	40	201	88	4.7	1.33
13	33	140	93	5.8	0.70
14	31	190	73	6.5	1.89
15	37	121	83	2.3	0.97
16	33	107	83	5.0	1.56
17	44	129	105	10.8	1.50
18	70	150	101	18.1	4.08
19	66	78	99	8.5	1.68
20	72	120	106	15.5	0.48
21	51	120	85	7.1	0.80
22	54	60	97	5.8	0.85
23	59	87	77	8.3	0.46
24	24	120	80	1.5	1.10

DATA FROM CHAPTER 10 (STUDY B): SALINE INFUSION.

'Periods' refer to sequential 30 minutes:

1-2 are the basal values, during 3-4 two litres of normal saline was infused, and 5-7 represent measurements after the infusion.

Subjects A-G are identical on the active and the control days.

SALINE (ACTIVE) DAY.

Period	1	2	3	4	5	6	7
Plasma ANF (pmol/l).							
A	4.5	5.1	6.8	10.4	11.6	9.1	8.4
B	2.9	2.7	3.4	5.0	5.8	4.6	3.1
C	3.3	3.0	4.4	8.3	7.4	7.2	8.6
D	1.4	1.0	1.8	2.3	1.8	2.1	1.8
E	3.8	3.5	5.5	5.3	6.7	4.8	4.4
F	3.6	3.4	4.7	4.9	6.4	6.3	5.8
G	7.2	2.5	4 7	4.7	5.1	7.6	5.1
Mean±SEM	3.8±0.7	3.0±0.5	4.5±0.6	5.8±1.0	6.4±1.1	6.0±0.9	5.3±1.0
Urine Sodium (micromols/min).							
A	168	195	237	283	231	249	230
B	80	87	225	410	476	463	525
C	34	22	53	120	156	186	150
D	124	177	213	129	126	108	108
E	196	209	214	155	220	265	320
F	154	143	215	337	162	297	420
G	51	44	63	139	204	192	225
Mean±SEM	115±23	125±28	174±30	224±44	225±44	251±42	282±56

Plasma Renin Activity (nmol/h/l).			
A	0.95	0.39	0.39
B	1.03	0.45	0.36
C	2.09	0.81	0.58
D	0.43	0.26	0.21
E	0.40	0.04	0.06
F	1.35	0.40	0.41
G	0.65	0.20	0.26
Mean±SEM	0.99±0.22	0.36±0.09	0.32±0.06

SALINE (ACTIVE) DAY CONTINUED.

Period	1	2	3	4	5	6	7
Plasma Aldosterone (pmol/l).							
A		35		115			88
B		550		260			150
C		540		280			150
D		280		88			75
E		340		200			85
F		200		100			70
G		1000		410			80
Mean±SEM		420±118		208±45			100±13
Total Plasma Protein (g/l).							
A		79		78			76
B		70		62			66
C		88		75			77
D		85		73			76
E		83		74			75
F		73		66			68
G		67		62			63
Mean±SEM		78±3		70±2			72±2
Mean Blood Pressure (mmHg).							
A	83	83		83			85
B	103	103		86			89
C	93	93		92			93
D	107	103		107			107
E	77	77		82			77
F	70	72		82			72
G	77	73		80			83
Mean±SEM	87±5	86±5		87±4			87±4
Plasma cGMP (nmol/l).							
A		5.9		5.6			4.1
B		3.6		4.3			4.0
C		1.5		3.4			4.5
D		3.9		3.9			3.9
E		7.7		10.1			8.3
F		4.6		4.9			3.4
G		1.2		2.7			5.6
Mean±SEM		4.1±0.9		5.0±0.9			4.8±0.6

DATA FROM CHAPTER 10, STUDY A, CONTINUED.

SALINE (CONTROL) DAY.

Period	1	2	3	4	5	6	7
Plasma ANF (pmol/l).							
A	5.5	4.2	5.3	3.8	4.9	4.3	3.6
B	6.8	4.5	6.8	5.4	5.2	5.2	5.8
C	2.8	2 9	1.5	1.2	0.7	1.8	1.6
D	2.5	1.9	2.0	1.6	1.5	2.2	2.3
E	4.0	4.6	4.3	3.0	2.9	3.2	3.2
F	5.3	5.1	4.7	5.5	4.3	4.6	2.9
G	7.9	6.5	4.8	5.6	5.1	6.5	6.1
Mean±SEM	5.0±0.8	4.2±0.6	4.2±0.7	3.7±0.7	3.5±0.7	4.0±0.6	3.6±0.6
Urine Sodium (micromol/min).							
A	193	258	157	226	104	205	245
B	45	60	89	90	140	98	100
C	90	55	93	60	127	90	93
D	190	191	187	91	111	101	110
E	320	250	250	220	200	210	230
F	232	145	132	135	153	171	116
G	97	79	63	49	41	48	48
Mean±SEM	167±36	148±33	139±25	124±27	125±18	132±24	135±28

Plasma Renin Activity (nmol/h/l).			
A	1.57	1.52	1.43
B	2.13	2.00	2.01
C	1.87	1.61	2.22
D	1.05	0.67	0.72
E	0.63	0.38	0.49
F	1.39	1.68	1.70
G	0.59	0.45	0.55
Mean±SEM	1.32±0.20	1.19±0.25	1.30±0.27

DATA FOR CHAPTER 11:

PATIENTS WITH CHRONIC RENAL FAILURE

WHO DO NOT REQUIRE DIALYSIS.

ID	Sex	Age (yr)	Creatinine Clearance (ml/min)	Daily Na+ Excretion (mmol)	Fractional Na+ Excretion (% CrCl)	Mean Blood Pressure (mmHg)	Plasma ANF (pmol/l)	PRA (nmol/h/l)
1	M	44	20	241	5.9	101	21.1	0.80
2	M	67	27	98	1.8	140	27.0	0.69
3	M	56	4	150	18.8	127	12.3	0.92
4	M	50	6	120	10.0	115	35.1	1.00
5	M	35	14	90	3.0	123	14.9	0.11
6	M	50	28	132	2.8	133	25.1	0.13
7	M	54	8	43	2.8	103	25.5	0.38
8	M	30	22	124	2.8	119	11.8	0.85
9	M	21	67	123	0.9	120	1.2	0.45
10	M	50	5	47	4.4	97	20.8	0.93
11	M	55	24	150	3.1	127	5.3	1.52
12	F	60	5	165	16.2	87	7.5	0.46
13	F	39	34	93	1.3	120	17.5	0.31
14	M	38	6	80	6.7	110	27.0	0.20
15	F	51	9	72	4.0	119	21.4	0.07
16	M	51	17	172	5.0	108	9.4	0.49
17	M	47	6	151	12.8	122	12.8	0.25
18	F	44	20	130	3.3	83	0.9	10.60
19	F	62	40	86	1.1	103	15.6	0.89
20	M	55	46	98	1.1	80	3.4	0.86
21	F	36	23	116	1.1	116	4.2	1.15
22	F	37	30	148	2.3	93	9.7	0.36
23	M	23	11	246	11.5	93	9.6	1.06
24	M	21	6	150	12.6	100	8.2	1.82
25	F	19	5	59	5.9	80	10.3	1.53
26	F	29	55	110	1.0	95	6.2	2.60
27	M	21	55	114	1.0	104	2.1	1.65
28	M	53	23	205	4.4	119	37.6	0.78
29	M	25	30	87	1.4	117	1.2	1.80
30	M	21	55	114	1.0	104	4.6	1.75
31	M	20	26	94	1.8	110	1.4	1.63
32	F	65	10	112	4.4	93	11.5	1.38
33	F	30	13	56	2.1	103	12.2	0.43
34	F	45	5	67	6.7	97	13.0	0.58
35	F	55	8	158	9.8	87	3.0	1.50
36	F	66	12	160	6.6	113	28.2	0.22

DATA FOR CHAPTER 12 (STUDY A):

BASAL DATA IN PATIENTS WHO REQUIRE HAEMODIALYSIS.

ID	Age (years)	Mean Blood Pressure (mmHg)	Plasma ANF (pmol/l)	Plasma Renin Activity (nmol/h/l)
1	35	101	15.6	0.55
2	63	109	33.1	1.35
3	25	101	12.3	0.24
4	29	114	19.1	0.09
5	51	100	33.1	0.55
6	61	114	124.0	1.80
7	40	98	24.1	1.68
8	48	104	61.0	1.26
9	33	95	22.7	1.22
10	40	105	35.0	1.72
11	45	99	28.8	1.55

EFFECTS OF A SINGLE HAEMODIALYSIS (STUDY B)

Patient.	ANF (pmol/l) PRE DIALYSIS	ANF (pmol/l) POST DIALYSIS	Plasma cGMP (nmol/l) PRE DIALYSIS	Plasma cGMP (nmol/l) POST DIALYSIS
1	50.3	30.1	14.5	9.9
2	68.1	51.9	18.3	16.8
3	69.5	26.6	20.6	14.8
4	27.6	20.7	14.8	10.6
5	27.6	19.1	13.5	7.1
6	26.3	13.3	21.1	7.7
7	18.8	14.6	9.0	9.9
8	27.6	14.2	12.2	10.9

	PRA (nmol/h/l)		Weight Loss (kg)
1	1.60	2.29	0.2
2	1.11	1.48	0.3
3	0.70	0.92	1.1
4	0.96	0.88	0.2
5	1.10	1.29	0.5
6	0.92	1.16	4.7
7	0.87	1.17	3.1
8	0.52	0.79	2.7

RENAL TRANSPLANT DATA (CHAPTER 13).

Key: Pl.Na+ = plasma sodium, Ur.Na+ = urine sodium, Fr.Na+ = fractional urinary sodium excretion, ANF = plasma atrial natriuretic factor, PRA = plasma renin activity, CGMP = plasma cyclic guanosine monophosphate, Aldo = plasma aldosterone, MBP = mean arterial blood pressure.

Day	Weight (kg)	Creatinine Clearance (ml/min)	Pl.Na+ (mmol)	24hrUr.Na+ (mmol)	Fr.Na+ (%)	ANF (pmol/l)	PRA (nmol/h/l)	MBP (mmHg)	Aldo (pmol/l)	cGMP (nmol/l)
Patient C.										
1	93	-	141	-	-	28.8	2.02	140	282	14.4
(Transplanted)										
2	93	7	139	168	12.0	31.4	0.89	140	270	14.0
3	90	8	135	113	7.3	42.5	2.06	125	255	14.0
4	93	6	131	71	6.0	46.7	2.29	127	276	15.0
5	93	10	139	150	7.6	28.3	1.53	107	250	18.0
6	91	8	128	183	12.5	35.7	1.56	103	264	15.6
7	91	8	134	176	11.5	24.3	2.41	113	235	20.0
8	91	12	141	228	9.4	25.6	3.07	92	218	23.6
9	88	16	143	190	5.8	23.1	2.39	107	196	12.8
10	83	24	147	170	5.1	20.1	1.91	110	224	10.0
11	83	-	146	-	-	12.9	1.78	113	237	7.6
12	88	41	141	95	1.2	11.6	0.78	110	216	6.4
13	88	64	144	223	1.8	17.2	0.62	101	202	8.0
Patient B.										
1	53	-	137	-	-	36.1	0.32	88	184	10.8
(Transplanted)										
2	53	-	131	-	-	34.1	0.26	117	176	9.2
3	51	40	131	647	8.6	26.9	0.67	123	199	4.2
4	50	45	132	260	3.0	14.6	0.33	117	211	4.7
5	50	40	137	256	3.2	20.1	0.22	150	192	6.8
6	49	50	134	186	1.9	8.4	0.13	127	180	2.5
7	49	48	134	76	0.8	4.5	0.23	120	164	4.5
8	48	52	133	96	1.0	5.5	0.23	123	178	6.0
9	48	54	135	44	0.4	7.4	0.41	106	160	3.8
10	48	50	137	155	1.6	6.5	0.46	120	182	1.6
11	48	55	134	120	1.1	3.4	0.36	90	171	1.1
12	47	51	132	90	0.9	2.0	1.09	92	176	0.9

Day	Weight	Cr.Cl	Pl.Na+	Ur.Na+	Fr.Na+	ANF	PRA	MBP	Aldo.	cGMP
Patient A.										
1	36	-	130	-	-	15.6	0.72	113	256	10.4
(Dialysis)										
2	36	-	130	-	-	11.0	1.18	100	280	4.6
(Transplant)										
3	39	4	137	146	19.0	34.1	0.61	118	245	8.3
4	40	10	138	84	4.3	30.2	0.27	122	228	7.2
5	40	42	138	167	2.0	23.7	0.27	135	205	8.3
6	38	59	140	424	3.5	9.4	0.34	107	220	5.5
7	37	78	138	340	2.9	9.1	0.43	100	208	4.6
8	37	57	138	180	2.2	6.5	0.81	90	219	3.8
9	38	66	141	99	0.7	3.8	1.91	92	230	2.6
Patient D (no pretransplant sample).										
1	60	-	135	-	-	35.0	2.24	103		
2	63	-	134	-	-	34.7	3.20	103		
3	63	-	129	-	-	50.3	1.60	87		
(Haemodialysis)										
4	63	-	133	-	-	30.2	2.29	80		
5	64	-	129	-	-	67.7	1.11	97		
(Haemodialysis)										
6	63	-	132	-	-	47.0	1.48	103		
7	66	-	128	-	-	83.5	0.79	107		
8	65	-	134	-	-	64.5	0.70	103		
9	65	-	133	-	-	27.3	0.92	107		
10	67	-	129	-	-	27.7	0.90	117		
11	68	5	132	11	1.2	24.8	1.06	132		
12	66	2	134	7	1.8	34.1	1.35	103		
13	67	2	133	3	0.8	24.5	0.61	100		
14	66	3	133	3	0.5	30.0	0.82	103		
15	65	7	133	7	0.5	46.1	0.67	93		
16	66	8	137	25	1.6	35.1	0.74	93		
17	64	14	134	8	0.3	30.2	0.50	80		
18	65	18	136	27	0.3	50.3	0.53	103		
(Frusemide)										
19	66	15	136	144	4.9	33.7	0.42	100		
20	66	13	140	204	7.8	58.1	0.52	83		
21	63	13	142	186	6.8	46.4	0.49	103		
22	65	13	137	82	3.2	46.0	0.53	103		
23	62	15	136	52	2.0	14.5	0.95	100		

DATA FOR CHAPTER 17:

ANF (AND PLACEBO) INFUSIONS NORMAL SUBJECTS

BASELINE DATA

Note that 'ANF' and 'Placebo' refer to data pertaining to active and control days on each subject. 24 hour urine values refer to day before the experimental day.

Patient	Sex	Age (yr)	Wt (kg)	Height (cm)	24 Hour Urine Values Albumin (mg)	Na+ (mmol)	cGMP (nmol)
1 ANF	M	29	65	166	34.2	200	435
Placebo					15.4	156	497
2 ANF	F	27	69	165	6.7	137	43
Placebo					10.0	133	76
3 ANF	M	27	79	180	14.2	120	267
Placebo					10.1	135	469
4 ANF	M	40	67	178	15.0	160	510
Placebo					14.2	157	391
5 ANF	M	31	78	180	21.0	167	396
Placebo					13.0	194	324
6 ANF	M	26	78	176	9.5	128	207
Placebo					8.6	189	519
7 ANF	M	25	95	188	7.5	152	450
Placebo					10.2	172	400

ANF INFUSION IN NORMALS CONTINUED.

Periods 1 and 2 are the basal hour, periods 3-6 refer to the ANF or placebo infusion, and periods 7 and 8 are the recovery hour.

A = ACTIVE and B = PLACEBO Infusions.

Period	1	2	3	4	5	6	7	8
Plasma Sodium (mmol/l).								
1A	133	134	130	138	137	138	140	133
1B	133	131	131	131	131	131	130	130
2A	133	134	133	133	134	134	133	134
2B	133	132	135	136	132	132	132	133
3A	138	138	136	136	133	133	134	133
3B	138	138	139	139	138	138	137	137
4A	136	136	138	138	138	138	138	136
4B	136	139	136	138	138	138	138	139
5A	137	138	141	134	138	138	138	138
5B	134	133	134	134	134	135	135	135
6A	136	138	138	139	140	140	140	140
6B	133	136	137	137	137	137	139	140
7A	133	135	134	135	135	134	135	134
7B	139	137	135	134	131	133	133	135
Mean±SEM								
A	135±1	136±1	136±1	136±1	136±1	137±1	137±1	135±1
B	135±1	135±1	136±1	136±1	136±1	137±1	137±1	135±1
Plasma Potassium (mmol/l).								
1A	3.3	4.3	4.0	4.4	4.0	4.5	3.9	4.3
1B	3.8	3.7	3.8	3.6	3.6	3.5	3.5	3.0
2A	4.2	4.9	4.9	4.2	4.9	4.7	4.5	4.9
2B	3.9	3.8	3 9	5.2	5.2	4.2	4.6	4.9
3A	4.2	5.0	4.5	4.6	4.3	4.9	3.7	3.7
3B	4.5	4.1	4.1	4.0	4.0	3.9	3.9	3.9
4A	4.0	3.8	3.8	3.8	3.8	3.9	3.8	3.8
4B	3.7	3.6	3.6	3.8	3.7	3.9	3.9	3.8
5A	4.4	4.4	4.9	4.6	4.2	4.2	3.8	4.0
5B	4.3	4.2	4.5	4.6	4.5	4.2	4.0	3.8
6A	4.1	4.0	4.2	3.9	4.2	3.7	3.7	3.7
6B	3.5	3.7	3.8	4.0	3.9	3.8	3.8	3.7
7A	4.1	3.6	4.1	4.0	4.1	4.5	4.4	4.3
7B	4.5	4.2	4.4	4.6	4.3	4.2	4.4	4.3
Mean±SEM								
A	4.0±0.2	4.3±0.3	4.3±0.2	4.2±0.1	4.2±0.2	4.4±0.4	4.0±0.1	4.1±0.1
B	4.0±0.2	3.9±0.1	4.0±0.1	4.3±0.2	4.2±0.2	4.0±0.1	4.0±0.1	3.9±0.2

ANF INFUSION IN NORMALS CONTINUED.

Period	1	2	3	4	5	6	7	8
Plasma Renin Activity (nmol/h/l).								
1A	0.97	0.97	0.91	0.66	0.63	0.57	0.52	0.71
1B	1.01	1.02	1.04	1.23	1.05	0.88	0.95	0.86
2A	0.52	0.67	0.55	0.46	0.49	0.46	0.37	0.40
2B	0.59	0.60	0.65	0.59	0.62	0.55	0.55	0.53
3A	1.74	1.05	1.24	0.59	0.54	0.46	0.36	0.38
3B	0.96	0.86	0.68	0.85	0.73	0.61	0.68	0.72
4A	1.00	0.86	0.57	0.52	0.47	0.43	0.62	0.60
4B	1.00	0.86	0.93	0.68	1.04	0.89	0.62	0.70
5A	0.45	0.46	0.39	0.38	0.23	0.23	0.24	0.24
5B	0.76	0.61	0.54	0.50	0.50	0.50	0.39	0.43
6A	1.40	1.49	1.65	0.89	0.67	0.64	0.60	0.70
6B	1.35	1.65	1.41	1.32	1.40	1.64	1.57	1.16
7A	1.28	1.24	1.55	0.98	0.78	0.70	0.72	1.09
7B	1.23	1.26	1.38	1.23	1.13	1.09	1.07	1.56
Mean±SEM								
A	1.05±0.18	0.96±0.13	0.98±0.19	0.64±0.08	0.54±0.07	0.50±0.06	0.49±0.06	0.59±0.11
B	0.99±0.10	0.98±0.14	0.95±0.13	0.91±0.13	0.92±0.12	0.88±0.15	0.83±0.15	0.85±0.15
Plasma Aldosterone (pmol/l).								
1A	184	100	83	<69	90			
1B	200	215	235	138	155			
2A	122	117	100	<69	<69			
2B	270	152	198	215	108			
3A	460	600	280	210	185			
3B	310	260	200	190	160			
4A	210	190	140	70	150			
4B	120	110	70	190	160			
5A	125	150	115	<69	<69			
5B	300	225	160	140	140			
6A	230	325	170	140	150			
6B	360	225	300	320	320			
7A	380	325	310	225	300			
7B	410	380	430	400	340			
Mean±SEM								
A	244±49	258±67	171±34	107±32	135±35			
B	281±37	223±32	228±43	228±37	198±35			

ANF INFUSION IN NORMALS CONTINUED.

Period	1	2	3	4	5	6	7	8
Pulse (beats per minute).								
1A	68	60	54	54	52	62	60	60
1B	50	52	45	47	46	48	49	49
2A	60	59	54	48	62	62	54	51
2B	70	65	60	60	62	60	68	60
3A	56	60	54	48	62	62	54	51
3B	52	52	52	52	52	52	54	54
4A	68	63	59	64	48	60	68	84
4B	66	68	52	60	68	64	60	60
5A	72	76	81	60	66	60	84	72
5B	64	56	64	66	65	68	68	68
6A	64	62	46	64	76	70	72	72
6B	56	62	60	72	68	64	66	66
7A	48	72	48	44	48	78	50	52
7B	60	48	52	48	60	48	60	60
Mean±SEM								
A	62±3	65±3	57±5	57±3	59±4	65±3	63±3	63±3
B	60±3	58±3	55±5	58±3	60±2	58±2	61±3	60±2
Mean Blood Pressure (mmHg).								
1A	106	104	99	100	101	99	101	92
1B	83	87	89	91	91	96	97	97
2A	87	70	77	58	79	68	59	85
2B	85	80	79	82	83	80	82	88
3A	93	87	77	89	91	89	103	115
3B	71	70	70	55	55	79	79	78
4A	92	94	87	93	96	96	90	100
4B	75	80	87	84	83	79	76	81
5A	82	86	75	73	68	71	74	77
5B	82	81	74	69	75	86	87	81
6A	79	79	79	79	79	74	73	82
6B	77	77	83	74	73	74	73	73
7A	83	76	80	80	80	74	73	76
7B	60	75	83	80	81	81	83	82
Mean±SEM								
A	89±3	85±4	82±3	82±5	85±5	82±5	82±6	90±5
B	76±3	79±2	81±3	76±5	77±4	82±3	82±3	83±3

ANF INFUSION IN NORMALS CONTINUED.

Period	1	2	3	4	5	6	7	8
Urine Sodium (micromol/minute).								
1A	170	216	260	343	336	436	300	290
1B	140	130	130	147	133	123	123	120
2A	153	170	180	220	230	240	246	230
2B	163	223	256	250	236	255	230	240
3A	90	90	92	94	88	112	115	112
3B	147	130	123	120	108	117	142	142
4A	172	201	239	417	495	602	406	246
4B	157	190	135	121	80	148	185	192
5A	295	297	229	219	252	285	330	402
5B	288	256	268	217	177	172	179	221
6A	108	100	150	197	288	301	343	279
6B	159	125	171	150	148	147	183	236
7A	69	66	95	127	146	170	167	194
7B	86	100	94	107	100	109	132	96
Mean±SEM								
A	151±28	163±31	178±28	231±43	262±50	307±64	272±39	250±34
B	162±23	165±22	168±27	159±20	140±20	153±19	168±14	178±22
Urine Potassium (micromol/minute).								
1A	50	58	57	66	60	69	41	48
1B	40	37	40	33	27	23	23	23
2A	130	113	93	80	63	53	43	37
2B	90	113	113	107	93	103	90	90
3A	60	116	124	98	50	27	16	14
3B	119	116	112	111	101	86	83	83
4A	88	93	84	75	63	67	94	50
4B	100	107	79	68	34	61	65	60
5A	122	146	139	132	97	81	62	70
5B	128	134	139	108	70	41	30	37
6A	116	84	100	78	84	50	40	40
6B	126	76	87	75	84	70	83	98
7A	75	69	54	63	62	72	57	58
7B	38	59	89	91	67	87	77	34
Mean±SEM								
A	92±12	97±11	93±12	85±9	68±6	60±7	50±9	45±7
B	92±14	92±13	88±17	85±11	68±11	67±17	64±10	61±11

ANF INFUSION IN NORMALS CONTINUED.

Period	1	2	3	4	5	6	7	8
Urine Volume (ml/min).								
1A	14.7	15.3	13.9	15.7	13.4	16.8	12.5	11.7
1B	10.4	10.3	10.6	11.0	10.7	10.0	11.3	10.4
2A	10.6	11.2	11.3	12.3	11.5	12.0	12.4	12.2
2B	10.2	13.3	12.8	12.6	11.8	12.4	11.4	12.1
3A	9.1	12.5	11.5	10.4	8.6	9.4	9.2	9.7
3B	9.9	9.3	7.7	6.3	6.4	7.1	7.5	7.5
4A	10.8	16.8	17.0	19.9	16.5	17.2	8.0	6.0
4B	8.3	19.1	16.9	15.1	3.3	8.8	18.1	11.3
5A	15.1	16.6	13.2	13.3	10.5	11.4	11.0	8.4
5B	16.0	15.1	14.9	10.8	9.3	9.8	9 3	11.5
6A	8.3	6.7	8 8	11.6	13.7	12.0	7.3	5.3
6B	6.1	11.3	13.2	7.6	7.5	5.2	10.2	9.5
7A	8.6	11.1	9.5	9.8	6.3	8.1	4.7	2.1
7B	4.9	6.1	6.6	9.6	4.5	6.7	5.1	2.3
Mean±SEM								
A	11.0±1.1	12.9±1.4	12.2±1.1	13.3±1.3	11.5±1.3	12.4±1.3	9.3±1.1	7.9±1.4
B	9.4±1.4	12.1±1.6	11.8±1.4	10.4±1.1	7.7±1.2	8.6±0.9	10.4±1.5	9.2±1.3
Plasma ANF (pmol/l).								
1A	4.9	3.5	11.3	16.5	17.4	18.0	5.0	2.4
1B	4.5	1.5	1.3	2.7	1.7	2.0	3.4	3.0
2A	2.9	1.5	6.4	14.0	15.5	13.1	2.9	3.2
2B	6.4	0.8	1.9	1.7	4.5	3.5	2.0	1.4
3A	0.9	1.2	7.3	8.0	12.2	13.2	7.1	0.9
3B	2.9	1.5	1.6	1.8	0.8	1.6	2.5	2.5
4A	3.2	3.0	27.3	31.5	26.7	20.5	7.5	3.9
4B	2.6	1.6	2.1	1.2	2.2	1.0	0.7	2.3
5A	3.2	2.0	12.8	15.5	21.9	22.1	5.6	3.2
5B	2.5	2.1	2.1	0.9	1.4	2.4	2.2	1.5
6A	0.3	0.3	7.3	13.4	25.5	26.2	2.6	0.3
6B	1.2	1.1	0.9	0.7	1.2	1.5	1.0	2.6
7A	2.2	1.8	7.0	14.7	17.0	23.0	7.2	3.8
7B	2.7	1.1	0.8	0.7	1.2	1.5	1.0	2.7
Mean±SEM								
A	2.5±0.6	1.9±0.4	11.3±2.8	16.2±2.8	19.5±2.0	19.4±1.9	5.4±0.8	2.5±0.5
B	2.7±0.7	1.4±0.2	1.5±0.2	1.4±0.3	1.9±0.5	1.9±0.3	1.8±0.4	2.3±0.2

ANF INFUSION IN NORMALS CONTINUED.

PERIOD	1	2	3	4	5	6	7	8
Glomerular Filtration Rate (ml/min).								
* uncorrected for surface area.								
1A	112	111	101	115	107	127	112	105
1B	106	102	104	108	104	107	108	116
2A	126	143	131	142	146	121	128	133
2B	104	141	135	137	128	136	114	116
3A	130	155	130	148	136	137	163	146
3B	177	152	129	143	140	141	141	141
4A	126	123	118	126	121	127	120	109
4B	118	129	117	114	120	135	141	116
5A	145	148	133	135	140	167	155	150
5B	146	144	119	126	128	125	128	135
6A	160	151	157	166	171	168	175	123
6B	151	149	154	148	147	119	150	149
7A	167	169	146	141	114	150	118	118
7B	142	132	122	139	110	144	137	132
Mean±SEM								
A	138±8	143±8	131±7	139±6	134±8	142±7	138±9	126±6
B	135±10	136±6	126±6	131±6	125±6	130±5	131±7	129±5
Effective Renal Plasma Flow (ml/min).								
* uncorrected for surface area.								
1A	527	543	490	482	464	513	465	535
1B	495	492	501	459	470	454	453	477
2A	533	582	512	491	449	451	469	526
2B	512	577	522	524	503	546	523	515
3A	544	544	447	433	424	435	411	444
3B	502	526	488	494	469	484	479	479
4A	534	503	480	509	493	476	427	517
4B	590	677	593	554	600	600	643	519
5A	765	715	629	557	438	509	522	621
5B	648	604	659	571	562	523	540	558
6A	701	683	671	592	575	595	642	537
6B	728	717	776	701	699	628	761	663
7A	589	727	610	614	547	520	514	769
7B	618	555	534	538	483	541	563	511
Mean±SEM								
A	600±36	614±35	548±33	525±25	484±22	500±20	492±29	564±39
B	528±8	592±31	581±39	549±29	476±52	539±23	566±40	531±24

ANF INFUSION IN NORMALS CONTINUED.

Period	1	2	3	4	5	6	7	8
Plasma Total Protein (g/l).								
1A	83	80	79	81	82	84	84	84
1B	76	69	69	71	71	69	71	68
2A	70	70	72	72	72	71	72	72
2B	72	69	71	70	70	70	71	70
3A	82	82	83	79	76	76	75	75
3B	74	74	74	75	76	73	75	75
4A	71	68	72	73	73	74	73	70
4B	69	70	72	71	72	71	73	72
5A	73	74	76	76	75	77	74	74
5B	76	73	76	75	75	74	74	74
6A	77	75	75	75	79	74	77	75
6B	79	77	75	75	74	73	75	74
7A	84	83	82	81	84	85	81	79
7B	79	78	78	79	81	81	79	83
Mean±SEM								
A	77±2	76±2	77±2	77±2	77±2	77±2	77±2	76±2
B	75±2	73±2	74±1	73±1	74±1	73±1	74±1	74±2

	Haemoglobin (g/dl)/ Haematocrit (%)		Plasma Volume (% Change)
	Period 2	Period 6	
1A	15.2/46	15.5/46	-1.9
1B	13.2/40	12.7/36	+10.9
2A	12.2/35	12.4/36	-3.1
2B	12.7/37	12.6/36	+2.4
3A	14.8/42	15.3/43	-4.9
3B	14.1/41	13.8/39	+5.6
4A	14.9/43	16.0/47	-13.4
4B	15.1/44	15.1/44	0.0
5A	14.4/42	14.7/42	-2.0
5B	14.9/44	14.2/42	+8.7
6A	14.1/42	14.8/44	-8.0
6B	13.5/39	13.4/39	+0.7
7A	14.2/44	15.4/49	-16.0
7B	14.0/42	14.1/42	-0.7
Mean±SEM			
A	14.3±0.3/42±1	14.9±0.5/44±1	
B	13.9±0.3/41±1	13.7±0.3/40±1	

ANF INFUSION IN NORMALS CONTINUED.

Period	1	2	3	4	5	6	7	8
Filtration Fraction (%).								
1A	21.3	20.4	20.6	23.9	23.1	24.7	24.0	19.6
1B	21.4	20.7	20.7	23.5	22.1	23.5	23.8	24.3
2A	23.6	24.5	25.5	28.9	32.5	26.8	27.3	25.2
2B	20.3	24.4	25.8	26.1	25.4	24.9	22.0	22.5
3A	23.9	28.5	29.1	34.1	32.0	31.4	39.7	32.8
3B	35.2	28.8	26.4	28.9	29.8	29.1	29.4	29.4
4A	23.6	24.4	24.5	24.7	24.5	26.6	28.1	21.1
4B	20.0	19.1	18.0	20.5	20.0	20.8	21.9	22.3
5A	18.9	20.6	21.1	24.2	31.9	32.8	29.6	24.2
5B	22.5	23.8	18.1	22.0	22.8	23.9	23.7	24.2
6A	22.8	22.1	23.3	28.0	29.7	28.2	27.3	22.6
6B	20.7	20.7	19.8	21.1	21.0	18.9	19.7	22.5
7A	28.3	23.2	23.9	22.7	20.8	28.8	22.9	15.3
7B	22.9	23.7	22.8	25.8	22.7	26.6	24.3	25.8
Mean±SEM								
A	23.2±1.1	23.4±1.1	24.0±1.1	26.6±1.5	27.8±1.9	28.5±1.1	28.4±2.1	23.0±2.0
B	22.2±2.0	23.0±1.2	21.7±1.3	24.0±1.2	23.4±1.2	23.9±1.3	23.5±1.1	24.4±0.9

Urine Albumin Excretion (microgram/minute).

Note that (only) subject 1 had an obvious increase of albuminuria: his GFR, 24 hour albumin excretion, fasting blood sugar and HbA1 were all normal.

1A	3.7	5.0	3.7	15.7	37.0	201.0	10.6	5.8
1B	17.6	12.6	11.3	11.3	9.3	9 3	11.0	13.6
2A	1.7	2.7	2.3	2.3	2.0	2.3	2.3	2.7
2B	5.6	1.3	2.7	7.0	13.0	5.0	5.0	6.7
3A	15.6	15.0	13.3	14.3	12.0	15.0	14.6	13.3
3B	3.0	3.0	2.5	2.0	2.5	2.3	2.4	2.4
4A	12.0	5.5	5.5	6.0	6.0	8.0	11.5	8.0
4B	15.0	9.0	6.0	5.0	4.0	7.0	6.0	5.5
5A	4.5	4.1	3.6	4.0	4.0	4.0	4.0	5.0
5B	14.4	17.7	16.4	10.2	11.2	10.2	9.7	14.9
6A	9.1	6.3	10.5	11.8	18.9	12.6	13.6	12.5
6B	10.4	3.4	3.9	3.8	12.1	4.6	6.6	8.1
7A	7.7	5.0	5.2	7.4	7.9	11.5	8.3	5.3
7B	12.2	5.5	5.5	6.2	3.6	5.0	4.6	4.7

DATA FOR CHAPTER 18:

ANF INFUSION IN THE NEPHROTIC SYNDROME.

'Periods' represent 30 minutes: 1-2 are basal measurements, 3-6 the ANF infusion, and 7-8 recovery. NS1-6 are the patients.

Period	1	2	3	4	5	6	7	8
Plasma Sodium (mmol/l).								
NS1	134	133	135	135	131	134	134	134
NS2	130	128	130	131	131	130	130	131
NS3	136	136	136	134	134	135	135	136
NS4	136	134	135	135	136	135	136	136
NS5	133	132	133	133	133	133	134	134
NS6	129	128	128	128	126	128	128	128
Mean±SEM	133±1	132±1	132±1	133±1	132±1	133±1	133±1	133±1
Plasma Potassium (mmol/l).								
NS1	4.3	4.4	4.5	4.6	4.1	4.0	3.9	3.9
NS2	5.7	6.1	6.2	5.6	5.3	4.8	4.5	4.5
NS3	3.8	4.2	4.1	3.9	3.8	3.9	3.9	3.7
NS4	4.0	4.0	4.0	4.2	4.2	3.8	4.3	3.9
NS5	4.2	4.2	4.6	4.6	3.6	3.6	3.9	3.9
NS6	4 4	4.4	4.2	4.3	3.8	4.0	3.9	4.0
Mean±SEM	4.4±0.3	4.6±0.3	4.6±0.3	4.5±0.2	4.1±0.2	4.0±0.2	4.1±0.1	4.0±0.1
Plasma ANF (pmol/l).								
NS1	3.3	3.3	9.8	31.0	34.7	36.3	4.3	4.3
NS2	3.3	2.8	8.4	10.6	21.5	18.9	7.7	5.6
NS3	5.7	6.6	15.2	35.3	38.0	30.6	9.3	6.7
NS4	3.9	2.7	3.8	10.9	15.5	24.1	4.7	1.7
NS5	7.3	6.2	26.1	33.4	30.1	28.2	7.2	4.2
NS6	3.3	2.3	7.1	14.8	16.9	13.2	4.4	2.4
Mean±SEM	4.5±0.7	4.0±0.8	11.7±3.2	22.7±4.7	26.1±3.8	25.2±3.3	6.3±0.8	4.2±0.8
Urinary Sodium (micromol/min).								
NS1	128	117	124	192	208	254	231	231
NS2	84	61	84	102	108	178	201	148
NS3	90	90	130	329	417	374	224	93
NS4	254	233	263	360	372	398	295	188
NS5	56	58	69	69	75	83	83	60
NS6	98	89	122	193	172	219	191	88
Mean±SEM	118±28	108±26	132±28	208±47	225±56	251±48	204±28	135±26

ANF INFUSION IN NEPHROTIC SYNDROME CONTINUED.

Period	1	2	3	4	5	6	7	8
Urinary Potassium (micromol/min).								
NS1	70	70	50	52	47	61	52	52
NS2	89	102	101	101	80	93	91	81
NS3	79	85	84	82	62	62	60	48
NS4	127	181	132	94	72	61	55	54
NS5	45	43	44	49	48	49	49	51
NS6	67	63	77	91	91	78	60	38
Mean±SEM	80±11	91±20	81±13	78±9	67±7	57±13	61±6	54±6
Urine Volume (ml/min).								
NS1	11.6	14.6	12.4	13.7	5.9	2.0	1.3	1.3
NS2	1.9	1.4	3.4	6.4	6.1	9.5	9.6	8.0
NS3	10.0	10.0	11.8	16.4	18.9	17.8	14.9	9.3
NS4	11.6	10.1	11.5	14.4	14.3	11.1	6.3	3.2
NS5	3.0	3.6	3.7	4.1	4.0	2.9	2.9	2.0
NS6	1.2	1.1	1.4	2.3	4.8	7.1	2.2	1.2
Mean±SEM	6.6±2.0	6.8±2.3	7.4±2.0	9.6±2.4	8.2±2.8	8.4±2.3	6.2±2.1	4.2±1.4
Pulse (beats/minute).								
NS1	84	82	80	62	70	64	70	68
NS2	60	56	49	44	50	48	50	64
NS3	68	76	60	64	64	64	64	64
NS4	64	78	72	72	76	64	64	68
NS5	81	80	84	84	84	100	104	110
NS6	48	42	42	44	42	50	76	76
Mean±SEM	68±5	69±7	65±7	62±6	56±12	58±12	71±7	75±7
Mean Blood Pressure (mmHg).								
NS1	86	85	79	87	87	67	81	87
NS2	97	103	109	105	102	108	104	103
NS3	106	83	87	104	101	105	98	109
NS4	93	93	96	93	91	88	98	87
NS5	82	80	80	80	79	78	84	87
NS6	107	109	113	127	117	108	117	110
Mean±SEM	95±4	92±5	94±6	99±7	96±5	92±7	97±5	97±5

ANF INFUSION IN NEPHROTIC SYNDROME CONTINUED.

Period	1	2	3	4	5	6	7	8
Plasma Renin Activity (nmol/h/l).								
NS1	1.28	1.10	1.45	0.91	0.97	0.73	1.03	1.03
NS2	0.77	0.77	0.67	0.71	0.61	0.64	0.61	0.61
NS3	0.79	0.83	0.80	0.78	0.74	0.59	0.52	0.46
NS4	1.13	1.03	0.87	0.72	0.52	0.65	0.62	0.56
NS5	1.25	1.28	1.01	1.01	0.79	0.60	0.58	0.58
NS6	1.53	1.41	1.38	0.96	0.99	0.86	0.56	0.74
Mean±SEM	1.13±0.11	1.07±0.10	1.03±0.13	0.94±0.10	0.77±0.05	0.68±0.04	0.65±0.08	0.66±0.08
Plasma Aldosterone (pmol/l)								
NS1	560	430		300		186		460
NS2	900	1150		780		380		250
NS3	140	100		68		<69		<69
NS4	<69	<69		<69		<69		<69
NS5	550	560		400		320		720
NS6	69	300		125		<69		75
Mean±SEM	376±139	429±165		285±112		165±64		263±111
Glomerular Filtration Rate (uncorrected for body area) (ml/min).								
NS1	155	165	187	192	171	177	185	185
NS2	53	48	50	51	54	66	62	63
NS3	162	190	181	173	179	220	160	160
NS4	172	180	167	191	207	161	139	120
NS5	17	17	19	22	18	17	17	17
NS6	102	86	98	96	100	118	100	71
Mean±SEM	110±26	114±30	117±29	121±30	122±30	111±25	111±25	103±25
Effective Renal Plasma Flow (uncorrected for surface area) (ml/min)								
NS1	447	485	464	423	384	412	555	555
NS2	179	195	169	174	166	166	208	188
NS3	810	836	802	721	724	620	674	710
NS4	1139	1205	1125	1007	1007	876	870	938
NS5	61	61	54	48	55	55	56	56
NS6	493	441	495	385	477	523	476	351
Mean±SEM	521±160	471±184	568±145	460±141	469±141	442±120	473±120	466±132

ANF INFUSION IN NEPHROTIC SYNDROME CONTINUED.

Period	1	2	3	4	5	6	7	8
Filtration Fraction (%).								
NS1	34.6	34.0	40.3	45.3	44.5	42.9	33.3	33.3
NS2	29.6	24.6	29.5	29.3	32.5	39.8	29.8	33.5
NS3	20.0	22.7	22.6	24.0	24.7	32.8	23.7	22.5
NS4	15.1	14.9	14.8	19.0	20.6	18.3	16.0	12.8
NS5	26.9	26.9	34.7	40.2	38.4	30.7	30.7	30.7
NS6	20.6	19.5	19.8	24.9	21.0	27.7	21.0	20.2
Mean±SEM	24.5±2.9	23.8±2.7	27.0±3.8	30.5±4.1	30.2±3.9	30.2±3.9	32.0±3.5	25.8±2.6
Plasma Total Protein (g/l).								
NS1	67	70	71	73	70	70	70	66
NS2	56	56	59	58	59	58	57	57
NS3	45	43	44	44	49	51	47	45
NS4	61	58	57	59	61	60	60	60
NS5	59	59	62	62	69	69	67	67
NS6	54	55	54	56	56	55	56	53
Mean±SEM	57±3	57±4	58±4	59±4	61±3	61±3	60±3	57±3
Plasma Albumin (g/l).								
NS1	30	29	29	22	22	26	26	26
NS2	30	31	26	29	25	27	27	27
NS3	21	20	21	19	21	23	21	22
NS4	26	25	25	25	23	25	25	23
NS5	23	24	24	25	28	27	27	28
NS6	19	19	19	20	20	19	20	20
Mean±SEM	25±2	25±2	24±1	23±1	23±1	24±1	24±1	24±1

	Haematocrit (%)/ Haemoglobin (g/dl).		Plasma Volume (% change).
	Period 2	Period 6	
NS1	50.5/17.4	48.0/17.2	+6.3
NS2	39.0/13.4	39.0/13.5	+0.7
NS3	48.4/16.3	51.5/17.4	-11.9
NS4	41.5/14.2	41.3/14.3	-0.4
NS5	32.0/ 9.9	36.0/11.6	-19.7
NS6	41.0/13.6	41.0/13.6	0.0
Mean±SEM	42.1±2.7/14.1±1	42.8±2.4/14.6±0.9	

ANF INFUSION IN THE NEPHROTIC SYNDROME CONTINUED.

Period	1	2	3	4	5	6	7	8
Urine Albumin (mg/min).								
NS1	1.6	2.3	2.6	3.0	4.2	3.1	2.1	2.1
NS2	1.9	1.9	2.7	4.3	4.5	7.0	3.4	6.4
NS3	3.1	3.5	4.3	8.9	13.3	17.6	13.4	6.5
NS4	9.1	11.1	12.0	12.7	15.7	13.9	17.0	5.5
NS5	3.5	5.9	6.2	6.7	7.7	7.7	7.9	7.9
NS6	5.6	4.6	6.7	6.4	10.2	14.1	9.6	4.2
Mean±SEM	4.1±1.1	4.9±1.4	5.8±1.4	7.0±1.4	9.3±1.9	10.6±2.2	8.9±2.3	5.4±0.8
Fractional Excretion of Albumin (% GFR).								
NS1	0.03	0.05	0.05	0.07	0.11	0.07	0.04	0.04
NS2	0.23	0.13	0.21	0.29	0.33	0.39	0.20	0.38
NS3	0.07	0.07	0.10	0.20	0.22	0.35	0.34	0.18
NS4	0.20	0.25	0.29	0.27	0.33	0.34	0.49	0.20
NS5	0.90	1.45	1.36	1.22	1.52	1.68	1.72	1.66
NS6	0.29	0.28	0.27	0.34	0.54	0.63	0.48	0.30
Mean±SEM	0.29±0.13	0.37±0.22	0.38±0.20	0.40±0.17	0.51±0.21	0.58±0.23	0.55±0.25	0.46±0.24
Plasma CGMP (nmol/l).								
NS1	3.1	4.6	6.0	11.2	9.3	9.0	6.6	6.6
NS2	3.2	3.2	4.7	5.3	9.6	8.1	7.3	4.7
NS3	2.4	2.7	3.7	4.8	5.9	8.9	6.8	4.7
NS4	2.5	3.2	3.5	5.8	5.0	4.8	5.1	4.2
NS5	5.1	4.7	6.2	7.4	12.5	8.4	7.0	7.6
NS6	2.4	2.2	2.6	8.9	5.0	5.3	5.6	2.1
Mean±SEM	3.1±0.4	3.4±0.4	4.5±0.6	7.2±1.0	7.9±1.2	7.4±0.8	6.4±0.4	4.0±1.0
Urine cGMP (pmol/min).								
NS1	196	219	562	1494	1302	439	412	412
NS2	99	130	185	374	203	306	361	288
NS3	264	334	498	1098	966	946	651	337
NS4	258	191	364	1402	1270	1566	1019	221
NS5	108	79	96	150	156	156	130	130
NS6	68	56	128	304	308	328	304	124
Mean±SEM	166±34	168±42	306±79	804±239	701±219	624±214	480±127	252±46

ANF INFUSION IN CHRONIC RENAL FAILURE (Chapter 19).

For key to periods, please see data for Chapter 17.

Period	1	2	3	4	5	6	7	8
Plasma Sodium (mmol/l)								
1 Active	139	139	141	141	142	142	140	140
2	140	140	140	140	140	140	140	140
3	139	138	140	140	138	139	138	140
4	135	135	135	134	134	133	134	135
5	140	139	136	137	136	134	135	134
6	139	139	139	140	140	139	140	140
7	131	130	130	129	132	130	130	130
8	134	132	131	131	131	131	130	131
Mean±SEM	137±1	136±1	136±1	136±1	137±1	136±2	136±2	136±2
1 Placebo	136	135	136	134	135	133	133	133
2	134	133	134	134	135	133	133	134
3	136	136	132	133	135	135	134	135
4	132	131	125	126	129	129	128	128
5	126	131	130	125	127	127	128	126
6	137	136	136	137	137	135	136	137
7	140	138	137	138	137	137	138	138
Mean±SEM	134±32	134±1	133±2	132±2	134±2	133±1	133±1	133±2
Plasma Potassium (mmol/l).								
1 Active	4.2	4.2	4.0	4.0	4.0	4.0	3.7	3.7
2	4.0	3.8	3.9	4.2	4.1	3.9	3.8	4.2
3	3.9	3.9	4.0	4.3	3.9	4.0	4.0	3.5
4	5.0	5.0	5.2	5.4	4.9	4.7	4.6	4.4
5	4.8	5.1	5.0	5.1	4.9	5.0	4.9	4.6
6	4.4	5.0	4.8	5.0	5.1	5.0	5.1	4.8
7	5.1	5.1	5.0	5.2	5.3	5.2	5.2	5.1
8	3.7	3.7	3.8	3.7	3.7	3.7	3.8	3.7
Mean±SEM	4.4±0.2	4.5±0.2	4.5±0.2	4.6±0.2	4.5±0.2	4.4±0.2	4.4±0.2	4.3±0.2
1 Placebo	3.5	3.6	3.5	3.5	3.6	3.6	3.6	3.6
2	3.8	3.8	4.0	4.2	4.1	4.0	3.8	4.2
3	4.3	4.5	4.4	4.5	4.2	4.5	4.0	4.3
4	5.2	5.4	5,4	5.5	6.1	5.6	5.6	5.6
5	5.1	5.7	5.4	4.9	4.9	4.9	5.4	4.8
6	3.8	3.8	4.2	4.0	4.1	4.2	4.2	3.9
7	4.9	4.4	4.1	4.9	4.3	4.1	4.5	4.4
Mean±SEM	4.4±0.3	4.5±0.3	4.4±0.3	4.5±0.3	4.5±0.3	4.4±0.3	4.4±0.3	4.4±0.3

ANF INFUSION IN CRF CONTINUED

Plasma Albumin (g/l).

1 Active	43	43	38	38	42	42	38	38
2	42	41	42	44	40	42	40	44
3	36	35	37	37	35	31	42	31
4	38	37	39	37	37	39	37	41
5	39	38	36	37	36	38	33	33
6	39	39	45	44	46	42	36	39
7	48	50	50	50	50	50	52	52
8	37	37	37	39	37	34	34	34
Mean±SEM	40±1	40±2	40±2	41±2	40±2	40±2	39±2	39±2
1 Placebo	42	41	40	40	40	40	40	40
2	40	39	43	41	41	41	43	42
3	43	43	41	46	41	41	44	43
4	40	38	38	32	37	38	37	37
5	45	43	44	40	40	41	40	41
6	44	44	44	43	45	45	45	43
7	42	38	40	41	39	37	38	41
Mean±SEM	42±1	41±1	42±1	40±1	40±1	40±1	40±1	40±1

Pulse (beats/minute).

1 Active	64	64	60	56	50	56	64	60
2	80	78	72	80	76	80	84	105
3	52	56	58	52	56	52	50	62
4	58	64	60	62	68	60	60	64
5	36	40	42	40	44	42	40	39
6	52	52	48	48	56	48	48	48
7	62	62	64	56	68	48	54	54
8	96	92	82	80	82	78	78	82
Mean±SEM	63±7	64±6	61±5	59±5	63±5	58±5	60±5	64±7
1 Placebo	72	64	62	62	64	62	60	68
2	68	64	64	64	56	64	70	76
3	68	64	64	60	60	58	52	64
4	52	52	56	56	52	56	58	64
5	50	52	48	52	60	48	52	48
6	52	56	56	52	52	60	60	60
7	96	80	76	82	76	96	82	82
Mean±SEM	65±6	62±4	61±3	61±4	60±3	63±6	62±4	66±4

ANF INFUSION IN CRF CONTINUED

Period	1	2	3	4	5	6	7	8
Mean Blood Pressure (mmHg).								
1 Active	80	97	73	80	72	85	75	78
2	87	91	90	97	98	97	99	88
3	89	96	83	81	80	81	85	83
4	73	80	86	71	83	97	80	88
5	114	110	112	113	112	113	98	103
6	105	108	111	110	109	106	107	107
7	107	83	90	83	93	84	87	93
8	83	104	92	104	97	103	100	97
Mean±SEM	92±5	96±4	92±5	92±6	93±5	96±4	91±4	92±4
1 Placebo	97	93	93	93	100	103	104	105
2	107	115	122	108	110	111	93	101
3	99	96	89	100	97	93	93	97
4	117	115	113	119	125	120	111	113
5	91	83	93	96	95	93	90	95
6	116	110	107	108	120	106	107	109
7	67	68	67	67	70	73	67	67
Mean±SEM	99±7	97±7	98±7	99±6	102±7	100±6	95±6	98±6
PRA (nmol/h/l).								
1 Active	2.08	1.73	1.50	1.59	1.37	1.47	1.01	0.91
2	1.35	1.21	1.17	1.11	0.97	0.97	0.95	0.75
3	3.49	2.82	2.55	2.19	1.86	1.77	1.84	1.74
4	0.50	0.51	0.40	0.60	0.43	0.44	0.46	0.38
5	0.15	0.31	0.27	0.22	0.31	I.21	0.19	0.18
6	0.38	0.31	0.18	0.24	0.12	0.16	0.13	0.07
7	1.28	0.78	0.61	0.33	0.43	0.49	0.26	0.30
8	2.26	2.27	1.86	2.43	1.99	1.82	2.10	1.71
Mean±SEM								
	1.34±0.42	1.24±0.33	1.07±0.30	1.09±0.31	0.94±0.26	0.92±0.24	0.87±0.27	0.76±0.23
1 Placebo	3.13	4.62	4.01	2.53	2.68	2.30	1.62	1.82
2	0.28	0.29	0.25	0.23	0.26	0.26	0.21	0.23
3	0.72	0.78	0.65	0.56	0.65	0.53	0.54	0.53
4	0.59	0.68	0.55	0.52	0.54	0.48	0.44	0.41
5	0.28	0.25	0.24	0.24	0.26	0.22	0.21	0.23
6	0.89	0.90	0.75	0.92	0.98	0.84	0.81	0.76
7	1.65	2.03	1.88	1.92	1.67	1.59	1.71	2.16
Mean±SEM								
	1.08±0.38	1.36±0.59	1.19±0.52	0.99±0.34	1.01±0.33	0.89±0.29	0.79±0.24	0.88±0.30

ANF INFUSION IN CRF CONTINUED

Period	1	2	3	4	5	6	7	8
Plasma Aldosterone (pmol/l).								
1 Active	760	780		610		650		450
2	3200	4600		2400		1080		1000
3	1920	2340		1120		780		790
4	920	900		1160		670		630
5	1000	680		780		610		1240
6	470	380		290		340		340
7	760	880		890		890		620
8	3310	2200		2600		1600		2650
Mean±SEM	1543±402	1595±498		1231±294		826±134		965±261
1 Placebo	790	800		660		560		560
2	680	590		740		670		430
3	730	670		650		680		660
4	720	820		1010		870		570
5	1110	1300		1880		1040		940
6	350	570		430		760		650
7	3250	3700		4500		4000		3750
Mean±SEM	1090±370	1207±425		1410±546		1226±467		1080±450
Plasma cGMP (nmol/l).								
1 Active	9.2	7.9	11.7	13.8	18.0	18.6	16.3	16.3
2	5.0	6.3	6.3	10.2	12.8	12.0	9.3	7.0
3	1.8	2.6	3.8	10.9	11.6	10.7	6.6	6.4
4	10.1	11.6	12.8	13.8	20.7	18.7	19.1	10.2
5	8.9	10.0	10.1	11.3	10.3	14.7	14.3	11.2
6	5.9	3.8	7.9	10.8	17.0	11.4	6.9	5.0
7	6.4	2.8	3.6	5.0	9.0	8.2	6.4	3.8
8	15.2	11.8	19.6	23.4	50.0	28.4	26.6	23.6
Mean±SEM	7.8±1.4	7.0±1.4	9.5±1.9	12.4±1.8	18.7±4.7	15.3±2.3	13.2±2.6	10.4±2.4
1 Placebo	5.4	5.0	6.1	6.2	9.2	9.4	9.9	9.8
2	12.4	12.6	11.0	9.1	9.7	10.6	10.5	10.6
3	4.0	4.3	4.6	4.0	2.5	3.1	3.8	4.3
4	7.8	9.8	5.8	5.8	8.3	6.2	5.7	4.7
5	5.7	5.2	3.5	1.3	3.0	5.9	3.4	2.5
6	4.8	4.0	2.0	1.8	2.2	1.5	2.7	2.7
7	6.1	6.4	3.8	5.0	4.0	3.2	4.0	5.6
Mean±SEM	6.1±1.2	6.4±1.3	5.2±1.1	4.9±1.0	5.6±1.3	5.7±1.3	5.7±1.2	5.7±1.2

Period	1	2	3	4	5	6	7	8
Urine cGMP (pmol/min).								
1 Active	73	73	137	137	186	186	107	107
2	121	88	92	242	190	267	220	142
3	102	117	231	454	498	534	355	238
4	97	63	74	124	245	190	106	72
5	64	91	89	184	152	150	142	97
6	378	315	446	774	856	799	590	226
7	290	202	340	605	682	683	389	311
8	132	172	170	329	422	360	503	273
Mean±SEM	157±40	140±30	197±47	356±84	404±92	396±82	302±66	183±32
1 Placebo	353	417	295	313	407	356	506	343
2	121	120	99	85	61	70	65	62
3	150	127	139	123	181	154	120	144
4	90	123	101	63	79	64	54	58
5	79	68	49	34	40	45	28	33
6	202	192	145	157	170	134	150	150
7	156	119	84	85	116	144	109	113
Mean±SEM	164±35	167±44	130±30	123±35	151±47	138±40	147±67	129±40
Plasma ANF (pmol/l).								
1 Active	8.2	8.1	32.7	34.1	34.7	30.7	9.8	9.8
2	5.0	3.7	6.4	11.5	10.8	14.2	5.4	5.2
3	5.7	3.7	7.4	11.9	17.5	22.0	4.2	2.4
4	7.6	8.0	24.0	21.3	17.9	20.7	4.7	1.4
5	25.7	18.8	53.1	79.3	57.3	86.0	17.6	9.9
6	15.8	18.2	28.5	31.0	59.8	39.1	9.0	8.9
7	3.9	5.0	-	-	-	-	-	-
8	11.2	11.0	77.0	93.0	90.0	95.0	17.3	18.0
Mean±SEM	11.2±2.8	10.2±2.4	32.7±9.5	40.3±12.3	41.1±11.0	44.0±12.4	9.7±2.2	7.7±2.1
(means during ANF infusion exclude patient 7 because data are incomplete)								
1 Placebo	7.8	7.9	10.2	8.2	10.1	11.1	12.0	10.1
2	16.3	14.1	9.7	10.1	10.0	13.2	13.1	16.7
3	8.0	4.8	6.0	6.4	4.9	7.4	4.3	4.3
4	22.2	16.9	12.6	12.3	13.2	13.5	13.8	18.4
5	16.4	14.1	13.8	13.4	15.8	12.9	16.3	16.7
6	5.4	5.0	5.6	4.2	3.5	3.2	2.0	2.5
7	8.5	11.5	7.2	8.2	6.5	7.5	8.0	6.2
Mean±SEM	12.1±2.4	10.6±1.8	9.3±1.2	9.0±1.2	9.1±1.7	9.8±1.5	9.9±2.0	10.7±2.5

ANF INFUSION IN CRF CONTINUED

Period	1	2	3	4	5	6	7	8
Urine Volume (ml/min).								
1 Active	2.3	2.3	2.8	2.8	3.3	3.3	2.0	2.0
2	4.8	4.0	4.9	5.8	4.7	5.3	5.5	4.1
3	4.9	5.5	6.6	8.0	8.0	8.8	6.8	7.0
4	1.8	1.8	1.9	2.7	3.3	3.1	3.0	2.1
5	1.8	2.9	2.9	3.1	3.4	3.2	2.8	2.9
6	8.9	8.2	9.5	10.0	10.0	9.3	6.0	3.4
7	6.1	7.3	10.0	8.8	8.6	11.5	6.4	4.7
8	3.4	3.1	3.4	4.3	4.3	3.7	4.5	3.5
Mean±SEM	4.2±0.9	4.4±0.8	5.3±1.1	5.7±1.0	6.0±1.0	6.1±1.2	4.6±0.7	3.7±0.6
1 Placebo	4.4	5.1	4.2	5.1	5.1	4.6	6.6	5.1
2	2.0	2.0	2.7	2.6	2.2	2.2	1.9	1.9
3	4.1	3.6	4.0	3.8	4.9	4.1	3.2	3.9
4	3.1	2.1	2.4	2.8	2.5	2.1	2.1	1.8
5	3.3	3.5	3.1	3.1	3.5	3.6	2.7	2.3
6	7.1	7.1	5.7	5.7	6.3	6.3	5.2	5.3
7	4.3	4.4	4.7	3.1	5.1	5.8	5.6	5.7
Mean±SEM	4.1±0.6	4.0±0.7	3.8±0.5	3.8±0.5	4.2±0.6	4.1±0.6	3.9±0.7	3.7±0.6
Urine Sodium (micromol/min).								
(For statistics results are expressed as % of basal period 2)								
1 Active	166	166	197	197	234	234	136	136
2	201	152	191	232	188	212	204	160
3	87	82	106	151	160	176	150	167
4	53	44	55	91	127	119	104	58
5	61	78	72	80	92	77	65	63
6	561	408	447	530	570	569	343	241
7	207	217	340	325	293	439	187	135
8	134	99	104	160	145	138	158	87
Mean±SEM	183±57	156±41	189±49	221±52	226±54	246±60	168±29	131±22
1 Placebo	79	77	50	46	51	46	73	66
2	178	180	157	151	108	108	91	93
3	122	91	97	83	97	75	52	62
4	229	224	132	97	81	70	57	37
5	147	116	87	84	84	79	49	42
6	206	206	120	120	152	152	84	84
7	215	179	228	163	215	204	196	206
Mean±SEM	168±21	153±22	124±22	106±16	113±21	105±21	86±19	84±22

ANF INFUSION IN CRF CONTINUED

Period	1	2	3	4	5	6	7	8
Urine Potassium (micromols/min).								
1 Active	39	39	42	42	40	40	28	27
2	67	50	58	68	56	59	58	50
3	70	66	65	73	70	74	64	68
4	36	32	37	48	55	48	42	30
5	29	37	36	37	41	39	37	35
6	95	62	66	64	62	59	39	28
7	63	64	84	98	95	106	70	73
8	74	56	54	67	64	55	67	53
Mean±SEM	59±8	51±5	55±6	62±7	60±6	60±8	51±6	46±6
1 Placebo	55	73	59	63	66	53	73	52
2	15	15	12	12	9	9	10	11
3	52	52	60	58	74	74	48	52
4	22	22	15	30	31	32	31	29
5	52	53	47	48	55	57	43	34
6	55	55	45	44	51	51	40	40
7	79	61	56	60	72	64	62	63
Mean±SEM	47±8	47±8	42±8	45±7	51±9	49±8	44±8	40±7
Urine Albumin (micrograms/min).								
(For statistics these are analysed as % of basal period 2)								
1 Active	350	360	630	580	750	750	760	520
2	240	190	190	335	145	175	345	250
3	725	615	620	1040	940	1145	950	700
4	730	680	730	990	1255	1060	1080	810
5	540	550	805	1055	1170	1155	855	690
6	105	70	120	540	720	580	169	105
7	975	780	1400	1495	1380	2195	1095	955
8	2290	1612	1875	2770	3065	2462	1900	1610
Mean±SEM	744±243	607±168	796±208	1101±272	1178±303	1190±276	894±187	705±164
1 Placebo	435	632	505	612	592	605	520	410
2	144	172	127	119	95	75	63	95
3	220	180	200	170	240	200	160	180
4	1190	916	888	1160	920	824	675	735
5	400	385	300	170	380	260	330	180
6	80	120	70	80	70	30	45	57
7	1640	1610	290	180	630	1160	700	1370
Mean±SEM	587±224	574±204	340±105	356±150	418±120	450±160	356±105	432±179

ANF INFUSION IN CRF CONTINUED

Period	1	2	3	4	5	6	7	8
(For statistics GFR and ERPF are analysed as % of basal period 2)								
GFR (ml/min: uncorrected for surface area)								
1 Active	13.0	13.0	13.0	13.0	15.0	15.0	11.0	11.0
2	13.0	11.0	15.0	14.0	13.0	17.0	12.0	9.0
3	44.4	47.6	68.0	55.4	56.4	53.7	41.8	51 1
4	13.1	12.5	12.5	14.1	17.7	13.3	15.0	12.6
5	8.6	11.2	11.4	11.6	12.3	9.5	9.3	10.0
6	66.0	54.0	59.0	67.0	56.0	72.0	47.0	41.0
7	32.8	35.9	74.9	65.5	55.6	62.5	48.8	55.0
8	13.5	10.8	12.5	14.3	15.0	10.5	15.1	12.5
Mean±SEM	25.6±7.2	24.5±6.5	33.3±10.1	31.9±9.1	30.1±7.6	31.7±9.3	25.0±6.2	25.3±7.1
1 Placebo	28.0	34.0	28.0	29.0	21.0	21.0	30.0	19.0
2	14.2	13.5	14.9	13.0	10.5	10.5	9.6	9.8
3	46.6	32.3	47.9	39.8	58.5	52.3	42.7	48.0
4	12.2	12.5	15.2	12.3	13.2	13.9	11.7	11.4
5	14.3	13.9	9.3	12.1	15.5	15.6	11.6	11.2
6	38.7	43.0	38.2	35.3	40.8	39.1	39.6	39.7
7	26.1	23.5	20.5	11.8	15.0	22.5	25.6	27.2
Mean±SEM	25.7±5.0	24.3±4.6	24.9±5.3	21.9±4.7	24.9±6.8	25.0±5.8	24.4±5.2	23.7±5.7
ERPF (ml/min: uncorrected for surface area).								
1 Active	28	28	27	27	28	28	20	20
2	73	51	51	60	46	77	57	51
3	147	158	148	151	144	151	139	175
4	35	28	33	33	36	30	32	30
5	16	21	20	18	20	19	18	20
6	223	175	158	179	129	198	149	129
7	(Failed for technical reasons)							
8	31	24	30	34	40	33	40	37
Mean±SEM	79±29	69±25	67±23	72±25	63±19	77±27	65±21	66±23
1 Placebo	121	168	132	141	104	109	154	95
2	29	26	25	25	22	21	22	22
3	131	82	104	123	165	146	128	151
4	24	21	24	21	31	28	30	28
5	46	47	41	54	63	74	52	50
6	157	150	135	134	143	132	106	113
7	117	100	67	44	55	74	78	92
Mean±SEM	89±21	85±22	75±18	78±20	83±21	81±19	81±19	79±18