ATRIAL NATRIURETIC PEPTIDE

IN

LIVER DISEASE WITH SODIUM RETENTION

Marios Zenon Panos
King's College School of Medicine & Dentistry

Thesis for the MD Degree, University of London

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ABSTRACT

Plasma concentration of atrial natriuretic peptide (ANP), measured by radioimmunoassay, was increased in patients with cirrhosis and ascites as compared to age matched healthy controls under basal conditions and throughout 24 hours of observation. Nocturnal natriuresis in patients, between midnight and 0800 hours, coincided with a marked reduction in the activity of the renin-aldosterone system. During this period there was significant correlation between urine sodium and plasma ANP concentration, suggesting that a). ANP may have a role in nocturnal natriuresis in cirrhosis and b). a reduction of renin-aldosterone in recumbency, may allow the natriuretic effect of ANP to become manifest.

In the course of therapeutic paracentesis for tense ascites, plasma ANP tended to rise initially, in parallel with an increase in cardiac output and a drop in right atrial pressure, findings consistent with atrial decompression. Investigation by 2D-echo-cardiography, confirmed the presence of compression of the right atrium in patients with tense ascites.

Plasma concentration of ANP in patients with paracetamol-induced fulminant hepatic failure was similar to that in healthy controls, but was increased in the presence of severe renal failure. Alterations in fluid balance, induced by haemodialysis and infusion of Human Albumin solution, resulted in appropriate changes of plasma ANP. These findings indicate that a). there is no deficiency of ANP in fulminant hepatic failure and b). known mechanisms of ANP release are not impaired.

In rats with carbon tetrachloride-induced cirrhosis, plasma ANP concentration was higher than in control animals. In cirrhotic rats with impaired sodium excretion *in-vivo*, there was diminished natriuretic response to infusion of synthetic ANP at physiological and patho-physiological concentrations, suggesting that in this animal model of cirrhosis, renal resistance to the natriuretic action of ANP may contribute to sodium retention.

To Irene, Zenon and George, for their support.

CONTENTS

Page

TABLES

Page

FIGURES

Page

Page

ACKNOWLEDGEMENTS

I am indebted to Dr Roger Williams and Dr David Westaby for allowing me to study their patients and for their supervision and guidance throughout this work. I am also indebted to the nurses of Annie Zunz and Todd wards of King's College Hospital, for their cooperation and kind care of the patients.

I also thank the following: Dr John Anderson of St Bartholomew's Hospital, for teaching me the method of radioimmunoassay for the atrial natriuretic peptide, at a time when few reliable assays were available and Professor Lesley Rees for allowing me to work in her laboratory; the late Dr JDH Slater and Nadia Payne of the Cobbold Laboratories, The Middlesex Hospital, for the assays of plasma renin activity and plasma aldosterone; Dr Christopher Gove of the Liver Unit, King's College Hospital, for advice and help with animal work; Dr John Chambers for echocardiographic studies; Drs John Firth and AEG Raine and Professor JGG Ledingham of the John Radcliffe Hospital, Oxford, for the isolated kidney perfusions; Dr Bernard Portmann for help with the histological examination of rat livers, and Claire Wicks for advice and help with the administration of low sodium diets; Mr Kypros H Nicolaides of the Harris Birthright Research Centre for Fetal Medicine, for providing samples of fetal blood; Sarah Lupton and Manos Stellakis for help with graphics.

The work that I personally carried out for this thesis included: the management of patients; the design of clinical trials and their execution, statistical analysis and interpretation of results; the proceedures of invasive instrumentation for haemodynamic studies, in the majority of cases; the administration and monitoring of compliance with

the low sodium diet; collection of all blood and urine samples; the radioimmunoassay of plasma for atrial natriuretic peptide; flame photometry for sodium and potassium estimation; experimental induction of cirrhosis in rats and the *in-vivo* studies of sodium handling in these. I assisted with some of the studies of isolated kidney perfusion and with all of the histological examinations of rat livers.

Clinical studies involving human subjects were performed with approval granted by the King's College Hospital Ethics Commitee.

Work involving animals was performed with permission from the Home Office, under licence no. PIL 70/06076.

ABBREVIATIONS

ANP = atrial natriuretic peptide

C = carbon

Cr = chromium

Ccl = creatinine clearance

cGMP= cyclic guanosine monophosphate

Δ = change

FHF = fulminant hepatic failure

Gp = group

HAS = human albumin solution

Hb = haemoglobin

I = iodine

IAP = intra-abdominal pressure

IVT = intravasscular blood transfusion

K = potassium

Na = sodium

PRA = plasma renin activity

PA = Plasma aldosterone concentration

pH = -log hydrogen ion concentration

pO2 = partial pressure of oxygen

SEM = standard error of the mean

UNa = urine sodium excretion

UV = urine flow rate

PUBLICATIONS FROM THIS WORK

This list does not include abstracts of less than 500 words

1. Panos MZ and Williams R. Feasibility of a short term severely restricted sodium diet with councelling support. *Ann Clin Biochem 1989;26:358-360.*

2. Panos MZ, Anderson JV, Payne N, Langley P, Rees L, Slater JDH, Williams R. Atrial natriuretic peptide and the renin-aldosterone system in patients with cirrhosis and ascites: Basal levels, changes during daily activity and nocturnal diuresis. *(Submitted).*

3. Panos MZ, Moore K, Vlavianos P, Chambers J, Anderson JV, Gimson AES, Slater JDH, Rees L, Westaby D, Williams R. Single, total paracentesis for tense ascites: sequential hemodynamic changes and right atrial size. *Hepatology. April 1990, In press.*

4. Panos MZ, Anderson JV, Forbes A, Payne N, Rees L, Slater JDH, Williams R. Human atrial natriuretic factor and renin-aldosterone in paracetamol-induced fulminant hepatic failure. *Gut. Accepted March 1990.*

5. Panos MZ, Gove CD, Firth J, Raine AEG, Ledingham JG, Westaby D, Williams R. Resistance to the natriuretic action of atrial natriuretic peptide on the isolated kidney of rats with experimental cirrhosis. *Clinical Science. Accepted March 1990.*

6. Firth J, Gove CD, Panos MZ, Raine AEG, Williams R, Ledingham JGG. Sodium handling in the isolated perfused kidney of the cirrhotic rat. *Clinical Science 1989;77:657-661.*

7. Panos MZ, Nicolaides KH, Anderson JV, Economides DL, Rees L, Williams R. Plasma atrial natriuretic peptide in human fetus: Response to Intravascular blood transfusion. *Am J Obst Gynecol 1989;161:357-361.*

AIMS OF THIS WORK

Sodium retention in association with chronic liver disease, as manifested in the formation of ascites, carries a poor prognosis. Ascites can be symptomatic and in a proportion of patients, is resistant to current treatment. The elucidation of pathogenetic mechanisms of sodium retention, may give rise to new approaches in therapy. A deficiency of, or resistance to the natriuretic action of a putative natriuretic factor, has been postulated as a contributory mechanism to sodium retention in cirrhosis.

The aims of this work were:

1. To measure circulating concentrations of the atrial natriuretic peptide (ANP) in patients with sodium retention in chronic liver disease and fulminant hepatic failure.
2. To examine the role of ANP and its possible interaction with the Renin-Aldosterone system, in patients with liver disease and sodium retention.
3. To investigate the possibility of "intrinsic" renal resistance to the natriuretic action of ANP in cirrhosis.

CHAPTER 1

INTRODUCTION

1.1 Ascites in cirrhosis

1.2 Pathogenesis of ascites

1.3 Theories of ascites formation

1.4 Efferent factors

1.5 Circulating natriuretic factors

1.5.1 Digoxin-like immunoreactive substance

1.5.2 Atrial Natriuretic Peptide

" When the liver is full of fluid and this overflows into the peritoneal cavity, so that the belly becomes full of water, death follows."

Hippocrates circa 400 B.C.

1.1 Ascites in cirrhosis

A significant proportion of patients with cirrhosis of the liver develop ascites at some stage of the disease (1). Ascites can be symptomatic and it's continued presence predisposes to the development of bacterial peritonitis (2,3). In about 10 per cent of patients ascites is refractory to treatment (1). The elucidation of the mechanisms of ascites formation and persistence is important, since it may give rise to new approaches in therapy.

1.1.1 Historical background

The coexistence of liver disease with fluid retention and the importance of the latter as a poor prognostic indicator was recognised by Hippocrates around 400 BC, who suggested that ascites originated from leakage of hepatic fluid into the peritoneal cavity (4). Erasistratus of Alexandria (circa 200 BC) postulated that the "narrowness of blood vessels going through the liver" was the major factor leading to peritoneal fluid accumulation (5). Galen (5) summised that "water in the skin (anasarka or dropsy) arises from too many humours; we hold that water in the skin is a cold and moist disease and it arises from abundancy of cold and moist humours". This view dominated physicians' thinking for 12 Centuries. In the treatment of ascites Graeco-Roman physicians employed abdominal paracentesis, though it was recognised as being hazardous. Celsus (born about 20 BC), gave a detailed description of how this should be done and suggested the use of a lead or bronze tube with a collar about the middle to avoid losing it in the patient's abdomen (6). Paul of Aegina (625-690 AD) gave another exact description of how the proceedure should be performed and warned that "sudden or

complete evacuation would immediately kill the patient" (7).

In the seventeenth century, Harvey's description of the circulation inspired the Oxford physiologist Richard Lower to perform a remarkable experiment in dogs, in which he ligated the inferior vena cava so producing ascites (8). In 1685 John Brown recorded an association between oedema and ascites in a patient with nodular cirrhosis (9). In the 1800's Thomas Sydenham deduced that "the terrible habit of swilling spiritous liquors" predisposed to the condition (10). Modern studies on the formation of ascites began in 1914 after C. Bolton induced ascites and described the pathological changes associated with passive venous congestion in the liver of animals, after partial occlusion of the inferior vena cava (11).

In the 1940's several groups of investigators documented the avid sodium retention and resultant positive sodium balance occurring in many patients with cirrhosis (12,13). Subsequent studies demonstrated an impaired renal excretory response to an acutely administered sodium chloride load (14,15) in patients with cirrhosis and ascites. In the 1950's the absence, or reversal of the normal diurnal rhythm of sodium excretion (with increased nocturnal natriuresis and diuresis) was documented in patients with cirrhosis (16-18). The advent of invasive haemodynamic monitoring in the 1950's made possible the first haemodynamic studies in humans, which demonstrated a progressively severe haemodynamic disturbance in patients with cirrhosis and cirrhosis with ascites (19-21).

An account of findings from studies between 1950 and 1989 is included in the discussion of current theories of ascites formation,

outlined below.

1.1.2 Clinical features

Patients with cirrhosis retain sodium avidly and frequently excrete urine which is virtually free of sodium (13-18). Extracellular fluid accumulates and eventually becomes manifest as clinically detectable ascites and oedema. The presence, or progression of ascites, is related to the degree of impairment of renal sodium handling although the ultimate determinant of whether fluid is retained is the net balance of dietary sodium intake and urinary excretion. Most studies have examined renal sodium handling in advanced liver disease. Naccarato et al (22), assessed sodium handling in patients with early cirrhosis. They examined the natriuretic response to acute saline administration in cirrhotic patients with no evidence of ascites or oedema and showed that a defect in sodium handling may occur early in the course of cirrhosis, before the development of ascites. At least in some patients, the abnormality in renal sodium handling may alternate between periods of sodium retention and spontaneous diuresis (23,24). The institution of a low sodium diet and bed rest have been noted to induce the latter phenomenon. A report by Bosch et al (25), indicates that these measures may produce the response in up to 28% of patients with cirrhosis and ascites. Patients likely to respond to these measures are those presenting for the first time, those with urine sodium excretion greater than 10 mmol per day and those with a normal glomerular filtration rate (26).

The primary abnormality causing fluid retention is a disturbance of sodium rather than water excretion (13,16,17). Many sodium retaining

patients with ascites and oedema can excrete urine of low osmolality when given a water load (23,27,28).

Despite extensive study, the mechanisms which mediate sodium retention in cirrhotic patients remain incompletely defined.

1.2 Pathogenesis of ascites

The pathogenesis of ascites involves changes in hepatic and intestinal lymph formation, changes in renal sodium handling and peritoneal factors (29). Hepatic venous outflow obstruction (intrahepatic hypertension) is a prerequisite for the development of ascites, in most cases of cirrhotic liver disease (30). Occasionally patients with cirrhosis develop ascites due to extrahepatic portal venous obstruction (31), or as a consequence of peritoneal disease. An examination of the pathogenetic factors which lead to the deranged sodium homeostasis in cirrhosis, is simplified by looking at "afferent" and "efferent" events. In a discussion of afferent events, consideration is given to the extracellular fluid translocations, or sequestration into serous spaces or interstitial fluid compartments. In addition, attention is paid to the detector element that recognises the degree of vascular volume alterations.

1.2.1 Afferent factors

1.2.1 (a). Splachnic circulation and splachnic lymph formation

Hepatic sinusoids are lined by a discontinuous endothelium and are highly permeable to protein (32). Hepatic lymph protein concentration is at least 90% of the simultaneous plasma value (33). Colloid osmotic pressure is thus not an effective determinant of net fluid flux across the hepatic sinusoids. Rather, fluid flux across the hepatic sinusoids

and into the space of Disse is determined almost entirely by changes in the hydraulic pressure gradient along the length of the sinusoids. The presence of intrahepatic sinusoidal hypertension causes transudation of plasma across the hepatic sinusoidal bed into the space of Disse, from which the fluid eventually enters the lymphatic vessels.

Several theories have been proposed to explain the elevation in sinusoidal pressures. Witte et al (34), postulated a two stage model of cirrhosis in which post-sinusoidal obstruction predominates early in the clinical course of the disease. Sinusoidal hypertension occurs due to the presence of an obstruction downstream from the hepatic sinusoids. If the capacity of the lymphatic system to return lymph to the systemic circulation is exceeded, protein rich fluid leaves the hepatic sinusoids, accumulates in the interstitium and eventually "weeps" from the surface of the liver into the peritoneal cavity (34). As cirrhosis progresses, Witte and associates postulated, presinusoidal obstruction occurs which in turn results in elevated hydrostatic pressures in the intestinal capillary bed. Since the intestinal capillaries are less permeable to protein than those of the liver, the ascites that forms late in the course of cirrhosis is usually of low protein content (34). A similar situation (low protein ascites) occurs in some cases of extrahepatic portal venous obstruction. Although this is an attractive model to explain the variability of ascitic fluid protein concentration in the ascites of cirrhosis, experimental evidence in support of this model is lacking.

Reynolds and colleagues (35), measured simultaneous portal venous pressures and wedged hepatic vein pressures in patients with alcoholic liver cirrhosis and found them to be essentially identical. They postulated that the equalisation of the two pressures, may be due to the reduction or loss of normal communications between sinusoids, so that sinusoidal pressure is transmitted downstream to the hepatic veins without first being "buffered" by the sinusoidal network that lies between portal and hepatic venous systems. Normally the sinusoidal network has many intercommunications that serve to dissipate elevations in portal pressure, before the hepatic veins are reached.

In patients with cirrhosis and sinusoidal hypertension there is increased hepatic lymph formation, that contributes to the formation of ascites, if the capacity to return lymph to the systemic circulation is exceeded (36). In healthy subjects 1.0-1.5 litres of lymph is returned to the systemic circulation via the thoracic duct per day (33). In patients with cirrhosis there is a five fold increase, up to 8-9 litres, and in cirrhosis with ascites it may be as high as 20 litres per day (34).

1.2.1 (b). Hypoalbuminaemia

The role of hypoalbuminaemia as a contributory event in the primary formation of ascites is not clear. Although decreased hepatic synthesis is present in many patients with cirrhosis, a significant proportion of patients have normal or increased rate of albumin synthesis (37). Dilutional effects and a shift from intravascular to extravascular pools contributes to the observed hypoalbuminaemia (37). In addition, there is evidence that increased intestinal venous pressure (as in portal

hypertension) decreases capillary surface area and may result in a selective reduction of permeability to macromolecules, such as albumin (38). In advanced cases of cirrhosis, reduced oncotic pressure due to hypoalbuminaemia, contributes to loss of fluid from the intravascular space (37).

1.3 Theories of ascites formation

The concepts of diminished effective volume or "Underfil" and of increased effective volume or "Overflow" theory, dominated thinking on the pathogenesis of ascites for the last two decades. Recently, a third theory was proposed, the "Peripheral arterial vasodilation hypothesis".

1.3.1 The "Underfil" theory

The "Underfil" theory was the first to be proposed and suggests that ascites formation occurs when a critical imbalance in Starling's forces across the hepatic and splachnic beds is reached. This imbalance results in an increase in the amount of lymph produced which exceeds the capacity of the thoracic duct for it's return to the systemic circulation. Lymph then "weeps" out into the peritoneal cavity, drawing out fluid and electrolytes, thus resulting in contraction of the circulating plasma volume. Another factor contributing to "Underfil" is the existence of a low systemic vascular resistance in patients with cirrhosis, which often occurs prior to ascites formation (39-42). This is partly due to vasodilatation of resistance vessels, caused by circulating vasodilator substances (43-46), which increase vascular bed capacity (39,40), and partly due to the formation of arterio-venous shunts or "fistulae" in the mesenteric, pulmonary and peripheral

circulations of patients with cirrhosis (47). Arterio-venous shunts appear to play an important role in causing the diminished peripheral vascular resistance and increased cardiac output of cirrhosis and probably contribute to activation of the sympathetic nervous system in these patients. This combination of haemodynamic and physical factors results in reduced "effective" plasma volume (i.e. plasma volume with access to central volume receptors situated in the heart and great vessels). The baroreceptors of the heart and great vessels set off compensatory mechanisms which include an increase in sympathetic nervous system activity (48), and increased activity of the renin-angiotensin-aldosterone system (28), resulting in a rise in circulating catecholamines and increased renal sodium and water retention. This theory may explain why fluid retention fails to modify the stimulus for continued sodium and water retention: Despite a progressive increase in total extracellular fluid volume, fluid is sequestered into one or more of the extravascular compartments without normalising "effective" blood volume. Thus, the stimulus for secondary sodium retention by the kidney persists.

Findings from studies using the head-out water immersion model to achieve an increase in central blood volume, without an increase in total plasma volume, support the "Underfil" theory. Epstein et al (49, 50), reported that water immersion corrected the anti-natriuresis in 15 of 26 patients with cirrhosis and ascites. Bichet et al (51), studied 8 patients with decompensated alcoholic cirrhosis and impaired ability to excrete a water load. Immersion produced significant increases in right

atrial pressure, pulmonary capillary wedge pressure, cardiac index and urine sodium excretion, with significant decreases in systemic vascular resistance, plasma renin activity, aldosterone, norepinephrine and arginine vasopressin (51). These findings support the hypothesis that, at least in "non-excretors", diminished "effective" vascular volume may be the non-osmotic mediator of arginine vasopressin release and of the activation of the sympathetic and renin-aldosterone systems (52,53). A significant proportion of patients with cirrhosis and ascites do not respond with a natriuresis to manoevres of volume expansion; Nicholls et al (54,55), reported that patients with the most severe decompensation of cirrhosis (tense ascites, marked resistance to diuretic therapy, greater impairment of water excretion, greater elevation of plasma renin activity and lower values for serum sodium, glomerular filtration rate and renal plasma flow) did not respond to immersion. It is possible that in these advanced cases, water immersion alone is not sufficient to normalise the haemodynamic and hormonal abnormalities.

Although evidence from water immersion studies is consistent with the "Underfil" theory (49-55), the patients studied all had advanced liver disease with well established ascites and relative hypotension, presumably due to extensive systemic arterio-venous shunting. It is possible that mechanisms operating in advanced disease may differ from those present early in the course of hepatic cirrhosis. Thus, primary renal sodium retention by other mechanisms at an earlier stage of the disease, can not be excluded.

Several inconsistencies have been noted in the "Underfil" theory. Measurements of plasma volume in cirrhotic patients have shown that

their plasma volume is expanded as compared to healthy controls (56,57). Although this has long been recognised, it was postulated that venous collaterals and expansion of the splachnic circulation accounted for the expansion in plasma volume and that "effective" or systemic vascular volume was actually diminished. Lieberman and Reynolds (58) used ^{131}I-albumin and ^{51}Cr-labelled erythrocytes to measure plasma volume in controls and patients with cirrhosis with, or without ascites. Values for plasma volume were significantly greater in both groups of patients, as compared to controls, although there was no difference in the values for patients with, and without ascites. Porta-caval shunting (i.e. decompression of the portal circulation) did not diminish plasma volume as measured by the above methods, indicating that splachnic pooling is unlikely to be responsible for the observed increase in plasma volume (58).

Sellars et al (59), studied 20 patients with cirrhosis who had never had oedema or ascites and reported initially that exchangeable sodium was within normal limits and that the renin-aldosterone system was normal. However, further analysis of their results revealed lower plasma renin activity and aldosterone concentration in patients with cirrhosis and no ascites, as compared to controls (60). Thus, it is possible that the renin-angiotensin-aldosterone system was suppressed in this group of patients, a finding that would be consistent with plasma volume expansion in the early stages of cirrhosis.

Based on these and other inconsistencies (61), Lieberman et al, proposed the "Overflow" theory of ascites formation (62).

1.3.2 The "Overflow" theory

The "Overflow" theory holds that the primary event in the formation of ascites is renal retention of sodium, leading to fluid retention and expansion of plasma volume with overflow into the extravascular space (62); raised hepatic pressure across the sinusoids, portal hypertenion and pooling in an expanded portal circulation, cause preferential localisation of fluid in the peritoneum. Both the "Underfil" and "Overflow" theories agree that transudation of ascites occurs due to alterations in hepatic and splachnic haemodynamics and an overwhelming of the lymphatic system. The issue is whether renal sodium retention is primary (Overflow) or secondary (Underfil).

Levy and Allotey (63,64) have provided the most convincing evidence in favour of the "Overflow" theory. Using a canine model of cirrhosis, they showed that sodium retention and expansion of plasma volume clearly precede the development of ascites. These changes were not related to hypotension, altered systemic haemodynamics, or hyperaldosteronism. However, a slight decrease in systolic pressure did occur, though not statistically significant, which could conceivably be the stimulus for renal retention of sodium and water (65,66).

The finding of early sodium retention in cirrhosis received support from the clinical study of Maccarato et al (22), who reported on eight patients with biopsy proven cirrhosis and eight controls. None of the patients had ever hed ascites or been on diuretics. Acute volume expansion with saline, caused a smaller increase in urine output and fractional excretion of sodium in patients. Proximal fractional sodium excretion was also diminished in patients. Further evidence in support

of a primary renal abnormality in early cirrhosis was provided by the same study; infusion of urine from cirrhotic patients into Wistar rats caused a significant decrease in urine output and absolute and fractional sodium excretion. Infusion of urine from controls did not have this effect. These results would be consistent with reduced production of a natriuretic factor or with the presence of an anti-natriuretic substance in the urine of patients with cirrhosis.

The role of intrahepatic hypertension as a possible stimulus for primary renal sodium retention was examined by Unikowsky et al (67). Biliary cirrhosis was induced in dogs by ligation of the common bile duct. Simultaneous side to side porta-caval anastomoses were created to maintain normal intrahepatic and portal pressures (68). Sodium retention and ascites formation occurred only in dogs with occluded porta-caval anastomoses (67). These findings are consistent with those of Orloff et al (69,70) and Campbell et al (71). The former, postulated that intra-hepatic hypertension causes sodium and water retention by stimulating aldosterone release. Other evidence (72) suggests that intrahepatic hypertension activates hepatic baroreceptors that cause increased efferent sympathetic activity in renal nerves, via an integrative site in the central nervous system. Efferent renal sympathetic nerves have been shown to be important in the renal handling of sodium (72). Thus a mechanism has been shown to exist whereby renal sodium retention may occur without the necessity of vascular underfil.

Sympathetic nervous system activation, as indicated by raised levels of circulating catecholamines, and activation of the renin-aldosterone system (53,73), occur in patients with cirrhotic ascites and

have been used as evidence against the "Overflow" theory of ascites formation (52,74). However, these findings are not inconsistent with the "Overflow" theory; Bosch et al (39), performed detailed haemodynamic and endocrine measurements in cirrhotic patients with and without ascites. The degree of renin-aldosterone activation and sodium retention correlated best with wedged hepatic venous pressures and not with systemic haemodynamic parameters, suggesting that hepatic haemodynamics and not plasma volume were the main influence on the renin-aldosterone system.

1.3.3 Peripheral arterial vasodilation hypothesis

The peripheral arterial vasodilation hypothesis was put forward by Schrier et al (75) as an alternative to the "Underfil" and "Overflow" theories, in order to account for phenomena that were inconsistent with either or both of these. For instance, while the occurrence of primary renal sodium and water retention and plasma volume expansion prior to ascites formation favours the "Overflow" hypothesis, increased cardiac output and low systemic vascular resistance (39,42), stimulation of the renin-aldosterone system (76-78) and vasopressin release (52), and sympathetic nervous system activation (53,55) is not consonant with primary volume expansion. The peripheral arterial vasodilation hypothesis is based on the observation that, in cirrhosis, peripheral arterial vasodilation occurs at an early stage (39-42). Arterial vasodilators and arteriovenous fistulae have been shown to result in sodium and water retention secondary to decreased filling of the arterial tree (43-46). An increase in cardiac output and stimulation of the renin-aldosterone system are common features of cirrhosis, arterio-

venous fistula (79) and drug induced peripheral arterial vasodilation (80). Specific to cirrhosis is the presence of portal hypertension which results in preferential localisation of fluid in the peritoneal cavity. The authors propose that peripheral arterial vasodilation due to increased circulating vasodilators (43-46) and arteriovenous shunts (47,79) is the initial event in intravascular underfilling, not because the intravascular blood volume is decreased, but because the intravascular compartment is enlarged. Vasodilation results in decreased cardiac afterload, or "run-off", which leads to an increase in cardiac output. This effect is detected by the cardiopulmonary volume receptors as underfil and several compensatory events are initiated: Increased activity of the sympathetic nervous system causes renal vasoconstriction and stimulation of the renin-aldosterone system and of vasopressin release, all of which increase renal sodium and water retention. Thus, increased plasma volume early in the course of cirrhosis, becomes compatible with decreased systemic vascular resistance and increased activity of the sympathetic nervous system. As plasma volume expansion occurs as a result of these compensatory mechanisms, renal haemodynamics are normalised and the renin-aldosterone system, circulating catecholamines and vasopressin, return to normal. Normal subjects and patients with moderate changes are able to excrete sodium loads normally i.e. "escape" (81). The mechanism for escape from a chronically administered sodium load, or from the experimental equivalent of exogenous administration of mineralocorticoid hormone, involves blood volume expansion, which leads to an increase in the filtered load and/or

decreased proximal reabsorption of sodium and water. The result is increased delivery of sodium and water to the distal renal tubule. These changes, along with a possible increase in a putative natriuretic factor, override the sodium retaining effect of aldosterone in the collecting duct, thus effecting "escape" (81-83). The authors of the Peripheral vasodilation hypothesis propose that when the degree of vasodilation is so severe that compensatory mechanisms are inadequate to normalise renal haemodynamics, the patient continues to retain sodium, is unable to "escape" and goes on to develop ascites (75).

1.4 Efferent factors

Initial attempts to explain the abnormalities of renal sodium handling focussed on the decrement in glomerular filtration rate which frequently occurs in patients with advanced liver disease. However, a decrease in glomerular filtration rate can not be the major determinant of these abnormalities since sodium retention often occurs when the glomerular filtration rate is normal or increased (23,84). The weight of evidence suggests that renal sodium retention accompanying cirrhosis is attributable primarily to enhanced tubular reabsorption, rather than to alterations in filtered load of sodium (23,81-84). Yet the precise nephron sites involved are the subject of controversy (23,77,83-85). Mediators of enhanced tubular reabsorption of sodium, and their relative importance, have not been fully identified. Possible mechanisms responsible include an increase in the activity of the renin-angiotensin-aldosterone system, abnormalities in renal prostaglandin synthesis and the renal kallikrein-kinin system, alterations in intra-renal blood flow distribution, an increase in sympathetic nervous

system activity, and a deficiency of, or renal resistance to a putative circulating natriuretic factor.

1.4.1 Hyperaldosteronism

Several investigators documented increased levels of aldosterone in the urine and plasma of patients with cirrhosis (73,76-78). Increased secretion of aldosterone by the adrenal gland was also demonstrated (73,77). Wilkinson et al (76), reported a hyperbolic relationship between the plasma level and urinary excretion of aldosterone and an inverse relationship between plasma aldosterone and urine sodium excretion. These findings led them to suggest that aldosterone may be a major determinant of sodium retention in cirrhosis and that there may be increased renal tubular sensitivity to aldosterone in cirrhosis.

Other investigators found a dissociation between urine sodium excretion and plasma aldosterone, suggesting that other factors may play an important role in sodium excretion in cirrhosis (73,77,86). Thus, renal sodium excretion has been shown to vary independently of plasma aldosterone in patients undergoing spontaneous diuresis (73,77), and after chronic oral loading of sodium (86). Water immersion caused a prompt and marked (66%) suppression of plasma aldosterone in 15 of 16 patients with cirrhosis (77). Although there was a similar degree of aldosterone suppression in all patients, there was no natriuretic response in 8. These data suggest that aldosterone may not be the primary or only determinant of impaired sodium excretion in cirrhosis. Results of further studies with the immersion model complement this conclusion: the administration of spironolactone (an aldosterone

antagonist) without immersion results in only a modest increase in sodium excretion, but increases dramatically when immersion is carried out during chronic administration of spironolactone (50). This finding suggests that the major contribution to natriuresis in these circumstances, is an enhanced delivery of filtrate, an explanation supported by the documentation of a concomitant kaliuresis and an increase in free water clearance (50). Collectively, the results from these studies suggest that hyperaldosteronism in cirrhosis may play a "permissive" role in sodium retention, by modifying the natriuretic effect of another factor or mechanism.

1.4.2 Renal prostaglandins

Alterations in prostaglandin release correlate with the natriuretic response to extracellular fluid volume expansion in normal man (87). This observation suggests that changes in prostaglandin synthesis may contribute to derangements in renal sodium handling. Inhibitors of prostaglandin synthetase (indomethacin and ibuprofen) have been shown to cause significant decrements in glomerular filtration rate and renal plasma flow in patients with alcoholic liver disease and to alter renal haemodynamics (88,89). These decrements vary directly with the degree of sodium retention induced, suggesting that the production of renal prostaglandins may serve a protective role on renal function, by preserving normal renal haemodynamics (89).

1.4.3 Kallikrein-kinin system

Zipser et al (90), reported a reduction in urinary kallikrein in patients with cirrhosis with or without ascites, which was independent of plasma aldosterone and urinary prostaglandin E excretion, suggesting

reduced activity of the renal kallikrein-kinin system in cirrhosis. Since bradykinin is believed to be a physiological renal vasodilator, it is possible that reduced bradykinin formation may contribute to the renal cortical vasoconstriction which has been documented in patients with cirrhotic ascites (88-91).

1.4.4 Sympathetic nervous system

It is well established that alterations in the input of cardiopulmonary receptors induce changes in renal sympathetic activity (92,93). Thus a decrease in blood volume can alter the afferent input from the cardiopulmonary receptors with a resultant increase in efferent sympathetic nervous activity (92-94). An increase in sympathetic tone could contribute to the anti-natriuresis of cirrhosis, by effecting a redistribution of blood flow within the kidney, leading to increased net reabsorption of filtrate (91). In addition, studies by Di Bona (95) provided evidence for a direct tubular effect of the renal sympathetic nerves on sodium handling, in the absence of alterations in renal haemodynamics. Thus, increased renal sympathetic activity due to diminished "effective" volume in cirrhosis, may contribute to sodium retention. Further evidence for enhanced sympathetic activity in cirrhosis was furnished by Nicholls et al (55), who reported increased concentrations of circulating catecholamines (with evidence for enhanced secretion and a normal metabolism) and by Bichet et al (53), who reported a correlation between circulating catecholamines and impaired sodium handling in cirrhosis.

1.5 Circulating natriuretic factors

A deficiency of a putative circulating natriuretic factor or defective function of such a factor, was proposed by Borst and de Vries, as a possible contributory mechanism to sodium retention in cirrhosis, as early as 1950 (17). Since then evidence has been accumulated, indicating that in healthy subjects, a circulating natriuretic factor or factors, depress renal tubular reabsorption in response to extracellular fluid volume expansion (96,97). It has been postulated that a deficiency or resistance to the natriuretic action of such factors may contribute to sodium retention in cirrhosis (82,83). Based on studies of mineralocorticoid "escape" in patients with cirrhosis, in which "escape" occurred in only a proportion of patients despite uniform suppression of the renin-aldosterone system, Wilkinson et al (83) postulated that the phenomenon of mineralocorticoid "escape", might be explained by an appropriate response to volume expansion by a putative natriuretic factor, and went on to suggest that "non-escapers" might be deficient in such a factor. In support of this explanation was a failure to induce natriuresis in rats by injection of urine from patients who had not "escaped", in contrast to a marked natriuresis induced after injection of urine from patients who had "escaped" (98).

1.5.1 Digoxin-like Immunoreactive Substance

For about 2 decades the existence of a circulating natriuretic substance with digoxin-like immunoreactivity, with vasoactive and Na/K ATPase inhibiting properties, has been postulated (22,97-99). The stimulus for its release appears to be volume expansion (97-99). It was hypothesised that a deficiency of this substance was relevant to fluid

and electrolyte disturbances in chronic liver disease (82,83,100). The inability to characterise satisfactorily the nature of this putative natriuretic hormone, complicates efforts to elucidate its role (101). Levels of digoxin-like immunoreactive substances (DGLIS), as measured by cross-reactivity to digoxin antibody, have been found to be high in patients with cirrhosis and ascites. Serum concentration of DGLIS correlates inversely with urine sodium excretion (102), a finding that would be compatible with resistance to its action rather than a deficiency. However, the interpretation of data on DGLIS in liver disease is complicated by the observation that its levels are raised in cholestasis without liver disease (103), and that bile acids, which account for almost 80% of the digoxin-like immunoreactivity found in bile as measured by one radio-immunoassay (104), can inhibit the activity of the Na/K ATPase (105).

1.5.2 Atrial Natriuretic Peptide

1.5.2 (a). Identification and structure

As early as 1847 the heart was attributed an important role in volume regulation, by inducing diuresis following the volume loading of water immersion (106) "... if the blood be thus driven from the external and internal parts, what becomes of the blood ? The heart and great vessels, it would seem, must be burdened. Such is to a degree the case; and it is perhaps the stimulus of this fullness and distension or its action on the elasticity of those great vessels and the heart that constitutes the reaction which leads forth the urine in abundant effusion". More than 100 years later, in 1956 Henry and Gauer demonstrated that it was distension of the atria which enhanced water

and sodium excretion upon an increase in central blood volume (107,108). In the same year, independently and unaware of physiologic research, B. Kisch, using electron microscopy, documented the presence of multiple dense granules in the cytoplasm of atrial muscle cells (109). In 1976 Marie et al (110), demonstrated that the degree of granularity of atrial myocytes was lower with increased dietary sodium and higher with sodium restriction. The connection between the physiological findings and these morphological features was not made until 1981, when de Bold reported a striking diuretic (and diuretic) activity when homogenates from rat atria were administered intravenously to control rats (111). Within 3 years of this observation, the atrial natriuretic peptide (ANP) was identified (112-118).

The process of biosynthesis of ANP in the cardiac atria resembles that of most secreted peptides: transcribed messenger RNA is translated to a 151-amino acid pre-prohormone ANP-151 with a hydrophobic signal peptide at the N-terminus, which is thought to expedite transport across the endoplasmic reticulum. Subsequent to cleavage of the signal peptide, the prohormone (ANP-126) is stored in secretory vesicles. ANP-126 is further processed enzymatically and secreted into the blood, where the biologically active C-terminal ANP 1-28 (or α-1-28 human ANP), has been defined as the predominant circulating hormone in man (113,118,119). Besides ANP 1-28 small amounts of β-1-56 human ANP (a dimer of α-1-28 human ANP) and γ-1-126 human ANP (the prohormone) may be present in the circulation (120,121). It was reported recently, that the pro-ANP gene may also be expressed in ventricular myocytes in ventricular hypertrophy and can be induced by the administration of dexamethasone (122,123).

Ventricular synthesis of ANP is active during fetal life, but practically disappears at birth (122). Although it can be activated experimentally, its contribution to the total secretion in the adult appears to be minimal (123). ANP 1-28 posesses a cysteine-cysteine disulfide cross-link, forming a 17-residue ring (Fig. 1), which is essential for biological activity (124). The estimated plasma elimination half-life of ANP is 2½ to 3 minutes (125). The circulating form in the rat also comprises 28 amino acids, with substitution of methionine by isoleucine at position 12 (124). All fully active peptides carry the C-terminal sequence -Phe-Arg-Tyr. Manipulation of the N-terminal extension, does not substantially alter the bioactivity of the peptide (126).

1.5.2 (b). Biological actions

Although the distribution of ANP binding sites is widespread (127), the kidney, blood vessels, adrenal cortex and the central nervous system have been identified as important target organs.

Kidney

Intravenous injection of synthetic ANP in animals or perfused kidneys causes a marked natriuresis and diuresis, with phosphaturia and magnesiuria (128), while potassium excretion is not much affected (129). Similar effects have been reported in human volunteers (130-134). Although the mechanism of ANP-induced natriuresis remains unclear, it seems unlikely that it is mediated by direct inhibition of the sodium pump. Several studies emphasize the importance of haemodynamic changes (135,136), with increased medullary blood flow and dilation of afferent and constriction of efferent glomerular arterioles (137), which

Fig. 1

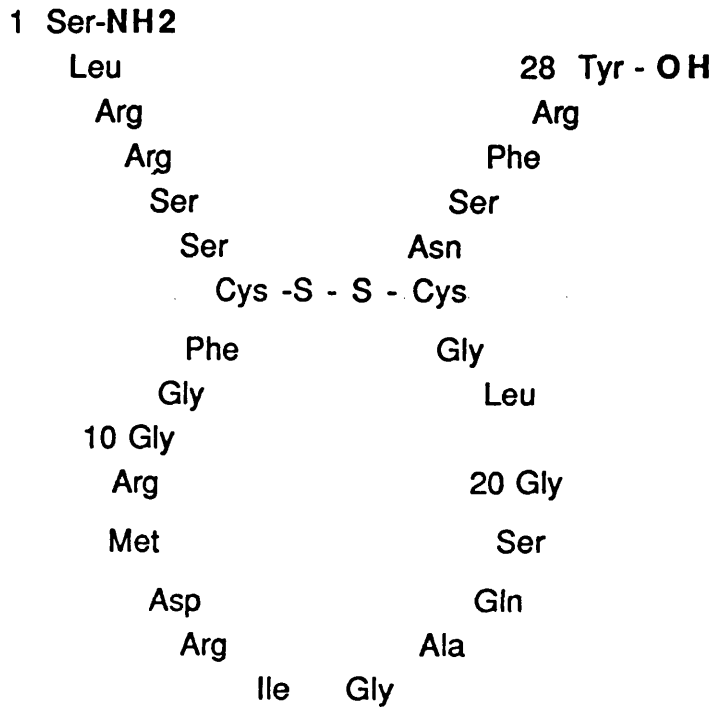


Fig. 1. Primary structure of human α-1-28 ANP.

result in increased filtration pressure. Permeability of glomerular capillaries may also be enhanced (138). A second site of action is the inner medullary collecting duct, where sodium reabsorption is inhibited by ANP (139). In addition, ANP has been shown to inhibit the effect of vasopressin on renal water reabsorption (140). This evidence is in keeping with results of studies of receptor distribution. Specific ANP receptors have been localised in the glomeruli, vasa recta and medullary collecting duct (141-143). ANP-induced stimulation of cyclic guanosine monophosphate (cGMP) production, which is believed to be the second messenger of its action (144), exhibits the same distribution within the nephron (145,146). The early studies on renal effects of ANP were performed with crude atrial extracts or with synthetic ANP at concentrations that were difficult to evaluate, because circulating blood levels could not be measured at that time. This led to some uncertainty as to whether ANP was physiologically important (147). A physiological role for ANP is supported by the following considerations: Infusion of ANP closely mimics the renal response to atrial distension, although there are understandable differences in the systemic haemodynamic response (148). Inactivation of circulating ANP by infusion of specific anti-ANP antibodies decreases diuresis, even at normal, non-stimulated plasma levels of ANP(148a). Further evidence in support of a physiological role for ANP came in 1987, from a study by Anderson et al (149), who reported significant natriuresis during a low rate of infusion of ANP, in which it was ascertained that the circulating concentration of ANP did not exceed the upper normal range.

Blood vessels

Relaxation of pre-contracted arteries has been used as one of the methods of bioassay during the original isolation of ANP (150). This endothelium independent effect requires binding of ANP to specific receptors of vascular smooth muscle cells (151,152). Activation of particulate guanylate cyclase and stimulated cGMP production probably mediates the vasorelaxing action (153). In its vascular effects ANP shows a relative selectivity to different vascular beds. *In-vivo,* the most pronounced changes have been seen in renal and splachnic regional blood flow (154,155). Present evidence with regard to the role of decreased peripheral resistance in the blood pressure lowering effect is controversial. Most studies have ascribed greater importance to the diminished cardiac output due to decreased venous return, rather than to a direct cardio-inhibitory effect (156,157). Infusion of ANP causes a comparable reduction in blood volume in nephrectomised and in normal rats (158,159), suggesting a fluid shift from the intravascular to the extravascular space, due to a direct effect on capillary permeability. Early studies showed that at pharmacological doses, ANP causes hypotension (155-157). More recently, Bolli et al (160), demonstrated the vasodilatory effect of ANP at concentrations within the normal range.

Adrenal cortex

The complexity of the involvement of ANP in body fluid homeostasis, is documented by its inhibitory action on aldosterone synthesis and secretion by cortical cells (161). This effect is receptor mediated and seems to be limited to the zona glomerulosa (162). ANP inhibits basal, and angiotensin II and ACTH stimulated aldosterone secretion, in a dose-

related manner (163,164). In addition to its direct suppressor effect on the secretion of aldosterone, ANP appears to suppress aldosterone indirectly, by decreasing plasma renin activity as shown by studies of *in-vivo* infusion of ANP in dogs (165). The suppression of renin release from juxtaglomerular cells incubated with ANP shows that this effect, is at least in part, direct (166).

It is unlikely that the inhibitory effect of ANP on aldosterone secretion is involved in the rapid natriuresis after ANP infusion, because the aldosterone effect on renal sodium handling occurs after a delay of 30-60 minutes. On the other hand, this mechanism may play a role in long-term homeostasis.

Central nervous system

The presence of ANP and its binding sites in the brain, evidenced by autoradiography (167), immunohistochemistry (168,169) and radio-immunoassay (170), suggests that it may have potential neuromodulator activity. Intracerebral infusion of ANP in the rat reduces both spontaneous and dehydration-induced or angiotensin II induced water intake (171,172). The multiple roles of ANP in fluid homeostasis are illustrated by its inhibitory effect on vasopressin release, which has been observed both *in-vitro*, with the hypothalamo-neurohypophyseal preparation (173,174) and *in-vivo*, when ANP was administered into the cerebral ventricles (174). Systemic administration of ANP does not seem to affect plasma vasopressin concentration (129).

1.5.2 (c). Control of ANP synthesis and secretion

It is now generally accepted that the immediate stimulus for ANP release is the excitation of atrial stretch receptors by distension of

the atrial wall, and this may be due to direct mechanical stretch (175), high perfusion pressure (176), or acute volume expansion (40). The majority of the peptide is released into the coronary sinus (178). The effect of most proceedures stimulating ANP release seems to be mediated by an increase in central blood volume and there is a close correlation between the plasma concentration of ANP and both the right and left atrial pressure (179,180). Increased dietary sodium intake (181) and infusion of saline (181,182), have been shown to stimulate ANP release in healthy volunteers. Water immersion, which increases central venous pressure by shifting extracellular fluid volume to the intra-thoracic venous bed, causes a prompt rise in plasma ANP concentration in healthy volunteers (183-185). Confirmatory evidence that stretch is the immediate stimulus for ANP release, comes from a study in which plasma ANP concentrations were measured before and during the relief of cardiac tamponade: plasma concentration of ANP increased as tamponade was relieved, in parallel with a drop in atrial pressure (186).

The role of other factors in ANP release is not clear. Pharmacological concentrations of catecholamines stimulate ANP release *in-vivo* (187), but in man such an effect was not observed during marked adrenergic discharge precipitated by insulin hypoglycaemia (188), pointing against a major role of sympathetic mechanisms in the release of ANP, under physiological conditions. Another possibility is that the adeno-hypophyseal axis may be involved in the regulation of ANP release. In rats, hypophysectomy resulted in a blunted ANP response to hypervolaemia, which was restored after re-implantation of the adenohypophysis (189).

1.5.2 (d). Clearance of ANP from the circulation

Clearance studies in man have shown that ANP is removed rapidly from the circulation, with a plasma elimination half-life of 2.5-3.0 minutes (125). The kidney is a major site of its metabolism (190). Recent experimental studies have clarified the nature of the renal clearance of ANP. It is apparent that, in addition to the importance of the kidney as a target organ, the kidney itself may have a profound influence on the clearance of ANP from the circulation. Maack et al (191), have shown that in the rat kidney more than 95% of ANP receptors are biologically silent, in that ANP binding occurs without a functional response or cGMP generation. They have termed these binding sites "clearance receptors". Blockade of these receptors by a ring-deleted analogue markedly increased plasma ANP concentration, suggesting an important clearance role for these receptors (191). The majority of ANP clearance is non-enzymatic, as indicated by the observation that ANP metabolites appear in the rat circulation only after 90% of the hormone is already cleared (192). Enzymatic breakdown of ANP is mainly by the kidney neutral endopeptidase 24.11, which has been shown to degrade ANP to inactive metabolites, *in-vitro*, with a high degree of specificity (193). Inhibition of neutral endopeptidase has been shown to prevent breakdown of ANP both *in-vitro* and *in-vivo*. More recently, infusion of an atriopeptidase inhibitor has been shown to raise circulating ANP concentration, with an increase in natriuresis, in normal volunteers and patients with heart failure (195).

Concentrations of plasma ANP increase with age in normal subjects (196) and are raised in various pathological situations: Congestive

cardiac failure (179), chronic anuric renal failure (197), essential hypertension (198) and during episodes of supraventricular tachycardia (199). In supraventricular tachycardia, plasma ANP correlates with atrial pressure, but not with heart rate (199,200), thus providing further evidence in support of a primary role for the former in the release of ANP.

At the time of commencing the work for the present thesis, in the second half of 1985, there had been no published reports on circulating concentrations of ANP in cirrhosis.

Chapter 2

METHODS

2.1 Radioimmunoassay for atrial natriuretic peptide

2.1.1 Introduction

Quantitative measurement of ANP has been a pre-requisite of the rapid progress that has occurred in this field. Two types of assay have been used, bioassays and competitive binding assays. Bioassay, either by measuring an *in-vivo* diuretic response or *in-vitro* vasorelaxation methods, was a valuable tool in the original isolation of atrial peptides and was used to quantitate bioactive ANP in atrial tissue and its release into incubation media (201). Bioassay did not prove sensitive enough to measure plasma concentrations of ANP. Competitive binding assays have been used extensively to measure ANP. Various radioimmunoassays have been described in the literature. The radioceptor assay, established in a few laboratories, is based either on the rat glomerular ANP receptors (202), or ANP receptors isolated from bovine adrenal cortex (203). When the plasma levels obtained by radioceptor assay and radioimmunoassay are compared, their correlation is strong (202,204). The radioceptor assay has the advantage of measuring only bioactive forms of ANP. Enzyme immunoassay, developed recently (205), uses acetyl-cholinesterase coupled to ANP as the labelled competitor and yields a comparable sensitivity to radioimmunoassay.

For the purposes of the present thesis, it was decided to use a straight forward equilibrium radioimmunoassay, since these tend to be robust and reliable, provided that a sufficiently sensitive antibody can be raised. The radioimmunoassay and the antibody used in the studies described here, was developed and validated in Professor SR Bloom's laboratory, at the Hammersmith Hospital.

Since the majority of the experimental work envisaged was to be concerned with human studies, the human 28 amino acid peptide (ANP 99-126), was used exclusively to manufacture both the radiolabel and antisera. The amino acid sequence of the human and rat peptides differ only by one amino acid and this takes the form of a "conservative" substitution of isoleucine for methionine (124). It has been shown that the the antiserum raised against the human peptide cross-reacts with with rat ANP, so allowing the radioimmunoassay which uses this antiserum to measure both human and rat ANP.

Iodine isotopes (^{125}I and ^{131}I) are most frequently used to radiolabel peptides since they are relatively easy to link covalently with tyrosine or histidine amino acid residues. ^{125}I is used far more widely than ^{131}I for two reasons. Its longer half life of 60 days (compared with 8 days for ^{131}I) allows several months to elapse between the preparation of one batch of labelled peptide and the next. The relatively low penetrative energy of the gamma radiation emitted by ^{125}I allows more efficient counting than the higher energy radiation emitted by ^{131}I. For these reasons ^{125}I was used to manufacture the radiolabel in the present studies.

A variety of methods exist for chemical linking of iodine to peptides. The use of oxidation by chloramine T (n-chloro-p-methyl benzene sulphonamide) (206) is one such method. In this reaction ^{125}I is oxidised by the chloramine T to $^{125}I^{+}$. This highly reactive radical binds to the aromatic ring of any tyrosine amino acids in the peptide.

The reaction is swift and is terminated by adding a reducing agent which destroys both the unreacted cloramine T and free $^{125}I^{+}$ ions. Alpha hANP has a single tyrosine moiety at the carboxyterminus and is thus well suited to such iodination.

All radioimmunoassays rely on the efficient separation of the free radiolabelled peptide from that bound to the antiserum at the end of the assay incubation period. Many methods can be used to undertake this separation. Coated charcoal separation (207) is a convenient, inexpensive method that is widely used. Wood charcoal is a non-specific adsorbent with many pores of about 60μ in diameter. An excess of dextran blocks the larger pores, so that the antigen-antibody complexes are too large to enter the charcoal granules, but free peptide does, becoming adsorbed onto the charcoal. This method of separation was used throughout the present work.

2.1.2 Method and Materials

(a). Antiserum

The antiserum used in the work described in this thesis was raised in Professor SR Bloom's laboratory, at the Hammersmith Hospital, in a lop-eared rabbit. The animal was immunised against synthetic alpha (1-28) human-ANP (Peninsula Laboratories), coupled to bovine serum albumin (208,209), in an emulsion of Freund's complete adjuvant (210-212). The antiserum (Y2), was shown to bind specifically to ANP, with no cross-reactivity against other vasoactive substances, including arginine vasopressin, synthetic ACTH (Synacthen), vasoactive intestinal polypeptide, glucagon, substance P, neurotensin and neuropeptide. The avidity constant of the antiserum is 2.7×10^{11} l/mol. Fragment binding

studies showed that the antiserum appeared to bind to the central portion of the ANP molecule preferentially (210).

(b). Iodination of ANP

Alpha h-ANP (6 nmol dissolved in 50µl of 0.1 M phosphate buffer) was iodinated using a modification of the chloramine T oxidation proceedure (206), with 0.4 nmol (approximately 1 mCi) of Na ^{125}I (IMS 30;Amersham International) in 10µl of diluent and 26 nmol of chloramine T in 25 µl of phosphate buffer. The reactants were mixed by bubbling air through the mixture. The reaction was terminated after 20 seconds by the addition of 6.3µmol of cysteine in phosphate buffer. Very mild reaction conditions were chosen to minimise oxidation of the single methionine residue. Furthermore an excess of alpha h-ANP was used in the reaction to ensure that the proportion of iodinated molecules which were also oxidised was as low as possible. Radiolabelled ANP was purified from the iodination reaction products by reverse phase high pressure liquid chromatography (HPLC) using a Techsil 5 micron column pre-equilibrated with 10% (v/v) acetonitrile in water containing 0.05% (v/v) trifluoroacetic acid. The column was eluted with this solvent mixture for 40 minutes followed by further elution with 27% (v/v) acetonitrile in water containing 0.05% (v/v) trifluoroacetic acid. The flow rate was 1 ml per minute. One ml fractions were collected and the radioactivity of each fraction was determined. The fractions of peak radioactivity were tested for immunoreactivity, pooled and diluted in a solution containing 10% lactose, 2.5% bovine serum albumin, 6mM cysteine hydrogen chloride and 0.01 M citric acid in 0.5 M acetic acid. This was then aliquoted, lyophilised, sealed in vacuo and stored at -20°C in the dark.

(c). Assay conditions

Assays were set up in duplicate polystyrene tubes. The buffer used was 0.06 M sodium potassium hydrogen phosphate buffer containing 7.7 mM sodium azide and 0.3% bovine serum albumin by weight, plus 10 mM EDTA. The final assay volume was 800µl.

After incubation at 4°C for 4 days, the antibody bound peptide was separated from free peptide by the addition of 4 mg of charcoal (Norit GSX, BDH Chemicals) together with 400 µg of dextran (Sigma Chemical Co) in 250 µl of assay buffer. The supernatant (containing peptide bound to antibody) was separated from the charcoal pellet (containing free peptide) after centrifugation at 800 x G at 4°C for 20 minutes. The bound and free fractions were counted on a gamma radiation counter (Nuclear Enterprises, NE 1600).

The maximum sensitivity of the assay was calculated by determining the smallest quantity of cold peptide that produced a change of the percentage of radioactivity bound to antibody that was greater than two standard deviations of the variation of the binding in the absence of cold peptide (zero standard).

2.1.3 Assay of α-1-28 ANP in human plasma

The following proceedures were used throughout the work for this thesis:

(i). Collection of blood samples, separation of plasma and storage

Blood samples were collected into 5 ml glass tubes containing 30mg of potassium EDTA. These tubes were stored in crushed ice for not more than 10 minutes before centrifuging at 800G for 8 minutes, at 4°C. The plasma was separated into polystyrene screw top pots and frozen on solid

carbon dioxide. The frozen samples were stored at -70°C until the time of assay.

(ii). Extraction of human plasma

Extraction under high ionic strength conditions, such as when plasma is dissolved in guanidine hydrochloride prior to extraction, increase the mean recovery of ANP-immunoreactivity by 12 per cent, as compared to "straight" plasma extraction (213). Hence, the following proceedure was adopted: Plasma samples were thawed and aliquots of 3 ml were added to 2g guanidine hydrochloride (Sigma Chemical Co., Poole, Dorset) and were then passed through a Sep-pak C-18 cartridge (Waters Millipore Ltd, Harrow, Middx) followed by 10ml water containing 0.05% (v/v) trifluoro-acetic acid. The retained peptides were then eluted from each cartridge into a polyethylene tube with 2 ml 60% (v/v) trifluoroacetic acid. The eluates were evaporated to dryness in a vacuum centrifuge and the resultant pellet reconstituted in 1.2 ml sodium potassium hydrogen phosphate buffer (60 mmol/l, pH 7.4) containing 0.3% (w/v) bovine serum albumin and 10 mmol/l EDTA (assay buffer).

Rat plasma was treated similarly. 100µl was first made up to 1 ml with 0.9% saline solution. The extraction method was identical to that used for human plasma except that the pellet was reconstituted in only 250µl of buffer.

(iii). Radioimmunoassay

The antiserum was used at a final dilution of 1:13 000 in an assay volume of 800µl buffer. Paired tubes containing 400µl reconstituted human plasma extract were assayed in duplicate. If high plasma concentrations were expected 100µl and 25µl aliquots of the extract were

Fig. 2

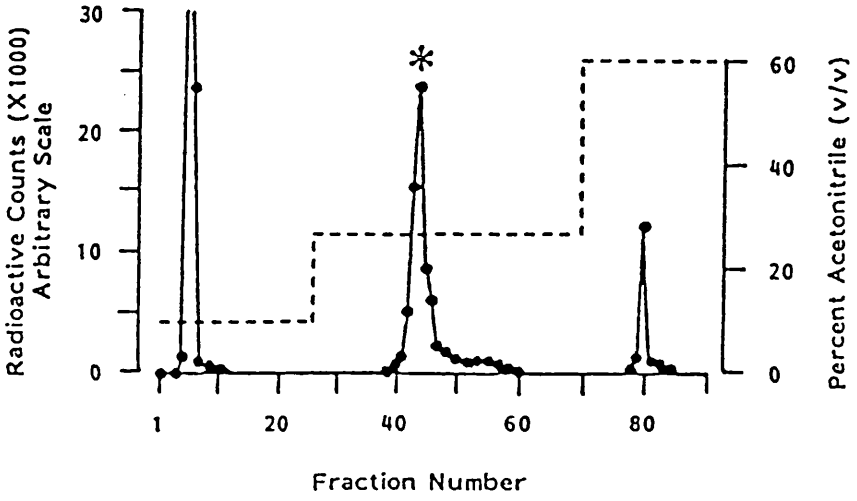


Fig. 2. Profile of radioactivity in the eluates from the HPLC purification of the iodination reaction products. Position of the immunoreactive peak marked *.

assayed in duplicate, in addition to the standard 400µl aliquots. Rat plasma samples were assayed in duplicate in 50, 25 and 25µl aliquots. Pure synthetic human α-1-28 ANP was used as standard. Recovery of synthetic human α-1-28 ANP added to plasma samples was 79±2% (mean±SEM, n=10). Assay sensitivity was 1 fmol per tube (equivalent to 1 pmol/l in plasma). The intra-assay variation was 8% and inter-assay variation 12%. No correction for extraction losses is made to the data presented throughout this thesis.

2.1.4 Results

Iodination of alpha h-ANP

The iodinated peptide was eluted from the HPLC column by a concentration of 27% (v/v) acetonitrile (Fig. 2). The specific activity of the radiolabel was 30.2 Bq/fmol (taking the efficiency of gamma counters as 70%). Its binding to excess antibody was 88-91% (n=10). In the absence of antibody (blank assay control) the apparent binding was 1.9-3.6% (n=10).

Validation of radioimmunoassay for rat and human plasma

Validation of the assay was carried out by the demonstration of parallelism on dilution, between plasma extracts and either pure synthetic human α-1-28 ANP or rat 1-28 ANP (Sigma Chemical Co.)(Fig. 3). In addition, gel chromatography and HPLC were performed on pooled plasma extracts from 30 normal subjects, 15 patients with chronic anuric renal failure and 10 subjects with congestive cardiac failure. Pooled samples from 5 lightly anaesthetised hypervolaemic rats were also used.

Fig. 3

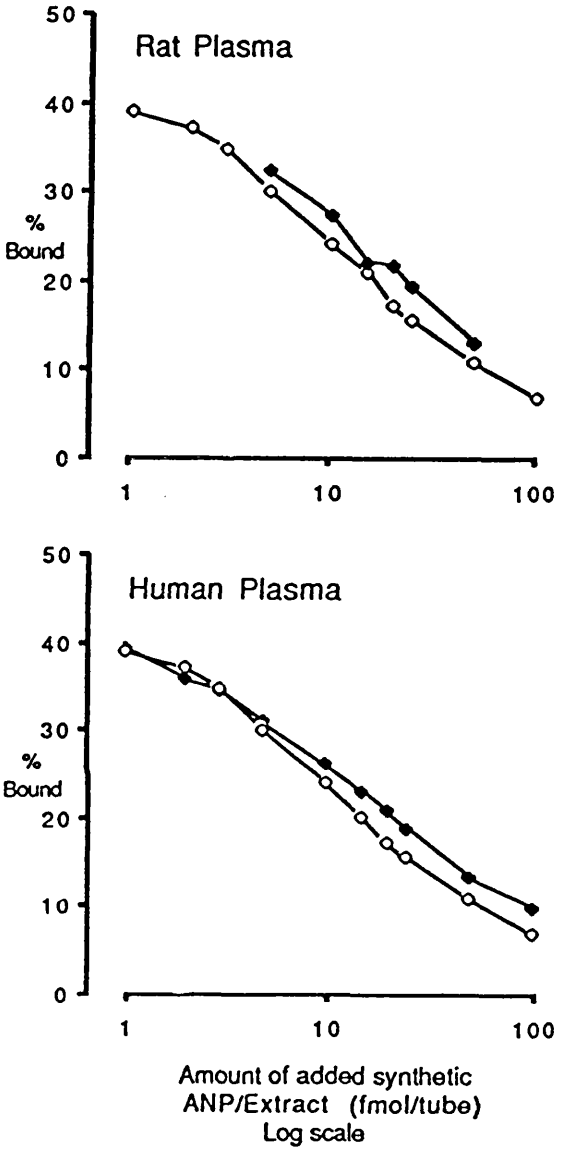
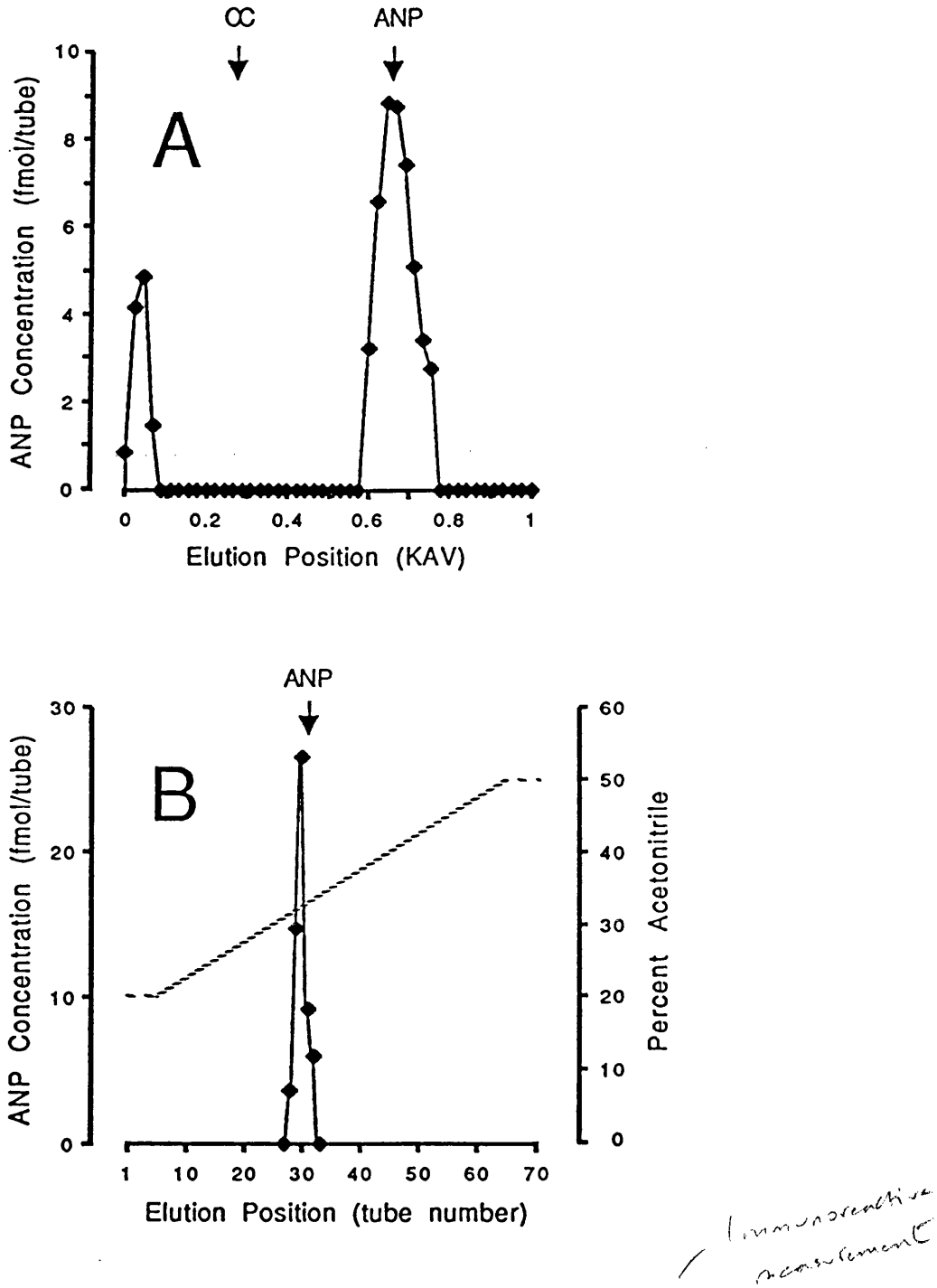


Fig. 3. Effect of serial dilution of hypervolaemic rat and human plasma extract on radioimmunoassay binding. Solid dots indicate the binding produced by the plasma-derived immuno-reactivity and open dots that of standard rat and human α 1-28 ANP, as appropriate.

Fig. 4



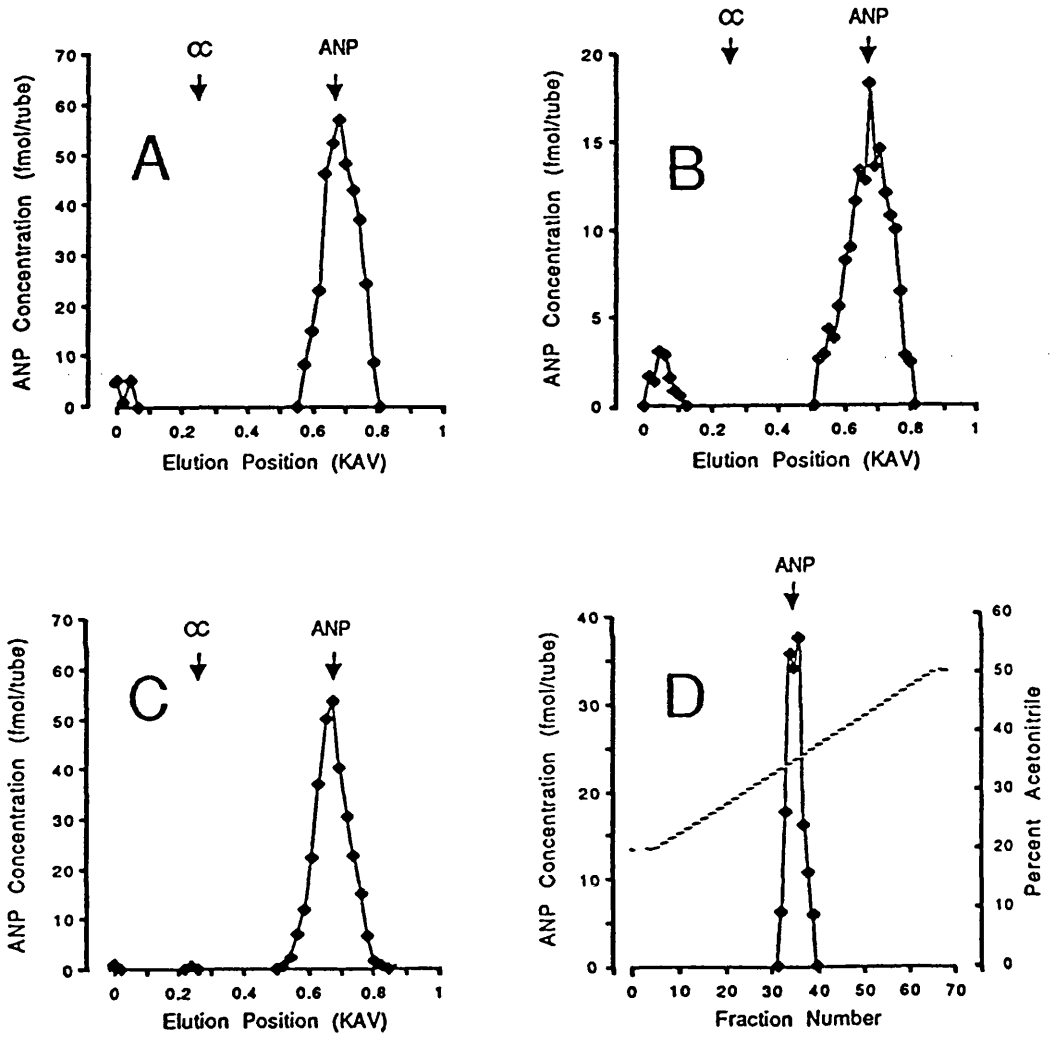
Sephadex G-50 gel permeation chromatographic profile (A) of rat plasma obtained after 50% acute blood volume expansion. The lower panel illustrates a high pressure liquid chromatographic (HPLC) profile (B) of similar plasma. Arrows indicate the elution positions of horse heart cytochrome C (CC) - used as as internal marker of molecular size - and of pure synthetic alpha rat 1-28 atrial natriuretic peptide (ANP). The dashed lines indicate the varying concentration of acetonitrile used to elute the HPLC columns.

Fractionation of ANP-like immunoreactivity in plasma extracts was undertaken on a Sephadex G-50 gel permeation column and by High Performance Liquid Chromatography. With each run of the gel-column, dextran blue, cytochrome C and Na ^{125}I were included as markers of molecular size and the void volume. The gel column was calibrated with synthetic rat 1-28 ANP and its elution position determined by radioimmmunoassay.

HPLC was performed with a Techsil C-18 reverse phase column (3.9x300mm, HPLC Technology Ltd). The column was eluted at a flow rate of 1 ml per minute and equilibrated with 20% acetonitrile in water containing 0.1% trifluoroacetic acid by volume. Following the addition of a sample the column was eluted with 20% acetonitrile in water containing 0.1% trifluoroacetic acid for 5 minutes and then by a linear gradient of 20% to 60% over 40 minutes. One ml fractions were collected. Synthetic rat 1-28 ANP was used as a standard marker in runs before and after the samples.

The majority of the ANP-immunoreactivity in rat plasma co-eluted with the synthetic rat standard both on G50 gel chromatography and on HPLC (Fig. 4). The ANP-immuno-reactivity in the pooled human plasma extracts also co-eluted with the synthetic human standard on both gel chromatography and on HPLC (Fig. 5). The source of the plasma (normal subjects, patients with renal failure or patients with congestive heart failure) did not appear to affect the chromatographic properties of the circulating form of the peptide.

Fig. 5



Sephadex G-50 gel (A,B,C) and high pressure liquid chromatograpy (D) chromatographic profiles of various pooled human plasma extracts. (A) Normal subjects' plasma extracts. (B) Renal failure patients' plasma extracts. (C&D) Congestive heart failure patients' extracts. The elution positions of horse heart cytochrome C (CC) - used as as internal marker of molecular size - and of pure synthetic alpha human 1-28 atrial natriuretic peptide (ANP) are indicated by the arrows. The dashed lines indicate the varying concentration of acetonitrile used to elute the HPLC columns).

2.1.5 Discussion

The above data indicate that this radioimmunoassay is specific and sensitive for alpha (1-28) h-ANP. The mild oxidation conditions used during iodination enabled the radiolabel to be purified in one stage by HPLC and avoided problems that appear to have been encountered by others (213) during the iodination of ANP.

On the basis of studies performed with ANP fragments, the antiserum appeared to bind to the central part of the ANP molecule. However, cross-reactivity data derived using peptide fragments must be interpreted with caution since a disulphide bridge within the peptide molecule suggests that it might have a folded tertiary structure (210) and the folding of the fragments may differ from that of the parent molecule.

It is important that the immunoreactivity measured by any radioimmunoassay is shown to have similar and binding properties to the synthetic substance used as standard. The ANP-immunoreactivity in plasma extracts not only exhibited parallel displacement on dilution with the standard but also co-eluted on gel chromatography and on HPLC with the standard. The similar results with extracts from the plasma of renal failure and heart failure patients implied that the extraction method could be used to measure circulating concentrations in pathological circumstances.

Many variants of the method of radioimmunoassay for ANP have been described. Consequently reported normal values in human plasma vary from as low as 1pmol/l (216) to as high as 60 pmol/l (217). A number of

studies have shown that direct radioimmunoassay on unextracted plasma introduces a gross positive bias to the results, as compared to those from assays using plasma which has been extracted prior to assay (197, 218, 219). The interference in the direct radioimmunoassay is probably due to a substance or substances that interfere with binding between the antiserum and the radiolabel during incubation which perhaps cause deiodination of the radiolabel itself (220), thus decreasing its availability and causing spurious inhibition of binding. Most laboratories that have incorporated an extraction step in their proceedures, report normal ranges ranging from 1-20 pmol/l (181,183,185,198,215,220). The normal range established for the present method ranges from 1 to 12 pmol/l (133,149,182,183,215).

2.2 Administration of a severely restricted sodium diet to healthy volunteers

2.2.1 Introduction

All subjects with chronic liver disease and sodium retention included in the present thesis were taking a severely restricted sodium diet as part of routine therapy. This was administered by the hospital diet kitchen, under strict supervision by the Unit dietitian. For comparison, it was necessary to include healthy controls, on a similar, severely restricted sodium intake. For the purpose of the present work, I had no access to a metabolic or diet kitchen, to provide meals for healthy volunteers. For this reason it was necessary to administer the diet to these subjects on an outpatient basis. Counselling methods based on help from dietitians, have been used

successfully to achieve mild to moderate sodium restriction (60-100 mmol daily) on an outpatient basis (221-226). For those studies in which severe sodium restriction (10-22 mmol daily) is necessary it has been considered that a metabolic kitchen is necessary to provide all meals (181,227). There were no published data evaluating the feasibility of a counselling-support method, for diets of severely restricted sodium intake (22 mmol daily) on an outpatient basis, before this study was undertaken.

2.2.2 Subjects and Methods

Thirteen healthy volunteers on no medication, eight male and five female, (age range 33-61 years, mean 47.4), all non-smokers, were recruited. Informed consent was obtained. There were seven medical or senior laboratory staff, four secretaries, one teacher and one train driver.

A 24 hour urine collection was taken while they were on their respective normal diets and following this, the subjects were instructed to follow a 22 mmol daily sodium intake diet for 5 days after having an interview with the Unit Dietitian and the author of this thesis for 30-45 minutes. During this time they were given dietary advice and detailed explanation of the sodium content of food items listed in a standard booklet based on an established computer analysis program (Diet 2000 - Dietary analysis System: B and W Electronics Systems Ltd, Southsea, Hants). Individual food preferences were discussed and the standard booklet modified accordingly. Topics covered at the initial interview included grocery shopping, food preparation, dining out and fast foods. Subjects were asked not to use salt

substitute preparations in the form of potassium chloride. Salt free bread was baked by the Unit dietitian and the subjects were asked to record in a diary all food and fluid intake during days 1-5 at or near the time of consumption, specifying standard portions and weight of food and fluid volume intake. They were encouraged to carry on their usual daily activities but to avoid strenuous exercise and during the study period all subjects were contacted daily either in person or by telephone in order to answer specific queries and ensure strict compliance with the diet. Queries were answered by referring to the computerised dietary analysis program.

Twenty four hour urine collections were carried out whilst on the normal diet and on days 4 and 5 of the low sodium diet. After an overnight fast each subject lay supine for 30 minutes in a quiet room and blood was taken without stasis at 0900 hours for electrolytes, creatinine and haematocrit; Arterial blood pressure was measured with a mercury column sphygmomanometer by the same observer (MZP) in all cases while patients remained supine (Korotkov Sounds II and V). Mean arterial pressure was determined by adding one third pulse pressure to the diastolic pressure. Subjects were weighed at the same time of day at the beginning of the study and on day 5. Sodium and potassium were measured by flame photometry and creatinine by standard autoanalyser. Dietary sodium, potassium and caloric intake for days 0 to 5 was calculated using the computer program stated above.

Statistical analysis was by the Paired't'test and results are expressed as mean$\pm$SEM.

2.2.3 Results

All subjects completed the study. Estimated mean daily sodium intake for days 1 to 5 was 17±1.26 mmol and results from 24 hour urinary collections show considerable reduction in urine sodium excretion over control values (Table 1). There was no difference in the urine sodium excretion on days 4 and 5 (20.5±3.86 v 18.09±3.82 mmol). Results shown in Table 1 on the low sodium diet represent mean values for days 4 and 5. There was no statistically significant difference in the 24 hour urinary sodium excretion between health care personnel and others. Mean arterial blood pressure was unchanged (66.5±1.75 v 66.69±2.20 mmHg). Average daily Potassium intake during the study period was 74.0±5.55 mmol. In twelve of thirteen subjects there was a mean weight loss of 1.66 kg (range 0.1-3.5) while one subject gained 0.5 kg. Mean daily caloric intake during the study was 1693.7±69.39 Calories. Two subjects experienced nocturnal leg cramps during the study. Ten subjects thought that retaining a degree of freedom in their choice of foods helped them comply with the diet although all found the diet unpalatable.

2.2.4 Discussion

The reliability of 24 hour urinary sodium excretion as a measure of sodium intake has been confirmed by numerous studies (221-224,181,228). The present study demonstrates that in small groups of highly motivated volunteers, for the purpose of short term clinical trials, severe restriction of dietary sodium can be achieved using a counselling-support method. In the present study the small rise in haematocrit, and the small decrease in plasma sodium and glomerular filtration rate as measured by creatinine clearance, provide indirect qualitative evidence

TABLE 1

Urinary sodium, potassium, creatinine clearance, plasma sodium, potassium and blood haematocrit on a normal and low sodium diet.

Diet	n	U Na mmol	UK mmol	Ccl ml/min	Pl Na mmol/l	Pl K mmol/l	Hct %
Normal salt	13	128.5 ± 11.9	72.2 ± 5.6	101.1 ± 4.6	140 ± 0.41	4.2 ± 0.15	43.1 ± 1.03
Low salt	13	19.2 ± 2.88***	6 10 ± 6.7	95.6 ± 4.6*	137 ± 0.78**	4.2 ± 0.20	44.2 ± 0.93*

Results are mean ± SEM. Statistical significance: *p <0.05, **p <0.001, ***p <0.0001.

Abbreviations
n = number of subjects
U = 24 hour urinary excretion
Na = sodium
K = potassium
Ccl = creatinine clearance
Pl = plasma
Hct = haematocrit

for the effectiveness of sodium restriction. These findings are comparable to those from an outpatient study by Solomon et al (228), in which all meals were provided by the investigators.

Weight loss, a prominent feature in most subjects, was likely to be mainly due to sodium and water depletion, and would be compatible with the observed changes in plasma sodium concentration, glomerular filtration rate and haematocrit. Although no attempt at caloric restriction was made in this study, estimated caloric intake was significantly lower than the recommended daily intake for moderately active individuals (229), and may have contributed to the weight loss observed. It is also possible that alterations in nitrogen balance may have been responsible for some of the changes observed. Such changes might have been prevented by appropriate supplementation. The success of the present method in achieving a severely restricted sodium intake was probably attributable to close monitoring of the volunteers by daily contact with the author and the Unit dietitian.

These results indicate that a 22 mmol sodium dietary restriction was achieved, on an outpatient basis. Ten of the thirteen volunteers went on to participate in studies comparing them to patients with cirrhosis and sodium retention, while the latter were on a similar diet, administered by the hospital diet kitchen.

CHAPTER 3

PLASMA ATRIAL NATRIURETIC PEPTIDE AND RENIN-ALDOSTERONE IN PATIENTS WITH CIRRHOSIS AND ASCITES

Basal levels, changes during daily activity and nocturnal diuresis.

3.1 Introduction

In normal subjects, urine volume flow and sodium excretion are subject to diurnal variation, with a reduction of both during the night (16,17). This pattern is either reversed or lost in patients with liver cirrhosis (16,17,230). In studies of patients with cirrhosis and ascites, during which patients remained supine over a 24 hour period, Bernardi et al (231,232), demonstrated a loss of the circadian pattern of plasma renin activity, aldosterone and norepinephrine concentration. Although no data on urine volume or composition were reported in these studies, the observations suggest that other factors, for instance posture (233,234) or changes of a putative humoral factor (17) may play a role in the nocturnal diuresis of cirrhosis. Studies of the effects of posture on the renin-aldosterone system and urine sodium excretion have been performed (233,234), but there have been no published studies relating these to the varying composition of urine during 24 hours of ordinary activity (as would be encountered in the clinical situation).

The observation that plasma concentrations of ANP increase in recumbency (228), its known natriuretic and diuretic properties, along with its suppressor action on the renin-aldosterone system (161-164), suggest that ANP could be involved in the phenomenon of nocturnal natriuresis and diuresis in cirrhosis. Previous studies measuring plasma concentration of the atrial natriuretic peptide (ANP) in cirrhosis, relied mainly on spot measurements (235-237). Since plasma levels of ANP are dependent on posture and the plasma elimination half-life of the peptide is only 2½-3 minutes (125),it seems essential to (a) investigate whether the circulating concentration of ANP under basal conditions

(at 0800 hours with subjects supine overnight) is representative of levels throughout a 24 hour period of ordinary activity, and (b) to relate more frequent plasma ANP estimations to the varying composition of the urine. In this study these concerns were addressed and in addition, the possible interaction between ANP and the renin-aldosterone system in the abnormal natriuretic and diuretic pattern of cirrhosis, was examined.

3.2 Subjects and Methods

3.2.1 Basal assessment

Twenty one patients with histologically proven cirrhosis (10 women, 11 men, aged 28-64, mean 51 years) and moderate to gross ascites and ten normal volunteers (5 women, 5 men, aged 33-60, mean 49 years) were studied. 19 patients were grade C (Pugh) and 2 grade B. In patients baseline mean serum bilirubin was 64±21.5 μmol/l, albumin 29.2±1.7 g/l, prothrombin time prolongation 5.5±1.0 seconds, serum creatinine 73.93±4.32 μmol/l, serum sodium 132±1.5 mmol/l and urine sodium excretion 5.1±2.03 mmol/day. All patients were hospitalised and were on a 22 mmol sodium diet. The diet was provided by the hospital diet kitchen for at least 5 days prior to entry. Diuretics were discontinued for a minimum of 6 days. Patients who gained weight in the last three days of this regime had been excluded from the study. Of 29 patients considered, 8 were excluded. A 24 hour urine collection was carried out between the fourth and fifth day of the low sodium diet for urine sodium and creatinine clearance estimation. Subjects with a history of heart failure, valvular heart disease, hypertension, cardiac arrhythmias or

thyroid disorders were excluded from the study. Patients with significant renal impairment (serum creatinine >130μmol/l), severe hyponatraemia (serum sodium < 126 mmol/l), evidence of hepatic encephalopathy or gastrointestinal bleeding in the previous four weeks were excluded. None of the subjects was receiving corticosteroid therapy, prostaglandin synthesis inhibitors, vasoactive drugs or drugs known to influence renal function.

Ten healthy volunteers were studied while on their respective normal diets and after 5 days on a 22 mmol sodium diet. The latter was administered on an out-patient basis, using a diary-counselling method, as described in Chapter 2 (238). A 24 hour urine collection was carried out while volunteers were on a normal diet and between the fourth and fifth day of the low sodium diet, for urine sodium and creatinine clearance estimation. On the same days, blood was taken after an overnight fast at 0800 hours without stasis, from a peripheral vein into chilled tubes containing EDTA, all subjects having remained supine for at least 8 hours, for ANP, plasma renin activity (PRA), aldosterone concentration (PA) and creatinine estimation. Samples were centrifuged within ten minutes of collection at 800 G for 8 minutes at 4°C and plasma, separated with care to exclude the buffy coat (219), was stored at -70°C until assay.

3.2.2 Changes during a 24 hour period

Eight ambulant patients, randomly selected from the 21 studied initially, (4 women, 4 men aged 28-61, mean 48 years) and all ten controls continued on the 22 mmol sodium diet and were investigated for

a further 24 hours. Seven patients were grade C (Pugh) and one grade B. On the day of study all subjects were asked to avoid strenuous activity, to take fluid during their waking hours at an approximate rate of 100ml per hour to a total of 1500 ml and to abstain from a specific list of alcohol and caffeine or xanthine containing beverages. All subjects were asked to remain mobile and to carry on their normal daily activities, to retire to bed at 2300 hours and to remain supine in bed (1 pillow) until 0800 the next day.

At 0800 hours a butterfly cannula (18G) was introduced into a peripheral vein of either forearm and was secured for the duration of the study. The cannula was maintained patent by injecting 100 units of heparin dissolved in 10ml of normal saline through the cannula, after each sample was taken. The first sample was taken at 0800 hours after an overnight fast with subjects having been supine for nine hours. Blood samples were then taken at 4 hourly intervals, within 10 minutes of 1200, 1600, 2000, 2400, 0400, and 0800 hours (7 time points), before meals and after each subject had been in the supine position for thirty minutes. After the final sample at 0800h subjects were asked to rise and to engage in non-strenuous activity and a further sample was taken at 0900h with subjects sitting quietly for 5 minutes. Blood was taken for plasma sodium, haematocrit, plasma atrial natriuretic peptide and renin-aldosterone estimations. Pulse and arterial pressure was recorded immediately before each venesection with subjects supine, except for the final 0900h sample when observations were taken in the sitting position. A standard mercury column sphygmomanometer was used (Korotkov sounds II and IV). Mean arterial pressure was derived by adding 1/3 pulse pressure

to diastolic pressure. Subjects were asked to empty their bladder completely after each venesection, the urine volume was measured and a 20 ml aliquot taken for sodium estimation. At 2400 and 0400 hours all subjects voided urine while supine, males into urine bottles and females into a bed pan. Special care was taken to collect urine before bowel actions. Each urine collection was timed to the nearest minute so that rates of excretion could be calculated.

Informed consent was obtained from all subjects and the study was approved by the King's College Hospital Ethics commitee.

3.2.3 Assays

Atrial natriuretic peptide was assayed after plasma extraction, as described in Chapter 2 (197,215). All samples were assayed together, in a single assay run. Plasma renin activity was measured by the generation of angiotensin I, by a modification of the method of Menard and Catt (239), and plasma aldosterone concentration with a Sorin radioimmunoassay kit (CIS, High Wycombe, Bucks) after solvent extraction of plasma (240). Serum creatinine and albumin were estimated by auto-analyser. Sodium and potassium were measured by flame photometry within two hours of collection. Haematocrit was measured in duplicate within a few minutes of blood sampling using a peripheral micro-haematocrit technique.

Results are expressed as mean±standard error. Statistical analysis was by the unpaired and paired Student's 't' test, as appropriate. Test of linear correlation was by the method of least squares analysis. Level of statistical significance was taken as $p<0.05$.

Fig. 6

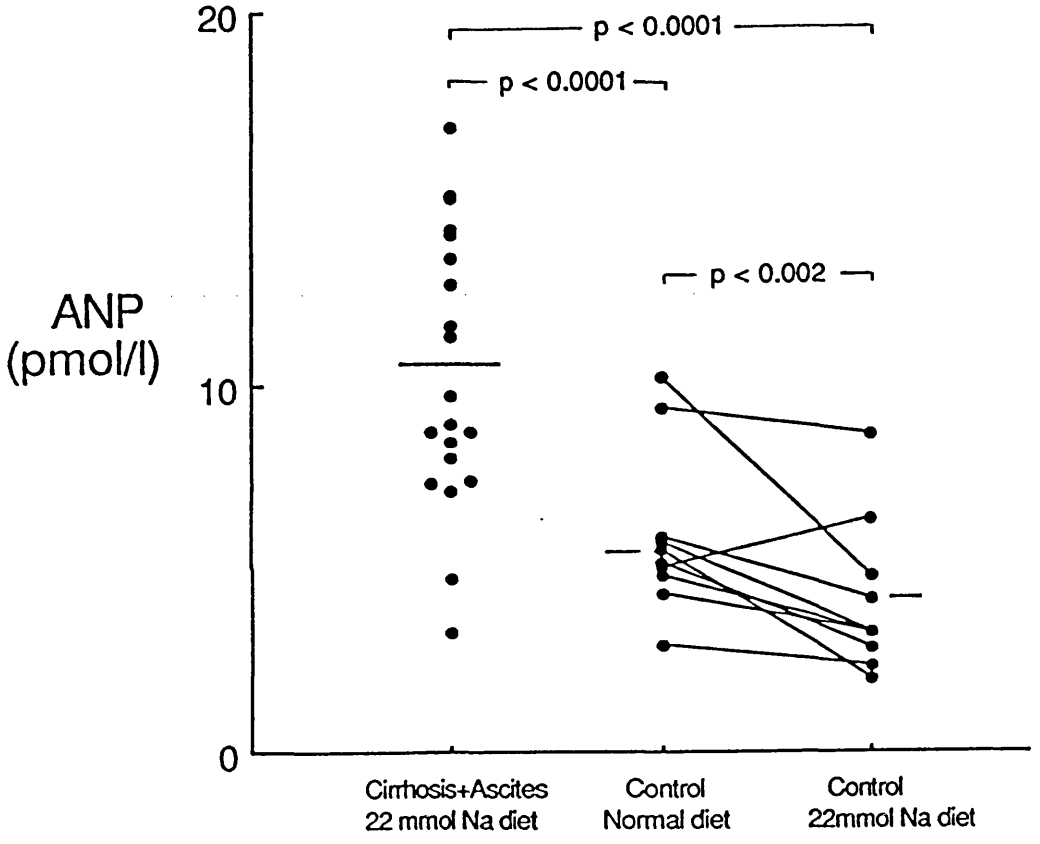


Fig. 6. Plasma atrial natriuretic peptide (ANP) concentration in 21 patients with cirrhosis and gross ascites on a 22 mmol sodium diet and ten age matched controls on normal (unrestricted) diet and after five days on a 22 mmol sodium diet.
Note: Student's unpaired and paired 't' test.

3.3 Results

3.3.1 Basal assessment

Baseline urine sodium excretion was lower in the 21 patients (5.1±2.03 mmol/day) as compared to controls on the fourth day of a 22 mmol sodium diet (19.3±4.14 $p<0.01$). There was no significant difference in creatinine clearance between controls (94.7±5.5 ml/min) and patients (82±7.9 ml/min), (Table 2). Baseline ANP levels at 0800h were higher in the 21 patients with cirrhosis and ascites (10.5±0.8 pmol/l) compared to the ten controls for both the unrestricted (5.8±0.58 pmol/l $p<0.0001$) and the 22 mmol sodium diets (4.1±0.64 pmol/l $p<0.0001$), (Fig. 6). Atrial natriuretic peptide concentration was lower in controls on the low sodium diet as compared to their respective normal diets ($p<0.02$). In the same group PRA was increased from 2.66±0.17 pmol/h/ml on the normal diet to 3.58±0.68 pmol/h/ml on the low sodium diet ($p<0.05$), PA increased from 272±23.1 to 397.3±32 pmol/l ($p<0.05$) and twenty four hour urinary sodium excretion dropped from 129±14 mmol to 19.3±4.14 mmol ($p<0.0001$).

Baseline plasma renin activity on the 22 mmol sodium diet was significantly higher in patients (8.17±1.2 pmol/h/ml) compared to controls (3.58±0.68 pmol/h/ml $p<0.01$). Similarly aldosterone was higher in patients (742±66.1 pmol/l) as compared to controls (397.3±32 pmol/l $p<0.01$). Within the patient group (n=21), there was no significant correlation between baseline ANP concentration, PRA, or PA at 0800 hours with 24 hour urine sodium excretion, serum creatinine or creatinine clearance.

TABLE 2

BASELINE BIOCHEMISTRY, HAEMATOCRIT (Hct), PROTHROMBIN TIME PROLONGATION IN SECONDS (PT), IN PATIENTS WITH CIRRHOSIS AND ASCITES AND CONTROL SUBJECTS ON THE 4th DAY OF A 22mmol SODIUM DIET INTAKE. SAMPLES AND MEASUREMENTS WERE TAKEN AT 0800 HOURS WITH SUBJECTS SUPINE AFTER AN OVERNIGHT FAST. VALUES ARE MEAN ± SEM.

	PT (secs) prolonged	Bil (μmol/l)	Alb (g/ l)	Hct (%)	Se Creat (μmol/l)	Creat Cl (ml/min)
Controls (n=10)	--	8.8±1.57	42.6±0.74	45.33±1.28	87.4±4.3	94.7±5.5
Patients (n=8)	6±0.94	67.5±20.1*	30.4±1.6***	32.08±2.22**	77.7±7.3	79.8±5.2*

	Se Na (mmol/l)	U Na (mmol/day)	U K (mmol/day)	Pulse (bpm)	MAP (mmHg)
Controls (n=10)	138±1.16	19.3±4.14	67.2±5.15	73.8±2.76	88.65±6.23
Patients (n=8)	135±1.3*	3.44±1.34**	30.2±5.9**	94.0±3.4***	77.01±3.41

Note: Student's t-test. * p <0.05; **p <0.01; ***p <0.001

3.3.2 Changes during 24 hours

As for the complete group of 21 patients, baseline ANP, PRA and PA at 0800h were all higher in the 8 patients as compared to controls ($p<0.0001$, $p<0.05$ and $p<0.001$ respectively) (Table 3). The difference in ANP concentration persisted when results for the entire 24 hour period were expressed as means of 6 time points. Mean ANP concentration for the control group was 3.91 ± 0.64 pmol/l and 9.45 ± 1.61 for the patient group ($p<0.0002$). The difference between patients and controls was even more significant when the mean 24 hour PRA and aldosterone levels were compared. Mean PRA in controls was 4.27 ± 0.26 compared to 11.4 ± 0.98 pmol/h/ml ($p<0.0002$) in patients and mean PA was 521.2 ± 39 in controls compared to 1074 ± 58 pmol/l ($p<0.0002$) in patients.

The mean circulating ANP concentration in controls during the 24 hours of study was not significantly different from values of samples taken at 0800h (3.91 ± 0.64 v 4.11 ± 0.64 pmol/l). Similarly there was no difference in the mean and 0800h ANP levels of patients with cirrhosis (9.45 ± 1.61 v 9.61 ± 1.33 pmol/l). There was a significant difference between the 0800h values and 24 hour means of PRA and aldosterone in the cirrhotic group 7.88 ± 1.22 v 11.4 ± 0.98 pmol/h/ml ($p<0.05$) and 836 ± 65 v 1074 ± 58 pmol/l ($p<0.05$) respectively, but not in the control group (PRA 3.58 ± 0.68 v mean 4.27 ± 0.26 pmol/h/ml and aldosterone 397.3 ± 32 v mean 521 ± 39.7 pmol/l ($p<0.1$).

Changes during 24 hours, within each group, were compared to the 0800h values at the beginning of the 24 hours. Circulating ANP concentration did not change significantly in either group, although in

Fig. 7

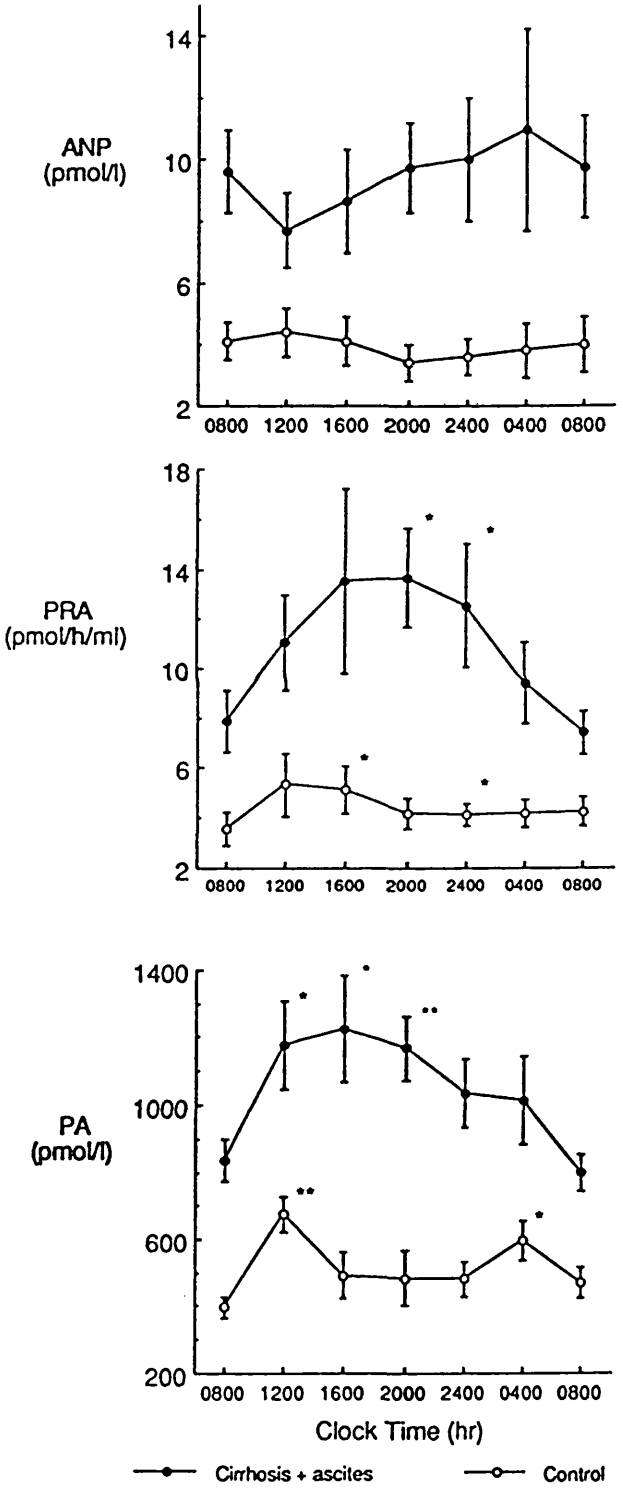


Fig. 7. Plasma concentration of ANP, plasma renin activity (PRA) and plasma aldosterone concentration (PA) in 6 patients with gross ascites and 10 controls during a 24 hour period. Results are mean±SEM.
Note: Paired 't'test. Values are compared to 0800 hours at the beginning of the 24 hour period. * $p < 0.05$ ** $p < 0.01$.

TABLE 3

PLASMA CONCENTRATION OF ATRIAL NATRIURETIC PEPTIDE (ANP), ALDOSTERONE (PA), PLASMA RENIN ACTIVITY (PRA), HEART RATE (HR), MEAN ARTERIAL PRESSURE (MAP), AND HAEMATOCRIT (Hct) IN CONTROL SUBJECTS AND PATIENTS WITH CIRRHOSIS AND ASCITES, DURING 24 HOURS OF ORDINARY ACTIVITY. VALUES EXPRESSED AS MEAN ±SEM.

		0800	1200	1600	2000	2400	0400	0800
Controls (n=10)	ANP (pmol/l)	4.11±0.6	4.4±0.8	4.1±0.8	3.4±0.6	3.6±0.6	3.8±0.9	4.0±0.9
	PRA (pmol/h/ml)	3.58±0.68	5.33±1.25	5.14±0.94*	4.18±0.62	4.13±0.43*	4.18±0.58	4.26±0.60
	PA (pmol/l)	397±32	675±52**	492±70	484±84	481±52	596±60*	469±47
	HR (bpm)	73.9±2.76	67.3±1.81	70.3±1.56	74.1±1.99	72.3±1.89	68.9±3.02	69.7±1.99
	MAP (mmHg)	88.6±6.23	90.34±4.55	83.8±4.11	92.0±5.32	85.6±3.79	83.34±3.8	82.1±3.89
	Hct (%) (n=8)	45.33±1.28	43.3±0.67	42.65±0.67	42.05±0.87	41.1±1.29	41.6±0.76	40.5±0.60
Patients (n=8)	ANP (pmol/l)	9.61±1.33	7.77±1.21	8.63±1.67	9.73±1.45	10.0±2.02	10.94±3.27	9.75±1.68
	PRA (pmol/h/ml)	7.88±1.22	11.04±1.95	13.5±3.71	13.65±1.98*	12.54±2.49*	9.41±1.65	7.41±0.88
	PA (pmol/l)	836±65	1176±131*	1224±158*	1168±95**	1032±101	1013±129	798±56
	HR (bpm)	94±3.44	91.2±3.9	94.6±4.27	91.77±3.16	93.1±4.43	90.0±3.59	90.6±4.64
	MAP (mmHg)	77.0±3.41	82.1±2.90	84.2±2.55*	82.3±5.14	81.77±4.69	81.3±4.4	82.3±3.81
	Hct (%)	32.1±2.2	34.1±2.5	33.1±2.54	33.1±2.9	33.1±2.25	30.33±2.3*	33.4±2.41

Note: Results compared to values at 0800 h at the beginning of the 24 hour period. Paired t-test. *p <0.05; ** <0.01.

patients there was greater variation (Fig. 7). PRA increased during the day in both groups, reaching a peak between 1200 and 2000 hours; the increase was greater in patients (Fig. 7). Plasma aldosterone concentration increased significantly between 0800 and 1200 in both groups but was sustained only in patients (Fig. 7). Haematocrit did not change during the day in controls though in patients there was a significant difference between 0800h and 0400h (Table 3).

Resting heart rate at 0800h was significantly increased in patients as compared to controls ($p<0.01$), and resting mean arterial pressure was not significantly different in the two groups ($p=0.09$), (Table 2). There were no significant changes of heart rate in either group. Mean arterial pressure did not change in the control group although in patients there was an increase at 1600h, as compared to 0800h ($p<0.05$), which coincided with peak levels of PRA and PA (Table 3).

3.3.3 Urine sodium excretion and urine flow

Inspection of the pattern of natriuresis and diuresis (Fig. 8), identified three distinct periods, from 24-0800h when subjects were supine (when urine sodium was higher in patients and lower in controls), 16-2400h (the period of lowest urine sodium excretion in patients and highest in controls) and 08-1600h. Data was analysed by dividing the 24 hour day into these three 8 hour periods. In controls, there was a significant difference in urine sodium excretion between 08-1600h (10.31 ± 2.92 µmol/min) and 16-2400h (19.34 ± 3.74 µmol/min $p<0.05$) and between 16-2400h and 24-0800h $p<0.001$). In patients, there was a significant difference between 08-1600h and 16-2400h (1.38 ± 0.39 and 0.63 ± 0.14 µmol/min respectively $p<0.05$) and an almost significant

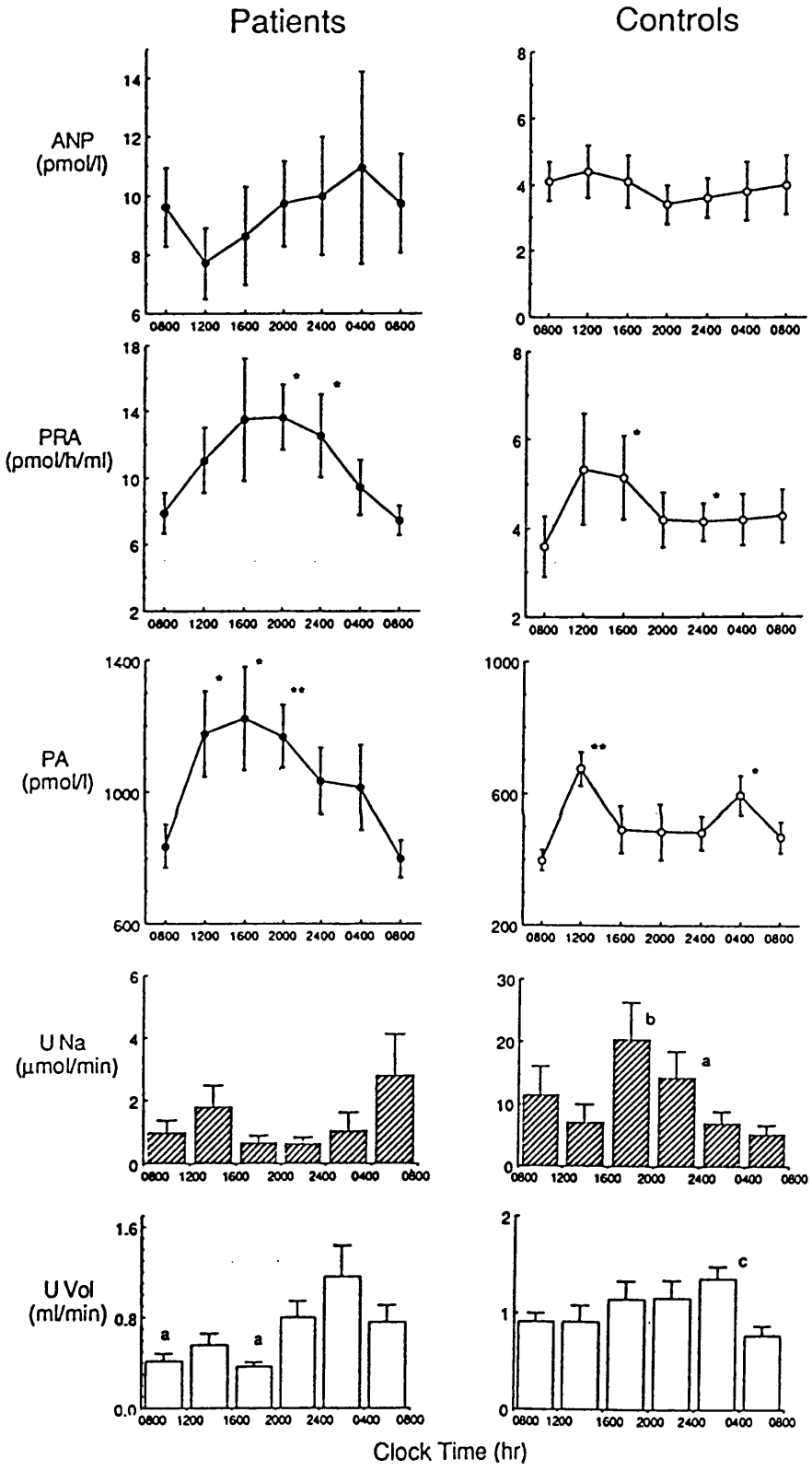


Fig. 8. Plasma ANP, PRA, PA, urine sodium excretion and urine flow rate at four hour intervals during a 24 hour period in patients (n=8) and controls (n=10). Results are mean±SEM. Note different scales.
Note: Paired 't' test. Values are compared to 04-0800 hours.
a = p <0.03, b = p <0.02, c = p <0.001.
* = p <0.05, ** = p <0.01

difference between 16-2400h and 24-0800h (0.63±0.14 and 1.85±0.71, p<0.08).

In patients, coinciding with the trend towards increased natriuresis between midnight and 0800h, was a drop in PRA and PA, with no significant change in ANP (Fig. 8). Comparison of circulating ANP with PRA and PA concentration for the period 24-0800h showed an inverse correlation between ANP and PA concentration (r -0.62, p <0.05) but not with PRA. The means of two measurements of PRA, PA, and ANP (at the beginning and end of each four hour period) were compared to urine sodium excretion for the corresponding period: In patients, there was a significant correlation between ANP concentration and urine sodium for the period 24-0800h (r 0.65, p <0.02) and for the 16-2400h period (r 0.54, p< 0.05) but not for the period 08-1600h (Fig. 9). When results for the entire 24 hour period were pooled, the significant correlation between ANP and urine sodium excretion was retained (r 0.65, p <0.05). When the ANP concentration at the end of each four hour period was plotted against urine sodium for the corresponding period, the correlation was even higher (r=0.79, p <0.005). There was no direct correlation between urine sodium excretion and PRA or PA during any of the three 8 hour periods, although there was a correlation between urine sodium excretion and the ratio of ANP/PA between 24-0800h (r 0.65, p <0.05), and 16-2400h (r 0.63, p<0.05). In controls, there was no correlation between urine sodium excretion and circulating atrial natriuretic peptide concentration, plasma renin activity or aldosterone concentration during any of the three 8 hour periods.

Fig. 9

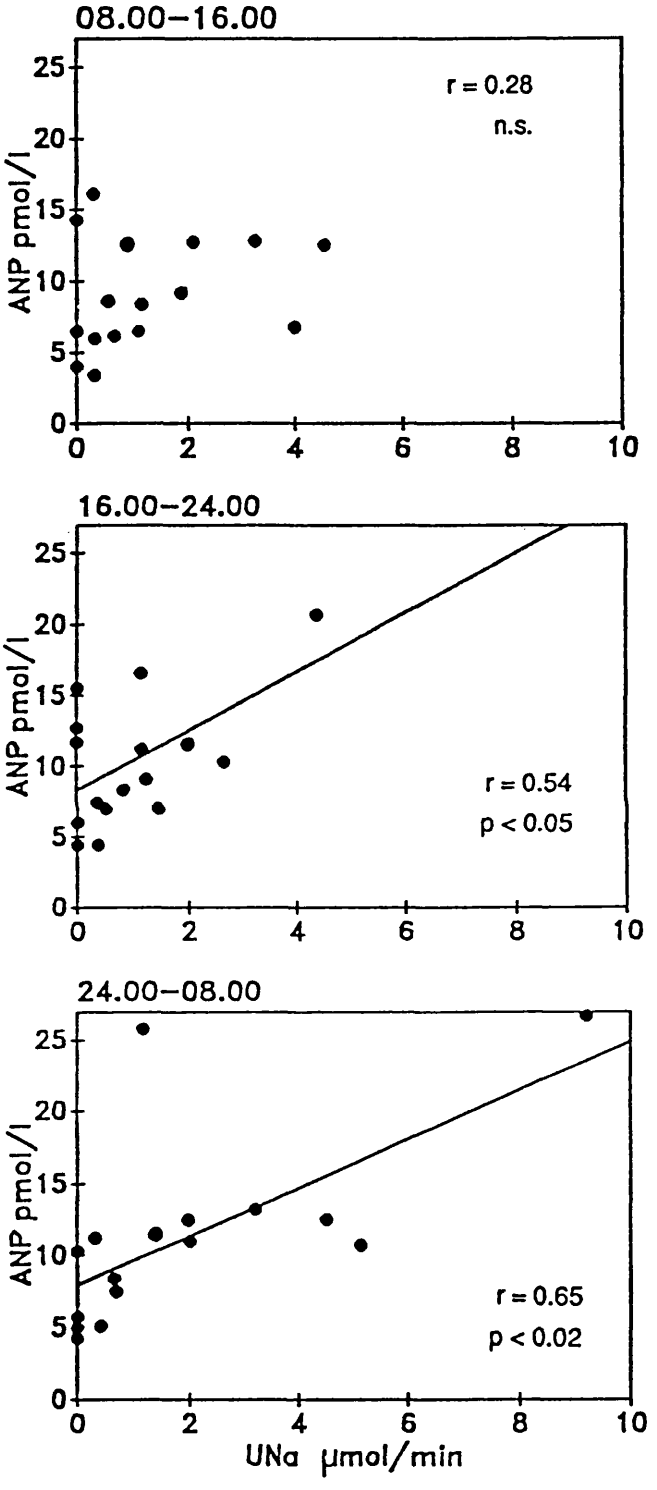


Fig. 9. Correlation of plasma ANP (mean of two estimations) with four hour urine sodium excretion, during three 8 hour periods. Test of linear correlation by the method of least squares analysis.

In controls, urine flow rate was higher during daytime, although the highest flow rate was between 24-0400h and the lowest between 04-0800h ($p < 0.001$). The reverse pattern was seen in patients (Fig. 8). When data were combined into three 8 hour periods, there was a significant difference between 08-1600h (0.49 ± 0.06 ml/min) and 24-0800h (0.97 ± 0.16 ml/min $p < 0.04$) in patients. There was no difference between any of the three periods in the control group. There was no correlation between plasma ANP, PA, PRA, ANP/PA, or ANP/PRA with urine flow rate for the period of 24-0800h.

3.3.4 Effect of posture and activity

On rising, after 1 hour of non-strenuous activity, mean plasma atrial natriuretic peptide concentration decreased significantly in patients ($p < 0.03$), but not in controls (Table 4). Plasma renin activity rose significantly in both patients ($p<0.01$) and controls ($p<0.01$). The increase in PRA was greater in patients (6.36 ± 1.31 pmol/h/ml) as compared to controls (1.82 ± 0.63 pmol/h/ml $p<0.05$). Similarly, the increase in plasma aldosterone concentration was greater in patients (350 ± 73 pmol/l) as compared to controls (130 ± 42 pmol/l, $p= 0.05$).

Paired samples for haematocrit estimation were available from 8 subjects in each group (Table 4). Haematocrit increased significantly both in controls ($p < 0.01$) and patients ($p < 0.01$). The increase was significantly greater in the patient group (2.93 ± 0.66 per cent) as compared to controls (1.50 ± 0.32 per cent, $p= 0.05$). Heart rate increased significantly in both groups and mean arterial pressure increased only in the control group (Table 4). Although not significant, m.a.p. may have fallen in the cirrhotic groups.

TABLE 4

PLASMA CONCENTRATION OF ATRIAL NATRIURETIC PEPTIDE (ANP), PLASMA RENIN ACTIVITY (PRA), ALDOSTERONE CONCENTRATION (PA), HAEMATOCRIT (Hct), HEART RATE (HR) AND MEAN ARTERIAL PRESSURE (MAP), AT 0800 hr WITH SUBJECTS FASTING HAVING BEEN SUPINE FOR 8 HOURS (SUP) AND AFTER 1 HOUR OF ORDINARY ACTIVITY (ACT).

		ANP (pmol/l)	PRA (pmol/h/ml)	PA (pmol/l)	HR (bpm)	MAP (mmHg)	Hct (%) (n=8)
Control (n=10)	SUP	4.0±0.90	4.26±0.60	469±47	69.7±1.9	82.41±3.89	40.5±0.6
	ACT	2.8±0.62	6.08±0.66***	599±65**	78.8±2.51**	87.41±4.18**	42.71±0.95***
Patients (n=8)	SUP	9.70±1.68	7.41±0.88	798±55	90.6±4.64	82.27±3.8	33.43±2.41
	ACT	7.34±1.24**	13.77±1.67***	1148±112**	102±3.53*	75.21±6.08	36.37±2.6***

Note: Paired t-test. * $p < 0.05$; ** $p < 0.03$; *** $p < 0.01$

3.4 Discussion

Since 1985, several studies have reported on circulating concentrations of atrial natriuretic peptide in cirrhosis, with conflicting results. Raised (235,241), normal (242,243) or low (237) concentrations of ANP have been reported. Factors which may account for these discordant results may be related to varying conditions under which patients were studied. For instance, in some studies patients were receiving diuretics at the time of study (237,241,244) and it is possible that these drugs influence circulating ANP concentration (245). In others, patient and control groups either were not matched for age (242), or no information was given in this regard (235). Matching for age is essential, since circulating ANP concentrations rise with age (196). Another consideration is the wide variability of dietary sodium intake administered in the above studies, which ranged between 40-200 mmol/day. Intake of sodium influences plasma ANP concentration (181). A further factor which may be responsible for the finding of normal or low concentrations, is the direct assay of plasma for ANP without prior extraction as in the study of Bonkovsky et al (237). A number of studies (218-220) have shown that assay sensitivity is greatly reduced and may fail to detect changes within or just above the physiological range, when plasma is not extracted prior to radioimmunoassay.

Studies which related circulating ANP levels to urine sodium excretion may also have been subject to some inaccuracy. Spot ANP measurements, usually at 0900 hours in the supine position, have been related to 24 hour urine sodium excretion or to excretion of sodium over

shorter periods. Gines et al (235), found elevated spot levels of ANP with no correlation to low urine sodium excretion between 0800-1600h. Hence, they postulated that ANP may be of little importance in the regulation of sodium excretion in cirrhosis. Since sodium excretion in cirrhosis is least during the day and greatest at night (16,17,230), it is likely that a relationship between ANP and natriuresis may have been obscured by their timing of urine collections, which coincides with the period of highest aldosterone concentration observed in the present study.

The present results show that concentrations of atrial natriuretic peptide at 0800 hours are increased in patients with cirrhosis and ascites on a 22 mmol sodium diet as compared to controls, whether the latter received unrestricted or 22 mmol sodium in their diet. Further, they demonstrate that raised levels persist throughout the day and that levels at 0800h in the supine position after an overnight fast are representative of mean levels throughout a 24 hour period of non-strenuous activity, even though they are subject to large variation during the day. Factors which may be responsible for raised circulating levels of ANP include increased secretion by the cardiac atria, as reported by Gines et al (235), or reduced extraction of ANP by the liver (246). Gines et al (235), reported that splachnic and peripheral extraction of ANP does not seem to be reduced in patients with cirrhosis and ascites. Their data needs to be interpreted with caution since in their study splachnic and hepatic blood flow was not measured. Hollister et al (246), determined human and canine plasma clearance of ANP by lung, liver and kidney, from arteriovenous differences in plasma ANP and

measured organ plasma flow. Hepatic plasma ANP clearance extraction ratio was 30% in humans and 36% in the dog and renal ANP clearance extraction ratio was 35% in humans and 42% in the dog. Thus it is possible that in cirrhosis, impaired extraction of ANP by the liver or kidney, may contribute to raised circulating levels of ANP.

The observation of a drop in plasma ANP concentration on assuming the upright posture in the present study, along with appropriate rises in response to central volume expansion reported from studies of water immersion (242) and peritoneovenous shunting (236), suggest that known mechanisms involved in the plasma release of ANP are not impaired in cirrhosis with ascites.

The finding of raised concentrations of plasma ANP in patients with cirrhosis and ascites has been used to argue in favour of the "Overflow" theory of ascites formation (235), by interpreting the rise in ANP as a secondary effect which acts as an index of increased plasma volume. On the other hand, the rise in the concentration of ANP in response to head-out water immersion (242) and peritoneovenous shunting (236), has been used to argue in support of the "Underfil" theory. However, both interpretations assume that extraction and metabolism of ANP by the liver and kidney in patients with cirrhosis is not impaired, even though this question has not been addressed directly in studies published to date. Moreover, it may not be appropriate to extrapolate from haemodynamic and hormonal findings in advanced cirrhosis with ascites, to explain the pathogenesis of ascites early in the course of the disease.

Plasma renin activity and aldosterone concentration were increased

in patients as compared to controls on either diet. Erect posture and activity during the day resulted in greater, more sustained stimulation of the renin-aldosterone system in patients, rendering the basal estimation at 0800h unrepresentative of the activity of this system over 24 hours. Thus measurements confined to basal conditions underestimate the activity of the renin-aldosterone system in patients with cirrhosis and ascites. The greater increase in haematocrit in patients on rising, would be consistent with greater loss of fluid from the intravascular compartment, through an expanded dependent vascular bed in patients, as compared to controls (233). A further possible contributory factor to the exaggerated response of the renin-aldosterone system in patients, is redistribution of blood flow within a low resistance vascular system, which may result in reduced renal blood flow (233). Our findings are in agreement with those from a study of postural changes by Bernardi et al (233), and go further to show that over-activity of the renin-aldosterone system in cirrhotic patients persists for most of the 24 hour day. These findings emphasize the importance of bed rest in the treatment of cirrhotic ascites.

In patients with cirrhosis, a posturally determined reduction in a putative circulating natriuretic factor, was proposed by Borst and de Vries in 1950 (17), as a possible explanation for the decrease in urine sodium excretion during daytime. The present results indicate that although there is a greater initial drop in circulating ANP concentration in patients on rising, mean levels do not change significantly during the day, despite large variations. This suggests

that sodium retention during daytime may be attributable to other anti-natriuretic influences, such as increased activity of the renin-aldosterone system, or adrenergic stimulation (233).

Previous studies have shown that in healthy adults, the high urine flow rate and sodium excretion during the day is not dependent on the sleep-work pattern or the pattern of food and water ingestion (16). In cirrhosis the pattern is reversed or abolished in more than three quarters of cases (16,230). The abnormality persists when patients are given food and water at equal intervals during the day or night (16) but seems to revert towards normal in some cases, when patients remain supine throughout a 24 hour period (17). Changes observed in the present study are necessarily composite of the effects of posture superimposed on those of any underlying diurnal rhythm. Nevertheless they are representative of the clinical situation. Bernardi et al (231,232), showed that under steady state conditions, with cirrhotic patients remaining supine, there is loss of diurnal variation in the activity of the renin-aldosterone system and of the adrenergic system. In the presence of such achronia, the influence of posture and activity assumes a more prominent role in determining the circulating levels of these hormones.

The finding of a significant correlation between circulating ANP concentration and urine sodium excretion when levels were estimated at 4 hourly intervals under the strictly monitored conditions of the present study, suggests that increased circulating levels of ANP may have a natriuretic role in cirrhosis and indicates that relating spot levels of ANP to 24 hour urine sodium excretion can be misleading. Correlation

between circulating ANP and urine sodium excretion was strongest between 24-0800h and coincided with a marked decrease in the activity of the renin-aldosterone system, suggesting that the latter may allow the natriuretic effect of the raised levels of ANP to become manifest. This interpretation is supported by the absence of a natriuresis between midnight and 0800h in controls, in whom there was no drop in aldosterone and plasma ANP remained low or normal. Even though in patients there was a decrease in plasma renin activity and aldosterone between midnight and 0800h, both remained above normal levels and this could account for the overall retention of sodium present in the cases studied. The present findings are consistent with the hypothesis proposed by Epstein et al (77), attributing a "permissive" role to aldosterone in the sodium retention of cirrhosis and concur with the previous finding of a dissociation between renal sodium excretion and plasma aldosterone, in patients undergoing spontaneous diuresis (73).

CHAPTER 4

Single, total paracentesis for tense ascites:
Haemodynamic and hormonal changes

"Sudden or complete evacuation of ascites will immediately kill the patient".

Paul of Aegina 625-690 A.D.

4.1 Introduction

In the 1950's, paracentesis was abandoned as a treatment for cirrhotic ascites largely due to fears that the rapid withdrawal of fluid followed by reaccumulation of ascites, would result in plasma volume depletion with life threatening hypovolaemia, hyponatraemia and renal impairment, as a consequence (247,248). Previous studies on the haemodynamic effects of paracentesis have yielded somewhat discordant results, with reports of no change (20), improvement (249) or initial improvement followed by deterioration (250). Albumin infusion during or following paracentesis may protect renal function by preventing hypovolaemia (251-255) although according to one report (256), up to 30% of patients treated with paracentesis plus albumin infusion develop some degree of hypovolaemia as evidenced by raised plasma renin activity.

In view of these conflicting findings, it seemed that further study of the haemodynamic changes associated with large volume paracentesis was warranted, both to confirm that plasma volume expansion with intravenous albumin is needed, and to derive guidelines as to the most appropriate timing for such plasma expansion. To determine the underlying mechanisms involved in the haemodynamic changes, echocardio-

graphic data on right atrial size together with measurements of plasma atrial natriuretic peptide (ANP) concentrations, plasma renin activity (PRA) and plasma aldosterone concentration (PA) were utilised.

4.2 Patients and Methods

23 patients (aged 23 to 72 years) with cirrhosis and gross ascites were studied. The diagnosis had been confirmed with liver biopsy in 14 cases and was based on clinical and laboratory data on the remainder. Aetiology was alcohol in 15 patients, autoimmune chronic active hepatitis in 2, chronic hepatitis B in 2, cryptogenic in 2, primary biliary cirrhosis in 1, and haemochromatosis in 1. Patients with severe renal impairment (serum creatinine >200 μmol /litre), hyponatraemia (serum sodium <125 mmol/litre), evidence of hepatic encephalopathy, sepsis or recent haemorrhage (within 15 days) were excluded. At the time of study 9 patients were classified as grade B (Pugh) and 14 grade C. Peripheral oedema was present in 10 patients.

All patients were on a 22 mmol daily sodium diet for at least 7 days prior to study and diuretics were stopped 5 days previously. The ascites was drained to dryness using a flexible sheath introducer (Arrow, Reading, Pennsylvania). In 8 patients, the paracentesis cannula was connected to a transducer via a three way tap and intra-abdominal pressure (IAP) was measured with the mid-axillary line as zero reference point at the beginning, at 2 hours and at the end of paracentesis.

Informed consent was obtained from all patients and the study was approved by the King's College Hospital Ethics Commitee.

4.2.1 Haemodynamic measurements

Patients were studied in the supine position. At the beginning of the study, a pulmonary artery flotation catheter (Ecosse, BMS, Glascow) was introduced via a subclavian vein. After a period of equilibration of 30 minutes measurements of heart rate (HR), right atrial pressure (RAP), pulmonary capillary wedge pressure (PCWP), and arterial blood pressure (mercury column sphygmomanometer, mean of three readings, Korotkov sounds II and IV) were made. Mean arterial pressure was derived by adding 1/3 pulse pressure to the diastolic pressure. Mean RAP and PCWP were derived electronically (Simonsen and Weel Ltd, series 9000; AKERS transducer, Sensonor 840, Norway), with the mid-axillary line as zero reference point. Haemodynamic measurements were taken at 30, 60, 90, 120, and 180 minutes from the start of paracentesis and at 6, 12, 24, 36 and 48 hours. Cardiac output was measured by a thermodilution technique using a computer (Edwards Laboratories, mean of 5 readings). Systemic vascular resistance (SVR) was calculated using the formula:

SVR= MAP-RAP x 79.9/CO, where SVR is in dynes sec cm^{-5}, MAP and RAP are in mmHg and CO in litres per minute.

Peripheral venous blood was taken without stasis for sodium, haematocrit, atrial natriuretic peptide (ANP) and plasma renin activity (PRA) and aldosterone (PA) estimation at 0, 2, 12, 24 and 48 hours from start of paracentesis from 12 of the 23 patients. Samples for ANP, PRA and PA were taken into chilled EDTA tubes, plasma separated within 10 minutes and stored at -70°C until assayed. Serum creatinine was measured at baseline, 24 and 48 hours. At 48 hours all patients

received 200ml of 20% albumin and commenced spironolactone 100mg daily. Serum electrolytes and creatinine were measured daily for 5 days.

4.2.2 Assays

Samples for ANP were assayed in duplicate after plasma extraction, in a single assay run, as described in Chapter 2 (197,210). PRA was measured by the generation of angiotensin I, by a modification of the method of Menard and Catt (239), and PA with a Sorin radioimmunoassay kit (CIS, High Wycombe, Bucks) after solvent extraction of plasma (240). Serum creatinine and albumin were estimated by autoanalyser and sodium by flame photometry.

4.2.3 Echocardiography

In the 8 patients in whom intra-abdominal pressure measurements were obtained, 2 D echo-cardiography was performed between 12 and 24 hours prior to paracentesis and in 2 cases, echocardiographic data was obtained again at 24 hours after completion of the paracentesis. A phased-array system (Hewlett-Packard model 77020A) was used with a 2.5 MHz probe. Images were recorded from all conventional approaches and the apical 4-chamber view was used for calculating atrial area. Care was taken to obtain a view in which neither atrial chamber was foreshortened or rotated. A frame was then frozen and the internal area of each atrial cavity planimetered using the on-line computer software package (mean of three measurements). Right atrial area was expressed as a ratio of the left atrial area. For comparison, echocardiography was performed on 8 age matched healthy volunteers (33-62 years) and 8 patients with cirrhosis (age range 40-66 years) and minimal ascites, while on a 22 mmol daily sodium diet.

Results are expressed as mean±standard error. Statistical analysis was by the paired or unpaired Student's 't' test, as appropriate and test of linear correlation by the method of least squares analysis. For multiple comparisons, the p value was multiplied by the number of comparisons performed, that is, by a factor of 9 for haemodynamic measurements and a factor of 4 for hormonal measurements.

4.3 Results

Baseline prolongation of prothrombin time (n=23) was 5.35±0.89 seconds, serum albumin 28.6±0.99 g/l, and total bilirubin 51.35±8.64 μmol/l. The mean volume of ascites removed was 10.30 litres (range 4-16 litres). In all except 1 patient, 8 litres or more were removed. Total time taken to complete the paracentesis ranged from 2 to 8 hours with a minimum and maximum drainage rate of 1.8 and 5.9 litres per hour respectively. In two patients haemodynamic measurements suggested the development of significant hypovolaemia during paracentesis, at 6 and 12 hours from the start of the procedure. In the first case, systolic blood pressure dropped by 10mmHg to 80 mmHg with a drop in PCWP from 8 to 3 mmHg and a rise in SVR from 896 to 1265 dynes s cm^{-5}. In the second case there was a drop of PCWP from 7 to 2mmHg, with a rise in SVR from 913 to 1184 dynes s cm^{-5}, and a drop in systolic blood pressure from 100 mmHg to 85mmHg. Neither patient complained of symptoms. Both patients responded promptly to infusion of 500 ml of intravenous colloid with a rise in PCWP (2 and 3 mmHg respectively) and cardiac output. There was no identifiable baseline haemodynamic or electrolyte abnormality which could have predicted the development of hypovolaemia. These 2 cases were excluded from the final analysis since

TABLE 5 **Haemodynamic changes recorded in the 21 of 23 patients not developing clinical hypovolaemia during single, total paracentesis. Results represented as mean ± standard error.**

	TIME									
	0 min	30 min	60 min	120 min	180 min	6 hours	12 hours	24 hours	36 hours	48 hours
HR bpm	92.7±2.7	90.2±2.7	90.5±2.7	90.5±2.7	90.1±3.0	90.8±3.1	90.2±3.4	89.8±2.1	90.9±3.0	90.6±4.3
RAP (mmHg)	9.3±0.8	7.6±0.8	7.4±0.8	7.4±0.8	7.0±0.8	6.4±0.7[b]	5.6±0.6[b]	6.3±0.7[b]	5.9±0.6	5.8±0.8[b]
PCWP (mmHg)	10.9±0.9	10.7±0.8	10.4±0.9	10.2±0.9	10.2±0.9	8.7±0.8[b]	7.2±0.8[c]	8.3±0.9[a]	6.7±0.7[b]	7.2±0.8[b]
MAP (mmHg)	89.1±3.0	88.0±3.0	81.3±2.6[a]	82.6±3.9[c]	82.6±3.9[c]	80.9±2.9[c]	81.5±2.1[a]	82.5±2.1	79.0±3.0[c]	80.4±3.7[c]
CO (*l*/min)	7.7±0.5	8.2±0.5	8.5±0.6[b]	8.7±0.6[b]	9.03±0.7[a]	8.7±0.6[b]	7.2±0.6	7.9±0.7	7.7±0.8	7.8±0.7
SVR (dyne. s.cm-5)	917±78	855±62	804±68[c]	755±59[b]	772±77	777±64	967±103	905±90	847±86	847±88

Note: HR = Heart Rate; RAP = right atrial pressure; PCWP = pulmonary capillary wedge pressure; MAP = mean arterial pressure; CO = cardiac output; and SVR = systemic vascular resistance.

Paired t-test: p values multiplied by 9. **a** = $p < 0.05$; **b** = < 0.02; **c** = $p < 0.002$. All values compared to baseline.

Fig. 10

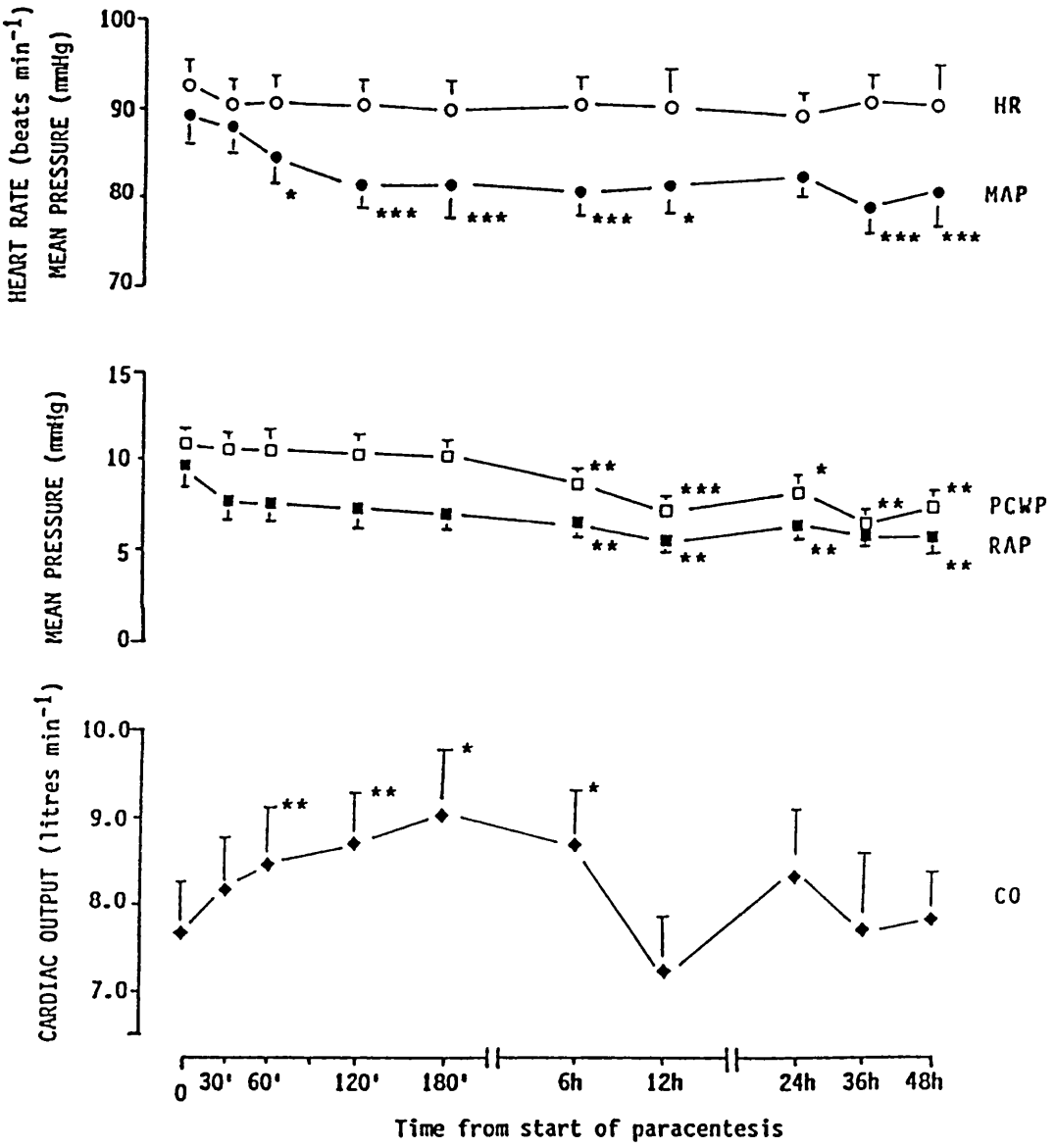


Fig. 10. Changes in heart rate (HR), mean arterial pressure (MAP), pulmonary capillary wedge pressure (PCWP), right atrial pressure (RAP), and cardiac output (CO) during single, total paracentesis for tense ascites (n=21).
Note: Paired 't'test. Results compared to baseline (p values multiplied by 9). * p <0.05, ** p <0.02, *** p <0.002 .

the infusion of colloid might have influenced subsequent haemodynamic changes. The subsequent clinical and haemodynamic course of these 2 patients was similar to the remaining 21 patients. Serum creatinine did not rise in either case up to 7 days after completion of the paracentesis.

Sequential haemodynamic measurements are shown in Table 5 and Fig. 10. Baseline CO was normal or increased in the 21 patients (range 3.2-12.8 litres/minute) included in the final analysis. The overall pattern of subsequent haemodynamic changes was similar in all patients despite the wide range in baseline values. During the first 3 hours of paracentesis there was a highly significant rise in cardiac output with a corresponding drop in RAP. PCWP remained unchanged for the first 3 hours corresponding to the period of sustained increase in cardiac output, after which time it dropped in parallel with the RAP and MAP. After 3 hours cardiac output decreased progressively reaching a nadir at 12 hours, coinciding with drops in RAP, PCWP and MAP. Between 12 and 24 hours there was a return to baseline cardiac output and SVR, but the RAP, PCWP and MAP continued to drop for 48 hours.

There was no significant difference between patients with or without peripheral oedema, in baseline measurements of RAP, PCWP, CO, SVR and MAP, or at any time point throughout the 48 hour period.

In the 12 patients in whom ANP, PRA, and PA measurements were made, mean volume of ascites removed was 10.5 litres (range 8-16 litres). Paracentesis time ranged from 3 to 5.5 hours. Baseline CO was normal or increased in all patients (range 3.2-12.8 l/minute), (Table 6). At 2 hours (mean volume of ascites removed 5.5 litres), there was a tendency

TABLE 6 **Haemodynamic changes in the 12 patients in whom ANP and PRA were measured. Results represented as mean ± standard error.**

	TIME								
	0 min	30 min	60 min	90 min	120 min	180 min	12 hours	24 hours	36 hours
HR bpm	94.83±4.25	92.17±4.09	92.92±4.27	93.92±4.17	92.0±4.21	93.0±4.20	96.1±4.13	96.1±4.11	98.0±4.5
RAP (mmHg)	9.25±1.37	8.0±0.76	7.70±0.99	6.90±1.03	6.6±0.92	5.91±1.0	6.05±0.84[b]	5.8±0.92	5.3±0.86[b]
PCWP (mmHg)	10.72±0.99	9.91±0.86	9.45±0.99	8.91±0.98	8.58±1.07	8.0±0.88	6.56±0.85[b]	6.72±1.10[b]	6.75±1.07[b]
MAP (mmHg)	87.86±2.71	86.35±3.31	82.72±3.17	79.75±3.06[b]	80.44±2.78[a]	78.79±2.9[a]	81.5±2.9	82.4±4.34	79.9±4.39[a]
CO (*l*/min)	7.6±0.76	8.59±0.76[b]	9.11±0.85[b]	9.09±0.90[b]	8.75±0.97[b]	8.79±0.97	7.08±0.75	7.6±0.99	7.79±0.82
IAP (mmHg) (n=8)	15.1±1.07	---	---	---	7.1±3.83[b]	---	*3.2±0.8[b]	---	---

Note: HR = Heart Rate; RAP = right atrial pressure; PCWP = pulmonary capillary wedge pressure; MAP = mean arterial pressure; CO = cardiac output; and IAP = intra-abdominal pressure.

* Measured at completion of paracentesis (3.5 - 5 hours).

Paired t-test: p value multiplied by 9. a = $p < 0.05$; b = $p < 0.02$. All values compared to baseline.

TABLE 7 **Changes in plasma atrial natriuretic peptide concentration (ANP), plasma renin activity (PRA) and plasma aldosterone concentration (PA) blood haematocrit (Hct), serum creatinine (Creat) and serum sodium in the 12 patients studied during paracentesis. Results are mean ± standard error.**

	TIME				
	0 min	120 min	12 hours	24 hours	48 hours
ANP (pmol/l)	8.09±1.18	10.25±1.2	7.24±0.87	5.9±0.84*a*	5.41±0.69[a]
PRA (pmol/h/ml)	10.85±2.06	9.21±2.48	10.92±2.04	13.18±2.35	16.6±2.76
PA (pmol/l)	950±147	824±59	759±166	914±117	1062±122 (n=8)
Hct (%)	30.33±2.03	29.61±2.16	31.0±2.25	32.05±2.33	31.5±2.31
Creat (μmol/l)	99.1±13.2	---	---	96.2±12.5	105.4±11.8
Sodium (mmol/l)	131.1±1.73	---	---	130±2.01	129.6±1.99

Note: Paired t-test: p values multiplied by 4. a = $p < 0.05$; Values compared to baseline.

Fig. 11

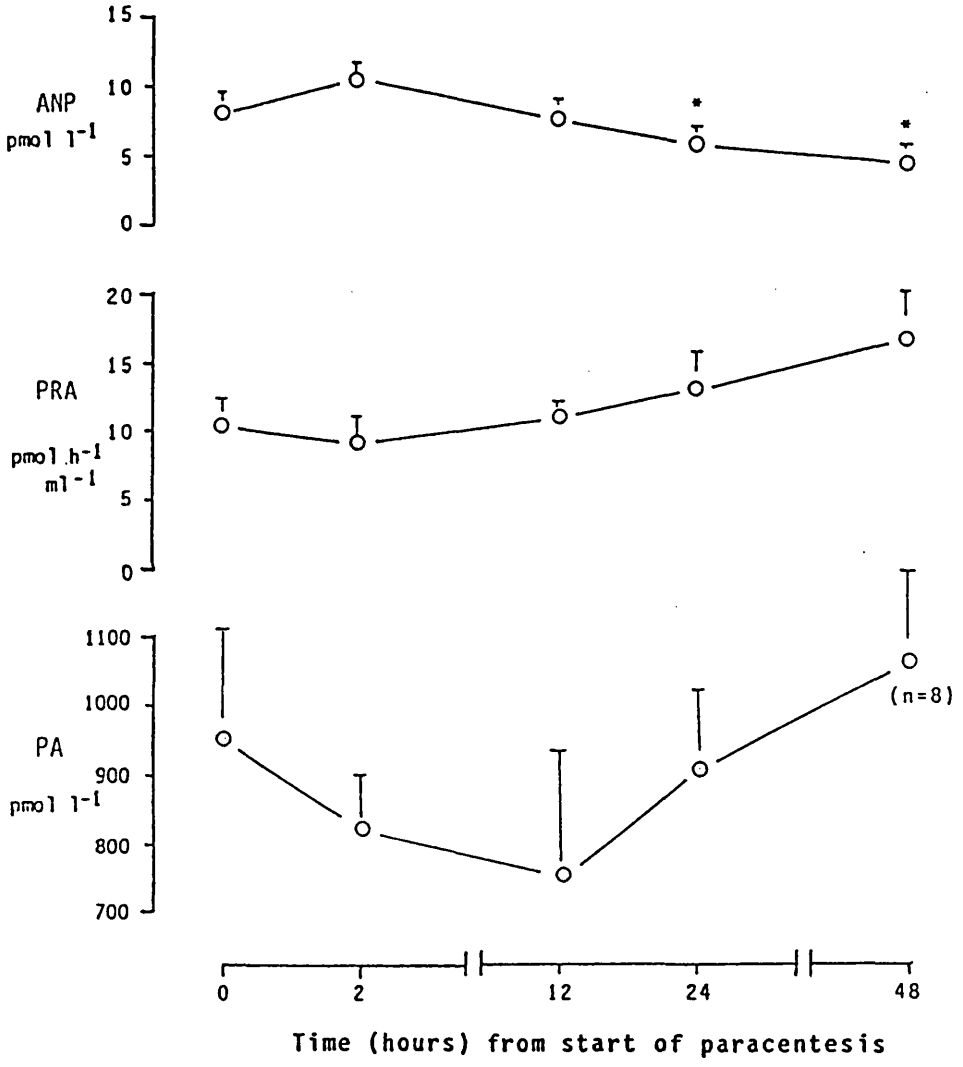


Fig. 11. Changes in plasma atrial natriuretic peptide (ANP), plasma renin activity (PRA) and plasma aldosterone concentration (PA) associated with paracentesis (n=12).
Note: Paired 't' test. Results compared to baseline (p values multiplied by 4). * p <0.05

for ANP to rise and for plasma aldosterone concentration and plasma renin activity to decrease (Table 7, Fig. 11). At 24 and 48 hours the ANP level was lower than baseline ($p<0.05$) and PRA and plasma aldosterone tended to increase. Compared to baseline, there was no significant change in haematocrit (n=12).

In the group of 21 patients, compared to baseline there was a tendency for serum creatinine to rise (85.5±7.0 to 91.9±7.8 μmol/l) and for serum sodium to drop (132.8±1.1 to 130.9±1.3 mmol/l) at 48 hours.

There were no life threatening complications in the first 48 hours of paracentesis and no deaths occurred in the first 3 weeks after paracentesis. Three patients complained of a feeling of fatigue in the first few hours of paracentesis which resolved within 12 hours. This symptom was not related to any identifiable haemodynamic deterioration.

Echocardiography

In none of the 8 patients with tense ascites studied was there any evidence of left or right ventricular wall motion, valvular abnormalities, or ventricular hypertrophy. In all cases ascitic fluid appeared to compress the right atrium (Fig. 12b). The RA/LA ratio was significantly lower (0.54±0.04) compared to age matched normal controls (0.84±0.04, $p<0.0001$) and patients with cirrhosis and minimal ascites (0.82+0.02, $p<0.0001$), (Fig. 12a). The baseline intra-abdominal pressure was significantly correlated with RAP (r 0.76, $p<0.05$). In the 2 patients in whom echocardiography was carried out before and after paracentesis the RA/LA ratio increased from 0.54 to 0.72 and from 0.35 to 0.70.

Fig. 12

Fig. 12a

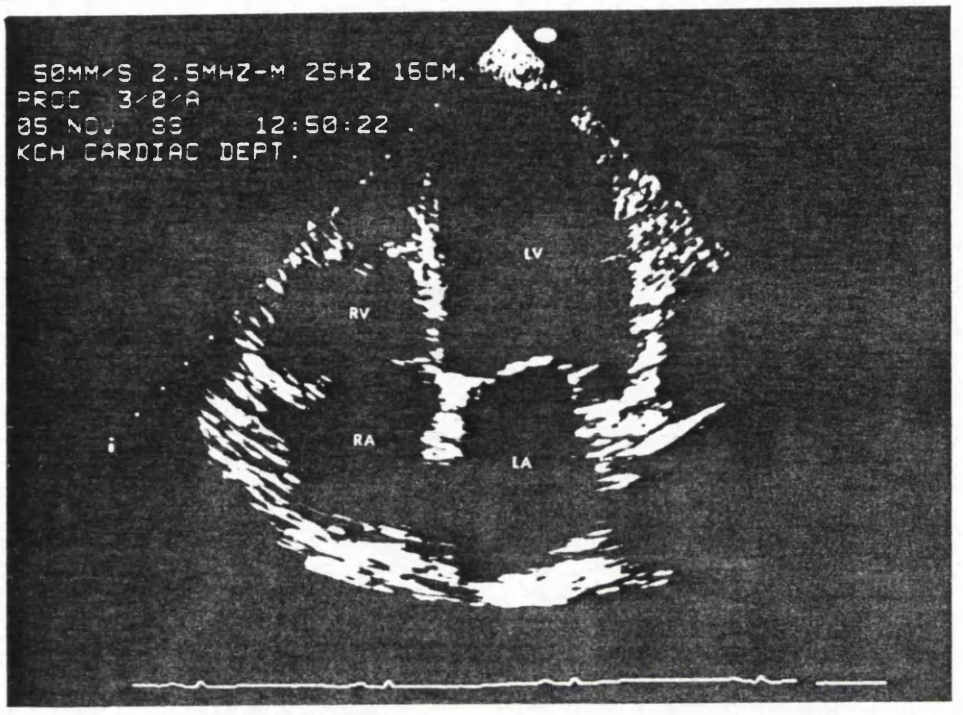


Fig. 12b

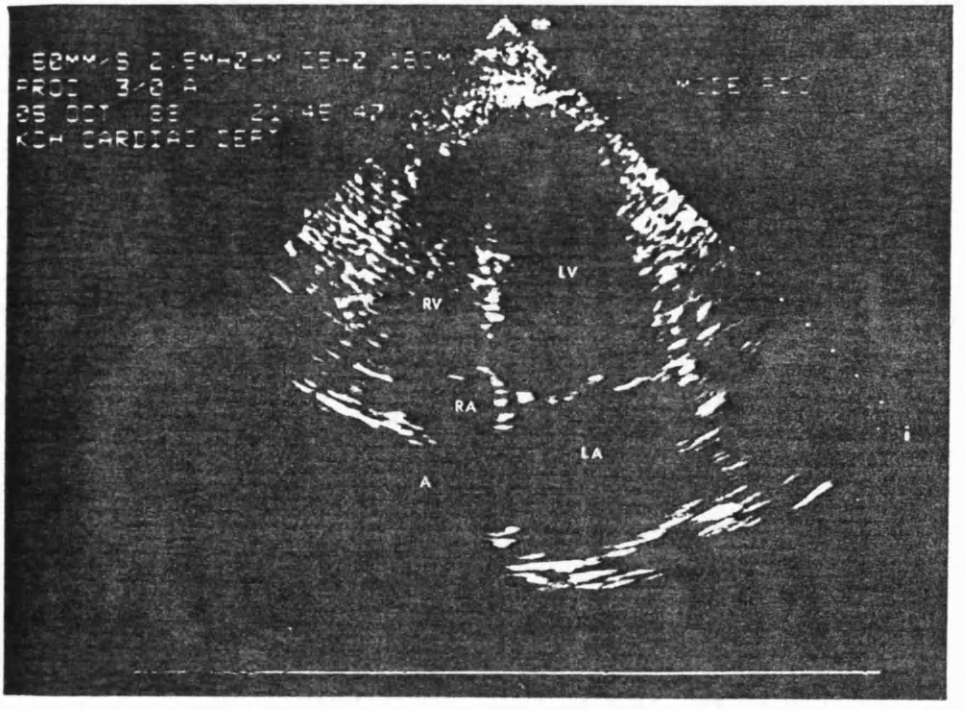


Fig. 12. Echocardiograms recorded from the apical 4 chamber approach.
Note: **RA**= Right Atrium, **RV** = Right Ventricle, **LA** = Left Atrium, **LV**= Left Ventricle, **A** = Ascites .
Fig. 12a. Patient with minimal ascites with a normal RA/LA ratio.
Fig. 12b. Patient with tense ascites showing compression of the right atrium with a reduced RA/LA ratio.

4.4 Discussion

Previous studies examined haemodynamic changes during staged withdrawal of ascitic fluid (250,252), or limited volume paracentesis (257), or have followed the haemodynamic changes for only up to 24 hours after the start of paracentesis (250,252). The present study examines the changes occurring over 48 hours, during rapid, total paracentesis of ascites volumes greater than 8 litres and incorporates echocardiographic data and hormonal measurements.

The known characteristics of ANP release render this peptide particularly useful in the interpretation of haemodynamic changes, since increases in its plasma concentration would be expected to occur through atrial stretch, independently of atrial pressure (which may be influenced by extrinsic compression), as has been shown in cases of cardiac tamponade (186).

Basal haemodynamic abnormalities in cirrhosis with ascites have been described previously (19,20,62,75,258). The present study suggests that the haemodynamic changes induced by large volume paracentesis are complex, involving both cardiac and peripheral circulatory mechanisms. The haemodynamic status of patients with tense ascites improved during the first 3 hours of paracentesis, after which there was a period of deterioration consistent with progressive systemic hypovolaemia. The consistent improvement in haemodynamic indices within the first 3 hours of paracentesis was accompanied by a tendency of plasma ANP to increase. The tendency of ANP to rise, with increasing cardiac output and unchanged pulmonary capillary wedge pressure, despite decreasing right atrial pressure during the first 3 hours, coupled with the echo-

cardiographic findings of right atrial compression before paracentesis, suggest that paracentesis resulted in distension of the right atrium as a result of relief of extrinsic compression and increased venous return. These results are in agreement with those of Guazzi et al (249), who reported a significant increase in cardiac output, stroke volume and right and left ventricular stroke work during paracentesis of up to 7 litres, in patients with a low baseline cardiac output. In addition, the present results suggest that even in patients with normal or increased resting cardiac output, venous return may be impaired due to inferior vena cava and right atrial compression by tense ascites. In such cases drainage of ascites may lead to increased venous return, with an increase in cardiac output and reflex inhibition of sympathetic vasoconstrictor tone (48,108,259). The decrease in mean arterial pressure after 60 minutes, was most likely due to the observed reduction in systemic vascular resistance, since cardiac output rose.

The haemodynamic changes after 3 hours and up to 12 hours, were probably determined by relative hypovolaemia, due to progressive re-accumulation of ascites. These findings suggest that for the rapidity of ascites drainage employed in the present study, the most appropriate time for commencing the infusion of plasma expanders - a therapy shown to protect from hypovolaemia and renal impairment (254,255) - would be between 3 and 12 hours.

Between 12 and 24 hours cardiac output and systemic vascular resistance returned to baseline, and RAP PCWP and MAP remained low or continued to decrease. Between 24 and 48 hours the findings were consistent with progressive hypovolaemia as indicated by a significant

decrease in plasma ANP and a tendency for PRA to rise. Serum creatinine change, though not significant, was compatible with slight deterioration of renal function and is in agreement with the findings of Simon et al (252), who reported a significant drop in endogenous creatinine clearance, 48 hours after staged, large volume paracentesis, without infusion of plasma expanders. The same authors found no change in ANP one hour and twenty four hours after completion of paracentesis, and infered that the early increase in cardiac output occurred without concomitant distension of the right atrium. However, early changes in ANP and the initial haemodynamic changes, would not have been detected in their study. The slower removal of ascites employed (252) and their use of non-extracted plasma for ANP assay (218-220) may account for apparent discrepancies with the present data.

In the present study, as in the study of Simon et al (252), there was no difference between patients with and without peripheral oedema, either in haemodynamic measurements or in electrolyte and creatinine estimations at any time point during or after the proceedure. It was previously suggested that the presence of peripheral oedema may protect intra-vascular volume by allowing mobilisation of fluid from the periphery (257,260). While this mechanism may be effective where up to 5 litres of ascites is drained, as in the study of Kao et al (257), the presence of peripheral oedema clearly does not prevent hypovolaemia when larger volume of ascites is drained. The present findings support the recommendation that infusion of plasma expanders should accompany paracentesis of greater than 5 litres, even in the presence of peripheral oedema (261).

CHAPTER 5

Atrial Natriuretic Peptide and Renin-Aldosterone in paracetamol-induced fulminant hepatic failure

5.1 Introduction

Volume homeostasis is frequently impaired in patients with fulminant hepatic failure, with sodium retention and an altered haemodynamic state of high cardiac output and a low systemic vascular resistance (98,262,263). Bernardi et al, reported high plasma renin and aldosterone concentrations in fulminant hepatic failure with no correlation between plasma aldosterone concentration and renal sodium excretion (264). Wilkinson et al (98), postulated that a deficiency of a putative circulating natriuretic factor, or resistance to such a factor, may contribute to the sodium retention. Circulating concentrations of the atrial natriuretic peptide (ANP), in fulminant hepatic failure, have not been reported previously. The known natriuretic property of ANP and its suppressor action on the renin-aldosterone system, prompted the present study, in order to investigate whether ANP could be the putative natriuretic factor postulated by Wilkinson et al.

In this study, plasma concentration of ANP, renin activity and aldosterone, were measured in patients with fulminant hepatic failure. The possible relationship of ANP to the renin-aldosterone system was examined by investigating the response of these factors to fluid volume and right atrial pressure (RAP) changes, induced by haemodialysis and by the infusion of 5% human albumin solution (HAS).

5.2 Patients and Methods

Thirty eight patients, 18 men and 20 women (age range 17 to 66 years) with fulminant hepatic failure (development of encephalopathy within 8 weeks of the onset of hepatic symptoms, in the absence of underlying liver disease) from paracetamol overdose were studied. Patients

were excluded if: a) there was a past history of heart disease or arrhythmias; b) if they were receiving inotropic support; c) if the mean arterial pressure (MAP) was less than 60 mmHg; d) if there was a greater than 15% change in pulse rate, MAP or right atrial pressure (RAP) on three occasions one hour apart in the three hours before study; e) if there was rapidly changing coma grade (265) in the three hours before study or if there were any clinical signs of cerebral oedema during the preceeding 24 hours ; f) if there was any evidence of haemorrhage. Central venous access was obtained via the subclavian or internal jugular vein at least two hours prior to entry into the study.

The patients were allocated to one of the following 3 groups: Group 1 (12 patients), comprised patients with no or only mild renal impairment, defined as: plasma creatinine <300 µmol/l and urine output >600ml/24 hours or 300ml/12h. Group 2 consisted of twenty one patients with severe renal failure, defined as: plasma creatinine >300 µmol /litre and urine output <100ml/ day. Group 3 comprised five patients who were recovering from fulminant hepatic failure following paracetamol overdose, with persisting severe renal failure requiring dialysis (predialysis plasma creatinine >500 µmol/litre and urine volume <100ml/24 hours), and no evidence of encephalopathy. From the latter group, blood samples were taken at least 18 hours after any previous dialysis. Group 3 was included as a "renal failure" control group. As controls, a group of twelve normal volunteers (age range 33 to 62 years) on a 22 mmol sodium daily restricted diet for 5 days prior to entry, had venous blood samples taken without stasis, for ANP at 0900 hours while semi-recumbent (trunk 45° to the horizontal) for 30 minutes, after an overnight fast. Their arterial blood pressure was

measured by a mercury column sphygmomanometer (mean of 3 readings, Korotkov sounds II and V). Mean arterial pressure was derived by adding 1/3 pulse pressure to the diastolic pressure.

To allow for stabilisation, patients were studied not less than 12 hours after admission. All patients were studied in the semi-recumbent position. The level of the right atrium, taken as corresponding to the surface marking of the fifth intercostal space in the mid-axillary line, was marked with permanent ink and the same reference point was used for all RAP estimations. All patients were receiving 5% Dextrose intravenously (1.5-2.0 litres daily) and saline infusion was not administered for at least 24 hours preceding the study. Potassium supplements were given intravenously to maintain plasma levels between 4.0-5.0 mmol/litre. None of the patients had received mannitol in the 24 hours prior to entry. Arterial pressure and RAP were measured via an indwelling intra-arterial cannula and a central venous line respectively (Simonsen and Weel Ltd, series 9000; Ames transducer AE 840). Mean arterial blood pressure and RAP was derived electronically (mean of 3 readings).

Baseline biochemistry, prothrombin time and grade of encephalopathy at the time of entry are shown in Table 8.

5.2.1 Basal assessment of ANP and hormonal status

At 0900h measurements of pulse rate, arterial blood pressure and RAP were taken and peripheral venous blood was drawn without stasis for ANP, renin, aldosterone, creatinine, sodium and potassium, bilirubin and prothrombin time estimation. Samples for ANP and renin-aldosterone were taken into chilled tubes containing EDTA and were centrifuged at 4°C for

TABLE 8

Serum biochemical measurements and prothrombin time (seconds prolonged) at the time of entry into the study for normal controls and three groups of patients with fulminant hepatic failure.

Group 1: No or mild renal failure; **Group 2**: Severe renal failure;
Group 3: Recovering fulminant hepatic failure with persisting severe renal failure.
Values expressed as median (range).

	Group 1 (n = 12)	Group 2 (n = 21)	Group 3 (n = 5)	Control (n = 12)
Creatinine (μmol/litre)	104 (53-259)	566 (337-1091)	719 (607-1100)	93 (57-103)
Sodium (mmol/l)	132 (129-137)	130 (129-133)	127 (112-133)	140 (138-143)
Bilirubin (μmol/l)	93 (51-138)	149* (80-265)	58 (37-162)	8 (3-15)
Albumin (g/litre)	35 (32-41)	36 (31-40)	36 (31-41)	43 (39-46)
Prothrombin time (secs. prolonged)	42 (25-92)	96* (29-186)	33 (1-48)	--
Encephalopathy (no. patients) Grade I-II	6	6	0	0
Grade III-IV	6	15	0	0

Note: Group 2 values compared to Group 3. * $p < 0.05$.

8 minutes within 10 minutes of collection. Plasma was separated and stored at -70°C until assayed.

In cases where urine could be obtained, a timed collection was taken between 0800-0900 hours, via an indwelling urinary catheter, for urine sodium estimation. In the case of 6 anuric patients in Group 2, urine was collected whenever available, over 2-24 hours.

5.2.2 Effect of fluid volume changes

The effect of haemodialysis was studied in fourteen patients from group 2. After one hour of baseline observation, measurements and peripheral venous blood samples were taken just before and 90 minutes after completion of haemodialysis, in order to allow circulating ANP concentration to reach a new steady state post-dialysis. Dialysis was carried out using a Biospal 2400S cartridge in a Monitral 5 dialysis unit. Patients developing hypotension during haemodialysis (systolic blood pressure <90 mmHg) received measured volumes of 5% Human Albumin Solution. For all patients 150ml 0.9% sodium chloride solution containing heparin, was used to "prime" the extracorporeal dialysis circuit and was accounted for in the final fluid balance estimation. Two patients developed cerebral oedema during dialysis which was abandoned and their data excluded from the final analysis.

The effect of plasma volume expansion was studied in six patients from group 1, who were being monitored by a pulmonary artery flotation catheter. Patients with a low RAP (≤2mmHg) and pulmonary capillary wedge pressure (≤5mmHg) for more than 30 minutes received an infusion of 900 ml of Human Albumin Solution over a period of 40 minutes, to a maximum RAP of 5-6mmHg or pulmonary capillary wedge pressure of 10-12 mmHg.

Venous blood samples were taken just before and 40 minutes after the end of the infusion. Urine was collected for the hour preceding the infusion and for a second hour after the albumin infusion was completed, for measurement of urine volume and sodium concentration .

5.2.3 Assays

All samples for ANP were assayed in a single assay run after plasma extraction, as described in Chapter 2 (197,210). Plasma renin activity (PRA) was measured by the generation of Angiotensin I, by a modification of the method of Menard and Catt (239), and aldosterone concentration with a Sorin radioimmunoassay kit (CIS, High Wycombe, Bucks) after solvent extraction of plasma (240). Serum creatinine was estimated by auto-analyser and plasma and urine sodium by flame photometry.

Results are expressed as median and range values. Statistical analysis was by the Wilcoxon two sample rank sum test and the Wilcoxon signed rank test for paired values, as appropriate. Tests of correlation were by the Spearman rank test. Level of statistical significance taken as $p < 0.05$.

5.3 Results

5.3.1 Basal assessment

There was no difference between ANP concentration in controls and patients with no or mild renal failure (Group 1), (2.0-9.0, median 4.15 and 1-28.6, median 6.1 pmol/l respectively). Concentrations were higher in patients with severe renal failure (group 2) when compared to controls or patients with no or mild renal failure (median 10.1, 1-25 pmol/l, $P<0.001$, Fig. 13). Values for RAP were similar in groups 1 and 2 (Table 9).

Fig. 13

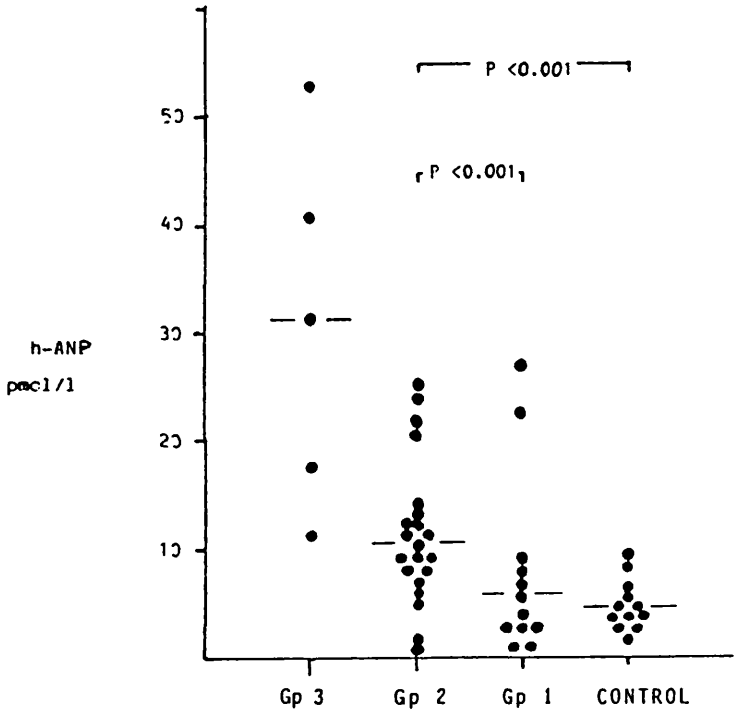


Fig. 13. Basal plasma concentrations of atrial natriuretic peptide (ANP) in fulminant hepatic failure with no or mild renal failure (Group 1), severe renal failure (Group 2) and patients recovering from fulminant hepatic failure with persisting severe renal failure (Group 3), and in control subjects (CONTROL).

TABLE 9

Basal levels of right atrial pressure (RAP), mean arterial pressure (MAP), plasma renin activity (PRA) and concentrations of plasma atrial natriuretic peptide (ANP), aldosterone (ALDO) and plasma creatinine (Creat). Values expressed as median (range) and are compared to normal controls.

	RAP (mmHg)	MAP (mmHg)	ANP (pmol/l)	PRA (pmol/h/ml)	ALDO (pmol/l)	Creat. (μmol/l)
Group 1 (n = 12)	5.25 (-0.5-16)	83 (60-93)	6.1 (1-28.6)	6.8 (1.39-22.4)	637* (202-1917)	104 (53-259)
Group 2 (n = 21)	6.12 (2-18)	90 (61-112.5)	10.1 (1-25)	19.8* (1.04-41.7)	2176** (199-6894)	566 (337-1091)
Group 3 (n = 5)	--	86.5 (80-93.5)	31 (11.2-53.2)	6.94* (1.12-14.7)	580 (196-1680)	719 (607-1100)
Control (n = 12)	--	77.8 (70-105)	4.15 (2-9)	2.86 (1.87-5.9)	368 (133-578)	93 (57-103)

*p <0.02, **p <0.01.

In Group 3, pre-dialysis ANP levels were even higher (11-53.2, median 31.5 pmol/l) when compared to controls (P<0.003).

There was a positive correlation between ANP and RAP levels (R 0.67, P <0.02) within group 1, but not within group 2 (predialysis). In group 1 cases, ANP levels correlated weakly with plasma creatinine (R 0.6, P<0.05). There was a positive correlation for pooled values of plasma ANP and creatinine for groups 1, 2 and 3 (R 0.50, P<0.002). There was no correlation between ANP and mean arterial pressure (MAP) in any group.

PRA tended to be higher in all patient groups compared to controls, although statistical significance was not reached for group 1 (0.05< P <0.1), (Table 2). There was an inverse correlation between PRA and MAP in patients in group 1 (R -0.78, P <0.007, Fig. 14), but not in group 2.

Aldosterone levels were raised in all patient groups compared to controls (Table 2). There was an inverse correlation between aldosterone and MAP levels in group 1 (R -0.73, P <0.02, Fig. 14), but not in group 2. There was a strong correlation between PRA and aldosterone concentration for combined values from all patient groups (R 0.978 P <0.0001).

Serum sodium tended to be lower in all three groups of patients, as compared to controls (Table 8).

Urine samples were available from only 6 patients in group 2 at 0900 hours, collected over periods ranging from 2-24 hours (total urine volume 5-80 ml). Median urine sodium excretion in these was 0.17 mmol/hour (range 0.06-0.90 mmol/h). Urine was available from all 12 patients in Group 1. Baseline urine sodium excretion was median 0.22 mmol/h (range 0.06-6.46 mmol/h). The distribution was skewed: In 10 of the 12 patients urine sodium was less than 0.4 mmol/h, in one 0.9 mmol/h and in the remaining

Fig. 14

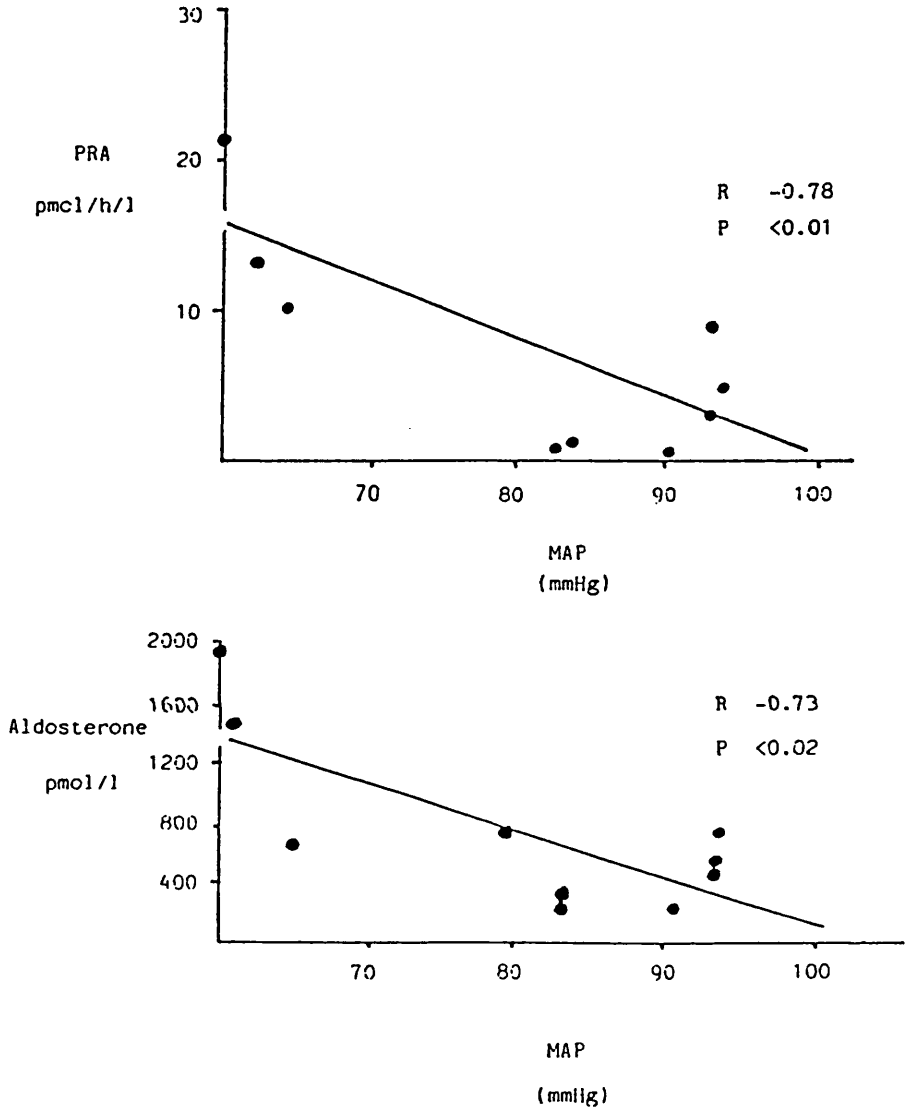


Fig. 14. Plasma renin activity (PRA), aldosterone concentration and mean arterial blood pressure (MAP) in fulminant hepatic failure with no or mild renal failure (group 1).

patient 6.46 mmol/h. There was no correlation between urine sodium excretion and ANP, PRA or aldosterone.

Response to volume changes

(i) Effect of haemodialysis:

Of the twelve patients who completed haemodialysis, 4 were in negative fluid balance, 6 in positive and 2 in approximate zero balance (+0.13 and +0.08 litres). Duration of haemodialysis was between 3.5 and 4.25 hours. Fluid volume change at completion, ranged from -1.35 to +1.0 litres, and change in RAP was -9.5 to +6.0 mmHg. Median pre- and post-dialysis plasma ANP levels were 9.67 (range 1-28.6) and 11.75 pmol/l (range 1-28.4) respectively. There was a positive correlation between change in RAP with change in ANP and between volume change and change in ANP (Fig. 15). Although PRA and aldosterone concentrations were decreased post-dialysis (median 23.8 to 18.3 pmol/l/h and 3621 to 2295 pmol/l respectively, P <0.03) these changes did not correlate with fluid volume or RAP change. There was no correlation between haemodialysis-induced change in ANP with change in PRA or aldosterone.

(ii) Response to albumin infusion:

Pre-infusion PRA and aldosterone concentration were markedly above the normal range (Tables 9 and 10). Haematocrit dropped significantly after albumin infusion, (median 40% range 28-44%, to 37% range 27-40% , P <0.05). There was a significant rise in RAP (P <0.05) and plasma ANP concentration (P <0.05) with a corresponding decrease in PRA (P <0.05, Table 10). Aldosterone concentration dropped in 5 patients and rose in one (not statistically significant) and there was no significant change in urine volume or sodium excretion (Table 10). Urine microscopy of pre-infusion

Fig. 15

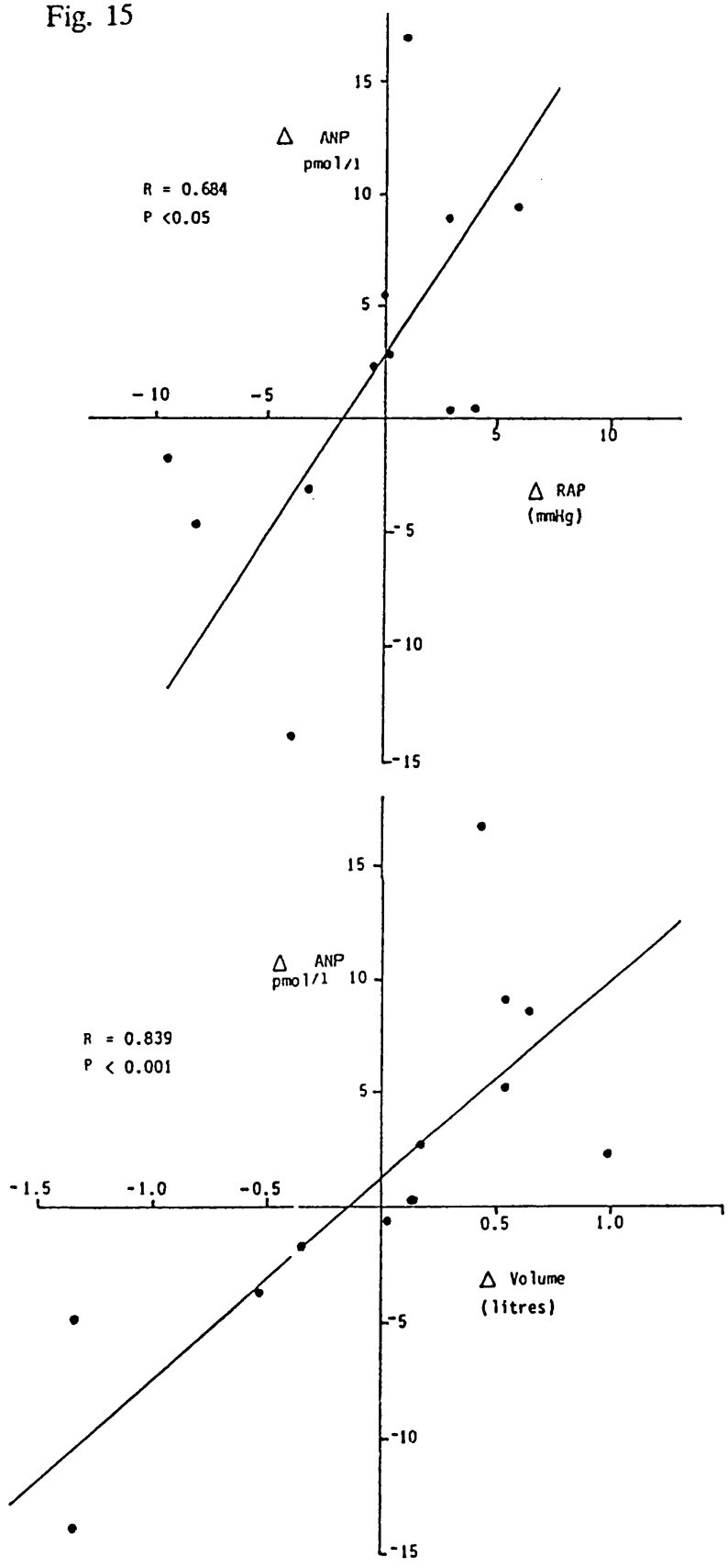


Fig. 15. Haemodialysis-induced net fluid volume balance (Δ Volume) and change in atrial natriuretic peptide concentration (Δ ANP) and changes in right atrial pressure (Δ RAP).

TABLE 10

Patients with no or mild renal failure before and after volume expansion with 900 ml of 5% human albumin solution (n = 6). Hourly urinary volume (Uvol) and sodium excretion (UNa). Other abbreviations as in Table 9. Values expressed as median (range).

	RAP (mmHg)	MAP (mmHg)	ANP (pmol/l)	PRA (pmol/h/ml)	ALDO (pmol/l)	UNa (mmol/h)	Uvol (ml/h)
Before	2 (-0.5-3)	62.5 (57-93.3)	3.75 (1-9.4)	13.45 (4.16-19.8)	693 (143-1830)	0.175 (0.06-6.46)	58 (30-102)
After	5.25* (3-7)	69.75 (58.5-93.3)	10.1* (6.2-23.3)	8.85* (1.39-17.7)	668 (202-1760)	0.42 (0.16-5.52)	80 (35-80)

*p <0.05.

samples revealed no casts in any of the 6 samples. Urine/plasma osmolality ratio was less than 1.09 in two cases and greater than 1.1 in four.

5.4 Discussion

Studies of the renin-aldosterone system and of electrolyte and water balance are usually carried out after a period of several days on a controlled sodium and potassium intake. Because of the rapid changes in the clinical condition of patients with fulminant hepatic failure, it is not possible to do this. Saline infusion is avoided in these patients because of their tendency to retain sodium. For this reason patients are compared to healthy controls on severe sodium restriction. Although the criteria for inclusion were such that only relatively stable patients were entered, these limitations must be considered when interpreting the present results. Since patients did not receive saline for 24 hours prior to the study, it would be difficult to identify those with inappropriate sodium retention on the basis of urine sodium excretion alone. Therefore the "dynamic" test of volume expansion was employed in six patients who were not oliguric (Group 1). Although specific tests to assess renal tubular function (urine acidification, excretion of amino acids and tubular proteins) were not performed in these 6 cases, the absence of casts in the urine suggests that acute tubular necrosis was not present at the time of study. The separation of patients into those with no or mild renal failure and severe renal failure according to plasma creatinine and urine volume was empirical, and was based on findings from a previous study by Wilkinson et al (262), in order to study patients at two ends of a continuous spectrum of renal impairment. In another study of paracetamol-induced fulminant hepatic failure with renal failure, Wilkinson et al (266),

reported that functional renal failure was present in about a third of cases, pre-renal uraemia in 10-15% and direct renal toxicity due to paracetamol causing acute tubular necrosis, in more than 50% of cases. It is likely that most patients in Group 2 belonged to the last category. Estimates of 24 hour urine sodium excretion from the data of Group 2 may be unreliable, since collections were available from only 6 of 21 patients and were collected over different lengths of time. In addition, in this group of patients, during a 24 hour period of observation, urine is often passed intermittently and urine volume may vary unpredictably from zero to a few millilitres per hour. However, it seems likely that the anuric patients of Group 2 would be retaining sodium.

As saline was not administered for 24 hours prior to study, it is possible that the observed hyponatraemia in patients, might be due to failure to generate free water, either due to inappropriately high levels of anti-diuretic hormone or marked proximal reabsorption of sodium.

The present results show that there is no deficiency of ANP in fulminant hepatic failure. In the presence of no or mild renal failure, basal ANP levels were similar to controls but in patients who had developed severe renal failure, they were raised. In patients recovering from fulminant hepatic failure with persisting severe renal failure ANP levels were even higher. These findings, along with the correlation of ANP to plasma creatinine concentration, suggest that the raised levels of ANP in fulminant hepatic failure are related to the presence of renal failure. The finding of similar RAP values in Groups 1 and 2 would be compatible with the hypothesis that in the presence of renal failure, factors other than increased atrial distension may be contributing to the raised

levels of ANP, although it is clearly possible that atrial volume may increase without a rise in atrial pressure. The importance of the kidney in the clearance of circulating ANP has been shown in previous studies of adults undergoing cardiac catheterisation, which showed significant extraction of circulating ANP by the kidney (267), and by receptor-distribution (191) and ANP clearance (191,192,268) studies in animals. Maack et al (191), have shown that in rat kidney more than 95% of ANP receptors are silent, in that ANP binding occurs without a functional response or cGMP generation. Blockade of these receptors by a ring-deleted analogue, markedly increased plasma ANP concentration, suggesting an important clearance role for these receptors. In the canine model, extraction of ANP by the kidney has been shown to be largely dependent on glomerular filtration rate (192). Marked renal vasoconstriction with reduced glomerular filtration rate, is common in fulminant hepatic failure (263), and thus may contribute to reduced extraction of ANP.

Basal aldosterone concentration and PRA was raised in all patient groups (269,270), although statistical significance was not reached in Group 1. In all groups aldosterone concentrations correlated well with PRA, suggesting that renin remains the major determinant of aldosterone levels in fulminant hepatic failure (271). In the absence of severe renal failure, PRA and aldosterone concentration were inversely proportional to MAP, supporting previous observations which suggested that stimulation of the renin-aldosterone system may be a homeostatic response to hypotension (264).

The correlation of changes in ANP with volume and right atrial pressure changes induced by haemodialysis, for positive and negative net

fluid balance, indicates that known mechanisms involved in the plasma release of ANP, are not impaired in fulminant hepatic failure. This was confirmed by the rise in circulating levels of ANP in response to albumin infusion in six patients with no or mild renal failure. The absence of natriuresis or diuresis in the latter, is compatible with the hypothesis that resistance to ANP may contribute to sodium retention. It is possible that over-activity of renin-aldosterone, which did not suppress to normal after volume expansion, may antagonise the action of the atrial natriuretic peptide or that there may be "intrinsic" renal resistance to the natriuretic action of ANP in fulminant hepatic failure. Further study of these aspects is required.

CHAPTER 6

Effect of infusion of synthetic ANP on the isolated kidney of rats

6.1 Introduction

Deficiency of, or resistance to a putative circulating natriuretic substance has been postulated as a possible mechanism, contributing to sodium retention in cirrhosis (17,98,235). Plasma concentration of atrial natriuretic peptide (ANP) is often increased in this condition, in the presence of sodium retention (Chapter 2, 235,273,274). In plasma, ANP seems to be present in its active form: Gerbes et al (273) and Arendt et al (275), reported that most circulating atrial natriuretic peptides in cirrhosis corresponded to the biologically active 3,080 dalton ANP1-28, and Jimenez et al (276), found no difference in the chromatographic pattern and biological activity of ANP in plasma of patients with cirrhosis and ascites, as compared to controls. These findings raise the possibility that in cirrhosis, there may be renal resistance to the natriuretic action of ANP. In support of this, Salerno et al (272) reported diminished natriuretic response to bolus injection of ANP in patients with cirrhosis and ascites, as compared to controls. Similar findings were reported by Lopez et al following the *in-vivo* infusion of ANP in cirrhotic rats (277). Koepke et al (278) also reported diminished natriuretic response to ANP in cirrhotic rats which reverted to normal, after renal sympathetic denervation.

In-vivo administration of ANP can not distinguish between direct and secondary renal effects, such as those mediated by the central nervous system (279), or those influenced by changes in arterial pressure (280). Therefore, apparent renal insensitivity to ANP *in-vivo*, may result from changes outside the kidney. In the present study we examined the natriuretic response to ANP of the isolated perfused kidney of rats with

experimentally induced cirrhosis. In this preparation, the immediate influence of external neural and humoral factors is removed, and perfusion pressure, a critical determinant of sensitivity to ANP (280), can be rigorously controlled.

6.2 Methods

6.2.1 Induction of cirrhosis

Eleven male Wistar rats were studied. Cirrhosis was induced in 6 animals by a modification of the method described by Proctor and Chatamra (281). The initial weights of the animals were 152±8.8g for the control (n=5) and 160±6.5g for the cirrhotic group (n=6). After an induction phase of 12-14 days during which rats were provided with drinking water containing phenobarbitone 350mg/l as their sole source of fluid, an initial dose of carbon tetrachloride (0.04ml) was administered by gavage under light ether anaesthesia. The response of individual rats to this was determined by the initial weight loss induced and the subsequent recovery during the following week. Increasing doses (to a maximum of 0.2ml) of carbon tetrachloride were administered at weekly intervals, the amount given to each rat being determined by the change in weight observed after the previous dose. The rats continued to have access only to water containing phenobarbitone. Within 12-14 weeks cirrhosis developed in all rats. This was reflected by an increase in Normotest clotting time of blood obtained by tail snip, median 40.6 seconds (range 29.6-43.7 s, n=5) in control animals and 43.5 s (range 35.1-49.7 s, n=6) in cirrhotic animals, and an increase in rat weight suggestive of ascites formation. After this time, no further doses of carbon tetrachloride were given and water free of phenobarbitone was provided. Sodium handling *in-vivo* was

assessed after 15 weeks and isolated renal perfusion performed between 16.5 and 29 weeks.

The control rats were age matched and received phenobarbitone throughout the 14 week period, in the same manner as the experimental animals.

6.2.2 Sodium excretion *in-vivo*

Rats were placed in individual metabolic cages 36 hours prior to study, fed a commercial chow diet (Beekay feeds; protein content 16.7%, sodium content 0.21%) and allowed free access to tap water. Under light ether anaesthesia, from which the animals recovered within a few minutes, 5 ml of a solution containing 2 mmol sodium chloride was administered by gavage. The rats were then immediately returned to their cages, where they had access to water but not to food. Urine collections were made under oil, every 2 hours, for 6 hours.

6.2.3 Effect of ANP infusion on the isolated kidney

Studies were carried out on the right kidney of the same animals on which the *in-vivo* studies had been performed. After the *in-vivo* studies, rats continued to receive free access to the same diet and water up to the time of the isolated kidney experiments. They were anaesthetised by intraperitoneal injection of pentobarbital sodium (60mg/kg body weight; Sigma) and the right kidney was perfused without ischaemia using the technique described by Nishiitsutsuji-Uwo et al (282). A double barrelled perfusion cannula was used, as previously described (280): perfusate passed into the kidney through the outer cannula; perfusion pressure within the renal artery was monitored directly through the fine inner

Fig. 16

CIRCUIT FOR ISOLATED KIDNEY PERFUSION

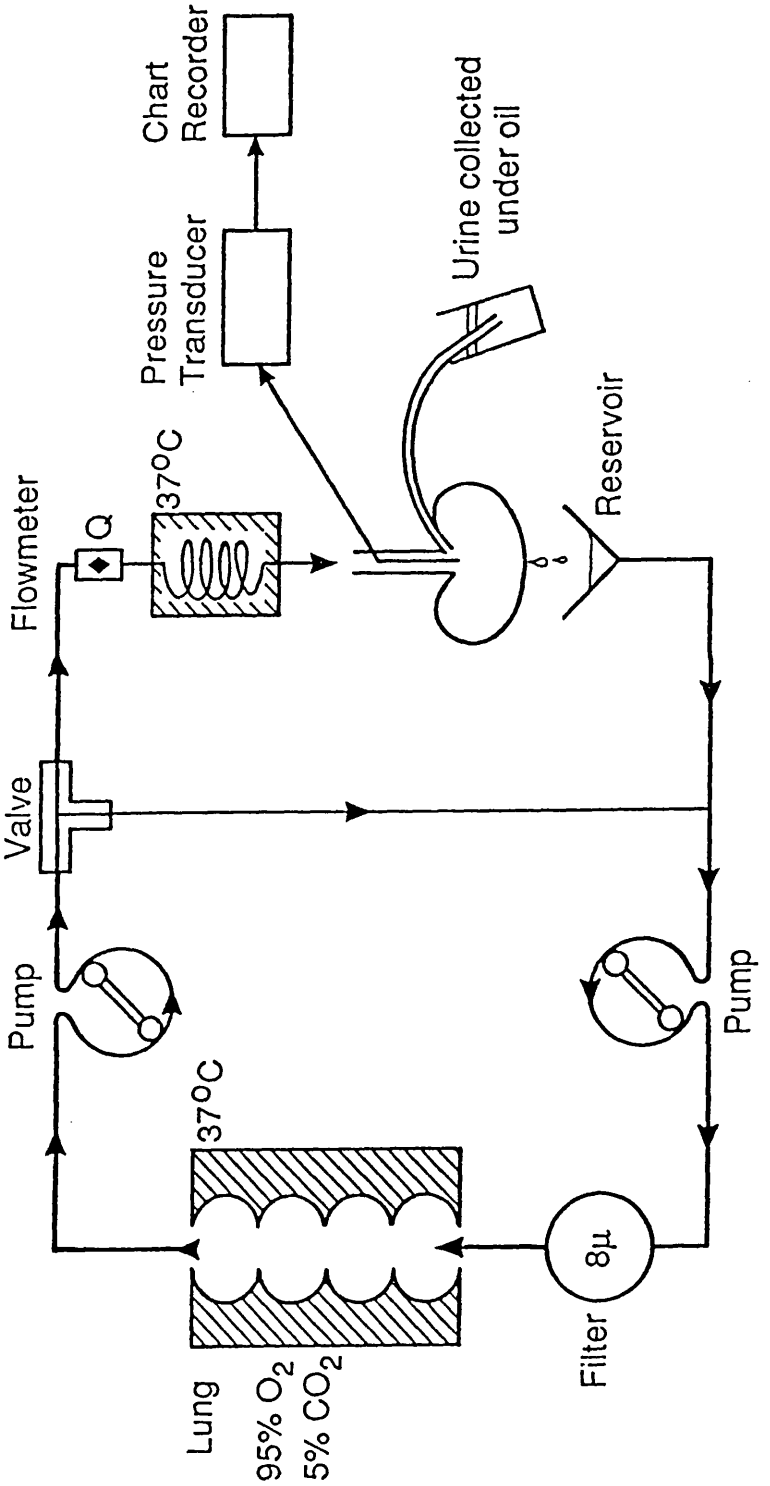


Fig. 16. Schematic representation of perfusion circuit for isolated kidney preparation.

cannula, which was also used to record intra-aortic pressure immediately before cannulation of the renal artery. The ureteric catheter consisted of a 12mm length of PP-10 tubing connected to a 10 cm length of PP-50 tubing (280). The perfusion medium contained 6.7% (w/v) dialysed bovine serum albumin fraction V (Miles) in Krebs-Henseleit buffer, glucose (5 mmol/l) and all 20 physiological amino acids in concentrations previously reported (283). The medium was continually gassed with a warmed and moistened mixture of 95% oxygen and 5% carbon dioxide. Temperature was controlled at 37°C and pH at 7.35-7.40. Perfusate flow was recorded by an in-line flow meter proximal to the arterial cannula (Jencons, GPEL16X).

Kidneys were perfused for a short period on open circuit to remove all traces of blood before being transferred to a recirculating unit with a capacity of approximately 120 ml (Fig. 16). ^{14}C-inulin (Amersham International) was then added to the perfusate to permit estimation of glomerular filtration rate by inulin clearance. Immediately after recirculation of medium had been established, a mannually operated valve was adjusted to maintain a perfusion pressure of 110 mmHg, which was kept constant throughout the experiment. This perfusion pressure was chosen on the basis of findings from a previous study which indicated that the natriuretic effect of ANP on the isolated kidney of normal Wistar rats is pressure dependent (280). After a 30 minute equilibration period, a 10 minute urine collection was taken to determine baseline function.

Synthetic 1-28 rat ANP (Sigma) was added in sequential boluses to the circuit reservoir to produce final perfusate concentrations of 10, 50, 200 and 1000 pmol/l. At each concentration a 10 minute urine collection

was taken after a 5 minute wash-out period to allow for urinary dead space (284). Perfusate samples were taken at the mid-point of each urine collection period. Perfusate volume was maintained constant by infusion of a mixture of Krebs-Henseleit buffer/water (50:50 v/v) as necessary.

All urine samples were collected under light paraffin oil in pre-weighed tubes and volume was determined by weight. When necessary, samples were stored at -20°C prior to analysis. Sodium was analysed by flame photometry (Instrumentation Laboratory, model 543) and ^{14}C-inulin activity was determined using a flat-plate scintillation counter.

All assays were performed in duplicate and the mean value was used in calculations. Inulin clearance, absolute sodium excretion and fractional sodium excretion were calculated by use of standard formulae. All data have been expressed per gram left kidney wet weight. Values reported are mean±SEM.

Atrial natriuretic peptide concentration in rat plasma was measured by radioimmunoassay after plasma extraction (Chapter 2), in 14 control and 26 cirrhotic animals, aged 16-28 weeks. These rats had been treated in an identical manner to the 11 animals described above. Blood was obtained by tail snip under light ether anaesthesia. The latter may cause a rise in ANP.

Statistical analysis was by the Student's 't' test, assuming unequal variance. Level of statistical significance was taken as $p < 0.05$.

6.2.4 Liver histology

Post-mortem histological examination was performed in all control and carbon tetrachloride treated rats. Routine paraffin sections of formalin fixed liver tissue were stained with haematoxylin and eosin and with the Gordon and Sweet's silver method for reticulin. The examiner of

Fig. 17

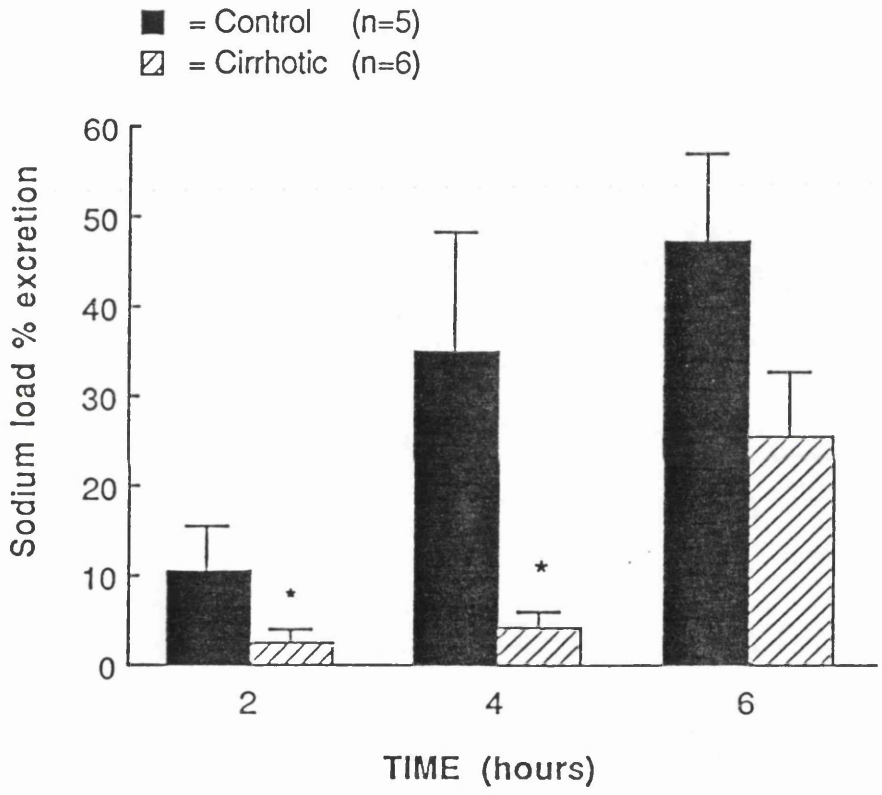


Fig. 17. Cumulative percent excretion of a 2 mmol sodium load administered by gavage, at 2, 4 and 6 hours. Values are mean±SEM. Control rats compared to cirrhotic. Student's 't' test. * p <0.05.

the sections was unaware of the treatment regime administered to the animals. Cirrhosis was diagnosed according to whether cell necrosis and/or loss was present, with bridging fibrosis and nodular regeneration with loss of the normal hepatic architecture.

6.3 Results

At the time of kidney perfusion, there were no significant differences in body weight (605.6±21 v 570.8±23 g), kidney weight (1.66±0.08 v 1.82±0.08g), or liver weight (17.9±0.58 v 19.46±1.44 g) between control and cirrhotic rats respectively. For technical reasons, *in-vivo* mean aortic pressure measurement was possible in only 3 control (median 92 mmHg, range 80-105 mmHg) and 5 cirrhotic (median 97 mmHg, range 92-115 mmHg) animals.

The proportion of the sodium load administered by gavage which was excreted was diminished in the cirrhotic rats as compared to controls at 2 and 4 hours ($p < 0.05$), although the difference failed to reach statistical significance at 6 hours (Fig. 17).

Inulin clearance and urine flow rate in isolated kidneys from cirrhotic rats perfused without ANP tended to be lower than in controls, but the differences were not statistically significant (Table 11). There was no statistically significant difference in the magnitude of the increase in inulin clearance or urine flow rate between the two groups at any concentration of ANP. Urine sodium excretion was not significantly different between control (8.41±1.48 µmol/min/gkw) and cirrhotic perfused kidneys (4.93±1.01 µmol/min/gkw $p=0.09$) in the absence of ANP and there was no difference in fractional excretion of sodium between the control (5.10±0.94%) and cirrhotic (3.32±0.57%) animals. The addition of ANP

TABLE 11

Effect of increasing concentrations of ANP on the isolated perfused kidney of control and cirrhotic rats. Values are expressed per gram kidney weight gkw, and are mean ± SEM. **Q** perfusate flow rate, **CIn** Inulin clearance, **V** urine flow rate. Student's t-test. Level of significance $p < 0.05$.

		ANP CONCENTRATION (pmol/l)				
		Baseline	10	50	200	1000
Q (ml/min/gkw)						
Control	(n = 5)	43.6 ± 2.36	44.0 ± 2.4	44.1 ± 2.62	44.3 ± 2.6	44.2 ± 2.5
Cirrhotic	(n = 4)*	44.0 ± 3.7	44.7 ± 3.7	44.8 ± 3.7	45.1 ± 3.7	45.0 ± 3.8
CIn (ml/min/g/kw)						
Control	(n = 5)	1.16 ± 0.05	1.18 ± 0.09	1.21 ± 0.05	1.26 ± 0.07	1.30 ± 0.08
Cirrhotic	(n = 6)	1.03 ± 0.05	1.02 ± 0.07	1.04 ± 0.07	1.10 ± 0.08	1.19 ± 0.10
V (µl/min/gkw)						
Control	(n = 5)	131 ± 24.0	169 ± 30.7	191 ± 31.0	220 ± 32.2	267 ± 35.0
Cirrhotic	(n = 6)	75.1 ± 19.8	93.0 ± 23.2	114 ± 25.1	143 ± 26.5	199 ± 38

* Note: For technical reasons data for perfusate flow rate were available from only 4 perfusions of kidneys from cirrhotic animals.

resulted in a smaller increase in absolute urine sodium excretion in the cirrhotic animals, as compared to controls, at concentrations of 10, 50 and 200 pmol/l (0.06±0.08 v 1.29±0.35, $p<0.02$; 0.49±0.08 v 1.82±0.42, $p<0.03$ and 1.42±0.16 v 3.23±0.73, $p<0.05$ μmol/min/gram kidney weight, respectively), but not at 1000 pmol/l (4.46±0.66 v 7.5±1.89 μmol/min/gkw, $p=0.10$), (Fig. 18). The increase in fractional excretion of sodium was also significantly smaller in the cirrhotic group at 10pmol/l, but the difference between the two groups failed to reach statistical significance at higher concentrations (Fig. 18).

Rat plasma ANP concentration was higher in rats with cirrhosis (103±4.36 pmol/l) as compared to control animals (89±4.1 pmol/l, $p<0.05$).

Liver histology:

The liver histology could be readily divided into two groups. (1) The liver of 5 rats, corresponding to those treated with phenobarbitone (control), showed well preserved architecture with a subtle loss of granularity and eosinophilic transformation of acinar zone 3 hepatocytes. (2) In 6 animals, corresponding to those treated with phenobarbitone plus carbon tetrachloride, there was complete destruction of the hepatic architecture due to annular fibrosis that delineated parenchymal nodules, usually smaller than a normal liver acinus. A large proportion of the fibrous septa were made of bands of confluent cell loss with numerous pigmented macrophages and sparse mixed inflammatory cells on a background of loose connective tissue. Eosinophilic necrotic hepatocytes were occasionally seen at their margins. The parenchymal nodules were devoid of hepatic venules, although the latter could still be identified within some of the post-necrotic bridging septa. Although there was slight individual

Fig. 18

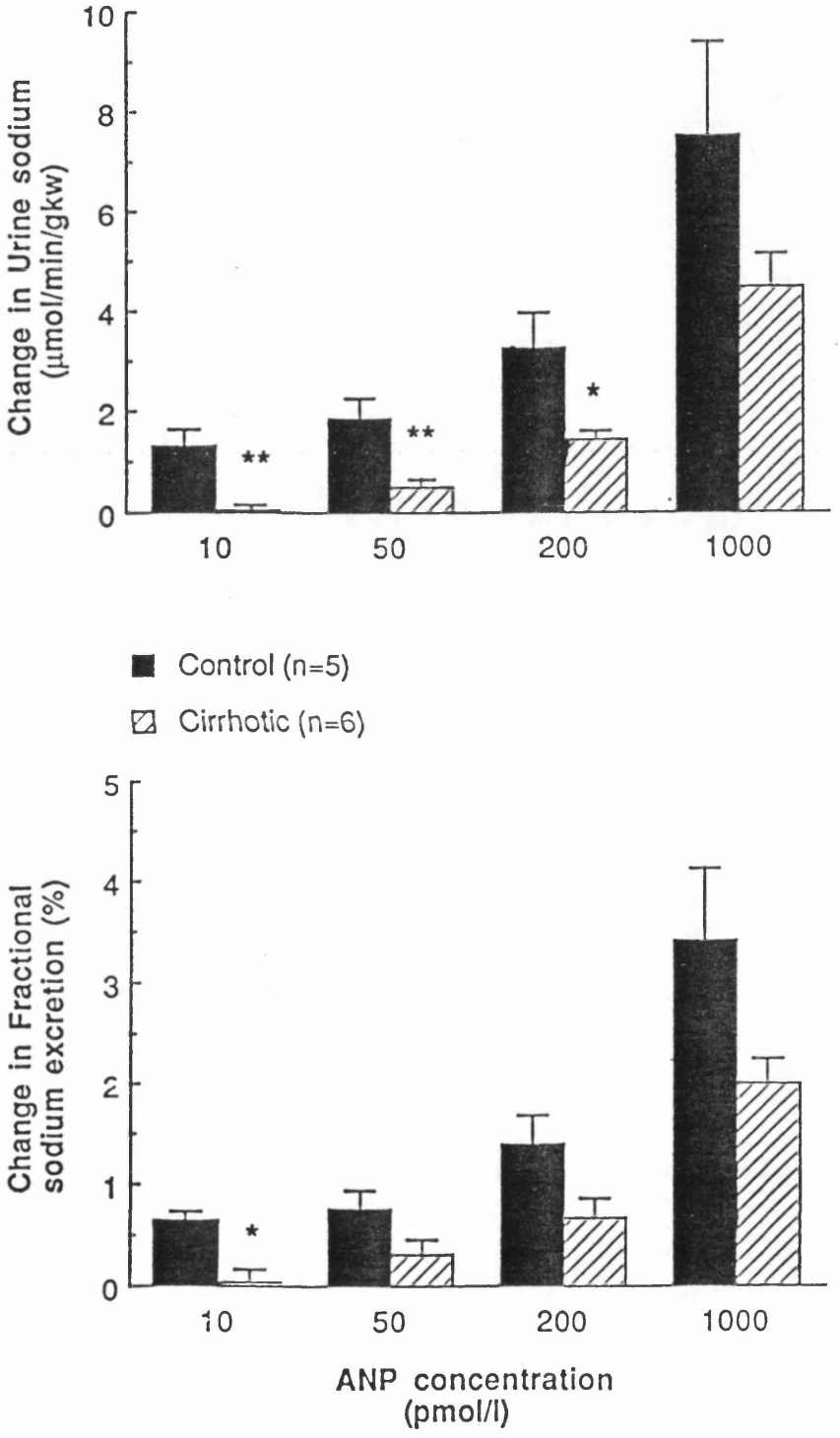


Fig. 18. Changes from baseline in absolute and fractional excretion of sodium in the isolated perfused kidney of control and cirrhotic rats, at increasing concentrations of ANP. Values are mean±SEM.
Student's 't' test. * p <0.05 ** p <0.03

variation in the thickness of the fibrous septa and the size of the nodules, all six livers fulfilled the histological criteria of an established micronodular cirrhosis.

6.4 Discussion

This study has demonstrated that in rats with carbon tetrachloride induced cirrhosis and impaired sodium excretion *in-vivo*, the isolated perfused kidney exhibits an impaired natriuretic response to infusion of ANP at physiological and pathophysiological concentrations. These findings suggest that in this model of cirrhosis, there is intrinsic renal resistance to the natriuretic action of ANP. This phenomenon may be specific, since in a previous study in which cirrhosis was induced by an identical method, we found a normal natriuretic response to stepwise increases in perfusion pressure between 90 and 150 mmHg (285).

Evidence that intrinsic renal abnormalities can be shown by the isolated perfusion technique has been provided using the Dahl hypertensive rat (286) and the rat model of nephrosis (287). In addition, the demonstration of appropriate responses by the isolated perfused kidney to dietary sodium intake manipulation and aldosterone infusion (288), provides further evidence that it is possible to demonstrate intrinsic renal changes in this model, despite the different haemodynamic patterns prevailing in perfused and native organs (289).

Previous *in-vivo* studies in rats have demonstrated that renal sympathetic nerve activity (290) and hyperaldosteronism (291) are important contributors to sodium retention in cirrhosis. Since in the present study the kidney was denervated and outside the immediate influence of humoral factors, our findings suggest that intrinsic renal

resistance to the natriuretic action of ANP may contribute to sodium retention in cirrhosis.

Possible mechanisms which may account for the observed resistance to the natriuretic action of ANP include 'down-regulation' or changes in renal receptors for ANP in the kidney of cirrhotic animals. Gerbes et al (292), recently reported a relative decrease in the ratio of biologically active B receptors to biologically inactive C receptors for ANP (191) in the renal glomerulus of rats with cirrhosis, which had been induced by ligation of the commom bile duct. Our findings are consistent with this observation. Another possibility would be an alteration in metabolism of ANP by kidney neutral endopeptidase (193,194,293). Alternatively there may be persistence of extrinsic influences on the kidney, such as that of aldosterone. Although the activity of the renin-aldosterone system was not measured in the present study, the previous finding of a normal natriuretic response in the isolated perfused kidney of cirrhotic rats with increasing perfusion pressure, after an identical equilibration period to that employed in the present study (285), suggests that aldosterone or other long acting circulating anti-natriuretic factors are unlikely to be contributing to the impaired natriuresis observed in the present circumstances. This interpretation is consistent with the *in-vivo* findings of Koepke et al (278). They reported no effect on the diuretic or natriuretic response to ANP by short term inhibition of the renin-aldosterone system, using captopril, in control and bile duct ligated cirrhotic rats. The same authors also reported that renal sympathetic denervation 7-10 days prior to study restored the natriuretic responsiveness of cirrhotic animals to ANP infusion (278). The latter

observation seems to be at variance with the findings of the present study, in which the denervated, isolated kidney exhibited a diminished natriuretic response to ANP. Two reasons may account for the discrepancy. Firstly, the use of different models of cirrhosis. Secondly, the timing of renal denervation: in the study by Koepke et al (278), the kidneys were denervated 7-10 days before study whereas in the present study the renal nerves were intact until the kidney was removed from the animal and experimentation was immediate.

A direct toxic effect of carbon tetrachloride on the kidney is unlikely to be responsible for the diminished natriuretic response in this model of cirrhosis, since there was no significant difference in natriuresis between control and cirrhotic rats when perfused in the absence of ANP. Moreover, in a previous study in which cirrhosis was induced in an identical manner (285), we found a normal natriuretic response to stepwise increases of perfusion pressure from 90 to 150 mmHg.

The present findings suggest that in addition to other anti-natriuretic influences that may exist *in-vivo* (Chapter 1, Chapter 3, 278, 290, 291), intrinsic renal resistance to the natriuretic action of ANP occurs in cirrhosis and may contribute to sodium retention.

CHAPTER 7

Epilogue

7.1 Summary of findings

7.1.1 Feasibility of severe sodium restriction

The feasibility of a administering a 22 mmol sodium diet to highly motivated volunteers, on an outpatient basis, has been demonstrated. Appropriate changes in urine sodium excretion, plasma sodium, haematocrit, plasma renin activity, aldosterone and ANP concentration, confirmed the effectiveness of this method. The implication of this finding is that short term clinical trials employing severe sodium restriction, can be performed reliably, without the use of metabolic or diet kitchens. Such facilities are expensive and are not widely available. Although financial costs were not evaluated in this work, it is likely that the administration of a low sodium diet by the counselling method would be the less expensive option.

7.1.2 Atrial natriuretic peptide in cirrhosis with ascites

Plasma concentration of atrial natriuretic peptide in patients with cirrhosis and sodium retention, was increased as compared to healthy controls. Despite large variation, levels of ANP remained higher throughout a 24 hour period of ordinary activity. Plasma renin activity and aldosterone exhibited a larger and more sustained increase with activity during the day in patients as compared to controls, indicating that the activity of this system may be underestimated in patients, if measurements are confined to basal conditions. These findings emphasize the importance of bed rest in the treatment of patients with ascites.

Estimations of plasma ANP and urine sodium excretion every 4 hours in patients with cirrhosis revealed a correlation between ANP and urine

sodium excretion. Correlation was highest between midnight and 0800 hours, when subjects were supine and coincided with a large drop in plasma renin activity and aldosterone concentration. This observation suggests that increased activity of the renin-aldosterone system during the day may antagonise the natriuretic action of ANP and that a reduction in the activity of the former during the night, may allow the natriuretic effect of ANP to become manifest.

An appropriate drop of ANP concentration in patients on rising, is in agreement with previous findings which indicate that known mechanisms of ANP release are not impaired in cirrhosis. A greater increase in haematocrit and a greater rise in plasma renin activity and aldosterone in patients on rising, is consistent with greater loss of fluid from the intravascular space in patients as compared to controls.

7.1.3 Changes in plasma atrial natriuretic peptide concentration during total paracentesis

The demonstration of appropriate changes in circulating ANP concentration to known stimuli in cirrhosis, suggested that measurement of circulating concentration of ANP might be employed to follow changes in atrial stretch and venous return. In this way sequential plasma ANP estimations along with plasma renin activity and aldosterone concentration assisted the interpretation of complex haemodynamic changes occuring during large volume paracentesis. Atrial distension and increased venous return, in the initial stages of paracentesis, was suggested by a rise in cardiac output and was supported by a tendency of ANP to rise, despite a drop in right atrial pressure. Investigation of patients by 2D echo-

cardiography confirmed the presence of right atrial compression in patients with tense ascites.

7.1.4 Atrial natriuretic peptide in fulminant hepatic failure

The possibility of a deficiency in circulating atrial natriuretic peptide, in patients with paracetamol-induced fulminant hepatic failure was investigated. Concentration of plasma ANP was similar in patients and healthy controls. Plasma ANP concentration was raised only in the presence of severe renal failure. Plasma ANP concentration changed appropriately to intravascular fluid volume changes, induced by haemodialysis and by the infusion of human albumin solution, suggesting that mechanisms of its release are not impaired in fulminant hepatic failure. The demonstration of an appropriate rise in circulating ANP without a significant increase in urine sodium excretion in patients with no or mild renal failure, is compatible with the hypothesis that in fulminant hepatic failure there may be resistance to the natriuretic action of ANP. This may be due to physiological antagonism by a stimulated renin-aldosterone system or due to intrinsic renal resistance to the natriuretic action of ANP.

7.1.5 Effect of ANP on the isolated kidney of rats with experimental cirrhosis

The finding of raised plasma concentrations of ANP in patients with cirrhosis and ascites and the failure to demonstrate increased natriuresis in response to plasma volume expansion in patients with fulminant hepatic failure, raised the possibility of renal resistance to the natriuretic action of ANP in patients with liver disease and sodium retention.

This study demonstrated that in rats with carbon tetrachloride-induced cirrhosis and impaired sodium excretion *in-vivo*, the isolated perfused kidney exhibits a diminished natriuretic response to infusion of ANP, at physiological and pathophysiological concentrations. These observations, along with the finding of raised plasma ANP concentration in the cirrhotic rats, indicate that in addition to other anti-natriuretic influences that may exist *in-vivo*, "intrinsic" renal resistance to the natriuretic action of ANP may contribute to sodium retention in cirrhosis.

7.2 Limitations of this work and future research

7.2.1 Patients with cirrhosis

In this work patients with cirrhosis and moderate to gross ascites were selected for study, since abnormalities in circulating ANP concentration, the mechanism of its release into plasma, or resistance to its natriuretic action, were held likely to be marked, and thus easily identifiable. By selecting this group of patients, observations have been restricted to patients with advanced disease.

Other approaches are needed. Measurements of circulating ANP in patients with cirrhosis without ascites, in conjunction with haemodynamic and plasma volume measurements, may yield information pertaining to the pathogenesis of ascites, early in the course of the disease. In this regard, detailed investigation of the response of ANP, the renin-aldosterone system and urine sodium excretion to dynamic tests, may yield more information on the complex interaction between the diseased liver and sodium homeostasis.

A number of questions are prompted by the findings of the work in this thesis, which may be answered by further research:

7.2.1 a). Reasons for raised circulating ANP concentrations in patients with cirrhosis and ascites merit further investigation. Determination of the extraction of ANP by the liver and kidney in patients with cirrhosis and sodium retention, with measurement of plasma flow to these organs, is essential for accurate estimation of extraction ratios. Estimation of extraction ratios in animal organs perfused in isolation, may yield additional information.

7.2.1 b). The role of the renin-aldosterone system in antagonising the natriuretic effect of ANP could be investigated further by examining the correlation of ANP to urine sodium excretion over 24 hours of ordinary activity in patients with cirrhosis and ascites, after cautious pharmacological inhibition of the renin-aldosterone system. Alternatively, the activity of the renin-aldosterone system could be manipulated in a physiological manner, through changes in posture. Evidence on the biological activity of ANP could be obtained by measuring cGMP in urine.

7.2.1 c). The advent of atriopeptidase inhibitors may offer an alternative mode of therapy for sodium retention associated with cirrhosis. Circulating concentrations of ANP have been shown to rise in response to infusion of an atriopeptidase inhibitor in patients with congestive cardiac failure, with measurable increases in urine sodium excretion (195). In cirrhosis and sodium retention, treatment with an atriopeptidase inhibitor, perhaps in conjuction with the aldosterone antagonist spironolactone, might prove to be an effective therapy. This hypothesis could be tested by a controlled clinical trial.

7.2.2 Haemodynamic changes during paracentesis

The deduction made earlier in this thesis from haemodynamic changes during paracentesis, that the appropriate time for therapeutic plasma expansion should be between 3 and 12 hours from start, should be examined prospectively by undertaking hormonal, haemodynamic and plasma volume measurements in patients receiving plasma expanders, at specified time points.

7.2.3 Fulminant hepatic failure

The absence of natriuresis in response to plasma volume expansion in patients with fulminant hepatic failure and no or mild renal failure, would be compatible with the hypothesis that in this situation there may be resistance to the natriuretic effect of ANP. The influence of the renin-aldosterone system and the possibility of renal resistance to the natriuretic action of ANP in fulminant hepatic failure could be examined further, in an animal model.

7.2.4 Diminished natriuresis in the isolated kidney

Diminished natriuretic response to infusion of ANP in the isolated kidney of rats with carbon tetrachloride-induced cirrhosis, indicated the presence of "intrinsic" renal resistance to the natriuretic action of ANP in this model of cirrhosis. The possibility that "down-regulation" of receptors for ANP in the kidney may be responsible for this finding, could be investigated. Equilibrium binding techniques, may be employed to examine whether ANP receptors in glomeruli of rats with experimental cirrhosis, are abundant or sparse.

A direct toxic effect of carbon tetrachloride on the kidney in this model of cirrhosis, although unlikely (since there was no difference in natriuresis between control and cirrhotic rats when kidneys were perfused in isolation, in the absence of ANP at perfusion pressures ranging from 90 to 150 mmHg), is nevertheless possible. This possibility could be examined by repeating the experimental protocol of infusion of ANP in the isolated kidney of rats with cirrhosis induced by another method, such as ligation of the common bile duct.

CHAPTER 8

ADDENDUM

Plasma atrial natriuretic peptide in the human fetus: Response to intravascular blood transfusion

8.1 Introduction

8.2 Patients and Methods

8.3 Results

8.4 Discussion

8.1 Introduction

Until the advent of ultrasound-guided cordocentesis (294), knowledge of fetal physiology and pathophysiology was derived from animal experiments and from studies in humans at hysterotomy or postpartum. Extrapolation of findings from these situations to the human fetus, is limited by inter-species variation and by changes in environmental conditions, which may differ from those prevailing in the human fetus, in its undisturbed condition, *in-utero*. Ultrasound-guided cordocentesis has made possible the assessment of fetal well being, through direct measurements on umbilical cord blood samples, with the minimum degree of disturbance to the fetal environment (294,296-298). Therapeutic blood transfusion *in-utero* is now possible by this method (299).

Prior to this study, atrial natriuretic peptide had been detected in umbilical cord blood post-partum (295), but it was not known whether the atrial natriuretic peptide (ANP) was present in the circulation of the human fetus or whether it might be involved in blood volume homeostasis in intrauterine life.

The aim of the present study was to examine whether ANP was present in the circulation of the human fetus, and if so, whether its circulating levels changed in response to fetal blood volume expansion.

8.2 Patients and Methods

Twenty four normotensive pregnant women (21-35 weeks gestation) aged 18 to 39 years, referred for intravascular blood transfusion (IVT) because of severe red cell iso-immunisation, were studied. None

of the fetuses were hydropic, as judged by ultrasound examination. Cordocentesis was performed as an outpatient procedure, without maternal fasting, sedation or fetal paralysis (294). Pure fetal blood (3ml) was aspirated from the umbilical vein into heparinised syringes for fetal haemoglobin, blood gases and ANP measurement just prior to IVT. Packed blood compatible with that of the mother (taken from the donor 24-36 hours previously) was infused into the fetal circulation (299). At the end of the transfusion the needle was flushed with 1ml of normal saline and after 1 minute, to allow for equilibration, a further sample of 1ml was aspirated and discarded and 0.2 ml obtained for haemoglobin measurement. In 12 cases a further 2.2ml of fetal blood was aspirated for ANP assay. The fetal heart rate was monitored throughout the transfusion. None of the procedures was complicated by fetal bradycardia. Transfusions were timed. In 7 cases, maternal blood was taken from an antecubital vein before and after fetal blood transfusion.

For comparison, blood was taken for ANP assay, from the umbilical cord vein at birth, of 10 neonates of normal full term pregnancies, within 5 minutes of delivery of the placenta. Plasma ANP concentration was also measured in 25 non-pregnant adult volunteers aged 18-32 years. Venous blood samples were taken from an antecubital vein at 0900 hours while sitting (30 minutes), after an overnight fast. Plasma from donor blood was separated just before transfusion and assayed for ANP.

All samples were collected into chilled heparinised tubes and were centrifuged at 4°C for eight minutes within 10 minutes of collection.

Plasma was stored at -20°C until assayed. Fetal haemoglobin (Hb) was measured on a Coulter Counter S-Plus system (Coulter Electronics Ltd, Luton UK), and blood oxygen tension (pO2) on Radiometer ABL 30 (Radiometer, Copenhagen).

Atrial natriuretic peptide immunoreactivity in plasma was measured following plasma extraction using a modification of the radio-immunoassay proceedure described in Chapter 2: A new rabbit anti-ANP antiserum was used (YB8) which increased the assay sensitivity to 0.3 fmol/tube, allowing smaller volume samples to be processed. This modified assay proceedure had been shown to detect amounts of ANP immunoreactivity in plasma extracts not significantly different from values measured with antiserum (Y2), in identical samples. Antiserum YB8 was used at a titre of 1 in 6,400 at which 50% binding occurred. The assay conditions were otherwise identical to those described earlier for antiserum Y2 (Chapter 2), except that a ten point standard curve of 0.25 to 25 fmol of synthetic human alpha ANP was used. Human plasma extracts caused displacement of binding in the YB8 assay in parallel with that caused by pure synthetic alpha human ANP. Fragment testing revealed antiserum YB8 to bind to the central part of the ANP molecule with negligible binding to carboxy- and amino- terminal ANP fragments. For adult samples, 3ml of plasma was extracted on Sep-Pak C-18 cartridges (Millipore-Waters Ltd). The retained peptides were eluted with 2ml of 60% acetonitrile containing 0.1% trifluroacatic acid, lyophilised in 1.2ml of radioimmunoassay buffer. Aliquots (200µl) were assayed in duplicate. One ml fetal plasma samples were treated similarly but reconstituted in 900µl of buffer. Three hundred

microlitre aliquots were assayed in duplicate and 100µl aliquots in singlet. The assay sensitivity was equivalent to 0.3 pmol/l in adult plasma and 1 pmol/l in the smaller volume fetal samples. Intra-assay variation was 9% and inter-assay variation 11% . Mean±SEM recovery of ANP immunoreactivity from the Sep-Pak extraction step was 79±2 % for the adult plasma samples and 74±4 % for the smaller fetal samples. Results are presented as measured, without correction for extraction recovery.

The volume of blood necessary for transfusion was calculated from the expected feto-placental blood volume (adjusted for gestation) and the pre-transfusion and donor haemoglobin concentrations (296). The feto-placental blood volume after each transfusion was calculated from the change in fetal haemoglobin concentration and the volume and haemoglobin concentration of the transfused blood (297). Rate of transfusion was calculated from the duration and volume of blood transfused. Since fetal haemoglobin and umbilical venous pO2 change with gestation, these were expressed as a difference from the normal means for gestation, ΔHb g/dl and ΔpO2 mmHg respectively (296,298).

Statistical analysis was by the Wilcoxon two sample rank sum test and by the Wilcoxon signed rank test for paired values, as appropriate. Tests of correlation were by the Spearman rank test. The level of statistical significance was taken as $P < 0.05$.

The study was performed with approval from the King's College Hospital Ethical Commitee.

Fig. 19

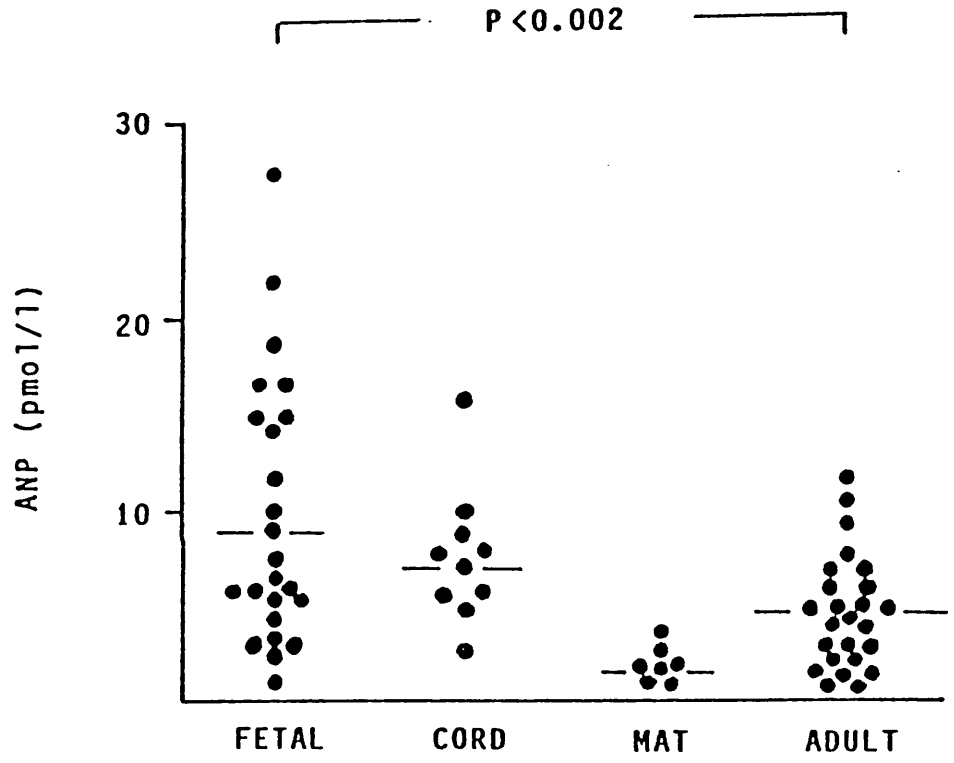


Fig. 19. Plasma atrial natriuretic peptide (ANP) concentration in fetal umbilical vein at 21-35 weeks gestation (FETAL), umbilical cord vein at full term delivery of normal neonates (CORD), maternal plasma (MAT), and normal 18-32 year old adults (ADULT).

8.3 Results

Intravascular blood transfusion was completed in all cases and there were no complications. Basal ANP concentration was significantly higher in the fetus (median 8.0 pmol/l, range 1-27.3) as compared to 18-32 year old non-pregnant adults (median 4.5 pmol/l, range 1-11.3, P <0.002), but not significantly different from post-delivery umbilical cord blood (median 7.35 pmol/l, range 2.7-15.5), (Fig. 19). There was no correlation between length of gestation and basal fetal ANP concentration. In the 7 cases where paired fetal and maternal blood samples were taken, fetal plasma ANP concentration (median 16.5, range 5.8-27.3) was higher than the corresponding maternal ANP concentration in all cases (median 1.5 pmol /l, range 1-3.3, p <0.01). There was no correlation between basal $\Delta pO2$ or ΔHb (median 2.4 mmHg, range -24.4 to 11.37 and median -3.2 g/dl, range -7.88 to 0.8 g/dl respectively) and basal ANP concentration, suggesting that oxygen tension or the degree of anaemia did not influence basal fetal ANP levels.

Atrial natriuretic peptide was not detectable in donor blood. Fetal transfusions lasted between 3 and 16 minutes (median 7.45) and the rate of transfusion was between 4.26 and 18.57 ml per minute (median 11.65). Volume of blood transfused expressed as a ratio of the estimated feto-placental volume was 0.29-1.37 (median 0.76). There was a significant increase in fetal ANP concentration post-transfusion (P <0.05), (Fig. 20), with a positive correlation between the rate of transfusion and change in ANP concentration (P <0.05). There was no correlation either between the volume of blood transfused or the rate

Fig. 20

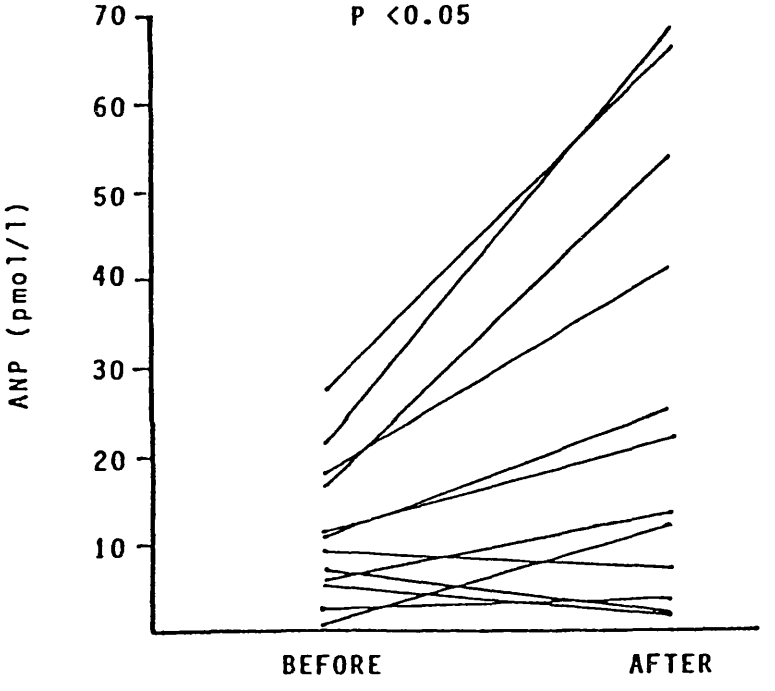


Fig. 20. Plasma atrial natriuretic peptide (ANP) concentrations before and immediately after intravascular blood transfusion in 12 fetuses with red cell iso-immunisation.

of expansion of the estimated feto-placental blood volume and the change in fetal ANP concentration. There was no significant difference in the plasma ANP concentration of the paired maternal samples taken before and after IVT (n=7).

8.4 Discussion::

It was reported in a previous study that ANP was detectable in human umbilical cord blood (295). This study concurs with this report and goes further to show that atrial natriuretic peptide is present in fetal plasma in considerable concentrations, as early as 21 weeks gestation. The demonstration of an increase in fetal plasma ANP concentration after fetal blood volume expansion, suggests that the mechanisms governing its release in the fetus are similar to those operating in the adult (180-184,218). Whether the observed rise in ANP after intravascular blood transfusion was enough to cause a natriuresis or an increase of venous capacitance in the fetus, is not shown by the present study and will require further investigation. However, in the ovine fetus intravascular infusion of saline has been shown to increase glomerular filtration rate and decrease fractional sodium reabsorption (300,301), findings consistent with previous reports of renal actions of ANP in adults (133,147,218). Studies of fetal volume homeostasis in animals have demonstrated that changes in maternal hydration cause acute changes in fetal blood volume, through transplacental water exchange, with corresponding changes in fetal arginine-vasopressin and urine and lung fluid flows (302,303). Collectively, these findings allow one to postulate that fetal ANP may act in a manner complementary to that of fluid conserving hormones, to

protect the fetal blood volume from over-expansion.

Concentrations of ANP in the fetus were higher than in maternal plasma and adult controls aged 18-32 years, but similar to those found in the umbilical cord blood of normal babies immediately post-delivery.

Even though no hydrops was noted by ultrasound, it is possible that some fetuses may have been on the verge of "high-output" heart failure and this might account for the relatively high plasma ANP concentrations in fetuses. However, the present results are consistent with the recent finding of a greater than two-fold concentration of ANP in the plasma of healthy ovine fetuses, compared to that of maternal plasma (300). Messenger RNA coding for the ANP prohormone is now known to be present in increased concentration in fetal cardiac ventricles compared with ventricles of normal adults (304), and it is possible that release of ANP from the fetal ventricle contributes to the higher plasma ANP concentrations found in the fetus.

The higher circulating levels of ANP in the fetus could be attributed either to an increase in secretion or reduced breakdown. The latter seems unlikely since the ovine fetus has been shown to have a rapid clearance rate of plasma ANP (301). On the other hand, haemodynamic and endocrine conditions in the fetus would favour increased secretion of ANP: fetal cardiac output is proportionately increased as compared to adults (302), and increased plasma concentrations of glucocorticoids, progesterone and aldosterone are known to be present in the fetal circulation. All these factors are known to potentiate ANP secretion (122,123,218).

The significant rise in fetal plasma ANP concentration post-transfusion did not correlate with volume of blood transfused or estimated feto-placental volume expansion. This finding contrasts with animal data from a study of healthy ovine fetuses: Ross et al, showed a positive correlation between volume of saline infused into the ovine fetal circulation and increase in ANP (300). Circulating ANP began rising 10-20 minutes after commencing the infusion and reached a maximum at 40 minutes, a similar delay to that observed in human adults (133,181-183). It is possible that the discrepancy between the present findings in the human fetus and those in the ovine fetus (300) is due to the timing of the post-transfusion fetal blood samples, which for ethical reasons were taken immediately after completion of transfusion, when maximal levels of ANP may not have been reached. The positive correlation between the rate of infusion and rise in ANP is in agreement with findings of previous studies on the effects of saline infusion given at varying rates in young adults (182). The increase in post-transfusion fetal ANP concentration, despite high basal levels, demonstrates that the fetus retains significant atrial natriuretic peptide reserves.

In conclusion, atrial natriuretic peptide is present in the human fetal circulation as early as 21 weeks gestation and its concentration rises in response to blood volume expansion. These findings support the hypothesis that plasma release of ANP in the fetus may play a role in fetal blood volume homeostasis.

REFERENCES:

1. Arroyo V, Epstein M, Gallus G, Gentilini P, Ring-Larsen H, Salerno F. Refractory ascites in cirrhosis: Mechanism and treatment. Gastroenterol Intl 1989;2:195-207.

2. Pinzello G, Virdone R, Lojacono F, et al. Is the acidity of ascitic fluid a reliable index in making the presumptive diagnosis of spontaneous bacterial peritonitis ? Hepatology 1986;6:244-247.

3. Conn HO. Complications of portal hypertension. In: Gitnick G ed. 1987, Current Hepatology 7, Year book Publications, Chicago:195-210.

4. Hippocrates. Cited in Atkinson M. Ascites in liver disease. Postgrad Med J 1956; 32:482.

5. Kuhn CG. (translator). The works of Galen, Vol. 14, p.746 and Vol. 16, p.447. Leipzig, 1827 and 1828.

6. Spencer WG. (translator). Celsus de Medicina, Vol. 7, p.15 and Vol. 3, pp. 21 and 14. Harvard, 1938.

7. Adams F. (translator). Paul of Aegina, Vol. 6, p.50. Sydenham Society, London, 1846.

8. Lower R. Traite´ du Coeur. (French translation from the Latin), p.121. Paris, 1679.

9. Brown J. The Philosophical Transaction of the Royal Society. III:248. London, 1685.

10. Sydenham Thomas. Tractatus de podagra et hydrope 8, London, 1683.

11. Bolton C. The pathological changes in the liver resulting from passive venous congestion experimentally induced. J Path Bact. 1914-15;19:258-266.

12. Farnsworth EB. Electrolyte partition in patients with edema of various origins. Sodium and chloride. Am J Med 1948;4:338-342.

13. Eisenmenger WJ, Blondheim SH, Bongiovanni AM, Kunkel HG. Electrolyte studies in patients with cirrhosis of the liver. J Clin Invest 1950;29:1491-1499.

14. Goodyer AVN, Relman AS, Lawrason FD, Epstein FH. Salt retention in cirrhosis of the liver. J Clin Invest 1950;29:973-981.

15. Papper S, Saxon L. The influence of intravenous infusion of sodium chloride solutions on the renal excretion of sodium in patients with cirrhosis of the liver. J Clin Invest 1956;35:728-735.

16. Goldman R. Studies in diurnal variation of water and electrolyte excretion: nocturnal diuresis of water and sodium in congestive cardiac failure and cirrhosis of the liver. J Clin Invest 1951;30:1191-1199.

17. Borst JGG, De Vries LA. The three types of "natural" diuresis. Lancet 1950;ii:1-6.

18. Jones RA, McDonald GO, Last JH. Reversal of diurnal variation in renal function in cases of cirrhosis and ascites. J Clin Invest 1952;31:326-334.

19. Kowalski HJ, Abelman WH. The cardiac output at rest in Laennec's cirrhosis. J Clin Invest 1953;32:1025-1033.

20. Kowalski HJ, Abelman WH, McNeely WF. The cardiac output in patients with cirrhosis of the liver and tense ascites with observations on the effect of paracentesis. J Clin Invest 1954;33:768-773.

21. Murray JF, Dawson AM, Sherlock S. Circulatory changes in chronic liver disease. Am J Med 1958;24:358-367.

22. Naccarato R, Messa P, D'Angelo A, et al. Renal handling of sodium and water in early chronic liver disease: evidence for a reduced natriuretic activity of the cirrhotic urinary extracts in rats. Gastroenterol 1981;81:205-210.

23. Klinger EL Jr, Vaamonde CA, Vaamonde LS, et al. Renal function changes in cirrhosis of the liver. Arch Int Med 1970;125:1010-1015.

24. Gabuzda GJ. Cirrhosis ascites and edema. Clinical course related to management. Gastroenterol 1970;58:546-553

25. Bosch J, Arroyo V,Rodes J, Burguera M, Teres J. Compensacion espontanea de la ascites en la cirrhosis hepatica. Revista Clin Espanola 1974;133:441-448.

26. Sherlock S. Ascites. In: Diseases of the liver and biliary system 7th edn, 1985, Blackwell:117-134.

27. Papper S, Saxon L. The diuretic response to administered water in patients with liver disease. II.Laennec's cirrhosis of the liver. Arch Int Med 1959;103:750-762.

28. Vaamonde CA. Renal water handling in liver disease. In: The kidney in liver disease, ed Epstein M. 2nd edn. 1983, New York, Elsevier:55-86

29. Boyer TD. Portal hypertension and its complications: bleeding esophageal varices, ascites and spontaneous bacterial peritonitis. In: Zakim D, Boyer TD. eds. Hepatology: A textbook of liver disease. 1983, Philadelphia: WB Saunders:464-499.

30. Orloff MJ. Pathogenesis and surgical treatment of intractable ascites associated with alcoholic cirrhosis. Ann NY Acad Sci 1970;170:213-38.

31. Webb LJ, Sherlock S. The aetiology, presentation and natural history of extrahepatic portal venous obstruction. Q J Med 1979;192:627-639.

32. Bennett HS, Luft JH, Hampton JC. Morphologic classification of vertebrate blood capillaries. Am J Physiol 1959;196:381-390.

33. Levy M, Seely JF. Pathophysiology of edema formation. In: Brenner MB, Rector FC, eds. The kidney. 2nd edn. 1981, Philadelphia: WB Saunders:723-776.

34. Witte MH, Witte CL, Dumont AE. Progress in liver disease: physiological factors involved in the causation of cirrhotic ascites. Gastroenterol 1971;61:742-750.

35. Reynolds TB, Ito S, Iwatsuki S. Measurement of portal pressure and its clinical application. Am J Med 1970;49:649-657.

36. Witte CL, Witte MH, Dumont AE. Lymph imbalance in the genesis and perpetuation of the ascites syndrome in hepatic cirrhosis. Gastroenterol 1980;78:1059-1068.

37. Dykes PW. The rates of distribution and catabolism of albumin in normal subjects and in patients with cirrhosis of the liver. Clin Sci 1968;34:161-183.

38. Granger DN, Taylor AE. Permeability of intestinal capillaries to endogenous macromolecules. Am J Physiol 1980;238:H457-H464.

39. Bosch J,Arroyo V, Betriu A, et al. Hepatic hemodynamics and the renin-angiotensin-aldosterone system in cirrhosis. Gastroenterol 1980;78:92-99.

40. Bredfeldt JE, Groszmann RJ. Hemodynamic aspects of portal hypertension. In: Epstein M, ed. The kidney in liver disease, 2nd edn. 1978, New York, Elsevier:281-292.

41. Vorobioff J, Bredfeldt JE, Groszmann RJ. Increased blood flow through the portal system in cirrhotic rats. Gastroenterol 1984;87;1120-1126.

42. Fernandez-Munoz D, Caramelo C, Santos JC, et al. Systemic and splachnic hemodynamic disturbances in conscious rats with experimental liver cirrhosis without ascites. Am J Physiol 1985;249:G316-G320.

43. Benoit JN, Barrowman JA, Harper SL, et al. Role of humoral factors in the intestinal hyperemia associated with chronic portal hypertension. Am J Physiol 1984;247:G486-G493.

44. Kravetz D, Arderiu M, Bosch J, et al. Hyperglucagonemia and hyperkinetic circulation after portocaval shunt in the rat. Am J Physiol 1987;252:G257-G261.

45. Hortnagl H, Singer EA, Lenz K, et al. Substance P is markedly increased in plasma of patients with hepatic coma. Lancet 1984;i:480-83.

46. Benoit JN, Granger DN. Splachnic hemodynamics in chronic liver portal hypertension. Semin Liver Dis 1986;6:287-298.

47. Dal Palu C, Donnaggio G, Dal Zotto I, Pessina AC. Arteriovenous shunts in cirrhotic patients studied with human serum albumin macroaggregates tagged with I^{131} (MAA ^{131}I). Scand J Gastroenterol 1968;3;425-431.

48. Neil E. Nervous control of the circulation II. Reflex influences on the cardiovascular system In: Folkow B and Neil E, eds. Circulation 1971, Oxford Univ Press:320-331.

49. Epstein M. Deranged sodium homeostasis in cirrhosis. Gastroenterol 1979;76:622-635.

50. Epstein M, Pins DS, Schneider N, Levinson R. Determinants of deranged sodium homeostasis in decompensated cirrhosis. J Lab Clin Med 1976;87:822-839.

51. Bichet DG, Groves BM, Schrier RW. Mechanisms of improvement of sodium and water excretion by immersion in decompensated cirrhotic patients. Kidney Int 1983;24:788-794.

52. Bichet DG, Szatalowicz V, Chaimovitz C, Schrier RW. Role of vasopressin in abnormal excretion in cirrhotic humans. Ann Intern Med 1982;96:413-417.

53. Bichet DG, Van Putten VJ, Schrier RW. Potential role of increased sympathetic activity in impaired sodium and water excretion in cirrhosis. N Engl J Med 1982;307:1552-1557.

54. Nicholls K, Shapiro M, Schrier R. Factors determining the renal response to water immersion of non-excretor cirrhotic patients. (Abstract). Kidney Int 1985;27:148.

55. Nicholls KM, Shapiro MD, Van Putten VJ, et al. Elevated plasma norepinephrine concentrations in cirrhosis: association with increased secretion rates, normal clearance rates and suppressibility by central volume expansion. Circ Res 1985;56:457-461.

56. Perera GA. The plasma volume in Laennec's cirrhosis of the liver. Ann Intern Med 1946;24:643-647.

57. Eisenberg S. Blood volume in patients with Laennec's cirrhosis of the liver as determined by radioactive chromium-tagged red cells. Am J Med 1956;20:189-195.

58. Lieberman FL, Reynolds TB. Plasma volume in cirrhosis of the liver: its relation to portal hypertension, ascites and renal failure. J Clin Invest 1967;46:1297-1308.

59. Sellars L, Shore AC, Wilkinson R, James OF, Robson V. Sodium status and the renin-angiotensin-aldosterone system in compensated liver disease. Eur J Clin Invest 1981;11:299-304.

60. Rocco VK, Ware AJ. Cirrhotic ascites. Pathophysiology, diagnosis and management. Ann Intern Med 1986;105:573-585.

61. Tristani FE, Cohn JN. Systemic and renal hemodynamics in oliguric hepatic failure: effect of volume expansion. J Clin Invest 1967;46:1894-1906.

62. Lieberman FL, Denison EK, Reynolds TB. The relationship of plasma volume, portal hypertension, ascites and renal sodium retention in cirrhosis: the overflow theory of ascites formation. Ann NY Acad Sci 1970;170:202-212.

63. Levy M. Sodium retention and ascites in dogs with experimental portal cirrhosis. Am J Physiol 1977;233:F572-F585

64. Levy M, Allotey JB. Temporal relationships between urinary salt retention and altered systemic haemodynamics in dogs with experimental cirrhosis. J Lab Clin Med 1978;92:560-569.

65. Better OS, Schrier RW. Disturbed volume homeostasis in patients with cirrhosis of the liver. Kidney Int 1983;23:303-311.

66. Alpern RJ. Renal sodium retention in liver disease. West J Med 1983;138:852-860.

67. Unikowsky B, Wexler MJ, Levy M. Dogs with experimental cirrhosis of the liver but without intrahepatic hypertension do not retain sodium or form ascites. J Clin Invest 1983;72:1594-1604.

68. Orloff MJ, Spitz BR, Wall MH, Thomas HS, Halasz NA. Experimental ascites: IV. Comparison of the effects of end-to-side and side to side portacaval shunts on intractable ascites. Surgery 1964;56:784-799.

69. Orloff MJ. Pathogenesis and surgical treatment of intractable ascites associated with alcoholic cirrhosis. Ann NY Acad Sci 1970;170:213-238.

70. Orloff MJ, Lipman CA, Noel SM, Halasz NA, Neesby T. Hepatic regulation of aldosterone secretion by a humoral mediator. Surgery 1965;58:225-247.

71. Campbell V, Greig PD, Cranford J, Langer B, Silverman M, Blendis LM. A comparison of acute reversible pre- and postsinusoidal portal hypertension on salt and water retention in the dog. Hepatology 1982;2:54-58.

72. Di Bona GF. Renal neural activity in hepatorenal syndrome. Kidney Int 1984;25:841-853.

73. Rossoff L Jr, Zia P, Reynolds T, Horton R. Studies of renin and aldosterone in cirrhotic patients with ascites. Gastroenterol 1975;69:698-705.

74. Epstein FM. Underfilling versus overflow in hepatic ascites N Engl J Med 1982;307:1577-1578.

75. Schrier RW, Arroyo V, Bernardi M, Epstein M, Henricksen JH, Rodes J. Peripheral arterial vasodilation hypothesis: A proposal for the initiation of renal sodium and water retention in cirrhosis. Hepatology 1988;8:1151-1157.

76. Wilkinson SP, Jowett TP, Slater JDH, Arroyo V, Moodie H, Williams R. Renal sodium retention in cirrhosis: relation to nephron site. Clin Sci 1979;56:169-177.

77. Epstein M, Levinson R, Sancho J, Haber E, Re R. Characterisation of renin-aldosterone system in decompensated cirrhosis. Circ Res 1977;41:818-829.

78. Bernardi M, Trevisani F, Santini C, et al. Plasma norepinephrine, weak neurotransmitters with renin activity during active tilting in liver cirrhosis: relationship with cardiovascular homeostasis and renal function. Hepatology 1983;3:54-64.

79. Epstein FH, Post RS, McDowell M. Effects of arteriovenous fistula on renal hemodynamics and electrolyte excretion. J Clin Invest 1953;32:233-240.

80. Laurence DR. Hypotensive and vasodilator drugs. In: Laurence DR and Bennett PN. Eds. Clinical Pharmacology, 1980 5th edn, Churchill and Livingstone:608-643.

81. Alexander EA, Doner DN, Auld RB, et al. Tubular reabsorption of sodium during acute and chronic volume expansion in man. J Clin Invest 1972;51:2370-2379.

82. Denison EK, Lieberman FL, Reynolds TB. 9-α-fluorohydrocortisone induced ascites in alcoholic liver disease. Gastroenterol 1971;61:497-503.

83. Wilkinson SP, Smith IK, Moodie H, Poston L, Williams R. Studies on mineralocorticoid "escape" in cirrhosis. Clin Sci 1979;56:401-406.

84. Chaimovitz C, Szylman P, Alroy G, Better OS. Mechanism of increased renal tubular sodium reabsorption in cirrhosis. Am J Med 1972;52:198-202

85. Epstein M, Ramachandran M, De Nunzio AG. Interrelationship of renal sodium and phosphate handling in cirrhosis. Min Electr Metab 1982;7:305-315.

86. Chonko AM, Bay WH, Stein JH, Ferris TF. The role of renin and aldosterone in the salt retention of edema. Am J Med 1977;63:881-889.

87. Epstein M, Lifschitz M, Hoffman DS, Stein JH. Relationship between renal prostaglandin E and renal sodium handling during water immersion in normal man. Circ Res 1979;45:71-80.

88. Boyer TD, Zia P, Reynolds TB. Effect of indomethacin and prostaglandin A1 on renal function and plasma renin activity in alcoholic liver disease. Gastroenterology 1979;77:215-222.

89. Zipser RD, Hoefs JC, Speckart PF, Zia PK, Horton R. Prostaglandins: modulators of renal function and pressor resistance in chronic liver disease. J Clin Endocr Metab 1979;48:895-900.

90. Zipser RD, Kerlin P, Hefs JC, Zia P, Barg A. Renal kallikrein secretion in alcoholic cirrhosis: relation to other vasoactive systems. Am J Gastroenterol 1981;75:183-187.

91. Stein JH, Boonjarern S, Wilson CB, Ferris TF. Alterations in intrarenal blood flow distribution. Methods of measurement and relationship to sodium balance. Circ Res 1973;61(suppl):32-33.

92. Di Bona GF. The functions of the renal nerves. Reviews Physiol Biochem Pharmacol 1982;94:75-94.

93. Thames MD. Neural control of renal function: contribution of cardiopulmonary baroreceptors to the control of the kidney. Fed Proc 1978;37:1209-1213.

94. Epstein M. Renal effects of head-out water immersion in man: implications for an understanding of volume homeostasis. Physiol Reviews 1978;58:529-581.

95. Di Bona GF. Neurogenic regulation of renal tubular sodium reabsorption. Am J Physiol 1977;233:F73-F81.

96. De Wardener HE, Clarkson EM. Concept of natriuretic hormone. Physiol Rev 1985;65:658-759.

97. Epstein M, Bricker NS, Bourgoignie JJ. The presence of a natriuretic factor in urine of normal men undergoing water immersion. Kidney Intl 1978;13:152-159.

98. Wilkinson SP, Moodie H, Alam A, Williams R. Renal sodium retention in cirrhosis and fulminant hepatic failure. Postgrad Med J 1975;51:527-531.

99. Buckalew VM, Gruber KA. Natriuretic hormone. In: Epstein M, ed. The kidney in liver disease 1st ed. New York: Elsevier/North-Holland Biomedical Press, 1983;479-499.

100. Kramer HJ, Natriuretic hormone - its possible role in fluid and electrolyte disturbances in chronic liver disease. Postgrad Med J 1975;51:532-540.

101. Epstein M. Natriuretic hormone and the sodium retention of cirrhosis. Gastroenterology 1981;81:395-399.

102. Yang SS, Korula J, Sundheimer J, Keyser A. Digoxin-like immunoreactive substances in chronic liver disease. Hepatology 1989;9:363-366.

103. Pudek MR, Seccombe DW, Humphries K. Digoxin-like immunoreactive substances and bile acids in the serum of patients with liver disease. Clin Chem 1986;32:2005-2006.

104. Vasdev SC, Longerich LL, Ittel TH, et al. Bile salts as endogenous digitalis-like factors. Clin Invest Med 1986;9:201-208.

105. Seda HWM, Gove CD, Hughes RD, Williams R. Inhibition of partially purified rat brain Na, K-dependent ATPase by bile acids, phenolic acids and endotoxin. Clin Sci 1984;66:415-420.

106. Hartshorne H. Water versus hydrotherapy or an essay on water and its true relations to medicine. Philadelphia: Lloyd P Smith, 1847;28.

107. Henry JP, Gauer O, Reeves IL. Evidence of the atrial location of receptors influencing urine flow. Circ Res 1956;4:85-90.

108. Gauer OH, Henry JP. Circulatory basis of fluid volume control. Physiol Rev 1963;43:423-481.

109. Kisch B. Electron microscopy of the atrium of the heart. Exp Med Surg 1956;14:99-112.

110. Marie JP, Guillemot H, Hatt PY. Le degre' de granulation des cardiocytes auriculaires. Etude planimetrique au cours de differents apports d'eau et de sodium chez le rat. Pathol Biol (Paris) 1976;24:549-554.

111. de Bold AJ, Borenstein HB, Veress AT, Sonnenberg HA. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci 1981;28:89-94.

112. Atlas SA, Laragh JH. Atrial natriuretic peptide: a new factor in hormonal control of blood pressure and electrolyte homeostasis. Annu Rev Med 1986;37:397-414.

113. Ballerman BJ, Brenner BM. Biologically active atrial peptides. J Clin Invest 1985;76:2041-2048.

114. Forsmann WG. Cardiac hormones. Review on the morphology, biochemistry and molecular biology of the endocrine heart. Eur J Clin Invest 1986;16:439-451.

115. Maack T, Kleinert HD. Renal and cardiovascular effects of atrial natriuretic factor. Biochem Pharmacol 1986;35:2057-2064.

116. Sagnella GA, MacGregor GA. Cardiac peptides and the control of sodium excretion. Nature 1984;309:666-667.

117. Thibault G, Garcia R, Gutkowska J, Genest J, Cantin M. Atrial natriuretic factor. A newly discovered hormone with significant clinical implications. Drugs 1986;31:369-375.

118. Needleman PJ, Greenwald JE. Atriopeptin: a cardiac hormone intimately involved in fluid, electrolyte and blood pressure homeostasis. N Engl J Med 1986;314:828-834.

119. Arendt RM, Stangl E, Zahringer J, Liebisch DC, Herz A. Demonstration and characterisation of α-human atrial natriuretic factor in human plasma. FEBS Lett 1985;189:57-62.

120. Arendt RM, Gerbes AL, Ritter D, Stangl E. Molecular weight heterogeneity of plasma-ANF in cardiovascular disease. Klin Wochenschr 1986;64(suppl 6):97-102.

121. Miyata A, Toshimori T, Hashiguchi T, Kangawa K, Matsuo H. Molecular forms of atrial natriuretic polypeptides circulating in human plasma. Biochem Biophys Res Commun 1987;142:461-467.

122. Day ML, Schwartz D, Wiegand RC, et al. Ventricular atriopeptin. Unmasking of messenger RNA and peptide synthesis by hypertrophy or dexamethasone. Hypertension 1987;9:485-491.

123. Cantin M, Ding J, Thibault G, Gotkowska J, Garcia R, Genest J. Atrial versus ventricular natriuretic factor. In: The American Society of Hypertension. Second World Congress on Biologically Active Atrial Peptides 1987:182.

124. Misono KS, Fukumi H, Grammer RT, Inagami T. Rat atrial natriuretic factor: complete amino acid sequence and disulfide linkage essential for biologic activity. Biochem Biophys Res Commun 1984;119:524-529.

125. Yandle TG, Richards AM, Nicholls MG, Cuneo R, Espiner EA, Livesay JH. Metabolic clearance rate and plasma half-life of alpha human atrial natriuretic peptide in man. Life Sci 1986;38:1827-1833.

126. Garcia R, Thibault, Seidah NG, et al. Structure-activity relationships of atrial natriuretic factor (ANF). II Effect of chain length modifications on vascular reactivity. Biochem Biophys Res Commun 1985;126:178-184.

127. Cantin M, Genest J. The heart and the atrial natriuretic factor. Endocrine Rev 1985;6:107-127.

128. Hammond TG, Hamarati A, Knox FG. Synthetic atrial natriuretic factor decreases renal tubular phosphate reabsorption in the rat. Am J Physiol 1985;249:F315-F318.

129. Murray RD, Itoh S, Inagami T, et al. Effects of synthetic atrial natriuretic factor in the isolated perfused rat kidney. Am J Physiol 1985;249:F603-F609.

130. Richards AM, Nicholls MG, Ikram H, Webster MWI, Yandle TG, Espiner EA. Renal, haemodynamic and hormonal effects of human alpha atrial natriuretic peptide in healthy volunteers. Lancet 1985;i:545-549.

131. Cuneo RC, Espiner EA, Nicholls MG, Yandle TG, Joyce SL, Gilchrist NL. Renal, haemodynamic and hormonal responses in normal man and effect of sodium intake. J Endocrin Metab 1986;63:946-953.

132. Weidmann P, Hassler L, Gnadinger MP, et al. Blood levels and renal effects of atrial natriuretic peptide in normal man. J Clin Invest 1986;77:734-742.

133. Anderson JV, Struthers A, Christofides N, Bloom SR. Atrial natriuretic peptide: an endogenous factor enhancing sodium excretion in man. Clin Sci 1986;70:327-331.

134. Cody RJ, Atlas SA, Laragh JH, et al. Atrial natriuretic factor in normal subjects and heart failure patients. Plasma levels and renal, hormonal and hemodynamic responses to peptide infusion. J Clin Invest 1986;78:1362-1374.

135. Maack T, Camargo MJF, Kleinert HD, Laragh JH, Atlas SA. Atrial natriuretic factor: structure and functional properties. Kidney Int 1985;27:607-615.

136. Oshima T, Currie MG, Geller DM, Needleman P. An atrial peptide is a potent vasodilator substance. Circ Res 1984;54:612-616.

137. Marin-Grez M, Fleming JT, Steinhausen M. Atrial natriuretic peptide causes pre-glomerular vasodilatation and post-glomerular vaso-constriction in rat kidney. Nature 1986;473-476.

138. Fried TA, McCoy RN, Osgood RW, Stein JH. Effect of atriopeptin II on determinants of glomerular filtration rate in the *in-vitro* perfused dog glomerulus. Am J Physiol 1986;250:F1119-F1122.

139. Sonnenberg H, Honrath U, Chong CK, Wilson DR. Atrial natriuretic factor inhibits sodium transport in the medullary collecting duct. Am J Physiol 1986;250:F963-F966.

140. Dillingham MA, Anderson RJ. Inhibition of vasopressin action by atrial natriuretic factor. Science 1986;231:1572-1573.

141. De Lean A, Vinay P, Cantin M. Distribution of atrial natriuretic factor receptors in dog kidney fractions. FEBS Lett 1985;193:239-241.

142. Ballermann BJ, Hoover RL, Karnovski MJ, Brenner BM. Physiologic regulation of atrial natriuretic peptide receptors in rat renal glomeruli. J Clin Invest 1985;76:2049-2056.

143. Murphy KMM, McLaughlin LL, Michener ML, Needleman P. Auto-radiographic localisation of atriopeptin III receptors in rat kidney. Eur J Pharmacol 1985;111:291-292.

144. Hamet P, Tremblay J, Pang SC, et al. Effects of native and synthetic atrial natriuretic factor on cyclic GMP. Biochem Biophys Res Commun 1984;123:515-517.

145. Tremblay J, Gerzer R, Vinay P, Pang SC, Beliveau R, Hamet P. The increase of cGMP by atrial natriuretic factor correlates with the distribution of particulate guanylate cyclase. FEBS Lett 1985;181:17-22.

146. Nonoguschi H, Knepper MA, Manganiello VC. Effects of atrial natriuretic factor on cyclic guanosine monophosphate and cyclic adenosine monophosphate accumulation in microdissected nephron segments from rats. J Clin Invest 1987;79:500-507.

147. Goetz KL. Atrial receptors, natriuretic peptides, and the kidney-Current understanding. Mayo Clin Proc 1986;61:600-603.

148. Goetz KL, Wang BC, Geer PJ, Sundet WD, Needleman P. Effect of atriopeptin infusion versus effects of left atrial stretch in awake dogs. Am J Physiol 1986;250:R221-R226.

149. Anderson JV, Donckier J, Payne NN, Beacham J, Slater JDH, Bloom SR. Atrial natriuretic peptide: evidence of action as a natriuretic hormone at physiological plasma concentrations in man. Clin Sci 1987;72:305-312.

150. Currie MG, Geller DM, Cole BR, et al. Purification and sequence analysis of bioactive atrial peptides (atriopeptins). Science 1984;223:67-69.

151. Napier MA, Vandlen RL, Alberts-Schonberg G, et al. Specific membrane receptors for atrial natriuretic factor in renal and vascular tissues. Proc Natl Acad Sci USA 1984;81:5946-5950.

152. Schiffrin EL, Chartier L, Thibault G, St-Louis J, Cantin M Genest J. Vascular and adrenal receptors for atrial natriuretic factor. Circ Res 1985;56:801-807.

153. Winquist RJ, Faison EP, Waldman SA, Schwartz K, Murad F, Rapoport RM. Atrial natriuretic factor elicits an endothelium-independent relaxation and activates particulate guanylate cyclase in vascular smooth muscle. Proc Natl Acad Sci USA 1984;81:5946-5950.

148a. Naruse M, Obana K, Naruse K, et al, Antisera to atrial natriuretic factor reduces urinary sodium excretion and increased plasma renin activity in rats. Biochem Biophys Res Commun 1985;132:954-960.

154. Garcia R, Thibault G, Gutkowska J, Cantin M, Genest J. Changes in regional blood flow induced by atrial natriuretic factor (ANF) in conscious rats. Life Sci 1985;36:1685-1692.

155. Caramelo C, Fernandez-Cruz A, Villamediana LM, et al. Systemic and haemodynamic effects of a synthetic atrial natriuretic peptide in conscious rats. Clin Sci 1986;71:323-325.

156. Lappe RW, Smits JFM, Todt JA, Debets JJM, Wendt RL. Failure of atriopeptin II to cause arterial vasodilation in the conscious rat. Circ Res 1985;56:606-612.

157. Breuhaus BA, Ssaneii HH, Brandt MA, Chimoskey JE. Atriopeptin II lowers cardiac output in conscious sheep. Am J Physiol 1985;249:R776-R780.

158. Almeida FA, Suzuki M, Maack T. Atrial natriuretic factor increases hematocrit and decreases plasma volume in nephrectomised rats. Life Sci 1986;39:1193-1199.

159. Fluckiger JP, Waeber B, Matsueda G, Delaloye B, Nussberger J, Brunner HR. Effect of atriopeptin III on hematocrit and volemia of nephrectomised rats. Am J Physiol 1986;251:H880-H883.

160. Bolli P, Muller FB, Linder L, et al. The vasodilator potency of atrial natriuretic peptide in man. Circulation 1987;75:221-228.

161. Atarashi K, Mulrow PJ, Franco-Saenz, Rapp J. Inhibition of aldosterone production by an atrial extract. Science 1984;224:992-994.

162. De Lean A, Racz K, Gutkowska J, Nguyen TT, Cantin M, Genest J. Specific receptor-mediated inhibition by synthetic atrial natriuretic factor of hormone-stimulated steroidogenesis in cultured bovine adrenal cells. Endocrinology 1984;115:1636-1638.

163. Anderson JV, Struthers AD, Payne NN, Slater JDH, Bloom SR. Atrial natriuretic peptide inhibits the aldosterone response to angiotensin II in man. Clin Sci 1986;70:507-512.

164. Chartier L, Schiffrin EL, Thibault G, Garcia R. Atrial natriuretic factor inhibits the stimulation of aldosterone secretion by angiotensin II, ACTH, and potassium in vitro and angiotensin II induced steroidogenesis *in-vivo*. Endocrinology 1984;115:2026-2028.

165. Maack T, Marion DN, Camargo MJ, et al. Effect of auriculin (atrial natriuretic factor) on blood pressure, renal function and the renin-aldosterone system in dogs. Am J Med 1984;77:1069-1075.

166. Kurtz A, Della Bruna R, Pfeilschifter J, Bauer C. Atrial natriuretic peptide inhibits renin release from juxtaglomerular cells by a cGMP-mediated process. Proc Natl Acad Sci USA 1986;83:4769-4773.

167. Quirion R, Dalpe M, De Lean A, Gutkowska J, Cantin M, Genest J. Atrial natriuretic factor (ANF) binding sites in brain and related structures. Peptides 1984;5:1167-1172.

168. Jacobowitz DM, Skofitsch G, Keiser HR, Eskay RL, Zamir N. Evidence for the existence of atrial natriuretic factor-containing neurons in the rat brain. Neurobiology 1985;40:92-94.

169. Saper CB, Standaert DG, Currie MG, Schwartz D, Geller DM, Needleman P. Atriopeptin-immunoreactive neurons in the brain: presence in cardiovascular regulating areas. Science 1985;227:1047-1049.

170. Tanaka I, Misono KS, Inagami T. Atrial natriuretic factor in rat hypothalamus, atria and plasma: determination by specific radioimmunoassay. Biochem Biophys Res Commun 1984;124:663-668.

171. Masotto C, Negro-Vilar A. Inhibition of spontaneous or angiotensin II stimulated water intake by atrial natriuretic factor. Brain Res Bull 1985;15:523-526.

172. Antunes-Rodrigues J, McCann SM, Rodgers LC, Samson WK. Atrial natriuretic factor inhibits dehydration- and angiotensin II-induced water intake in the conscious, unrestrained rat. Proc Natl Acad Sci USA 1985;82:8720-8723.

173. Januszewicz P, Thibault G, Garcia R, Gutkowska J, Genest J, Cantin M. Effect of synthetic atrial natriuretic factor on arginine vasopressin release by the rat hypothalamo-neurohypophyseal complex in organ culture. Biochem Biophys Res Commun 1986;134:652-658.

174. Poole CJM, Carter DA, Vallejo M, Lightman SL. Atrial natriuretic factor inhibits the stimulated *in-vivo* and *in-vitro* release of vasopressin and oxytocin in the rat. J Endocrinol 1987;112:97-102.

175. Ledsome JR, Wilson N, Courneya CA, Rankin AJ. Release of atrial natriuretic peptide by atrial distension. Can J Physiol Pharmacol 1985;63:739-742.

176. Dietz JR. Release of atrial natriuretic factor from rat heart-lung preparations by atrial distension. Am J Physiol 1984;247:R1093-R1096.

177. Lang RE, Tholken H, Ganten D, Luft FC, Ruskoaho H, Unger T. Atrial natriuretic factor: a circulating hormone stimulated by volume loading. Nature 1985;314:264-266.

178. Sugawara A, Nakao K, Morii N, et al. α-human atrial natriuretic polypeptide is released from the heart and circulates in the body. Biochem Biophys Res Commun 1985;129:439-446.

179. Raine AEG, Erne P, Burgisser E, Muller FB, Bolli P, Burkart F, Buhler FR. Atrial natriuretic peptide and atrial pressure in patients with congestive heart failure. N Engl J Med 1986;315:533-537.

180. Sato F, Kamoi K, Wakiya Y, et al. Relationship between atrial natriuretic peptide levels and atrial pressure in man. J Endocrinol Metab 1986;63:823-827.

181. Sagnella GA, Markandu ND, Shore AC, MacGregor GA. Effects of changes in dietary sodium intake and saline infusion on immunoreactive atrial natriuretic peptide in human plasma. Lancet 1985;ii:1208-1211.

182. Anderson JV, Donckier J, McKenna WJ, Bloom SR. The plasma release of atrial natriuretic peptide in man. Clin Sci 1986;71:151-155.

183. Anderson JV, Millar ND, O'Hare JP, Mackenzie JC, Corrall RJM, Bloom SR. Atrial natriuretic peptide: physiological release during water immersion in man. Clin Sci 1986;71:319-322.

184. Gerbes AL, Arendt RM, Schnizer W, et al. Regulation of atrial natriuretic factor release in man: effect of water immersion. Klin Wochenschr 1986;64:666-667.

185. Epstein M, Loutzenheiser R, Friedland E, Aceto RM, Camargo MIF, Atlas SA. Relationship of increased atrial natriuretic factor and renal sodium handling during immersion-induced central hypervolemia in normal humans. J Clin Invest 1987;79:738-745.

186. Koller PT, Grekin RJ, Nicklas JM. Paradoxical response of plasma atrial natriuretic hormone to pericardiocentesis in cardiac tamponade. Am J Cardiol 1987;59:491-492.

187. Rankin AJ, Wilson N, Ledsome JR. Influence of isoproterenol on plasma immunoreactive atrial natriuretic peptide and plasma vasopressin in the anesthetised rabbit. Pflugers Arch 1987;408:124-128.

188. Fisher BM, Baylis PH, Frier BM. Plasma oxytocin, arginine vasopressin and atrial natriuretic peptide responses to insulin-induced hypoglycaemia in man. Clin Endocrinol 1987;26:179-185.

189. Zamir N, Haass M, Dave JR, Zukowska-Grojec Z. Anterior pituitary gland modulates the release of atrial natriuretic peptides from cardiac atria. Proc Natl Acad Sci USA 1987;84:541-545.

190. Espiner EA, Nicholls MG, Yandle TG, Crozier TG, Cuneo RC, McCormick D, Ikram H. Studies on the secretion, metabolism and action of atrial natriuretic peptide in man. J Hypertens 1986;4:S85-S91.

191. Maack T, Suzuki M, Almeida FA, Nussensveig D, Scarborough RM, McEnroe GA, Lewicki JA. Physiological role of silent receptors of atrial natriuretic factor. Science 1987;238:675-678.

192. Condra CL, Leidy EA, Bunting P, Colton CD, Nutt RF, Rosenblatt M, Jacobs JW. Clearance and early hydrolysis of atrial natriuretic factor *in-vivo*. Structural analysis of cleavage sites and design of an analogue that inhibits hormone cleavage. J Clin Invest 1988;81:1348-1354.

193. Johnson GR, Arik L, Foster CJ. Metabolism of ^{125}I-atrial natriuretic factor by vascular smooth muscle cells. Evidence for a peptidase that specifically removes the COOH-terminal tripeptide. J Biol Chem 1989;264:11637-11642.

194. Olins GM, Krieter PA, Trapani AJ, Spear KL, Bovy PR. Specific inhibitors of endopeptidase 24.11 inhibit the metabolism of atrial natriuretic peptides *in-vitro* and *in-vivo*. Mol Cell Endocrinol 1989;61:201-208.

195. Northridge DB, Jardine AG, Alabaster CT, et al. Effects of UK 69 578: A novel atriopeptidase inhibitor. Lancet 1989;ii:591-593.

196. Larochelle P, Cusson JR, Gutkowska J, Schiffrin EL, Hamet P, Kuchel O, Genest J, Cantin M. Plasma atrial natriuretic factor concentrations in essential and renovascular hypertension. Br Med J 1987;294:1249-1252.

197. Anderson JV, Raine AEG, Proudler A, Bloom SR. Effect of haemodialysis on plasma concentrations of atrial natriuretic peptide in adult patients with chronic renal failure. J Endocr 1986;110:193-196.

198. Sagnella GA, Markandu ND, Shore AC, MacGregor GA. Raised circulating levels of atrial natriuretic peptides in essential hypertension. Lancet 1986;i:179-181.

199. Nicklas JM, DiCarlo LA, Koller PT, et al. Plasma levels of atrial natriuretic factor increase during supraventricular tachycardia. Am Heart J 1986;112:923-928.

200. Roy D, Paillard F, Cassidy D, et al. Atrial natriuretic factor during atrial fibrillation and supraventricular tachycardia. Am J Coll Cardiol 1987;9:509-514.

201. Sonnenberg H, Veress AT. Cellular mechanism of release of atrial natriuretic factor. Biochem Biophys Res Commun 1984;124:443-449.

202. Ballerman BJ. A highly sensitive radioceptor assay for atrial natriuretic peptide in rat plasma. In: The American Society of Hypertension. Second World Congress on Biologically active Peptides 1987:208.

203. Burgisser E, Raine AEG, Erne P, Kamber P, Buhler FR. Human cardiac plasma concentrations of atrial natriuretic peptide quantified by radioceptor assay. Biochem Biophys Res Commun 1985;133:1201-1209.

204. Sagnella GA, Buckley MG, Markandu ND, MacGregor GA. Atrial natriuretic peptide in human plasma: comparison of radioceptor vs radioimmunoassay. In: The American Society of Hypertension. Second World Congress on Biologically Active Atrial Peptides 1987:208.

205. McLaughlin LL, Wei Y, Stockman PT, Leahy KM, Needleman P. Development, validation and application of an enzyme immunoassay (EIA) of atriopeptin. Biochem Biophys Res Commun 1987;144:469-476.

206. Hunter WM, Greenwood FC. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (London) 1962;194: 495-496.

207. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ. Coated charcoal immunoassay for insulin. J Cli Endocr Metab 1965;25:1375-1384.

208. Arimura A, Sato H, Coy DH, Worobec RB, Schally AV, Yanaihara N, Hashimoto T, Yanaihara C, Sukura N. The antigenic determinant of the LH-releasing hormone of three different antisera. Acta Endocrinol 1975;78:222-231.

209. Goodfriend TL, Levine L, Fasman GD. Antibodies to bradykinin and angiotensin: a use of carbodiimides in immunology. Science 1964;144:1344-1346.

210. Anderson JV, Christofides ND, Vinas P, Wharton J, Varndell IM, Polak JM, Bloom SR. Radioimmunoassay of alpha rat atrial natriuretic peptide. Neuropeptides 1986;7:159-173.

211. Mitchison NA. The carrier effect in the secondary response to hapten-protein conjugates. I. Measurement of the effect with transferred cells and objections to the local environment hypothesis. Eur J Immunol 1971;1:10-18.

212. Freund J. The effect of paraffin oil and mycobacteria on antibody formation and sensitisation. Am J Clin Pathol 1951;21:645-656.

213. Anderson JV. Atrial Natriuretic Peptide. MD Thesis 1988. Univ of Cambridge 1988, Ch 2, pp 36-90.

214. Gutkowska J, Thibault G, Januszewicz P, et al. Direct radioimmunoassay of atrial natriuretic factor. Biochem Biophys Res Commun 1984;122:593-601.

215. Nakao K, Sugawara A, Morii N, et al. Radioimmunoassay for alpha human and rat atrial natriuretic polypeptide. Biochem Biophys Res Commun 1984;124:815-821.

216. Tikkanen I, Fyhrquist F, Metsarinne K, Leidenius R. Plasma atrial natriuretic peptide in cardiac disease and during infusion in healthy volunteers. Lancet 1985;ii:66-69.

217. Naruse M, Naruse K, Obana K, et al. Immunoreactive alpha-human atrial natriuretic polypeptide in human plasma. Peptides 1986;7:141-145.

218. Cernacek P, Crawhall JC, Levy M. Atrial natriuretic peptide: Blood levels in human disease and their measurement. Clin Biochem 1988;21:5-17.

219. Richards AM, Tonolo G, McIntyre GD, Leckie BJ, Robertson IS. Radioimmunoassay for plasma alpha atrial natriuretic peptide: A comparison of direct and pre-extracted methods. J Hypertension 1987;5:227-236.

220. Nishiuchi T, Saito H, Yamasaki Y, Saito S. Radioimmunoassay for atrial natriuretic peptide: method and results in normal subjects and patients with with various diseases. Clin Chim Acta 1986;159:45-47.

221. MacGregor GA, Markandu ND, Best FE, et al. Double-blind randomised crossover trial of moderate sodium restriction in essential hypertension. Lancet 1982;i:351-355.

222. Thaler BI, Paulin JM, Phelan EL et al. A pilot study to test the feasibility of salt restriction in a community. NZ Med J 1982;95:839-842.

223. Pietinen P, Tuomilehto J. Estimating sodium intake in epidemiological studies. In: Kesteloot H, Joosens JV, eds. Epidemiology of arterial blood pressure. The Hague: Martinus Nijhoff, 1982:29-44.

224. Lang CL, Weinberger MH, Miller JZ. Dietary councelling results in effective sodium restriction. J Am Diet Assoc 1985;85:477-479.

225. Caggiula AW, Wing RR, Nowalk MP, et al. The measurement of sodium and potassium intake. Am J Clin Nutr 1985;42:391-398.

226. MacGregor GA, Markandu ND, Singer DRJ, et al. Moderate sodium restriction with angiotensin converting enzyme inhibitor in essential hypertension: a double blind study. Br Med J 1987;294:531-534.

227. Richards AM, Tonolo G, Cleland GF, et al. Plasma atrial natriuretic peptide concentrations during exercise in sodium replete and sodium deplete normal man. Clin Sci 1987;72:159-164.

228. Solomon LR, Atherton JC, Bobinski H, et al. Effect of dietary sodium chloride and posture on plasma immunoreactive atrial natriuretic peptide concentrations in man. Clin Sci 1987;72:201-208.

229. Department of Health and Social Security Report on Health and Social Subjects. Recommended daily amounts of food energy and nutrients for groups of people in the United Kingdom. London HMSO, 1981:15.

230. Papper, Rosenbaum JD. Abnormalities in the excretion of water and sodium in "compensated" cirrhosis of the liver. J Lab Clin Med 1952;523-530.

231. Bernardi M, de Palma R, Trevisani F, Santini C, Capani F, Baraldini M, Gasbarrini G. Chronobiological study of factors affecting plasma aldosterone concentration in cirrhosis. Gastroenterology 1986;91:683-691.

232. Bernardi M, Trevisani F, de Palma R, Ligabue A, Capani F, Baraldini M, Gasbarrini G. Chronobiological evaluation of sympathoadrenergic function in cirrhosis. Gastroenterology 1987;93:1178-1186.

233. Bernardi M, Santini C, Trevisani F, Baraldini M, Ligabue A, Gasbarrini G. Renal function impairment induced by change in posture in patients with cirrhosis and ascites. Gut 1985;26:629-635.

234. Karnad DR, Tembulkar P, Abraham P, Desai NK. Head-down tilt as a physiological diuretic in normal controls and in patients with fluid retaining ascites. Lancet 1987;ii:525-527.

235. Gines P, Jimenez W, Arroyo V, Navasa M, Lopez C, Tito L, Serra A, Bosch J, Sanz G, Rivera F, Rodes J. Atrial natriuretic factor in cirrhosis with ascites: Plasma levels, cardiac release and splachnic extraction. Hepatology 1988;8:636-642.

236. Klepetko W, Muller Ch, Hartter E, Miholics J, Scharz Ch, Voloszcuk W, Moeschl P. Plasma atrial natriuretic factor in cirrhotic patients with ascites. Effect of peritoneovenous shunt implantation. Gastroenterology 1988;95:764-770.

237. Bonkovski HL, Hartle DK, Mellen BG, Kutner M, Galambos JT. Plasma concentrations of immunoreactive atrial natriuretic peptide in hospitalised cirrhotic and non cirrhotic patients: Evidence for a role of deficient atrial natriuretic peptide in pathogenesis of cirrhotic ascites. Am J Gastroenterol 1988;83:531-535.

238. Panos MZ, Williams R. Feasibility of a severely restricted sodium diet by a councelling method. Ann Clin Biochem 1989;26:358-360.

239. Menard J, Catt KJ. Measurement of renin activity, concentration and substrate in rat plasma by radioimmunoassay of angiotensin I. Endocrinology 1972;90:422-430.

240. Jones JC, Cauter GD, MacGregor GA. Interference by plasma metabolites in a direct radioimmunoassay for plasma aldosterone. Ann Clin Biochem 1981;18:54-59.

241. Brabant G, Juppner H, Kirschner M, et al. Human atrial natriuretic peptide (ANP) for the treatment of patients with liver cirrhosis and ascites. Klin Wochenschr 1986;64(Suppl VI):108-111.

242. Gerbes A, Wernze H, Arendt RM, Riedel A, Sauerbruch T, Paumgartner G. Atrial natriuretic factor and renin-aldosterone in volume regulation of patients with cirrhosis. Hepatology 1989;9:417-422.

243. Shenker Y, Sider RS, Ostafin EA, et al. Plasma levels of immunoreactive atrial natriuretic factor in healthy subjects and in patients with edema. J Clin Invest 1985;76:1684-1687.

244. Henricksen JH, Schuten HJ, Bendtsen F, et al. Circulating atrial natriuretic peptide (ANP) and central blood volume (CBV) in cirrhosis. Liver 1986;6:361-368.

245. Kimura T, Abe K, Ota K, et al. Effects of acute water load, hypertonic saline infusion and furosemide administration on atrial natriuretic peptide and vasopressin release in humans. J Clin Endocr Metab 1986;62:1003-1010.

246. Hollister AS, Rodeheffer RJ, White FJ, Potts JR, Imada T, Inagami T. Clearance of atrial natriuretic factor by lung, liver, and kidney in human subjects and the dog. J Clin Invest 1989;83:623-628.

247. Liebowitz HR. Hazards of abdominal paracentesis in the cirrhotic patient. Part III, NY State J Med 1962;62:2223-2229.

248. Nelson WP, Rosenbaum JD, Strauss MB. Hyponatraemia in hepatic cirrhosis following paracentesis. J Clin Invest 1951;30:738-744.

249. Guazzi M, Polese A, Magrini F, Olivari MT. Negative influences of ascites on the cardiac function of cirrhotic patients. Am J Med 1975;59:165-170.

250. Knauer CM, Lowe HM. Haemodynamics in the cirrhotic patient during paracentesis. N Engl J Med 1967;276:491-496.

251. Quintero E, Gines P, Arroyo V, Rimola A, Bori F, Planas R, Viver J, Cabrera J. Paracentesis versus diuretics in the treatment of cirrhotics with tense ascites. Lancet 1985;1:611-612.

252. Simon DM, McCain JR, Bonkovski HL, Wells JO, Hartle D, Galambos JT. Effects of therapeutic paracentesis on systemic and hepatic hemodynamics and on renal function. Hepatology 1987;7:423-429.

253. Salerno F, Badalamenti S, Incerti P, et al. Repeated paracentesis and IV albumin infusion to treat "tense" ascites in cirrhotic patients: a safe alternative therapy. J Hepatol 1987;5:102-108.

254. Gines P, Tito Ll, Arroyo V, et al. Randomised comparative study of therapeutic paracentesis with and without intravenous albumin in cirrhosis. Gastroenterology 1988;94:1493-1502.

255. Gines P, Arroyo V, Quintero E, et al. Comparison of paracentesis and diuretics in the treatment of cirrhotics with tense ascites: Results of a randomised study. Gastroenterology 1987;93:234-241.

256. Badalamenti S, Gines P, Tito Ll, Panes J, Planas R, Llach J, Jimenez W, Rimola A, Arroyo V, Rodes J. Hypovolemia in cirrhotics treated with paracentesis plus IV albumin: incidence, clinical relevance, and predictive factors. J Hepatol 1987;5(Suppl):S7.

257. Kao HW, Rakov NE, Savage E, Reynolds TB. The effect of large volume paracentesis on plasma volume- a cause of hypovolemia? Hepatology 1985;5:403-407.

258. Ring-Larsen H, Hesse B, Henricksen JH, Christensen NJ. Sympathetic nervous activity and systemic hemodynamics in cirrhosis: Plasma norepinephrine concentration, hepatic extraction, and renal release. Hepatology 1982;2:304-310.

259. Paintal AS. A study of right and left atrial receptors. J Physiol 1953;120:596-610.

260. Pockros PJ, Reynolds TB. Rapid diuresis in patients with chronic liver disease: the importance of peripheral edema. Gastroenterology 1986;90:1827-1833.

261. Panos M, Westaby D, Williams R. Treating the ascites of cirrhosis. Br Med J 1988;297:698-699.

262. Wilkinson SP, Arroyo VA, Moodie H, Blendis LM, Williams R. Abnormalities of sodium excretion and other disorders of renal function in fulminant hepatic failure. Gut 1976;17:501-505.

263. Guarner F, Hughes RD, Gimson AES, Williams R. Renal function in fulminant hepatic failure: Haemodynamics and renal prostaglandins. Gut 1987;28:1643-1647.

264. Bernardi M, Wilkinson SP, Wernze H, Spech HJ, Muller G, Poston L, Williams R. The renin-angiotensin-aldosterone system in fulminant hepatic failure. Scand J Gastroenterol 1983;18:369-375.

265. Trey C, Davidson V. The management of fulminant hepatic failure. In: Popper H, Schaffner F, eds. Progress in liver diseases, Vol 3. New York: Grune and Stratton, 1970:282-298.

266. Wilkinson SP, Blendis LM, Williams R. Frequency and type of renal and electrolyte disorders in fulminant hepatic failure. Br Med J 1974;1:186-189.

267. Richards AM, Cleland JGF, Tonolo G, McIntyre GD, Leckie BJ, Dargie HJ, Ball SG, Robertson JIS. Plasma α natriuretic peptide in cardiac impairment. Br Med J 1986;293:409-412.

268. Woods RL. Contribution of the kidney to metabolic clearance of atrial natriuretic peptide. Am J Physiol 1988;55:E934-E935.

269. Strong CG, Tapia HR, Hunt J. Metabolism of renin activity in three canine models: Isolated liver, acute hepatic necrosis and chronic toxic hepatitis. Clin Sci Mol Med 1973;45:229S-233S.

270. Coppage WS, Island DP, Cooner AE, Liddle G. The metabolism of aldosterone in normal subjects and in patients with hepatic cirrhosis. J Clin Invest 1962;41:1672-1680.

271. Laragh JH, Angers M, Kelly WG, Lieberman S. Hypotensive agents and pressor substances: the effect of epinephrine, angiotensin II and others on the secretory rate of aldosterone. J Am Med Assoc 1960;174:234-240.

272. Salerno F, Badalamenti S, Incerti P, Capozza L, Mainardi L. Renal response to atrial natriuretic peptide in patients with advanced liver cirrhosis. Hepatology 1988;8:21-26.

273. Gerbes AL, Arendt RM, Ritter D, Jungst D, Zahringer J, Paumgartner G. Plasma atrial natriuretic factor in patients with cirrhosis. N Engl J Med 1985;313:1609-1610.

274. Fukui H, Tsujii T, Matsamura M, Okamoto S, Morita T, Koiozumi M, Morimura M, Kojima H, Hokaze Y, Fujimoto M, Yamao J. Plasma levels of atrial natriuretic peptide in patients with liver cirrhosis and its relation to ascites and renal function. Gastroenterologia Japonica 1989;24:149-155.

275. Arendt RM, Gerbes AL, Ritter D, Stangl E, Bach P, Zahringer J. Atrial natriuretic factor in patients with arterial hypertension, heart failure or cirrhosis of the liver. J Hypertension 1986;4 (Suppl 2):S131-S135.

276. Jimenez W, Gutkowska J, Gines P, Rivera F, Arroyo V, Rodes J. Chromatographic pattern and biological activity of atrial natriuretic factor (ANF) in cirrhosis with ascites. J Hepatol 1989;9 (Suppl 1):S171.

277. Lopez C, Jimenez W, Arroyo V, Gaya J, Rivera F, Rodes J. Infusion of atrial natriuretic factor (ANF) produces a diminished natriuretic response in cirrhotic rats with ascites. J Hepatol 1988;6 (Suppl.3): P13-P14.

278. Koepke JP, Jones S, Di Bona GF. Renal nerves mediate blunted natriuresis to atrial natriuretic peptide in cirrhotic rats. Am J Physiol 1987;252:R1019-R1023.

279. Lee J, Feng JQ, Malvin RL, Huang BS, Grekin RJ. Centrally administered atrial natriuretic factor increases renal water excretion. Am J Physiol 1987;252:F1011-F1015.

280. Firth JD, Raine AEG, Ledingham JGG. Low concentrations of ANP cause pressure-dependent natriuresis in the isolated kidney. Am J Physiol 1988;255:F391-F396.

281. Proctor E and Chatamra K. High yield micronodular cirrhosis in the rat. Gastroenterology 1982;83:1183-1190.

282. Nishiitsutsuji-Uwo JM, Ross BD, Krebs HA. Metabolic activities of the isolated perfused rat kidney. Biochem J 1967;103:852-862.

283. Epstein FH, Brosnan JT, Tange JD, Ross BD. Improved function with amino acids in the isolated perfused kidney. Am J Physiol 1982; 243: F284-F292.

284. Maack T. Renal clearance and isolated kidney perfusion techniques. Kidn Intl 1986;30:142-151.

285. Firth JD, Gove C, Panos MZ, Raine AEG, Williams R, Ledingham JGG. Sodium handling in the isolated perfused kidney of the cirrhotic rat. Clin Sci 1989;77:657-661.

286. Maude DL, Kao-Lo G. Salt excretion and vascular resistance of perfused kidneys of Dahl rats. Hypertension 1982;4:532-537.

287. Firth JD, Raine AEG, Ledingham JGG. Abnormal sodium handling occurs in the isolated perfused kidney of the nephrotic rat. Clin Sci 1989;76:387-395.

288. Harrap SB, Clark SA, Fraser R, Towrie A, Brown AJ, Lever AF. Effects of sodium intake and aldosterone on the renal pressure-natriuresis. Am J Physiol 1988;254:F697-F703.

289. Maack T. Physiological evaluation of the isolated perfused rat kidney. Am J Physiol 1980;238:F71-F78.

290. Di Bona GF, Herman PJ, Sawin LL. Neural control of renal function in edema forming states. Am J Physiol 1988;254:R1017-R1024.

291. Jimenez W, Martinez-Pardo A, Arroyo V, Bruix J, Rimola A, Gaya J, Rivera F, Rodes J. Temporal relationship between hyperaldosteronism, sodium retention and ascites formation in rats with experimental cirrhosis. Hepatology 1985;5:245-250.

292. Gerbes AL, Kollenda MC, Reichen J, Vakil N, Vollmar AM, Scarborough RM. Bile duct ligated rats with ascites exhibit increased density of two types of binding sites for atrial natriuretic factor (ANF) on glomerular membranes. J Hepatology 1989;9 (Suppl I):S35.

293. Tripodo MC, Cole FE, MacPhee AA, Pegram BL. Biologic mechanisms of atrial natriuretic factor. J Lab Clin Med 1986;109:112-119.

294. Nicolaides KH, Rodeck CH, Soothill PW, Campbell S. Ultrasound guided sampling of umbilical cord to assess fetal well being. Lancet 1986;ii:1065-1067.

295. Yamaji T, Hirai N, Ishibashi M, et al. Atrial natriuretic peptide in umbilical cord blood: Evidence for a circulating hormone in human fetus. J Endocrinol Metab 1986;63:1414-1417.

296. Nicolaides KH, Clewell W, Rodeck CH. Measurement of human feto-placental blood volume in erythroblastosis fetalis. Am J Obst Gynecol 1987;157:50-53.

297. Nicolaides KH, Soothill PW, Clewell WH, Rodeck CH, Mibashan RS, Campbell S. Fetal haemoglobin measurement in the assessment of red cell iso-immunisation. Lancet 1988;i:1073-1075.

298. Soothill PW, Nicolaides KH, Rodeck CH, Campbell S. The effect of gestational age on fetal and intervillous blood gas and acid-base values in human pregnancy. Fetal Therapy 1986;i:166-173.

299. Nicolaides KH, Soothill PW, Rodeck CH, Clewell W. Rhesus disease: Intravascular blood transfusion by cordocentesis. Fetal Therapy 1986;i:185-192.

300. Ross MG, Ervin MG, Lam RW, Castro L, Leake RD, Fisher DA. Plasma atrial natriuretic peptide response to volume expansion in the ovine fetus. Am J Obstet Gynecol 1987;157:1292-1297.

301. Ervin MG, Ross MG, Lam RW et al. Atrial natriuretic peptide: ovine fetal cardiovascular responses and arginine-vasopressin secretion. Clin Res. 1987;35:233A.

302. Hurley JK, Kirkpatrick SE, Pitlick PT, Friedeman WF, Mendoza SA. Renal responses of the fetal lamb to fetal or maternal volume expansion. Circ Res 1977;40:557-560.

303. Ross MG, Leake RD, Ervin MG, Fisher DA. Fetal lung fluid response to maternal hyperosmolality. Pediatr Pulmonol. 1986;250:E253-E258.

304. Tsuchimochi H, Kurimoto F, Ieki K et al. Atrial natriuretic peptide distribution in fetal and failed adult human hearts. Circulation 1988;78:920-927.

305. Walker AM. Physiological control of the fetal cardiovascular system. In: Beard RW, Nathaniels ZPW, Eds. Fetal Physiology and Medicine Butterworths 1984:287-316.