STUDIES ON THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN CIRRHOSIS OF THE LIVER

Alastair John MacGilchrist, M.B., Ch.B. (Glasgow), M.R.C.P. (U.K.)

Thesis submitted for the degree of Doctor of Medicine

to the

University of Glasgow

from the

Department of Materia Medica,

University of Glasgow,

Stobhill General Hospital,

Glasgow.

July 1988

PREFACE

This thesis describes research undertaken during my appointment as Registrar in the University Department of Materia Medica, Stobhill General Hospital, Glasgow. None of the work has been published, but some of it has been presented to learned societies. I have been fortunate in having the co-operation and collaboration of a number of colleagues and friends. They are formally acknowledged. Except where stated, the work of this thesis has been personally carried out by me. The writing of this thesis is entirely my own work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure 4.1.

Plasma noradrenaline concentrations in control subjects and cirrhotic patients who have never had ascites (Group I), those now free of ascites on diuretics (Group II) and those currently with ascites (Group III). Horizontal lines represent median values.

Figure 4.2.

Plasma renin activity in control subjects and cirrhotic patients who have never had ascites (Group I), those now free of ascites on diuretics (Group II) and those currently with ascites (Group III). Horizontal lines represent median values.

Figure 4.3.

Plasma noradrenaline concentrations in cirrhotic patients graded according to Pugh (184) as mild (A), moderate (B) or severe (C). Horizontal lines represent median values.

Figure 4.4.

"Life table" survival graph of cirrhotic patients after one year according to plasma noradrenaline on presentation: (a) < 5 nmol.l^{-1}; (b) 5-10 nmol.l^{-1}; (c) > 10 nmol.l^{-1}

Figure 4.5.

Plasma ANP in control subjects and cirrhotic patients who have never had ascites (Group I), those now free of ascites on diuretics (Group II) and those currently with ascites (Group III). Horizontal lines represent median values.

Figure 4.6.

Plasma ANP in cirrhotic patients graded according to Pugh (184) as mild (A), moderate (B) or severe (C). Horizontal lines represent median values.

Figure 5.1.

Standing-to-lying ratio in mild cirrhosis (◇), severe cirrhosis (◆) and controls (▲). Horizontal bars represent median values.

Figure 5.2.

Valsalva Ratio in mild cirrhosis (◇), severe cirrhosis (◆) and controls (▲). Horizontal bars represent median values.

Figure 5.3.

Maximum change in heart rate during facial immersion in water in mild cirrhosis (◇), severe cirrhosis (◆) and controls (▲). Horizontal bars represent median values.

Figure 5.4.

Maximum change in heart rate during deep breathing in mild cirrhosis (◇), severe cirrhosis (◆) and controls (▲). Horizontal bars represent median values.

Figure 5.5.

Blood pressure changes during isometric exercise and the cold pressor test in mild cirrhosis (◇), severe cirrhosis (◆) and controls (▲). Upper lines represent systolic BP and lower lines diastolic BP.

Figure 5.6.

Changes in systolic and diastolic blood pressure in response to maximal and timed dynamic exercise. The bars represent mean values with standard deviations indicated by error bars.

Figure 5.7.

Heart rate increase in response to maximal and timed dynamic exercise. The bars represent mean values with standard deviations indicated by error bars.

Figure 6.1.

Rationale for a quadratic fit for human dose response curves.

Figure 6.2.

Increase in systolic blood pressure in response to noradrenaline, constructed from the means of the individual log PD_{10} and log PD_{20} values for each group.

Figure 6.3.

Increase in mean arterial pressure in response to angiotensin II, constructed from the means of the individual log PD_{10} and log PD_{20} values for each group.

Figure 6.4.

Increase in mean arterial pressure in response to phenylephrine in a representative matched pair of subjects.

Figure 6.5.

Increase in mean arterial pressure in response to phenylephrine, constructed from the means of the individual log PD_{10} and log PD_{20} values for both groups. (Horizontal bars represent standard deviation).

Figure 6.6.

Increase in systolic blood pressure in response to alphamethylnoradrenaline in a representative matched pair of subjects.

Figure 6.7.

Increase in systolic blood pressure in response to alphamethylnoradrenaline, constructed from the means of the individual log PD_{10} and log PD_{20} values for both groups. (Horizontal bars represent standard deviation).

Figure 6.8.

Increase in heart rate in response to isoprenaline in a representative matched pair of subjects.

Figure 6.9.

Increase in heart rate in response to isoprenaline, constructed from the means of the individual log CD_{10} and log CD_{20} values for both groups. (Horizontal bars represent standard deviation).

Figure 7.1.

Bmax for platelet $alpha_2$ receptors (Error bars represent mean and standard deviation).

Figure 7.2.

K_D for platelet $alpha_2$ receptors (Error bars represent mean and standard deviation).

Figure 7.3.

Bmax for lymphocyte $beta_2$ receptors (Error bars represent mean and standard deviation).

Figure 7.4.

K_D for lymphocyte $beta_2$ receptors (Error bars represent mean and standard deviation).

ACKNOWLEDGMENTS

I am indebted to many people within the Department of Materia Medica at Stobhill Hospital who have made this thesis possible, and whose assistance I wish to acknowledge. Professor John Reid was responsible for the original idea to study the sympathetic nervous system in cirrhosis, and throughout my studies provided an invaluable source of expert guidance, constructive criticism and encouragement. He and Dr. T.J. Thomson permitted me sufficient freedom from my clinical duties to carry out the experiments, and Dr. Thomson and Dr. John Forrest kindly allowed me access to their patients with liver disease. The staff of the Clinical Pharmacology Research Unit helped me to perform the clinical studies. Dr. Laurie Howes initiated the catecholamine assays, many of which were performed by Mrs. C. Hawksby. Dr. N. Deighton was of great assistance in the adrenoceptor studies, and the ANP assay was performed by Miss J. Brown of the Department of Clinical Pharmacology, Royal Infirmary, Edinburgh. Dr. David Sumner and Miss K. Howie gave much invaluable advice regarding the statistical analyses. Mrs. J. Hamilton carried out the typing patiently and faultlessly. To all these individuals, and to the many others whom I have not methioned by name, I wish to register my sincere gratitude. I also wish to acknowledge the support of my wife, who suffered my preoccupation with great patience.

SUMMARY

This thesis is a result of the observation that plasma noradrenaline is increased in cirrhosis with ascites. Three specific questions were addressed: is this increase the result of increased spillover of noradrenaline into plasma (and thus increased activity of the sympathetic nervous system) or of reduced clearance from plasma? What are the implications of sympathetic activation for the various theories of ascites formation? Why does sympathetic overactivity fail to correct the reduced peripheral vascular resistance characteristic of cirrhosis?

The kinetics of exogenous tritiated noradrenaline were measured, using an HPLC separation of the radiolabelled noradrenaline in plasma, in 14 cirrhotics with ascites and 13 controls. The cirrhotics had a marked increase in noradrenaline spillover with plasma clearance also slightly increased. Therefore the increased plasma noradrenaline in cirrhosis is due to increased sympathetic activity.

Noradrenaline, dihydroxyphenylglycol (DHPG), renin and atrial natriuretic peptide (ANP) were then measured in peripheral venous blood in 41 cirrhotic patients and 34 control patients. Noradrenaline was increased in cirrhotics both with and without ascites, in contrast to renin which was only increased in those cirrhotics with ascites. Therefore sympathetic overactivity in cirrhosis appears to precede the development of ascites. This supports the "underfilling" theory of ascites, which

suggests that the effective central intravascular volume is reduced in cirrhosis. The degree of sympathetic activity was shown to parallel the degree of hepatic impairment, and plasma noradrenaline was a useful prognostic indicator in cirrhosis. This may prove of clinical value in assessing the timing of hepatic transplantation.

It has been speculated that a deficiency of ANP may contribute to the sodium retention of ascites. In fact plasma ANP was not reduced, but was marginally increased in the presence of ascites. This suggests that ANP is responding appropriately to sodium overload occurring for other reasons.

Despite this sympathetic overactivity, patients with cirrhosis demonstrate reduced peripheral vascular resistance and cutaneous vasodilatation. A series of experiments was performed to try to identify the site of this "block" to normal sympathetic vasoconstriction. One theoretical possibility would be an autonomic neuropathy, particularly in alcoholic liver disease. Autonomic function was assessed by a standard battery of parasympathetic and sympathetic tests in 20 cirrhotics and 20 controls. The parasympathetically-mediated heart rate responses to deep breathing, to facial immersion in water and to the Valsalva manoeuvre were all reduced in cirrhotics with severe hepatic impairment. The sympathetically-mediated blood pressure response to isometric exercise was also reduced. However, the absence of clinical evidence of neuropathy in these

patients suggested that the changes demonstrated might be a result of impaired vascular responsiveness rather than neuropathic damage.

To consider this question, the blood pressure responses to steady state intravenous infusions of noradrenaline and angiotensin II were measured in 20 cirrhotics and 20 controls. Dose response curves were constructed from a minimum of 4 doses using a quadratic fit, allowing calculation of individual PD_{20}, the dose of agent required to raise blood pressure by 20 mmHg. The rise in blood pressure was attenuated to both noradrenaline and angiotensin II in the cirrhotics with severe disease. Therefore the site of the "block" is distal to the sympathetic nerves. The attenuated response to both sympathetic and non-sympathetic vasoconstrictors could indicate a direct vascular abnormality. Alternatively, there could be parallel desensitisation to the effects of noradrenaline and angiotensin II, both of which are frequently raised in cirrhosis.

The question of sympathetic desensitisation was therefore considered in more detail by examining the cardiovascular responses to selective sympathetic agonists in 10 cirrhotics with severe disease and 10 controls, using similar methods to the previous experiment. The blood pressure responses to phenylephrine (an $alpha_1$ agonist) and alphamethylnoradrenaline (an $alpha_2$ agonist) were both impaired in the cirrhotic group, in contrast to the heart

rate response to isoprenaline (a beta agonist) which was normal. There is thus no generalised sympathetic desensitisation, but all responses mediated by the peripheral vasculature are impaired, pointing to a defect at this site.

Such a defect could involve the adrenergic receptors, by prior occupancy of the receptor site either by the increased circulating noradrenaline or by some catecholamine-like substance (c.f. the "false neurotransmitter" theory) or by "down-regulation" of the receptor number in response to sustained sympathetic overactivity. The $alpha_1$ adrenoceptor on peripheral vessels is inaccessible to study in man, and therefore as a compromise the $alpha_2$ receptors on platelets were studied in 10 cirrhotics and 10 controls. The method involved incubation of the isolated platelets with tritiated yohimbine, measurement of free and bound radioactivity, and calculation of receptor affinity and number by Scatchard analysis. In fact both the number and affinity of the platelet $alpha_2$ receptors were normal, i.e. there was no down-regulation nor receptor site interference. If this reflects the $alpha_1$ adrenoceptor on peripheral vessels, it would suggest a "post-receptor" defect within the vascular smooth muscle cell itself. This probable localisation should assist in the on-going search for the vasodilator(s) responsible for the reduced vascular resistance.

Beta adrenoceptors were also assessed, using the $beta_2$

receptor on circulating lymphocytes. The radioligand employed was [^{131}I] iodocyanopindolol. Again, normal receptor number and affinity were demonstrated, which is in keeping with the normal heart rate response to the beta agonist isoprenaline. This study therefore does not support the hypothesis that down-regulation of beta receptors contributes to reduced efficacy of the beta adrenergic antagonist propranolol in variceal haemorrhage in "decompensated" cirrhosis.

The studies described above are in keeping with, but do not provide proof of, the following hypothesis: that in cirrhosis one or more vasodilatory substances accumulate, perhaps due to failure of hepatic inactivation, which act at an intracellular level on the peripheral vasculature. The resulting vasodilatation causes peripheral blood pooling with relative underfilling of the central vascular space. A baroreceptor-mediated increase in sympathetic activity, and possibly other neurohumoral mechanisms, ensues. This sympathetic activation could be one of the factors which cause sodium retention leading to ascites, and ultimately renal vasoconstriction leading to functional renal failure. It appears that the sympathetic nervous system does play a significant role in the complications of cirrhosis of the liver.

CHAPTER 1

BACKGROUND AND SCOPE OF THE THESIS

1.1. INTRODUCTION

This thesis explores aspects of the sympathetic nervous system in cirrhosis of the liver. Firstly, can the measurement of the degree of sympathetic activity shed any light on the continuing controversy regarding the pathogenesis of ascites? Secondly, does the sympathetic nervous system contribute to the sodium retention and renal vasoconstriction which are fundamental to the pathogenesis of ascites and functional renal failure in cirrhosis, respectively? Thirdly, how are the normal sympathetic control mechanisms altered in the presence of the still unexplained circulatory disturbance of cirrhosis? The first and last of these topics constitute the basis for the experiments in this thesis, in Chapters 3-4 and 5-7 respectively. The contribution of sympathetic activity to sodium retention and renal vasoconstriction has been extensively studied by others and their findings are discussed in this background chapter.

The principal theories of the pathogenesis of ascites are discussed, followed by the contribution to the debate of studies measuring or manipulating the plasma volume both directly and indirectly. The latter involves assessment of the renin-angiotensin-aldosterone system, the sympathetic nervous system and vasopressin. The mechanism of functional renal failure in cirrhosis, probably the extreme end of a spectrum which incorporates sodium retention and ascites, is discussed with emphasis on the role of imbalance

of the neurohumoral control of renal blood flow. Next, the circulatory disturbance of cirrhosis is described followed by a discussion of its possible causes, with emphasis on alterations in the normal sympathetic control mechanisms. The review is prefaced by some historical background.

1.2. THE PATHOGENESIS OF ASCITES

1.2.1. History

The association of ascites with liver disease has been recognised for over two thousand years, the word itself being derived from the Greek for a fluid-filled bag. The first theory of its formation is attributed to Hippocrates, who stated that "when the liver is full of fluid and this overflows into the peritoneal cavity, so that the belly becomes full of water, death follows" (c400 BC). His choice of words reminds us that there is little new in medicine, since Lieberman's alternative theory of ascites formation, which revitalised interest in this area, has become known as the "overflow" theory (1).

The contributions to our knowledge from the days of the ancient Greeks until the mid-twentieth century were infrequent but noteworthy. Thus Erasistratus of Alexandria (c250 BC) recognised the significance of portal hypertension when he stated that the "narrowness of the blood vessels going through the liver" was the major factor leading to peritoneal fluid accumulation, and modern teaching echoes the warning of Paul of Aegina in the seventh century AD that

sudden or complete paracentesis (described in 20 BC by Celsus) would immediately kill the patient.

In 1685 a surgeon at St. Thomas' Hospital called John Brown reported a patient with nodular cirrhosis who had ascites and oedema to the Royal Society (2). It may come as a surprise to modern investigators that by then an appropriate animal model had already been developed. Richard Lower, an Oxford physiologist, produced ascites in dogs by ligating the inferior vena cava. Two centuries later, two famous physicians on either side of the Atlantic - Thomas Sydenham in England and Austin Flint in New York - recognised alcohol as the principal aetiological factor. The latter also commented on the development of oliguria in some cases (3).

The spark which ignited the intense interest this subject has received in the past 40 years was the invention of the flame photometer. Sodium balance studies became possible and in the late 1940s and early 1950s several groups described avid sodium retention in ascites (4,5,6,7). Papper subsequently demonstrated the impaired renal excretory response to a saline load (8). It was apparent that the explanation for this sodium retention held the key to understanding ascites. Two apparently opposing theories have been developed, and, despite a vast amount of information, the debate has continued into the 1980s. Indeed, the cynic might argue that the words which introduce a review of the situation by Atkinson in 1956 are equally

applicable 30 years later "... our understanding of its [ascites'] pathogenesis is not in keeping with the frequency of its occurrence. In recent years knowledge of various underlying abnormalities has increased without any clear conception of their sequence of events in the pathogenesis of ascites emerging" (9).

1.2.2. The Traditional ("Underfilling") Theory

The Starling hypothesis states that to maintain equilibrium between intravascular and extravascular fluid volumes, the difference between the capillary and interstitial hydrostatic pressure must be equal to the difference between the capillary and interstitial colloid osmotic pressure (10). In cirrhosis the architectural disturbance within the liver impairs portal venous flow causing portal venous hypertension. Thus there is a rise in capillary hydrostatic pressure within the portal-splanchnic circulation. Also in cirrhosis, the reduced synthesis of albumin causes a reduction in capillary colloid osmotic pressure. These two factors disturb the Starling equilibrium and favour the exudation of fluid from the portal-splanchnic circulation into the interstitium. The exact site of this fluid leakage is unknown, although the relatively high protein content of the ascitic fluid suggests that a major site is the liver itself, since hepatic sinusoids are lined by discontinuous endothelium allowing easier passage of plasma proteins into the hepatic

interstitium than elsewhere (11). Whatever the site, the interstitial fluid will initially be returned to the circulation via the lymphatic system, and indeed the flow of lymph in the thoracic duct is greatly increased in cirrhosis (12). However, once the increased formation of interstitial fluid exceeds the capacity of the lymphatic system to return it to the circulation, the fluid will accumulate in the peritoneal cavity and form ascites (13).

The traditional theory proposes that the consequence of this fluid shift is intravascular volume depletion ("underfilling") which will activate various neurohumoral homeostatic mechanisms, principally the renin-angiotensin-aldosterone system, the sympathetic nervous system and vasopressin. These prompt the kidney to retain sodium and water, which is therefore seen as a secondary phenomenon.

1.2.3. The "Overflow" Theory

The underfilling theory was challenged in the late 1960s by Lieberman and his colleagues in Los Angeles. They demonstrated convincingly that plasma volume, far from being reduced in cirrhosis, was in fact increased (14). They also demonstrated that resolution of ascites does not cause a rise in plasma volume (15), that paracentesis does not cause a fall in plasma volume (1) and that plasma volume expansion by a sodium retaining hormone results in the formation of ascites (1). On the basis of these observations they proposed the "overflow" theory. This

states that ascites formation in cirrhosis is a consequence of plasma volume expansion, aided by renal sodium retention, which is a primary rather than a secondary phenomenon (1).

There is some support for this theory both in experimental animals and in man. The strongest support comes from the observation that in dogs with experimental cirrhosis, urinary salt retention precedes ascites formation (16). In man, three groups have demonstrated that in contrast to the increase in plasma renin activity which would be expected if the circulation were underfilled, levels in plasma are in fact normal or even low in most cirrhotics with ascites (17,18,19).

1.2.4. The measurement and manipulation of plasma volume

An important concept relevant to the debate over plasma volume is that of the "effective extracellular fluid (ECF) volume". This refers not to the total ECF volume nor even to the total intravascular volume, but to that volume which is physiologically perceived by the afferent receptors, e.g. baroreceptors, which activate the neurohumoral mechanisms. For example, sodium excretion alters with change in posture - a manoeuvre which does not alter total intravascular volume, but presumably does alter the physiologically perceived "effective" volume. If the trigger for sodium retention in cirrhosis is a reduction in the effective rather than the total ECF volume, then the increase in total plasma volume demonstrated by Lieberman

does not automatically disprove the underfilling theory.

Papper introduced this concept to cirrhosis in 1958 (20). He observed that intravenous infusions of the patient's own ascitic fluid caused increased excretion of sodium (20). This confirmed that ascitic fluid is physiologically ineffective while it remains in the peritoneal cavity, and does not contribute to the effective ECF volume. Next, the concept of reduced effective ECF volume in cirrhosis was extended to the intravascular compartment. It was suggested that much of the increased plasma volume demonstrated by Lieberman was "pooled" in the portal-splanchnic circulation as a result of portal hypertension. The increased portal pressure leads to portasystemic shunting via new collateral vessel formation (21) and the increase in splanchnic blood flow in cirrhotic rats with portal hypertension and portasystemic shunting approaches 50 per cent (22). However, at least in experimental animals, the increase in plasma volume in cirrhosis affects both the splanchnic and non-splanchnic circulation (16).

A second possible site of blood pooling is in the peripheral systemic circulation as a result of peripheral (principally cutaneous) vasodilatation. This is a characteristic feature of cirrhosis and as a consequence of the reduction in peripheral vascular resistance, patients with cirrhosis tend to have a low systemic blood pressure despite an increase in cardiac output (23,24). The cause

of this vasodilatation is unknown.

A third possible contribution to a discrepancy between effective and total ECF volume is arteriovenous anastomoses (25). These occur in the mesenteric, pulmonary and peripheral circulations of patients with advanced cirrhosis (26).

Effective ECF volume is a concept rather than a measurable entity and therefore the hypothesis that it is reduced in cirrhosis cannot be tested by direct measurement. Instead, investigators have attempted to assess the effect of intravascular volume expansion. It has already been pointed out that intravenous re-infusion of ascitic fluid increases renal sodium excretion (20). Other studies of intravenous plasma volume expansion give conflicting results. In the same paper (20) infusion of hypotonic saline increased urinary sodium excretion, and volume expansion may cause an increase in glomerular filtration rate and natriuresis, accompanied by suppression of the renin-angiotensin-aldosterone system (27). On the other hand volume expansion with dextrose increased renal blood flow in only 13 of 21 cirrhotics, there being no effect in the 8 cirrhotics who had an increased cardiac output (28).

Two further means of volume expansion need to be considered, both of which lend strong support to the underfilling theory. The first is the peritoneo-venous shunt pioneered by Le Veen (29). As its name

suggests, this returns ascitic fluid to the systemic venous circulation via a one way valve. All authors agree that the insertion of such a shunt leads to a natriuresis (30,31,32). However, cirrhotic dogs continue to retain sodium during liberal salt intake even after ascites has been mobilised using a Le Veen shunt (33).

Finally, Epstein has applied the model of head-out water immersion to the study of ascites (34). Immersion to the neck causes a prompt redistribution of circulating blood volume with relative central hypervolaemia, increasing cardiac output by 30% and central blood volume by 700 ml. These changes are similar to those induced by an intravenous saline load, without any change in plasma composition nor any increase in body weight (35). Thus this means of expanding the ECF will expand its "effective" compartment in a more physiological manner than those described previously. Epstein found that in 32 cirrhotics with ascites, immersion resulted in marked natriuresis in the majority, with a 20-fold increase in the rate of sodium excretion (34), markedly in excess of the natriuresis seen in normal subjects. Also, the manoeuvre, which does not alter glomerular filtration rate in normal subjects, greatly improved it in the cirrhotic patients (17). Epstein interpreted these observations as suggesting that immersion tends to "normalise" the diminished "effective" volume of cirrhotic subjects and thus strongly support the "underfilling"

theory. However, others have argued the opposite, namely that an exaggerated response to volume expansion indicates that the ECF was already expanded (36). One of the drawbacks of the head-out water immersion model is that it decreases sytemic vascular resistance, thus increasing the compliance of the system. This could explain its failure to consistently provoke a natriuresis in the hands of the Denver group (37). They subsequently demonstrated similar results to Epstein when a noradrenaline infusion was added to the model to maintain vascular tone during the immersion period (38).

Also of relevance to this debate is the concept of mineralocorticoid escape in cirrhosis. This is the term applied to the phenomenon whereby in normal individuals, mineralocorticoid administration causes only temporary sodium retention, following which a natriuresis occurs, i.e. they "escape" from the sodium-retaining effects (39). This does not occur in the majority of cirrhotics (40,41). These studies were interpreted as supporting a primary renal defect prompting sodium retention, i.e. support for the overflow theory. This was based on the presumption that the mechanism of mineralocorticoid escape was expansion of ECF (41). However, an alternative mechanism is reduced sodium retention in the proximal renal tubules to compensate for the mineralocorticoid induced distal tubule sodium retention (42). Therefore an alternative interpretation for the

failure of this escape in cirrhotics could be that sodium retention is at least partially from the proximal rather than distal tubule.

It can been seen that both direct measurement and manipulation of the plasma volume in cirrhosis have failed to conclusively answer the debate regarding the pathogenesis of ascites. What light can studies of the indirect determinants of plasma volume shed on the problem?

1.2.5. The Renin-Angiotensin-Aldosterone System

Traditionally, the renin-angiotensin-aldosterone system (RAAS) has been seen as the principal mediator of sodium retention in ascites. Early studies found increased plasma concentrations of renin (43), angiotensin (44) and aldosterone (45) in cirrhosis with ascites, and these findings were confirmed by many others. The stimulus to the system was presumed to be vascular underfilling. However, subsequently studies which excluded dietary salt restriction, diuretic therapy and paracentesis as causes of a high renin state found the RAAS to be activated in only a minority of cirrhotics with ascites (17,18,19). On the afferent side, this is indirect evidence against a reduced effective ECF as this would be expected to stimulate the RAAS. On the efferent side, it might appear at first glance as evidence that aldosterone is not the principal mediator of sodium retention in these patients. Certainly Epstein found a dissociation between suppression of plasma

aldosterone and the absence of a natriuresis (17). However, spironolactone - an aldosterone antagonist - effectively induces a diuresis in most cirrhotics, even without activated RAAS. Wilkinson proposed that this might be explained by an increase in the renal tubular sensitivity to aldosterone (46).

All authors agree that when RAAS activation does occur in cirrhosis, it is usually in the more advanced cases, and by the time renal failure intervenes, it is universal. Indeed Arroyo and his associates have shown the poor prognosis associated with RAAS activation in cirrhosis, with a six month survival of only 50% (47).

In conclusion there is evidence that the RAAS is activated in some but not all cirrhotics. Aldosterone may play a central role in sodium retention even in those cirrhotics without raised levels via raised renal tubular sensitivity, but the conflicting evidence on this point has justified the search for other possible mediators of sodium retention.

1.2.6. The sympathetic nervous system

Since the degree of activation of the sympathetic nervous system depends on baroreceptors an index of sympathetic activity would be an attractive indirect measure of effective ECF volume. The first such index to be considered was plasma noradrenaline concentration.

Initial attempts at noradrenaline estimation in liver

disease yielded conflicting results (48,49,50,51). This was probably the result of problems with the bioassay and fluorimetric techniques of measurement. Subsequently more sensitive assays using radioenzymatic methods have been developed (52). The first report of the use of such an assay in liver disease showed decreased catecholamine levels (53) but since then several investigators have found elevation of noradrenaline levels in cirrhosis (54,55,56). The Danish group demonstrated a mean plasma noradrenaline level of 2.7 $nmol.l^{-1}$ in 8 cirrhotics with severe liver disease, half of whom had ascites, as compared to 0.3 $nmol.l^{-1}$ in healthy controls (54). Bichet and colleagues studied 26 patients with alcoholic cirrhosis and ascites, and found plasma noradrenaline concentrations of 4.1 $nmol.l^{-1}$ compared with a control value of 1.5 $nmol.l^{-1}$ (55). Arroyo studied a sufficiently large group of cirrhotics (65 patients) to allow subdivision into groups according to presence of ascites and renal failure. He demonstrated not only elevated noradrenaline levels in cirrhotics vs controls, but within the cirrhotic group demonstrated the highest noradrenaline values in those cirrhotics with both ascites and renal failure (6.8 $nmol.l^{-1}$), followed by those with ascites but without renal failure (3.0 $nmol.l^{-1}$), who in turn had higher values than in cirrhosis without ascites (2.0 $nmol.l^{-1}$) (56). Does this increase in noradrenaline concentration represent increased sympathetic activity in cirrhosis? Plasma noradrenaline

concentration depends on both its release from sympathetic nerve endings and its rate of clearance from plasma. Therefore the increased plasma noradrenaline in cirrhosis might result from impaired clearance due to hepatic dysfunction. This problem is addressed in this thesis by studying the kinetics of radiolabelled noradrenaline (Chapter 3).

If there were an increase in sympathetic activity in cirrhosis, as most workers have assumed, this raises two questions with regard to the pathogenesis of ascites. Firstly, is sympathetic overactivity only present in established, severe ascites as with the renin-angiotensin-aldosterone system, or does it precede the development of ascites? The former situation is compatible with the overflow theory, whereas the latter would suggest vascular underfilling. This question may be answered by studying sympathetic activity in a large number of cirrhotics with a spectrum of disease severity (Chapter 4).

Secondly, does the increased sympathetic activity play a part in the development of ascites itself? There is now strong evidence to suggest it does, by both direct and indirect means. Both proximal and distal renal tubules are richly innervated by sympathetic nerves in a variety of mammals (57,58). The work of Bello-Reuss using initially micropuncture studies of rat kidneys (59) and subsequently an isolated rabbit proximal convoluted tubule preparation (60) provides strong evidence for sympathetically-mediated

sodium reabsorption at the proximal tubule. This effect is not mediated by the renin-angiotensin-aldosterone system or by renal prostaglandins (61). In addition sympathetic stimulation will have indirect effects on sodium retention. Beta-adrenoceptor mediated sympathetic neural activity promotes renin release from the juxtaglomerular apparatus which will stimulate angiotensin and aldosterone secretion, resulting in sodium reabsorption from the distal tubule. Renal sodium retention could also result from a reduction in renal blood flow and/or glomerular filtration rate, both of which may result from alpha-adrenoceptor mediated sympathetic activation (42). Glomerular filtration rate is only rarely sufficiently reduced to be a major cause of sodium retention in ascites and such a situation implies the development of functional renal failure (62). However renal blood flow is reduced in cirrhosis even in the absence of overt functional renal failure (63). The relationship between renal blood flow and sympathetic activity is discussed in more detail in the section on functional renal failure (Section 1.3.).

Thus the increased sympathetic activity in cirrhosis is associated with renal sympathetic activation which can cause renal sodium retention directly via an effect on the proximal tubule and indirectly by renin-angiotensin-aldosterone stimulation and possibly by altering renal blood flow.

1.2.7. Vasopressin

Despite avid sodium retention, hyponatraemia is common in cirrhosis - in one study 40% of all cirrhotics had a serum sodium concentration below 130 $mmol.l^{-1}$ (64). Therefore these patients must retain water in greater excess, i.e. they have impaired free water excretion. Free water reabsorption takes place in the distal convoluted tubules and collecting ducts of the kidney under the control of the antidiuretic hormone arginine vasopressin (65). Much of the work on the role of vasopressin in abnormal water excretion in cirrhosis comes from Robert Schrier and his co-workers in Denver over the past decade, following the development of a sensitive radioimmunoassay. They found that in experimental cirrhosis in rats (66) and in cirrhotic man (67) impaired free water clearance was associated with increased plasma vasopressin concentrations, suggesting a principal role for this hormone in mediating the water retention of cirrhosis. They had previously demonstrated that vasopressin may be released not only in response to osmotic stimuli via hypothalamic osmoreceptors but also to non-osmotic stimuli such as low and high pressure baroreceptors, acting on parasympathetic pathways (68). Thus vasopressin can be viewed as another indirect hormonal indicator of the state of filling of the vasculature, with increased levels supporting the underfilling theory of ascites (69).

Vasopressin may not, however, be the sole mediator of

water retention in cirrhosis. The decreased delivery of filtrate to the distal nephron which results from reduction in glomerular filtration rate and avid proximal tubular reabsorption may itself impair free water clearance (70). Increased water excretion induced by head-out water immersion may (37) or may not (71) be accompanied by suppression of plasma vasopressin. The insertion of a peritoneovenous shunt for ascites corrects the hyponatraemia and increases free water clearance before any fall in the elevated vasopressin concentration occurs (72) supporting the theory that increased delivery of filtrate to the distal nephron is more important than a reduction in vasopressin levels in promoting free water excretion in cirrhosis.

1.2.8. Therapy

The debate regarding the pathogenesis of ascites has direct bearing on its therapy. There is no doubt about the avid sodium retention in these patients and strict dietary sodium restriction to 40 mmol per day or less is required. The avoidance of medications which either contain large amounts of sodium (e.g. certain antacids and antibiotics) or themselves promote sodium retention, whether by aldosterone-like actions (carbenoxolone) or prostaglandin antagonism (non steroidal anti-inflammatory drugs), is important. Bed rest combined with dietary sodium restriction alone will result in natriuresis and diuresis in a significant

proportion of patients (73). It has traditionally been taught that further treatment involved diuretics, principally with the aldosterone antagonist spironolactone. Opinion is divided as to the efficacy of this agent. Wilkinson (36) cited the response seen in the great majority of patients as evidence for a fundamental role for aldosterone in the development of sodium retention. On the contrary, Epstein (74) found that spironolactone only elicited a modest natriuretic response, greatly augmented by water immersion, which suggests that the contribution of the distal nephron to sodium retention is small. Clinical experience favours the former situation with several controlled trials showing ninety per cent response to spironolactone (75,76) especially in high doses of up to 1g/day (77). The principal alternative is the loop diuretic, frusemide. Although more potent than spironolactone in other conditions, it only provokes a diuresis in 50% of ascitic patients (76). Spironolactone is therefore the diuretic of choice. Frusemide may however be successful when added to spironolactone in patients unresponsive to the latter alone (78,79), although this increases the risk of complications (80).

Other therapeutic options have been considered. Other diuretics have been assessed, singly or in combination, namely metolazone, either alone (81) or in combination with spironolactone (82) or frusemide (83); amiloride (84); ethacrynic acid (85) and muzolinine (86). None has been

superior to high or moderate doses of spironolactone, with or without the addition of frusemide (25).

The introduction of angiotensin-converting enzyme (ACE) inhibitors has proved valuable in the treatment of cardiac failure and hypertension. These agents therefore had some appeal in the therapy of resistant ascites where the renin-angiotensin-aldosterone system is invariably activated. Initial case reports of a favourable effect of captopril (87,88) were countered by worries about systemic hypotension and impaired renal function (89,90) and confusion (91). Captopril appears to cause an average 20% fall in mean arterial pressure and glomerular filtration rate (92). This effect could be predicted by earlier work using the experimental angiotensin II antagonist, saralasin, which provoked severe hypotension in cirrhotic patients, indicating that in these cases, maintenance of systemic blood pressure is dependent on circulating angiotensin II (93). Several groups subsequently confirmed this finding (94,95,96). Thus it is unlikely that the ACE inhibitors will have a therapeutic role in resistant ascites.

All authors emphasise the importance of a gradual rather than a sudden diuresis, recommending weight loss of only 0.5 kg per day (1kg/day where peripheral oedema is present). This is because the maximum rate of mobilisation of ascites is 900 ml per day (97,98). The dangers of overdiuresis are electrolyte disturbance, especially hypokalaemia, and intravascular volume depletion which may

precipitate encephalopathy (80), and functional renal failure respectively. Functional renal failure is considered in more detail below (Section 1.3.).

For similar reasons, paracentesis of large volumes has long been considered contra-indicated in these patients - rapid reaccumulation of ascites precipitating hypovolaemia, renal failure, encephalopathy and death. This cautious view has recently been challenged by the Spanish group under Professor Rodes. In their hands paracentesis of up to 3 litres daily was not associated with any more risk of renal insufficiency or electrolyte disturbance than traditional therapy with diuretics, provided plasma volume was replaced by albumin infusions (99). Two other groups have recently confirmed the safety of paracentesis (100,101).

An extension of the paracentesis/albumin infusion concept is that of the intravenous reinfusion of ascitic fluid itself. This was employed as a research tool as long ago as 1958 (20). It can be achieved on a temporary basis by an external pump arrangement employing ultrafiltration (Rhodiascit) which has very limited clinical applications (102). A more permanent solution is the peritoneovenous shunt (29) which returns ascitic fluid to the superior vena cava via a tunnelled catheter with a one-way valve, and is effective in initiating a natriuresis (30). However, it is plagued by complications including shunt blockage, septicaemia and disseminated intravascular coagulation. Its use is therefore limited to the rare patient with truly

resistant ascites and preserved hepatic function (79).

What are the implications of these treatments for the theories of ascites described above? The efficacy of the paracentesis/albumin infusion regime supports the underfilling theory, while the success of judicious diuretic therapy supports the overflow theory. The greater efficacy of spironolactone than other diuretics favours the distal tubule as the principal site of sodium reabsorption. It has been argued that the frequent precipitation of renal failure by diuretics favours an already underfilled intravascular compartment (69), but this would not apply if such complications only occurred where too rapid a diuresis (exceeding the mobilisation capacity of the ascites) had occurred. The natriuresis which follows the insertion of the peritoneovenous shunt is accompanied by depression of all markers of intravascular volume depletion, i.e. renin, noradrenaline and vasopressin (103) which provides strong support for the underfilling theory.

1.2.9. Conclusion

It seems likely that the two theories of ascites formation are in fact complimentary rather than conflicting. Most of the studies whose results favour vascular underfilling have been carried out on patients with fully established ascites. Most of the studies supporting the "overflow" theory have concentrated on the sodium retention occurring at the commencement of, or even preceding, the

formation of ascites. Although in the past five years the weight of evidence has moved against the overflow theory, largely as a result of studies on the sympathetic nervous system and on the head-out water immersion model, it is inconceivable that all the diverse evidence for the overflow theory is "wrong". The basic flaw in the original underfilling theory was that the intravascular volume depletion was said to result from loss of fluid into the peritoneal cavity. Ascites cannot be caused by ascites alone! There requires to be some initiating mechanism. The modified underfilling theory, which cites peripheral vasodilatation as the cause for underfilling despite increased cardiac output and blood volume is more credible. The two basic flaws in the overflow theory as the sole explanation for events have until now been the lack of the precipitating factor which initiates sodium retention de novo, and the obvious underfilling present later in the natural history of the disease process. Interestingly, the proponents of the overflow theory have pointed out that the sympathetic nervous system could be stimulated by the increase in hepatic venous pressure in cirrhosis, via hepatic baroreceptors (104). Thus sympathetic activation could precede vascular underfilling and could conceivably be the missing trigger for sodium retention. Better and Schrier in an attempt to formulate a unifying hypothesis incorporating both theories, suggest a central role for activation of the sympathetic nervous

system (105).

1.3. FUNCTIONAL RENAL FAILURE

Some patients with advanced liver disease develop "unexplained renal failure in the absence of any clinical or anatomical evidence of other known causes of renal failure" (106). This is variously known as functional renal failure (107), the hepatorenal syndrome (106,108) and hepatic nephropathy (109). I will use the first of these terms and confine the discussion to the development of renal impairment complicating cirrhosis, i.e. excluding renal problems in fulminant hepatic failure and obstructive jaundice. In the context of cirrhosis, functional renal failure represents the extreme end of the spectrum which incorporates sodium retention and ascites (105).

The absence of pathological damage to the kidney in this condition is fundamental, as confirmed by the successful transplantation of such kidneys, where they function normally in recipients (110). This suggests that the primary abnormality is a circulatory one, either disturbed intrarenal or extrarenal haemodynamics. In 1970, Epstein demonstrated reduced renal blood flow, particularly to the renal cortex in fifteen cirrhotics with functional renal impairment (111). The following year Kew showed that blood flow to the renal cortex was reduced in thirty three cirrhotics without biochemical renal impairment (63). Ring-Larsen found progressive reduction in renal blood flow

in cirrhotics without ascites, those with ascites, and those with renal impairment (64). Therefore functional renal failure appears to be closely linked to reduced renal blood flow.

The control of renal blood flow is extremely complex. Of particular interest in the context of this thesis is the role of renal sympathetic activity. Although the renal vasculature is richly innervated by sympathetic nerves (58), it has not been conclusively proven that these sympathetic nerves directly regulate renal blood flow (113). Nevertheless, sympathetic stimulation in animal models, e.g. chronic bile duct-ligated dogs, causes renal vasoconstriction via alpha adrenoceptor activation (114), and renal sympathetic activity certainly modulates renal blood flow indirectly, via beta-adrenoceptor mediated stimulation of the renin-angiotensin system.

Peripheral plasma noradrenaline concentration is significantly higher in functional renal failure than in cirrhotics with ascites but without renal impairment (56). Regional studies are divided as to whether this sympathetic activity is (115) or is not (116) primarily of renal origin. Alpha adrenergic blockade by infusion of phentolamine directly into the renal artery in four patients failed to significantly improve renal perfusion (111). However, the study was clouded by significant arterial hypotension following phentolamine. Baldus therefore repeated the experiment with the inclusion of plasma expansion to prevent

arterial hypotension, but with the same results (117). However, dihydroergocristine, an alternative alpha adrenergic antagonist, did improve renal blood flow (118). The question of the contribution of increased renal sympathetic activity to renal vasoconstriction in cirrhosis therefore remains open.

There are other hormonal factors involved in the control of renal blood flow. Although angiotensin II, a renal vasoconstrictor, is probably increased in functional renal failure (19), its inhibition both directly, using the antagonist saralasin (36,95) and indirectly, by beta adrenergic blockade (119) fails to improve renal blood flow. However, the oral angiotensin converting enzyme inhibitor, captopril, has a renal vasodilating effect both in normal and cirrhotic subjects (92). Unfortunately this is of little practical value as it selectively dilates the efferent glomerular arteriole which may lower the glomerular filtration rate, and can cause marked systemic hypotension when used in cirrhosis. In fact activation of the renin angiotensin system may result from decreased renal blood flow. The suppression of plasma renin activity by dopamine-induced renal vasodilatation supports this theory (120).

Renal prostaglandins play an important role in maintaining normal renal blood flow. In an elegant clinical study, Arroyo demonstrated that in cirrhotics with ascites, urinary PGE_2 (a vasodilatory renal prostaglandin)

and plasma noradrenaline are both increased, but when functional renal failure ensues, urinary PGE_2 falls while plasma noradrenaline increases further (56). Others (121,122) have confirmed these findings which suggests that in ascites without functional renal failure, the tendency to renal vasoconstriction from sympathetic activation is prevented by a compensatory increase in vasodilating prostaglandins, while in functional renal failure this mechanism fails, renal prostaglandin secretion falls and vasoconstriction ensues (123).

The practical implication of these findings for the cirrhotic patient is that drugs which inhibit prostaglandin synthesis - principally non steroidal anti-inflammatory drugs - have a deleterious effect. They cause both sodium and water retention and reduced renal blood flow (124,125). A new non steroidal anti-inflammatory drug, sulindac, appears to spare the renal prostaglandins in normal subjects but there is controversy as to whether it does (126) or does not (127) impair renal blood flow in cirrhosis.

Another hormone system with renal vasodilatory properties is the kallikrein-kinin system. Plasma bradykinin (128), and urinary kallikrein (129,130,131) are reduced in cirrhotics, particularly with functional renal failure. One group found urinary kallikrein to be increased in cirrhosis with ascites alone, but decreased in patients with ascites and renal failure (121) analogous to the pattern with renal prostaglandins. The significance of

these observations, and the inter-relationships between the kinin system and the other hormones controlling renal blood flow, remain to be determined.

An alternative explanation for the renal vasoconstriction of functional renal failure, other than imbalance between the normal neurohumoral control mechanisms, would be the presence in cirrhosis of a pathological renal vasoconstrictor. One such agent is endotoxin. This degradation product of intestinal bacteria normally inactivated by hepatic Kuppfer cells may reach the systemic circulation in cirrhosis. Wilkinson found that endotoxaemia was associated with the development of renal failure in both fulminant hepatic failure (132) and cirrhosis (133) and showed a possible benefit from polymixin B, which has an anti-endotoxin effect (134). Unfortunately this agent is too nephrotoxic for general clinical use. However, endotoxin is only present in a minority of cases of functional renal failure and cannot be its sole explanation.

Gastrointestinal peptides have potentially harmful vasoactive effects if released into the systemic circulation because of impaired hepatic metabolism or portasystemic anastomoses. Vasoactive intestinal peptide (VIP) is an attractive candidate in this regard, as it may cause systemic vasodilatation and renal vasoconstriction, i.e. the haemodynamic profile of advanced cirrhosis. There are conflicting reports regarding the plasma concentration of VIP in cirrhosis. An initial report showed an increase

(135) but a much larger study failed to confirm this (136). However, this latter group did identify a subgroup of patients with high levels in fulminant hepatic failure, which corresponded to those patients with a lower systemic blood pressure (137), results supported by more recent work in cirrhosis (138). VIP levels do not correlate with renal blood flow and it is unlikely to play a significant role in most causes of functional renal failure. The possibility remains that some of the other GI hormones elevated in fulminant hepatic failure - which include insulin, glucagon, pancreatic polypeptide and enteroglucagon (137) - or indeed other, as yet unidentified, agents which the failing liver fails to inactivate, may have a deleterious effect on renal blood flow.

What are the implications for the management of functional renal failure in cirrhosis?

The cirrhotic patient who develops renal impairment should have known causes of renal damage identified and treated, diuretic therapy discontinued and any pre-renal uraemia corrected, which will often require a trial of intravenous fluid loading. If these measures fail, the patient has established functional renal failure. Haemodialysis should be considered if spontaneous improvement in hepatic function is anticipated or if the patient is awaiting hepatic transplantation. Otherwise it should be avoided as it holds no longterm prospects of improvement. Pharmacological intervention remains of no proven benefit. In particular

sympathetic blockade, both of alpha (111,117) and beta (119) adrenoceptors, angiotensin blockade (92) and octapressin, a synthetic vasopressin analogue with renal vasodilatory properties (139) have not improved renal blood flow. Dopamine (120) and aminophylline (140) have improved renal blood flow but have yet to be shown to have clinical value. The prognosis reflects the underlying severity of the liver disease in most cases and although the mortality approaches 100%, few die of uraemia in isolation.

1.4. CIRCULATORY CHANGES IN CIRRHOSIS

The remainder of this background chapter considers the abnormal circulation of cirrhosis, whose disturbed sympathetic control is currently poorly understood.

The patient with cirrhosis typically has a "hyperdynamic" circulation comprising increased plasma volume, increased cardiac output, tachycardia, cutaneous vasodilatation, reduced peripheral vascular resistance and systemic hypotension (23,141). These features account for the florid complexion, bounding pulse, flow murmurs and possibly cutaneous vascular manifestations such as palmar erythema and spider naevi (142).

Although this circulatory disturbance has been recognised for over thirty years, it has received only sporadic attempts to identify its cause. Nevertheless, it may be of primary importance in triggering the formation of ascites, via stimulation of neurohumoral homeostatic

mechanisms such as the sympathetic nervous system which promote sodium retention.

The studies which approach this problem fall into four categories. Firstly, there are those which simply measure the haemodynamic disturbance. Secondly there are those which seek to measure the effect of altering the circulation by physiological means such as changes in posture or reflex autonomic stimulation. Thirdly, some studies consider the cardiovascular responses to exogenous vasoactive substances such as noradrenaline and angiotensin II. Finally there are studies which seek to alter the circulation by blockade of the normal vasomotor control systems.

In the first category there is general agreement. The initial observation of increased cardiac output, reduced peripheral vascular resistance and reduced systemic blood pressure was made by Kowalski in 1953 (141) and confirmed by Murray in 1958 (23). The suggestion that the same abnormalities persisted or were even more marked in terminal liver failure (143) were also confirmed (144). Kontos identified the site of the reduced vascular resistance as being principally within the skin and/or skeletal muscle (24).

Stimulation of sympathetic activity by exercise caused an appropriate increase in cardiac output in cirrhosis, the greatest increase being in those individuals with supranormal resting values (145). This is supported by more recent work indicating normal increases in plasma noradrenaline and heart rate during exercise in cirrhosis

(146). Sympathetic activity can also be stimulated by changes in posture. Ring-Larsen found similar changes in heart rate, blood pressure, renal blood flow and plasma noradrenaline concentrations in cirrhotic patients as in controls during tilting, suggesting normal neurovascular reactivity (147). However, other studies do not support this contention. The haemodynamic response to sympathetic stimulation by head-up tilting was abnormal in cirrhosis in that the heart rate and blood pressure rose only transiently and subsequently fell, in contrast to a sustained rise in control subjects (148,149). Also, head-up tilting caused significantly less peripheral vasoconstriction and tachycardia in cirrhosis as measured by changes in forearm blood flow (150). This group also used changes in forearm blood flow to measure cardiovascular responsiveness to a number of other autonomic stimuli, including application of ice, stressful mental arithmetic and lower body negative pressure (151). They found that in each case the response was reduced in the cirrhotic patients.

Parasympathetic cardiovascular responses have also been considered in a number of studies. Their interpretation requires consideration of the role of alcohol, since vagal neuropathy is described in chronic alcoholics (152). Autonomic neuropathy is said to be more common in alcoholics with than without chronic liver disease (153) but the groups were not matched for duration and severity of their alcoholism. Vagal function was impaired in cirrhosis only

if of alcoholic origin (154). However, an earlier study found the heart rate response to the Valsalva manoeuvre - a parasympathetic effect - to be impaired in non-alcoholic cirrhosis (151).

The third category of experimental evidence to be considered concerns the cardiovascular response to the infusion of vasoactive agents, both in experimental animals and in man. In both situations the evidence to date is conflicting. In carbon tetrachloride-induced cirrhosis in rats some workers have found decreased pressor responses to angiotensin II but not to noradrenaline (155). Others using the same model have found decreased pressor responses to both agents (156,157). In dogs with chronic bile duct ligation, a model with a similar haemodynamic disturbance to cirrhotic man, the cardiovascular response to angiotensin was markedly reduced, but to noradrenaline only slightly so (158). The pressor response to vasopressin, another non-sympathetic vasoconstrictor was impaired in cirrhotic rats (159).

In human studies of cirrhosis the cardiovascular response to the administration of exogenous noradrenaline is variously reported as markedly reduced (151), minimally reduced (160), normal (144,161) or even enhanced (162). Angiotensin II administration has yielded equally confusing results with reports that responsiveness in cirrhosis is decreased (160,161,163), normal (151) or increased (162). The injection of tyramine, which promotes release of

endogenous noradrenaline caused a less marked rise in blood pressure in cirrhotics than controls (144). The rise in heart rate in response to isoprenaline, a sympathetic stimulant which acts selectively on beta adrenergic receptors, is reported to be reduced in cirrhosis (162,164). There are no studies reporting the effect of selective alpha adrenoceptor stimulation.

The final group of studies to consider are those which examine the effect of blockade of the normal vasoactive systems. Parasympathetic blockade by atropine causes a less marked rise in heart rate in cirrhosis than controls (162) but this effect is limited to alcohol-induced cirrhosis (154). Blockade of both sympathetic alpha adrenoceptor action by phentolamine (111) and angiotensin-mediated responses by captopril, an angiotensin converting enzyme inhibitor (92) or saralasin, an angiotensin II antagonist (93) can cause profound hypotension. This suggests that the increased activity of these two vasomotor control systems is an attempt to maintain a normal blood pressure. Sympathetic beta adrenoceptor blockade has been widely studied in cirrhosis since the observation that propranolol lowers portal pressure (165) and may reduce the risk of rebleeding from oesophageal varices (166). As regards the effect on the systemic circulation, the reduction in cardiac output and blood pressure in cirrhosis has not been specifically compared to normal values, but appears to be of the expected order of magnitude. Finally

reduction in sympathetic activity by clonidine (a centrally-acting alpha$_2$ adrenoceptor agonist) reduces plasma noradrenaline, cardiac output and mean arterial pressure in cirrhosis (167,168).

Although these studies define the nature of the haemodynamic disturbance, and hint at abnormal autonomic circulatory control, no clear picture of the nature of the autonomic disturbance emerges. In some instances, the autonomic tests are too imprecise to define the site of the abnormal response. The conflicting results of the studies administering vasoactive substances reflect widely diverging and often poorly validated techniques. Autonomic neuropathy can best be assessed by a battery of cardiovascular function tests, which are applied to cirrhosis in Chapter 5. Pharmacological studies have now provided valid, reproducible techniques of measuring the cardiovascular responses to infusions of vasoactive substances, and these are applied to cirrhosis in Chapter 6. The question of whether abnormal autonomic circulatory control reflects changes in the number or function of adrenergic receptors, which had not previously been studied, is addressed in Chapter 7.

It is appropriate to consider the mechanisms, other than disturbed autonomic function, which have been proposed to explain the circulatory changes in cirrhosis. One possibility is failed hepatic inactivation of vasoactive gastrointestinal peptides. VIP is a systemic vasodilator,

but as already explained when discussing renal vasoconstriction (Section 1.3.), is only elevated in a minority of cirrhotics (138). An alternative vasodilatory gut peptide is substance P, which has recently been reported as markedly elevated in the plasma of cirrhotics (169). Other potential vasodilators are the enkephalins, which are elevated in cirrhosis due to reduced hepatic metabolism (170).

Atrial natriuretic peptide (ANP) is a potent systemic vasodilator. It has variously been reported in cirrhosis as being increased (171,172,173,174), normal (175,176,177) or reduced (178). If it is reduced, this might contribute to the sodium retention of ascites. Alternatively the sodium retention occurring for other reasons might be expected to stimulate its release, hence causing an increase in plasma levels. The conflicting results to date probably largely reflect initial methodological problems with sampling and assay techniques. These have now been resolved and ANP measurements in cirrhosis are reported in Chapter 4.

Another group of vasodilating agents increased in cirrhosis are the prostaglandins. Renal prostaglandins with a vasodilator effect, e.g. prostaglandin E_2, are increased in cirrhotics without functional renal failure (56). Increased systemic production of prostacyclin, a powerful vasodilator, has been demonstrated in cirrhosis (179). Also, prostaglandin E has been

shown to contribute to the pressor resistance to angiotensin, since this could be partially reversed by pretreatment with indomethacin, a prostaglandin inhibitor (180).

An alternative hypothesis for the abnormal vasodilatation of cirrhosis is the so called "false neurotransmitter" theory. This proposes that the neurotransmitters which maintain vascular tone, e.g. noradrenaline, are displaced from their receptors by chemically similar agents. One such agent is octopamine (181), though this was only elevated in two out of ten cirrhotics (182). The studies in Chapter 7 of adrenoceptor number and affinity consider the possibility of interference at the receptor binding site.

In summary, the abnormal systemic circulation in cirrhosis is unexplained. It seems possible that the primary defect is that which causes a reduction in peripheral vascular resistance. This in turn allows underfilling of the intravascular space despite an increase in cardiac output. This stimulates sympathetic activation, the renin-angiotensin-aldosterone system and non osmotic vasopressin release. For reasons yet to be determined these fail to correct the vascular abnormalities, although striving to maintain them, such that pharmacological blockade precipitates severe hypotension. The continual overactivity of these neurohumoral homeostatic mechanisms causes sodium and water retention and hence ascites.

CHAPTER 2

GENERAL METHODOLOGY

2.1. PATIENTS

2.1.1. Liver Disease

The 41 subjects with liver disease used in these studies were drawn from patients attending Stobhill General Hospital, Glasgow. In the majority of cases, they had histologically proven cirrhosis. In addition, a number of cases have been included with presumed cirrhosis. These are patients in whom liver biopsy was contraindicated by either coagulopathy or lack of patient consent. To be included in any of the studies, such cases required to have clinical evidence of chronic liver disease, with abnormal biochemical liver function tests plus portal hypertension indicated by either oesophageal varices (endoscopically proven) or splenomegaly (palpable spleen on abdominal examination confirmed on isotope scanning), with these findings persisting for longer than six months.

Cirrhosis of any aetiology was included, although the majority were of alcoholic origin. The effects of alcohol on autonomic function have been taken into account in the interpretation of each study.

The severity of the liver disease has been graded according to the method of Child (183) as modified by Pugh (184). This utilises the three laboratory tests which give an indication of hepatocellular function, i.e. serum albumin, serum bilirubin and prothrombin time, and two readily assessible clinical features, i.e. ascites and encephalopathy (see Table 2.1.).

TABLE 2.1.

PUGH'S GRADING OF LIVER DISEASE SEVERITY (184)

	1	2	3
Encephalopathy (grade)	0	1 and 2	3 and 4
Ascites	None	Slight	Moderate or severe
Bilirubin ($umol.l^{-1}$)	< 34	34-51	> 51
Prothrombin time (seconds prolonged)	⩽ 3	4-6	> 6
Albumin ($g.l^{-1}$)	> 35	28-35	< 28
Bilirubin in PBC ($umol.l^{-1}$)	< 68	68-170	> 170

Cumulative Score 5 - 6: Grade A (mild)
7 - 9: Grade B (moderate)
10 -15: Grade C (severe)

The cirrhotic patients studied represent most of those presenting to the two physicians with an interest in gastroenterology in Stobhill Hospital. There were no secondary referrals from outwith the hospital's catchment area.

2.1.2. Control Subjects

Choice of control groups is particularly critical to studies on the sympathetic nervous system. Plasma catecholamine concentrations rise with age (185,186), and are lower in hospital or laboratory staff familiar with study techniques than in "naive" subjects (187). Also, certain disease states are associated with raised catecholamine levels, including hypertension, cardiac failure, thyroid disease and chronic obstructive airways disease. For the studies of autonomic function, diabetes mellitus and neoplasia must also be excluded. To satisfy these criteria the control subjects used were drawn from patients attending the gastroenterology clinic with non-hepatic disorders (principally peptic ulcer disease and irritable bowel syndrome/diverticular disease) and from patients awaiting minor elective surgery (principally hernia repair or varicose vein ligation). The latter group were studied as in-patients prior to their surgery, allowing for matching of dietary sodium intake.

Patients with peptic ulcer disease were included as controls despite the reported increase in plasma

catecholamines in this condition (188). Plasma noradrenaline levels in our peptic ulcer patients (2.7 ± 1.9 $nmol.l^{-1}$, n=8) were no different from our control patients with other non-hepatic disorders (2.5 ± 1.2, n=26; p = 0.75).

2.2. LABORATORY CONDITIONS

All studies were carried out in the Clinical Investigation Unit of the University Department of Materia Medica. The studies were performed by one investigator (the author) on all occasions, with a research nurse in attendance. The environmental conditions were constant, with the ambient temperature maintained at 21°C. In each study the timing of the procedure was constant (usually 9 a.m. commencement). Wherever possible subjects were studied in tandem, one cirrhotic plus one control patient on each study day. On all study days, the subjects abstained from caffeine-containing beverages, alcohol, tobacco and all medication. The details of fasting or non-fasting state, and of duration of abstinence from alcohol and medication prior to the study day, varied between studies and is described in the appropriate chapters.

2.3. HANDLING AND PREPARATION OF BLOOD SAMPLES

2.3.1. Catecholamines

The same procedure for catecholamine measurement was adhered to in all studies. The subjects had an indwelling

cannula inserted into an antecubital vein, and patency maintained with heparinised saline (1 ml containing 20 units heparin). After a minimum of 30 minutes rest in a supine position and after verification of a stable blood pressure and heart rate, blood was drawn from the cannula and immediately placed in cooled lithium heparin tubes on ice. This was centrifuged immediately at 4°C at 1000 rpm for 10 minutes. The plasma was separated and stored in plastic tubes at -70°C.

2.3.2. Plasma Renin Activity

As above, with the exception that 5 ml of blood only were required, placed in an E.D.T.A. tube and the plasma stored at -20°C.

2.3.3. Atrial Natriuretic Peptide

As above, with the exception that to the lithium heparin tubes prior to the insertion of the blood, had been added aprotinin, 20 KIU for a 10 ml blood sample.

2.3.4. Adrenoceptor Studies

120 ml blood (cirrhotic patients) or 60 ml (control patients) were drawn from an intravenous cannula after 30 minutes supine rest and placed in universal containers containing 5% sodium citrate (18 ml blood to 2 ml citrate). The samples were removed for immediate analysis (see Chapter 7).

2.4. ANALYTICAL METHODS

2.4.1. Plasma noradrenaline and plasma dihydroxyphenylethylene glycol (DHPG)

Noradrenaline and its dihydroxymetabolite DHPG were measured simultaneously using high performance liquid chromatography with electrochemical detection (189).

Plasma collected and stored as outlined in 2.3.1. was mixed with 4 mg of alpha-methylnoradrenaline (as an internal standard) and the pH adjusted to 8.6 using 500 ul of 1.5 M Tris. The sample was mixed with acid-washed alumina (50 mg) for 30 minutes. After the alumina had settled the plasma was aspirated and the alumina washed twice with 10 ml distilled water. The catecholamines were then eluted by vigorously mixing the alumina with 250 ul of 0.2 M perchloric acid. A 200 ul aliquot of the eluate was injected into the liquid chromatograph.

The chromatographic system consisted of a Pye-Unicam LC-XPS pump, a precolumn (40 x 1 mm) packed with Li Chroprep (Merck) and a 25 cm x 4.0 mm I.D. reversed-phase column. The column was packed with Spherisorb 5u silica (Phase Separating). The mobile phase was 70 mM NaH_2PO_4 (pH 3.0) (Fisons) with 1.85 mM octanesulphonic acid (Fisons) and 13.4 mM EDTA (BDH, Anala R). The flow rate was 1 $ml.min^{-1}$ and the column effluent was passed through a TL3 cell (Bioanalytical Systems) with a glassy carbon electrode where the catecholamines were detected using an LC4 amperometric detector (Bioanalytical Systems). The applied potential

was set at +0.66 V relative to the Ag/AgCl reference electrode. The sensitivity of the detector was set at 1.10 nA/V and the background current was 1.2 nA. The sensitivity of the system (twice background level) was 30 pg for noradrenaline and DHPG. The within-day coefficients of variation were 3.4% for noradrenaline and 4.1% for DHPG, and between day coefficients of variation were 4.6% for noradrenaline and 8.9% for DHPG.

A typical chromatogram is illustrated in Figure 2.1. The plasma concentration of the catecholamine is calculated by the "peak height ratio" method:

$$\text{plasma noradrenaline } (\text{nmol.l}^{-1}) = \frac{\text{height of noradrenaline}}{\text{height of internal standard peak}} \times \frac{\mathbf{X}}{\text{volume of plasma (ml)}}$$

where **X** = amount of internal standard (ng) added, divided by the ratio of peak height of noradrenaline internal standard on a chromatogram of an extracted standard, divided by molecular weight of noradrenaline x 10^{-3}.

2.4.2. Plasma Renin Activity

Plasma renin activity was determined by a specific radioimmunoassay of angiotensin I formed upon cleavage by renin of its substrate angiotensinogen (190). The limit of

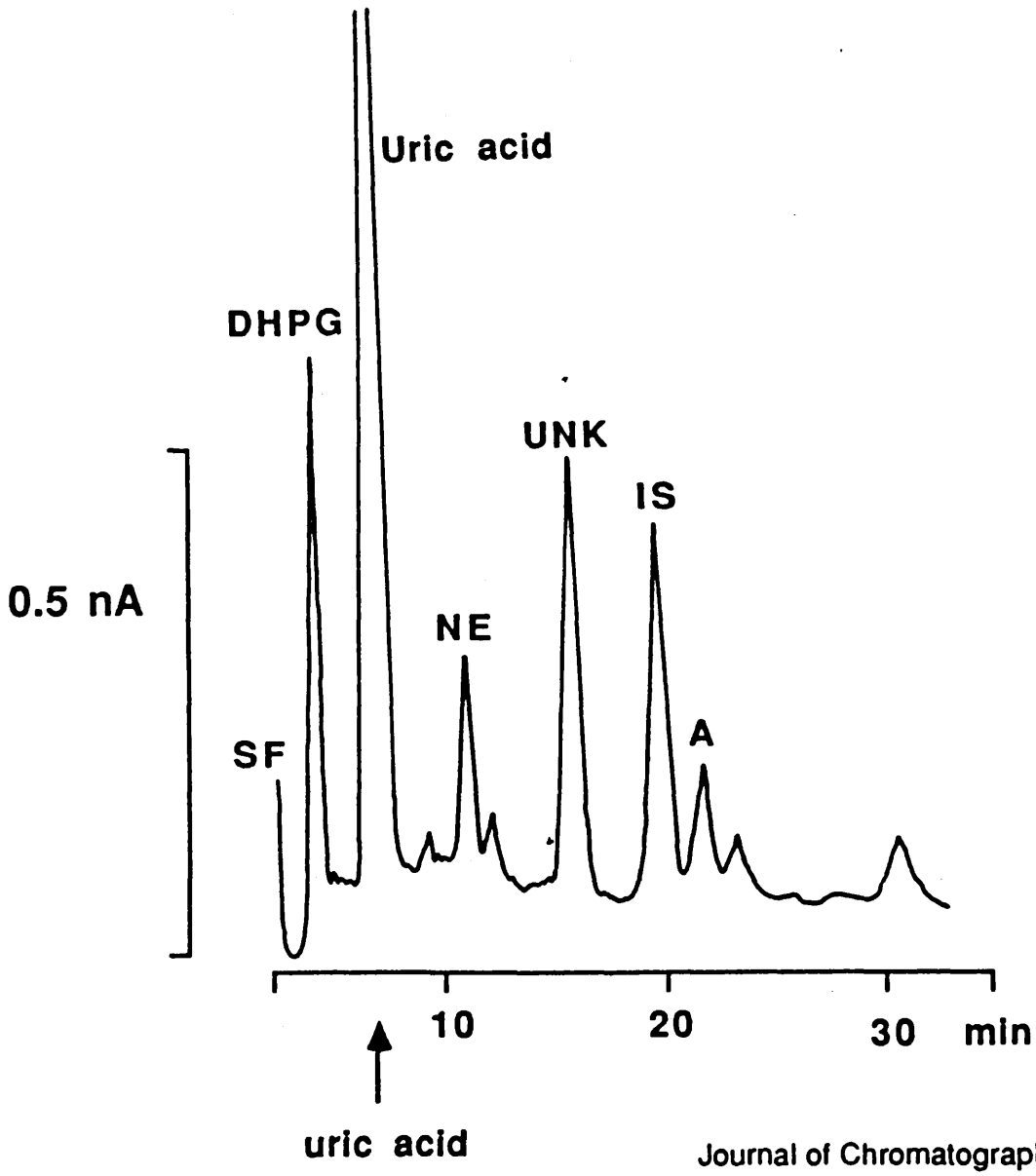

Figure 2.1.

Chromatogram from plasma following HPLC with electrochemical detection for simultaneous determination of noradrenaline and DHPG.

detection of the assay was 0.1 ng AI $ml^{-1}hr^{-1}$ and the inter and intra assay variations were 7.0% and 5.5% respectively.

2.4.3. Atrial Natriuretic Peptide

Extraction

Atrial natriuretic peptide (ANP) was extracted from plasma, using Water's Sep Pac C18 cartridges. The adsorbed ANP was eluted in an ethanol solvent. Eluates were dried down in oxygen free nitrogen at 40°C and residues were reconstituted in assay buffer.

Radioimmunoassay

ANP antibody was raised in New Zealand white rabbits against synthetic human 1-28 ANP. To evoke a greater immunogenic response the ANP immunoglobulin was conjugated using carbon di-inide as a coupling agent (191). The resulting antisera were used at 1-11,000 dilution to give 50% binding in the assay system. ANP ^{125}I label was purchased from Amersham International and used in the assay at a concentration of 4pg/tube. The diluent used throughout the assay was sodium hydrogen phosphate buffer 0.1M, pH 7.4, containing 0.25% egg albumin.

The non equilibrium assay system was used along with dextran coated charcoal to separate the free from the antibody "bound" radioactivity. The sensitivity of the assay was 10 pg/100 ul. Intra and interassay coefficients of variation was 4.9% and 8.2% respectively. The extraction

recovered was 90-95%.

2.4.4. Biochemical and Haematological Analysis

Urea and electrolytes and biochemical liver function tests were measured in the Department of Biochemistry at Stobhill Hospital by standard autoanalyser techniques (TECHNICON). Full blood count including platelets was measured by the Haematology Department using a Coulter counter. Prothrombin time was compared to a standard control sample, and the result expressed as the number of seconds by which the study sample exceeded the control sample (normal < 3 secs).

2.5. [^{3}H]NORADRENALINE METHODOLOGY

2.5.1. Introduction

The spillover of noradrenaline into plasma and its clearance from plasma were calculated using a method developed in the Department of Materia Medica (192). The rationale behind this development and the methodology employed will be discussed in some detail.

Plasma noradrenaline concentration depends not only on the spillover into plasma from sympathetic nerve endings but also on its clearance from plasma by uptake into sympathetic nerves, uptake into other tissues or by metabolism principally by the catechol-O-methyltransferase (COMT) system. The spillover rate is therefore a better guide to sympathetic activity than simply plasma noradrenaline.

Spillover and clearance can be calculated using steady state kinetics of 1-[^{3}H] noradrenaline (193). This method involves the intravenous infusion of [^{3}H] noradrenaline to steady state in a resting individual, and calculation of amount of radioactivity in plasma extracted on alumina. Although it has previously been assumed that all such radioactivity is due to [^{3}H] noradrenaline, there are theoretical grounds to suspect that [^{3}H] metabolites of noradrenaline may also form. For example, DHPG is an important metabolite of noradrenaline cleared from plasma by uptake into sympathetic nerves (194), and a substantial proportion of [^{3}H] noradrenaline infused intravenously is cleared in this manner (195). A method was therefore developed which introduced a high performance liquid chromatographic (HPLC) separation of plasma [^{3}H] noradrenaline.

2.5.2. Composition of plasma radioactivity during [^{3}H] noradrenaline infusion

Methods

L-[7,8-^{3}H] noradrenaline (1.1-1.85 TBq mmol^{-1}) (Amersham) was prepared for human use by ultrafiltration. Radiochemical purity was checked by thin layer chromatography and found to be greater than 95%. 4MBq of [^{3}H] noradrenaline, diluted to 55 ml in normal saline with 500 mg of ascorbic acid was infused at a rate of 3.6 x 10^4 Bq min^{-1} (0.458 ml min^{-1}) using a Braun perfusor Mark 4 pump

into a peripheral vein of six healthy male volunteers who had been resting supine for at least 30 minutes. Ten mls of blood were collected from the opposite arm into chilled lithium heparin tubes at regular intervals during the two hour infusion. An additional 12 ml of blood were taken at 30 min for comparisons between the amount of radioactivity in plasma, the amount recovered on alumina and the amount recovered from the HPLC column.

The blood was centrifuged at 1000 g for 10 min ($4^{o}C$) and the plasma separated. Five ml of plasma from each sample was added to 50 mg of alumina, 4 ml of 1 mol l^{-1} Tris EDTA (pH 8.7) and 235 pmol of dihydroxybenzylamine (DHBA) which was used as a cold internal standard. The samples were mixed on a rotary mixer for 15 min then washed twice with distilled water. Catechols were eluted from the alumina by the addition of 230 ul of 0.2 mol l^{-1} perchloric acid. 20 ul of this was assayed by HPLC with electrochemical detection to determine the recovery of internal standard in each sample, and the remainder was injected onto the HPLC to separate out [^{3}H] noradrenaline. The alumina eluate from the additional 30 min sample was counted by liquid scintillation spectrometry without separation on the HPLC column. This allowed a comparison of the total amount of radioactivity recovered from the column with the amount recovered on alumina. A 1 ml plasma sample was also counted from the 30 min time point without preparation on alumina, to determine the total plasma

radioactivity.

Alumina eluates from the 30 and 90 min time points of 6 subjects were separated by HPLC to allow simultaneous isolation of [^{3}H] noradrenaline, [^{3}H] DHPG and [^{3}H] dihydroxymandelic acid ([^{3}H] DOMA) another hydroxymetabolite of noradrenaline. The mobile phase was 70 mmol l^{-1} KH_2PO4 (Fisons, HPLC grade) with 13.4 mmol l^{-1} EDTA (Analar, BDH) and 1.85 mmol.l^{-1} octane sulphonic acid (Fisons, HPLC grade). The column was 25 cm x 4 mm internal diameter packed within the laboratory with spherisorb 5u ODS. The flow rate was 1 ml min^{-1}. Under these conditions, the retention times of DHPG, noradrenaline and DOMA determined by electrochemical detection (ED) were 5.6, 10.5 and 24.0 min respectively. The sensitivity of this system for the assay of plasma noradrenaline and DHPG was 20 pg, and the between assay coefficients of variation were 4.6% and 8.9% respectively. DOMA was much less electrochemically active than noradrenaline and DHPG and therefore not normally detectable in plasma. For the separation of plasma radioactivity fractions, the outflow tube from the column was disconnected from the electrochemical detector and connected to a Gilson Fraction collector. Fractions were collected at 1 min intervals for 30 min (the usual run time of the system) to allow comparative plots of the radioactivity that eluted from the column at 30 and 90 min in the 6 selected subjects. At all other time points, only [^{3}H] noradrenaline radioactivity was collected. This was

performed on a slightly different system using a 10 cm x 4 mm internal diameter column packed with 3u ODS spherisorb (Waters Novapak C_{18}) allowing a shorter run time (15 min). In this system, [^{3}H] DHPG eluted in the solvent front, [^{3}H] noradrenaline had a retention time of 6.4 min and [^{3}H] DOMA a retention time of 11.0 min. The column eluate was collected for 2.5 min from the time corresponding with the start of a cold noradrenaline standard peak. Injections of standard solutions of [^{3}H] noradrenaline followed by 1-minute fraction collections were performed on this system to ensure that the [^{3}H] noradrenaline eluated over a similar time to cold noradrenaline. Standard injections of 15 pmol of cold noradrenaline were made each day to check its retention time. HPLC eluate fractions were collected into glass vials and 20 ml of scintillant (Optiphase, Fisons) added. The vials were counted on a liquid scintillation counter at an efficiency of 20-30%. HPLC eluate fractions taken prior to and after each run were counted to determine the level of background radiation, typically 30-35 c.p.m. [^{3}H] noradrenaline radioactivity per ml of plasma was calculated after the subtraction of background radioactivity and correction for the recovery of the cold internal standard (DHBA) from alumina, which averaged 53.9 $\pm$ 5.5% (n=85).

In two subjects, an additional 16 ml of plasma was collected at the 90 min time point and the plasma from the two subjects pooled and divided into six 5 ml aliquots.

Each of these aliquots was then processed as described above to determine plasma [^{3}H] noradrenaline levels. This allowed the calculation of the within-day coefficient of variation of the procedure.

Results

Injections of [^{3}H] noradrenaline were eluted from the column over a similar time course to injections of cold noradrenaline standards, with the exception that the peak was followed by a small tail of radioactivity lasting 5 min.

The recovery of [^{3}H] noradrenaline injected onto the column was 75.7 ± 3.1% (n=6) when using a 2.5 ml collection fraction. After 30 min of [^{3}H] noradrenaline infusion the percentage of total plasma radioactivity recovered on alumina was 50.1 ± 10.6% (n=7) and that eluted from the column was 80.3 ± 4.8% (n=6). This value is slightly higher than the calculated recovery of [^{3}H] noradrenaline from the column because the "tails" of the major peaks of radioactivity in the plasma samples were included.

Figure 2.2. shows a representative plot of the 1 minute fraction that eluted from the column following the injection of a 30 minute sample, compared to a corresponding electrochemically detected chromatogram of plasma from the same subject. Three major peaks of radioactivity were found, having the same retention times as DHPG, noradrenaline and DOMA. At 30 min, the proportions due to these three peaks were 32.3 ± 11.5%, 57.2 ± 13.2% and 4.9 ±

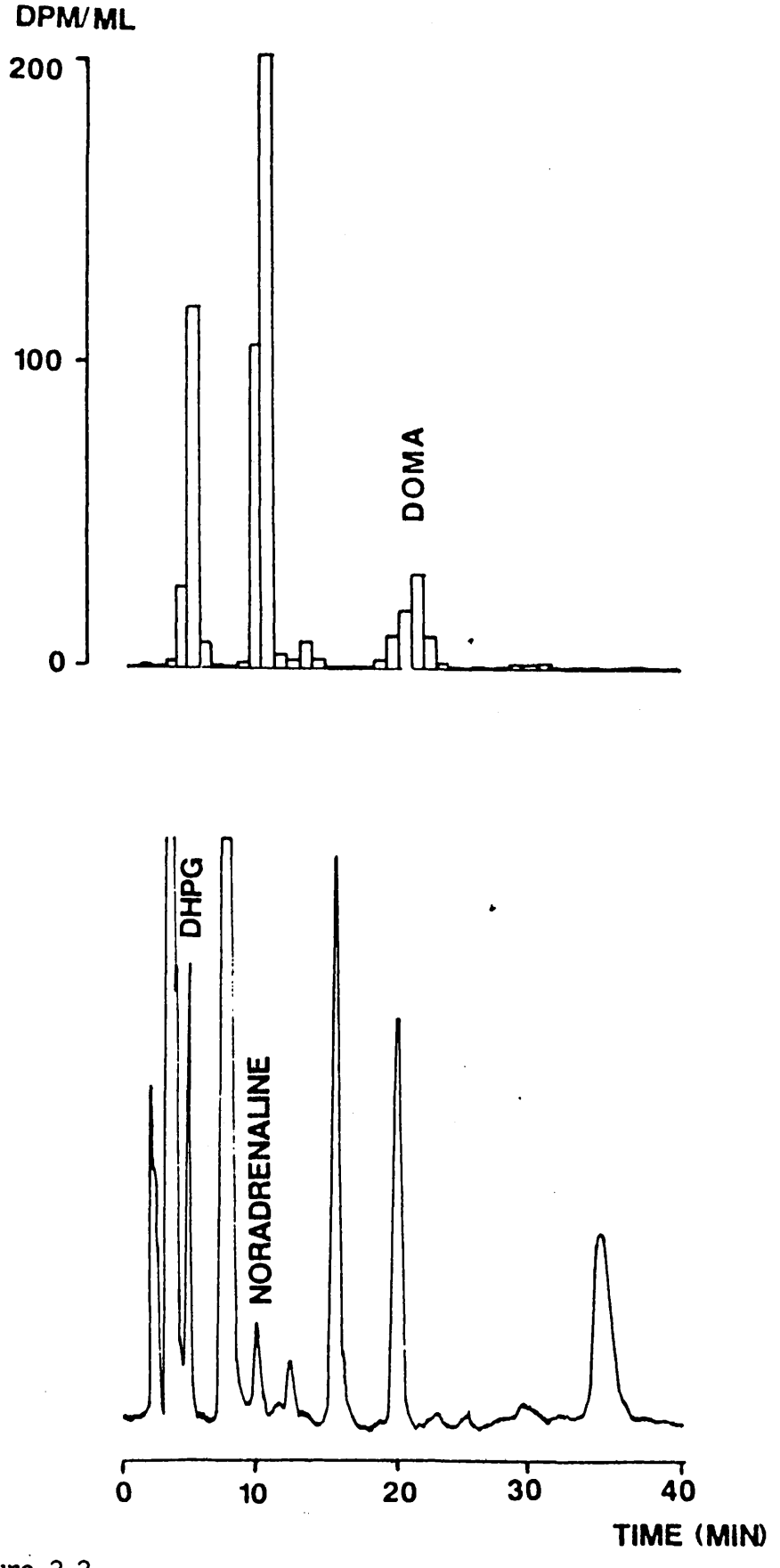

Figure 2.2.

Comparison of the radioactivity (collected in 1 min fractions) eluted after injection of plasma extracted in alumina (top) with an electrochemically detected chromatogram from the same patient using the same system (bottom).

6.0% respectively (n=6). After 90 mins of [^{3}H] noradrenaline infusion the proportion due to DHPG rose to 45.9 ± 9.5% ($p < 0.05$, Student's paired t test) while the proportion due to [^{3}H] noradrenaline fell to 44.4 ± 10.4% ($p < 0.005$). The proportion due to [^{3}H] DOMA remained unchanged (5.1 ± 4.8%).

Figure 2.3. shows the mean accumulation of plasma [^{3}H] noradrenaline radioactivity during the 110 min constant infusion. This reached a plateau after 20-30 min, although in some subjects it fluctuated substantially during the infusion. The within-day coefficient of variation for the determination of [^{3}H] noradrenaline radioactivity concentration was 3.9% (n=6).

Discussion

These experiments suggested that a substantial proportion of the radioactivity recovered from alumina following [^{3}H] noradrenaline infusion was not [^{3}H] noradrenaline itself. The lack of any detectable impurities in the stock [^{3}H] noradrenaline on thin layer chromatography and the fact that the principal second peak of the chromatogram had the same retention time as [^{3}H] DHPG, prompted speculation that [^{3}H] DHPG was present in plasma during the [^{3}H] noradrenaline infusion. The increase in the proportion of radioactivity from this second peak as the infusion progressed could reflect the slower elimination of DHPG compared with noradrenaline (192).

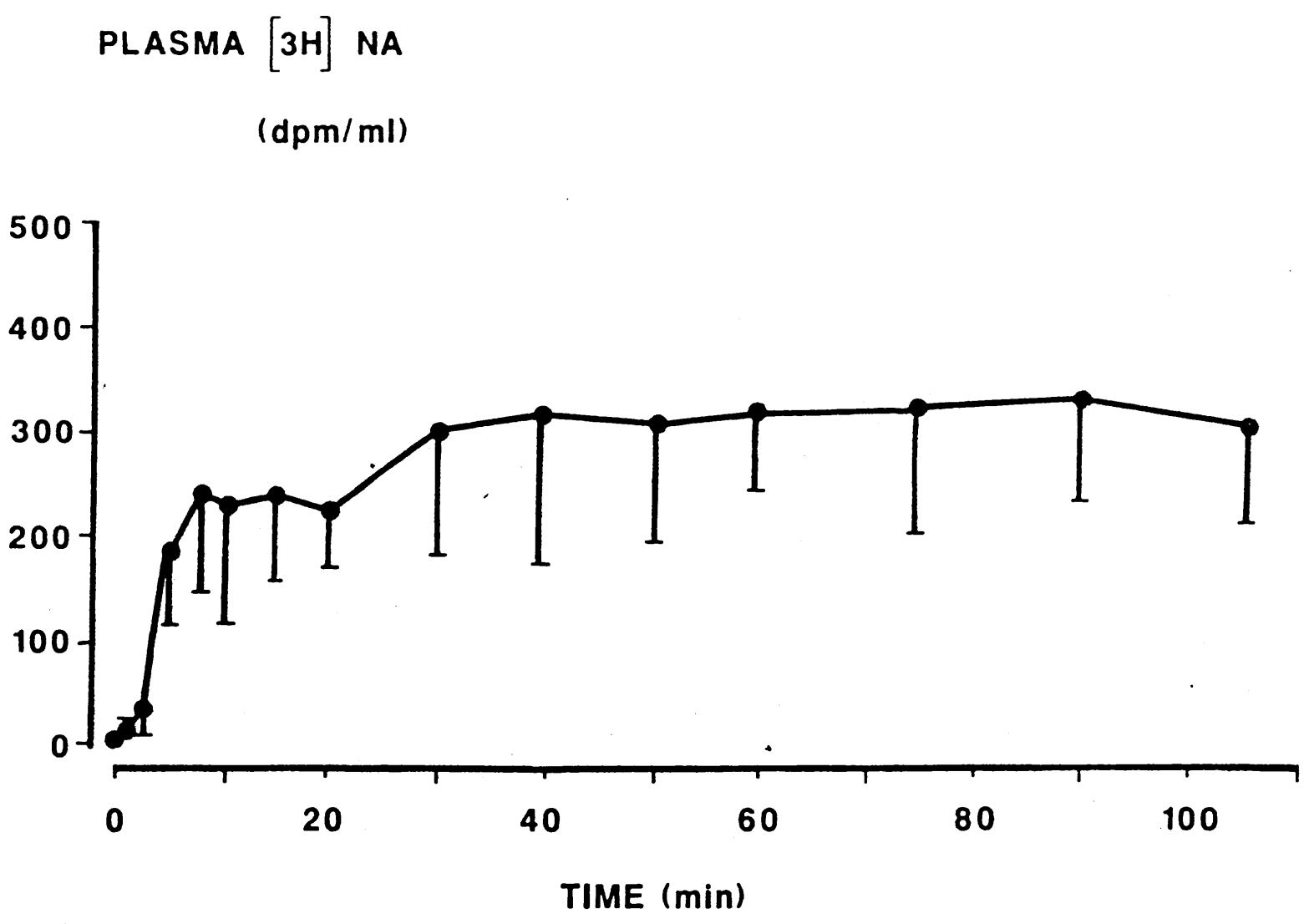

Figure 2.3.

Mean plasma [^{3}H] noradrenaline during 110 min infusion in 8 healthy subjects.

However, other workers have been unable to confirm these findings (196,197). Eisenhofer (196) adopted a similar HPLC technique but could only detect small amounts of [^{3}H] DHPG (3.9% of plasma radioactivity). McCance (197) also found that [^{3}H] DHPG could be detected in plasma and accumulated as the infusion progressed but with values much lower than those found by ourselves. Although Eisenhofer and McCance showed similar percentages of [^{3}H] metabolites in plasma McCance concluded that HPLC separation was unnecessary, while Eisenhofer recommended that it improved the accuracy of the technique. The principal difference in our study was the nature and source of the [^{3}H] noradrenaline stock. Our group used 7,8 labelled noradrenaline from Amersham plc while other investigators have used ring labelled noradrenaline from New England Nuclear (NEN). My colleagues have since undertaken further studies to determine whether there was any impurity in the Amersham preparation (198). A sample of the infusion solution was extracted on alumina and injected into the HPLC system. A peak corresponding to the retention time of DHPG was found in the infusion solution and also in the stock [^{3}H] noradrenaline (14.5% and 15% of total radioactivity respectively). Thin layer chromatography was again performed on the stock [^{3}H] noradrenaline (Amersham) and the radioactivity from the region of the plate subjected to HPLC separation, after the radioactivity had been eluted from the silica by washing with 1 mg ml^{-1} sodium meta-bisulphide in

100% ethanol and extracted on alumina. Again, two peaks of radioactivity with retention times the same as noradrenaline and DHPG were demonstrated.

Thus the stock from Amersham appears to contain an impurity which was not resolved from [^{3}H] noradrenaline using the thin layer chromatography technique recommended by the manufacturers and which co-chromatogaraphed with DHPG in our HPLC system.

Nevertheless, Eisenhofer and our own group were in agreement that, due to their slower elimination, even small concentrations of [^{3}H] dihydroxy metabolites could accumulate during a prolonged infusion. Therefore separation of [^{3}H] noradrenaline from its [^{3}H] metabolites by HPLC after alumina extraction offers a more accurate assessment of [^{3}H] noradrenaline kinetics than previous methods which measured total tritium content of the alumina extract without further purification (196).

This problem of contamination did not come to light until after the experiments on [^{3}H] noradrenaline kinetics in cirrhosis, described in Chapter 3, had been completed. These were carried out using the same batch of [^{3}H] noradrenaline from Amersham as the experiments described above. Since the amount of contamination appears to be approximately 15% of the total radioactivity, the values of spillover and clearance obtained have been reduced by this figure (see equations below).

2.5.3. [^{3}H] Noradrenaline kinetics

Having developed the method outlined above, the values for noradrenaline spillover and clearance using this technique were determined.

Methods

Eight normal male subjects aged 25-41 years were studied at least 1 hour following a light breakfast. Cannulae were inserted into antecubital veins of both arms and they then rested supine in a quiet room for at least 30 mins. Two MBq of [^{3}H] noradrenaline, diluted to 55 ml in sodium chloride solution with 500 mg ascorbic acid, were infused at a rate of 3.6×10^4 Bq/min (0.916 ml min^{-1}) using the same Braun pump. Blood for plasma [^{3}H] noradrenaline assay was removed at 40, 47.5 and 55 minutes. Additional 10 ml blood samples were taken at 40 and 55 mins for the assay of unlabelled plasma noradrenaline levels by HPLC with electrochemical detection (see Section 2.4.1.). Plasma [^{3}H] noradrenaline radioactivity was determined as outlined in 2.5.2.1. using the second HPLC column.

[^{3}H] noradrenaline plasma concentration was calculated from [^{3}H] noradrenaline radioactivity as follows. The efficiency of the scintillation counter was checked for each sample (from the Quench factor supplied and a previously determined Quench curve), allowing determination of [^{3}H] noradrenaline disintegrations per minute (dpm) from the counts per minute, from which the background count was

subtracted. Corrections were then made for:

i) Recovery from alumina (ratio of peak height of internal standard to unextracted standard on HPLC chromatogram)

ii) Recovery from HPLC column (i.e. 75.7% - see 2.5.2.).

iii) Adjustment for volume of plasma removed for internal standard recovery experiment. Using the shorter column, the volume of perchlorate added to the alumina was 130 ul, 120 ul of which were recovered and 20 ul were used for recovery experiment. Therefore this adjustment is 100/130 = 0.77

iv) Volume of plasma used (typically 4-5 ml).

This represents the plasma [^{3}H] noradrenaline concentration in dpm.ml^{-1}. Finally the [^{3}H] noradrenaline infusion rate was calculated from an aliquot of the infusion solution adjusted to the pump rate.

Noradrenaline spillover and clearance can then be calculated from the following formulae (193)

(1) specific radioactivity of plasma $= \dfrac{\text{plasma } [^3\text{H}] \text{ NA concentration}}{\text{endogenous plasma NA concentration}}$

(2) NA spillover rate $= \dfrac{[^3\text{H}] \text{ NA infusion rate}}{\text{specific radioactivity of plasma NA}}$

(3) NA plasma clearance $= \dfrac{[^3\text{H}] \text{ NA infusion rate}}{\text{plasma } [^3\text{H}] \text{ NA concentration}}$

Traditionally, the results of spillover and clearance are expressed corrected for body surface area. This was calculated from an individual's height and weight using a nomogram (Geigy scientific tables).

Results

In the eight normal subjects the resting endogenous plasma noradrenaline level was 0.70 ± 0.23 $nmol.l^{-1}$ (mean ± SD), noradrenaline spillover was 2.48 ± 1.40 $nmol\ min^{-1}m^{-2}$ and noradrenaline plasma clearance was 3.69 ± 1.82 $l\ min^{-1}m^{-2}$.

Discussion

The values obtained for noradrenaline spillover and plasma clearance by this method are higher than those reported by Esler (193) whose method did not include separation of plasma radioactivity. This suggests that some [^{3}H] dihydroxy metabolites may be formed during the infusion, since an overestimate of plasma [^{3}H] noradrenaline radioactivity will result in an underestimate of both clearance and spillover. However the impurities in the Amersham [^{3}H] noradrenaline render valid comparisons between the methods difficult. Certainly the NEN product, whose purity is checked by HPLC at source is likely to contain less impurities than the Amersham product. In addition, there is reason to believe that in the rabbit model the amount of [^{3}H] DHPG formed during [^{3}H] noradrenaline

infusion using ring-labelled product (as in the NEN product) is 40% less than if using the chain-labelled variety (as in the Amersham product). (199).

In conclusion, although the ideal method for the determination of noradrenaline spillover into plasma remains a matter of debate, the method developed in our laboratory, namely the introduction of HPLC to separate [^{3}H] noradrenaline in plasma from [^{3}H] dihydroxy metabolites or contaminants appears to be both valid and worthwhile.

2.6. STATISTICAL ANALYSIS

The details of the statistical analyses are indicated in the individual chapters. In general, the normality or otherwise of the distribution of the data was determined by correlation with the normal scores calculated using MINITAB (200). If the distribution was not normal, a logarithmic transformation was undertaken. If this also failed to satisfy the criteria for normal distribution, a non-parametric test was employed. For comparison between 2 groups, the Student's t-test was used for parametric and the Mann Whitney U test for non-parametric analysis (for unpaired data in all cases). Where more than 2 groups were being compared, a one-way analysis of variance was undertaken, or in the case of non-parametric data, a Kruskall-Wallis analysis of variance. Where appropriate, both the p values obtained and the 95% confidence intervals are quoted (201).

Correlations were determined by linear regression and, in the case of multiple comparisons, stepwise regression analysis or general linear modelling was employed, using MINITAB (200) or RUMMAGE (202).

2.7. ETHICAL CONSIDERATIONS

All the studies described in this thesis were approved by the Research and Ethical Committee of the Greater Glasgow Health Board, Units North 1, 2 and 3. All participants received written and oral explanations of the studies to be undertaken and gave written consent. The studies involving the administration of radioisotopes were approved by the appropriate committee of the Department of Health and Social Security.

CHAPTER 3

NORADRENALINE KINETICS IN CIRRHOSIS

3.1. INTRODUCTION

The reported increase in plasma noradrenaline concentration in cirrhosis (54,55,56) has been interpreted as evidence that sympathetic activity is increased in cirrhosis thus supporting the traditional "underfilling" theory of ascites formation (69,203). However, before such a conclusion can be drawn, the kinetics of noradrenaline in plasma deserve closer scrutiny.

When a sympathetic nerve fires, some noradrenaline is released from storage granules in the nerve ending into the synaptic cleft. Most of this is taken back up into the nerve ending, but a small proportion escapes into the circulation. This constitutes the majority of noradrenaline found in plasma, with a small contribution from the adrenal medulla. Noradrenaline in plasma has three possible fates. It may be taken back into neuronal tissue (uptake 1) or other tissues (uptake 2) by specific transport mechanisms, or undergo metabolism. The principal method of metabolism is via the catechol-O-methyl transferase system of which the liver is a rich source. It is therefore possible that the increased noradrenaline in plasma in cirrhosis results not from increased release, or "spillover" into plasma, and by implication, increased sympathetic activity, but from reduced clearance from plasma due to impaired hepatic enzyme activity.

Plasma noradrenaline spillover and clearance can be

estimated by the intravenous infusion to steady state of a sub-pressor dose of l-noradrenaline labelled with the radioisotope tritium [^{3}H] (193). A modification of this method which includes high performance liquid chromatography (HPLC) to separate plasma [^{3}H] noradrenaline from other contaminants or metabolites is described in the previous chapter. The present study utilised this method to measure noradrenaline spillover into plasma and its clearance from plasma in cirrhosis with ascites.

3.2. METHODS

3.2.1. Subjects

Fourteen patients with cirrhosis and clinically obvious ascites were studied. The diagnosis of cirrhosis was histological in nine cases and clinical in four (see Chapter 2 for clinical criteria for cirrhosis). The ascites was confirmed to be a transudate (protein content < 30 gl^{-1}) in all cases by diagnostic paracentesis. Hepatoma was excluded by serum alphafetoprotein < 4ul^{-1}. The cirrhosis was alcohol-induced in all but two cases which resulted from chronic active hepatitis. There were 10 males and 4 females with a mean age of 56 years (range 39 to 77). None had bled from oesophageal varices or other source in the previous three months. Two patients had clinically evident hepatic encephalopathy. Thirteen control subjects were studied. They were otherwise healthy patients awaiting hospital admission to undergo minor

elective surgery, principally hernia repairs or varicose vein surgery. None had any clinical or biochemical evidence of liver disease. There were 9 males and 4 females with a mean age of 51 years (range 39 to 59). No subject (control or cirrhotic) had systemic hypertension (BP > 160/90), cardiac failure, obstructive airways disease or diabetes mellitus.

3.2.2. Study Outline

All subjects were studied as in-patients. Daily sodium intake was restricted to 50 mmol for three days in both groups. Those cirrhotics previously on diuretic therapy (usually spironolactone, amiloride and/or frusemide) had these drugs stopped at least 72 hours prior to study. No alcohol was permitted for one week prior to study. Urine was collected for 24 hours on two occasions for measurement of creatinine clearance and urinary sodium and potassium excretion. Serum was collected on the study day for serum sodium, potassium, chloride, bicarbonate, urea, creatinine, albumin, bilirubin and prothrombin time.

On the study day the subjects fasted and avoided caffeine-containing beverages from 2400 hours the previous evening. In-dwelling cannulae were inserted in an antecubital vein in both forearms and subjects then rested supine in a quiet room for the remainder of the study. After a minimum of 30 minutes, blood pressure and heart rate were measured using a semiautomatic sphygmomanometer

(Sentron, Bard). Recordings were taken at 1 min intervals until 5 consecutive readings varied by less than 5 mmHg. The mean of these readings was then recorded.

2 MBq of 1-[^{3}H] noradrenaline (Amersham International) was added to 55 ml sodium chloride solution (0.9%) and 500 mg ascorbic acid. This was then infused via one intravenous cannula over one hour using a Braun perfuser pump at a rate of 3.6×10^4 Bq min^{-1} (0.916 $ml.min^{-1}$). At 40, 47.5 and 55 min blood was drawn from the opposite arm for the determination of [^{3}H] noradrenaline radioactivity in plasma. A further 10 ml blood was removed at 40 and 55 min for estimation of cold (endogenous) plasma noradrenaline and dihydroxyphenylglycol (DHPG) concentration. The laboratory methods employed in these analyses are described in detail in Chapter 2 (Sections 2.4.1. and 2.5.3.).

3.2.3. Statistical Analysis

The majority of the data, including liver function tests, plasma noradrenaline and the spillover and clearance values were not normally distributed, but became so following logarithmic transformation. All these results are therefore quoted as the medians (with ranges in parentheses) and comparisons between groups are carried out by an unpaired Student's t test on a logarithmic transformation of the data. The haemodynamic data did satisfy a normal distribution and are therefore quoted as means and standard deviations, with comparison by unpaired t

test on untransformed data. Correlations were calculated by linear regression and stepwise linear modelling using the MINITAB computer programme (200).

3.3. RESULTS

The biochemical and haemodynamic results are summarised in Tables 3.1. and 3.2. respectively. The noradrenaline kinetic results are detailed in Table 3.3. and summarised in Table 3.4. Figures 3.1. to 3.3. illustrate plasma noradrenaline, noradrenaline spillover and plasma noradrenaline clearance respectively.

The cirrhotic patients had biochemical evidence of severe liver disease with ten of the fourteen being Pugh's Grade C (184). They demonstrated reduced renal excretion of sodium, potassium and free water (the latter indicated by their significant hyponatraemia), impaired renal function with elevation of serum creatinine, reduced creatinine clearance and low urine volumes compared to controls. Although they had increased heart rates compared to controls, blood pressure was similar in both groups.

These cirrhotics demonstrated a highly significant increase in plasma noradrenaline spillover which closely followed the expected increase in endogenous plasma noradrenaline concentration. The clearance of noradrenaline in plasma was slightly but significantly increased in the cirrhotic group.

Both noradrenaline and DHPG are strongly correlated

TABLE 3.1 BIOCHEMICAL MEASUREMENTS IN SERUM AND URINE

Values quoted are medians, with ranges in parenthesis. Comparisons are by unpaired Student's t-test following a logarithmic transformation in all cases except serum sodium and prothrombin time, where log transformation was unhelpful and Mann Whitney U test employed, and serum albumin and urinary volume, where normal distribution of the data permitted a t-test on untransformed data.

	Cirrhosis + Ascites (n=14)	Controls (n=13)	P
Serum			
Sodium ($mmol.l^{-1}$)	134(114-140)	140(136-145)	< 0.001
Urea ($mmol.l^{-1}$)	6.2(1.7-19.6)	5.2(3.3-6.5)	NS
Creatinine ($umol.l^{-1}$)	101(60-429)	92(60-115)	< 0.05
Bilirubin ($umol.l^{-1}$)	71(8-505)	9(7-17)	< 0.001
Albumin ($g.l^{-1}$)	27(21-40)	43(39-46)	< 0.001
Prothrombin time (seconds prolonged)	6(0-25)	0(0-1)	< 0.001
Urine			
Volume ($ml24hr^{-1}$)	552(400-1337)	1990(1288-3334)	< 0.001
Sodium ($mmol24hr^{-1}$)	20(3-64)	45(23-105)	< 0.001
Potassium ($mmol24hr^{-1}$)	21(8-56)	47(19-73)	< 0.01
Creatinine clearance ($ml.min^{-1}$)	46(7-104)	93(73-233)	< 0.001

TABLE 3.2. BLOOD PRESSURE AND HEART RATE

Values quoted are mean ± SD and comparisons are by Student's t-test for unpaired data.

	Cirrhosis+Ascites (n=14)	Controls (n=13)	P
Heart rate (min^{-1})	84 ± 12	64 ± 7	< 0.005
Systolic BP (mmHg)	115 ± 16	117 ± 11	NS (p=0.57)
Diastolic BP (mmHg)	64 ± 18	71 ± 7	NS (p=0.16)

TABLE 3.3. PLASMA NORADRENALINE, SPILLOVER AND CLEARANCE

Subject No.	Plasma noradrenaline ($nmol.l^{-1}$)		Noradrenaline spillover ($nmol.min^{-1}m^{-2}$)		Plasma noradrenaline clearance ($l.min^{-1}m^{-2}$)	
	Cirrhotics	Controls	Cirrhotics	Controls	Cirrhotics	Controls
1	4.6	2.2	16.9	8.5	3.7	3.8
2	17.1	2.9	61.6	3.2	3.6	1.1
3	2.0	2.3	8.3	5.3	4.3	2.3
4	3.4	1.8	10.1	3.7	3.0	2.0
5	3.7	2.2	21.4	4.7	5.8	2.1
6	10.8	1.4	37.1	1.6	3.4	1.2
7	6.7	3.4	5.7	4.8	0.8	1.4
8	11.5	1.9	38.0	3.9	3.3	2.1
9	6.7	1.8	11.1	4.7	1.6	2.7
10	3.2	3.7	8.5	4.7	2.7	1.3
11	4.7	1.0	27.9	2.5	6.0	2.6
12	5.0	1.5	22.1	3.7	4.4	2.5
13	2.1	1.6	12.0	2.8	5.9	1.8
14	9.4	-	9.1	-	1.0	-

TABLE 3.4. NORADRENALINE KINETICS SUMMARY

Values are median (range) and comparison is by unpaired Student's t-test on a logarithmic transformation.

	Cirrhotics (n=14)	Controls (n=13)	P
Plasma noradrenaline ($nmol.l^{-1}$)	4.9 (2.0-17.1)	1.9 (1.0-3.7)	< 0.0005
Noradrenaline spillover ($nmol.min^{-1}m^{-2}$)	14.5 (5.7-61.6)	3.9 (1.6-8.5)	< 0.0001
Plasma noradrenaline Clearance ($l.min^{-1}m^{-2}$)	3.5 (0.8-6.0)	2.1 (1.1-3.8)	< 0.01

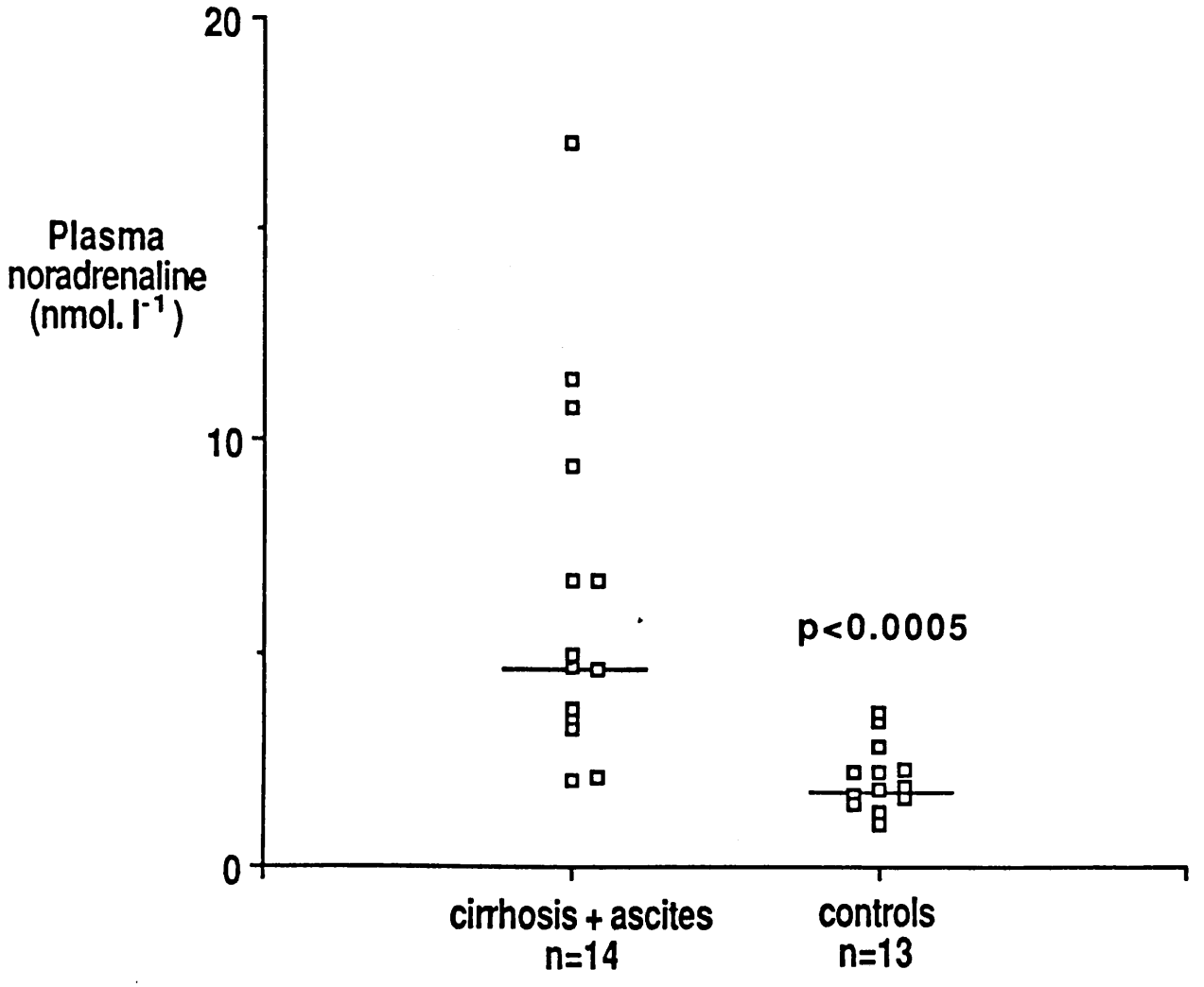

Figure 3.1.

Plasma noradrenaline concentrations in 14 cirrhotics with ascites and 13 controls (horizontal bar represents the median value).

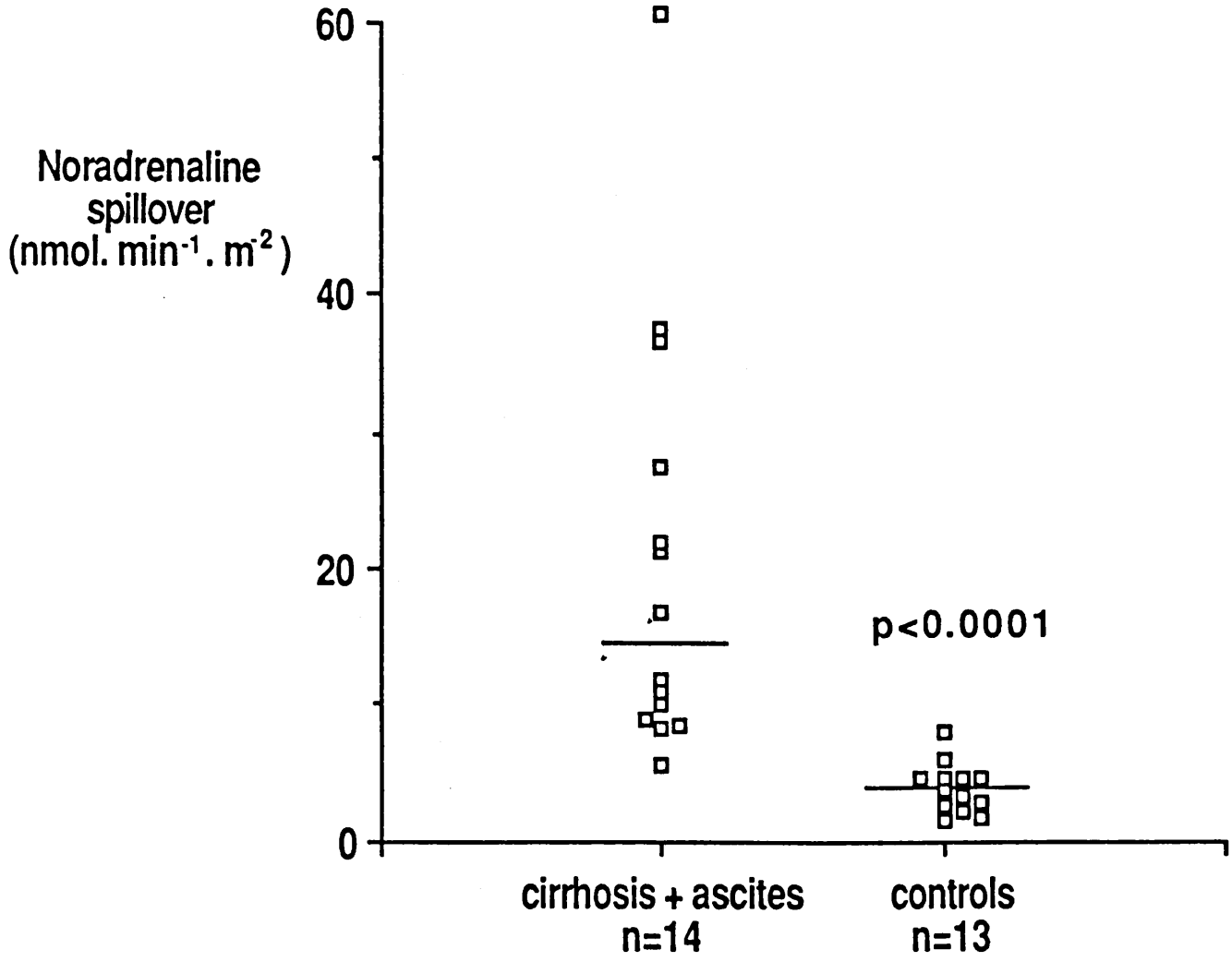

Figure 3.2.

Noradrenaline spillover in 14 cirrhotics with ascites and 13 controls (horizontal bar represents the median value).

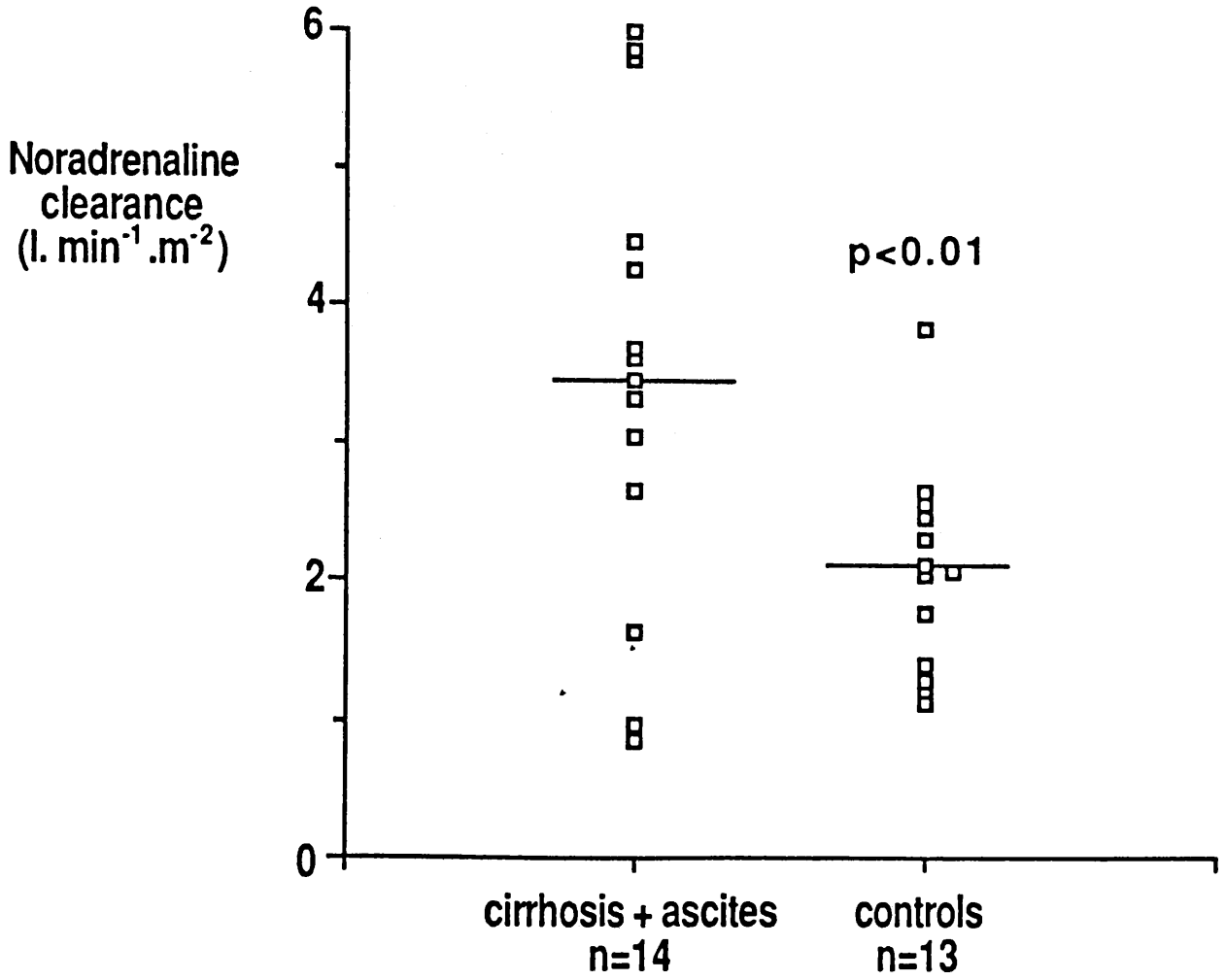

Figure 3.3.

Noradrenaline plasma clearance in 14 cirrhotics with ascites and 13 controls (horizontal bar represents the median value).

with spillover ($r = 0.83$ and 0.64 respectively, both $p < 0.01$) but not at all with clearance (Figures 3.4. and 3.5.). Within the cirrhotic group, there was no correlation between noradrenaline spillover and the severity of liver disease as judged by serum bilirubin, albumin, prothrombin time or numerical Pugh's score nor was there any link with the degree of haemodynamic disturbance, indicated by heart rate and systemic blood pressure. There was, however, a weak negative correlation between noradrenaline spillover and glomerular filtration rate indicated by both serum creatinine ($r = 0.42$, $p < 0.05$) and creatinine clearance ($r = -0.53$, $p < 0.05$).

Plasma noradrenaline clearance in this study was negatively correlated with numerical Pugh's score ($r = -0.72$, $p < 0.01$) and also with serum bilirubin ($r = -0.54$, $p < 0.05$), although not with other markers of hepatic impairment. As with spillover, there was no relationship with the systemic haemodynamic state. Clearance was also higher in these cirrhotics with less marked reductions in serum sodium ($r = 0.69$, $p < 0.01$) and sodium and potassium excretion ($r = 0.4$, $p < 0.05$ and $r = 0.66$, $p < 0.01$ respectively). There was no correlation, however, with the various indices of glomerular filtration.

3.4. DISCUSSION

This study demonstrates that the spillover of noradrenaline into plasma is increased in cirrhosis with

ascites. This is in agreement with two other studies measuring plasma noradrenaline kinetics in cirrhosis, both of which were published after this work was undertaken (116,204). There is therefore compelling evidence that global sympathetic activity is increased in cirrhosis with ascites. This is likely to be a vascular baroreceptor-mediated response to a reduction in the effective extracellular fluid volume and therefore supports the traditional "underfilling" theory of ascites. However, sympathetic overactivity could also be an hepatic baroreceptor-mediated response to the increased intrasinusoidal pressure which occurs in cirrhosis (104). Also, these patients all had advanced disease with well-established fluid retention and do not necessarily reflect the situation earlier in the evolution of ascites.

The lack of any correlation between noradrenaline spillover and the indices of hepatic impairment is likely to reflect the latter's poor ability to discriminate between degrees of disease severity, particularly in a group of cirrhotics all as severely ill as in this study. The weak correlation between increased sympathetic activity, as indicated by noradrenaline spillover and impaired renal function is in keeping with the work of Arroyo (56) who found that the highest plasma noradrenaline concentrations were in those cirrhotics who had developed functional renal failure. Such increased sympathetic activity could contribute to the renal vasoconstriction

which is thought to be important in the development of functional renal failure (63,111).

The increase in noradrenaline plasma clearance is a little surprising. Noradrenaline is largely metabolised but not released by the liver (205) which has a high content of the catecholamine degrading enzyme catechol-O-methyl-transferase (206). Also, the high splanchnic extraction of noradrenaline (68%) (207) mneans that splanchnic clearance - which accounts for 30% of the total body turnover of noradrenaline (207) - is dependent on liver blood flow, which is usually reduced in cirrhosis (208). Therefore one might predict a reduction in plasma noradrenaline clearance in cirrhosis. However, an uncontrolled regional study which suggested no major reduction in hepatic noradrenaline clearance in cirrhosis (147) has since been confirmed in two controlled studies (115,207) both of which demonstrated only a slight reduction in hepatic noradrenaline clearance.

An increase in noradrenaline plasma clearance in cirrhosis was also reported by one (116) but not the other (204) of the two previous studies measuring [^{3}H] noradrenaline kinetics. It is likely that any slight reduction in hepatic clearance is more than compensated by increased regional clearance elsewhere. Willett (116) proposed that the site might be the pulmonary circulation, since pulmonary blood flow is increased along with the increase in cardiac output in cirrhosis (23) and pulmonary clearance accounts for 41% of the total clearance of

noradrenaline (209). However, this assumes that the extraction of noradrenaline across the pulmonary bed remains constant, which seems unlikely in view of the extensive pulmonary arteriovenous shunting in advanced cirrhosis (210). The renal clearance of noradrenaline is not increased in cirrhosis (116,147). The site of increased noradrenaline clearance therefore remains a matter of speculation.

The negative correlation between noradrenaline plasma clearance and the indices of liver disease severity, principally Pugh's score, is in contrast to the work of Willett (116) who found that the increased clearance was only apparent in the most ill cirrhotics. The present study suggests that the increase in clearance is not simply a reflection of deteriorating hepatic function.

The increased plasma clearance of noradrenaline means that measurements of plasma noradrenaline alone will slightly underestimate the degree of sympathetic activity. Nevertheless, the increase in plasma noradrenaline concentration in cirrhosis (54,55,56) is seen to be due to an increase in spillover into plasma rather than any reduction in clearance. As such, plasma noradrenaline is still a useful guide to sympathetic activity in cirrhosis, as indicated by the close correlation between plasma noradrenaline and spillover in this study.

CHAPTER 4

SYMPATHETIC ACTIVITY AND ATRIAL NATRIURETIC PEPTIDE IN CIRRHOSIS

4.1. INTRODUCTION

In the previous chapter sympathetic activity was found to be increased in patients with cirrhosis and ascites. The increased concentration of noradrenaline in plasma in these patients was the result of increased spillover of noradrenaline into the circulation from sympathetic nerve endings. As noradrenaline plasma clearance was in fact increased in these cirrhotic patients, plasma noradrenaline is likely to underestimate the degree of sympathetic activation. Nevertheless it remains a valid approximation of sympathetic activity, and could be used as such in a large scale study, where detailed kinetic studies are impractical.

Another catecholamine detectable in plasma is dihydroxy-phenylethylene glycol (DHPG), the major dihydroxy-metabolite of noradrenaline in mammals (211) formed by oxidative deamination of noradrenaline within noradrenergic neurones. Plasma free DHPG is dependent upon noradrenergic impulse flow (212,213) and is thus a useful index of sympathetic activity. Indeed its prolonged half-life makes it less prone than noradrenaline to rapid fluctuation, and may make it a better index of sympathetic activity over a prolonged time, although it has no advantages over noradrenaline as an acute sympathetic index (214).

This study describes the measurement of these two catecholamines in a wide range of cirrhotic patients of varying aetiologies, duration and severity, with and without

ascites, to further assess the contribution of the sympathetic nervous system in cirrhosis. In addition, plasma renin activity has been measured as an appropriate guide to the state of the renin-angiotensin-aldosterone system. Finally, most cases have also measured the plasma concentrations of immunoreactive atrial natriuretic peptide (ANP), which may be of relevance both to the sodium retention and vasodilatation of severe cirrhosis. Previous workers have found conflicting results with one report of a reduced level of plasma ANP in cirrhosis (178) three reports of no change (174-177) and four reports of increased plasma levels (171-174). There is clearly scope for further study of plasma ANP levels in cirrhotic man.

4.2. METHODS

4.2.1. Subjects

Fortyone patients with cirrhosis were studied on 62 occasions over a 20 month period from March 1985 until October 1986. Every patient with chronic liver disease attending the 2 gastroenterologists in the hospital was considered for study. To be included they had to have cirrhosis, either confirmed histologically (30 cases) or diagnosed on clinical grounds (11 cases) - see Chapter 2 for the criteria for a clinical diagnosis of cirrhosis. Fifteen patients were studied on more than one occasion, 9 twice and 6 three times. Patients were eligible to be re-studied if either more than 6 months had elapsed since the

previous study, or the severity of the patient's liver disease had changed significantly. This latter criteria was defined as a shift from one to another of the 3 grades of disease severity (A, B and C) as defined by Pugh (184). Eleven of the repeat studies were performed after a gap of 6 months or more and 10 following a change in the patient's condition (2 deteriorations, 8 improvements).

There were 29 males and 12 females. Their ages ranged from 33-78 years (mean). The duration of the liver disease (from diagnosis) ranged from 1 month to 12 years (median 24 months). The diagnosis was alcoholic cirrhosis in the majority, with a handful of other aetiologies (Table 4.1.). All but one of the 41 cirrhotic patients had established portal hypertension indicated either by the endoscopic demonstration of oesophageal varices, or clinically evident splenomegaly, confirmed by isotope scanning. Alcohol consumption was assessed in an approximate manner, on each visit. Thirtysix of the subjects were teetotal at the time of study. Twentyseven of these had formerly drunk alcohol to excess, but not within the preceding 3 months. Twentysix of the subjects were still drinking regularly, 23 of these consuming more than one drink per day. No subject was suffering from acute alcohol withdrawal. Nineteen subjects were smokers, 21 non-smokers and in one case the smoking habit was not recorded.

Twentyseven of the studies were performed when the subjects were in-patients, the remaining 35 on an outpatient

TABLE 4.1. DIAGNOSES IN THE CIRRHOTIC PATIENTS

DIAGNOSIS	NUMBER (%) Male	Female	Total
Alcoholic Liver Disease	22 (76)	6 (50)	28 (68)
Chronic Active Hepatitis	4 (14)	2 (17)	6 (15)
Primary Biliary Cirrhosis	1 (3)	1 (8)	2 (5)
Haemochromatosis	1 (3)	0	1 (2)
Alpha-1-antitrypsin Deficiency	0	1 (8)	1 (2)
Cryptogenic Cirrhosis	1 (3)	2 (17)	3 (7)
	29 (100)	12 (100)	41 (100)

basis. On 26 of the study days the patients had recently been receiving diuretic therapy, mainly spironolactone or amiloride, with a few subjects receiving frusemide. On the remaining 36 study days they were not on diuretics. In addition 26 of the 62 subjects studied were receiving other medication, either H_2 receptor antagonists, corticosteroids, lactulose and/or neomycin.

None of the cirrhotic patients had any clinically evident cause for haemodynamic disturbance other than their liver disease. In particular none had bled from oesophageal varices (or other source) within the previous 10 days and none was receiving intravenous fluid replacement.

Thirtyfour control subjects were studied. As outlined in Chapter 2, these consisted either of patients attending the gastroenterology clinic with non-hepatic disorders (principally peptic ulcer disease, gastro-oesophageal reflux, diverticular disease and the irritable bowel syndrome) or patients being admitted for minor surgical procedures, principally hernia repair and varicose vein ligation. There were 21 males and 13 females. Their ages ranged from 39 to 75 years (mean 55). Seventeen were teetotal, none of whom were reformed alcoholics. Of the 17 who drank alcohol, 15 took less than one drink per day. There were 9 smokers and 23 non-smokers, the smoking habit having not been recorded in 2 cases. Sixteen subjects were in-patients when studied and 18 outpatients. Ten subjects had received medication in the recent past, principally H_2

receptor antagonists. None of the control subjects had been receiving diuretic therapy.

Conditions known to alter sympathetic activity or plasma catecholamine levels, i.e. systemic hypertension, cardiac failure, thyroid disease and overt diabetes mellitus were excluded from both the cirrhotic and the control group. (Some of the cirrhotic patients had impaired glucose tolerance, but required no therapy).

4.2.2. Study Design

The cirrhotic subjects studied as in-patients received a strictly controlled salt intake of 50 mmol per day, and any diuretic therapy was discontinued 72 hours prior to the study day. Those studied as outpatients were either receiving an unrestricted salt intake, or a "no added salt" diet (approximately 80-100 mmol Na^+ per day). If these patients required diuretic therapy, this was omitted on the study day only. The in-patients were studied fasted. The outpatients took a light breakfast least 1 hour prior to the study. All subjects abstained from tea, coffee, alcohol, tobacco and all medication on the study day. In the cirrhotic patients, the presence or absence of ascites and hepatic encephalopathy were noted. Ascites was graded as absent, mild or severe. Encephalopathy was graded from 0 (absent) to 4 (unconscious) according to Trey (215). An intravenous cannula was inserted into an antecubital vein. After a minimum of 30 mins heart rate and blood pressure

were recorded. When the systolic pressure varied by less than 5 mmHg on 5 consecutive readings, the subjects were judged to be at steady state and the mean of the 5 readings recorded. Blood was then drawn from the indwelling cannula for measurement of bilirubin, albumin, prothrombin time, sodium, potassium, urea, creatinine, noradrenaline, DHPG, plasma renin activity and atrial natriuretic peptide. The precise method of collection and the laboratory assay methods are described in Chapter 2 (sections 2.3. and 2.4.). This study was of course often undertaken in conjunction with some of the experiments described in other chapters.

4.2.3. Statistical analysis

The statistical analysis was carried out using the MINITAB 85 computer programme (200). The distribution of each variable was assessed by correlation with a derived set of normal scores. Comparison between cirrhotic and control groups were made using Student's unpaired t test, with or without a logarithmic transformation, depending on the distribution of the variable being assessed. Where a logarithmic transformation failed to render the data suitable for parametric analysis, the Mann Whitney U test was employed. Where the cirrhotic group were subdivided one way analysis of variance was used. Correlations were calculated by simple linear regression and by stepwise regression analysis.

4.3. RESULTS

The liver function tests, heart rate and blood pressure, plasma noradrenaline and DHPG, plasma renin activity and ANP in the cirrhotic and control groups are summarised in Table 4.2. Noradrenaline, DHPG and renin were all significantly raised in the cirrhotic patients who also demonstrate impaired hepatic function by their raised bilirubin, decreased albumin and prolonged prothrombin time, slightly impaired glomerular filtration by their raised serum creatinine and impaired free water clearance by their reduced serum sodium concentration. The group as a whole showed little haemodynamic disturbance, with increased heart rate and lowered diastolic BP but no alteration in systolic pressure.

The increase in plasma noradrenaline concentration was unrelated to the duration of liver disease ($r = -0.17$) or to its aetiology (analysis of variance (AOV) on the diagnostic categories $F = 1.42$). Plasma noradrenaline concentrations were similar whether or not the cirrhotic patients had recently been taking diuretics or other medication (diuretics 4.7 ± 2.5 v no diuretics 4.5 ± 2.8 $nmol.l^{-1}$, NS) or alcohol (AOV on 4 grades of consumption, $F = 1.45$). Noradrenaline tended to be higher in those cirrhotics on sodium restriction (5.9 ± 3.2 $nmol.l^{-1}$) than those with unrestricted sodium intake (4.3 ± 2.3 $nmol.l^{-1}$) although this was not significant ($p = 0.15$). Consideration of these potential confounding variables with respect to the

TABLE 4.2. LIVER AND RENAL FUNCTION, HEART RATE AND BLOOD PRESSURE, PLASMA CATECHOLAMINES, RENIN AND ANP IN CIRRHOTIC AND CONTROL PATIENTS

DHPG = dihydroxyphenylglycol, PRA = plasma renin activity and ANP = atrial natriuretic peptide. Values quoted are median (range) or mean ± SD with comparison by unpaired t-test with (t-log) or without (t) logarithmic transformation, or by Mann Whitney U test (MWU), according to distribution.

	CIRRHOSIS n=62	CONTROLS n=34	
Bilirubin ($umol.l^{-1}$)	36.5 (4-600)	8.0 (5-17)	$p < 0.001$ (t-log)
Albumin ($g.l^{-1}$)	32.1 ± 6.2	40.8 ± 2.9	$p < 0.001$ (t)
Prothrombin time (secs prolonged)	4 (0-30)	0 (0-3)	$p < 0.001$ (MWU)
Serum sodium ($mmol.l^{-1}$)	139 (114-144)	141 (136-145)	$p < 0.001$ (MWU)
Serum creatinine ($umol.l^{-1}$)	91 (46-429)	85 (53-120)	$p < 0.05$ (t-log)
Heart rate (min^{-1})	78 ± 13	67 ± 11	$p < 0.001$ (t)
Systolic BP (mmHg)	120 ± 16	127 ± 16	NS $p = 0.07$ (t)
Diastolic BP (mmHg)	67 ± 11	73 ± 9	$p < 0.01$ (t)
Plasma noradrenaline ($nmol.l^{-1}$)	4.3 (0.9-17.4)	1.9 (0.7-5.3)	$p < 0.001$ (t-log)
Plasma DHPG ($nmol.l^{-1}$	14.6 ± 5.8 n=50	9.0 ± 3.3 n=30	$p < 0.001$ (t)
PRA ($ngAIml^{-1}hr^{-1}$)	11.5 (0.9-419) n=50	3.0(0.7-17.1) n-22	$p < 0.001$ (t-log)
ANP ($pg.ml^{-1}$)	37.7(8.1-238) n=13	33.3(5.6-81.2) n=25	ns $p=0.12$ (t-log)

raised DHPG concentrations yielded similar results with the exception that the increase in the sodium-restricted group (16.2 ± 6.5 v 12.5 ± 4.0 nmol.l^{-1}) was significant, $p < 0.02$.

Subdivision of the cirrhotic group according to the presence or absence of ascites reveals that although the increase in noradrenaline and DHPH levels is greatest in those patients with ascites, they are also significantly increased in cirrhotic patients who have never had ascites (Table 4.3., Figure 4.1.). In contrast plasma renin activity is only significantly increased in those patients with ascites (currently or formerly), values in the non-ascitic patients being similar to control subjects (Table 4.4., Figure 4.2.).

Plasma noradrenaline and DHPG are both positively correlated to liver disease severity as indicated by Pugh's score and in the case of DHPG also to serum albumin and prothrombin time (Table 4.5.). The progressive increase in noradrenaline in relation to Pugh's grades is illustrated in Figure 4.3. This trend is supported by the limited information available on plasma catecholamine concentrations as disease severity alters in a few patients studied on several occasions. In the one patient who deteriorated, both noradrenaline and DHPG rose. In ten patients who improved, plasma noradrenaline fell in five cases, remained the same in one and rose (minimally) in four, while DHPG results in seven of these patients showed a fall on five

TABLE 4.3. PLASMA CATECHOLAMINES, RENIN AND ANP IN RELATION TO ASCITES

Subdivided into those who have never had ascites (Group I), those now free of ascites on diuretics (Group II) and those currently with ascites (Group III).
Values are median (range) and comparison between groups is by one way analysis of variance.

	CONTROLS	CIRRHOSIS		
		I	II	III
Plasma noradrenaline ($nmol.l^{-1}$)	1.9 (0.7 - 5.3) n=34	3.8 (0.9 - 10.4) n=17	4.2 (1.8 - 9.5) n=22	4.7 (1.4 - 17.4) n=23
Plasma DHPG ($nmol.l^{-1}$)	8.8 (3.2 -17.1) n=30	12.0 (4.4 - 20.2) n=14	13.7 (8.3 -31.5) n=19	14.7 (7.2 - 28.6) n=17
PRA ($ngAIml^{1}hr^{-1}$)	3.0 (0.7 -17.1) n=32	4.5 (0.9 - 52.0) n=14	20.1 (1.2 - 118.0) n=19	32.0 (3.2 - 419.0) n=17
ANP ($pg.ml^{-1}$	33.3 (5.6 -81.2) n=25	29.2 (8.1 -67.2) n=10	37.6 (16.1 - 163.0) n=12	50.0 (20.0 - 238.0) n=11

In the case of plasma noradrenaline and DHPG all three groups of cirrhotic patients including those who have never had ascites, have significantly higher values than controls. In contrast PRA is only significantly raised in those cirrhotics with ascites (currently or formerly) whereas cirrhotics who have never had ascites have values similar to control subjects. The differences in ANP concentrations among the groups just fails to achieve significance ($F=2.5$, $p = 0.064$).

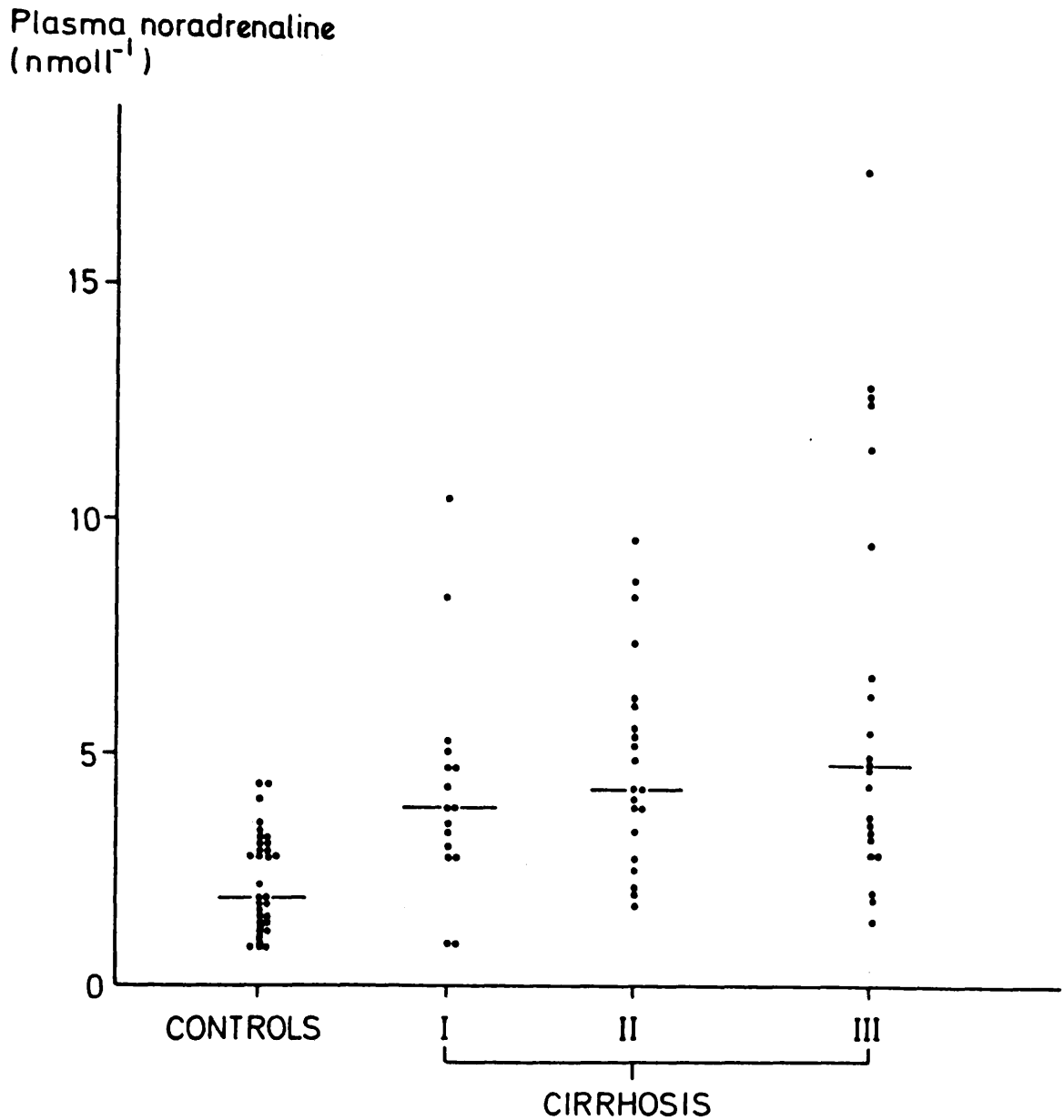

Figure 4.1.

Plasma noradrenaline concentrations in control subjects and cirrhotic patients who have never had ascites (Group I), those now free of ascites on diuretics (Group II) and those currently with ascites (Group III). Horizontal lines represent median values.

TABLE 4.4. CORRELATION COEFFICIENTS FOR PLASMA CATECHOLAMINES

Related to liver function tests, heart rate and blood pressure, plasma renin activity (PRA) and atrial natriuretic peptide (ANP).

	r	
	Noradrenaline	DHPG
Bilirubin	0.25	0.29
Albumin	-0.34 $p < 0.01$	-0.56* $p < 0.001$
Prothrombin time	0.26	0.42* $p < 0.001$
Pugh's score	0.52* $p < 0.001$	0.43* $p < 0.001$
Heart rate	0.34 $p < 0.01$	0.20
Systolic BP	-0.15	-0.07
Diastolic BP	-0.16	-0.22
Serum sodium	-0.23	-0.21
Creatinine	0.38* $p < 0.005$	0.16
PRA	0.34 $p < 0.01$	0.27
ANP	0.60* $p < 0.001$	0.23

11 variables, therefore significance level is $0.05 \div 11 = 0.005$ approximately; corresponding r value for 62 subjects is 0.35.

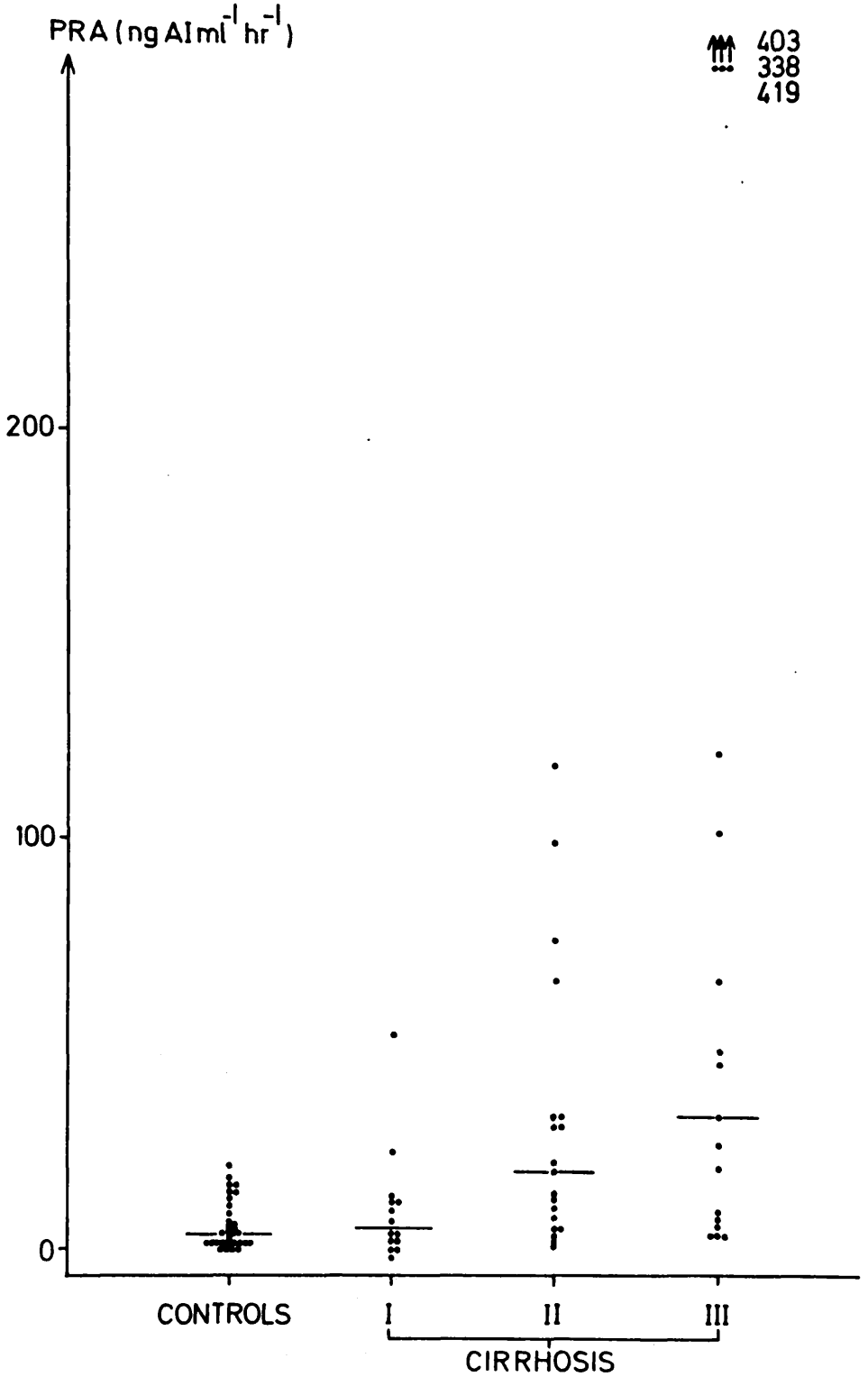

Figure 4.2.

Plasma renin activity in control subjects and cirrhotic patients who have never had ascites (Group I), those now free of ascites on diuretics (Group II) and those currently with ascites (Group III). Horizontal lines represent median values.

TABLE 5.1. LIVER FUNCTION TESTS, BLOOD PRESSURE, HEART RATE AND PLASMA NORADRENALINE

Group 1 represents the mild cirrhotics (Pugh's grade A) Group 2 the severe cirrhotics (Pugh's grade B or C) and Group 3 the controls.
Values are mean ± SD and comparison is by one way analysis of variance.

	GROUP 1 (n=10)	GROUP 2 (n=10)	GROUP 3 (n=20)	1 v 2	1 v 3	2 v 3
Bilirubin ($umol.l^{-1}$)	15.2 ± 7.9	45.8 ± 36.2	9.5 ± 3.1	$p < 0.005$	NS	$p < 0.001$
Albumin ($g.l^{-1}$)	38.4 ± 3.3	31.6 ± 5.9	40.1 ± 2.9	$p < 0.005$	NS	$p < 0.001$
Prothrombin time (seconds prolonged)	1.6 ± 1.4	4.8 ± 1.5	0.5 ± 0.8	$p < 0.001$	NS	$p < 0.001$
Systolic BP (mmHg)	127 ± 12	115 ± 12	129 ± 14	NS	NS	$p < 0.05$
Diastolic BP (mmHg)	68 ± 6	67 ± 9	74 ± 8	NS	NS	NS
Heart Rate (min^{-1})	70.3 ± 8.9	77.9 ± 11.8	65.4 ± 8.6	NS	NS	$p < 0.05$
Plasma Noradrenaline ($nmol.l^{-1}$)	3.27± 1.5	4.56± 1.8	2.19± 1.1	NS	NS	$p < 0.001$

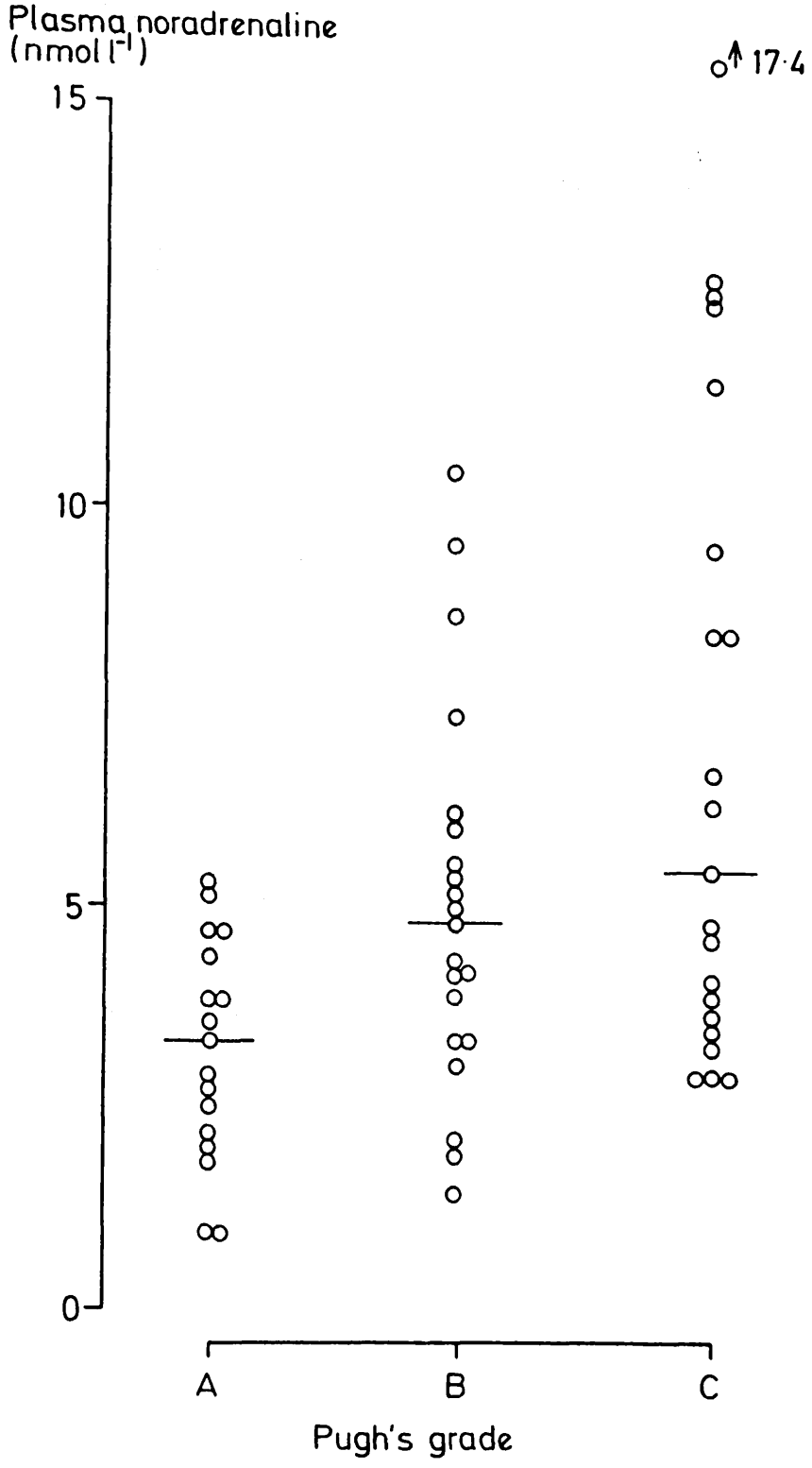

Figure 4.3.

Plasma noradrenaline concentrations in cirrhotic patients graded according to Pugh (184) as mild (A), moderate (B) or severe (C). Horizontal lines represent median values.

occasions. One patient with cirrhosis and ascites studied on three occasions persistently had noradrenaline values in excess of DHPG values, a pattern usually only seen in phaeochromocytoma.

Other correlations are indicated in Table 4.5. Plasma noradrenaline (but not DHPG) was related to impaired renal function, although this result is artifically weighted by two patients with functional renal failure who had very high noradrenaline concentrations. Interestingly, the third patient studied with functional renal failure had low values of both noradrenaline and DHPG. There was no correlation whatsoever between plasma noradrenaline or DHPG and serum sodium, heart rate or blood pressure. Multiple regression analysis failed to identify any independent factors among these correlations.

The significance of plasma noradrenaline as a prognostic indicator is illustrated in Figure 4.4. The 41 patients studied have been followed up for 12 to 27 months. The noradrenaline concentration in the 26 survivors (median 3.5, range 0.9-9.5) is significantly lower than in the 15 patients who have died (median 6.4, range 2.8 - 17.4). None of the four patients with noradrenaline concentrations greater than 10 $mmol.l^{-1}$ on first measurement survived six months. Of the ten patients with noradrenaline between 5 and 10 $mmol.l^{-1}$, 50% were still alive at one year, while the corresponding figure for the 26 patients with noradrenaline below 5 $mmol.l^{-1}$ is 88%.

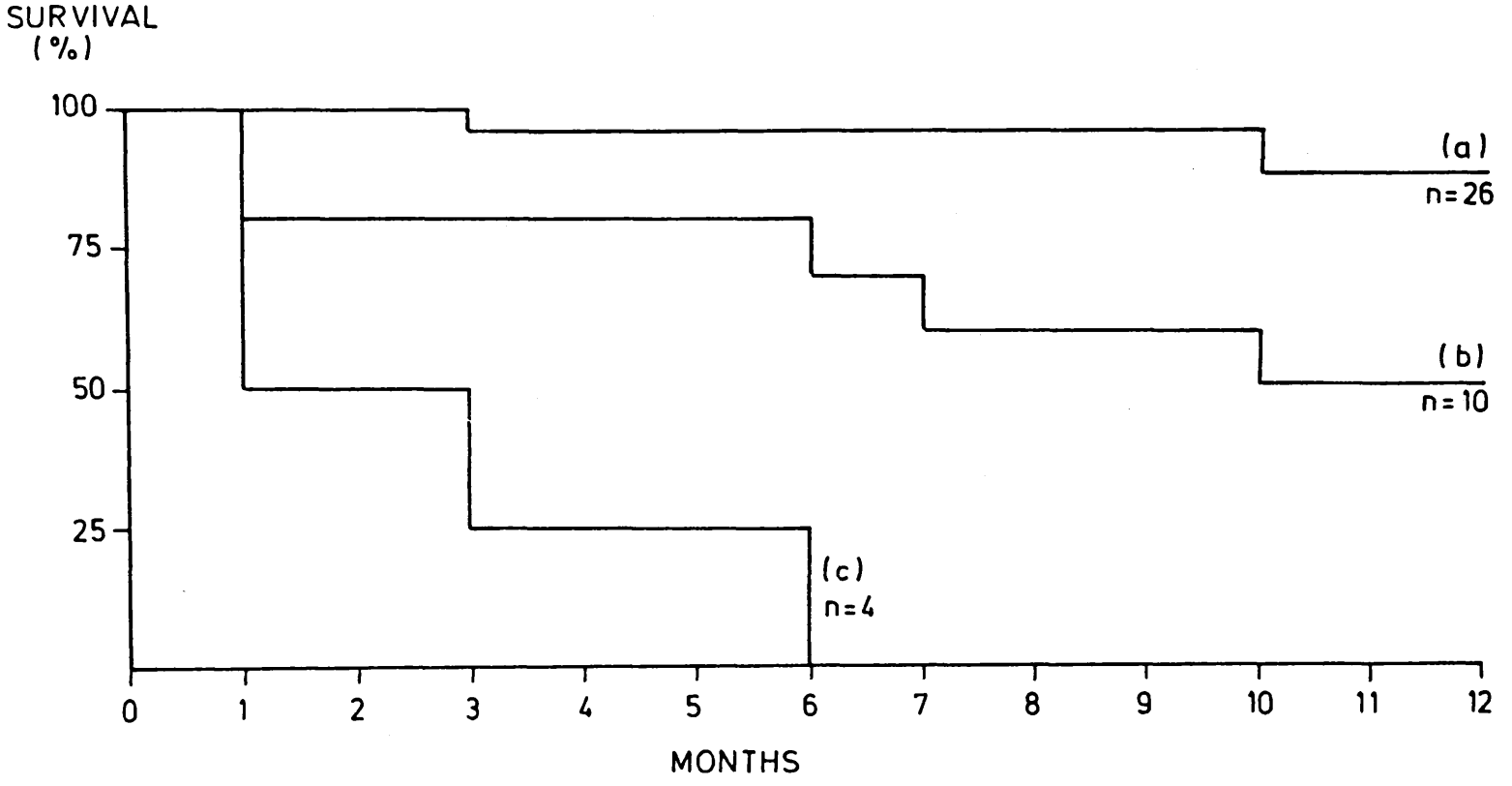

Figure 4.4.

"Life table" survival graph of cirrhotic patients after one year according to plasma noradrenaline on presentation:
(a) < 5 $nmol.l^{-1}$; (b) 5-10 $nmol.l^{-1}$; (c) > 10 $nmol.l^{-1}$

Plasma ANP concentrations were not significantly different in the cirrhotic group as a whole compared to controls (Table 4.1.). There is a tendency towards higher concentrations in the patients with ascites than in those who have never had ascites, although the majority of both groups still fall within the range of the normal controls, and analysis of variance just fails to detect a significant difference between the groups (Table 4.3., Figure 4.5.). Similarly there is a non-significant trend towards progressively increasing concentrations of ANP with increasing disease severity as indicated by Pugh's grade (Figure 4.6.).

DISCUSSION

Before discussing the significance of the results of this study, three technical matters require consideration. Firstly, what is the optimum sampling site for noradrenaline, arterial or venous, central or peripheral? In theory, arterial or central venous levels should give a better indication of total sympathetic activity. Sympathetic activity is organ specific and varies widely from one site to another. Antecubital venous noradrenaline is largely derived from skeletal muscle (216). However, noradrenaline levels in antecubital veins are similar to those in the right atrium in normal subjects (195) and to the femoral vein in both normal and cirrhotic subjects (217). In the latter paper, both antecubital and femoral venous noradrenaline

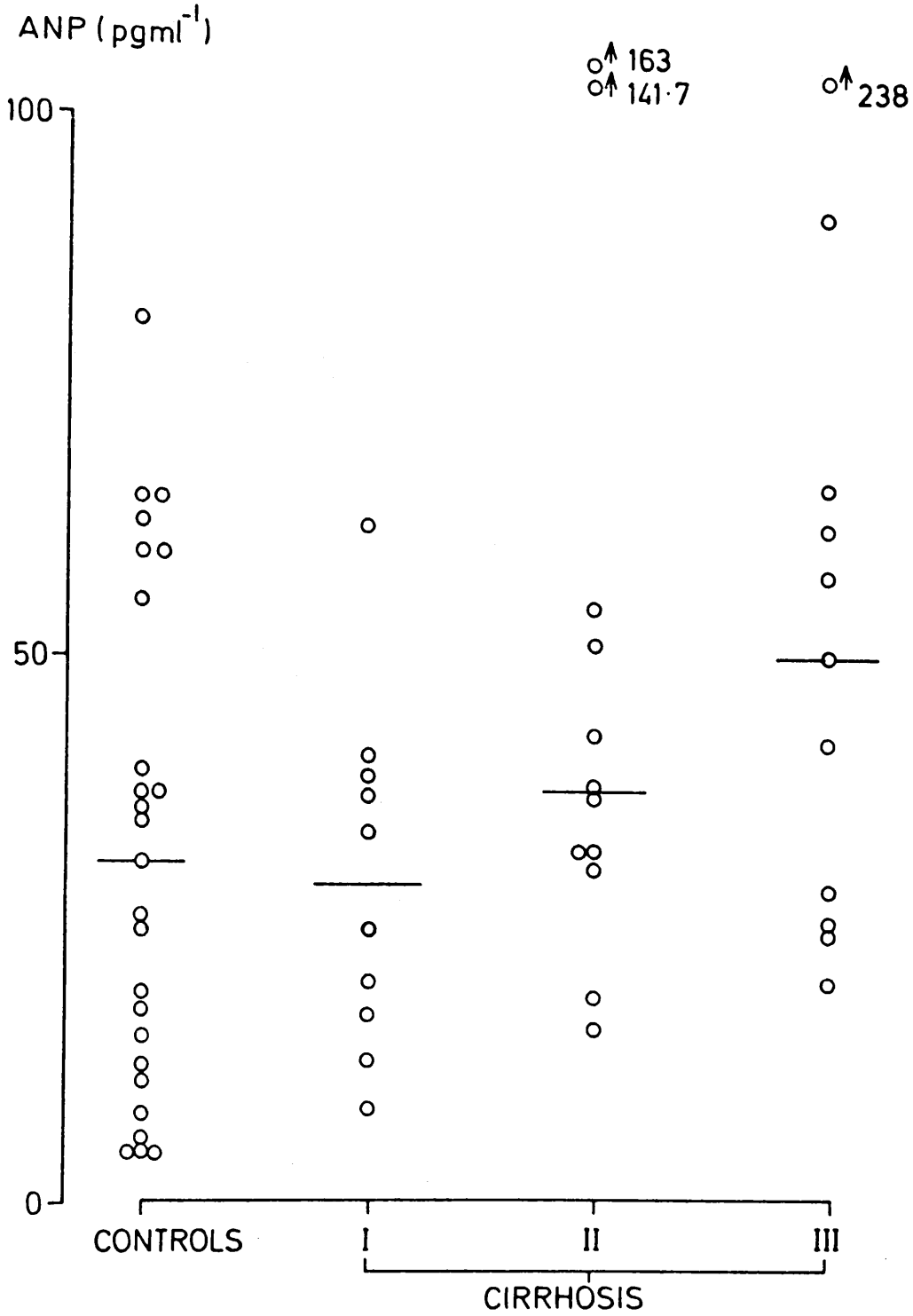

Figure 4.5.

Plasma ANP in control subjects and cirrhotic patients who have never had ascites (Group I), those now free of ascites on diuretics (Group II) and those currently with ascites (Group III). Horizontal lines represent median values.

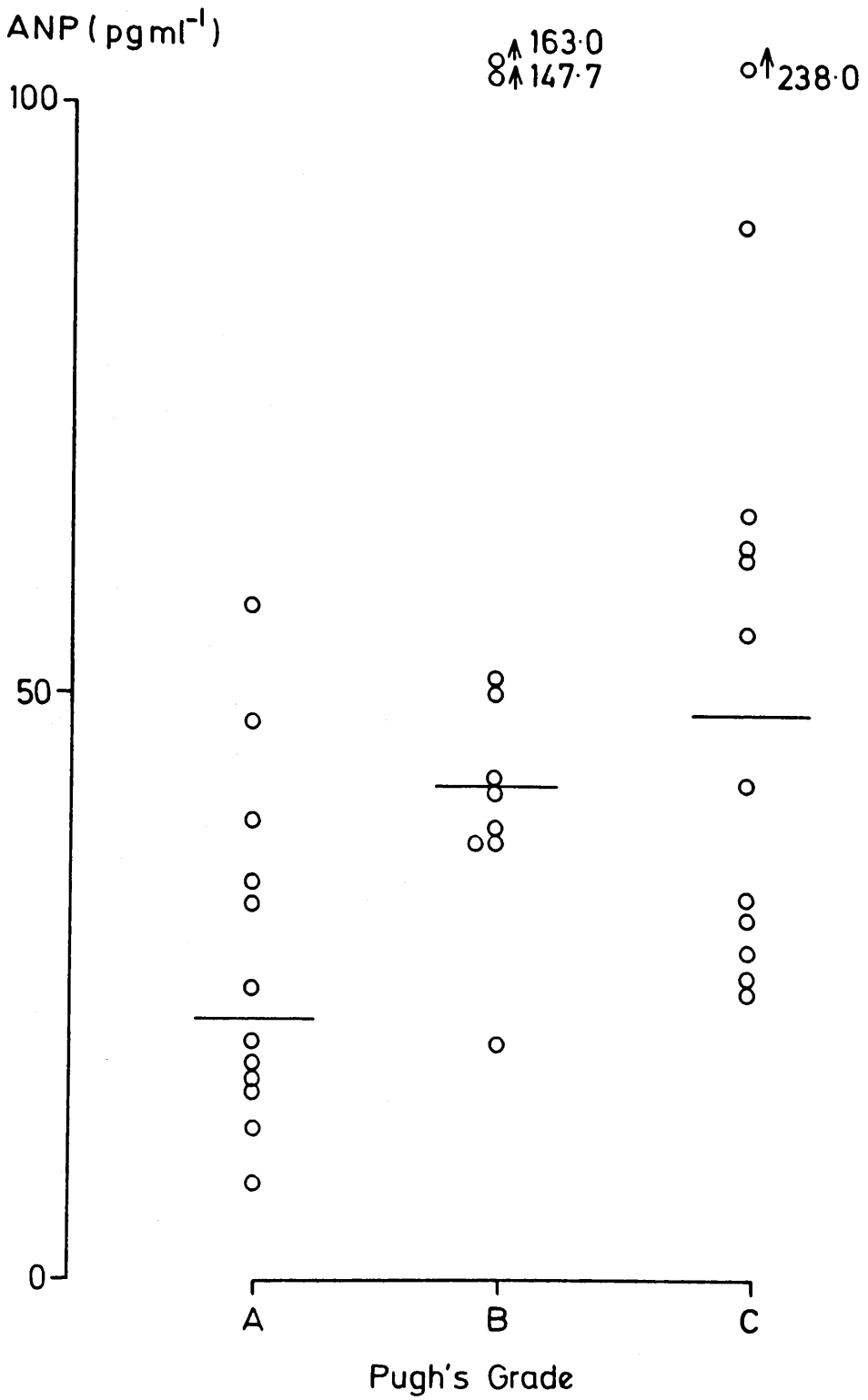

Figure 4.6.

Plasma ANP in cirrhotic patients graded according to Pugh (184) as mild (A), moderate (B) or severe (C). Horizontal lines represent median values.

concentrations correlated closely with arterial values. It therefore appears that antecubital venous noradrenaline levels may be suitable for comparison between cirrhotic and control groups, obviating the need to expose those patients with coagulation defects to arterial or central venous sampling.

Secondly, should adrenaline as well as noradrenaline concentrations be measured? Plasma adrenaline, derived from the adrenal medulla which responds to similar stimuli which activate sympathetic nerves, might be expected to give useful additional information. However, two studies which demonstrated increased plasma noradrnaline in cirrhosis found no such increase in venous adrenaline unless functional renal failure had supervened (55,56). Increased levels of adrenaline in venous blood (54) could not subsequently be confirmed on arterial samples (217), the optimum site for adrenaline measurement (218). More recently two groups have found elevated adrenaline levels in cirrhosis in venous (146) and arterial (207) blood. In all these studies the differences in plasma noradrenaline were more striking and there seems little to be gained from the addition of adrenaline measurements.

Thirdly, should urinary as well as plasma catecholamines be measured? No, since the complexity of renal handling of catecholamines renders the interpretation of urinary catecholamines difficult (219) and the concentrations of catecholamines in plasma are more

reproducible than those in urine providing the many variables which influence plasma levels are taken into consideration (220).

This study confirms the increase in plasma noradrenaline concentration in cirrhosis noted in the previous chapter and by numerous others (54,55,56,116,203,207). Since the clearance of noradrenaline from plasma is not reduced in cirrhosis, as indicated in the previous chapter and supported by others (116,203), there must be an increase in overall sympathetic activity in cirrhosis. The parallel increase in plasma DHPG is further evidence of this increased sympathetic activity, which does not appear to be due to such confounding variables as alcohol and diuretic intake. The degree of sympathetic activation seems to depend on the severity of the disease process rather than its duration or a particular aetiology. The progressive rise in noradrenaline as the severity of the hepatic impairment increases is a feature common to all studies in this field (54,55,56).

However, the situation in the "compensated" patient who has not developed ascites is more controversial. Henriksen found normal plasma noradrenaline concentrations in this group (115) but Arroyo showed plasma noradrenaline in cirrhotic patients without ascites to be increased by fifty per cent over control values (56). The Denver group did not address this question specifically, but did note that

their cirrhotic subjects with unimpaired free water clearance (probably equivalent to a "compensated" state) had noradrenaline values no greater than control subjects (55). The current study is in agreement with that of Arroyo with increased sympathetic activity in cirrhotic patients who have never had ascites. This is despite the fact that plasma renin activity in this group was not increased, again in keeping with Arroyo's work (56) and several others (17,18,19) often cited in support of the overflow theory. If the increased sympathetic activity in the pre-ascitic cirrhotic group is in response to baroreceptor stimulation, this represents strong evidence in favour of a reduced effective circulating volume in these patients.

This study was unable to confirm any relationship between the haemodynamic status - as indicated by heart rate and blood pressure - and the degree of sympathetic activity. Although this could be argued as evidence against the sympathetic activation being in response to systemic haemodynamic disturbance, heart rate and blood pressure are relatively crude measures. In fact, Henriksen was able to demonstrate significant correlations between noradrenaline and more direct indices of central haemodynanics such as stroke volume and cardiac output, when no such correlations were evident with either heart rate or blood pressure (221). However, one potential mechanism of sympathetic activation independent of the state of systemic vascular filling would be direct stimulation of the sympathetic nervous system in

response to increased hepatic sinusoidal pressure. Such hepatic baroreceptors have been demonstrated in non-cirrhotic dogs (104) but not in man, either healthy or cirrhotic.

Further evaluation of the stimulus to sympathetic activity depends on invasive studies, which will yield information on regional sympathetic activity. Such invasive procedures were outwith the scope of the current study, and indeed published data on this subject are sparse. Plasma noradrenaline correlated closely to wedged hepatic venous pressure ($r = 0.86$) in one study (54), supported by a similar correlation with wedged minus free hepatic venous pressure ($r = 0.75$), a better index of portal pressure, in a subsequent study from the same investigators (115). However, such correlations between either systemic or portosplanchnic haemodynamics do not prove a causal link, since both might be indicators of some other factor in deteriorating liver function, e.g. accumulation of vasodilator substances.

Regional sympathetic studies have been undertaken by two centres, with conflicting results. The Danish group, measuring renal arterial-venous differences in catecholamine concentrations, found a net renal release and suggested that renal sympathetic drive was a major contribution to total sympathetic activity (115). In contrast Willett and co-workers studying net renal release by [^{3}H] noradrenaline kinetics concluded that renal sympathetic activity merely

reflected overall sympathetic activation (116). The agreement that renal sympathetic activity is increased has important implications for the clinical effects of sympathetic stimulation, irrespective of its cause. Renal sympathetic activation will result in sodium retention from the proximal tubule, probably by a direct effect (60,61), which might explain the failure of cirrhotic patients to "escape" from the sodium-retaining effects of mineralocorticoid administration (41). Renal sympathetic activation will also cause sodium retention from the distal tubule, indirectly, by stimulation of the renin-angiotensin-aldosterone system. Thirdly, renal sympathetic stimulation will cause renal vascular constriction, particularly to the renal cortex, which might contribute to the functional renal failure which can complicate cirrhosis with ascites (56,111,222).

The theoretical link between sympathetic overactivity and development of ascites and renal failure in cirrhosis may be relevant to the apparent value of plasma noradrenaline as a prognostic indicator which this study has demonstrated. A significantly increased plasma noradrenaline concentration indicates a very poor prognosis in these patients. Other studies have demonstrated an equally poor survival with increased plasma renin (47) and decreased serum sodium (64). Interestingly, a recent study of 141 cirrhotic patients over a six year period found that plasma noradrenaline was one of only six variables out of a

possible 42 measured which were independent predictors of survival. The other were mean arterial pressure, urinary sodium excretion, ascitic protein concentration, serum urea and serum albumin (223). The advent of hepatic transplantation means that such prognostic indicators are of vital importance in assessing the correct timing of transplantation, which is perhaps the most taxing problem facing hepatologists today.

This study also considers the significance of ANP in cirrhosis. ANP is a natriuretic peptide (or group of peptides) derived from the right atrium (224). This discovery rejuvenated an older theory that the sodium retention of ascites might result from the lack of some undefined natriuretic factor (225). However, the current study demonstrated no reduction in plasma ANP levels, even when ascites was present. Indeed, although the differences are just outwith statistical significance, there is a clear trend towards higher levels of ANP in the cirrhotics with ascites and more severe hepatic dysfunction. These findings are in keeping with reports in the literature that ANP levels are either normal (176,177) or high (171-174,226) in patients with cirrhosis. Only one group have found reduced plasma levels of ANP in cirrhosis (178), although this has been reported in dogs with experimental cirrhosis (227). The cardiac content of ANP is reduced in cirrhotic rats (228), but this could represent either decreased synthesis of ANP, or depletion of ANP stores as a result of

increased release! It is possible that methodological problems with sample collection and assay have contributed to these variable results, since reliable radioimmunoassays have only become available in the past three years (229,230). The consensus view would appear to be that a deficiency of ANP is not responsible for the sodium retention of cirrhosis, and the current study supports this conclusion. Further evidence against lack of ANP being significant in ascites is the response to infusion of exogenous ANP in these patients. Numbers studied so far have been small, but the natriuretic response seems to be modest and incomplete (231,232). It remains possible that in cirrhosis the kidney has in some way developed resistance to the natriuretic action of ANP.

ANP in supraphysiological concentrations is also a vasodilator (233). Although the majority of cirrhotic patients have ANP levels in plasma which are only slightly elevated, there were a few patients in this study with high enough concentrations for ANP to be a contributory factor to the systemic vasodilatation which occurs and whose significance is considered in more detail in subsequent chapters.

What are the implications of normal or raised plasma ANP levels for the theories of ascites formation? A regional study has suggested that any increase in plasma ANP in cirrhosis is due to increased cardiac release rather than impaired hepatic clearance (173). The only proven stimulus

to ANP release is atrial stretch, and therefore normal or raised plasma levels would seem to favour an increase rather than a reduction in the effective circulating volume. Attempts to expand the effective circulating volume in cirrhosis, e.g. by head-out water immersion, or by insertion of peritoneovenous shunts, result in marked rises in plasma ANP, both in dogs (227) and in man (176,234), which does suggest initial volume depletion. However, this effect is also seen in normal subjects, albeit to a lesser degree (235,236). It has also been suggested that factors other than atrial stretch might stimulate ANP release, for example the sympathetic nervous system (237), which could explain the increased ANP in cirrhosis with ascites even with central volume depletion (171). However, recent work in normal subjects does not support a direct sympathetic role in ANP release (238) and Wernze has argued that his failure to show any correlation between plasma noradrenaline and plasma ANP is evidence against a direct relationship in cirrhosis (176). The present study, examining many more subjects, did demonstrate a correlation between plasma noradrenaline and ANP in cirrhosis ($r = 0.60$, $p < 0.001$). Dietary sodium intake also influences ANP levels, and this study could be criticised as the dietary sodium varied amongst the cirrhotic patients. In fact, although in normal subjects increased sodium intake promotes ANP release, the cirrhotics on sodium restriction exhibited higher levels (median 40.0 v 33.2 $pgml^{-1}$, $p = 0.08$), presumably since these were the

patients with ascites. Plasma ANP concentration cannot be used as a categorical indication of the state of vascular filling in cirrhosis until more is known about the interaction with the other neurohumoral disturbances.

In summary, this chapter shows increased sympathetic activity in cirrhosis both when ascites is present and in the pre-ascitic stage, which tends to support the underfilling theory of ascites formation. The degree of sympathetic activity is related to the extent of liver damage and significantly raised plasma noradrenaline is a useful indication of poor prognosis. Plasma levels of ANP are not reduced in cirrhosis, with or without ascites, therefore deficiency of ANP is not a factor in the sodium retention in these patients.

CHAPTER 5

AUTONOMIC FUNCTION IN CIRRHOSIS

5.1. INTRODUCTION

There is a well documented haemodynamic disturbance in cirrhosis characterised by a reduced blood pressure despite increased heart rate and increased cardiac output, indicating a fall in peripheral systemic vascular resistance (23,24). The question has been raised as to whether autonomic dysfunction may contribute to this (239). Autonomic damage might be expected in some cirrhotics, as the majority in the UK are alcohol-induced, and autonomic neuropathy, especially of vagal origin, is seen in chronic alcoholics (152). At the time this study was undertaken, there was no published work examining the effect of cirrhosis on autonomic function. It has been suggested that the best way of assessing autonomic function clinically is by performing a battery of both sympathetic and parasympathetic tests in each individual (240). We therefore examined autonomic function in this way in a heterogeneous group of 20 cirrhotics, subdivided into those with mild and those with moderate to severe liver damage, and 20 patient controls.

5.2. METHODS

5.2.1. Subjects

Twenty patients with hepatic cirrhosis were studied. The cirrhosis was confirmed histologically in 15 cases and in the remainder a clinical diagnosis of cirrhosis was made (see Chapter 2, Section 2.1.1. for the criteria for

clinically-defined cirrhosis). These patients were divided into two groups according to their degree of liver impairment. Group 1 consisted of 10 patients with Pugh's Grade A disease and group 2 consisted of 10 patients with Pugh's Grade B or C. The cirrhosis was due to alcohol in 12 cases (5 group 1, 7 group 2), primary biliary cirrhosis in 2 (1 in each group) and one each of haemochromatosis (Group 1) alpha-1-antitrypsin (Group 1) and cryptogenic (Group 2). The duration of liver disease ranged from 2 to 98 months in Group 1 (median 34 months) and from 1 to 138 months in Group 2 (median 20.5 months).

These were compared to twenty control patients (Group 3) with non hepatic disorders who had no clinical or biochemical evidence of liver disease. No subject in either group had hypertension, cardiac failure, diabetes mellitus, thyroid disease or malignancy.

The mean age in Group 1 was 55.6 years (range 33-65), in Group 2, 56.4 years (41-73) and in controls 55.3 years (39-75). The sex ratio (male: female) was 8:2 in Group 1, 5:5 in Group 2 and 13:7 in controls.

Although medication was kept to a minimum, several patients had received diuretic therapy within 3 months of the study. One cirrhotic patient in Group 1 had taken frusemide and spironolactone. In Group 2, 7 patients in total had taken diuretics (1 frusemide alone, 4 spironolactone or amiloride, and 2 both classes of diuretics). Two cirrhotics (both Group 1) were taking

azathioprine with or without prednisolone; 1 cirrhotic (Group 1) and 5 control patients were taking a variety of ulcer healing agents and 4 cirrhotics (1 in Group 1 and 3 in Group 2) were taking lactulose and/or neomycin.

Despite the prevalence of alcoholic liver disease, only 7 cirrhotic patients (3 in Group 1, 4 in Group 2) had been drinking alcohol in excess of 10 g per day during the 3 months prior to study, with 5 of the 20 control patients consuming this quantity also. Three cirrhotics in Group 1, 4 in Group 2 and 8 control patients smoked.

5.2.2. Study Design

No alcohol was taken during the 7 days preceding the study, and all diuretic therapy was stopped for at least 3 days. On the study day the patients fasted and abstained from caffeine-containing beverages and tobacco. An indwelling cannula was inserted into an antecubital vein, and the subject rested supine for a minimum of 30 minutes. Heart rate and blood pressure (BP) were recorded using a semi-automatic sphygmomanometer (Sentron, Bard Biomedical) and blood drawn from the cannula for measurement of urea and electrolytes, albumin, bilirubin, prothrombin time, noradrenaline and plasma renin activity. After a light breakfast they rested for a further hour then the following tests of autonomic function were performed. BP was again recorded by semi-automatic sphygmomanometer. Heart rate and/or RR intervals were recorded using 3 praecordial

electrocardiograph electrodes linked to a multichannel recorder (Grass Polygraph model 7B1B; Quiney, Mass. USA). This in turn was connected to a Nodecrest computer terminal programmed to directly analyse the RR interval data.

5.2.3. Parasympathetic tests

Standing to lying test (241)

The patient stood erect for 5 minutes, then lay supine while the heart rate was recorded continuously. The standing to lying ratio (SLR) which was calculated as the mean of the 10 RR intervals immediately preceding lying down divided by the shortest RR interval during the 30 seconds after lying down was performed in duplicate. The transient increase in heart rate on lying down is a measure of vagal withdrawal, and the normal SLR is > 1.07 (241).

Valsalva manoeuvre (242)

The patient, after becoming familiar with the instrument, maintained forced expiration to hold a column of mercury in a modified sphygmomanometer at 50 mmHg for 15 seconds. The heart rate was recorded during and for 1 minute after the manoeuvre. The test was performed in duplicate. The longest RR interval following the manoeuvre divided by the shortest RR interval during the manoeuvre is known as the Valsalva Ratio (VR) which reflects parasympathetic function (243). The normal VR is > 1.20 (244).

"Diving" test (245)

The heart rate was recorded for 30 seconds with the patient seated with face held 4 cm above a basin of cold water (temperature 18-20°C). On instruction he lowered his face into the water, taking care to avoid a large inspiration or Valsalva manoeuvre. The patient then held this position for 30 seconds, or as long as he/she was able, while the heart rate was recorded continuously. The test was performed in duplicate. The profound bradycardia which ensues is parasympathetically mediated (246). The maximum reduction in heart rate is calculated from the longest RR interval during immersion compared to the mean of the 5 RR intervals preceding immersion.

Heart rate variation with deep breathing (247)

After 10 minutes supine rest the patient took maximum inspiration and expiration at a rate of 6 respiratory cycles per minute. The heart rate was recorded continuously for 1 minute and the maximum variation in heart rate during the last respiratory cycle was noted, calculated from the longest and shortest RR intervals during that cycle. The normal response, indicating parasympathetic tone, is a variation of 15 or more beats per minute (240).

5.2.4. Sympathetic tests

Forearm isometric exercise (248)

After 10 minutes rest in a sitting position, the heart

rate and BP were recorded and blood drawn for plasma noradrenaline assay. The patient then squeezed a modified sphygmomanometer inflated at 60 mmHg to record his maximum handgrip. He then maintained the column of mercury at 30% of his maximum for 2 minutes. Heart rate and BP were recorded from the opposite arm at 1 and 2 minutes, and blood for noradrenaline assay drawn immediately after the procedure. The sympathetically mediated rise in diastolic blood pressure normally exceeds 15 mmHg (240).

Cold pressor test (249)

After 10 minutes seated rest, heart rate and BP were recorded and blood drawn for plasma noradrenaline assay. The patient then inserted his right hand in a bucket of iced water ($0^{o}C$) and maintained this position for 2 minutes. HR and BP were recorded from the opposite arm at 1 and 2 minutes, and blood for noradrenaline assay drawn immediately after the procedure.

Dynamic exercise tests

A modification of the Balke-Ware exercise test was employed (250). The patient was familiarised with a bicycle ergometer (Tuntura, Pugh). Commencing with a workload of 25 watts, the patient cycled upright continuously against a progressively increasing workload, at increments of 25 watts every minute. The test was stopped when the patient achieved a heart rate of 210 minus age in

years, or when symptoms required the patient to stop. Both of these end points were regarded as representing maximal exercise capacity. BP and plasma noradrenaline were measured before and immediately after the exercise and heart rate recorded by ECG at the end of each minute. After a 15 minute rest, the test was repeated, on this occasion the patient cycling for 5 minutes at a workload equal to 50% of the maximum workload achieved (referred to as "timed exercise").

The order of the tests was standing to lying, forearm isometric exercise, Valsalva manoeuvre, diving test, cold pressor test, heart rate variation with deep breathing and finally the 2 dynamic exercise tests. All the tests with the exception of those of dynamic exercise had been previously validated within the Department of Materia Medica (251), and shown capable of detecting subtle differences between groups (252). The graded bicycle exercise test was chosen because of the frailty of some of the cirrhotic patients.

5.2.5. Statistical methods

The results of the tests of parasympathetic function, with the exception of the standing to lying test, were not normally distributed even after a logarithmic transformation and therefore non parametric statistical methods were employed. Comparison between the 3 groups was made using the Kruskall-Wallis one way analysis of variance. The

sympathetic data, which were normally distributed were analysed by repeated measures analysis of variance where more than 2 time points were recorded. Comparison between the 3 groups was by one way analysis of variance. Correlations were obtained by linear regression and general linear modelling using Minitab (200) and Rummage (202) computer programs.

5.3. RESULTS

5.3.1. Liver function tests, plasma noradrenaline and systemic haemodynamics

The liver function tests, blood pressure, heart rate and plasma noradrenaline are recorded in Table 5.1. The bilirubin, albumin and prothrombin time were of course markedly abnormal in the cirrhotics with severe liver damage. The severe cirrhotics had higher plasma noradrenaline concentrations, faster heart rate and lower systolic BP than controls.

5.3.2. Parasympathetic tests

The parasympathetic results are summarised in Table 5.2. and illustrated in Figures 5.1.-5.4. As can be seen from the numbers, not all patients completed all tests. In the standing to lying test, electrical interference with the ECG tracing prevented accurate measurement of the RR intervals in 4 cases, one each in Groups 1 and 2 and 2 in group 3. Eight patients

TABLE 5.1. LIVER FUNCTION TESTS, BLOOD PRESSURE, HEART RATE AND PLASMA NORADRENALINE

Group 1 represents the mild cirrhosis (Pugh's grade A) Group 2 the severe cirrhotics (Pugh's grade B or C) and Group 3 the controls.
Values are mean ± SD and comparison is by one way analysis of variance.

	GROUP 1 (n=10)	GROUP 2 (n=10)	GROUP 3 (n=20)	1 v 2	1 v 3	2 v 3
Bilirubin ($umol.l^{-1}$)	15.2 ± 7.9	45.8 ± 36.2	9.5 ± 3.1	$p < 0.005$	NS	$p < 0.001$
Albumin ($g.l^{-1}$)	38.4 ± 3.3	31.6 ± 5.9	40.1 ± 2.9	$p < 0.005$	NS	$p < 0.001$
Prothrombin time (seconds prolonged)	1.6 ± 1.4	4.8 ± 1.5	0.5 ± 0.8	$p < 0.001$	NS	$p < 0.001$
Systolic BP (mmHg)	127 ± 12	115 ± 12	129 ± 14	NS	NS	$p < 0.05$
Diastolic BP (mmHg)	68 ± 6	67 ± 9	74 ± 8	NS	NS	NS
Heart Rate (min^{-1})	70.3 ± 8.9	77.9 ± 11.8	65.4 ± 8.6	NS	NS	$p < 0.05$
Plasma Noradrenaline ($nmol.l^{-1}$)	3.27± 1.5	4.56± 1.8	2.19± 1.1	NS	NS	$p < 0.001$

TABLE 5.2. PARASYMPATHETIC TESTS

Group 1 represents the mild cirrhotics (Pugh's grade A) Group 2 the severe cirrhotics (Pugh's grade B or C) and Group 3 the controls.
Values are median (range) and comparison is by Kruskall-Wallis one way analysis of variance.

	GROUP 1	GROUP 2	GROUP 3	1 v 2	1 v 3	2 v 3
Standing to lying ratio	1.19 (1.05-1.35)	1.18 (1.08-1.22)	1.17 (1.06-1.31)	NS	NS	NS
Valsalva ratio	1.42 (1.29-2.66)	1.37 (1.17-1.56)	1.70 (1.21-2.40)	NS	NS	$p < 0.05$
Change in heart rate on facial immersion in water (min^{-1})	21.6 (3.5 -36.5)	11.2 (0 -17.0)	18.5 (9 -56.4)	NS	NS	$p < 0.05$
Heart rate variation on deep breathing (min^{-1})	13.2 (5-34)	10.5 (2-18)	15.5 (7-46)	NS	NS	$p < 0.05$

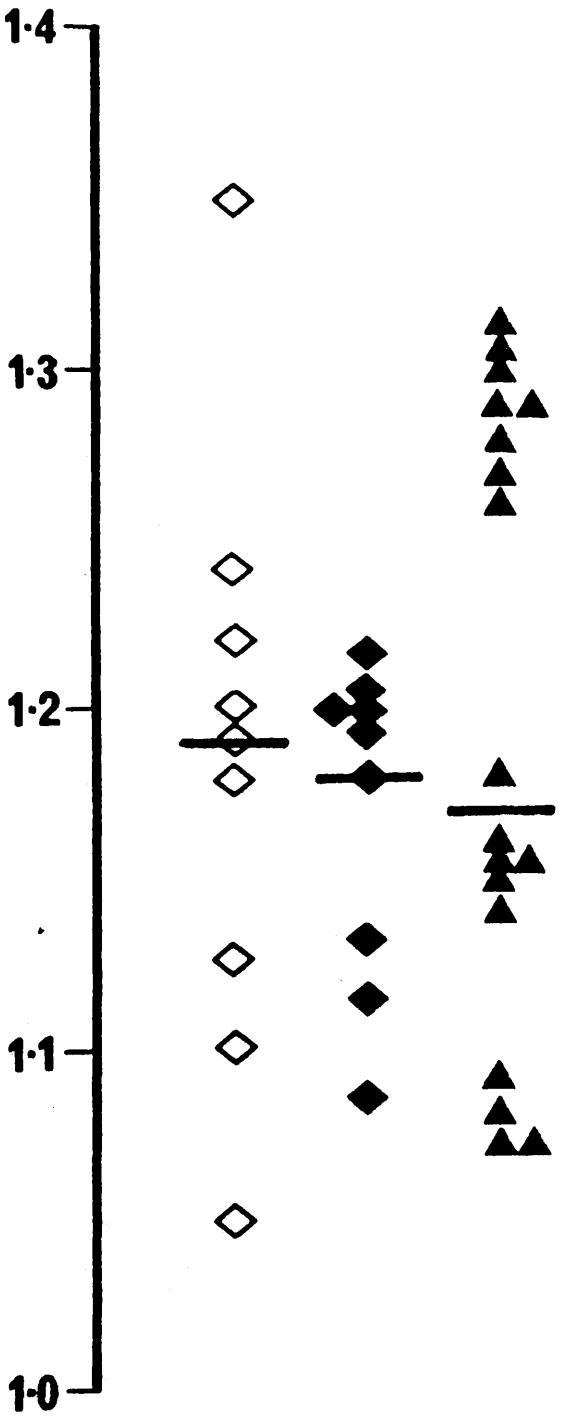

Figure 5.1.

Standing-to-lying ratio in mild cirrhosis (◇), severe cirrhosis (◆) and controls (▲). Horizontal bars represent median values.

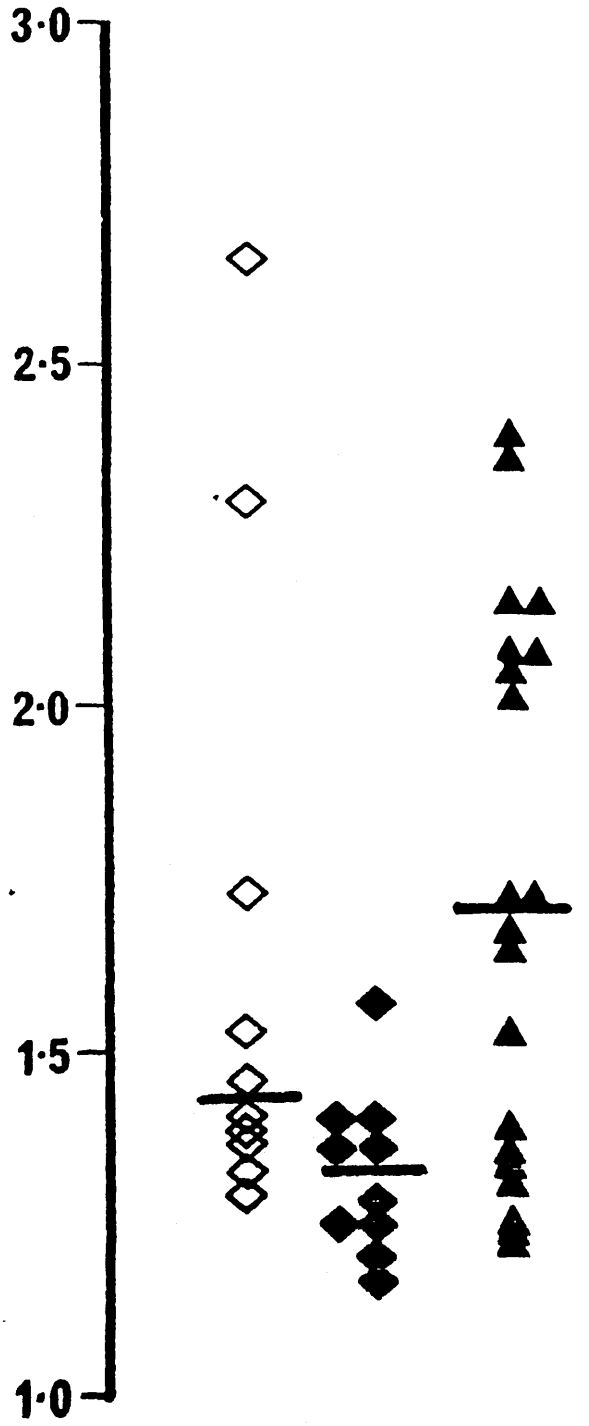

Figure 5.2.

Valsalva Ratio in mild cirrhosis (◇), severe cirrhosis (◆) and controls (▲). Horizontal bars represent median values.

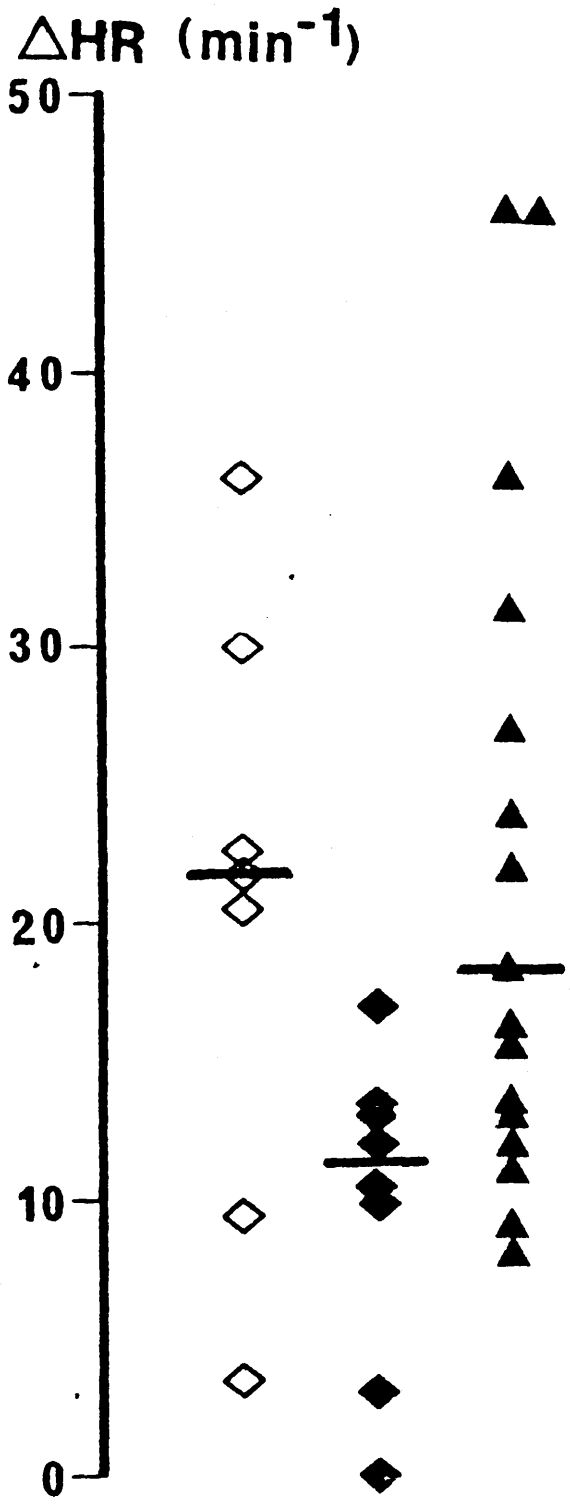

Figure 5.3.

Maximum change in heart rate during facial immersion in water in mild cirrhosis (◇), severe cirrhosis (◆) and controls (▲). Horizontal bars represent median values.

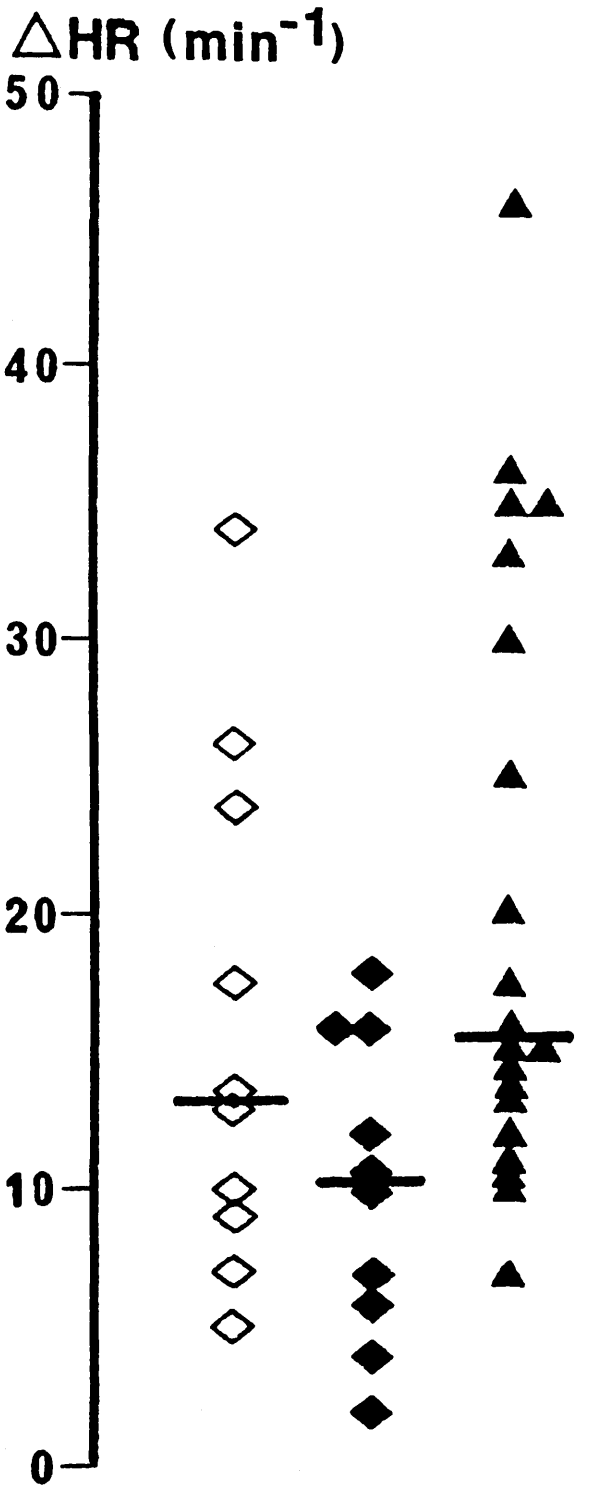

Figure 5.4.

Maximum change in heart rate during deep breathing in mild cirrhosis (◇), severe cirrhosis (◆) and controls (▲). Horizontal bars represent median values.

were unable to carry out the diving test (3 in Group 1, 2 in Group 2 and 3 in Group 3). In each case, they were unable to maintain facial immersion for sufficient time to analyse the HR response. There was no difference in the standing to lying ratio in the 3 groups, significant reduction in the Valsalva ratio, the facial immersion-induced bradycardia and the heart rate variation on deep breathing in the severe cirrhotic group compared to the control group, with no significant differences in the severe v mild cirrhotics, nor in mild cirrhotics v controls. Linear regression analysis and linear modelling revealed no significant correlations between the results of the various parasympathetic tests and recent alcohol consumption, nature or duration of liver disease, diuretic therapy or plasma noradrenaline. The only test affected by baseline haemodynamics was the diving test which was most abnormal in those patients with the lowest systolic BP ($r = 0.54$, $p < 0.01$), but this effect was not independent of liver disease severity. There was a weak correlation between the diving and deep breathing tests and severity of liver disease as indicated by prothrombin time ($r = -0.49$, $p < 0.05$) and serum albumin ($r = 0.54$, $p < 0.01$) respectively.

5.3.3. Sympathetic tests

The results of isometric exercise and the cold pressor tests are given in Tables 5.3. and 5.4. respectively. The

TABLE 5.3. FOREARM ISOMETRIC EXERCISE

Group 1 represents the mild cirrhotics (Pugh's grade A) Group 2 the severe cirrhotics (Pugh's grade B or C) and Group 3 the controls.
Values are mean ± SD. The comparisons listed are the mean differences (with 95% confidence intervals) between the groups. Analysis is by one way analysis of variance.

	GROUP 1	GROUP 2	GROUP 3	1 v 2	1 v 3	2 v 3
Heart Rate (min^{-1})						
Baseline	68.9 ± 8.0	76.2 ± 15.1	66.0 ± 9.2	-	-	-
Δ	10.6 ± 7.1	8.6 ± 4.2	11.5 ± 6.4	2.0 (-4.6,8.6)	-0.9 (-6.6,4.8)	-2.9 (-8.6,2.8)
Systolic BP (mmHg)						
Baseline	133 ± 14	116 ± 19	132 ± 16	-	-	-
Δ	23.8 ± 20.5	14.3 ± 9.3	29.8 ± 12.5	9.5 (0.7,18.3)	-6.0 (-14.8,2.8)	-15.5* (-24.3,-6.7)
Diastolic BP (mmHg)						
Baseline	70 ± 7	64 ± 15	77 ± 12	-	-	-
Δ	15.8 ± 10.7	7.6 ± 5.9	19.9 ± 9.8	8.2 (-1.3,17.7)	-4.1 (-11.7,3.5)	-13.3* (-20.9,-5.7)
Plasma noradrenaline ($nmol.l^{-1}$)						
Baseline	3.2 ± 1.5	5.8 ± 2.7	3.1 ± 1.5	-	-	-
Δ	0.5 ± 1.1	0.2 ± 1.0	0.4 ± 0.8	0.4 (-0.3,1.1)	0.1 (-0.5,0.7)	-0.2 (-0.8,0.4)

* $p < 0.01$.

TABLE 5.4. COLD PRESSOR TEST

Group 1 represents the mild cirrhotics (Pugh's grade A) Group 2 the severe cirrhotics (Pugh's grade B or C) and Group 3 the controls.
Values are mean ± SD. The comparisons listed are the mean differences (with 95% confidence intervals) between the groups. One way analysis of variance reveals no significant differences between the groups.

	GROUP 1	GROUP 2	GROUP 3	1 v 2	1 v 3	2 v 3
Heart Rate (min^{-1})						
Baseline	72.3 ± 11.1	76.9 ± 15.2	67.5 ± 8.8	-	-	-
Δ	7.7 ± 5.8	8.6 ± 7.0	7.3 ± 8.8	-0.9 (-6.2,4.4)	0.4 (-4.2,5.0)	1.3 (-3.3,5.9)
Systolic BP (mmHg)						
Baseline	134 ± 15	123 ± 17	134 ± 12	-	-	-
Δ	22.5 ± 9.6	17.0 ± 11.0	19.2 ± 10.0	5.5 (-7.9,18.9)	3.3 (-8.3,14.9)	-2.2 (-13.8,9.4)
Diastolic BP (mmHg)						
Baseline	75 ± 5	68 ± 13	82 ± 10	-	-	-
Δ	12.2 ± 11.8	9.3 ± 10.6	13.4 ± 10.8	2.9 (-6.1,11.9)	-1.2 (-9.0,6.6)	-4.1 (-11.9,3.7)
Plasma noradrenaline ($nmol.l^{-1}$)						
Baseline	3.9 ± 2.5	6.4 ± 2.6	3.8 ± 1.7	-	-	-
Δ	0.4 ± 0.6	0.8 ± 0.8	0.8 ± 0.9	-0.4 (-1.1,0.4)	-0.4 (-1.1,0.4)	0 (-0.7,0.6)

Maximal and timed dynamic exercise results are given in Tables 5.5. and 5.6. respectively. 4 of the cirrhotics and 4 control subjects were unable to satisfactorily complete the dynamic exercise tests.

The blood pressure rise in response to forearm isometric exercise was markedly reduced in the severe cirrhotics (14/8 mmHg) compared to controls (30/20 mmHg). Although isometric exercise increased the heart rate and marginally raised plasma noradrenaline in all 3 groups, the differences between the groups were not significant. During the cold pressor test the blood pressure, heart rate and plasma noradrenaline increased in all 3 groups, but there were no significant differences between the groups. The pattern of blood pressure response to isometric exercise and the cold pressor test is illustrated in Figure 5.5.

All three groups demonstrated a marked rise in plasma noradrenaline during dynamic exercise. When exercised to their maximum, the control subjects' systolic pressure rose markedly while the diastolic pressure fell slightly. The severe cirrhotics demonstrated a smaller rise in systolic pressure, without any fall in diastolic pressure. Although these differences were not significant, the increase in the pulse pressure reflecting both systolic and diastolic changes was significantly smaller in the severe cirrhotics (15 ± 17 v 37 ± 21 mmHg, $p < 0.05$). This was not apparent in the mild cirrhotic group. The blood pressure response to a period of timed, sub-maximal exercise was similar in

TABLE 5.5. DYNAMIC EXERCISE: TO MAXIMAL EXERCISE CAPACITY

Group 1 represents the mild cirrhotics (Pugh's grade A) Group 2 the severe cirrhotics (Pugh's grade B or C) and Group 3 the controls.
Values are mean ± SD. The comparisons listed are the mean differences (with 95% confidence intervals) between the groups. One way analysis of variance reveals no significant differences between the groups.

	GROUP 1	GROUP 2	GROUP 3	1 v 2	1 v 3	2 v 3
Heart Rate (min^{-1})						
Baseline	77 ± 12	86 ± 20	78 ± 12	-	-	-
Δ	65 ± 21	40 ± 30	60 ± 19	25 (2,48)	5 (-14,24)	-20 (-40,0)
Systolic BP (mmHg)						
Baseline	136 ± 13	129 ± 14	130 ± 13	-	-	-
Δ	28 ± 23	18 ± 14	34 ± 21	10 (-10,20)	-6 (-23,9)	-16 (-34,2)
Diastolic BP (mmHg)						
Baseline	75 ± 6	75 ± 12	88 ± 9	-	-	-
Δ	7 ± 10	4 ± 11	-4 ± 11	3 (-9,15)	11 (1,21)	8 (-3,19)
Plasma noradrenaline ($nmol.l^{-1}$)						
Baseline	6.0 ± 3.4	10.7 ± 4.8	4.3 ± 2.0	-	-	-
Δ	4.9 ± 2.7	5.9 ± 3.7	8.0 ± 5.7	-1.0 (-6.0,4.0)	-3.1 (-7.4,1.2)	-2.1 (-6.4,2.2)

TABLE 5.6. DYNAMIC EXERCISE: 5 MINS AT 50% MAXIMUM WORK LOAD

Group 1 represents the mild cirrhotics (Pugh's grade A) Group 2 the severe cirrhotics (Pugh's grade B or C) and Group 3 the controls.
Values are mean ± SD. The comparisons listed are the mean differences (with 95% confidence intervals) between the groups. Analysis is by one way analysis of variance.

	GROUP 1	GROUP 2	GROUP 3	1 v 2	1 v 3	2 v 3
Heart Rate (min^{-1})						
Baseline	89 ± 10	89 ± 18	86 ± 12	-	-	-
Δ	14 ± 16	19 ± 12	34 ± 12	-5 (-23,13)	-20* (-33,-7)	-15* (-30,0)
Systolic BP (mmHg)						
Baseline	128 ± 18	124 ± 10	122 ± 32	-	-	-
Δ	31 ± 19	37 ± 27	41 ± 41	-6 (-27,15)	-10 (-27,7)	-4 (-23,15)
Diastolic BP (mmHg)						
Baseline	80 ± 9	73 ± 8	84 ± 10	-	-	-
Δ	2 ± 9	1 ± 12	5 ± 12	1 (-10,12)	-3 (-12,6)	-4 (-14,6)
Plasma noradrenaline ($nmol.l^{-1}$)						
Baseline	7.0 ± 4.6	10.6 ± 4.9	4.8 ± 2.0	-	-	-
Δ	3.0 ± 2.6	3.6 ± 3.2	2.9 ± 1.7	-0.6 (-3.0,1.8)	0.1 (-1.9,2.1)	0.7 (-1.3,2.7)

* $p < 0.05$.

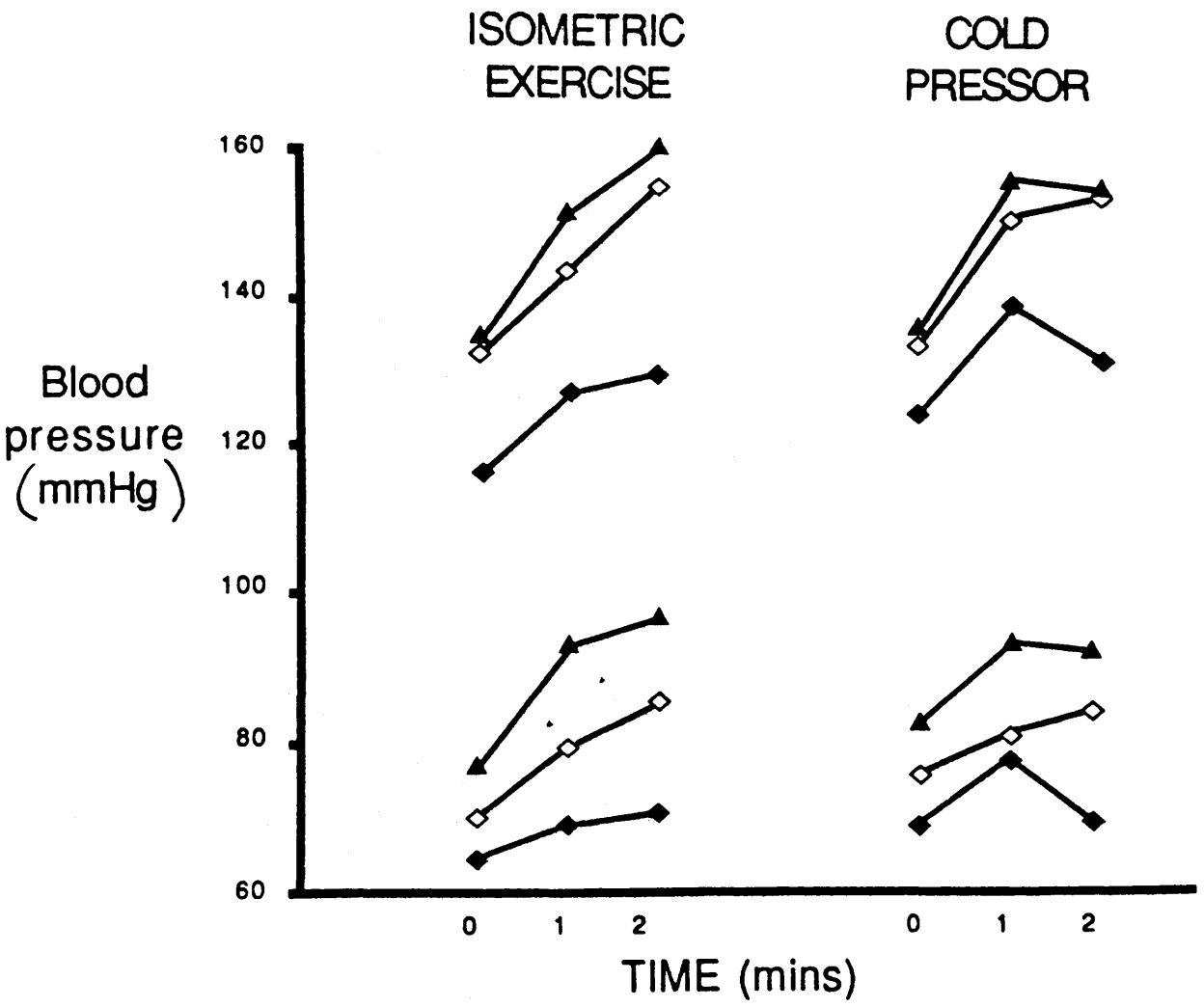

Figure 5.5.

Blood pressure changes during isometric exercise and the cold pressor test in mild cirrhosis (◇), severe cirrhosis (◆) and controls (▲). Upper lines represent systolic BP and lower lines diastolic BP.

all 3 groups with a marked rise in systolic pressure and no significant change in diastolic pressure. These blood pressure changes are illustrated in Figure 5.6.

The increase in heart rate was less pronounced in the cirrhotics than the controls, significantly so during the submaximal exercise. This is illustrated in Figure 5.7.

The cirrhotics with severe liver disease tolerated the bicycle exercise less well than the other 2 groups. Thus the maximal workload achieved in the severe cirrhotic group was limited by symptoms in all cases (usually fatigue) whereas 2 of the mild cirrhotic group and 6 of the control group achieved their target heart rate. The maximal workload achieved by the severe cirrhotics (92 $\pm$ 30 watts) was less than mild cirrhotics (136 $\pm$ 33 watts) or controls (130 $\pm$ 36 watts) and thus the maximal exercise test was of shorter duration in the severe cirrhotic group. These differences mean that the total energy expended during maximal exercise was less in the cirrhotics with severe disease (11.3 $\pm$ 6.6 joules) than those with mild liver disease (26.8 $\pm$ 11.5 joules) or controls (28.9 $\pm$ 18.6 joules), although this difference was not significant. There was a similar, although less marked, trend during the timed exercise.

5.4. DISCUSSION

The cirrhotics with the most severe liver damage consistently show impaired parasympathetic responses. This

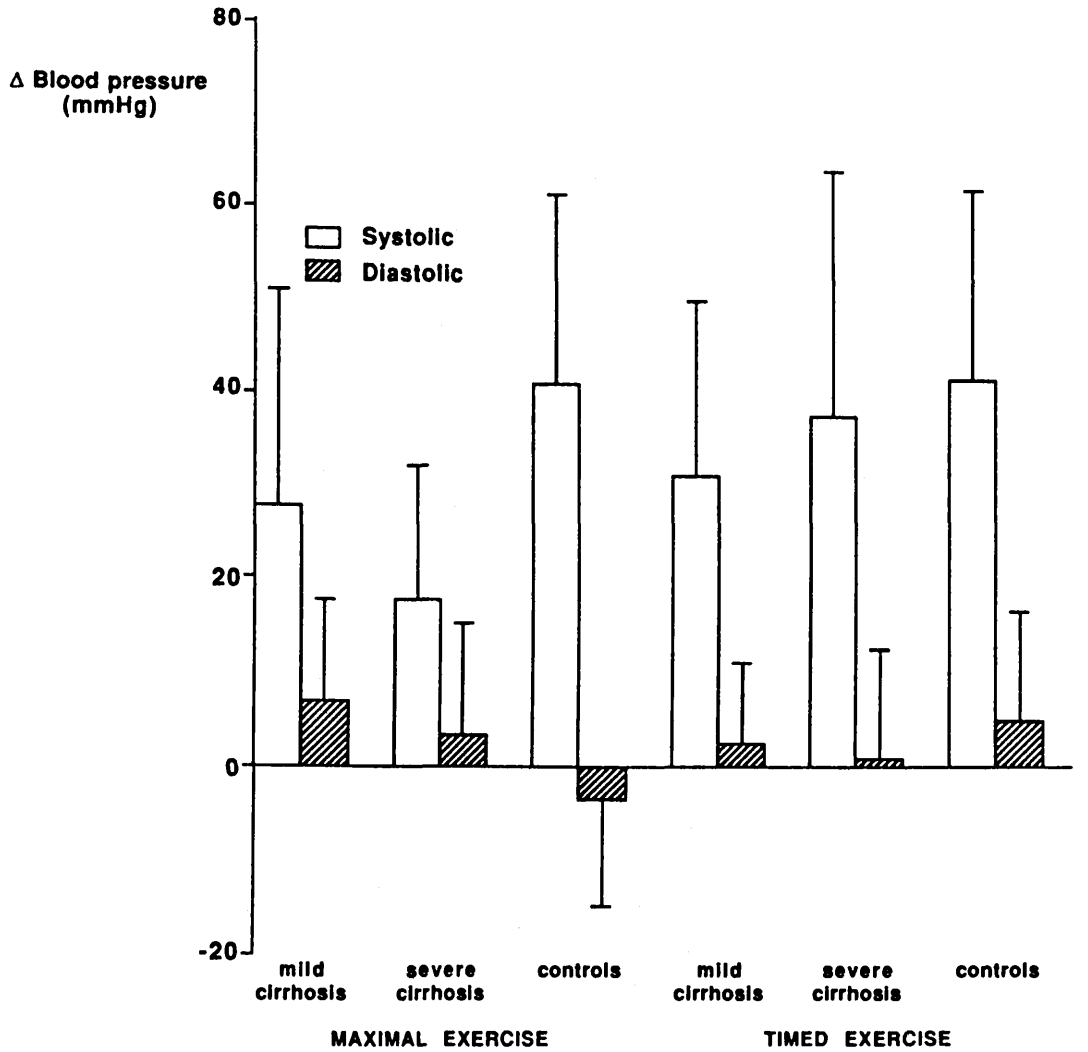

Figure 5.6.

Changes in systolic and diastolic blood pressure in response to maximal and timed dynamic exercise. The bars represent mean values with standard deviations indicated by error bars.

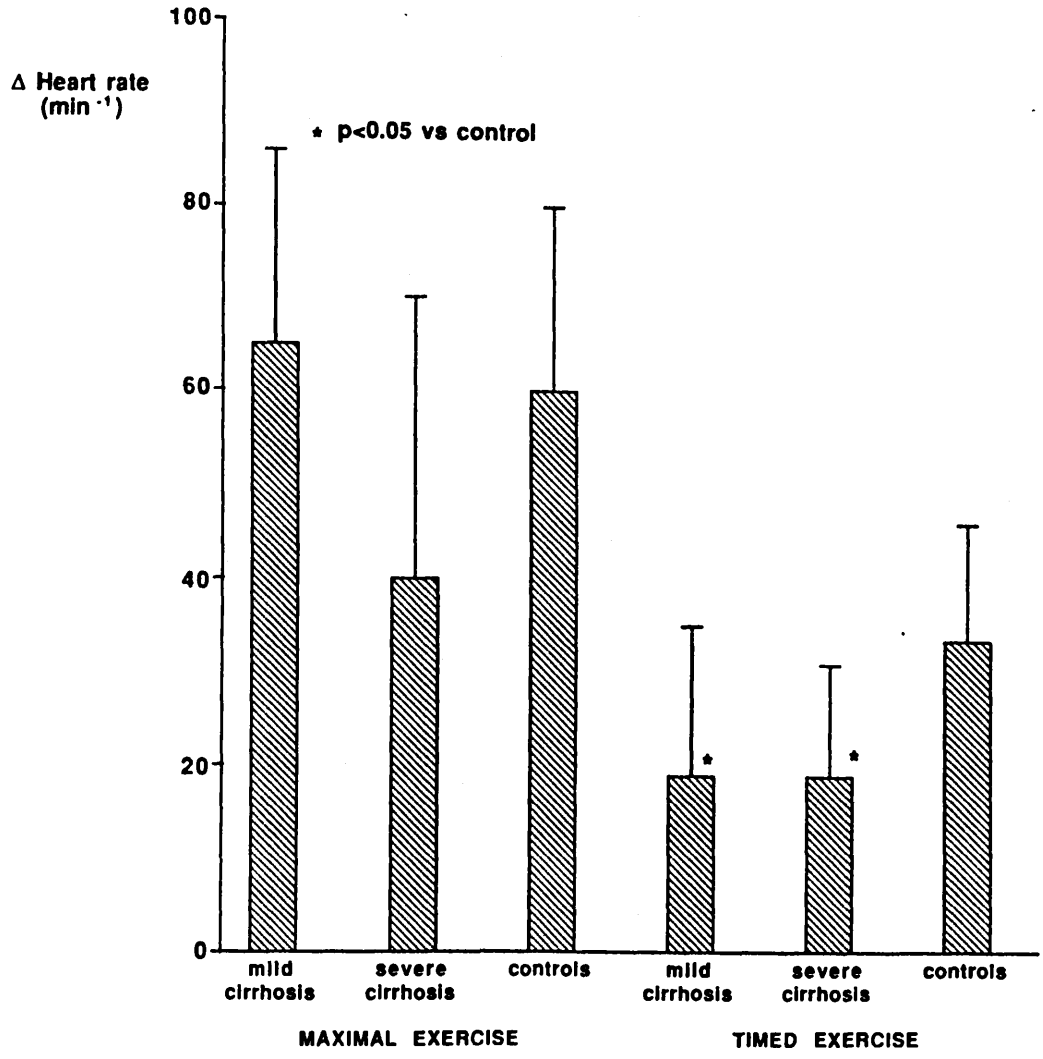

Figure 5.7.

Heart rate increase in response to maximal and timed dynamic exercise. The bars represent mean values with standard deviations indicated by error bars.

appears to be independent of the duration of liver disease and of the underlying aetiology. In particular the responses in alcoholic liver disease were no different from cirrhosis of other origins. In addition, the analysis did not suggest alcohol consumption was a confounding variable, although the alcohol history must be interpreted with caution. Although more of the severe cirrhotic group had taken diuretics within three months, linear regression analysis showed no correlation between the parasympathetic responses and diuretic ingestion. There is no published evidence of a direct effect of diuretic therapy on parasympathetic function. The severe cirrhotics have, as expected, lower systolic blood pressure and faster heart rates than controls. However, there was no evidence that these differences had a confounding effect on the parasympathetic results. Despite the significant differences demonstrated between the groups, the cirrhotic patients did not have marked parasymapthetic failure, the great majority of the results falling within the established "normal range" (240). This is in keeping with the absence of any symptoms of autonomic failure in the cirrhotic group.

There is little published evidence concerning parasympathetic function in cirrhosis. Barter found that autonomic neuropathy was more common in alcoholics with than without liver disease (153). However, it was not clear whether this was simply a reflection of heavier and more prolonged alcohol consumption in the liver disease group,

who were very different from the patients in the present study in that over 90% had clinical evidence of peripheral neuropathy. Vagal autonomic neuropathy is well recognised in chronic alcoholics (152). The present study does however seem to confirm that liver damage is an important contributing factor to the development of abnormal parasympathetic responses. Other evidence of vagal neuropathy comes from Decaux (154) who found that 16 of 25 alcoholic cirrhotics had vagal impairment in contrast to nine patients with non-alcoholic cirrhosis, of whom none had evidence of vagal damage. They demonstrated a correlation between vagal neuropathy and hyponatraemia and proposed that autonomic impairment could explain the impaired free water excretion in ascites by stimulating hypothalamic release of vasopressin (154). Lenz also found an impaired heart rate response to atropine in cirrhotics with hepatic encephalopathy, suggesting a reduced parasympathetic tone (162). These studies in conjunction with the present work, support a minor role for impaired parasympathetic function as a contributory factor to the haemodynamic disturbances of severe cirrhosis.

In respect of the sympathetic tests, there is a striking reduction in the pressor response to isometric exercise in the severe cirrhotic group. Although this could represent a sympathetic autonomic neuropathy, the result is best explained by a reduction in the vascular response to the sympathetic stimulus. The degree of

sympathetic activation was similar in all three groups as indicated by the rise in plasma noradrenaline concentrations. Also, by analogy to the autonomic neuropathy of diabetes, where vagal damage invariably precedes sympathetic damage (240), it seems improbable that a sympathetic neuropathy would develop in the absence of marked parasympathetic failure.

The response to cold pressor stimulation was similar in all 3 groups. This could indicate efferent sympathetic activity (249). Alternatively, the lack of any rise in plasma noradrenaline during the test in any of the groups may indicate that it is an insufficient stimulus of sympathetic activity to detect minor differences between groups. Interestingly, in the severe cirrhotic group, although the initial rise in blood pressure is similar to the other groups, they are less well able to sustain this increase (Figure 5.5.). This effect is similar to that reported by Bernardi (148) who found that in response to head-up tilting, cirrhotics initially showed a normal rise in blood pressure, but that this was not sustained despite maintaining an increased plasma noradrenaline. They interpreted this result as supporting some interference with vascular receptors, a possibility considered further in Chapter 7.

There is some agreement between the findings of the present study and the few published studies which consider sympathetic responses. The response of cirrhotics to

sustained head-up tilt is impaired in terms of both vasoconstriction (150) and blood pressure (148) although Ring-Larsen could not confirm the latter observation (147). Cardiovascular responses to such sympathetic stimuli as application of ice, stressful mental arithmetic and lower body negative pressure are impaired in cirrhosis (151).

What conclusions can be drawn from the bicycle exercise experiments? Firstly, the ability of the sympathetic nervous system to respond to a marked stimulus such as dynamic exercise is unimpaired even in severe cirrhosis where sympathetic activity is already increased. This was apparent even in those cirrhotics too ill to exercise to any great extent. Secondly, the blood pressure response to exercise is largely intact. The only difference demonstrated in cirrhosis is a less marked increase in pulse pressure during maximal exercise. This could reflect the peripheral vasodilatation of the ill cirrhotics, with limited ability to increase this further during exercise. The published evidence is divided as to whether the vasodilatation of cirrhosis does (24) or does not (150) affect skeletal muscle as well as skin. The apparent failure of the cirrhotic group to increase their heart rate during exercise to the same extent as the control group could indicate some impairment of beta-adrenoceptor-mediated sympathetic responses, a possibility considered further in Chapters 6 and 7.

Two previous studies have explored the response to

dynamic exercise in cirrhosis. Bayley found normal or supranormal increases in cardiac output during exercise (145). Ratge exercised 30 patients with alcoholic liver disease using a bicycle ergometer (146). He found that the increases in plasma noradrenaline and heart rate were similar to control values. This contrasts with the possible impairment of heart rate response demonstrated in the present study. These studies suggest a relatively normal response to dynamic exercise. The extent of the sympathetic stimulation during dynamic exercise may be sufficient to overcome any competitive interference with the sympathetic response, e.g. by vasoactive amines.

In summary, parasympathetic responses are mildly impaired in severe cirrhosis, independently of the nature of the liver damage, alcohol consumption and diuretic intake. Evidence from other studies suggests a possible, although minor, role for this reduced parasympathetic activity in the haemodynamic disturbance of cirrhosis, and also a possible role in permitting excessive antidiuretic hormone secretion in ascites. Sympathetic responses to dynamic exercise are largely intact, but to isometric exercise are clearly impaired. This may reflect abnormal vascular responsiveness rather than neuropathy itself. This question is explored in the next chapter.

CHAPTER 6

CARDIOVASCULAR REACTIVITY IN CIRRHOSIS

6.1. INTRODUCTION

The preceding chapters have demonstrated that there is a global increase in sympathetic activity in cirrhosis. Despite this, most patients with cirrhosis have reduced peripheral vascular resistance (141) as indicated by low systemic blood pressure despite increased cardiac output (23). This is in keeping with the clinical appearance of these patients whose palmar erythema, warm extremities, capillary pulsations and cutaneous spider naevi suggest cutaneous vasodilatation (24). The fact that the increased sympathetic activity, whatever its explanation, fails to correct the apparent reduction in peripheral vascular resistance suggests some interference with the normal adrenoceptor-mediated control of vascular tone. The reduced pressor response to such sympathetic stimuli as forearm isometric exercise demonstrated in the previous chapter is in keeping with this proposal.

Previous attempts to address this problem by assessing neurovascular reactivity in response to various pressor agents have yielded conflicting results (144,151,160,162,163) probably because of variability of the methods employed. A method to assess the cardiovascular response to vasoactive agents has been developed and validated within the Department of Materia Medica at Glasgow University (253) which employs construction of dose-response curves by infusing a range of doses of the agents. This chapter describes the first application of this

technique, which has been widely used in clinical pharmacology, to patients with cirrhosis. The aim of both studies was to localise the defect responsible for the impaired sympathetic control of vascular tone.

6.2. VASCULAR REACTIVITY TO NORADRENALINE AND ANGIOTENSIN II (STUDY A)

6.2.1. Methods

Patients

Twenty patients with cirrhosis (histological proof in 14, clinical diagnosis in 6 - see Chapter 2 for clinical definition of cirrhosis) were subdivided into "compensated" (Pugh's Grade A; Group 1, n=10) and "decompensated" (Pugh's Grade B or C, Group 2, n=10). The demographic details including diuretic, smoking and alcohol ingestion are listed in Table 6.1. and the nature and duration of liver disease in Table 6.2. Two patients had ascites and four encephalopathy (all Grade 1-2). No patient had gastrointestinal bleeding within 1 month of study. These were compared to an equal number of control subjects with non hepatic disorders (see Chapter 2), whose demographic details are also listed in Table 6.1. Each cirrhotic patient was matched with a control subject of the same sex and age (within 5 years) and wherever possible also matched for alcohol and tobacco consumption.

TABLE 6.1.

STUDY A: DEMOGRAPHIC DETAILS

	CIRRHOSIS		CONTROLS
	GROUP 1 (Pugh's A)	GROUP 2 (Pugh's B/C)	GROUP 3
Number	10	10	20
Mean age (years)	55.7	58.3	56.0
(Range)	(33-65)	(43-77)	(41-75)
Sex (M:F)	8:2	6:4	14:6
Smokers	1	4	8
Recent alcohol ingestion*	2	4	9
Recent diuretic therapy+	1	7	0

* refers to preceding 3 months, details of daily consumption as follows: Group 1: 0-10g (1), 20 g (1); Group 2: 0-10 g (1), 30 g (1), 200 g (2, both of whom had had no alcohol for 4 weeks); Group 3: 0-10 g (7), 20 g (1), 50 g (1).

+ during preceding 3 months, no subject received diuretics within 72 hours of study.

TABLE 6.2.

Study A: NATURE AND DURATION OF LIVER DISEASE

	CIRRHOSIS GROUP 1 (Pugh's A)	CIRRHOSIS GROUP 2 (Pugh's B/C)	Total
Diagnosis			
Alcoholic liver disease	5	7	12
Chronic active hepatitis	2	1	3
Primary biliary cirrhosis	1	1	2
Haemochromatosis	1	0	1
Cryptogenic cirrhosis	1	1	2
	10	10	20
Duration			
Mean duration (months)	31	40	
(range)	(6-96)	(1-120)	

Study Design

The subjects abstained from alcohol for 24 hours and from caffeine-containing beverages and smoking for 10 hours preceding the study. All medication was omitted on the study day, diuretics having been stopped 72 hours previously. Thirty minutes following a light breakfast an intravenous cannula was inserted into an antecubital vein and flushed with 1 ml heparinised saline. After 30 minutes supine rest, blood was taken from the cannula for plasma catecholamines, plasma renin activity, liver function tests and prothrombin time. Blood pressure and heart rate were then measured by semi-automatic sphygmomanometer (Sentron, Bard) every 2 minutes for 20 minutes. The subject was regarded as being at steady state when the systolic blood pressure varied by less than 5 mmHg on 5 consecutive readings. The values were then averaged to obtain baseline heart rate and blood pressure.

The blood pressure responses to progressively increasing infusion rates of noradrenaline were then determined. 1:1000 noradrenaline tartrate solution containing 2 $mg.ml^{-1}$ sodium metabisulphide (Levophed, Winthrop) was diluted in 0.9% sodium chloride solution to a final concentration of 0.1 ug kg^{-1} subject's weight ml^{-1}, immediately prior to infusion. This solution was then infused at increasing rates ranging from 0.1 to 3 ml min^{-1} (0.01 to 0.3 ug noradrenaline $kg^{-1}min^{-1}$) using a Braun Perfusor 4 infusion pump. The total volume of solution

infused did not exceed 30 ml per subject. At least four dose levels were investigated in each subject. Blood pressure and heart rate were recorded at 1 minute intervals for 10 minutes at each infusion rate. The average blood pressure for the last 5 minutes of the infusion by which time steady state had been reached (254), were calculated. The infusion was stopped if the blood pressure rose by more than 45 mmHg systolic or 30 mmHg diastolic above pre-infusion values or if the subject experienced unpleasant symptoms, e.g. headache.

After a 1 hour drug-free period, the above procedure was repeated substituting angiotensin II for noradrenaline. Angiotensin II (Hypertensin, Ciba-Geigy) was diluted in 0.9% sodium chloride solution to a final concentration of 10 ng kg^{-1} subject's weight $ml.^{-1}$. This solution was infused at increasing rates (a minimum of 4) varying from 0.025 to 6 ml min^{-1} (2.5 to 60 $ng.kg^{-1}min^{-1}$). The total volume of solution infused did not exceed 60 ml. The same procedure for recording blood pressure and heart rate was followed. A trained nurse and a doctor (the author) were in attendance throughout the study period.

The average rise in systolic, diastolic and mean arterial blood presure was calculated for each dose of noradrenaline and angiotensin II, and the rise in pressure plotted against the log of the dose (noradrenaline: $\log_e$ of dose in ng kg^{-1} min^{-1}, angiotensin II: $\log_e$ of dose in ng $kg^{-1}min^{-1}$ x 10). A dose response curve for each subject

was constructed using a quadratic fit. This was based on the assumption that in man one is likely to be exploring the lower end of a sigmoid dose response curve, whose shape resembles one limb of a parabola, which is the shape of curve constructed using a quadratic fit (see Figure 5.1.) (253). Other mathematical models to construct dose response curves to pressor agents in man have been proposed, including a linear fit of the dose of agent against the blood pressure and a linear fit of the log of the dose against the log of the blood pressure (255). It was found that in the case of noradrenaline in all subjects the quadratic fit was mathematically more closely correlated to the values than either of these options (by comparing correlation coefficients derived from least squares regression analysis). In the case of angiotensin II, the quadratic fit gave a correlation at least as good as the alternatives in the majority of cases and therefore this fit was adopted for analysis.

From these curves the doses of noradrenaline and angiotensin II (and their logarithmic transformations) required to raise blood pressure by 10 and 20 mmHg were calculated in each individual (referred to as PD_{10} and PD_{20} respectively). The log PD_{10} and PD_{20} values were compared between the 3 groups, by one way analysis of variance and correlations were derived from linear regression analysis.

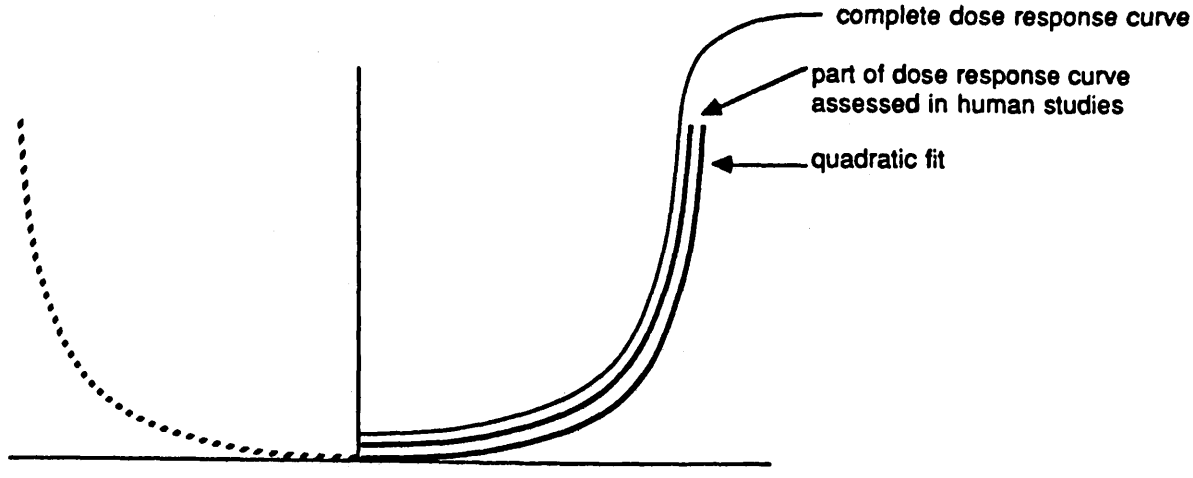

Figure 6.1.

Rationale for a quadratic fit for human dose response curves.

6.2.2. Results

The results of the biochemical tests of liver function, plasma catecholamine levels and plasma renin activity are summarised in Table 6.3. and the baseline haemodynamic data similarly in Table 6.4. Plasma noradrenaline and plasma dihydroxyphenylethylene glycol (DHPG), were significantly elevated in the severe cirrhotic group, reflecting enhanced sympathetic activity (see Chapter 3). Heart rate was significantly higher in the cirrhotics with severe disease and there was a reduction in systolic pressure in the severe cirrhotics, significant at the 5% level in comparison to mild cirrhosis and showing a clear trend (as indicated by the confidence intervals) in comparison to controls. Diastolic pressure did not differ significantly between the 3 groups.

The systolic blood pressure responses to noradrenaline are listed in Table 6.5. and the mean arterial pressure responses to angiotensin in Table 6.6. (Noradrenaline raises systolic but not diastolic pressure, whereas angiotensin raises both systolic and diastolic pressures, hence the decision to examine mean pressure responses to angiotensin).

The severe cirrhotic group demonstrate impaired pressor responses to infused noradrenaline, as demonstrated by the significant increase in log PD_{10} and log PD_{20}. The values in the cirrhotics with mild disease are similar to those of controls, indicating no impairment of pressor response in this group.

TABLE 6.3. STUDY A: LIVER FUNCTION TESTS, PLASMA CATECHOLAMINES AND PLASMA RENIN ACTIVITY

Group 1 = mild cirrhosis, Group 2 = severe cirrhosis, Group 3 = controls.
Group values are median (range). Comparisons are represented by the mean difference and 95% confidence intervals (calculated as a log transformation and re-converted).
Analysis is by Kruskall-Wallis one way analysis of variance.
(NA = noradrenaline; PRA = plasma renin activity)

	GROUP 1	GROUP 2	GROUP 3	1 v 2	1 v 3	2 v 3
Bilirubin ($\mu mol.l^{-1}$)	14 (4-30)	41 (17-600)	8 (5-17)	-3.6 ** (-5.7,-1.5)	1.7 (-0.2,3.6)	6.1 *** (4.2,8.0)
Albumin ($g.l^{-1}$)	39 (35-43)	33.5 (22-41	40 (36-46)	1.3 ** (0.1,2.4)	-0.2 (-1.0,0.7)	-1.9 ** (-3.4,-0.4)
Prothrombin Time (secs prolonged)	1 (0-5)	5 (2-15)	0 (0-3)	- **	-	- **
Plasma NA ($nmol.l^{-1}$)	2.8 (0.9-4.3)	5.4 (3.3-10.4)	2.85 (1.2-4.6)	-2.2 ** (-3.8,-0.7)	-1.0 (-2.5,0.4)	2.1 ** (0.7,3.6)
Plasma DHPG ($nmol.l^{-1}$)	10.5 (8.3-14.2)	18.4 (9.4-31.5)	9.7 (5.0-17.2)	-1.7 * (-3.1,-0.3)	0.4 (-1.0,1.8)	2.0 ** (0.3,3.7)
PRA ($ngAIml^{-1}hr^{-1}$)	3.4 (0.4-8.5)	4.1 (1.2-410.4)	2.6 (0.4-13.4)	-4.2 (-9.8,1.4)	1.3 (-2.8,4.6)	5.8 ** (0.5,10.1)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

TABLE 6.4. STUDY A: BASELINE HEART RATE AND BLOOD PRESSURE

Study A. Summary of baseline haemodynamic data in mild cirrhosis (Group 1, n=10), severe cirrhosis (Group 2, n=10) and controls (Group 3, n=20).
Group values are mean (SD). Comparisons are represented by mean differences (95% confidence intervals). Analysis is by one way analysis of variance.

	GROUP 1	GROUP 2	GROUP 3	1 v 2	1 v 3	2 v 3
Heart Rate (min^{-1})	67 ± 9	83 ± 7	65 ± 10	16 * (6,26)	-2 (-11,7)	-17 * (-26,-8)
Systolic BP (mmHg)	131 ± 18	115 ± 17	126 ± 15	-16 * (-31,-1)	-5 (-18,8)	11 (-2,24)
Diastolic BP (mmHg)	73 ± 7	69 ± 10	73 ± 10	-4 (-12,4)	0 (-7,7)	4 (-3,11)

* $p < 0.05$

TABLE 6.5. SYSTOLIC BLOOD PRESSURE RESPONSES TO NORADRENALINE

Group 1 = mild cirrhosis, Group 2 = severe cirrhosis, Group 3 = controls. Group values are mean ± SD and comparisons between groups are mean difference (95% confidence intervals). Comparison is by one way analysis of variance on the log values.

	GROUP 1	GROUP 2	GROUP 3	1 v 2	1 v 3	2 v 3
PD_{10} ($ngkg^{-1}min^{-1}$)	43	66	46	23 (3,43)	3 (-14,20)	-20 (-37,-3)
Log PD_{10}	3.67± 0.43	4.19± 0.32	3.76± 0.43	0.52 * (0.11,0.93)	0.09 (-0.27,0.45)	-0.43 * (-0.79,-0.07)
PD_{20} ($ngkg^{-1}min^{-1}$)	72	127	84	55 (17,93)	12 (-21,45)	-43 (-76,-10)
Log PD_{20}	4.18± 0.49	4.78± 0.36	4.36± 0.37	0.66 ** (0.21,0.99)	0.18 (-0.16,0.52)	-0.42 ** (-0.76,-0.08)

* $p < 0.02$; ** $p < 0.01$

TABLE 6.6. MEAN ARTERIAL PRESSURE RESPONSES TO ANGIOTENSIN II

Group 1 = mild cirrhosis, Group 2 = severe cirrhosis, Group 3 = controls. Group values are mean ± SD and comparisons between groups are mean difference (95% confidence intervals) Comparison is by one way analysis of variance on the log values.

	GROUP 1	GROUP 2	GROUP 3	1 v 2	1 v 3	2 v 3
PD_{10} ($ngkg^{-1}min^{-1}$)	5.2	15.5	3.9	10.3 (0.6,20.0)	-1.2 (-9.8,7.3)	-11.5 (-20.1,-3.0)
Log PD_{10}	1.46± 0.59	2.17± 1.05	1.19± 0.64	0.71 (-0.04,1.46)	-0.27 (-0.93,0.39)	-0.98 * (-1.64,-0.38)
PD_{20} ($ngkg^{-1}min^{1}$)	12.7	9.1	26.8	-3.6 (3.4,50.2)	-30.4 (-24.2,17.0)	(-51.0,-9.8)
Log PD_{20}	2.37± 0.62	3.14± 1.06	1.97± 0.74	0.77 (0.05,1.59)	-0.40 (-1.13,0.33)	-1.17 * (-1.90,-0.44)

* $p < 0.01$

There was an impaired pressor response to the infusion of angiotensin II in the severe cirrhotic group. The log PD_{10} and log PD_{20} values were significantly higher in this group compared to controls. The magnitude of the change was rather greater than that seen in the noradrenaline infusions. It should also be noted that, although not statistically significant, the PD_{20} and particularly PD_{10} values in the cirrhotics with mild disease were higher than the control values. Two of the mild cirrhotics had obviously impaired pressor responses, despite being indistinguishable from the rest of their group in terms of baseline haemodynamic data or catecholamine values.

The dose response curves of a representative subject from the cirrhotic group with severe disease and those of her paired control subject are illustrated in Figures 6.2. (noradrenaline) and 6.3. (angiotensin II). For both agents the response curve for the cirrhotic lies to the right of that of the control, indicating decreased vascular reactivity in the cirrhotic subject. The mean responses in the 3 groups are illustrated in Figure 6.4. (noradrenaline) and Figure 6.5. (angiotensin II). The standard deviation bars are omitted for clarity. Again the shift to the right in the severe cirrhotics, indicating decreased vascular reactivity in this group, is demonstrated for both noradrenaline and angiotensin.

There was a weak but significant correlation between the degree of hepatic impairment, as judged by the

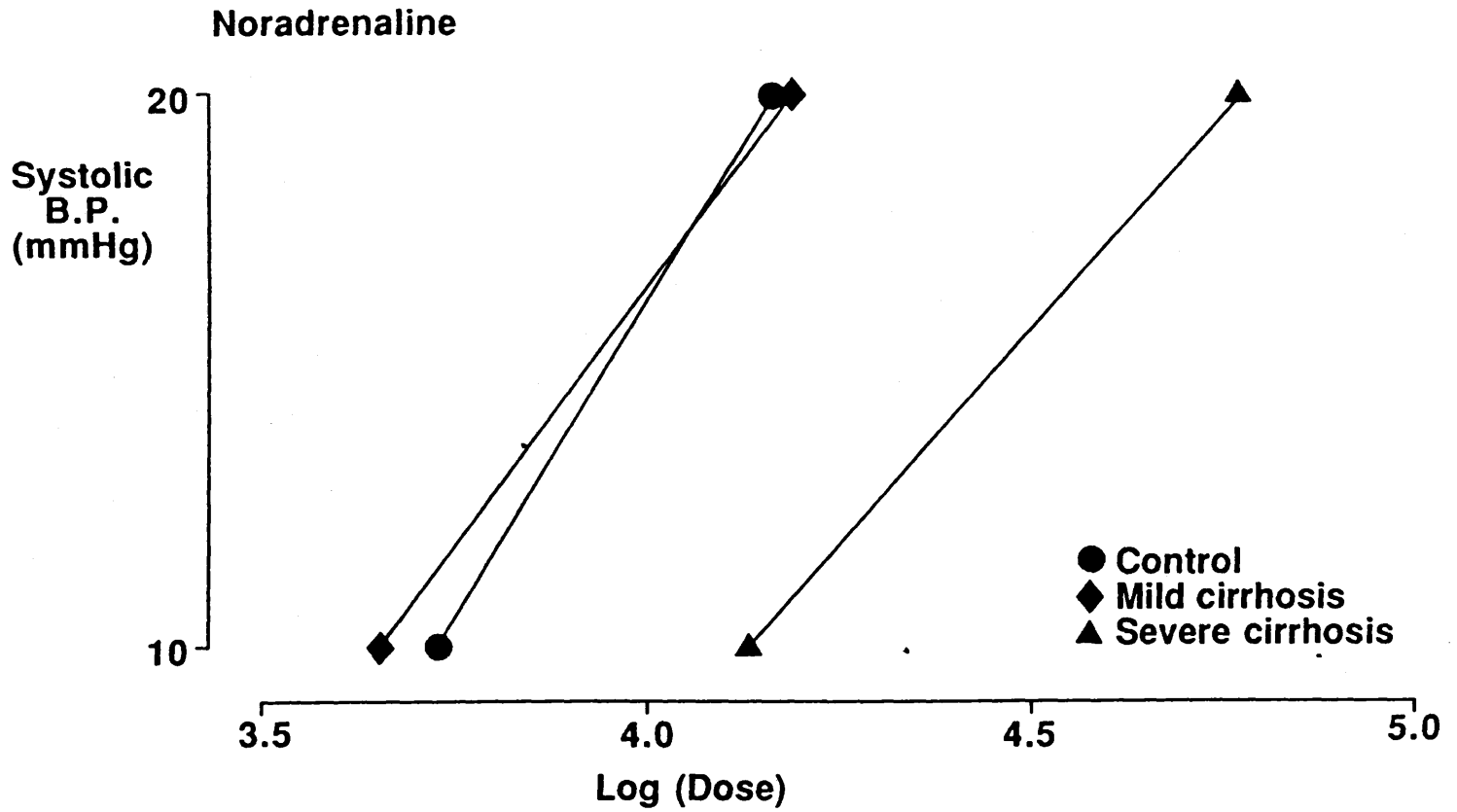

Figure 6.2.

Increase in systolic blood pressure in response to noradrenaline, constructed from the means of the individual log PD_{10} and log PD_{20} values for each group.

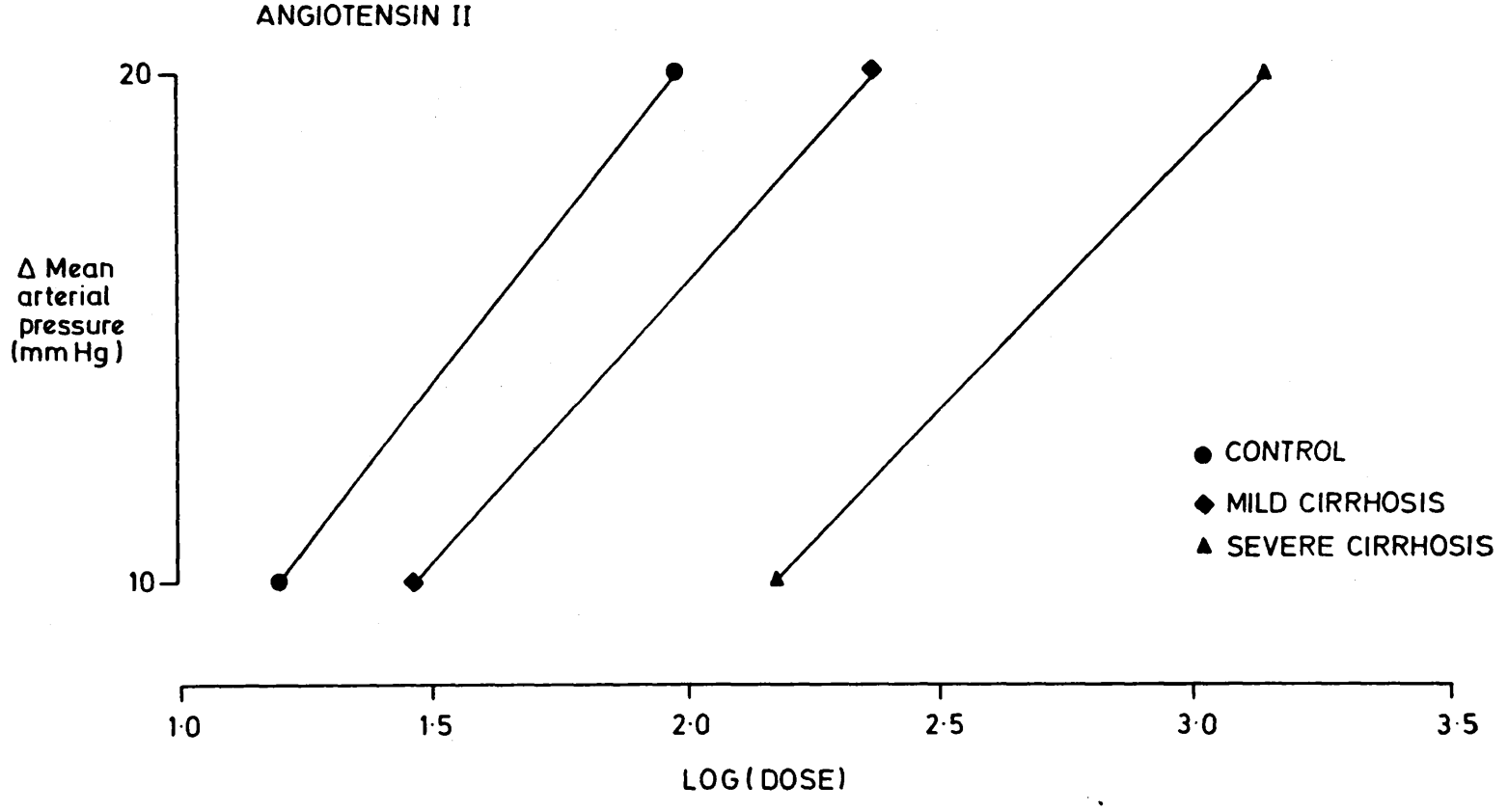

Figure 6.3.

Increase in mean arterial pressure in response to angiotensin II, constructed from the means of the individual log PD_{10} and log PD_{20} values for each group.

negative correlation between baseline systolic blood pressure and PD_{20} for noradrenaline ($r = -0.50$, $p < 0.025$) and between baseline diastolic blood pressure and PD_{20} for angiotensin ($r = -0.46$; $p < 0.05$). Thus the lower the starting blood pressure, the greater the impairment of adrenergic reactivity. The pressor responses to the 2 agents were themselves significantly correlated ($r = 0.56$. $p < 0.02$). Stepwise linear regression of these various factors failed to identify any one as being independently related to the pressor responses. Interestingly, there was no correlation between the pressor responses to noradrenaline and resting plasma noradrenaline concentration ($r = 0.14$).

6.2.3. Discussion

This study has demonstrated that there is reduced peripheral vascular reactivity to both noradrenaline, an adrenoceptor mediated vasoconstrictor, and to angiotensin II, a non-adrenergic vasoconstrictor, in patients with cirrhosis. In the case of noradrenaline, this is only apparent in those individuals with severe hepatic impairment, but in the case of angiotensin, reduced vascular responsiveness has also been demonstrated in some patients with apparently mild disease. This is the first study in man to find reduced reactivity to both agents.

The few previous studies which have examined the effects of these agents on the systemic vasculature have

yielded conflicting results. Mashford gave bolus injections of noradrenaline to cirrhotics, some of whom were stable, and some in liver failure (144). The resulting increase in blood pressure was greater in the more ill patients, in contrast to the present study. However, the numbers studied were few, there were no control data and the authors themselves drew no conclusions. Laragh and his co-workers (160,163) gave prolonged infusions (up to 6 days) of both noradrenaline and angiotensin to a group of cirrhotics. The doses employed were variable but in general higher than in the present study. They found pressor sensitivity to angiotensin to be diminished in their cirrhotic patients. There was a suggestion of reduced pressor sensitivity to noradrenaline, but this was not significant. In a controlled study of 21 cirrhotic patients, Lunzer demonstrated convincing evidence of a reduction in peripheral vascular responsiveness to noradrenaline, when intravenous noradrenaline (10 ug min^{-1}) reduced forearm blood flow in controls but not in cirrhotics (151). However, angiotensin II (2 ug min^{-1}) failed to alter limb blood flow in either controls or cirrhotics. More recently Lenz reported that infusions of noradrenaline (0.4 ug $kg^{-1}min^{-1}$) and angiotensin (20 ng $kg^{-1}min^{-1}$) yielded similar rises in blood pressure in patients with cirrhosis and hepatic encephalopathy and in control subjects (162).

The present study has the advantage of employing a range of doses of both agents to allow construction of a

dose response curve, rather than relying on a single bolus response. With this exception, it is difficult to explain the conflicting results between the present study and that of Lenz. The lack of effect of angiotensin on forearm blood flow in the study of Lunzer (151) may reflect insufficient sensitivy of the technique, since no effect was seen in either control or cirrhotic patients. The earlier studies described differ too greatly in methodology to allow direct comparison with the present study.

Data from 2 other sources should be considered, namely the results of exogenous infusions of such agents in experimental animals, and attempts to measure the effect of modulating endogenous catecholamines. Animal studies have yielded uniform results with respect to angiotensin. Resistance to the pressor effect of exogenous angiotensin II has been observed in the bile duct ligated dog (158) and in rats with carbon tetrachloride-induced cirrhosis (155-157). Regarding noradrenaline infusions, Finberg found only alishgly reduced pressor response in the bile-duct ligated dog (158) and Murray found no such reduction in the rat model (155). However two other groups have recently reported reduced pressor responses to noradrenaline using the same rat model (156,157). Assessing adrenergic responses by stimulating the sympathetic nervous system is a more physiological, though less readily controlled approach than exogenous infusions. The blood pressure response to isometric exercise was clearly blunted in severe cirrhosis

(Chapter 5). The suggestion of decreased peripheral adrenergic responsiveness is supported by the work of Lunzer on forearm blood flow. He demonstrated less peripheral vasoconstriction in cirrhotics than controls in response to head-up tilting (150) and to sympathetic stimulation by application of ice and lower body negative pressure (151). Two other groups have examined the effect of posture on blood pressure in cirrhosis, with conflicting results. Eight cirrhotics were unable to sustain the normal rise in blood pressure on prolonged standing, despite raising their plasma noradrenaline levels by a similar amount as control subjects, suggesting an inadequate vascular response to adrenergic stimulation (148). The same group subsequently confirmed these findings in a larger series (149). However, Ring-Larsen, measuring intra-arterial pressure in response to 60^{o} head-up tilt, found no postural change either in controls or cirrhotics (147). They interpreted the lack of any fall in blood pressure as evidence of normal neurovascular reactivity.

Potential confounding variables in the present study include diuretic therapy, alcohol and smoking. Although the 72 hour diuretic-free period will have allowed washout of the drug itself, the effects of certain diuretics (e.g. thiazides) on the vascular tree may persist for months (256) and spironolactone, the principal diuretic employed in these patients, is reported to reduce vascular reactivity to noradrenaline (257,258). This is a difficult problem to

resolve, since it was deemed ethically unacceptable to stop diuretic therapy for longer than 72 hours in these ill patients. It is worth noting, however, that within the group of severe cirrhotics (in whom the diuretic question arises) the vascular reactivity is similar in the 7 who had taken diuretics (log PD_{20} 4.79 ± 0.4) and the 3 who had not (log PD_{20} 4.75 ± 0.3). Ingestion of alcohol for 4 days is known to reduce vascular reactivity to noradrenaline, but not angiotensin (259). The long term effects are unknown. However, only 2 of the cirrhotics in this study had been drinking heavily within 3 months, and in both instances had abstained for 1 month prior to study, having been hospitalised during this period. Smoking, also known to reduce vascular reactivity was taken into account during the matching of cirrhotic-control pairs.

In view of the above, it seems likely that vascular reactivity to both noradrenaline and angiotensin II is indeed impaired in cirrhosis with severe liver impairment. This suggests an abnormality of vascular smooth muscle causing a generalised decrease in vascular reactivity. However "desensitisation" of the vasculature to the increased sympathetic activity would be an alternative explanation, if in addition there was a parallel but separate angiotensin desensitisation. To further complicate the issue it has recently been suggested that the two systems are not entirely independent in man (260) and therefore the possibility that overactivity of one system

influences sensitivity to the other ("heterologous desensitisation") must be borne in mind.

The next section explores the question of sympathetic desensitisation in more detail by examining cardiovascular responsiveness to selective adrenoceptor agonists.

6.3. CARDIOVASCULAR RESPONSIVENESS TO SELECTIVE ADRENOCEPTOR AGONISTS (STUDY B)

6.3.1. Introduction

Having demonstrated in the previous study (6.2.) that cirrhotic patients with severe, but not mild, impairment of hepatic function have decreased vascular reactivity to both sympathetic and non sympathetic stimulation, the next experiment was designed to assess whether the decreased response was limited to the peripheral vasculature, or was a reflection of generalised sympathetic desensitisation. To achieve this we selected 10 cirrhotic patients with severe liver disease and infused a series of adrenoceptor agonists with different affinities for the alpha and beta subtypes - phenylephrine (selective $alpha_1$ agonist), alphamethylnoradrenaline (preferential $alpha_2$ agonist) and isoprenaline ($beta_1$ and $beta_2$ agonist).

6.3.2. Methods

Patients

Ten "decompensated" cirrhotic patients (Pugh's grade B

TABLE 6.7. STUDY B: DEMOGRAPHIC DETAILS

	CIRRHOSIS (Pugh's B/C)	CONTROLS
Number	10	10
Mean age (years)	56.5	57.7
Sex (M:F)	7:3	7:3
Smokers	5	4
Recent alcohol ingestion*	2	6
Recent diuretic therapy+	6	0

* refers to preceding 3 months, details of daily consumption as follows: cirrhotics: 50 g (1), 150 g (1, who had had no alcohol for 8 weeks prior to study); controls: < 10 g (3), 10-15 g (3).

+ during preceding 3 months, no subject received diuretics within 72 hours of study.

or C) and ten control patients from similar sources to those outlined in 6.2., were studied. The cirrhosis was confirmed histologically in 4 cases and diagnosed on clinical grounds in the remaining 6. The aetiology of the cirrhosis was alcoholic liver disease in 7 cases, chronic active hepatitis in 2 and cryptogenic in 1. The mean duration of liver disease was 30.5 months with a range of 2 to 145 months. No patient had bled from oesophageal varices or elsewhere in the preceding month. Two patients had encephalopathy (grade 1) but none had ascites. The diagnoses in the control patients were as follows: peptic ulcer disease (6) and 1 each of hiatus hernia, inguinal hernia, varicose veins and diverticular disease (all of these inactive, on no therapy). Demographic details, including alcohol and smoking history and diuretic therapy are listed in Table 6.7.

The cirrhotic patients were matched with a control of the same sex and age (within 5 years) and in 9 of the 10 pairs, they were studied on the same day.

Study Designs

An identical format to that outlined in the previous study was adopted. For this study the sequence of agonist administration was randomly allocated and the drug-free period between infusions was 45 minutes. Phenylephrine hydrochloride was diluted in 0.9% sodium chloride solution to a final concentration of 2 ug kg^{-1}

subject's weight ml^{-1} and infused at a minimum of 4 rates ranging from 0.25 to 3.0 $ml.min^{-1}$ (0.5 - 6.0 ug $kg^{-1}min^{-1}$). Alphamethylnoradrenaline was similarly diluted, to 2 concentrations: a) 0.1 ug kg^{-1} ml^{-1} and b) 0.5 ug kg^{-1} ml^{-1}. Solution (a) was infused at rates varying from 0.2 to 2.0 ml min^{-1}, and solution (b) from 0.5 to 1.0 ml min^{-1}. This allowed doses ranging from 0.02 to 0.5 ug kg^{-1} min^{-1}. Isoprenaline was similarly diluted to a concentration of 50 ng kg^{-1} ml^{-1} and infused at rates varying from 0.2 to 1.0 ml min^{-1} (10 to 50 ng kg^{-1} min^{-1}). Each infusion dose step lasted 10 min and the heart rate and blood pressure recorded during the last 5 minutes, when steady state was reached (254). As in the previous study, the infusion was discontinued if the systolic blood pressure rose by > 45 mmHg or the diastolic by > 30 mmHg. In the case of isoprenaline the maximum rise in heart rate permitted was 45 min^{-1}.

As in the case of noradrenaline and angiotensin II, dose response curves were constructed from the $\log_e$ dose of agent v blood pressure for phenylephrine and alphamethylnoradrenaline, and v heart rate for isoprenaline. In all three agonist infusions, the quadratically fitted curve was best suited to the individual data points and this curve used to calculate dose of agonist required to raise blood pressure by 10 and 20 mmHg (PD_{10} and PD_{20} respectively) or heart rate by 10 and 20 min^{-1} (CD_{10} and CD_{20} respectively). The logarithmic transformations of

PD_{10}, PD_{20}, CD_{10} and CD_{20} were compared between the 2 groups by Student's unpaired t test. Correlations were calculated by linear regression.

6.3.3. Results

The results of the biochemical tests of liver function, plasma catecholamine levels and plasma renin activity are summarised in Table 6.8. and the baseline haemodynamic data similarly in Table 6.9. There is significant elevation of plasma noradrenaline and DHPG indicating enhanced sympathetic activity. The resting heart rate is increased in the cirrhotics. In this particular group the systolic blood pressure shows no reduction, while the 7 mmHg reduction in diastolic pressure, is just outwith the 5% significance level, but with a clear trend illustrated in the confidence intervals.

The mean arterial pressure responses to phenylephrine are listed in Table 6.10. The systolic pressure responses to alphamethylnoradrenaline are listed in Table 6.11 and the heart rate responses to isoprenaline in Table 6.12.

The PD_{10} and PD_{20} values for the phenylephrine infusions are higher in the cirrhotics than in the controls, although this is only significant for log PD_{20}. Therefore vascular reactivity to an $alpha_1$ adrenoceptor agonist is reduced in severe cirrhosis. A representative pair of subjects is illustrated in Figure

TABLE 6.8. STUDY B: LIVER FUNCTION TESTS, PLASMA CATECHOLAMINES AND PLASMA RENIN ACTIVITY

Values quoted are median (range) and comparison is by Mann Whitney U test. The mean difference and 95% confidence intervals were calculated from a log transformation and reconverted.

	Cirrhosis (n=10)	Controls (n=10)	Mean Difference	95% Confidence Intervals	P
Bilirubin ($\mu mol.l^{-1}$)	36.5 (12-123)	6 (4-10)	6.7	4.7,8.7	<0.001
Albumin ($g.l^{-1}$)	31.5 (21-41)	40.5 (35-44)	-1.3	-2.5,-0.2	<0.001
Prothrombin Time (secs prolonged)	7 (0-11)	0 (0-2)	- (0-3)	-	<0.001
Plasma NA ($nmol.l^{-1}$)	4.0 (1.5-10.0)	2.5 (0.8-3.3)	2.0	0.4,3.6	<0.01
Plasma DHPG ($nmol.l^{-1}$)	12.6 (5.0-31.5)	7.0 (5.0-13.2)	1.8	0.3,3.3	<0.01
PRA ($ngAIml^{-1}hr^{-1}$)	27.5 (1.2,99.4)	2.8 (0.8-10.6)	6.5	3.3,9.8	< 0.005

TABLE 6.9. STUDY B: BASELINE HEART RATE AND BLOOD PRESSURE

Values quoted are mean ± SD, plus mean differences and 95% confidence intervals. Analysis is by Student's t-test for unpaired data.

	Cirrhosis (n=10)	Controls (n=10)	Mean Difference	95% Confidence Intervals	P
Heart Rate (min^{-1})	78 ± 15	58 ± 7	-20	-33,-6	< 0.002
Systolic BP (mmHg)	126 ± 15	126 ± 13	1	-13,14	< 0.88
Diastolic BP (mmHg)	67 ± 10	74 ± 6	7	-0.2,15	< 0.08

TABLE 6.10. MEAN ARTERIAL PRESSURE RESPONSES TO PHENYLEPHRINE

Values are mean ± SD, Comparison by Student's t-test for unpaired data on the log values.

	Cirrhosis	Controls	Mean Difference	95% Confidence Intervals	P
PD_{10} ($ugkg^{-1}min^{-1}$)	1.25	0.97	-0.28	-0.67,0.11	
Log PD_{10}	4.86 ± 0.67	4.54 ± 0.28	-0.32	-0.79,0.15	0.29
PD_{20} ($ugkg^{-1}min^{-1}$)	2.34	1.51	-0.84	-1.68,0	
Log PD_{20}	5.35 ± 0.49	4.95 ± 0.35	-0.40	-0.79,-0.01	<0.05

TABLE 6.11. SYSTOLIC BLOOD PRESSURE RESPONSES TO ALPHAMETHYLNORADRENALINE

Values are mean ± SD, Comparison by Student's t-test for unpaired data on log values

	Cirrhosis	Controls	Mean Difference	95% Confidence Intervals	P
PD_{10} ($ugkg^{-1}min^{-1}$)	0.24	0.17	-0.07	-0.17,0.13	
Log PD_{10}	3.08 ± 0.48	2.66 ± 0.62	-0.42	-0.93,0.09	0.12
PD_{20} ($ugkg^{-1}min^{-1}$)	0.59	0.36	-0.23	-0.42,-0.04	
Log PD_{20}	4.05 ± 0.26	3.44 ± 0.55	-0.61	-1.08,-0.14	0.001

TABLE 6.12. HEART RATE RESPONSES TO ISOPRENALINE

Values are mean ± SD, Comparison by Student's t-test for unpaired data on the log values.

	Cirrhosis	Controls	Mean Difference	95% Confidence Intervals	P
CD_{10} ($ngkg^{-1}min^{-1}$)	11.3	12.4	-1.1	-3.0,5.2	
Log CD_{10}	2.38± 0.31	2.42± 0.47	0.04	-0.30,0.38	0.84
CD_{20} ($ngkg^{-1}min^{-1}$)	17.6	20.6	3.0	-3.7,9.7	
Log CD_{20}	2.81± 0.38	2.94± 0.45	0.13	-0.23.0.494	0.49

6.4. and the mean phenylephrine responses in Figure 6.5.

For alphamethylnoradrenaline, systolic pressure only has been analysed, since it has no consistent effect on diastolic pressure in either group. There is a trend towards higher PD_{10} values in the cirrhotic group and at the PD_{20} level, this increase in cirrhosis is highly significant ($p < 0.001$). Therefore vascular reactivity to an alpha adrenoceptor agonist is also significantly reduced in severe cirrhosis. A representative pair of subjects is illustrated in Figure 6.6 and the mean values in Figure 6.7.

In contrast, there is no difference between the CD_{10} or CD_{20} values for isoprenaline in the control and cirrhotic groups, nor do the confidence intervals show any trend towards a difference. It appears that the chronotropic response to beta adrenoceptor stimulation is not reduced (or enhanced) in cirrhosis. A representative subject pair is illustrated in Figure 6.8 and the mean values are shown in Figure 6.9.

6.3.4. Discussion

This study has demonstrated that alpha adrenoceptor-mediated pressor responses are impaired in cirrhosis but beta adrenoceptor-mediated chronotropic responses are not.

This is the first time that the function of specific alpha adrenoceptor subtypes has been studied in cirrhosis, either in man or in experimental animals. The impairment of the pressor response to both phenylephrine (a selective

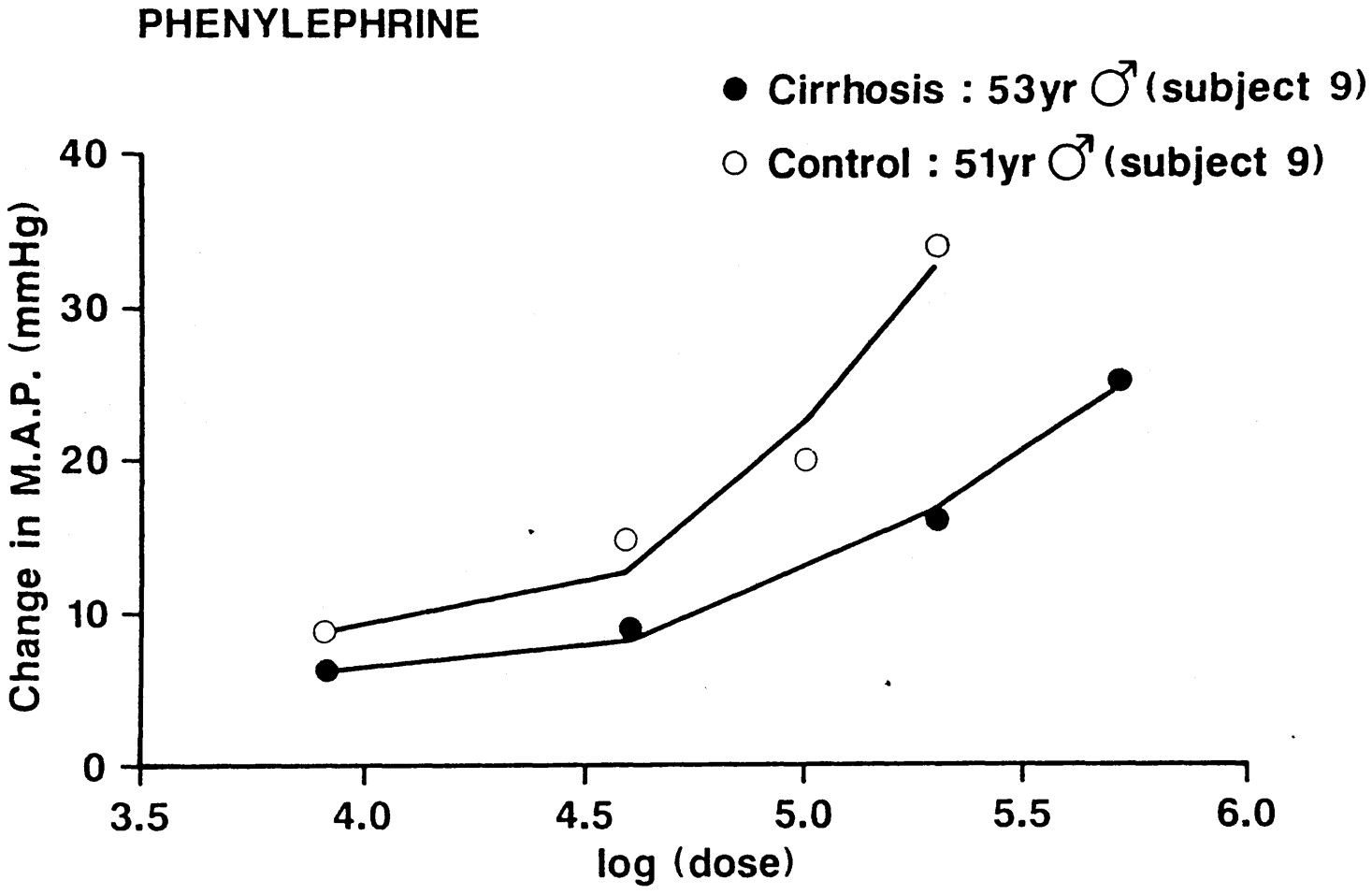

Figure 6.4.

Increase in mean arterial pressure in response to phenylephrine in a representative matched pair of subjects.

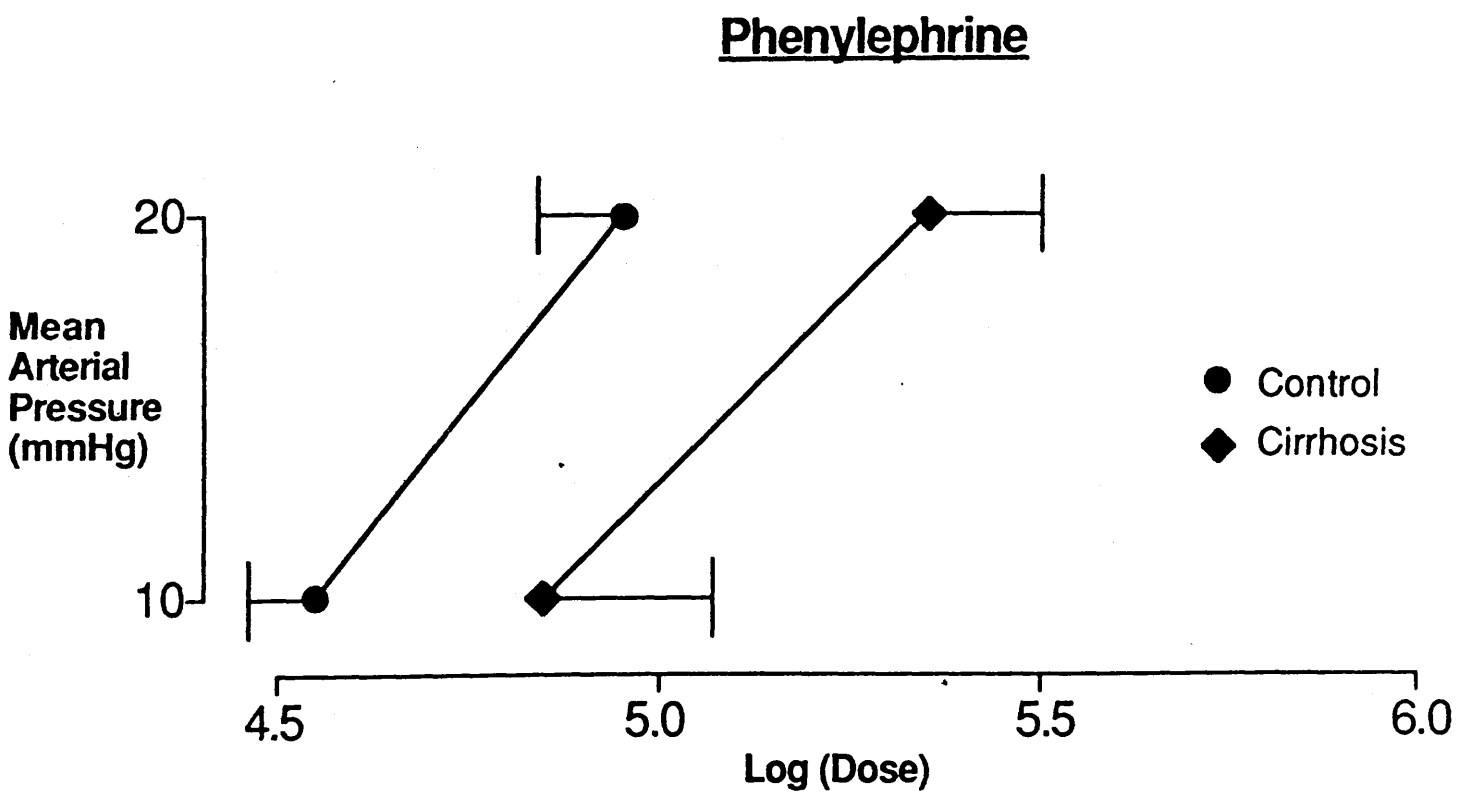

Figure 6.5.

Increase in mean arterial pressure in response to phenylephrine, constructed from the means of the individual log PD_{10} and log PD_{20} values for both groups. (Horizontal bars represent standard deviation).

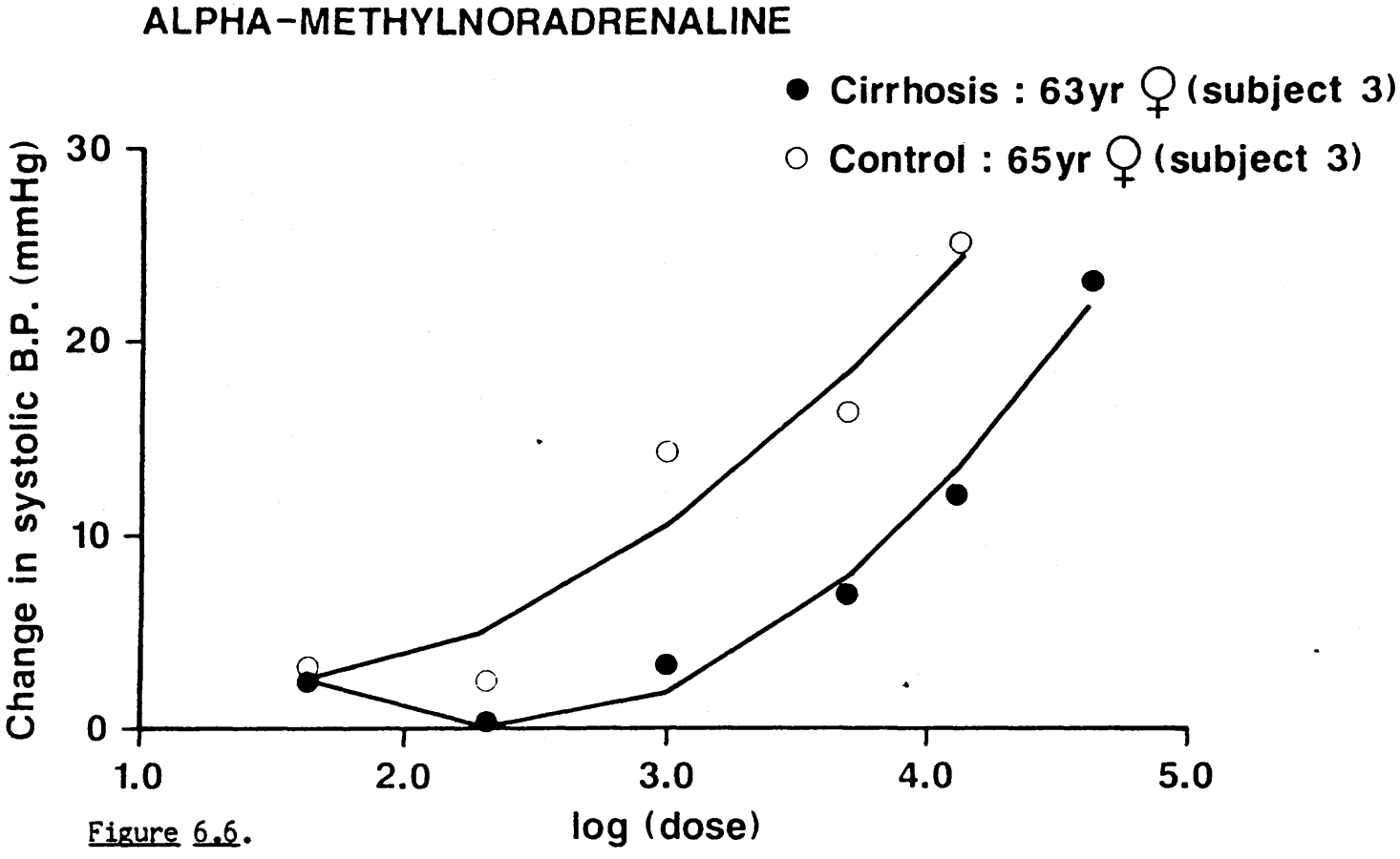

Figure 6.6.

Increase in systolic blood pressure in response to alphamethylnoradrenaline in a representative matched pair of subjects.

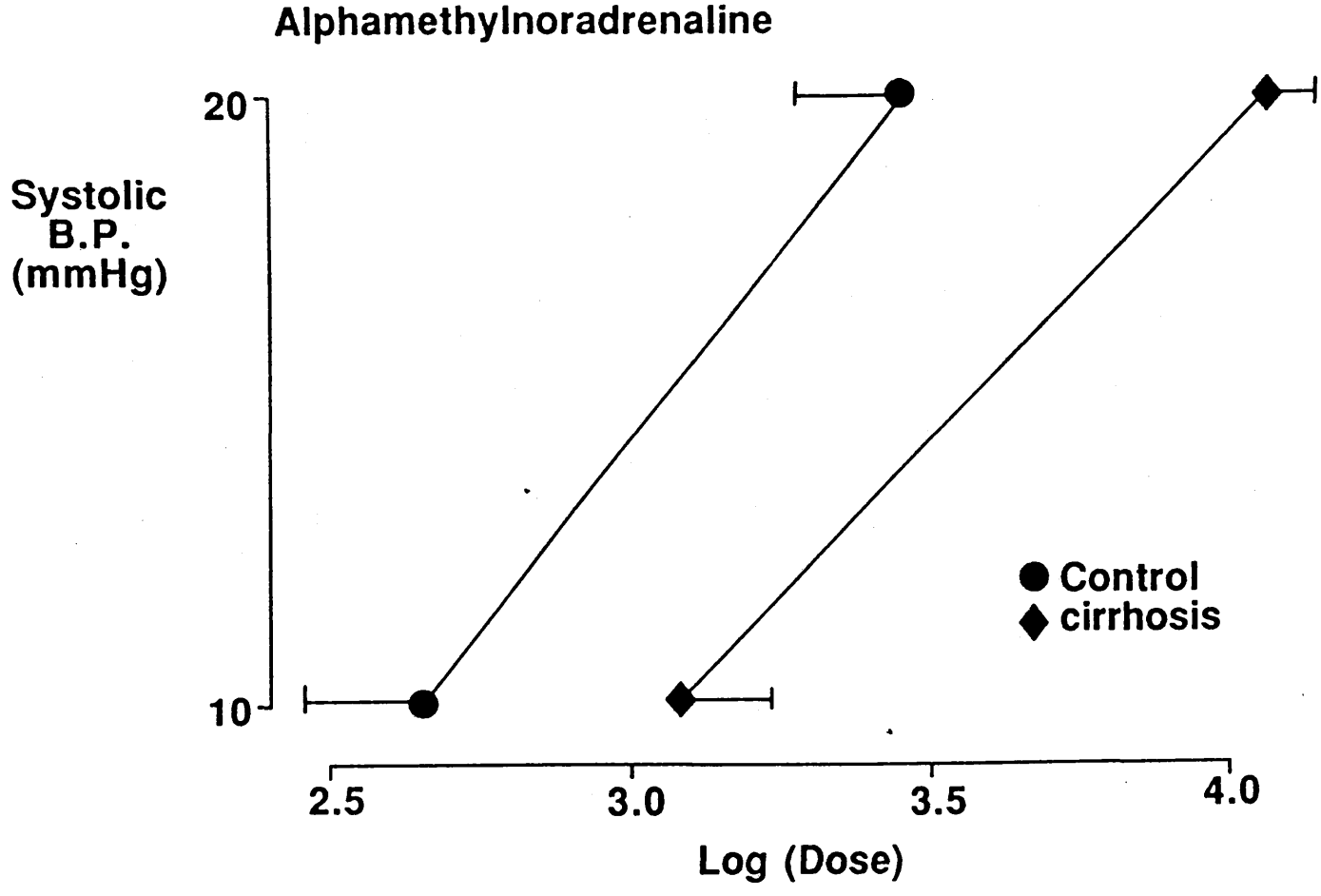

Figure 6.7.

Increase in systolic blood pressure in response to alphamethylnoradrenaline, constructed from the means of the individual log PD_{10} and log PD_{20} values for both groups. (Horizontal bars represent standard deviation).

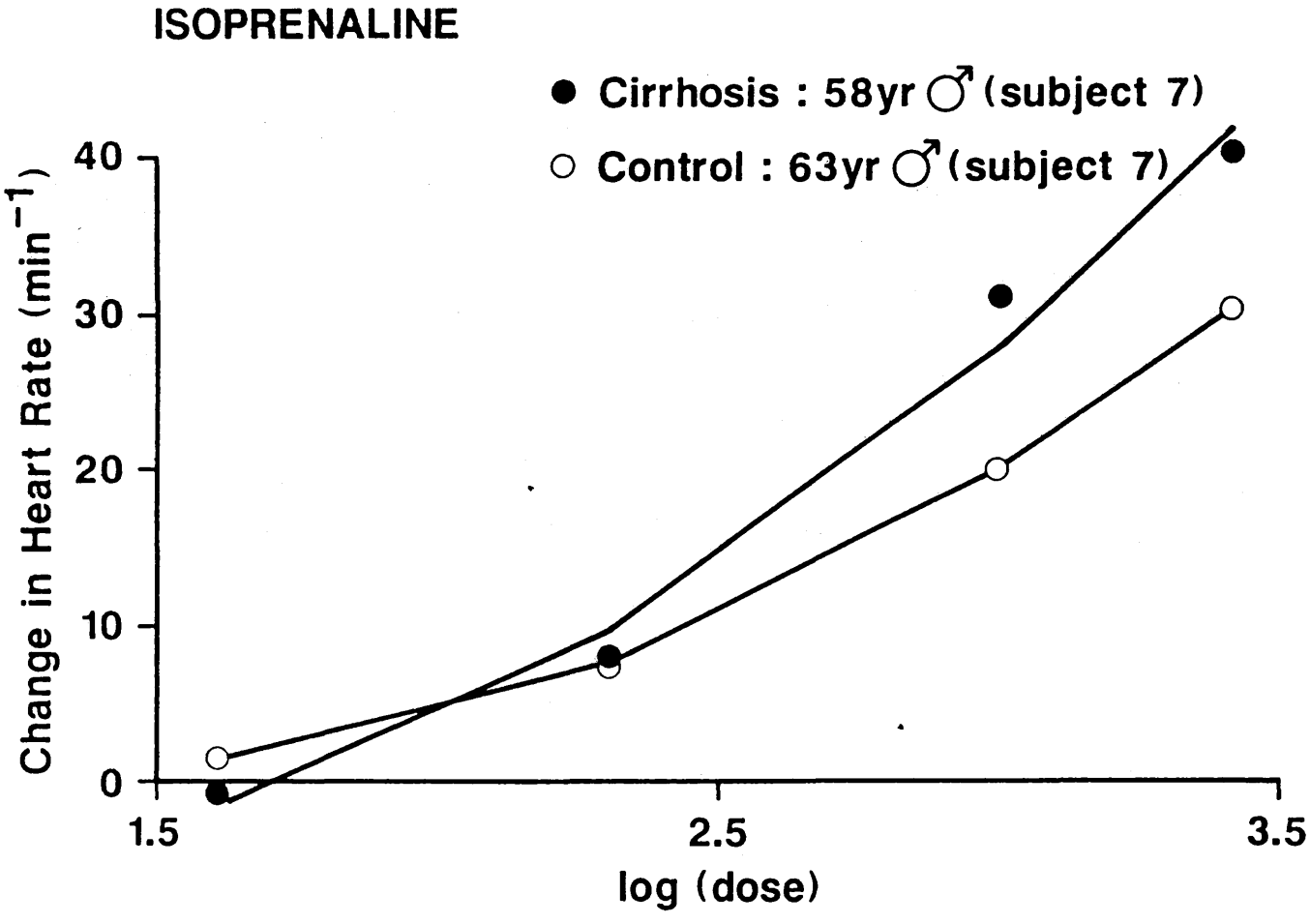

Figure 6.8.

Increase in heart rate in response to isoprenaline in a representative matched pair of subjects.

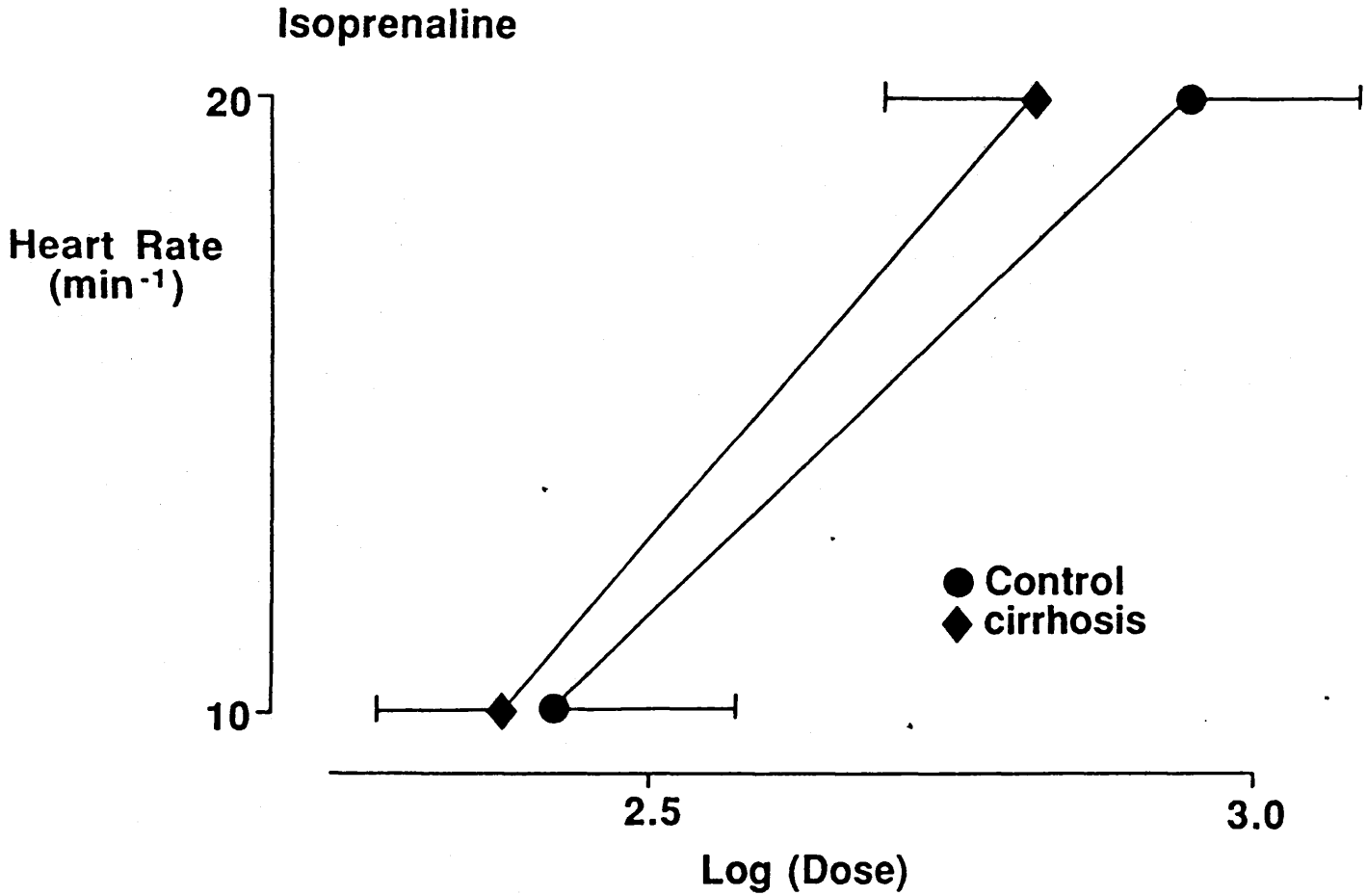

Figure 6.9.

Increase in heart rate in response to isoprenaline, constructed from the means of the individual log CD_{10} and log CD_{20} values for both groups. (Horizontal bars represent standard deviation).

alpha$_1$ agonist) and alphamethylnoradrenaline (a preferential alpha$_2$ agonist) supports the finding of the previous study (Section 6.2.) of impaired pressor response to noradrenaline (a mixed alpha$_1$ and alpha$_2$ agonist) in cirrhosis.

Two other groups have recently considered beta-adrenoceptor mediated responses (162,164). In contrast to the present study, both groups reported a decrease in the sensitivity to isoprenaline in cirrhosis. These studies differed from the present one in the disease population studied, the choice of control group and the methodology employed. In both the reported studies, the cirrhotic patients appeared to have a more severe degree of liver failure. All 11 patients studied by Lenz had ascites and hepatic encephalopathy, which proved fatal in 6 of the cases (162). All but 3 of the 13 alcholic cirrhotics studied by Lebrec's group (164) had ascites and 7 were in Pugh's grade C. In the present study, the degree of jaundice, hypoalbuminaemia and coagulopathy placed 4 of the cirrhotics in Pugh's grade C and the remaining 6 in Pugh's grade B. However, none of these had ascites at the time of study and only 2 had encephalopathy, which was mild in both cases. Therefore, this study may not have detected a difference if this only becomes apparent in the most severely ill patients. Nevertheless, the grade of disease in the present study was sufficient to detect significant differences in alpha adrenoceptor responses, without any trend whatsoever to impaired isoprenaline sensitivity.

This does imply some difference in alpha and beta adrenoceptor responses in moderately severe cirrhosis.

The differences in methodology are more difficult to compare. In the Lenz study, the heart rate response to a single bolus of isoprenaline was assessed. For reasons outlined in 6.2.4. this may be a less reliable method than constructing dose-response curves from multiple dosing. The latter method was employed by Lebrec and co-workers, using multiple bolus injections of increasing doses of isoprenaline, in contrast to the stepwise series of infusions to steady state employed in the present study. Both methods have their advocates (261,253). However, the manner in which the dose response was analysed by Lebrec is open to criticism. Firstly, the dose response graph was constructed by a linear plot of the log dose against heart rate. It has been suggested by several workers that a quadratic fit of log dose v response is more appropriate (253,262). Secondly, the increment in heart rate chosen for analysis was 25 min^{-1} (CD_{25}), but only 4 of their 13 cirrhotics achieved this increase at the doses used. In the other 9 cases the value has been obtained by extrapolation. In view of the inevitable degree of error in the construction of such graphs, the degree of extrapolation raises doubts as to the validity of the interpretation (which could have been avoided by adopting a lower response, e.g. CD_{15}).

Finally, it has been pointed out previously that the

choice of control group is critical in catecholamine studies (187) and neither of the published papers is ideal in this respect. The control group studied by Lenz were considerably younger (range 19 to 32 years) than the cirrhotics (range 41-65 years). The sensitivity to isoprenaline is well known to decrease with age (263-265). Lebrec studied only 5 control subjects.

Notwithstanding the above criticisms, the reduction in isoprenaline sensitivity in these 2 studies was striking and they reached the same conclusions by widely differing techniques. There is clearly scope for further work on beta adrenergic responses in cirrhosis with severe liver damage.

In conclusion, the reduced alpha adrenergic pressor response, which is a peripheral vascular effect, in conjunction with the normal beta adrenergic chronotropic response, which is principally a cardiac effect, indicate that the reduced vascular reactivity is <u>not</u> part of a generalised sympathetic desensitisation.

6.4. <u>BARORECEPTOR FUNCTION</u>

In the previous chapter the blood pressure response to reflex sympathetic stimulation in the form of isometric exercise was blunted in cirrhosis. This was in keeping with the demonstration that the alteration in limb blood flow in response to such autonomic stimuli as lower body negative pressure and Valsalva manoeuvre is also reduced in

cirrhosis (151). These particular reflexes require an intact baroreceptor response, and it has therefore been deemed important to establish whether baroreceptor reflexes are normal or not in cirrhosis (162).

The alpha$_1$ agonist used in the previous study, phenylephrine, is sufficiently selective to have no direct cardiac effects. Therefore the bradycardia which accompanies its infusion is a baroreceptor mediated response to the rise in blood pressure. The reduction in heart rate for a given rise in blood pressure during phenyelphrine infusion is therefore a measure of baroreceptor responsiveness. This can be calculated as $\Delta HR/\Delta BP$. The values for this ratio in the 10 cirrhotics and 10 controls in the previous study are 0.74 ± 0.49 and 0.38 ± 0.25 respectively. It is apparent that the heart rate response in cirrhosis is not reduced. Indeed, although the difference between cirrhotic and control groups did not achieve statistical significance, there is a suggestion that the responsiveness is actually increased in cirrhosis (95% confidence interval of difference -0.01 to 0.73). If subsequent work were to verify this it might indicate a disproportionate increase in sympathetic activation in response to even a slight drop in blood pressure. This could explain the increased sympathetic activity in these patients, as judged by plasma noradrenaline concentrations, without a significant fall in blood pressure. The lack of any impairment of baroreceptor function in cirrhosis is

supported by the work of Lenz exploring cardiovascular responses to sympathetic and non sympathetic vasoconstriction (162). Clearly the impaired reflex autonomic responses result from defects elsewhere in the reflex pathway, as would be anticipated by the similar impairment of cardiovascular responses in response to exogenous catecholamine infusions (Sections 6.2. and 6.3.).

6.5. GENERAL DISCUSSION AND CONCLUSIONS

The experiments in this chapter have been concerned with the cardiovascular responses to a variety of types of sympathetic stimulants, or agonists. Appreciation of their significance requires some knowledge of selective adrenoceptor subtypes and their actions.

The actions of the sympathetic nervous system result from the release of 2 catecholamines - adrenaline (from the adrenal medulla) and noradrenaline (from post ganglionic sympathetic nerve endings). The cardiovascular (and other) responses to these catecholamines, whether acting as a hormone (adrenaline) or a neurotransmitter (noradrenaline), are mediated by highly specific cell surface receptors - adrenoceptors. There are different types of adrenoceptors which mediate characteristic and different cellular responses (266). The two main types of adrenoceptor are alpha and beta, which have been subdivided into $alpha_1$ and $alpha_2$ receptors (267) and $beta_1$ and $beta_2$ receptors (268). Stimulation of $alpha_1$ receptors causes peripheral

vasoconstriction. Pre-synaptic alpha$_2$ receptors outwith the central nervous system provide a negative feedback loop by inhibiting noradrenaline release. However, there are also post-synaptic alpha$_2$ receptors, which like alpha$_1$, cause vasoconstriction. Clasically beta$_1$ receptors are predominantly cardiac where they exert both an inotropic and a chronotropic effect. Beta$_2$ receptors located on peripheral vessels cause vasodilatation when stimulated.

Noradrenaline itself has both alpha and beta effects, but is more potent as an alpha agonist. Hence the rise in blood pressure seen during the noradrenaline infusion in Section 6.2. is predominantly the result of peripheral vasoconstriction. However, there is also a beta agonist effect. This is demonstrated by the lack of a reflex bradycardia in response to the rise in blood pressure, due to a positive chronotropic effect on the heart.

These experiments have attempted to explain the anomaly of decreased peripheral vascular tone despite increased sympathetic activity. The blood pressure response to sympathetic stimulation by exogenous noradrenaline infusion is impaired in "decompensated" cirrhosis. This is in keeping with the blunted response to certain reflex sympathetic stimuli, e.g. isometric exercise, as demonstrated in the previous chapter. The impaired response to exogenous noradrenaline supports the impression from that chapter that the reflex sympathetic abnormalities are the result of impaired vascular responsiveness rather

than actual autonomic neuronal damage (see Section 5.4.). The previous suggestion (151) that these reflex abnormalities might be due to baroreceptor dysfunction has been disproven; baroreceptor responsiveness, as indicated by the reflex bradycardia in response to a rise in blood pressure, is normal, or slightly enhanced. The impaired vascular responsiveness to angiotensin II as well as noradrenaline could indicate a common defect at the site of vascular smooth muscle, or parallel separate "desensitisation" to both systems, which are both frequently over active in cirrhosis. The possibility of generalised sympathetic desensitisation seems remote in the light of the normal heart rate response to beta-adrenergic stimulation with isoprenaline. What the four agents with impaired cardiovascular responses - noradrenaline, angiotensin, phenylephrine and alphamethylnoradrenaline - have in common is a principal or sole action on the peripheral vasculature. The evidence is therefore accumulating that the site of the defect causing the impaired neurovascular reactivity is at the smooth muscle of the peripheral vascular tree. Whether this is at the cell membrane receptors, or elsewhere, is the subject of the next chapter.

CHAPTER 7

STUDIES OF ADRENERGIC RECEPTORS IN CIRRHOSIS

7.1. STUDIES OF PLATELET ALPHA$_2$ ADRENOCEPTORS

7.1.1. Introduction

The studies described in the preceding chapters have demonstrated that in cirrhotic patients with severe liver damage there is a reduction in peripheral vascular responsiveness to sympathetic stimulation. This hypothesis could be examined further by direct assessment of the adrenoceptors themselves. Clearly the particular adrenoceptors of most interest are the alpha$_1$ receptors on small peripheral resistance vessels, since these are the principal sympathetic mediators of vascular tone. However, they are not accessible for study in in vivo experiments on human subjects, nor is there an accessible model for human alpha$_1$ receptors. Animal studies on isolated vessels would be an alternative, but there is no ideal animal model of cirrhosis, nor could the behaviour of animal adrenoceptors be taken automatically to represent the human situation, since there are considerable inter-species variations.

A compromise would be to study the alpha$_2$ adrenoceptors present on circulating platelets. These are readily accessible for study in the human subject and may give some indication of overall alpha adrenoceptor status. This study describes the assessment of the number and affinity of platelet alpha$_2$ adrenoceptors in cirrhosis.

7.1.2. Study A ("Unwashed" platelets)

Subjects and Study Design

Ten patients with cirrhosis and ten control subjects were studied. The cirrhotic group comprised 7 males and 3 females, aged 41-78 years (mean 56.5). The cirrhosis was of alcoholic origin in 7 patients, with 2 cases of chronic active hepatitis and one cryptogenic cirrhosis. The control group consisted of 7 male and 3 female patients with non-hepatic disorders, aged 39-77 years (mean 55). None of the cirrhotics had taken alcohol within one month of study. All medications were omitted on the study day, diuretics having been stopped 72 hours previously. The studies were performed in pairs matched for sex and age (within 5 years). Subjects were studied in the morning, at least one hour after a light breakfast. After the insertion of an indwelling cannula in a peripheral vein, subjects rested for 30 minutes. Blood pressure and heart rate were recorded by semi automatic sphygmomanometer, and blood was drawn from the cannula for liver function tests, plasma catecholamines and renin (for details of sampling and analysis, see Chapter 2). A further 120 ml blood (60 ml for controls) was collected and anticoagulated with citrate (1 volume 3.28% citrate to 9 volumes of blood) for platelet analysis.

Preparation of platelets for binding studies

The whole blood was centrifuged at 180 g for 15 mins at 20°C. The platelet-rich plasma was harvested and spun at

1700 g for 15 mins at 4°C to produce a platelet pellet. This was then resuspended in 0.1% EDTA, 150 mM sodium chloride (pH 7.4) to give a platelet concentration of 100,000 platelets/ul as determined by Coulter counting.

Alpha$_2$ adrenoceptor binding assay on intact platelets

Binding studies on intact platelets were performed according to the method of Newman (269), as modified by Motulsky (270). Aliquots of platelet suspension (0.8 ml) were incubated for 20 mins at 25°C with 6 concentrations of [^{3}H] yohimbine (Amersham 90 Ci/mmol) 1.2 - 18 nM in duplicate. The non specific binding was determined in the presence of 1 uM phentolamine. Incubations were terminated by filtration with 20 ml of ice cold Tris HCl (50 mM pH 7.4) through Whatman GF/C filters, using a millipore multiport filtration apparatus set at 30 cm.Hg. The filters were dried overnight at room temperature and the [^{3}H] bound radioactivity was determined by liquid scintillation counting at an efficiency of 36%. Saturation binding isotherms were analysed by plotting free/bound radioactivity vs free using least squares fitting to obtain values for the antagonist dissociation constant, K_D (nM) and the maximum number of binding sites, Bmax (fmoles/10^9 platelets).

Results

It proved impossible to construct satisfactory

saturation binding isotherms by least squares fitting in 2 of the cirrhotics and 4 of the control subjects due to variability of their data. The Bmax was increased in the 8 cirrhotics (48.6 ± 20.9 v 28.4 ± 9.1 fmoles/10^9 platelets, $p < 0.05$ by unpaired t-test). K_D was similar in cirrhotics (10.3 ± 7.2 $nmol.l^{-1}$) and controls (13.4 ± 8.1 $nmol.l^{-1}$).

Discussion

The apparent increase in $alpha_2$ adrenoceptor number in cirrhosis, where sympathetic activity and circulating noradrenaline are increased, and where pressor responsiveness to alpha adrenoceptor stimulation is reduced, is surprising. It was noted that the K_D values obtained in both groups were considerably higher than those seen in previous studies of young, healthy volunteers. It was postulated that the reduced affinity that this represents might be due to higher concentrations of circulating noradrenaline in these patients adhering to binding sites on the platelets and thereby interfering with the binding of the ligand in vitro. It was therefore decided to repeat the above study, with the inclusion of a step to "wash off" any retained noradrenaline prior to incubation with [^{3}H] yohimbine.

7.1.3. Study B ("Washed" platelets)

Subjects and Study Design

Eleven cirrhotics were studied, 8 of whom had participated in the previous study. On this occasion there were 9 males and 2 females, aged 41-65 years (mean 55). All but one had alcoholic liver disease, the remaining diagnosis being chronic active hepatitis. Eleven control subjects were studied, with no overlap from the previous study. Four were patients with non hepatic disorders, the remaining 7 comprising members of staff. There were 6 males and 5 females, aged 39-62 years (mean 52).

The study design was identical to that described for the previous study.

Preparation of platelets and alpha$_2$ binding assay

The preparation of platelets was as described in the previous experiment with one exception: after resuspension in the platelet buffer, whose composition was unaltered, the suspension was recentrifuged and resuspended twice more to remove any retained catecholamines (271). Thereafter the pellet was resuspended in the buffer to a final concentration of 100,000 platelets/ul as previously.

The binding assay on the intact platelets was performed in an identical manner to that described above.

Results

The cirrhotic patients had biochemical evidence of

severe hepatic impairment indicated by a median bilirubin of 45 umol.l^{-1} (range 11-136), a median albumin of 31g.l^{-1} (range 25-40) and a median prolongation of prothrombin time of 5 seconds (range 1-15). This placed 2 of the cirrhotics in Pugh's grade A, 5 in grade B and 4 in grade C. However, none of the cirrhotics had ascites, and only 1 had mild encephalopathy. The results of K_D, Bmax, heart rate, blood pressure and catecholamine levels are indicated in Table 7.1. The haemodynamic picture and catecholamine concentrations follow a similar pattern to previous studies, with lower blood pressure and higher plasma concentrations of both noradrenaline and DHPG.

Wide intra subject variability prevented construction of satisfactory saturation binding isotherms in 8 of the 22 subjects (5 cirrhotics and 3 controls). There was no difference in either Bmax or K_D between the remaining 6 cirrhotics and 8 controls (Figures 7.1. and 7.2.).

Linear regression analysis of the cirrhotic patients only, revealed a significant negative correlation between Bmax and plasma bilirubin ($r = -0.85$, $p < 0.05$ using log bilirubin values), but not other indices of disease severity, plasma catecholamine concentrations or haemodynamic indices. The affinity appeared to be greater in those patients with the most severe liver disease, as indicated by correlation coefficients of -0.86 ($p < 0.05$), 0.92 ($p < 0.01$) and -0.86 ($p < 0.05$) between K_D and bilirubin, albumin and Pugh's score, respectively. Again,

TABLE 7.1. K_D, B_{max}, HEART RATE, BLOOD PRESSURE AND PLASMA CATECHOLAMINES IN PLATELET ALPHA$_2$ ADRENOCEPTOR STUDY (B)

Analysis is by unpaired Student's t-test.

	CIRRHOTICS		CONTROLS					
	Mean	SD	Mean	SD	Mean difference	95% confidence interval	P	Significance
K_D (nmol.l^{-1})	4.6	2.7	5.5	2.6	-0.9	-3.61,2.62	.56	NS
B_{max} (fmoles/10^9 platelets)	24.9	8.4	22.1	5.3	2.8	-3.98,9.58	.47	NS
SBP (mmHg)	117	9	127	17	-10	-21.9,1.9	.11	"trend"
DBP (mmHg)	64	9	74	9	-10	-18.7,-1.3	.02	<.05
HR (min^{-1})	74	13	69	6	5	-3.8,13.8	.28	NS
NA (nmol.l^{-1})	4.7	3	2.8	1.1	1.9	-0.11,3.91	.06	"trend"
DHPG (nmol.l^{-1})	11.3	3.9	7.5	2.7	3.8	0.6,7.0	.02	<.05

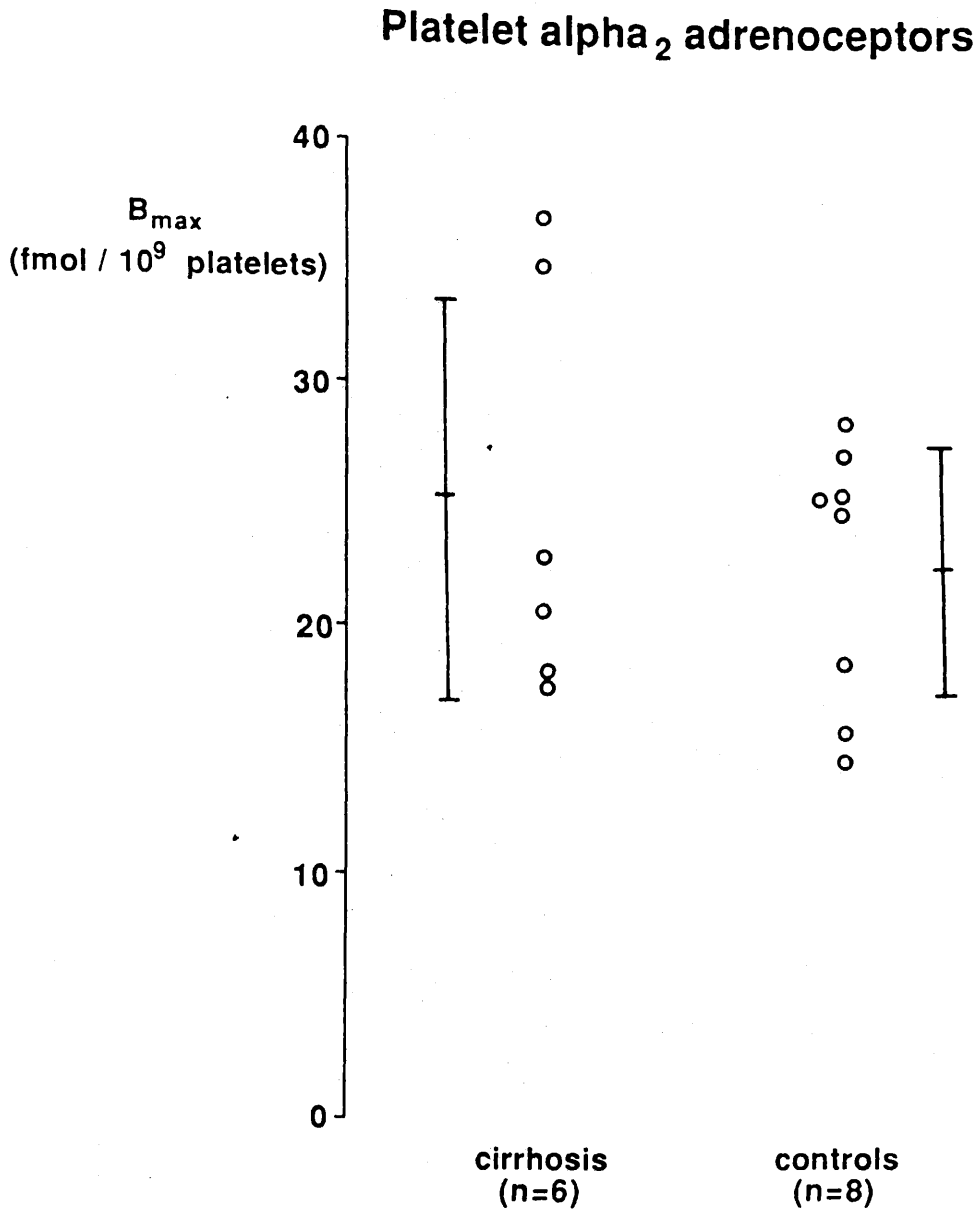

Figure 7.1.

Bmax for platelet alpha$_2$ receptors (Error bars represent mean and standard deviation).

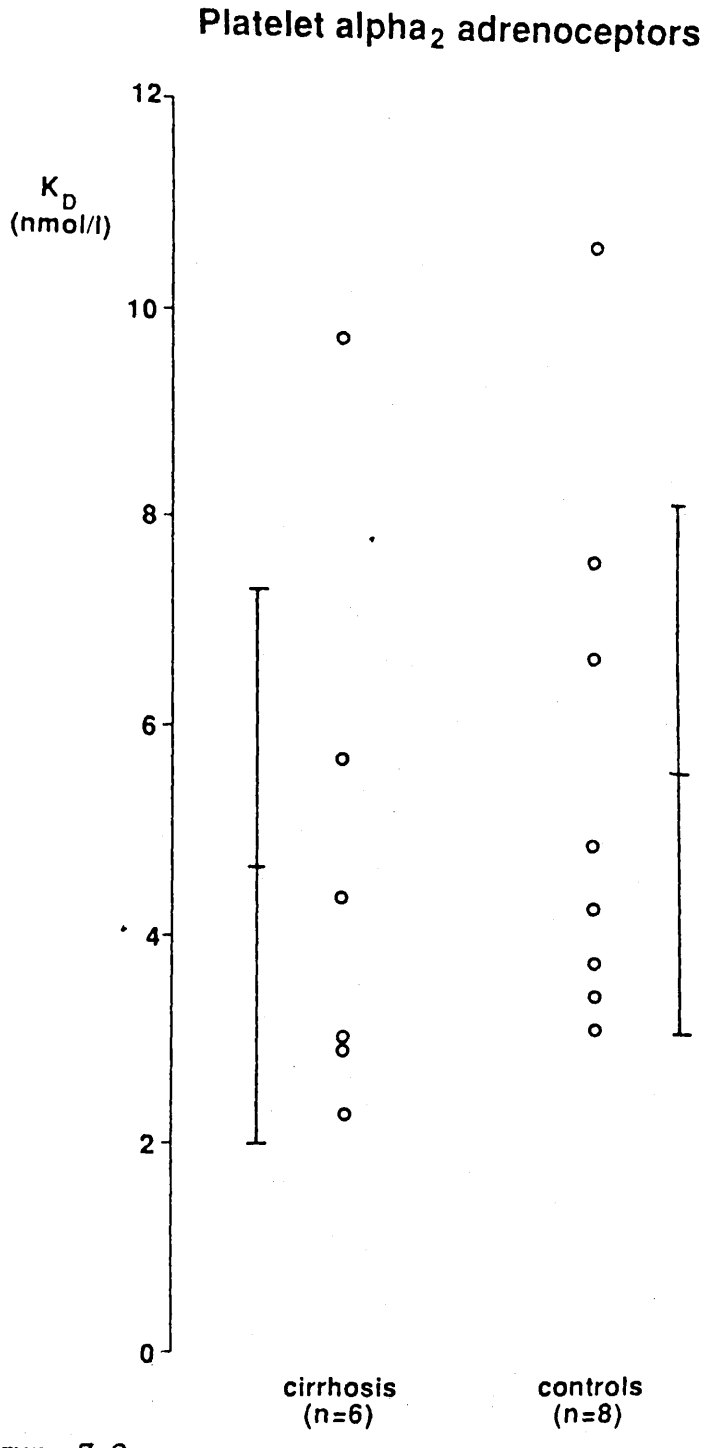

Figure 7.2.

K_D for platelet alpha$_2$ receptors (Error bars represent mean and standard deviation).

K_D was unrelated to plasma catecholamine levels and haemodynamic indices.

The differences in K_D and Bmax between Study A ("unwashed" platelets) and Study B ("washed" platelets) are given in Table 7.2. The washing has lowered both K_D and Bmax in both groups although this only achieves significance in the cirrhotic group.

7.1.4. Discussion

These studies provide no evidence of any reduction in the number of platelet alpha$_2$ receptors in cirrhosis, as indicated by the values of Bmax. The exclusion of some subjects from the analysis due to unsatisfactory saturation binding isotherms is unlikely to have biased the result, as the degree of hepatic impairment, sympathetic activation and haemodynamic disturbance in these individuals was no different from the rest of the group. Also, despite the reduced numbers remaining for analysis, the chance of the study missing a true difference (of one standard deviation or more) is less than 20%, and the confidence intervals show no trend towards any difference. The negative result is therefore likely to be a valid one. The alpha$_2$ receptor on platelets is not necessarily representative of the alpha$_2$ receptor on peripheral vascular smooth muscle. However, if so, this study suggests that the decreased cardiovascular responsiveness in cirrhosis is not due to down-regulation of receptor number, despite the sympathetic over-activity.

TABLE 7.2. EFFECT OF "WASHING" PLATELETS ON K_D AND B_{max} IN CIRRHOTICS AND CONTROLS

Values are mean + SD, comparison by unpaired Student's t-test.

	"Unwashed" Platelets (Study A)	"Washed" Platelets (Study B)	Mean Difference	95% Confidence Interval	P	Significance
CIRRHOSIS	n=8	n=6				
K_D ($nmol.l^{-1}$)	13.4 ± 8.1	4.6 ± 2.7	8.8	0.7,16.9	0.03	< 0.05
B_{max} (fmoles/10^9 platelets)	48.6 ± 20.9	24.9 ± 8.3	23.7	2.3,45.1	0.02	< 0.05
CONTROLS	n=6	n=8				
K_D ($nmol.l^{-1}$)	10.3 ± 7.2	5.4 ± 2.5	4.9	-0.9,10.7	0.11	"trend"
B_{max} (fmoles/10^9 platelets)	28.4 ± 9.1	22.1 ± 5.2	6.3	-1.7,14.3	0.14	"trend"

It is tempting to ascribe pathological significance to the reduction in K_D by washing the platelets. In particular, this might be seen as evidence that the reduced cardiovascular responsiveness in cirrhosis was due to some interference at the receptor site, e.g. by circulating catecholamines or by some catecholamine-like substance which the damaged liver has failed to initiate, analagous to the "false neurotransmitter" theory of hepatic encephalopathy (148). However, washing the platelets also reduced K_D in the control subjects, and the lack of any difference in K_D between the cirrhotic and control groups for both "washed" and "unwashed" platelets makes it unlikely that interference at the receptor site is the explanation for the decreased vascular reactivity in cirrhosis. However, since K_D is a measure of the affinity of the receptor for the ligand in vitro, this does not absolutely exclude interference at the receptor site in vivo.

There are no previous human or animal studies which consider alpha adrenoceptor number or affinity in cirrhosis. There is however some indirect support for these results from work on cirrhotic rats, which suggested that the reduced pressor effect of angiotensin II in cirrhosis was not due to a defect at the cell membrane receptor (155). Previous studies examining the effect of sympathetic stimulation on platelet $alpha_2$ receptors in situations other than cirrhosis have found a similar lack of down-regulation as in the present study, both in rabbits (272) and in man

(271). Although alpha adrenoceptor function (rather than number) can be assessed in man by in vitro platelet aggregation in response to e.g. adrenaline, this test cannot be used in cirrhosis, because the thrombocytopoenia in these patients impairs platelet aggregation irrespective of adrenoceptor function.

In conclusion, these studies, in conjunction with the impaired pressor responses to noradrenaline, phenylephrine, alphamethylnoradrenaline and angiotensin II, all of which raise blood pressure principally by peripheral vasoconstriction (see Chapter 6) provide at least circumstantial evidence for a post-receptor defect within vascular smooth muscle cells themselves as the explanation for the decreased vascular reactivity in cirrhosis.

7.2. STUDY OF LYMPHOCYTE BETA$_2$ ADRENOCEPTORS

7.2.1. Introduction

In addition to studying platelet alpha$_2$ adrenoceptors the opportunity was taken to study a beta adrenoceptor in cirrhosis, namely the beta$_2$ receptor on circulating lymphocytes. This would provide a useful comparison with the alpha receptor study, and also provide evidence for or against the beta agonist infusion study in Chapter 6. In addition it later became of more significance in the light of a report claiming that down-regulation of beta receptors might explain the decreased efficacy of propranolol in preventing oesophageal variceal rebleeding in severe

cirrhosis (273).

7.2.2. Methods

Subjects and study design

The criteria for selection of cirrhotic patients and controls, and the conditions under which venous blood was collected, were the same as for the platelet adrenoceptor studies. Thirteen cirrhotic patients and 13 controls were studied, 10 of each group comprising the participants in the platelet study. There were 9 males and 4 females in the cirrhotic group, aged 41-78 years (mean 57 years). The cirrhosis was alcohol-induced in 9 patients, with 2 cases each of chronic active hepatitis and cryptogenic cirrhosis. The control group also comprised 9 males and 4 females, aged 41-78 years.

Preparation of lymphocyte membranes

After collection of platelet rich plasma the remaining red cells from 120 mls blood were used to isolate lymphocytes according to the method of Boyum (274). Blood was diluted with Hanks balanced salt solution (Gibco, Scotland) and carefully layered onto a Ficoll/Hypaque solution (6%10%) (Pharmacia, Uppsala, Sweden) and the samples centrifuged at 400 g for 40 mins (20^{o}C). The lymphocyte band was harvested by aspiration (consisting of at least 85% small lymphocytes) and a broken cell lysate prepared according to a modification of the method of Aarons

(275). The lymphocyte band was resuspended in 30 nM NaCl and centrifuged at 180 g for 10 mins at 4°C to remove remaining red blood cells, by hypotonic lysis. The pellet was resuspended in ice cold distilled water, homogenised for 5 secs in a Brinkman Polytron at setting 4 (Brinkman Instruments Inc. Westbury, N.Y.) and centrifuged at 40,000 g for 45 mins at 4°C. The pellet was resuspended in 2 ml of ice cold assay buffer (150 mM NaCl with 12.5 mM $MgCl_2$, 1.5 mM EDTA buffered with 50 mM Tris HCl pH 7.4) and stored frozen at -70°C until assayed. On the day of the assay samples were thawed and an additional wash performed by resuspending the pellet in 30 ml of ice cold assay buffer followed by centrifugation at 40,000 g for 30 mins. The pellet was resuspended in 5 ml of ice cold assay buffer, homogenised for 5 secs (Brinkman Polytron setting 3) and used immediately for binding studies.

Beta adrenoceptor assay on lymphocyte membranes

Aliquots (100 ul) of membrane preparation were incubated with 8 concentrations of [^{125}I] iodocyanopindolol (ICYP) (Amersham UK) 10-150 pM according to the method of Brodde (276). Incubations were for 60 mins at 25°C and terminated by addition of 10 ml of incubation buffer and vacuum filtration over Whatman GFB glass fibre filters. The radioactivity of the wet filters was determined in a gamma counter (Berthold model LB2100) at an efficiency of 80%. Non specific binding was defined as radioactivity

bound to membranes which was not displaced by 1 uM propranolol. Specific binding was defined as total radioactivity minus non-specific binding. The equilibrium binding constant, K_D (pM) and maximum number of binding sites, Bmax (fmoles/mg protein) were estimated by Scatchard analysis. Protein concentration was measured using the method of Lowry (277).

7.2.3. Results

The median bilirubin in the cirrhotic group was 34 $umol.l^{-1}$ (range 12-123), albumin 31 $g.l^{-1}$ (21-41) and prothrombin time 4 seconds prolonged (0-11). Most of the cirrhotic group had liver disease of moderate severity with 9 Pugh's grade B, 3 grade C and 1 grade A. Two had ascites and 2 mild encephalopathy.

The K_D, Bmax, heart rate, blood pressure and catecholamine values are summarised in Table 7.3. The haemodynamic picture, as expected, reveals lower blood pressure and faster heart rates in the cirrhotic patients. The K_D and Bmax values were essentially the same in the cirrhotics as the controls, despite significant elevation of both noradrenaline and DHPG levels in plasma (Figures 7.3. and 7.4.).

Linear regression analysis of the cirrhotic patients revealed no relationship between receptor number (Bmax) and severity of liver disease, heart rate, blood pressure and plasma catecholamine levels. The only significant

TABLE 7.3. K_D, B_{max}, HEART RATE, BLOOD PRESSURE AND PLASMA CATECHOLAMINES IN LYMPHOCYTE BETA$_2$ ADRENOCEPTOR STUDY

Analysis is by unpaired Student's t-test.

	CIRRHOTICS		CONTROLS					
	Mean	SD	Mean	SD	Mean difference	95% confidence interval	P	Significance
K_D (pmol.l^{-1})	49.6	14.1	55.3	29.8	-5.7	-26.9,15.5	.65	NS
B_{max} (fmoles/10^9 protein)	24	7.6	27.2	12.3	-3.2	-12.6,6.2	.55	NS
SBP (mmHg)	123	15	130	16	-7	-17.7,3.7	.25	NS
DBP	67	8	76	8	-9	-16.0,-2.0	.02	<.05
HR (min^{-1})	80	15	61	7	19	7.6,30.4	.0004	<.0005
NA (nmol.l^{-1})	4.4	1.7	2.6	1	1.8	.07,3.53	.02	<.05
DHPG (nmol.l^{-1})	14.9	7.3	7.9	2.3	7	2.1,11.9	.003	<.005

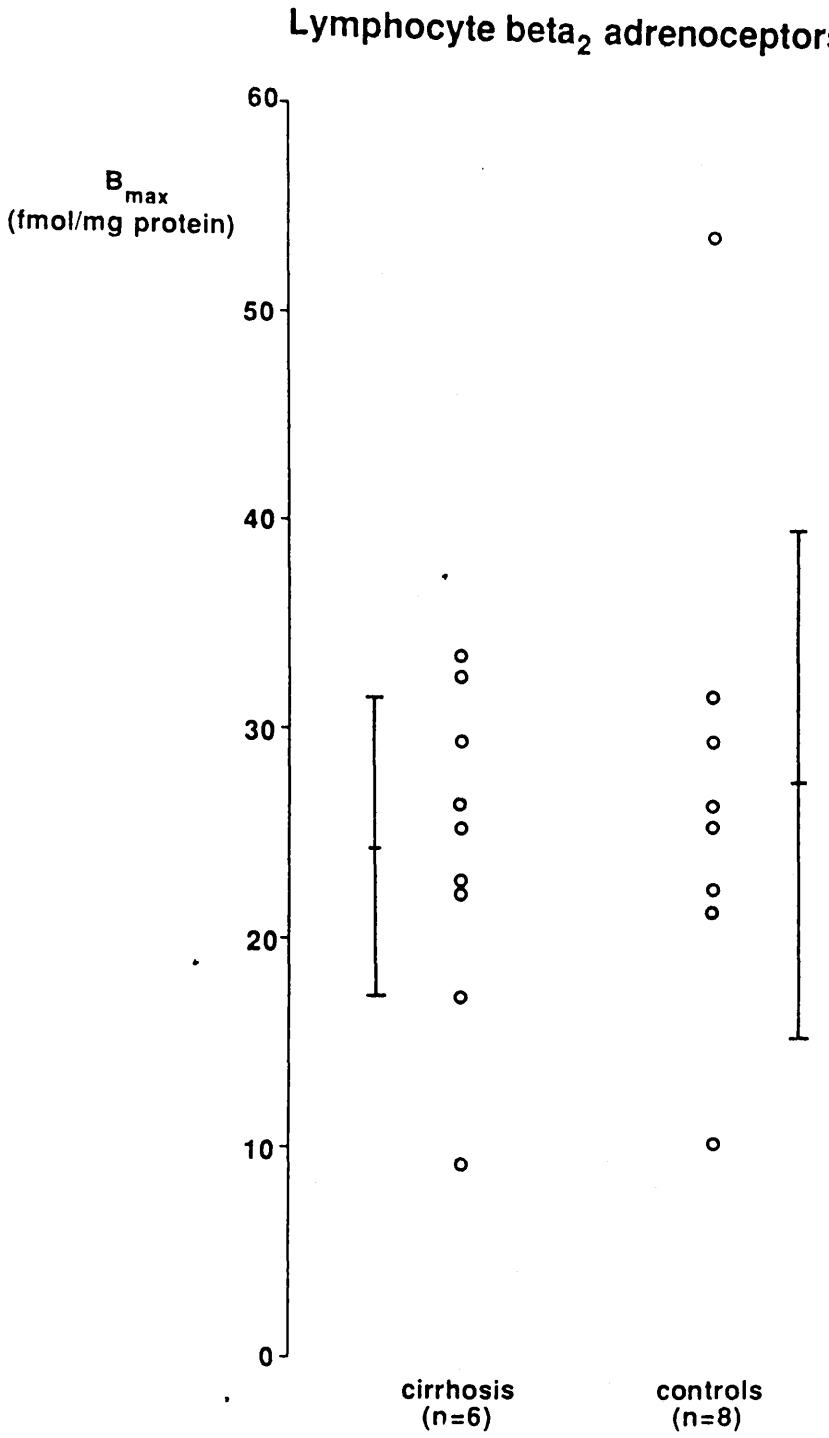

Figure 7.3.

Bmax for lymphocyte $beta_2$ receptors (Error bars represent mean and standard deviation).

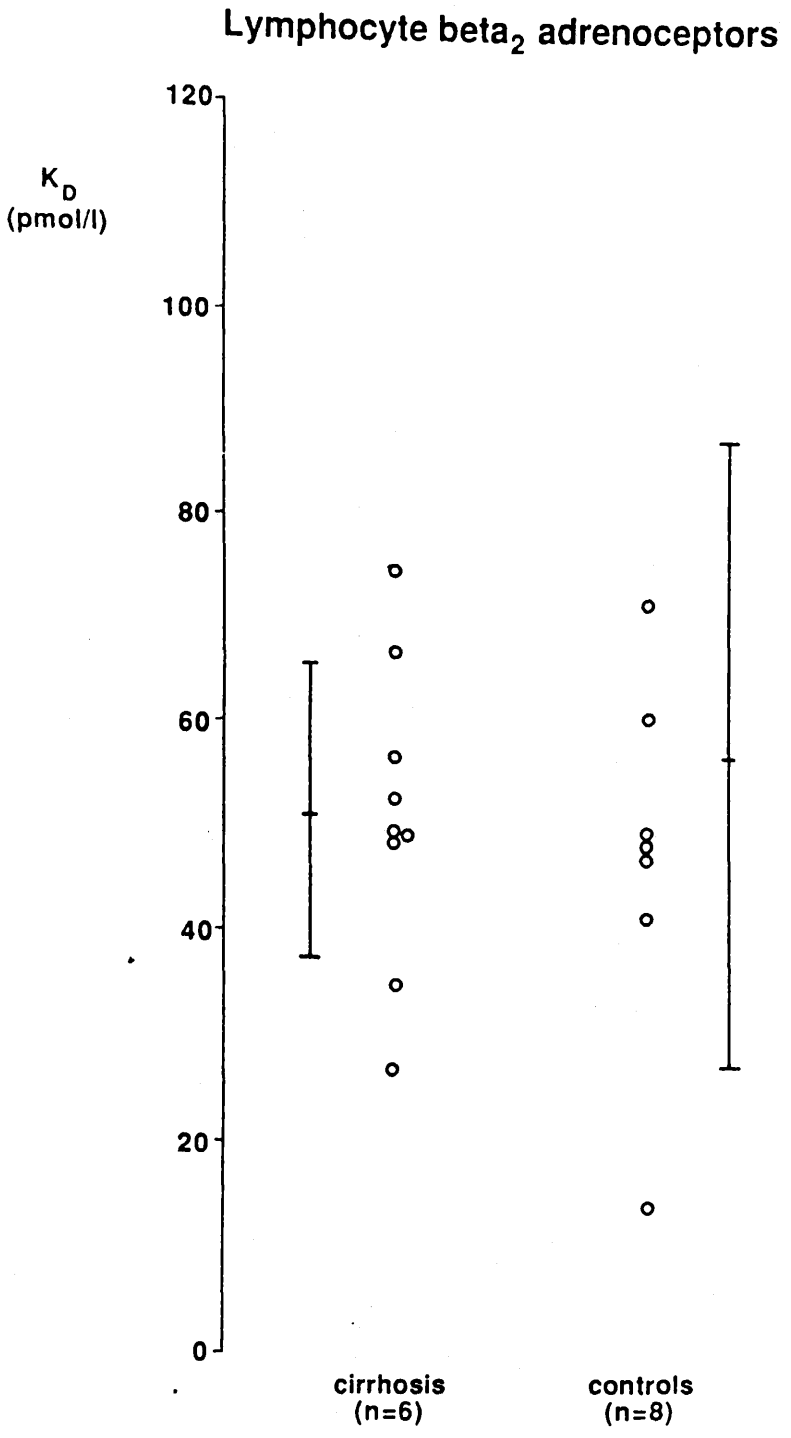

Figure 7.4.

K_D for lymphocyte $beta_2$ receptors (Error bars represent mean and standard deviation).

correlation to emerge from the data on receptor affinity was a reduction in affinity in patients with the most elevated plasma bilirubin levels ($r = 0.79$, $p < 0.02$), the opposite trend to that seen with the affinity of the platelet alpha receptors.

7.2.4. Discussion

The lack of any reduction in Bmax argues against down-regulation of the lymphocyte $beta_2$ adrenoeptors in moderately severe cirrhosis in this situation. As in the platelet studies, a number of subjects could not be included as their Scatchard plots were too unreliable to calculate K_D or Bmax with confidence. This applied to 4 cirrhotics and 5 controls, i.e. the receptor analysis was performed on 9 cirrhotics and 8 controls. Does this alter the validity of the data? As in the platelet study, the group of cirrhotics with valid results are indistinguishable from those excluded in terms of catecholamine levels, heart rate, blood pressure and liver function tests. Even with the reduced number of results, the power of the study to detect a difference of 1 standard deviation exceeds 80%. Finally, the confidence intervals of the difference in the mean for K_D and Bmax do not indicate any trend. It therefore seems likely that the negative conclusion is valid.

During the course of this study Gerbes and co-workers in Munich published the results of a similar study (273). In a similar number of cirrhotics of comparable disease

severity, they also found no change in density or affinity of lymphocyte $beta_2$ adrenoceptors. However they reported that in a subgroup of 4 patients with severe ascites, there was a reduction in receptor density. This was interpreted as evidence of down-regulation in this group. Since only 2 of my cirrhotics had ascites, the studies do not necessarily contradict each other. However, neither of my ascitic patients had low Bmax values. One important difference in the two studies is the question of diuretic therapy. None of the subjects in the present study had taken diuretics within 3 days of the sampling (although 6 had been on diuretics prior to this). In contrast, most of the cirrhotics in the Munich study were on continuing diuretic therapy at the time of study. Since diuretics are known to reduce lymphocyte beta adrenoceptor density (278), this is a possible confounding factor in the Munich study. Although beta adrenoceptor status in severe ascites remains a matter of debate, cirrhosis severe enough to significantly increase sympathetic activity, lower blood pressure and increase heart rate does not cause lymphocyte $beta_2$ adrenoceptor down-regulation. This supports the normal heart rate response to isoprenaline, a beta agonist, in cirrhosis (Chapter 6). This is also in keeping with the clinical status of cirrhotic patients, where the sympathetic over-activity, although unable to correct the reduced blood pressure, does result in an appropriate increase in heart rate - a beta adrenoceptor-mediated response. The present

study is therefore unable to confirm the hypothesis of the Munich group, that the reported decreased efficacy of propranolol to prevent rebleeding from oesophageal varices in severe cirrhosis is due to down-regulation of beta adrenoceptors in this group.

CHAPTER 8

CONCLUSIONS AND IMPLICATIONS

CONCLUSIONS AND IMPLICATIONS

The work described in this thesis was inspired by the observation that plasma noradrenaline concentrations are increased in cirrhosis. The first experiment demonstrated that this increase was the result of increased spillover of noradrenaline into the circulation from sympathetic nerve endings, and hence represents an overall increase in the activity of the sympathetic nervous system in cirrhosis. Surprisingly, the clearance of noradrenaline from plasma was slightly increased in cirrhosis. This observation, which has been confirmed by others, remains unexplained. The answer lies in regional studies of sympathetic activity which are outwith the scope of this thesis.

Having established that plasma noradrenaline is a reasonable indicator of the degree of sympathetic activity in cirrhosis, plasma noradrenaline was measured in 40 patients with cirrhosis of varying duration, severity and aetiology. The degree of sympathetic activation related to the severity of the liver impairment, in agreement with other recent work. There has been much enthusiasm in the current medical literature regarding the significance of the sympathetic nervous system in the pathogenesis of ascites. The early studies indicating overactivity of the renin-angiotensin-aldosterone system in cirrhosis were greeted with similar enthusiasm, only for later work to demonstrate that the latter system is only activated in a minority of cirrhotics with ascites, and is normal or even suppressed

earlier in the disease process. The increased sympathetic activity which was not due to confounding variables such as the ingestion of diuretics or alcohol was present even in cirrhotic patients who had never had ascites. Therefore the stimulus to sympathetic activity is not simply vascular underfilling consequent to the fluid shifts of ascites.

The potential candidates for the stimulus to sympathetic activity are increased hepatic sinusoidal pressure and systemic vascular underfilling. The former could occur as portal hypertension develops and influences sympathetic activity by activating hepatic baroreceptors. Thus sympathetic activity could increase without reduced systemic intravascular volume. If this were the case, increased sympathetic activity in cirrhotic patients without ascites would not disprove the so called "overflow" theory of ascites. However this hepatic baroreceptor reflex has only been demonstrated in non cirrhotic dogs, not in cirrhotic man, and most authors have taken sympathetic overactivity as an indication of reduced effective blood volume and therefore supporting the traditional, or "underfilling" theory of ascites. The possible reasons for reduced effective blood volume include splanchnic pooling of blood due to portal hypertension, systemic vasodilatation, and arteriovenous shunting.

In addition to providing valuable indirect evidence on the state of vascular filling in cirrhosis, this sympathetic overactivity may itself be important in the renal sodium

retention which is critical to the formation of ascites. Renal sympathetic stimulation causes sodium reabsorption from the proximal tubule. This may be important in the early development of ascites and could explain the phenomenon of failed mineralocorticoid escape in cirrhosis. However in established ascites the success of the aldosterone antagonist spironolactone testifies to the importance of distal tubular reabsorption of sodium.

This study also demonstrated that high concentrations of plasma noradrenaline carry a poor prognosis. This is supported by other workers who found plasma noradrenaline to be a powerful and independent prognostic indicator. Most severely decompensated cirrhotic patients also have activation of the renin-angiotensin-aldosterone system, which is likewise associated with a poor prognosis. These findings might be explained by the adverse effects of both these systems on renal blood flow. There is some evidence that the functional renal failure which can complicate cirrhosis with ascites, and which is probably the result of renal cortical vasoconstriction, is the result of an imbalance between renal vasoconstrictors (e.g. sympathetic nervous system and angiotensin II) and renal vasodilators (e.g. renal prostaglandins and bradykinin).

The recent identification of atrial natriuretic peptide (ANP) led to speculation that deficiency of such a natriuretic substance might contribute to the sodium

retention of ascites. However, in the presence of ascites, ANP levels are modestly increased rather than decreased. This presumably reflects a reflex response (as yet undefined) to sodium retention occurring by other mechanisms, and suggests that ANP deficiency is not a cause of sodium retention in ascites. Although it has potent vasodilatory properties, the levels found in cirrhosis do not suggest ANP as a major cause of the systemic vasodilatation of cirrhosis, although it may be important in some individuals.

The second half of this thesis concerns the haemodynamic disturbance of cirrhosis. Patients with cirrhosis have reduced vascular resistance, indicated by systemic hypotension despite increased cardiac output. The question of whether autonomic neuropathy, in the conventional sense, might contribute to this haemodynamic disturbance was considered. Parasympathetic cardiovascular responses were impaired in severe cirrhosis, and this did not appear to be an alcohol effect, but the changes were mild and were not associated with the clinical manifestations of neuropathy. Reflex sympathetic response to isometric exercise was markedly reduced, but it was not clear whether this was due to an abnormality within the reflex arc or at the level of the target organ, i.e. vascular smooth muscle. Experiments infusing exogenous sympathetic and non sympathetic vasoactive agents clarified the situation. Firstly baroreceptor function was found to

be normal, or even slightly enhanced. Secondly, the pressor response to noradrenaline, the neurotransmitter of the sympathetic nervous system was impaired in cirrhosis. This confirmed the suspicion that the impairment of reflex sympathetic responses was due to a defect beyond the reflex arc. It also confirmed that the anomaly of vasodilatation despite increased sympathetic activity was due to some form of block in the normal sympathetically-induced vasoconstriction. The remaining experiments in the thesis were designed to try to identify the site of this block.

The reduced pressor response to angiotensin as well as to noradrenaline suggested a general reduction in vascular reactivity, but could represent parallel desensitisation of the vascular tree to the effects of the two vasoconstricting systems, both of which are commonly over-active in severe cirrhosis. The demonstration of impaired cardiovascular responses to two further selective alpha agonists (phenylephrine and alphamethylnoradrenaline) but not to a beta agonist (isoprenaline) indicated that the reduced pressor response to noradrenaline was not part of a generalised sympathetic desensitisation. The fact that the response to isoprenaline, which is principally a cardiac effect, was normal unlike the other four agents, which all exert their effect principally on the peripheral vasculature, localised the impaired sympathetic response to vascular smooth muscle.

The next question to be considered was whether this

defect involved the adrenergic receptors which mediate the vasoconstrictor response. The adrenoceptor status might be altered in three ways. Firstly the high concentrations of circulating catecholamines might occupy the receptors and prevent further activation. Secondly the receptors might be occupied by catecholamine-like substances which the cirrhotic liver has failed to inactivate ("false neurotransmitters"). Thirdly the increased sympathetic activity could lead to a reduction in the number of receptors, i.e. "down regulation". Unfortunately the receptor most relevant, i.e. the $alpha_1$ receptor on vascular smooth muscle, is not accessible to study in man. As a compromise an alpha receptor which is accessible, namely the $alpha_2$ receptor on platelets, was studied. In fact radioligand binding studies revealed that both the number of receptors and their affinity for the ligand were normal in cirrhosis. Beta adrenoceptor status was also studied using circulating lymphocytes and found to be normal. This is in keeping with the normal chronotropic response to beta stimulation by isoprenaline. Prior occupancy of receptor sites by circulating catecholamines or false neurotransmitters seems unlikely in view of the normal affinity of the alpha receptors. The lack of any down regulation of platelet $alpha_2$ receptors does not necessarily imply the same for vascular $alpha_1$ receptors. This is a problem which cannot be resolved with human studies at present and would require work using one of the animal

models of cirrhosis. If there is indeed no down regulation of vascular alpha$_1$ receptors, this would suggest that the defect causing impaired sympathetic response lies within the smooth muscle cell itself - i.e. a "post-receptor" defect. The reduced angiotensin reactivity has also been shown to be a post-receptor defect in experimental animals. It seems likely that one common defect at the intracellular level is responsible for the impaired response to both systems.

What are the implications of the localisation of this defect in vascular responsiveness? It suggests that the vasodilatation of cirrhosis is due to local effects on vascular smooth muscle and that future research should concentrate on attempting to identify possible pharmacological agents responsible. Candidates include substances escaping into the systemic circulation from the portal system via shunts or a failing liver. These could be physiological e.g. gut peptides, or pathological, e.g. endotoxins. Other candidates include enkephalins and systemic prostaglandins, e.g. prostacyclin. ANP is unlikely to be responsible as it only reached vasodilatory concentrations in a few patients. Better understanding of the mechanisms of vasodilatation of these various agents may allow selection of those likely to cause a defect at the site identified. The studies in this thesis indicate that certain other potential explanations for the vasodilatation must be considered less likely. These include a decrease in physiological vasoconstrictors, and interference by false

neurotransmitters.

Is the cause of the vasodilatation important? Although this thesis does not specifically consider this question, it seems likely that the peripheral vasodilatation may be a principal cause for sympathetic overactivity, which in turn could initiate sodium retention and ascites.

In summary, the studies described in this thesis have examined certain aspects of the sympathetic nervous system in cirrhosis of the liver. They have established that sympathetic activity is increased in cirrhosis, especially where severe liver impairment is present, but also earlier in the disease process. They considered the question of vasodilatation in cirrhosis despite sympathetic overactivity, established that there was impaired sympathetic vascular responsiveness both by reflex stimulation and exogenous agonist infusions, and accumulated indirect evidence suggesting that this is likely to be a post-receptor defect in vascular smooth muscle. The cause of this defect (and hence the vasodilatation) may hold the key to the initiation of ascites, of which the sympathetic nervous system appears to be an important mediator.

REFERENCES

1. 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.

2. Brown J. A remarkable account of a liver, appearing glandulous to the eye. Philos Trans R Soc 1685; 15-16: 1265-1268.

3. Flint A. Clinical report on hydroperitoneum, based on an analysis of forty six cases. Am J Med Sci 1863; 45: 306-339.

4. Farnsworth EB, Krakusin JB. Electrolyte partition in patients with edema of various origins. Qualitative and quantitative definitions of cations and anions in hepatic cirrhosis. J Lab Clin Med 1948; 33: 1545-1554.

5. Faloon WW, Eckhardt, RD, Cooper AM, Davidson CS. The effect of human serum albumin, mercurial diuretics and a low sodium diet on sodium excretion in patients with cirrhosis of the liver. J Clin Invest 1949; 28: 595-602.

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

7. Papper S, Rosenbaum JD. Abnormalities in the excretion of water and sodium in "compensated" cirrhosis of the liver. J Lab Clin Med 1952; 40: 523-530.

8. 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: 723-731.

9. Atkinson M. Ascites in liver disease. Postgrad Med J 1956; 32: 482-485.

10. Starling EH. On the absorption of fluids from the connective tissue spaces. J Physiol (London) 1896; 19: 312.

11. Witte CL, Witte MH, Dumont AE. Lymph protein in hepatic cirrhosis and experimental hepatic and portal venous hypertension. Trans Am Surg Assoc 1968; 86: 256-259.

12. Dumont AE, Mulholland JH. Flow rate and composition of thoracic duct lymph in patients with cirrhosis. N Engl J Med 1960; 263: 471-474.

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

14. 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, 1297-1306.

15. Lieberman FL, Ito S, Reynolds TB. Effective plasma volume in cirrhosis with ascites. Evidence that a decreased value does not account for renal sodium retention, a spontaneous reduction in glomerular filtration rate (GFR) and a fall in GFR during drug-induced diuresis. J Clin Invest 1969; 48: 975-981.

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

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

18. Wernze H, Spech HJ, Muller G. Studies on the activity of the renin-angiotensin-aldosterone system (RAAS) in patients with cirrhosis of the liver. Klin Wschr 1978; 56, 389-397.

19. Wilkinson SP, Smith IK, Williams R. Changes in plasma renin activity in cirrhosis: a reappraisal based on studies in 67 patients. Hypertension 1979; 1: 125-129.

20. Papper S. The role of the kidney in Laennec's cirrhosis of the liver. Medicine (Baltimore) 1958; 37, 299-316.

21. Groszman R, Kotelanski B, Cohn JN, Khatri IM. Quantitation of portasystemic shunting from the splenic and mesenteric beds in alcoholic liver disease. Am J Med 1972; 53, 715-722.

22. Bredfelt JE, Groszman, RJ. Haemodynamics of portal hypertension. In: Epstein M, ed. The kidney in liver disease. New York: Elsevier, 1983; 281-292.

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

24. Kontos HA, Shapiro W, Mauck HP, Patterson JL. General and regional circulatory alterations in cirrhosis of the liver. Am J Med 1964; 37, 526-534.

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

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

27. Vlahcevic ZR, Adham NF, Jick H, Moore EW, Chalmers TC. Renal effects of acute expansion of plasma volume in cirrhosis. N Engl J Med 1965; 272: 387-390.

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

29. Le Veen HH, Christoudias G, Moon IP, Luft R, Falk G, Grosberg S. Peritoneovenous shunting for ascites. Ann Surg 1974; 180: 580-590.

30. Le Veen HH, Wapnick S, Grosberg S, Kinney MJ. Further experiences with peritoneovenous shunt for ascites. Ann Surg 1976; 184: 574-579.

31. Witte MH, Witte CL, Jacobs S, Kut R. Peritoneovenous (Le Veen) shunt. J A M A 1978; 239, 31-33.

32. Berkowitz HD, Mullen JL, Miller LD, Rosato EF. Improved renal function and inhibition of renin and aldosterone secretion following peritoneovenous (Le Veen) shunt. Surgery 1978; 84, 120-126.

33. Levy M, Wexler MJ, McCaffrey C. Sodium retention in dogs with experimental cirrhosis following removal of ascites by continuous peritoneovenous shunting. J Lab Clin Med 1979; 94, 933-946.

34. Epstein M. Renal sodium handling in cirrhosis. In: Epstein M, ed. The kidney in liver disease. New York: Elsevier, 1983; 25-53.

35. Epstein M, Pins DS, Arrington R, De Nunzio AG, Engstrom R. Comparison of water immersion and saline infusion as a means of inducing volume expansion in man. J Appl Physiol 1975; 39: 66-70.

36. Wilkinson SP, Williams R. Renin-angiotensin-aldosterone system in cirrhosis. Gut 1980; 21: 545-554.

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

38. Shapiro MD, Nicholls KM, Groves BM et al. Inter-relationship between cardiac output and vascular resistance as determinants of effective arterial blood volume in cirrhotic patients. Kidney Int 1985; 28: 206-211.

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

40. Denison EK, Lieberman FL, Reynolds TB. 9-α-fluoro-hydrocortisone-induced ascites in alcoholic liver disease. Gastroenterology 1971; 61, 497-503.

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

42. Skorecki KL, Brenner BM. Body fluid homeostasis in congestive heart failure and cirrhosis with ascites. Am J Med 1982; 72: 323-338.

43. Brown JJ, Davies DL, Lever AF, Robertson JIS. Variations in plasma renin concentrations in several phyhsiological and pathological states. Can Med Assoc J 1964; 90: 201-206.

44. Massani ZM, Finkelman S, Worcel M, Agrest A, Palacini AC. Angiotensin blood levels in hypertensive and non-hypertensive diseases. Clin Sci 1966; 30: 473-483.

45. Bongiovanni AM, Eisenmenger WJ. Adrenal cortical metabolism in chronic liver disease. J Clin Endocrinol 1951; 11: 152-172.

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

47. Arroyo J, Bosch J, Gaya J et al. Plasma renin activity and urinary sodium excretion as prognostic indicators in non-azotemic cirrhosis with ascites. Ann Intern Med 1981; 94: 198-201.

48. Shaldon C, Beacock JH, Walker RM. The portal venous content of adrenaline and noradrenaline in portal hypertension. Lancet 1961; 1: 957-961.

49. Siegel JH, Harrison RC. Portal venous catecholamines in portal hypertension. Lancet 1963; 2: 1357-1358.

50. Evans CS, Kay AW. Catecholamines in portal venous blood in portal hypertension. Lancet 1964; 2: 387-388.

51. Joly JG, Leduc J, Bernie J et al. Catecholamine levels in portal, hepatic and systemic venous blood in portal hypertension. Lancet 1967; 2: 121-123.

52. Engelman K, Portnoy B. A sensitive double-isotope derivative assay for norepinephrine and epinephrine. Normal resting human plasma levels. Circ Res 1970; 26: 53-57.

53. Ciplea A, Bubuianu E. Cerebrospinal fluid, blood and ascites catecholamines in hepatic cirrhosis. Physiologie 1979; 16: 19-24.

54. Henricksen JH, Christensen NJ, Ring-Larsen H. Noradrenaline and adrenaline concentrations in various vascular beds in patients with cirrhosis. Relation to haemodynamics. Clin Physiol 1981; 1: 293-304.

55. 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.

56. Arroyo V, Planas R, Gaya J et al. Sympathetic nervous activity, renin-angiotensin system and renal excretion of prostaglandin E_2 in cirrhosis. Relationship to functional renal failure and sodium and water excretion. Eur J Clin Invest 1983; 13: 271-278.

57. Muller J, Barajas L. Electron microscopic and histochemical evidence for a tubular innervation in the renal cortex of the monkey. J Ultrastruc Res 1972; 41: 533-549.

58. Barajas L, Muller J. The innervation of the juxtaglomerular apparatus and surrounding tubules: a quantitative analysis by serial section electron microscopy. J Ultrastruc Res 1973; 43: 107-132.

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

60. Bello-Reuss E. Effect of catecholamines on fluid reabsorption by the isolated proximal convoluted tubule. Am J Physiol 1980; 238: F347-F352.

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

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

63. Kew MC, Brunt PW, Varma RR, Hourigan KJ, Williams HS, Sherlock S. Renal and intrarenal blood flow in cirrhosis of the liver. Lancet 1971; 2: 504-509.

64. Arroyo V, Rodes J, Gutierrez-Lizarraga MA. Prognostic value of spontaneous hyponatraemia in cirrhosis with ascites. Dig Dis 1976; 21: 249-256.

65. Verney EB. The antidiuretic hormone and the factors which determine its release. Proc Royal Soc London Ser B 1947; 135: 25-106.

66. Linas SL, Anderson RJ, Guggenheim SJ, Robertson GL, Berl T. Role of vasopressin in impaired water excretion in conscious rats with experimental cirrhosis. Kidney Int 1981; 20: 173-180.

67. Bichet D, Szatalowicz, V, Chaimovitz C, Schrier RW. Role of vasopressin in abnormal water excretion in cirrhotic patients. Ann Intern Med 1982; 96: 413-417.

68. Schrier RW, Berl T, Anderson RJ. Osmotic and non-osmotic control of vasopressin release. Am J Physiol 1979; 236: F321-F332.

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

70. Epstein M. Derangements of renal water handling in liver disease. Gastroenterology 1985; 89: 1415-1425.

71. Epstein M, Weitzman RE, Preston S, De Nunzio AG. Relationship between plasma arginine vasopressin and renal water handling in decompensated cirrhosis. Min Elect Metab 1984; 10: 155-165.

72. Blendix LM, Reznick RK, Langer B, Taylor BR, Seif S. Hyponatraemia and ADH secretion in hepatic ascites. Gut 1982; 23: A449.

73. Sherlock S. Diseases of the liver and biliary system (6th edition) Oxford: Blackwell Scientific Publications, 1982; 121-129.

74. Epstein M, Pins DS, Schneider N, Levinson R. Determinants of deranged sodium and water homeostasis in decompensated cirrhosis. J Lab Clin Med 1976; 87: 822-827.

75. Fogel MR, Sawhney UK, Neal EA, Miller RG, Knauer CM, Gregory PB. Diuresis in the ascitic patient: a randomised controlled trial of three regimens. J Clin Gastroenterol 1981; 3 (Suppl.1): 73-80.

76. Perez-Aguso RM, Arroyo V, Planas R, et al. Randomised comparative study of efficacy of furosemide venous spironolactone in non-azotaemic cirrhosis with ascites: relationship between the diuretic response and the activity of the renin-aldosterone system. Gastroenterology 1984; 84: 961-968.

77. Campra JL, Reynolds TB. Effectiveness of high dose spironolactone therapy in patients with chronic liver disease and relatively refractory ascites. Am J Dig Dis 1978; 23: 1025-1030.

78. Eggert RC. Spironolactone diuresis in patients with cirrhosis and ascites. Br Med J 1970; 4: 401-403.

79. Arroyo V, Gines P, Rodes J. Treatment of ascites in patients with cirrhosis of the liver. J Hepatol 1986; 2: 504-512.

80. Sherlock S, Senewiratne B, Scott A, Walker JG. Complications of diuretic therapy in hepatic cirrhosis. Lancet 1966; 1: 1049-1053.

81. Hillenbrand P, Sherlock S. Use of metolazone in the treatment of ascites due to liver disease. Br Med J 1971; 4: 266-270.

82. Lang GR, Westenfelder C, Nascimento L, Dhupelia VB, Arrida JA, Kane RE. Metolazone and spironolactone in cirrhosis and the nephrotic syndrome. Clin Pharmacol Ther 1977; 21: 234-243.

83. Epstein M, Lepp BA, Hoffman DS, Levinson R. Potentiation of furosemide by metolazone in refractory oedema. Curr Ther Res 1977; 21: 656-667.

84. Senewiratne B, Sherlock S. Amiloride ("MK870") in patients with ascites due to cirrhosis of the liver. Lancet 1968; 1: 120-122.

85. Lieberman FL, Reynolds TB. The use of ethacrynic acid in patients with cirrhosis and ascites. Gastroenterology 1965; 49: 531-538.

86. Bernardi M, De Palma R, Trevisani F, et al. Effects of a new loop diuretic (muzolimine) in cirrhosis with ascites: comparison with furosemide. Hepatology 1986; 6: 400-405.

87. Espiner EA, Nicholls MG. Hormones and fluid retention in cirrhosis. Lancet 1982; 2: 501-502.

88. Shepherd AN, Neligan P, Hayes PC. Captopril and resistant ascites. Lancet 1983; 1: 1391.

89. Schlienger JL, Imbs JL, Chabrier G, Doppoel M, Imler M. Tratement de l'ascite cirrhotique. Absence d'effect favorable du captopril. Nouve Presse Med 1982; 11: 1570.

90. Ring T. Captopril and resistant ascites: a word of caution. Lancet 1982; 2: 165.

91. Jorgensen F, Badskjaer J, Nordin H. Captopril and resistant ascites. Lancet 1983; 2: 405.

92. Pariente EA, Bataille C, Bercoff E, Lebrec D. Acute effects of captopril on systemic and renal haemodynamics and on renal function in cirrhotic patients with ascites. Gastroenterology 1985; 88: 1255-1259.

93. Shroeder ET, Anderson GH, Goldman SH, Streeten DHP. Effect of blockade of angiotensin II on blood pressure, renin and aldosterone in cirrhosis. Kidney Int. 1976; 9: 511-519.

94. Hata T, Ogihara T, Mikami H, Nakamaru M, Mandai T, Kumahara Y. Blood pressure response to (1-sarcosine, 8-isoleucine) angiotensin II in patients with liver cirrhosis and ascites. Jpn Circ J 1979; 43: 37-41.

95. Arroyo V, Boxch J, Mauri M, Ribera F, Navarro-Lopez F, Rodes J. Effect of angiotensin II blockade on systemic and hepatic haemodynamics and on the renin angiotensin aldosterone system in cirrhosis with ascites. Eur J Clin Invest. 1981; 11: 221-229.

96. Saruta T, Eguchi T, Saito I. Angiotensin antagonists in liver disease. In: Epstein M, ed. The kidney in liver disease. New York: Elsevier. 1983; 441-450.

97. Shear L, Ching S, Gabuzda GJ. Compartmentalisation of ascites and edema in patients with hepatic cirrhosis. N Engl J Med 1982; 282: 1391-1396.

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

99. Quintero E, Gines P, Arroyo V et al. Paracentesis versus diuretics in the treatment of cirrhotics with tense ascites. Lancet 1985; 1: 611-612.

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

101. Salerno F, Badalamenti S, Tempini S et al. Paracentesis versus diuretics in the treatment of cirrhotic ascites. Preliminary results of a multicentre randomised controlled trial. Verona: Associacione Italiana per lo studio del Fagata Proc 1986.

102. Parbhoo SP, Ajdukiewicz A, Sherlock S. Treatment of ascites by continuous ultrafiltration and reinfusion of protein concentrate. Lancet 1974; 1: 949-950.

103. Blendis LM, Greig PD, Langer B, Baigrie RS, Ruse J, Taylor B. The renal and haemodynamic effects of the peritoneovenous shunt for intractable hepatic ascites. Gastroenterology 1979; 77: 250-257.

104. Kostreva DR, Castaner A, Kampine JP. Reflex effects of hepatic baroreceptors on renal and cardiac sympathetic nerve activity. Am J Physiol 1980; 238: R390-R394.

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

106. Papper S. The hepatorenal syndrome. Clin Nephrol 1975; 4 41-44.

107. Vesin P, Rueff B, Traverso H, Hirsch-Maric H, Cattan R. L'insufficence renale fonctionelle du cirrhotique ascitique. Etude critique du role des diuretiques. Bull Mem Hop Paris 1962; 113: 787-795.

108. Helwig JG, Schutz CB. A liver kidney syndrome: clinical, pathological and experimental studies. Surg Gynaecol Obstet 1932; 55: 570-580.

109. Leading article. Hepatorenal syndrome or hepatic nephropathy? Lancet 1980; 1: 801-803.

110. Koppel MH, Coburn JW, Mims MM, Goldstein H, Boyle JD, Rubini ME. Transplantation of cadaveric kidneys from patients with hepatorenal syndrome. Evidence for functional nature of renal failure in advanced liver disease. N Engl J Med 1969; 280: 1367-1371.

111. Epstein M, Berk D, Hollenberg N et al. Renal failure in patients with liver cirrhosis. The role of active vasoconstriction. Am J Med 1970; 49: 175-185.

112. Ring-Larsen H. Renal blood flow in cirrhosis: relation to systemic and portal haemodynamics and liver function. Scand J Clin Lab Invest 1977; 37: 635-642.

113. Bomzon A. Sympathetic contgrol of the renal circulation. J Auton Pharmacol 1983; 3: 37-46.

114. Bomzon L, Rosendorff C, Scriven DRL, Farr J. The effect of noradrenaline, adrenergic blocking agents and tyramine on the intrarenal distribution of blood flow in the baboon. Cardiovasc Res 1975; 9: 314-322.

115. Henricksen JH, Ring-Larsen H, Kanstrup I-L, Christensen NJ. Splanchnic and renal elimination and release of catecholamines in cirrhosis. Evidence of enhanced sympathetic nervous activity in patients with decompensated cirrhosis. Gut 1984; 25: 1034-1043.

116. Willett I, Esler M, Burke F, Leonard P, Dudley F. Total and renal sympathetic nervous activity in alcoholic cirrhosis. J Hepatol 1985; 1: 639-648.

117. Baldus WP. Etiology and management of renal failure in cirrhosis and portal hypertension. Ann NY Acad Sci 1970; 170: 267-276.

118. Gatta A, Merkel C, Milani L, Zuin R, Ruol A. Enhanced renal sympathetic tone in liver cirrhosis: evaluation by intrarenal administration of dihydroergocristine. Nephron 1982; 30: 364-367.

119. Wilkinson SP, Bernardi M, Smith IK, Jowett JP, Slater JDH, Williams R. Effects of beta adrenergic blocking drugs on the renin-aldosterone system, sodium excretion and renal haemodynamics in cirrhosis with ascites. Gastroenterology 1977; 73: 659-663.

120. Barnardo DE, Summerskill WHJ, Strong CG, Baldus WP. Renal function, renin activity and endogenous vasoactive substances in cirrhosis. Am J Dig Dis 1970; 15: 419-425.

121. Perez-Ayuso RM, Arroyo V, Camps J et al. Renal kallikrein excretion in cirrhotics with ascites: relationship to renal haemodynamics. Hepatology 1984; 4: 247-252.

122. Guarner C, Colina I, Guarner F, Corzo J, Prieto J, Vilardel F. Renal prostaglandins in cirrhosis of the liver. Clin Sci 1986; 70: 477-484.

123. Arroyo V, Gines, P, Rimola A, Gaya J. Renal function abnormalities, prostaglandins, and effects of non-steroidal anti-inflammatory drugs in cirrhosis with ascites. An overview with emphasis on pathogenesis. Am J Med 1986; 81 (2B): 104-122.

124. Boyer TD, Reynolds TB. The effect of indomethacin on renal blood flow and creatinine clearance in patients with cirrhosis. Gastroenterology 1976; 70: 121A.

125. Levy M, Wexler MJ, Fechner C. Renal perfusion in dogs with experimental cirrhosis: role of prostaglandins. Am J Physiol 1983; 245: F521-F529.

126. Quintero E, Gines P, Arroyo V et al. Sulindac reduces the urinary excretion of prostaglandins and impairs renal function in cirrhosis with ascites. Nephron 1986; 42: 298-303.

127. Laffi G, Daskalopoulos G, Kronberg I, Hsueh W, Gentilini P, Zipser RD. Effects of sulindac and ibuprofen in patients with cirrhosis and ascites. Gastroenterology 1986; 90: 182-187.

128. Wong PY, Talamo RC, Williams GH. Kallikrein-kinin and renin-angiotensin systems in functional renal failure of cirrhosis of the liver. Gastroenterology 1977; 73: 1114-1118.

129. Zipser RD, Kerlin P, Hoefs JC, Zia P, Barg A. Renal kallikrein excretion in alcoholic cirrhosis. Relationship to other vasoactive systems. Am J Gastroenterol 1981; 75: 183-187.

130. Hattori K, Hasumura Y, Takeuchi J. Role of renal kallikrein in the derangement of sodium and water excretion

in cirrhotic patients. Scand J Gastroenterol 1984; 19: 844-848.

131. Kawasaki H, Murawaki Y, Hirayama C. Urinary kallikrein excretion in chronic liver disease and effect of indomethacin. Am J Gastroenterol 1986; 81: 67-70.

132. Wilkinson SP, Gazzard BG, Arroyo V, Moodie H, Williams R. Relation of renal impairment and haemorrhagic diathesis to endotoxaemia in fulminant hepatic failure. Lancet 1974; 1: 521-524.

133. Wilkinson SP, Moodie H, Stamakis JD, Kakker VV, Williams R. Endotoxaemia and renal failure in cirrhosis and obstructive jaundice. Br Med J 1976; 2: 1415-1418.

134. Wilkinson SP. Endotoxins and liver disease. Scand J Gastroenterol 1977; 12: 385-386.

135. Said SI, Hirose T, Kilamura S, Siegel SR. Vasoactive intestinal peptide (VIP): mediator of haemodynamic and respiratory changes in liver cirrhosis? J Clin Invest 1971; 50: 80A.

136. Elias E, Mitchell SJ, Bloom SR. Vasoactive intestinal peptide in cirrhosis. Lancet 1975; 2: 1312.

137. Sullivan SN, Chase RA, Christofides ND, Bloom SR, Williams R. The gut hormone profile of fulminant hepatic failure. Am J Gastroenterol 1981; 76: 338-341.

138. Henriksen JH, Staun-Olsen P, Mogensen B, Fahrenkrug J. Circulating endogenous vasoactive intestinal peptide (VIP) in patients with uraemia and liver cirrhosis. Eur J Clin Invest 1986; 16: 211-216.

139. Kew MC, Varma RR, Samson DJ, Sherlock S. The effect of octapressin on renal and intrarenal blood flow in cirrhosis of the liver. Gut 1972; 13: 293-296.

140. Milani L, Merkel C, Gatta A. Renal effects of aminophylline in hepatic cirrhosis. Eur J Clin Pharmacol 1983; 24: 757-760.

141. Kowalski HJ, Abelmann WH. Cardiac output at rest in Laennec's cirrhosis. J Clin Invest 1953; 32: 1025-1033.

142. Martini GA, Baltzer G, Arndt H. Some aspects of circulatory disturbances in cirrhosis of the liver. Prog Liver Dis 1972; 1: 231-250.

143. Hecker R, Sherlock S. Electrolyte and circulatory changes in terminal liver failure. Lancet 1956; 2: 1121-1125.

144. Mashford ML, Mahon WA, Chalmers TC. Studies of the cardiovascular system in the hypertension of liver failure. N Engl J Med 1962; 267: 1071-1074.

145. Bayley TJ, Segel N, Bishop JM. The circulatory changes in patients with cirrhosis of the liver at rest and during exercise. Clin Sci 1964; 26: 227-235.

146. Ratge D, Brugger G, Wehr M, Bode JC, Wisser H. Catecholamines in the plasma and urine of patients with alcoholic liver damage under resting and exercise conditions. J Clin Chem Clin Biochem 1985; 23: 447-452.

147. Ring-Larsen H, Hesse B, Henriksen JH, Christensen NJ. Sympathetic nervous activity and renal and systemic haemodynamics in cirrhosis: plasma norepinephrine concentration, hepatic extraction and renal release. Hepatology 1982; 2: 304-310.

148. Bernardi M, Trevisani F, Santini C, Ligabue L, Capelli M, Gasbarnini G. Impairment of blood pressure control in patients with liver cirrhosis during tilting: study on adrenergic and renin-angiotensin systems. Digestion 1982; 25: 124-130.

149. Bernardi M, Trevisani F, Santini C et al. Plasma norepinephrine, weak neurotransmitters and renin activity during active tilting in liver cirrhosis: relationship with cardiovascular homeostasis and renal function. Hepatology 1983; 3: 56-64.

150. Lunzer MR, Newman SP, Sherlock S. Skeletal muscle blood flow and neurovascular reactivity in liver disease. Gut 1973; 14: 354-359.

151. Lunzer MR, Manghani KK, Newman SP, Sherlock S, Bernard A, Ginsburg J. Impaired cardiovascular responsiveness in liver disease. Lancet 1975; 2: 382-385.

152. Duncan G, Johnson RH, Lambie DG, Whiteside EA. Evidence of vagal neuropathy in chronic alcoholics. Lancet 1980; 2: 1053-1057.

153. Barter F, Tanner R. Autonomic neuropathy and alcoholic liver disease. Gut 1985; 26: A1139.

154. Decaux G, Cauchie P, Soupart A, Kruger M, Delwiche F. Role of vagal neuropathy in the hyponatraemia of alcoholic cirrhosis. Br Med J 1986; 293: 1534-1536.

155. Murray BM, Paller MS. Decreased pressor reactivity to angiotensin II in cirrhotic rats. Evidence for a post-receptor defect in angiotensin action. Circ Res 1985; 57: 424-431.

156. Villamediana LM, Dieguez G, Sankos JC et al. Response to norepinephrine is not decreased in cirrhotic rats. Eur J Clin Invest 1986; 16: A27.

157. Weinbroum A, Blendis LM, Poucell S, Bomzon A. Temporal relationships between cirrhosis, portal hypertension and systemic haemodymnamics. J Hepatol 1986; 3 (Suppl.1): S173.

158. Finberg JPM, Syrop HA, Better OS. Blunted pressor response to angiotensin and sympathetic amines in the bile duct-ligated dog. Clin Sci 1981; 61: 535-539.

159. Murray BM, Paller MS. Pressor resistance to vasopressin in sodium depletion, potassium depletion and cirrhosis. Am J Physiol 1986; 251: R525-R530.

160. Ames RP, Borkowski AJ, Sicinski AM, Laragh JH. Prolonged infusions of angiotensin II and norepinephrine and blood pressure, electrolyte balance and aldosterone and cortisol secretion in normal man and in cirrhosis with ascites. J Clin Invest 1965; 44: 1171-1186.

161. Johnston CI, Jose AD. Reduced vascular response to angiotensin II in secondary hyperaldosteronism. J Clin Invest 1963; 42: 1411-1420.

162. Lenz K, Hortnagl H, Magometschnigg D, Kleinberger G, Drunl W, Laggner A. Function of the autonomic nervous system in patients with hepatic encephalopathy. Hepatology 1985; 5: 831-836.

163. Laragh JG, Cannon PJ, Bentzel CJ, Scinski AM, Meltzer JI. Angiotensin II, norepinephrine and renal transport of electrolytes and water in normal man and in cirrhosis with ascites. J Clin Invest 1963; 42: 1179-1192.

164. Ramond M-J, Cornoy E, Lebrec D. Alteration in isoprenaline sensitivity in patients with cirrhosis: evidence of an abnormality of the sympathetic nervous activity. Br J Clin Pharmacol 1986; 21: 191-196.

165. Lebrec D, Nouel O, Corbic M, Benhamou J-P. Propranolol - a medical treatment for portal hypertension. Lancet 1980; 2: 180-182.

166. Lebrec D, Poynard T, Hillon P, Benhamou J-P. Propranolol for prevention of recurrent gastrointestinal bleeding in patients with cirrhosis. N Engl J Med 1981; 305: 11371-1374.

167.Willett IR, Esler M, Jennings G, Dudley FJ. Sympathetic tone modulates portal venous pressure in alcoholic cirrhosis. Lancet 1986; 2: 939-943.

168. Moreau R, Lee SS, Hadengue A, Braillon A, Lebrec D.

Haemodynamic effects of a clonidine-induced decrease in sympathetic tone in patients with cirrhosis. Hepatology 1987; 7: 149-154.

169. Hortnagl H, Singer E, Lenz K, Kleinberger G, Lochs H. Substance P is markedly increased in plasma of patients with hepatic coma. Lancet 1984; 1: 480-483.

170. Thornton JR, Dean H, Losowsky MS. Is ascites caused by impaired hepatic inactivation of circulating endogenous opioid peptides. Gut 1986; 27: A1250.

171. Fernandez-Cruz A, Marco J, Cuadrado LM, et al. Plasma levels of atrial natriuretic peptide in cirrhotic patients. Lancet 1985; 2: 1439-1440.

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

173. Gines P, Jimenez W, Navason M, et al. Atrial natriuretic factor (ANF) in cirrhosis: plasma levels, cardiac release and splanchnic extraction. J Hepatol 1986; 3(Suppl.1): S30.

174. Vinel JP, Denoyel P, Chabrier P, Cales P, Pascal JP. Relationships between atrial natriuretic factor, plasma renin activity and plasma volume in cirrhotic patients with and without ascites. Gastroenterology 1987; 92: 1789.

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

176. Wernze H, Burghardt W. Atrial natriuretic peptide, the sympathetic nervous system and decompensated cirrhosis. Lancet 1986; 1: 331.

177. Burghardt W, Diehl K-L, Wernze H. Atrial natriuretic peptide is not increased in compensated and decompensated cirrhosis: relation to sodium excretion, plasma catecholamines, renin and aldosterone. J Hepatol 1986; 3(Suppl.1): S31.

178. Simon D, Bonkovsky H, Hartle D, McCain R, Wells J, Galambos J. Atrial natriuretic peptides (ANP) in cirrhosis: effect of therapeutic paracentesis. Gastroenterology 1987; 92: 1777.

179. Guarner F, Guarner C, Prieto J et al. Increased synthesis of systemic prostacyclin in cirrhotic patients. Gastroenterology 1986; 90: 687-694.

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

181. Nespoli A, Chiara O, Clement MG, Dugnino G, Berilacqua G, Aguggini G. The cardiorespiratory impairment in cirrhosis and sepsis. An experimental interpretation using octopamine infusion. Circ Shock 1983; 10: 15-30.

182. Hortnagl H, Lochs H, Kleinberger G. Plasma catecholamines in hepatic coma and liver cirrhosis: role of octopamine. Klin Wschr 1981; 59: 1159-1164.

183. Child CG III. The hepatic circulation and portal hypertension. Philadelphia: WB Saunders 1954.

184. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973; 60: 646-649.

185. Sever PS, Birch M, Osikowska B, Tunbridge RDG. Plasma noradrenaline in essential hypertension. Lancet 1977; 1: 1078-1081.

186. MacGilchrist AJ, Howes LG, Hawksby C, Reid JL. The effects of aging on plasma noradrenaline clearance and spillover rate using tritiated noradrenaline kinetics. Clin Sci 1987; 72(Suppl.16): 73p.

187. Rubin PC, Reid JL. Strategies for dynamic assessment of sympathetic activity from plasma catecholamine measurements. In: Ziegler MG, Lake CR, eds. Norepinephrine. Baltimore/London: Williams and Wilkins, 1984; 209-216.

188. Christensen NJ, Brandsborg O, Brandsborg M, Lovgren NA. Elevated plasma noradrenaline concentration in duodenal ulcer patients are not normalised by vagotomy. J Clin Endocrin Metabl 1979; 49: 331-334.

189. Howes LG, Miller S, Reid JL. Simultaneous assay of 3,4-dihydroxyphenylethylene glycol and norepinephrine in human plasma by high performance liquid chromatography with electrochemical detection. J Chromatogr 1985; 338: 401-403.

190. Derkx FHM, Tan-Tjiong HL, Man in't Veld AJ, Schalekamp MPA, Schalekamp B. Activation of inactive plasma renin by plasma and tissue kallikreins. Clin Sci 1979; 57: 351-357.

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

192. Howes LG, MacGilchrist AJ, Hawksby C, Sumner D, Reid JL. An improved approach for the determination of plasma

[^{3}H] noradrenaline kinetics using high performance liquid chromatography. Clin Sci 1984; 71: 211-215.

193. Esler M, Jackman G, Bobik A, et al. Determination of norepinephrine apparent release rate and clearance in humans. Life Sci 1979; 25: 1461-1470.

194. Brown MJ. Simultaneous assay of noradrenaline and its deaminated metabolite, dihydroxyphenylglycol, in plasma: a simplified approach to the exclusion of phaeochromocytoma in patients with borderline elevation of plasma noradrenaline concentration. Eur J Clin Pharmacol 1984; 14: 67-72.

195. Esler M. Assessment of sympathetic nervous function in humans with noradrenaline plasma kinetics. Clin Sci 1982; 62: 247-254.

196. Eisenhofer G, Goldstein DS, Stull R, Ropchak A, Keiser HR, Kopin IJ. Dihydroxyphenylglycol and dihydroxymandelic acid during intravenous infusions of noradrenaline. Clin Sci 1987; 73: 123-124.

197. McCance AJ, Forfar GC. Determination of plasma [^{3}H] noradrenaline kinetics in man. Separation of [^{3}H] noradrenaline metabolites from the parent amine is unnecessary. Clin Sci 1987; 73(Suppl.17): 53p.

198. Howes LG, Rowe PR, Reid JL. Dihydroxyphenylglycol and dihydroxymandelic acid during intravenous infusions of noradrenaline (Authors' reply). Clin Sci 1987; 73: 125-126.

199. Grohmann H, Henseling M, Cassis L, Trendelenburg U. Errors introduced by a tritium label in position 8 of catecholamines. Naunyn Schmiedeberg's Arch Pharmacol 1986; 332: 332-334.

200. Ryan BF, Joiner BL, Ryan TA. Minitab (2nd edition) Boston: Daxby Press 1985.

201. Bulpitt CJ. Confidence intervals. Lancet 1987; 1: 494-497.

202. Scott DT, Bryce GR, Carter MW. Rummage. Brigham Young University of Utah. 1980.

203. Henriksen JH. The "overflow" theory of ascites formation: a fading concept? Scand J Gastroenterol 1983; 18: 833-837.

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

205. Brown MJ, Jenner DA, Allison DJ, Dollery CT. Variations in individual organ release of noradrenaline measured by an improved radioenzymatic technique: limitations of peripheral venous measurements in the assessment of sympathetic nervous activity. Clin Sci 1981; 61: 585-590.

206. Axelrod J, Tomchick R. Enzymatic O-methylation of epinephrine and other catechols. J Biol Chem 1958; 233: 702-705.

207. Keller U, Gerber PPG, Buhler FR, Stauffacher W. Role of the splanchnic bed in extracting circulating adrenaline and noradrenaline in normal subjects and in patients with cirrhosis of the liver. Clin Sci 1984; 67: 45-49.

208. Reynolds TB. The role of haemodynamic measurements in portosystemic shunting. Arch Surg 1984; 108: 276-278.

209. Esler M, Jennings G, Korner P, et al. Total and organ-specific noradrenaline plasma kinetics in essential hypertension. Clin Exp Hyp Theor Prac 1984; A6: 507-521.

210. Hutchison DCS, Sapru RP, Sumerling MD, Donaldson GWK, Richmond J. Cirrhosis, cyanosis and polycythaemia: multiple pulmonary arteriovenous anastomoses. Am J Med 1968; 45: 139-151.

211. Tarlov SR, Langer SZ. The fate of [^{3}H] norepinephrine released from isolated atria and vas deferens: effect of field stimulation. J Pharmacol Exp Ther 1971; 179: 186-197.

212. Jackman G, Snell J, Skews H, Bobik A. Effects of noradrenergic neuronal activity on 3,4-dihydroxy-phenylethylene glycol (DHPG) levels. Quantitation by high performance liquid chromatography with electrochemical detection. Life Sci 1982; 31: 923-929.

213. Vlachakis N, Kogosov E, Yoneda S, Alexander N, Maronde R. Plasma levels of free and total catecholamines and two deaminated metabolites in man - rapid deconjugation by heat in acid. Clin Chim Acta 1984; 137: 199-209.

214. Howes LG, Hawksby C, Reid JL. Comparison of plasma 3,4-dihydroxyphenylethylene glycol (DHPG) and norepinephrine levels as indices of sympathetic activity in man. Eur J Clin Invest 1986; 16: 18-21.

215. Trey C, Burns DG, Saunders SJ. Treatment of hepatic coma by exchange blood transfusion. N Engl J Med 1966; 274: 473-487.

216. Wallin BG, Sundlof G. A quantitative study of muscle nerve sympathetic activity in resting normotensive and hypertensive subjects. Hypertension 1979; 1: 67-77.

217. Henriksen JH, Ring-Larsen H, Christensen NJ. Catecholamines in plasma from artery, cubital vein and femoral vein in patients with cirrhosis. Significance of a sampling site. Scand J Clin Lab Invest 1986; 46: 39-44.

218. Halter JB, Pflug AE, Tolas AG. Atrial venous differences of plasma catecholamines in man. Metabolism 1980; 29: 9-12.

219. Kuchel O, Thann Bui N. Conjugation of norepinephrine and other catecholamines. In: Ziegler M, Lake CR, eds. Norepinephrine. Baltimore/London: Williams and Wilkins, 1984; 250-270.

220. Lake CR, Chernow B, Feuerstein G, Goldstein DS, Ziegler MG. The sympathetic nervous system in man: its evaluation and the measurement of plasma NE. In: Ziegler MG, Lake CR, eds. Norepinephrine. Baltimore/London: Williams and Wilkins, 1984; 1-26.

221. Henriksen JH, Ring-Larsen H, Christensen NJ. Circulating noradrenaline and central haemodynamics in patients with cirrhosis. Scand J Gastroenterol 1985; 20: 1185-1190.

222. Ring-Larsen H. Hepatic nephropathy, related to haemodynamics. Liver 1983; 3: 265-289.

223. Llach J, Gines P, Tito L et al. Prognostic factors in cirrhotics with ascites. J Hepatol 1986; 3(Suppl.1): S56.

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

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

226. 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.

227. Witte CL, Martinez AP, Witte MH. Plasma atriopeptin before and after peritoneojugular venous shunt for hepatogenic ascites. N Engl J Med 1987; 316: 487.

228. Jimenez W, Martinez-Pardo A, Arroyo V, Gaya J, Rivera F, Rodes J. Atrial natriuretic factor: reduced cardiac content in cirrhotic rats with ascites. Am J Physiol 1986; 250: F749-F752.

229. 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.

230. Gutkowska J, Bourassa M, Roy D, et al. Immunoreactive atrial natriuretic factor (IR-ANF) in human plasma. Biochem Biophys Res Commun 1985; 128: 1350-1357.

231. Fyhrquist F, Totterman K-J, Tikkanen I. Infusion of atrial natriuretic peptide in liver cirrhosis with ascites. Lancet 1985; 2: 1439.

232. Salerno F, Badalamenti S, Incerti PL, Mainardi L, Capozza L. Reduced natriuretic response to atrial natriuretic peptide (ANP) in patients with advanced liver cirrhosis. J Hepatol 1986; 3(Suppl.1): S80.

233. Garcia R, Thibault G, Cantin M, Genest J. Effect of a purified atrial natriuretic factor on rat and rabbit vascular strips and vascular beds. Am J Physiol 1984; 247: R34-R39.

234. Gerbes AL, Arendt RM, Zahringer J, Paumgartner G. Atrial natriuretic peptide, the sympathetic nervous system and decompensated cirrhosis. Lancet 1986; 1: 331.

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

236. Ogihara T, Shima J, Hara H, et al. Significant increase in plasma immunoreactive atrial natriuretic polypeptide concentration during head-out water immersion. Life Sci 1986; 38: 2413-2418.

237. Currie MG, Newman WH. Evidence for α-1-adrenergic receptor regulation of atriopeptin release from the isolated rat heart. Biochem Biophys Res Commun 1986; 137: 94-100.

238. Uehlinger DE, Zaman T, Weidmann P, Shaw S, Gnadinger MP. Pressure dependence of atrial natriuretic peptide during norepinephrine infusion in humans. Hypertension 1987; 10: 249-253.

239. Henriksen JH, Ring-Larsen H, Christensen NJ. Sympathetic nervous activity in cirrhosis: a survey of plasma catecholamine studies. J Hepatol 1984; 1: 55-65.

240. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. Br Med J 1982; 285: 916-918.

241. Bellavere F, Ewing DJ. Autonomic control of the immediate heart rate response to lying down. Clin Sci 1982; 62: 57-64.

242. Sharpey-Schafer EP. Effects of Valsalva's manoeuvre on the normal and failing circulation. Br Med J 1955; 1: 693-695.

243. Leon DF, Shaver JA, Leonard JJ. Reflex heart rate control in man. Am Heart J 1970; 80: 729-739.

244. Ewing DJ, Campbell IW, Burt AA, Clarke BJ. Vascular reflexes in diabetic autonomic neuropathy. Lancet 1973; 2: 1354-1356.

245. Brick K. Circulating responses to immersing the face in water. J Appl Physiol 1966; 21: 33-36.

246. Finley JP, Bonet JF, Waxman MB. Autonomic pathways responsible for bradycardia on facial immersion. J Appl Physiol: Respir Environ Exerc Physiol 1979; 47: 1218-1222.

247. Wheeler T, Watkins PJ. Cardiac denervation in diabetes. Br Med J 1973; 4: 584-586.

248. Ewing DJ, Irving JB, Kerr F, Wildsmith JAW, Clarke BF. Cardiovascular responses to sustained handgrip in normal subjects and in patients with diabetes mellitus: a test of autonomic function. Clin Sci Mol Med 1974; 46: 295-306.

249. Hines EA, Brown GE. The cold pressor test for measuring the reactability of the blood pressure: data concerning 571 normal and hypertensive subjects. Am Heart J 1936; 11: 1-9.

250. Balke B, Ware RW. An experimental study of "physical fitness" in Air Force personnel. U S Armed Forces Med J 1959; 10: 675.

251. Campbell BC, Sturani A, Reid JL. Evidence of parasympathetic activity of the angiotensin converting enzyme inhibitor, captopril in normotensive man. Clin Sci 1985; 68: 49-56.

252. Ajayi AA, Campbell BC, Howie CA, Reid JL. Acute and chronic effects of converting enzyme inhibitors on reflex control of heart rate in normotensive man. J Hypertens 1985; 3, 47-53.

253. Sumner DJ, Elliott HL, Reid JL. Analysis of the pressor dose response. Clin Pharmacol Ther 1982; 32: 450-458.

254. Sumner DJ, Elliott HL, Reid JL. A pragmatic approach to the pressor dose response as an index of vascular reactivity and adrenoceptor function in man. Br J Clin Pharmacol 1987; 23: 505-510.

255. Sumner DJ, Elliott HL. The pressor dose-response in clinical cardiovascular pharmacology. Br J Clin Pharmacol

1987; 23: 499-503.

256. Alexsandrow D, Wysnacka W, Gajewski J. Influence of chlorothiazide upon arterial responsiveness to norepinephrine in hypertensive subjects. N Engl J Med 1959; 261: 1052-1055.

257. Mendlovitz M, Naftchi NE, Gitlow SE, Wolf RL. The effect of spironolactone on digital vascular reactivity in essential hypertension. Am Heart J 1968; 76: 795-798.

258. Oka M, Manku MS. Spironolactone inhibits vascular reactivity by a prostaglandin-related mechanism unconnected with aldosterone. Prostaglandins Med 1981; 7: 305-319.

259. Howes LG, Reid JL. Decreased vascular responsiveness to noradrenaline following regular ethanol consumption. Br J Clin Pharmacol 1985; 20: 669-674.

260. Struthers AD, Pai MS. Evidence for an interaction between noradrenaline and angiotensin II in man. Scott Med J 1984; 31: 268.

261. George CF, Connolly ME, Fenyvesi T, Briant R, Dollery CT. Intravenously administered isoproterenol sulfate dose-response curves in man. Arch Intern Med 1982; 130: 361-364.

262. Nathan MA, Reid DJ. Baroreceptor sensitivity - a new method of assessment. In Sleight P, ed. Arterial baroreceptors and hypertension. New York: Oxford University Press, Inc. 1980; 462-469.

263. Cookson DU, Reed CE. A comparison of the effects of isoproterenol in the normal and asthmatic subject. A preliminary report. Am Rev Resp Dis 1963; 88: 636-643.

264. Cryer PE. Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system. N Engl J Med 1980; 303: 436-444.

265. Fitzgerald D, Doyle V, Kelly JG, O'Malley K. Cardiac sensitivity to isoprenaline, lymphocyte beta-adrenoceptors and age. Clin Sci 1984; 66: 697-699.

266. Ahlquist RP. A study of adrenotropic receptors. Am J Physiol 1948; 153: 586-600.

267. Berthelson S, Pettinger WA. A functional basis for classification of α-adrenergic receptors. Life Sci 1977; 21: 596-606.

268. Lands AM, Arnold A, McAuliff JP, Luduena FP, Brown TG. Differentiation of receptor systems activated by sympathomimetic amines. Nature 1967; 214: 597-598.

269. Newman KD, Williams LT, Bishopric NH, Lefkowitz RJ. Identification of α-adrenergic receptors in human platelets by [^{3}H] dihydroergocryptine binding. J Clin Invest 1978; 61: 395-402.

270. Motulsky HJ, Shattil SJ, Insel PA. Characterisation of alpha$_2$-adrenergic receptors on human platelets using [^{3}H] yohimbine. Biochem Biophys Res Commun 1980; 97: 1562-1570.

271. Karliner JS, Motulsky HJ, Insel PA. Apparent "down regulation" of human platelet alpha$_2$ adrenergic receptors is due to retained agonist. Mol Pharmacol 1982; 21: 36-43.

272. Hamilton CA, Deighton NM, Reid JL. Rapid and reversible desensitisation of vascular and platelet alpha$_2$ adrenoceptors. Naunyn-Schmiedeberg's Arch Pharmacol 1987; 335: 534-540.

273. Gerbes AL, Remien J, Jungst D, Sauerbruch T, Paumgartner G. Evidence for down-regulation of beta-2-adrenoceptors in cirrhotic patients with severe ascites. Lancet 1986; 1: 1409-1411.

274. Boyum A. Isolation of mononuclear cells by one centrifugation and granulocytes by combining centrifugation and sedimentation at 1 g. Scand J Clin Lab Invest 1968; 21(Suppl.97): 77-89.

275. Aarons RD, Nies AS, Gerber JG, Molinoff PB. Decreased beta adrenergic receptor density on human lymphocytes following chronic treatment with agonists. J Pharmacol Exp Ther 1983; 224: 1-6.

276. Brodde OE. Endogenous and exogenous regulation of human alpha and beta adrenergic receptors. J Rec Res 1983; 3: 151-162.

277. Lowry OH, Roxbrough NJ, Farr AL, Randal FRJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193: 265-275.

278. Middeke M, Remien J, Kirzinger S, Holzgreve H. Adrenergic hyposensitivity during long-term diuretic therapy - a possible explanation for the antihypertensive effect of diuretics? Eur J Clin Pharmacol 1985; 109: 401-403.

APPENDIX

PRESENTATIONS TO LEARNED SOCIETIES

The following presentations to learned societies have arisen from the work described in this thesis. Where the abstracts of the presentations have been published, the reference is included in parentheses.

1. An improved technique for the measurement of [^{3}H] noradrenaline kinetics using high performance liquid chromatography.
   A.J. MacGilchrist, L.G. Howes, C. Hawksby, D. Sumner and J.L. Reid.
   European Society for Clinical Investigation, Scheveningen, Holland. March 1986 (Acta Toxicologica Pharmacologica 1986; 16(2): A58.

2. Impairment of autonomic responses in chronic liver disease.
   A.J. MacGilchrist and J.L. Reid.
   Clinical Autonomic Research Society, London. September 1986. (Journal of the Autonomic Nervous System 1988; 22: in press).

3. The use of [^{3}H] noradrenaline kinetics to assess sympathetic activity: high performance liquid chromatography improves the technique.
   A.J. MacGilchrist, L.G. Howes, C. Hawksby and J.L. Reid.
   Clinical Autonomic Research Society, London. September 1986. (Journal of the Autonomic Nervous System 1988; 22: in press).

4. Decreased vascular responsiveness to noradrenaline in severe chronic liver disease.
   A.J. MacGilchrist, J.L. Reid and T.J. Thomson.
   Glasgow Gastroenterology Club, September 1986.

5. Autonomic responses in cirrhosis.
   A.J. MacGilchrist and J.L. Reid.
   Scottish Society of Experimental Medicine, Dundee. October 1986. (Scottish Medical Journal 1986; 31: 267).

6. Studies on the sympathetic nervous system in cirrhosis.
   A.J. MacGilchrist.
   Registrars Research Prize Meeting, Royal College of Physicians & Surgeons of Glasgow. November 1986.

7. Impaired vascular responsiveness in cirrhosis of the liver.
   A.J. MacGilchrist, D.J. Sumner and J.L. Reid.
   Medical Research Society, London. January 1987 (Clinical Science 1987; 72(Suppl.16): 34p).

8. Sympathetic activation in cirrhosis with ascites.
A.J. MacGilchrist, J.L. Reid and T.J. Thomson.
Joint Meeting of the Caledonian Society of Gastroenterology, Ulster Society of Gastroenterology and the Glasgow Gastroenterology Club, Edinburgh. February 1987.

9. Is the reduced vascular tone in cirrhosis due to "desensitisation" of sympathetic nervous activity?
A.J. MacGilchrist, D.J. Sumner, J.L. Reid and T.J. Thomson.
Jubilee Meeting of the British Society of Gastroenterology, London. September 1987 (Gut 1987; 28: A1349).

10. Is sympathetic activity really increased in cirrhosis with ascites?
A.J. MacGilchrist, L.G. Howes, C. Hawksby and J.L. Reid.
Jubilee Meeting of the British Society of Gastroenterology, London. September 1987 (Gut 1987; 28: A1394).

11. Reduced sympathetic reactivity in cirrhosis: evidence for a post-receptor, vascular defect.
A.J. MacGilchrist, N. Deighton, D.J. Sumner and J.L. Reid. British Society of Gastroenterology, Leicester. March 1988. (Gut 1988; 29: A 725).

12. Studies of adrenergic receptors in cirrhosis.
A.J. MacGilchrist, N. Deighton and J.L. Reid.
20th Congress of the European Association for Gastroenterology and Endoscopy, Lake Garda, Italy. April 1988.