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**Juan Rodés, Robert W. Schrier**

# Ascites & Renal Dysfunction in Liver Disease

SECOND EDITION

## Pathogenesis Diagnosis & Treatment

Blackwell Publishing

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## Pathogenesis, Diagnosis, and Treatment

Dedicated to our wives, Nuria, Joana, Paula, and Barbara, in recognition of their contribution to our scientific careers.

# Ascites and Renal Dysfunction in Liver Disease

## Pathogenesis, Diagnosis, and Treatment

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# Preface to the Second Edition

It has been six years since we published the first edition of *Ascites and Renal Dysfunction in Liver Disease*. Since then, significant advances have been made in the pathogenesis of circulatory and renal dysfunction that occur in the setting of chronic liver diseases, particularly cirrhosis. Specifically, the role of vasodilatory factors, particularly nitric oxide, has been investigated extensively. Moreover, there is increased recognition of the mechanistic role of impaired heart function on the circulatory dysfunction of liver failure. In this second edition of *Ascites and Renal Dysfunction in Liver Disease*, these advances in pathogenetics are described in specific chapters.

Besides this increased knowledge on pathophysiology, major advances have been made in the clinical management of renal dysfunction in liver disease. A new therapeutic method, transjugular intravenous portosystemic shunts, has emerged for patients with ascites refractory to diuretic therapy. A large number of nonrandomized studies (as well as several randomized trials) have been published concerning the effects of this therapeutic approach. For the first time ever, an effective treatment has been described to treat hepatorenal syndrome in patients with cirrhosis, namely administration of vasoconstrictor drugs. Moreover, there are studies showing how hepatorenal syndrome can be effectively prevented in specific settings such as spontaneous bacterial peritonitis and alcoholic hepatitis. Finally, specific antagonists of the V2 vasopressin receptor are in advanced stages of clinical development. These drugs might prove to be useful in the management and prevention of dilutional hyponatremia, a complication for which there is currently no effective therapy. All these new topics, as well as other topics on the management of liver disease, are covered in this second edition.

The layout and look of the book have changed from the previous edition. The book has been divided into two sections: the first (Parts 1, 2 and 3) describes the pathophysiology of circulatory and renal abnormalities, whilst the second (Parts 4–7) relates to clinical management of patients. We hope this will make the book easier to read when looking for either pathogenic factors or answers to clinical questions.

Finally, we would like to acknowledge the work of the authors of the chapters, who are internationally recognised specialists in their fields and have done a tremendous job in summarizing the different topics inside the page limits. We thank both Nicki van Berckel and Janet Darling for their administrative assistance, and Blackwell Publishing for making the book appealing to the readers.

We hope that this second edition of *Ascites and Renal Dysfunction in Liver Disease* will be helpful not only to clinical researchers interested in complications of cirrhosis, but also to those clinicians – whether they be gastroenterologists, transplant hepatologists, nephrologists, or internists – caring for patients with liver diseases.

*P. Ginès*
*V. Arroyo*
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*2005*

# Part 1
# Regulation of Extracellular Fluid Volume and Renal and Splanchnic Circulation

# Chapter 1
# Extracellular Fluid Volume Homeostasis

Brian D. Poole, William T. Abraham, and Robert W. Schrier

The development of ascites is the most common complication in patients with compensated cirrhosis, occurring in 58% of patients within 10 years of diagnosis. Ascites develops in the context of an increase in the extracellular fluid volume (ECF) and therefore it is essential to understand the regulation of body fluid volume to appreciate its pathogenesis. Knowledge of the intrarenal and extrarenal factors governing renal sodium excretion is crucial to understanding because the sodium ion is the principal determinant of ECF volume. In normal individuals, if the ECF is expanded by the administration of isotonic saline the kidney will excrete the excess sodium and water in the urine and return the ECF to normal values. However, in pathogenic disease states such as congestive heart failure (CHF) and cirrhosis the kidneys continue to retain sodium and water even in the presence of an increased ECF volume. In these edematous disorders the integrity of the kidney as the primary organ controlling ECF volume remains intact because transplantation of the kidney from an edematous, cirrhotic patient to a subject with normal liver function totally reverses the renal sodium and water retention (1). Moreover, transplantation of a normal liver into a cirrhotic patient with ascites and edema has been shown to abolish the renal sodium and water retention (2). Thus, the kidney must be responding to extrarenal signals from the afferent limb of a volume regulatory system in these edematous disorders. The study of these edematous states has led to a unifying hypothesis of body fluid volume regulation (3–8). This chapter will review the afferent and efferent mechanisms that contribute to extracellular fluid volume homeostasis in health and disease.

## Regulation of sodium excretion

Due to active transport processes, the sodium ion is primarily located in the ECF and, along with its major anions chloride and bicarbonate, constitutes more than 90% of the extracellular solute. Therefore, because sodium and its anions are the major osmotically active substances in the ECF, they are the major determinants of the ECF volume. With a positive sodium balance, the ECF volume will increase secondary to osmotically driven movement of water into the extracellular space. Sodium balance is determined by the equilibrium between sodium intake, extrarenal sodium loss, and renal sodium excretion. Practically, renal sodium excretion is the major determinant of sodium balance, given the ability of the kidney to excrete large amounts of sodium in response to a sodium load. In addition, sodium loading, by increasing serum osmolality, stimulates the hypothalamic thirst center leading to increased fluid intake as well as the osmotic release of arginine vasopressin (AVP). The release of AVP from the posterior pituitary decreases water excretion by increasing the permeability of the collecting duct epithelium to water. If the increase in ECF volume is sufficient to alter the Starling forces governing the transfer of fluid from the vascular to the interstitial compartment then edema results.

One of the major regulators of sodium excretion is the mineralocorticoid hormone aldosterone. Aldosterone is produced in the zona glomerulosa of the adrenal gland and acts to increase sodium reabsorption by increasing the number of epithelial sodium channels in the cortical collecting duct. In states of volume depletion, the renin–angiotensin–aldosterone system (RAAS) is stimulated causing an increase in sodium reabsorption that leads to expansion of the ECF. With expansion, the stimulus for aldosterone secretion is removed and sodium reabsorption is diminished, thereby stabilizing volume status. In states of mineralocorticoid excess such as primary hyperaldosteronism there is unregulated secretion of aldosterone leading to an increase in sodium reabsorption with resultant volume expansion and hypertension. The effect of aldosterone to cause renal sodium retention can be overridden, however, by the phenomenon of aldosterone escape. In this circumstance the ECF reaches a new, higher steady state, but does not continue to expand despite increased levels of aldosterone. This has been postulated to be mediated by hemodynamic mechanisms whereby an increase in renal artery pressure secondary to expansion of the ECF causes a pressure natriuresis. The increase in renal artery pressure subsequently increases the glomerular filtration rate (GFR) and the fractional excretion of sodium ($Fe_{Na}$). Recently it was reported that the chief molecular target of the escape phenomenon is the thiazide-sensitive NaCl

cotransporter. In a rat model of aldosterone infusion coupled with a high sodium diet, it was found that levels of the epithelial sodium channel were unchanged during the escape phenomenon, but the amount of the thiazide-sensitive NaCl cotransporter was significantly diminished. Therefore it appears that the so-called pressure natriuresis is mediated at least in part by downregulation of the thiazide-sensitive NaCl cotransporter.

Homeostasis of the ECF is also mediated by the hormones atrial natriuretic peptide (ANP) and, as mentioned previously, AVP. ANP has been shown to be released from the myocardium in response to volume expansion and it has two major actions contributing to maintenance of volume status. It is a direct vasodilator that can lower systemic blood pressure and it also increases the urinary excretion of sodium and water. The natriuresis appears to be mediated by an increase in GFR secondary to afferent arteriole vasodilation coupled with efferent arteriole vasoconstriction. Furthermore, ANP has been shown to directly decrease tubular sodium reabsorption. In another rat model of hyperaldosteronism, it has been shown that the level of ANP increases coinciding with an increase in sodium excretion. Therefore the authors postulate that ANP may also mediate aldosterone escape. It is not known whether ANP has an effect on the thiazide-sensitive NaCl cotransporter.

AVP is the chief regulator of renal water excretion and as such can be expected to have a major role in ECF volume regulation. It is known that in edematous disorders like CHF and cirrhosis there are inappropriate levels of AVP relative to plasma osmolality that results in water retention and hyponatremia. However, it has been shown that there is also counterregulation of this system. In rats administered AVP plus a water load it was shown that after an initial period of water retention there was subsequently an increase in urine volume that corresponded to a downregulation of the renal water channel aquaporin 2 despite continued elevated levels of AVP. Therefore, the authors conclude there is a vasopressin-independent downregulation of aquaporin 2 and therefore a limit on water reabsorption in this model.

Unlike in conditions such as primary hyperaldosteronism where there is an escape from continued sodium reabsorption despite elevated levels of aldosterone, in the edematous disorders there is impaired escape and continuous sodium and water reabsorption. It seems that the difference in these disorders is in how the kidney is responding to the afferent limb of the volume regulatory system.

## Afferent mechanisms governing extracellular fluid volume homeostasis

An increase in sodium and water intake is associated with an expansion of the extracellular fluid volume. This includes expansion of the interstitial fluid and plasma components of total body fluid volume. Under normal circumstances, this expansion of total body fluid volume results in an increase in renal sodium and water excretion followed by restoration of the normal extracellular fluid volume. However, in patients with edematous disorders, avid sodium and water retention persists despite expansion of total extracellular fluid and blood volume. Thus, the afferent volume receptors governing extracellular fluid volume must not primarily sense total extracellular fluid or blood volume. In such instances, there must be some body fluid compartment that is still inadequately filled even in the presence of expansion of these body fluid compartments.

### Effective blood volume and the concept of arterial underfilling

John Peters first coined the term *effective blood volume* in allusion to the component of blood or body fluid volume to which the volume regulatory system responds by altering the renal excretion of sodium and water (9). Peters suggested that extrarenal signals that enhance tubular sodium and water reabsorption by the otherwise normal kidney are initiated by this decrease in effective blood volume in the setting of cardiac failure or cirrhosis. In support of this claim is the observation that renal sodium and water retention can occur in patients with cardiac or liver failure before any decrease in glomerular filtration rate.

Borst and deVries (10) first suggested cardiac output as the primary regulator of renal sodium and water excretion, thus constituting effective blood volume. While this notion is attractive, there exist several states of sodium and water retention that are associated with an augmented rather than a decreased cardiac output. For example, a significant increase in cardiac output may occur in the presence of avid renal sodium and water retention and expansion of extracellular fluid volume in association with cirrhosis, high-output cardiac failure, pregnancy, and large arteriovenous fistulae. Hence, cardiac output must not constitute the sole or primary determinant of effective blood volume.

The unifying hypothesis of body fluid volume regulation suggests that the relative integrity or *fullness* of the arterial circulation constitutes the primary afferent signal through which the kidneys either increase or decrease their excretion of sodium and water (3–8). This theory explains how an increase in the volume of blood on the venous side of the circulation may cause a rise in total blood volume, whereas a decrease in the relative volume of blood in the arterial circulation may promote continued renal sodium and water retention. A reduction in cardiac output is one way in which a decrease in arterial circulatory integrity may occur. However, as mentioned above, diminished cardiac output cannot be the only afferent signal for underfilling of the arterial circulation.

The unifying hypothesis of body fluid volume regulation proposes peripheral arterial vascular resistance and the compliance of the arterial vasculature as the second major determinant of the fullness of the arterial circulation (3–8). Thus, peripheral arterial vasodilation may provide another afferent signal for arterial underfilling, which causes renal sodium and water retention.

In summary, either a decrease in cardiac output or peripheral arterial vasodilation may constitute the afferent signal for arterial underfilling with resultant renal sodium and water retention that leads to expansion of the total blood volume. The afferent receptors or sensors of arterial underfilling must be responsive to small changes in effective arterial blood volume since the steady-state arterial blood pressure is not a sensitive index of the presence of arterial underfilling. For example, the rapidity of the compensatory response to arterial underfilling may obscure a fall in blood pressure until this efferent response becomes inadequate to maintain effective arterial blood volume. The mechanisms involved in this volume regulatory system are summarized in Figs 1.1 and 1.2, and the sensors of arterial underfilling are discussed next.

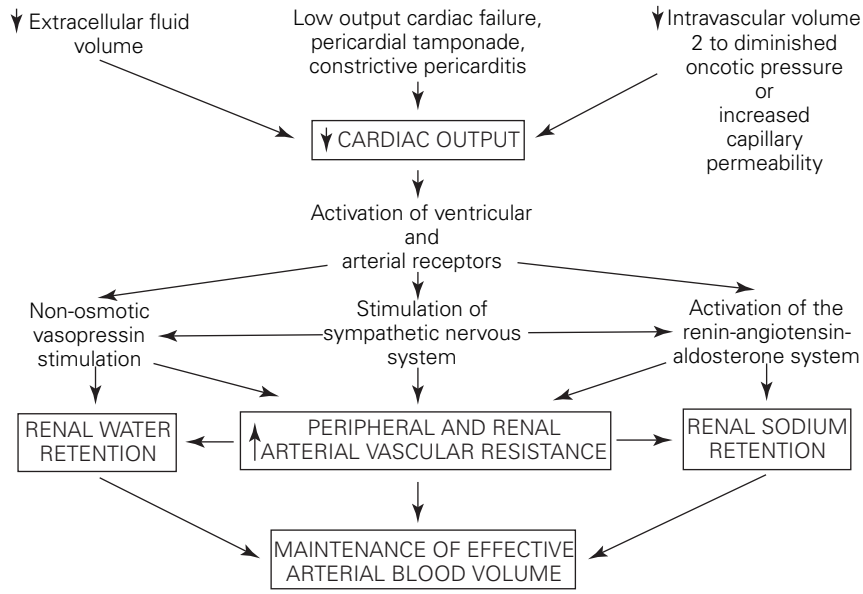


**Figure 1.1** Mechanism by which decreased cardiac output results in renal sodium and water retention and peripheral and renal vasoconstriction. (Reproduced with permission from Schrier R, Niederberger M. Paradoxes of body fluid volume regulation in health and disease: a unifying hypothesis. West J Med 1994; 16:393–407.)

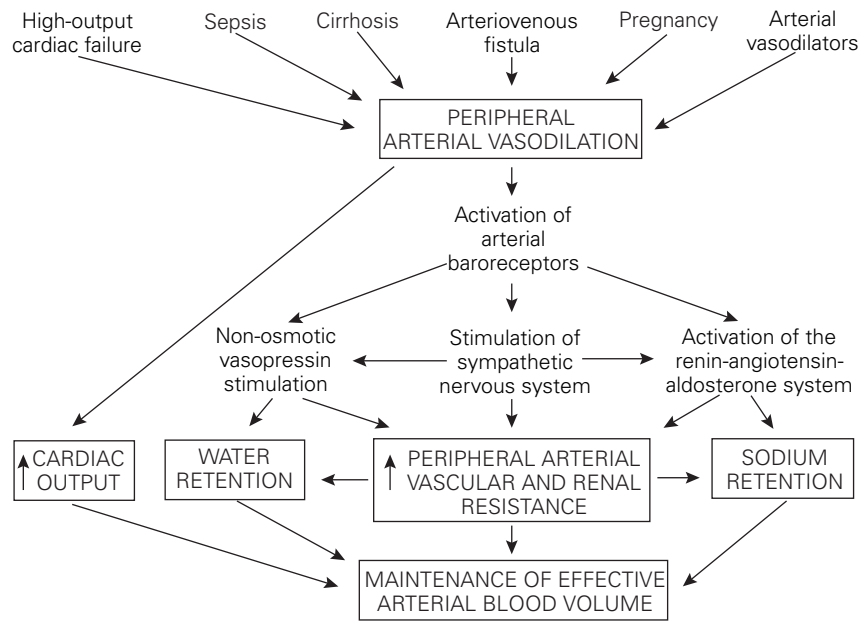


**Figure 1.2** Mechanism by which peripheral arterial vasodilation results in renal sodium and water retention, increased cardiac output, and peripheral and renal vasoconstriction. (Reproduced with permission from Schrier R, Niederberger M. Paradoxes of body fluid volume regulation in health and disease: a unifying hypothesis. West J Med 1994; 16:393–407.)

## Sensors of arterial underfilling

### *High-pressure baroreceptors*

Afferent receptors for this volume regulatory system must reside in the arterial vascular compartment. In this regard, high-pressure baroreceptors in the left ventricle, carotid sinus, aortic arch, and juxtaglomerular apparatus have been implicated as the primary afferent receptors involved in the regulation of renal sodium and water excretion and extracellular fluid volume homeostasis (11–19). The presence of volume-sensitive receptors in the arterial circulation in humans was initially suggested by observations made in patients with traumatic arteriovenous fistulae (20). In such patients, closure of the fistulae results in a decrease in the rate of emptying of the arterial blood into the venous circulation, as demonstrated by closure-induced increases in diastolic arterial pressure and decreases in cardiac output. This increase in arterial fullness produces an immediate increase in renal sodium excretion without changes in either glomerular filtration rate or renal blood flow (20).

Various denervation experiments also implicate high-pressure volume receptors, and thus the integrity of the arterial circulation, as primary afferent receptors in modulating renal sodium and water excretion. In these studies, pharmacological or surgical interruption of sympathetic afferent neural pathways arising from high-pressure areas inhibited the natriuretic response to volume expansion (21–27). In addition, reduction of pressure or stretch at the carotid sinus has been shown to activate the sympathetic nervous system and to cause renal sodium and water retention (28,29). High-pressure baroreceptors also appear to be important factors in regulating the non-osmotic release of vasopressin and thus renal water excretion (30,31).

The juxtaglomerular apparatus is a high-pressure receptor located in the afferent arterioles within the kidney. It responds to decreased stretch or increased renal sympathetic activity with enhanced secretion of renin (28). Thus, this renal baroreceptor is an important factor in the control of angiotensin II formation and aldosterone secretion and ultimately in the regulation of renal sodium excretion.

### *Low-pressure baroreceptors*

The low-pressure baroreceptors of the thorax, including the atria, right ventricle, and pulmonary vessels, may also contribute to extracellular fluid volume homeostasis. Loading of these volume-sensitive receptors results in enhanced cardiac release of natriuretic peptides (32) and suppression of non-osmotic vasopressin release from the neurohypophysis (33). Since patients with advanced cardiac failure exhibit avid sodium and water retention and activation of neurohormonal vasoconstrictor systems—including enhanced non-osmotic vasopressin release—despite elevated atrial pressures and increased circulating concentrations of the natriuretic peptides, high-pressure baroreceptors must predominate over these low-pressure ones. This observation also supports the primacy of the arterial circulation as the determinant of extracellular fluid volume homeostasis.

### *Cardiac and pulmonary chemoreceptors*

In the heart and lungs, both vagal and sympathetic afferent nerve endings respond to a variety of exogenous and endogenous chemical substances, including capsaicin, phenyldiguanidine, bradykinin, substance P, and prostaglandins (34–36). Since substances such as bradykinin and prostaglandins may circulate at increased concentrations in subjects with edematous disorders (37), it is possible that altered central nervous system input from chemically sensitive cardiac and/or pulmonary afferents contributes to the sodium and water retention characteristic of these disease states. This possibility may have important implications for the treatment of some sodium-retaining disorders. For example, in heart failure, commonly prescribed medications such as angiotensin-converting enzyme inhibitors may alter circulating bradykinin and prostaglandin levels, thus potentially influencing cardiopulmonary chemoreceptor activity. At the present time, however, the exact role of these cardiac and pulmonary chemoreceptors in body fluid volume regulation remains unknown.

### *Hepatic receptors*

Conceptually, the liver should be in an ideal position to monitor dietary sodium intake and thus adjust urinary sodium excretion. In support of this notion, infusion of saline into the portal circulation has been reported to result in a greater natriuresis when compared with peripheral venous saline administration (38,39). Similarly, the increase in urinary sodium excretion has been shown to be greater when the sodium load is given orally than when it is given intravenously (40–42). Moreover, the pathophysiological retention of sodium in patients with severe liver disease is also consistent with an important role for the liver in the control of sodium excretion. However, the experimental evidence in favor of hepatic sodium or volume receptors remains controversial since some investigators have been unable to confirm the above observations of increased sodium excretion in response to portal vein or gastric sodium loading (43–45).

In summary, the afferent mechanisms for sodium and water retention appear to be preferentially localized to the arterial or high-pressure side of the circulation, where arterial fullness may serve as the primary determinant of the renal response. Reflexes emanating from low-pressure cardiopulmonary receptors may also be altered so as to influence renal sodium and water handling in

heart failure. In this regard, increases in atrial pressure also stimulate the release of the natriuretic peptides and inhibit vasopressin release, which may be important attenuating factors in renal sodium and water retention. At the present time, the role of cardiac and pulmonary chemoreceptors and possibly hepatic volume receptors and osmoreceptors remains unclear.

## Efferent mechanisms involved in extracellular fluid volume homeostasis

The kidney alters the amount of dietary sodium excreted in response to signals from high-pressure and low-pressure volume receptors in the circulation. These receptors may affect renal function by altering renal sympathetic nerve activity and by altering levels of circulating hormones with vasoactive (renal hemodynamic) and nonvasoactive (direct sodium- and/or water-retaining) effects on the kidney. In addition to the sympathetic neurotransmitter norepinephrine, angiotensin II, aldosterone, arginine vasopressin, and other vasoconstrictor hormones may contribute to renal sodium and water retention. Nitric oxide, vasodilating prostaglandins, bradykinin, and the natriuretic peptides may play important counterregulatory roles attenuating both the renal vasoconstriction and antinatriuresis caused by norepinephrine, angiotensin II, and other vasoconstrictor hormones.

### Renal hemodynamics

The glomerular filtration rate is usually normal early in the course of arterial underfilling and is reduced only as the disease state becomes more advanced. Renal vascular resistance, however, is often increased early, with a concomitant decrease in renal blood flow (46,47). Thus, the ratio of glomerular filtration rate to renal blood flow, or the filtration fraction, is often increased in such patients. This increased filtration fraction is a consequence of predominant constriction of the efferent arterioles within the kidney. These changes in renal hemodynamics alter the hydrostatic and oncotic forces in the peritubular capillaries to favor increased proximal tubular reabsorption of sodium and water. These renal hemodynamic changes are primarily mediated by the neurohormonal response to arterial underfilling.

### The neurohormonal response to arterial underfilling

Arterial underfilling secondary to a diminished cardiac output or to peripheral arterial vasodilation elicits a series of initially compensatory neuroendocrine responses in order to maintain the integrity of the arterial circulation by promoting increased cardiac inotropy, peripheral vasoconstriction, and expansion of the extracellular fluid volume through renal vasoconstriction and renal sodium and water retention (Figs 1.1 and 1.2). The three major neurohormonal vasoconstrictor responses to arterial underfilling are activation of the sympathetic nervous system and the RAAS, and the non-osmotic release of vasopressin.

Baroreceptor activation of the sympathetic nervous system appears to be the primary integrator of the hormonal vasoconstrictor systems involved in renal sodium and water retention, since the non-osmotic release of vasopressin involves sympathetic stimulation of the supraoptic and paraventricular nuclei of the hypothalamus (48), and activation of the RAAS involves renal β-adrenergic stimulation (49). In addition, the renin-angiotensin system may provide positive feedback stimulation of the sympathetic nervous system and non-osmotic vasopressin release (50), thus indicating that these vasoconstrictor systems may be co-regulated in various pathophysiological states. The effects of these neurohormonal systems on renal hemodynamics and tubular sodium and water handling are discussed below.

#### *The sympathetic nervous system*

The sympathetic nervous system is unquestionably activated in patients with arterial underfilling. In edematous states such as heart failure and cirrhosis, this sympathetic activity has been documented by both indirect (51–61) and direct (62,63) measures. For example, Leimbach *et al*. (62) in the case of heart failure and Floras *et al*. (63) in the case of cirrhosis have demonstrated increased central sympathetic outflow to skeletal muscle using direct intraneuronal recordings of the peroneal nerve. Similarly, employing continuous infusion of tritiated norepinephrine in patients with mild to moderate heart failure or cirrhosis, whole-body norepinephrine kinetics studies have shown increased norepinephrine secretion rates and normal norepinephrine clearance rates, compatible with activation of the sympathetic nervous system (55,61). Finally, using similar techniques, renal sympathetic activation has been demonstrated in patients with such edematous disorders as heart failure (51). Significantly, the degree of activation of the sympathetic nervous system strongly correlates with disease severity and poor prognosis in both heart failure and cirrhosis (64,65).

Through renal vasoconstriction, stimulation of the RAAS, and direct effects on the proximal convoluted tubule, enhanced renal sympathetic activity may contribute to the avid sodium and water retention associated with arterial underfilling. Indeed, intrarenal adrenergic blockade has been shown to cause a natriuresis in experimental animals and humans with heart failure or cirrhosis (21,66,67). In the rat, renal nerve stimulation has been demonstrated to produce an approximately 25% reduction in sodium excretion and urine volume (68).

The diminished renal sodium excretion that accompanies renal nerve stimulation may be mediated by at least two mechanisms. Studies performed in rats have demonstrated that norepinephrine-induced efferent arteriolar constriction alters peritubular hemodynamic forces in favor of increased tubular sodium reabsorption (69). As previously mentioned, the increase in filtration fraction with a normal or only slightly reduced glomerular filtration rate that is often seen in edematous patients is due to efferent arteriolar constriction. Constriction of the efferent arterioles in such states has been confirmed by renal micropuncture studies performed in rats (70) and is at least partially mediated by increased renal sympathetic activity and also by angiotensin II. Thus, efferent arteriolar constriction in states of arterial underfilling shifts the balance of hemodynamic forces in the peritubular capillaries in favor of enhanced proximal tubular sodium reabsorption.

In addition, renal nerves have been shown to exert a direct influence on sodium reabsorption in the proximal convoluted tubule (66,68). Bello-Reuss *et al*. (68) demonstrated this direct effect of renal nerve activation to enhance proximal tubular sodium reabsorption in whole-kidney and individual nephron studies in the rat. In these animals, renal nerve stimulation produced an increase in the tubular fluid to plasma inulin concentration ratio in the late proximal tubule, an outcome of increased fractional sodium and water reabsorption in this segment of the nephron (68). Hence, increased renal nerve activity may promote sodium retention by a mechanism independent of changes in renal hemodynamics.

### *The renin–angiotensin–aldosterone system*

The RAAS is also activated in response to arterial underfilling, as assessed by plasma renin activity and plasma aldosterone concentration (71–73). Moreover, activation of the RAAS is associated with hyponatremia and an unfavorable prognosis in edematous disorders (74,75). Angiotensin II may contribute to sodium and water retention through direct and indirect effects on proximal tubular sodium reabsorption and by stimulating the release of aldosterone from the adrenal gland. Angiotensin II causes renal efferent vasoconstriction, resulting in decreased renal blood flow and an increased filtration fraction. As with renal nerve stimulation, this results in increased peritubular capillary oncotic pressure and reduced peritubular capillary hydrostatic pressure, which favor the reabsorption of sodium and water in the proximal tubule (70,76). In addition, angiotensin II has been shown to have a direct effect of enhancing sodium reabsorption in the proximal tubule (77). Finally, angiotensin II enhances aldosterone secretion by the adrenal gland, which promotes tubular sodium reabsorption in the cortical and medullary ducts.

A role for aldosterone in the renal sodium retention of human heart failure has been demonstrated (78). The effect of spironolactone on urinary sodium excretion was examined in patients with mild to moderate heart failure, who were withdrawn from all medications prior to study. Sodium was retained in all subjects throughout the period prior to aldosterone antagonism (Fig. 1.3). On an average sodium intake of 97 ± 8 mmol/day, the average sodium excretion before spironolactone was 76 ± 8 mmol/day. During therapy with spironolactone, all heart failure patients demonstrated a significant increase in urinary sodium excretion to 131 ± 13 mmol/day. Moreover, the urine sodium concentration to potassium concentration ratio significantly increased during spironolactone administration, consistent with a decrease in aldosterone action in

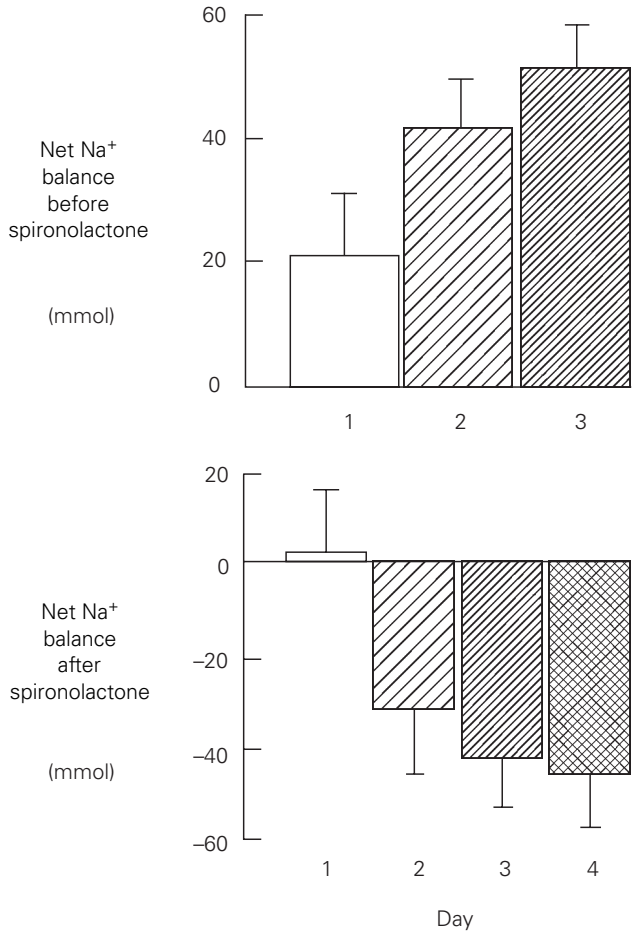


**Figure 1.3** Reversal of sodium retention in heart failure patients during aldosterone antagonism. (Top) Net cumulative positive sodium balance, by day, for the period before spironolactone administration. (Bottom) Net cumulative negative sodium balance with spironolactone 400 mg/day. $P < 0.01$ for increase in sodium excretion with aldosterone antagonism. (Reproduced with permission from Hensen J, Abraham WT, Durr JA *et al*. Aldosterone in congestive heart failure: analysis of determinants and role in sodium retention. Am J Nephrol 1991; 11:441.)

the distal nephron. Similarly, there also have been reports of natriuresis occurring in cirrhosis after the administration of spironolactone (79). The near-uniform response to spironolactone in cirrhosis suggests that the high plasma levels of aldosterone frequently seen in these subjects contribute to the increased distal sodium reabsorption.

### *The non-osmotic release of vasopressin*

Elevated plasma vasopressin levels have been demonstrated in patients with heart failure and cirrhosis and correlate with the clinical and hemodynamic severity of disease and with the serum sodium concentration (80–89). Through the use of a single intravenous bolus technique, we determined vasopressin clearance to be normal in six patients with mild to moderate heart failure (unpublished observations). Moreover, plasma vasopressin concentrations are inappropriately elevated in hyponatremic patients with heart failure or cirrhosis, and these levels fail to suppress normally with acute water loading (82,84,85), suggesting that the enhanced release of vasopressin in these settings is due to non-osmotic stimulation. As already suggested, baroreceptor activation of the sympathetic nervous system probably mediates this non-osmotic release of vasopressin in states of arterial underfilling.

Arginine vasopressin, via stimulation of its renal or $V_2$ receptor, enhances water reabsorption in the cortical and medullary collecting ducts. Two lines of evidence implicate non-osmotic vasopressin release in the abnormal water retention seen in the edematous disorders. First, in animal models of heart failure, the absence of a pituitary source of vasopressin is associated with normal or near-normal water excretion (17,90). This observation was first made by Anderson and colleagues in the dog during acute thoracic vena caval constriction (17). In these animals, acute removal of the pituitary source of vasopressin by surgical hypophysectomy virtually abolished the defect in water excretion. Abnormal water excretion occurring in the rat with high-output cardiac failure due to aortocaval fistula also appears to be the result of abnormal vasopressin release, since the defect is not demonstrable in rats with central diabetes insipidus (90). The second line of evidence supporting a role for vasopressin in the water retention of heart failure and cirrhosis may be found in studies of selective $V_2$ receptor antagonists. These agents have been shown to reverse the impairment in water excretion in animal models of cardiac failure and cirrhosis and in human heart failure (91–95). Thus, while diminished fluid delivery to the distal diluting segment may also contribute to the abnormal water excretion seen in states of arterial underfilling, increased vasopressin appears to exert the predominant effect.

In summary, baroreceptor activation of the three major neurohormonal vasoconstrictor systems is involved in the avid renal sodium and water retention characteristic of the edematous disorders. Increased adrenergic nervous system activity in response to arterial underfilling appears to orchestrate this neurohormonal response. Renal nerves, angiotensin II, aldosterone, and vasopressin all may play a role as important effector mechanisms in the abnormal retention of sodium and water.

While the aforementioned neuroendocrine systems conspire to promote sodium and water retention in states of arterial underfilling, counterregulatory vasodilatory or natriuretic substances may attenuate, to some degree, this neurohormonal vasoconstrictor activation. Chief among these are the natriuretic peptides and vasodilating prostaglandins.

### *The natriuretic peptides*

The natriuretic peptides, including atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), circulate at increased concentrations in patients with heart failure (96–98) and in some patients with cirrhosis (99,100). These peptide hormones possess natriuretic, vasorelaxant, and renin-, aldosterone-, and possibly vasopressin- and sympathoinhibiting properties (101–106). In normal humans, ANP and BNP increase glomerular filtration rate and urinary sodium excretion with no change or only a slight fall in renal blood flow (107,108). The changes in renal hemodynamics are probably mediated by afferent arteriolar vasodilation with constriction of the efferent arterioles, as discerned by micropuncture studies in the rat (109,110). In addition to increasing glomerular filtration rate and filtered sodium load as a mechanism of their natriuretic effect, ANP and BNP are specific inhibitors of sodium reabsorption in the collecting tubule (111–113).

Despite the above observations, the natriuretic effects of these peptide hormones are blunted in states of arterial underfilling such as heart failure and cirrhosis (107,114–116). Possible mechanisms for natriuretic peptide resistance in heart failure and cirrhosis include: (i) downregulation of renal natriuretic peptide receptors; (ii) secretion of biologically inactive, immunoreactive ANP or BNP; (iii) enhanced renal neutral endopeptidase activity that degrades natriuretic peptides, thus limiting the delivery of ANP and BNP to distal nephron receptor sites; (iv) hyperaldosteronism causing an increased sodium reabsorption in the distal renal tubule; (v) intracellular mechanisms, including increased phosphodiesterase activity; and (vi) diminished delivery of sodium to the distal renal tubule site of natriuretic peptide action. According to the unifying hypothesis of body fluid volume regulation, arterial underfilling results in renal vasoconstriction, decreased renal perfusion pressure, and activation of the sympathetic and renin–angiotensin systems. These renal hemodynamic and neurohormonal changes then decrease the glomerular filtration rate and

increase proximal tubular sodium reabsorption, thereby resulting in diminished distal tubular sodium delivery that may explain the blunted natriuretic response to ANP and BNP (3–8). This notion is supported by several observations. In sodium-retaining patients with heart failure, a strong positive correlation between levels of plasma ANP and urinary cyclic guanosine monophosphate [the second messenger for the natriuretic effect of ANP *in vivo* (117)] has been reported, supporting the active biological responsiveness of renal ANP receptors in heart failure (118). Further, in cirrhosis, maneuvers that increase distal tubular sodium delivery have been shown to reverse ANP resistance (119). Finally, distal tubular sodium delivery has been reported to be the most potent predictor of renal responsiveness to BNP in heart failure patients (Fig. 1.4) (115).

### *Renal prostaglandins*

In normal subjects and in intact animals, renal prostaglandins do not regulate renal sodium excretion or renal hemodynamics to any significant extent (120,121). In patients with heart failure or cirrhosis, vasodilating prostaglandins appear to play an important role in the maintenance of renal blood flow and glomerular filtration. For example, inhibition of prostaglandin synthesis in decompensated cirrhotic patients decreases renal blood flow, glomerular filtration rate, sodium excretion, and solute-free water excretion and impairs the natriuretic response to furosemide or spironolactone (122,123). Infusion of prostaglandin $E_1$ has been shown to reverse these decreases in renal hemodynamics observed after prostaglandin inhibition (123). Similar observations have been made in patients with chronic heart failure (124). These findings support a counterregulatory role for vasodilating prostaglandins in the regulation of body fluid volume in patients with heart failure and cirrhosis.

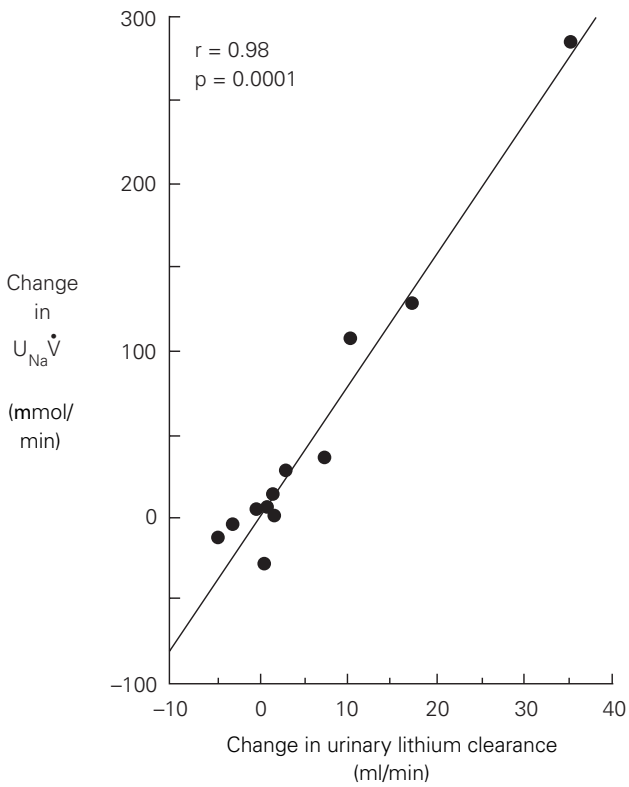


**Figure 1.4** Correlation between the natriuretic response to infused brain natriuretic peptide and the change in distal tubular sodium delivery in heart failure patients. $U_{Na}\dot{V}$, urinary sodium excretion. (Reproduced with permission from Abraham WT, Lowes BD, Ferguson DA *et al*. Systemic hemodynamic, neurohormonal, and renal excretory effects of a steady-state infusion of human brain natriuretic peptide in patients with decompensated chronic heart failure. J Card Fail 1998; 4:1.)

## Summary

The various neurohormonal systems activated in response to diminished effective arterial blood volume influence changes in renal hemodynamics and directly affect tubular sodium and water handling, resulting in an avid sodium- and water-retaining state in an attempt to restore the integrity of the arterial circulation. Activation of neurohormonal vasoconstrictor systems appears to be mediated primarily by high-pressure baroreceptor stimulation of the sympathetic nervous system, leading to activation of the RAAS and the non-osmotic release of vasopressin, in response to arterial underfilling. Counterregulatory vasodilator and natriuretic hormones, such as the natriuretic peptides and vasodilating prostaglandins, are also activated in edematous states such as heart failure and cirrhosis. These hormones may serve to attenuate to some degree the antinatriuretic and antidiuretic effects of vasoconstrictor hormone activation.

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# Chapter 2
# Physiology of the Renal Circulation

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## Introduction

The kidneys receive approximately 20% of cardiac output in the normal human or mammal while at rest. This is a surprisingly large percentage of cardiac output when one considers that the two kidneys only constitute 0.5% of total body mass, implying that the blood flow per gram of tissue is about 40-fold higher in the kidney than the average of the rest of the body (1). This means that the total renal blood flow in the human is at least 1 l/min with a plasma flow rate of approximately 600 ml/min and a glomerular filtration rate (GFR) of about 100 ml/min. These quantitative realities imply at least two things: since all organs observe the same mean arterial pressure in essence, renal vascular resistance must be extraordinarily low. Second, since such a large volume of ultrafiltrate is formed, 150 liters of protein-free ultrafiltrate leaving the plasma volume each day, the process of glomerular filtration must be highly regulated. The latter conclusion is implied from the fact that total body water in such an individual is probably no more than 40–50 liters and impairments in glomerular ultrafiltration regulation or wide swings in glomerular filtration rate might overload the capacity of the tubules to reabsorb this large volume of ultrafiltrate since 98–99% is absorbed on a normal day (2). In fact, total body volume status is very tightly regulated with swings much less than half a liter each day noted in the normal human on a reasonably fixed diet.

First, let us add to the question about "why does the kidney receive such a large percentage of cardiac output?" Low vascular resistance can be the consequence of a more dilated resistance vessel, usually at the precapillary or afferent arteriolar level. However, electron photomicrographs and standard light microscopy would suggest that the range of diameters for the afferent arteriole, the major preglomerular resistance, is very similar to the diameters of precapillary resistance vessels in skeletal muscle, mesentery, and other organs. Therefore, the reason for the low vascular resistance in the kidney derives from the very large number of afferent arterioles closely packed into the kidney. In fact, there are probably at least a million afferent arteriolar-glomerular-tubular units per kidney. The reason for the low vascular resistance, therefore, is that all renal blood flow is via the glomerulus and there are a large number of nephron units so that the low vascular resistance is a consequence of a large number of resistances in parallel intensely packed in the organ.

The preglomerular vasculature in the kidney is reasonably complex with the renal artery branching into interlobular vessels, and arcuate vessels which connect these interlobular arteries, which then branch into intralobular vessels and later afferent arteriolar units, each of which is connected to a glomerular capillary. Therefore, the total vascular resistance of an individual vascular nephron unit is the summation of all resistances, preglomerular, the efferent arteriole which separates glomerular capillary from the peritubular network, and the resistances supplied by the peritubular and venular capillaries prior to the renal vein. The distribution of resistances in a nephron vascular unit is similar to that of other organs in the sense that approximately 50% of vascular resistance between aorta and the glomerular capillary resides between the aorta and the glomerular capillary. Presumably, a fairly high percentage of this preglomerular resistance is proximal to the afferent arteriole although, as we will see, the afferent arteriole functions as the major regulator of vascular resistance in most circumstances. The kidney vasculature is unique in one other aspect. The kidney vasculature is divided into two distinct capillary beds, the first being the glomerulus, the series of structures between the afferent arteriole and the efferent arteriole which are constituted by up to 20 or more parallel capillary units that freely communicate with one another and contain no measurable smooth muscle in the capillary system. This unit is dominantly a unidirectional filtering unit (3). These capillaries are supported by mesangial cells which supply physical support for the capillary units in the glomerulus and also exhibit contractile properties which may modify the architecture of the capillary units but probably do not significantly increase resistance to blood flow. In the secondary peritubular capillary network, fluid flux is also unidirectional with reabsorption of solutes and water.

The species in which glomerular capillary pressure has been directly and consistently monitored is the rat. Based upon directly measured assessments, the mean glomerular capillary pressure in the rat under anesthetic conditions is approximately 45–50 mmHg, while aortic mean arterial pressure is approximately 100–110 mmHg. Experimental data would suggest that there is no significant pressure drop along the glomerular capillary, implying (3,4) that the glomerular capillary vasculature supplies no significant percentage of total vascular resistance. The efferent arteriole contributes substantially to total vascular resistance of the nephron vascular unit, approximately 30–35%. Peritubular capillary pressure is usually approximately equal to tubular pressure or 10–15 mmHg. The renal vein pressure is usually in the order of 3–4 mmHg. Therefore, the major regulation of renal vascular resistance logically must take place in the preglomerular vasculature. Experimental studies would suggest that regulation of blood flow in response to changes in blood pressure occurs primarily at or near the afferent arteriole (5,6). Vascular resistances proximal to the afferent arteriole can change but usually in response to other neurohumoral factors. The contribution of these larger vessels to autoregulation, although important, is probably of lesser primary significance than is the afferent arteriole.

## Autoregulation of renal blood flow

Excellent studies demonstrating highly efficient autoregulation of renal blood flow date back at least 30–40 years (5–8). Experimental studies in the anesthetized and unanesthetized dog and rat have suggested nearly 100% efficiency of autoregulation of blood flow in response to changes in blood pressure between mean pressures of 50 mmHg and 140–150 mmHg. Investigators have concluded that the variation in vascular resistance which occurs in response to changes in systemic and renal artery pressure occurs predominantly in preglomerular vessels (5,6). There are a variety of conditions in which autoregulation of renal blood flow can be impaired which include massive volume expansion, partial ureteral obstruction with elevation of intratubular and intrarenal pressures, administration of certain potent diuretics such as furosemide or bumetanide, vasodilators, and in certain disease conditions such as acute renal failure (9,10). Studies in the rat have also evaluated autoregulation of GFR and renal blood flow while glomerular pressure was monitored by direct pressure measurements (6). Results verified the efficiency of autoregulation of renal blood flow but also attested to the fact that GFR is almost equally well autoregulated. These phenomena are accomplished with a relative constancy of glomerular capillary pressure, as a result of appropriate vasodilation of the afferent arteriole in response to lowering of systemic and renal artery pressure, but also to some extent due to the behavior of the efferent arteriole which, at the lower limits of systemic blood pressure, increases resistance slightly to sustain or maintain glomerular capillary pressure. The rapid component of autoregulation at the afferent arteriole appears to be dominantly myogenic in character (11). However, there appear to be slower components which are unique to the kidney and they involve participation of the tubuloglomerular feedback (TGF) system, a system that will be described in greater detail later in this chapter. Most organs in the body exhibit highly efficient myogenic autoregulation of blood flow, but the additional impact of TGF appears to permit the kidney to exhibit the most efficient autoregulatory processes (12). Since the major reason for oxygen utilization in the kidney is tubular reabsorption of NaCl and other solutes and secretion of other molecules, it is logical that mechanisms contributing to the autoregulation of renal blood flow should be linked in some way to the tubular reabsorptive process. This linkage is probably mediated via the activity of the TGF system.

The arteriovenous oxygen difference in the kidney is exceedingly small, in part because of the high blood flow rate and the relatively small oxygen utilization relative to blood flow rate. The high renal blood flow rate is required teleologically for the high rate of glomerular ultrafiltration. In the nonfiltering, nonreabsorbing kidney the nutrient requirements necessary to sustain viable cell function are no more than 5–10% of normal renal blood flow (13). However, with normal filtration rates oxygen utilization is somewhat higher. These initial observations would suggest that the kidney is living in a condition of oxygen excess whereby more oxygen is supplied than is needed for both active transport and sustaining nutrient activity of the cells. However, at the same time studies from clinical experience suggest that the kidney is in fact not protected from hypoxia or ischemia, and that acute renal failure does occur as a result of a variety of hemodynamic insults. Recent studies utilizing $pO_2$ electrodes have also demonstrated that there is a significant compartmentalization of oxygen within the kidney cortex and medulla which is relatively unaffected by large variations in systemic $pO_2$. Studies by Schurek and coworkers have demonstrated the normal $pO_2$ of the kidney cortex is much lower than systemic $pO_2$, in the range of 50–65 mmHg (14). Medullary $pO_2$ is much lower in the range of 25–40 mmHg, suggesting significant compartmental heterogeneity within both the cortex and the medulla (15). These relatively low values for oxygen tension remain relatively constant even when systemic $pO_2$ is raised to 550 mmHg, suggesting significant compartmentalization of gas mixtures within the kidney (14). It should be recalled that the $pCO_2$ in the kidney cortex is also elevated above systemic $CO_2$ at approximately 60–65 mmHg (16). This latter finding is a reflection of the

high rate of proton secretion by renal epithelial cells and the acidification of the urine resulting in high rates of $CO_2$ production.

This relatively low cortical and medullary $pO_2$ is accomplished by a significant preglomerular arteriolar–venous diffusion shunt pathway in soluble oxygen (14). The density of the arteriolar networks in the kidney as previously described is quite high since there are normally a million nephrons within the normal human kidney. It is presumed that as $pO_2$ is elevated in the systemic circulation and in the renal artery, arteriolar oxygen diffuses into the exiting blood prior to the glomerulus and prior to the cortical filtration process such that, regardless of $pO_2$ in the systemic blood, oxygen tension remains relatively low in both the cortex and the medulla. This finding makes some sense since the site for erythropoietin production is located within the kidney and the generation of this hormone which regulates red cell production should be sensitive to variations in oxygenation (17). Since the $pO_2$ is lower within the kidney than previously predicted, probably similar to the capillary $pO_2$ in other organs, the cortex and particularly the medulla may be living on the edge of hypoxia and dependent upon the oxygen demands placed upon the tubule for reabsorption of solutes and water.

Regulation of medullary $pO_2$ would be quite important since the normal value is on the edge of aerobic/anaerobic metabolism. Recent studies have demonstrated that inhibitors of nitric oxide synthase (NOS), L-NMMA, when infused into the isolated perfused kidney, further decrease medullary $pO_2$, suggesting either that nitric oxide (NO) is linked to the regulation of vascular resistance in the medulla or that NO somehow influences transport rates or NaCl delivery to the macula densa thick ascending limb (15). It is possible that there are also more complex interactions between NO and oxygen since both gases, oxygen and NO radical, bind to various ferroporphryin enzyme systems and may regulate in some direct or indirect manner the vascular resistance in medullary blood vessels (18). Mechanisms whereby oxygen utilization and tubular reabsorption may contribute to the regulation of renal blood flow also relate to the role of the tubuloglomerular feedback system described in the next section.

## Tubuloglomerular feedback system

The renal system which filters 150 l/day and reabsorbs 148 l of these solutes and water must be highly coordinated. If there was no relationship between reabsorptive and ultrafiltration processes and they were not coordinated, large swings in extracellular volume and total body water might occur. It has been known for several years that part of the coordination of the reabsorptive filtration processes is mediated by a process called glomerular tubular balance, a term coined by Homer Smith (2), whereby increases or decreases in flow into each nephron segment, proximal tubule, loop of Henle, and distal tubule are accompanied by a near proportional increase or decrease in the rate of tubular reabsorption, maintaining fractional reabsorption nearly constant. However, one can obviously deduce that such a system which guarantees a forward, positive feedback relationship resulting in constancy of fractional reabsorption will not produce full coordination between the processes of filtration and reabsorption. Glomerulotubular balance as the single regulatory mechanism would make excretion of solutes and water totally proportional to the filtration rate.

For the past three or four decades it has been recognized that another coordinating system exists called the TGF system, a mechanism which is intrinsic to the kidney and to single nephrons, which regulates the filtered load in relationship to the delivery or reabsorption of NaCl in the more distal segments of the nephron (12,19,20). The afferent or affecter signal of this system appears to reside in and around the macula densa, a specialized distal tubular cell which is in close physical contiguity to the glomerulus to the vascular pole afferent and efferent arterioles of the same nephron (21).

Demonstration of this system was accomplished by microperfusion techniques in which purposely the distal tubule was isolated from the proximal nephron by blockade of the nephron (19,20). When the distal or late proximal tubule is perfused at varying rates with NaCl-containing solutions, it can be demonstrated that the increased perfusion rate is associated with a reduction in filtration rate of that nephron unit. When the flow rate is reduced to the distal nephron below normal ambient levels, a rise in filtration rate occurs presumably as a result of an intrinsic system transmitting information to the vascular pole and vascular resistances primarily the afferent arteriole. These initial studies, which might be considered open loop assessments of the TGF system, demonstrated that various factors modulated the activity or the gain and the maximum response of this system. When the perfusion rates increased to at least twice normal, the reduction in nephron filtration was approximately 30–50% in euvolemic condition under these open loop systems purposely separating the affecter signal from the effector response mechanism. It was clear that volume status on a chronic basis modulates the response of the system with volume expansion diminishing the magnitude of that response.

However, the importance or the physiological relevance of this system relates to its operation in and around the normal flow rate. Recent studies from our laboratory and others have attempted to estimate the gain and efficiency of the system by examining the TGF system in a closed loop system in which the proximal and distal portions of each nephron are in constant

communication (22). This system utilizes free-flowing nephrons with videometric flow of velocitometry measuring the ambient flow rate in the tubule while flow rate distal to this measurement is purposely altered by either addition or subtraction of fluid from the nephron. This technique measures the integrated effects of both glomerulotubular balance and TGF functions on the flow rate. Utilizing the open loop microperfusion approach, the contribution of glomerulotubular balance is not assessed, but the maximum capacity of the system is quite accurately characterized. The current closed loop on-line technique permits evaluation of the behavior of the feedback system in and around its ambient flow rate. These studies suggest that the gain or the efficiency around the normal flow rate of the single nephron is really quite high, around 70–75%. Whole kidney integrated operation of the TGF is probably even higher because of some cross-communication of information among nephrons via transmission along the afferent arteriole. This means that as distal fluid delivery increased, there was approximately 70–75% compensation via reduction in flow rate proximal to the perturbation. This was mediated by an alteration in nephron filtration rate. There was symmetrical efficiency to either addition or withdrawal of fluid from the nephron. Peak efficiency was always in and around the normal flow. Utilizing these techniques and others, it was suggested that this feedback system exhibits an oscillatory behavior with the rate of about 2–3 cycles/min, suggesting a system with a high gain or efficiency and a defined time delay between the regulated flow rates and the proximal tubule and the sensing segment located at the macula densa in the early distal tubule (23).

There have also been *in vitro* demonstrations of the existence of the TGF system utilizing perfusion of dissected glomeruli with their macula densa segments (24). Perfusion with high NaCl concentration fluids results in afferent arteriolar constriction. When low NaCl solutions are utilized, the afferent preglomerular vessels vasodilate. This relationship as it is *in vivo* is inhibited by high concentrations of furosemide, a diuretic that inhibits the $Na^+$-$Cl^-$-$Cl^-$-$K^+$ symporter in the distal tubular segments. This finding further suggests that a signal related to the transport of NaCl is mediating the afferent signal, which eventually communicates to the vascular segments, resulting in changes in afferent arteriolar diameter. From a teleological standpoint, the TGF system functions to maintain distal flow rate relatively constant despite variations in proximal reabsorption and in GFR in an effort to prevent the capacity of the distal tubular reabsorption from being overwhelmed or exceeded, thereby avoiding inordinate extracellular volume losses into the urine. In addition, this system operates as an adjunctive mechanism to the autoregulation of renal blood flow (11). Surges in renal blood flow related to increases in systemic blood pressure will result in increased flow to the macula densa, thereby eliciting slightly time-delayed vasoconstriction at the afferent arteriole, augmenting existing myogenic mechanisms operating to maintain renal blood flow constant.

Questions arise as to what are the practical day-to-day functions of the TGF system and how does this system influence renal function and the regulation of GFR (25,26). Certainly, all investigators who examined the issue have agreed that diabetes mellitus and hyperglycemia result in a dampening or diminished homeostatic efficiency of the TGF system (27,28). From a teleological standpoint this would result in further or inordinate losses of salt and water during episodes of hyperglycemia because of the relative inability of the kidney to control the filtered load in spite of high distal NaCl and volume delivery.

Although NO and NOS activity will be discussed in a later portion of this chapter, it is also clear that NO is a modulator of TGF activity. Nonselective inhibition of NO generation resulted in significant increases in the homeostatic efficiency of the feedback system (29). There is evidence that the NOS which is participating in this process is the neuronal or brain NOS system located primarily in the macula densa cell segment and, in part, in the efferent arteriole (30). It is interesting to speculate that various pathophysiological conditions which have been characterized as relative NO activity deficiency states could therefore be associated with heightened TGF activity. Certain forms of hereditary hypertension such as salt-sensitive hypertension and the spontaneously hypertensive rat exhibit either heightened TGF activity or diminished capacity to suppress TGF activity when submitted to a exceedingly high NaCl intake (31,32).

In addition, angiotensin II (AII), possibly related to changes in volume status, exerts important modulator influences on the behavior of this system (33). The search for a mediator has been frustrated by complexity of the neurohumoral interactions. A firm conclusion cannot be stated at this juncture, but it is clear that adenosine plays a critical role and may serve as a major mediator of this system (34). It is also possible that other substances which are regulated by NO, such as the local cytochrome P450s which metabolize arachidonic acid to vasoactive products, may modulate or possibly mediate TGF responses (35). Another important pathophysiological condition in which TGF systems appear to be importantly activated are forms of acute renal failure. In a normal animal inhibition of proximal tubular reabsorption elicits feedback responses by increasing distal delivery of NaCl and sodium bicarbonate resulting in significant reductions in nephron filtration rate (36). In a similar fashion, nephrotoxic insults to the kidney with heavy metals elicit TGF responses which appear to persist and contribute to the reduction in GFR observed following major proximal tubular injuries (37).

With our present state of understanding, there is no doubt that the TGF system participates in the regulation of GFR and as an adjunct to autoregulation of renal blood flow. However, the role of this system may be adaptation or deactivation over time. Certainly, during such normal processes as growth, pregnancy, and chronic alterations in NaCl intake, adaptations must take place that allow the TGF system to operate efficiently at the normal flow rate. Temporal adaptation is a normal phenomenon (38,39) and certain pathophysiological conditions may contribute to salt retention or hypertension due to associated abnormalities in the adaptation of normal intrinsic feedback systems. These temporal adaptations may depend upon specific neurohumoral alterations within the kidney milieu. Further investigations are required to define the specific mechanisms whereby adaptation does or does not take place.

## The major intrarenal vasoconstrictor systems

### Angiotensin II

AII is a small octapeptide of approximately 1000 molecular weight. This is probably the first hormone for which there was early evidence of a major role in the regulation of the renal circulation (4). Investigators have been aware for years that AII was generated in the kidney and that the hormone exerted significant pressor effects when injected into the systemic circulation (40). However, over the years it has become appreciated that AII exerts multiple renal responses which are both direct and indirect, and these effects are not confined to effects of AII on vascular smooth muscle cells.

AII increases proximal reabsorption (41,42) and AII receptor blockade reduces absolute and fractional proximal reabsorption during chronic salt depletion (43). AII exerts rather complex effects on glomerular ultrafiltration by increasing both afferent and efferent arteriolar resistances and increasing glomerular hydrostatic pressure gradient, in part because of a somewhat greater effect on the efferent arteriole, and by decreasing the glomerular ultrafiltration coefficient (LpA) (4). Because of the balance between increased glomerular pressure and the negative influences (decreased plasma flow and decreased LpA), modest influences of AII may result in maintenance of GFR in spite of reductions in plasma flow resulting in an increased filtration fraction. Initial studies were based upon exogenous infusions of AII (4). However, later studies demonstrated that during chronic salt depletion and in models of congestive heart failure, endogenous AII generation resulted in almost identical changes in glomerular hemodynamics (43,44). Studies on glomerular hemodynamics utilizing AII infusion or chronic salt depletion suggested that there were target cells within the glomerulus other than vascular smooth muscle which responded to AII. These include glomerular mesangial cells, which exhibit modest contractual properties, and probably the glomerular visceral epithelial cell acting in concert to mediate reductions in the glomerular ultrafiltration coefficient.

AII effects on tubular reabsorption are complex and both direct and indirect. Obviously, AII generates aldosterone from the adrenal cortex, which in turn influences collecting duct sodium reabsorption and potassium and proton secretion. In addition, AII exerts biphasic and contrasting effects on proximal tubular reabsorption and lower doses (high pM) stimulate proximal sodium reabsorption and high nM doses inhibit reabsorption, in all probability via differing signal transduction pathways (41). There are recent data suggesting modest direct effects of AII on tubular reabsorption in such disparate segments as the thick ascending limb and the cortical distal tubule (45–47).

AII is generated locally within the kidney and recent reports suggest rather high proximal tubular luminal concentrations of this hormone (48). In fact, all of the components of the renin–angiotensin system are present within the kidney, generating rather remarkably large quantities of this peptide. However, the receptors within the kidney are even more numerous, such that much of the AII measured by radioimmunoassay may be bound to receptors and the actual free concentration within the interstitium is undoubtedly somewhat lower. The location of AII receptors appears also to be very important. Rather low levels of AII in blood (pM) appear to exert significant biological effects within the circulation, while concentrations of AII within the kidney are up to 1000-fold higher (48).

#### *Angiotensin interactions*

The effects of AII on certain effector cells are modified not only by the density and type of AII receptor but also by the capacity of local antagonistic hormonal systems to modify the effects of AII. For example, prostaglandins and NO are vasodilators, which function as naturally occurring antagonists of AII activity, especially on vascular smooth muscle cells. In fact, most of the initial confusion on whether AII acted at both the afferent and efferent arterioles is probably related to the capacity of these alternate hormonal systems to inhibit or modify the activity of AII as a vasoconstrictor. This interaction is further complicated by the fact that certain types of cyclooxygenase-derived prostaglandins stimulate renin as does NO (48), generating a rather consistent pattern in which certain hormones antagonize AII at effector cells, yet stimulate the generation of the AII via effects on the enzyme, renin. Acute inhibition of NO synthase (49) and of cyclooxygenase (50) magnifies the effects of AII. However, chronic inhibition of these systems exerts more complex effects

because of their capacity to decrease renin activity, and therefore AII generation (51).

### Renal adrenergic activity

Renal nerve stimulation, increases in circulating norepinephrine or both result in renal vasoconstriction (52–54). However, analysis of norepinephrine effects on the kidney vasculature are complicated by the fact that multiple adrenergic receptor subtypes reside within the kidney. Each of the $\alpha_1$, $\alpha_2$, and β-adrenoreceptors have been subdivided into a variety of subtypes that exert particular functions with differing *in situ* localizations within the kidney. It is generally recognized that adrenergic renal vasoconstriction is dominantly produced by the activity of α-adrenoreceptors. However, the concerted effect of multiple adrenoreceptor stimulation may be necessary for the full expression of norepinephrine activity. This is in part related to the fact that $\alpha_1$ receptor stimulation probably is less effective as a single vasoconstrictor agent in the kidney than it is in the systemic circulation (55). Renal nerve stimulation also results in β receptor activation, which in turn stimulates renin and generates AII.

It is difficult to analyze the effects of renal adrenergic activity without discussing interactions with other systems. Studies have demonstrated that much of the vasoconstrictor effects of modest frequency renal nerve stimulation are mediated via the actions of AII (52). Blockade with AII receptor blockers or angiotensin-converting enzyme (ACE) inhibitors remove about 75% of the vasoconstrictor effects of 3 Hz frequency nerve stimulation. Alternatively, acute renal denervation does not produce a major increase in renal plasma flow unless angiotensin activity has been inhibited by ACE inhibitors or by AII receptor blockade (56). Subacute renal denervation does result in a modest increase in nephron plasma flow but also appears to be associated with heightened sensitivity to AII, in part based upon increases in AII receptor number in glomeruli (57).

The effects of $\alpha_2$-adrenoreceptor stimulation are even more complex. In the innervated kidney administration of $\alpha_2$ agonists results in vasodilation, primarily as a result of prejunctional $\alpha_2$-adrenoreceptor stimulation and decreased norepinephrine release (58). However, in the denervated kidney $\alpha_2$-adrenoreceptor stimulation reduces nephron filtration rate primarily by producing a reduction in the glomerular ultrafiltration coefficient. AII and $\alpha_2$-adrenoreceptors interact in a positive or synergistic fashion in that the effects of $\alpha_2$-adrenergic agonists are enhanced by high local AII activity in the kidney and are blocked by AII receptor blockers (59). In a similar fashion, low levels of $\alpha_2$-adrenergic agonists, which do not affect glomerular hemodynamics, appear to amplify the effects of AII on glomerular ultrafiltration. In summary, the significant vasoconstrictor effects of norepinephrine infusion and renal nerve stimulation depend upon activation of a variety of adrenergic receptors, $\alpha_1$, $\alpha_2$, and β, and may involve concurrent activities of the other major renal vasoconstrictor system such as AII.

## Endothelin

The endothelin family includes three 21 amino acid peptides (ET1, ET2, ET3) (60,61). Endothelins are cleaved from proendothelins both in the intracellular and extracellular compartment by endothelin-converting enzymes (62,63). Among the three peptides, the major renal isoform is ET-1 (64–67). ET-1 is produced by endothelial cells, mesangial cells, and epithelial cells in the glomerulus as well as by tubular cells. Endothelin is recognized as one of the most potent vasoconstrictors known (60). Administration of ET-1 is associated with significant reductions in GFR and renal plasma flow. Micropuncture studies have characterized the effects of endothelin at the single nephron level (68–70). These studies demonstrate that endothelin reduces nephron filtration rate due to reductions in nephron plasma flow and the ultrafiltration coefficient. The reduction in nephron plasma flow is secondary to an increase in both afferent and efferent arteriolar resistances. Using the hydronephrotic kidney preparation, endothelin was demonstrated to have similar effects on preglomerular and efferent arteriolar vessels of cortical and juxtamedullary nephrons. Interestingly, the effects on preglomerular and efferent arterioles are mediated through a different receptor mechanism (ETA for the preglomerular vessels and ETB for efferent arterioles) (71).

The effects of endothelin-1 are mediated through two different receptors, ETA and ETB (72,73). One very important and unusual characteristic of ET-1 is the fact that it remains associated with its receptor for a very long period of time (up to 2 h after endocytosis in the case of ET-1 and ETA) leading to a prolonged biological effect (74). Increased sensitivity of the renal vasculature to ET-1 results from the increased density of receptors in the renal vasculature (75,76). Human kidneys have a predominance of ETB receptor over ETA, ETA receptors being localized in the vasculature and ETB in renal tubules and medulla (77,78).

Three major categories of stimuli lead to increased renal production of ET-1: (i) vasoconstrictor/thrombogenic agents, (ii) physical factors, and (iii) inflammatory cytokines (79). AII, vasopressin, 8-epi-prostaglandin $F_2\alpha$ and thrombin are among the vasoconstrictors/thrombogenic agents (80). Mechanical strain, low levels of shear stress, and pressure without cell distortion constitute the physical factors (81–84). Tumor necrosis factor-α and interleukin-1 stimulate ET-1 production in mesangial cells (85,86). ET-1 production is inhibited by nitric oxide, pros-

tacyclin, atrial natriuretic factor, prostaglandin $E_2$, bradykinin, and heparin (87–90).

ET-1-induced vasoconstriction is mediated by increased intracellular calcium concentration secondary to release from intracellular stores by the inositol triphosphate pathway and by receptor-operated and voltage-gated channels in the plasma membrane (91).

There are significant interactions between endothelin and the other vasoconstrictors and vasodilators which modulate effects in the renal vasculature. Of great interest, endothelin stimulates, through the ETB receptor, the production of NO in endothelial cells, which limits the effects of endothelin on vascular smooth muscle cells (92,93). The production of vasodilation and vasoconstriction through two different receptors (ETB and ETA) makes the design and interpretation of the effects of endothelin antagonists or receptor blockers a confusing but potentially very interesting field of research (94).

Endothelin has important interactions with AII, nitric oxide, and the prostaglandin system (95). Some of these interactions are reciprocal, as in the case of ET-1 and AII. ET-1 modulates renin secretion, although important differences exist between *in vitro* and *in vivo* conditions (91). In *in vivo* situations, the vasoconstrictor effects of ET-1 seem to activate the renin–angiotensin system. While ET-1 modulates renin secretion, AII increases ET-1 release from endothelial cells (79).

ET-1 activates phospholipase $A_2$ and prostaglandin endoperoxide synthase-2 leading to increases in prostaglandin generation. In endothelial cells, ET-1 stimulates the production of $PGI_2$, $PGE_2$, and thromboxane $A_2$ ($TxA_2$) (96). In mesangial cells, ET-1 induces $PGH_2$ leading to marked increases in $PGE_2$ generation (97). Production of these vasodilatory prostaglandins blocks some of the vasoconstrictor effects of ET-1, as clearly demonstrated by the enhanced vasoconstriction observed after the administration of nonsteroidal anti-inflammatory drugs (NSAIDs) (98,99).

As mentioned previously, ET-1 stimulates constitutive NOS to increase NO generation in endothelial cells and mesangial cells. Increased NO generation decreases vasoconstriction and reduces mesangial cell contraction.

## Prostaglandins

Prostaglandins are derived from the metabolism of arachidonic acid (100–102). In the presence of phospholipase $A_2$, arachidonic acid is freed from membrane phospholipids and can be converted to the various prostaglandins in the presence of the enzyme prostaglandin endoperoxide synthase or cyclooxygenase (COX). Two isoforms of COX have been demonstrated: COX-1 and COX-2 (101–103). COX-1 is expressed constitutively in most cells throughout the organism and is responsible for the generation of prostaglandins in response to various hormones. In contrast, COX-2 is not detectable under normal conditions but can be induced in the presence of various cytokines and inflammatory processes with the exception of the macula densa where neuronal NOS and COX-2 are constitutively expressed. Induction of COX-2 leads to increased production of prostaglandins over a long period of time. This increase in prostaglandin generation after immune injury may be critical to maintain renal function (RPF and GFR) in the various renal inflammatory conditions (104,105). The individual characteristics of COX-1 and COX-2 suggest that COX-1 is actively involved in the minute-to-minute regulation of renal blood flow and sodium excretion while COX-2 is an unlikely participant in this acute regulatory process. However, COX-2 seems to play an important role in decreasing along with NO the increase in afferent arteriolar tone during increases in macula densa sodium chloride. Interestingly, the upregulation of COX-2 during salt restriction suggests the possibility that this enzyme is also involved in the regulation of sodium and water homeostasis (106). COX-1 and COX-2 convert arachidonic acid into the unstable products endoperoxides $PGG_2$ and $PGH_2$, which are then converted in the presence of the various synthases and reductases into the different prostaglandins ($PGI_2$, $PGE_2$, $TxA_2$, $PGF_{2\alpha}$) (100–102).

Prostacyclin ($PGI_2$) is the most abundant prostaglandin in the renal cortex and is synthesized by glomeruli and arterioles (107,108). $PGE_2$ is the most abundant prostaglandin produced by the tubules but is also produced in the glomerulus. Systemic and intrarenal administration of $PGI_2$ and $PGE_2$ lead to renal vasodilation with reductions in both afferent and efferent resistances (109,110). The increase in RPF is associated with variable changes in GFR, probably as a reflection of the impact of prostaglandin administration on blood pressure and other neurohumoral systems (111).

Both $PGE_2$ and $PGI_2$ bind to their specific receptor (112). $PGE_2$ binds to the EP receptor that is the most abundant prostanoid receptor in the kidney. There are four different subtypes of EP receptors localized throughout the entire kidney, including epithelial cells, endothelial cells, mesangial cells, and vascular smooth muscle cells (112). Signaling pathways vary between the receptor types and include phosphatidylinositol hydrolysis with receptor-operated calcium mobilization, and pertussis toxin $G_i$ and $G_S$ leading to activation or inhibition of adenylcyclase. $PGI_2$ activates the $PGI_2$ receptor IP found throughout the cortex and the medulla. IP receptor activation is coupled to generation of intracellular cyclic AMP.

In contrast with the vasodilatory effect of $PGE_2$ and $PGI_2$, the kidney also synthesizes $TxA_2$, which is a potent vasoconstrictor (113–114). $TxA_2$ is produced in very small quantities under normal conditions and its site of production is the glomerular mesangial cell and podocyte.

$TxA_2$ binds to its receptor (Tpa) present in intrarenal arteries and glomeruli leading to increases in intracellular inositol triphosphate ($IP_3$) and mobilization of calcium from intracellular stores with afferent and efferent vasoconstriction. Increases in $TxA_2$ levels have been found during nephritis, cyclosporin toxicity, and renal transplant rejection.

Prostaglandins are not major regulators of GFR or renal plasma flow under normal conditions. As demonstrated both in experimental animals and humans, acute and chronic blockade of prostaglandin synthesis with NSAIDs does not modify GFR or renal plasma flow in euvolemic animals or normal volunteers (115–123). In contrast, conditions associated with increased prostaglandin levels or activity demonstrate significant reductions in renal plasma flow and GFR after administration of NSAIDs (124–126). Among the classical examples are the dramatic reductions in GFR, RPF, and sodium excretion observed after administration of NSAID in patients with cirrhosis and ascites (127–131). Increased prostaglandin levels can be primary or secondary (132). Decreased intravascular volume or decreased effective arterial blood volume conditions (i.e. congestive heart failure, cirrhosis, nephrosis, sepsis, hypotension) are secondary causes that stimulate prostaglandin generation via increased adrenergic activity and AII levels. Primary causes include various conditions such as chronic renal failure, obstructive nephropathy, cyclosporin, and glomerulonephritis. Primary causes are not associated with reductions in effective arterial blood volume or increased AII or adrenergic activity.

There has been great interest recently in the potential beneficial effects of COX-2 inhibitors vs. the traditional nonspecific COX-1 and COX-2 inhibitors such as the NSAIDs. COX-2 inhibitors constitute ideal agents for the management of chronic inflammatory diseases such as rheumatoid arthritis or osteoarthritis with reduced risk of gastrointestinal side-effects. However, these new agents offer no benefit compared with traditional NSAIDs in terms of renal protection in individuals with volume depletion, high angiotensin II states, liver, heart, or renal disease. COX-2 inhibitors do not alter renal function in normal healthy individuals but reduce GFR and RPF in patients under 'stress conditions' (133–134).

There is a two-way interaction between prostaglandins and the renin–angiotensin system. $PGI_2$ plays a critical role in renin secretion such that it is well established that administration of NSAIDs is associated with a reduction in renin secretion (135–138). AII stimulates phospholipase $A_2$, increasing free arachidonic acid and prostaglandin generation. As mentioned earlier, endothelin is another important stimulus for phospholipase $A_2$ and through this mechanism stimulates prostaglandin generation. Prostaglandins also interact with the L-arginine NO system. Recent studies also suggest the presence of a two-way interaction between prostaglandin and NO by which NO regulates prostaglandin production and prostaglandins can modulate the induction of NOS (139–143).

## Other eicosanoids: CYP450 metabolites

Arachidonic acid can be metabolized by CYP450 monooxygenase to epoxyeicosatrienoic acids (EETs) that are hydrolyzed to dihydroxyeicosatrienoic acids (DHETs) and HETEs (144,145). CYP450 epoxygenase enzymes primarily form EETs and DHETs while HETEs are primarily formed via CYP450 hydroxylase enzymes. These enzymes are distributed throughout the kidney vasculature and tubules. Of interest, epoxygenase and hydrolase enzyme activities are modulated by humoral factors including angiotensin II, endothelin, parathyroid hormone, and epidermal growth factor (144), low salt diet, and disease conditions such as hypertension and diabetes mellitus (145).

One of the major products of the CYP450 hydroxylase is 20 HETE, which constitutes a potent vasoconstrictor of preglomerular vessels and may contribute significantly to the autoregulatory response of the afferent arteriole (146). No specific receptor has been identified so far for 20 HETE, the response of which is associated with membrane depolarization and increases in intracellular calcium (147). Increases in AII and endothelin activity lead to increases in 20 HETE.

Reductions in 20 HETE activity constitute an important mechanism of action of NO in the renal vasculature. NO binds to the heme moiety of the CYP450 enzyme and inhibits the activity of this enzyme (148). Such inhibition decreases 20 HETE production, leading to vasodilation. Attempts to quantify the role of inhibition of CYP450 enzymes as a mechanism to explain NO-induced vasodilation suggest that 75% of the vasodilatory effect of NO is mediated by inhibiting the generation of 20 HETE, the other 25% depending on increases in cGMP production.

CYP450 epoxygenase metabolites 11,12-EET and 14-15-EET vasodilate preglomerular arterioles, while 5,6-EET and 8,9-EET cause vasodilation or vasoconstriction dependent on where COX converts these products into prostaglandins or thromboxane-like compounds (147). These two CYP450 pathways, HETE and EET, seem to be closely linked since activators such as AII and endothelin stimulate both, thereby limiting important vasoconstriction.

## L-Arginine nitric oxide system

NO is derived from the amino acid L-arginine in the presence of the enzyme NOS and various cofactors (149–151). Two major families of NOS isoforms have been described, the constitutive type NOS and the inducible type NOS (iNOS). Endothelial NOS (eNOS) and neuronal NOS

(nNOS) are considered the constitutive NOS, which require intracellular $Ca^{2+}$ mobilization for activation. Hormonal activation of eNOS stimulates NO production, which is critical to maintain vessel tone. The kidney contains all three enzyme isoforms (eNOS, nNOS, and iNOS) (152). eNOS is found in glomeruli and renal vessels while nNOS is localized at the level of the macula densa and possibly efferent arteriole. Localization of eNOS and nNOS suggests that both enzymes play an important role in the regulation of renal blood flow and GFR. iNOS has been detected in almost all the structures, both vascular and tubular, within the kidney.

A large number of studies demonstrate an important role of NO in the regulation of renal blood flow and GFR (149,150). All these studies, however, have utilized various inhibitors of NOS to define the role of NO in the regulation of renal function. These studies demonstrate that NOS blockade is associated with reduction in renal blood flow secondary to increases in both afferent and efferent arteriolar resistances (49,153–155). These studies also demonstrate that NOS inhibition reduces the ultrafiltration coefficient, which in combination with the reduction in blood flow leads to variable reductions in GFR. Interestingly, the effects of NOS blockade modify glomerular hemodynamics in a manner very similar to the infusion of AII. These findings suggest that production of NO is important to negate the effect of major intrarenal vasoconstrictors (49,153,155,156). As mentioned previously, there are important interactions between NO, AII, and renal nerves. Interestingly, once again the interaction between NO and AII is a two-way interaction by which NO is important in renin generation at the same time that NO negates the effects of AII on the renal vasculature.

Generation of large amounts of NO for a prolonged period of time by iNOS makes this enzyme an unlikely participant in the minute-to-minute regulation of renal function. However, increased activity of this enzyme can reduce renal blood flow by modifying the effects of eNOS as postulated in a rat model of sepsis (157). This study demonstrates that induction of iNOS after lipopolysaccharide injection produces renal vasoconstriction through NO autoinhibition and suppression of the normal eNOS response to hormonal stimuli. This provocative finding opens the possibility that iNOS may also influence renal blood flow under certain disease conditions.

### *AII/NO interaction*

Following acute NOS blockade, systemic blood pressure increases in parallel with renal vascular resistance (49). However, the glomerular hemodynamic alterations are completely eliminated or reversed by concurrent administration of $AT_1$ AII receptor blockers while the systemic blood pressure is unaffected. These results suggest major differences in the interactions of AII and NO within the kidney vs. the systemic vasculature. The correction of glomerular hemodynamics during NOS blockade by $AT_1$ receptor blockers was not the result of restoration of NO generating capacity, suggesting that the net effects on glomerular hemodynamics result from a balance of NO and AII activity and that NO functions primarily in the kidney as a tonic antagonist of AII. This functional antagonism appears to occur at the level of the glomerulus in the vasculature and, in addition, at the level of the proximal tubule. Losartan, an $AT_1$ receptor blocker, can reverse the effects of NOS blockade on proximal tubular reabsorption. In addition, NO modulates the generation of AII by a variety of mechanisms (48).

The effects of acute NOS blockade can also be modified by renal adrenergic innervation. Subacute renal denervation of the kidney eliminates the effects of nonselective NOS blockers on glomerular hemodynamics, although generation of both NO and AII within the kidney appears to be unaffected (158). The effects of denervation can be reversed by the concurrent administration of $\alpha_2$-adrenergic agonists, thereby completely restoring the normal glomerular hemodynamic response to NOS blockade (renal vasoconstriction, reductions in plasma flow, filtration rate and the glomerular ultrafiltration coefficient) (159). In innervated kidneys the effects of denervation could also be duplicated by yohimbine, an $\alpha_2$-adrenergic agonist, resulting in elimination of the glomerular and tubular effects of acute NOS inhibition. Given the rapidity of the restoration of these responses, it seems unlikely that the quantity of NOS enzyme actually changed as a result of either removal or restoration of $\alpha_2$-adrenergic activity. These studies then suggest a complex three-way interaction between NO, AII, and $\alpha_2$-adrenergic activity. As we have previously stated, $\alpha_2$-adrenoreceptors can magnify the effects of AII at effector cells (59), but at the same time $\alpha_2$-adrenergic stimulation is purported to decrease renin activity (160). This pattern is parallel to those of AII and NO, whereby NO plays an antagonistic role at the level of effector cells, yet NO promotes the activity of renin and the generation of AII.

AII can exert its effects within the kidney via receptors other than the $AT_1$ receptor. Although the $AT_2$ receptors are not readily demonstrable in the adult rat kidney, there is physiological and pharmacological evidence that the $AT_2$ system does exert modulating influences. In chronic salt depletion it appears that $AT_2$ receptors modulate the generation of prostaglandins in response to AII and may play a role in activating NOS and generating cGMP (161).

## Renal kallikrein–kinin system

Kallikreins exist in two major types: plasma and glandular (162). Kallikreins, which are serine proteases, convert low-molecular-weight or high-molecular-weight

kininogen to bradykinin, lysyl-bradykinin or methionyl-lysyl-bradykinin. Kinins are degraded by kininases (I and II). The kidney is rich in tissular kallikrein and all the different components of this system (kallikrein, kininogen, kinin binding sites, and kininases), although they are localized in the distal segment of the nephron (163–167). The proximal tubule and the vascular endothelial are rich in kininases.

Intrarenal administration of bradykinin and kallikrein produces renal vasodilation, suggesting a potential role for these substances in the regulation of renal blood flow (168,169). However, the presence of large amounts of kininases both in the vascular endothelium and proximal tubule makes this possibility very unlikely (170,171). Localization of the renal kallikrein–kinin system to the distal nephron added to the natriuretic effect of kinins, which suggests that this system is more involved in the regulation of sodium and water excretion than modulation of renal blood flow (172). In spite of its unlikely effect on renal blood flow, the renal kallikrein–kinin system interacts with the renin–angiotensin system, the prostaglandin and NO systems. Studies suggest an important role for renin in the activity of the kallikrein–kinin system, since renin suppression is associated with reduction in urinary kallikrein and kinin excretion (173). Bradykinin activates phospholipase $A_2$ leading to increased prostaglandin generation, and bradykinin is also a stimulus for eNOS leading to increased NO generation (174).

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# Chapter 3
# Physiology of the Gastrointestinal Circulation

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## Introduction

The gastrointestinal circulation is markedly affected during the onset and progression of liver disease. The maintenance of adequate blood flow through this vascular bed is not only essential to ensure optimal function of the gastrointestinal tract, but it also contributes to the health and well-being of the liver, which receives over half of its blood supply from veins draining the gastrointestinal tract. The portal hypertension that often accompanies chronic liver disease is known to profoundly alter the mechanisms that regulate blood flow and transcapillary fluid exchange within the gastrointestinal tract. The resulting changes in blood flow, intravascular pressures, and capillary filtration in the gastrointestinal tract serve only to worsen the cardiovascular dysfunction in chronic liver disease. This chapter is focused on the physiology of the gastrointestinal circulation, with special emphasis given to factors involved in the regulation of blood flow and transcapillary exchange.

## Vascular anatomy, distribution of intestinal blood flow

The vascular supply to the gastrointestinal mucosa is particularly well suited for the absorptive and secretory functions of this tissue in that it can accommodate a high rate of blood flow, has a large exchange surface area, and permits easy permeation of nutrients and water, yet largely retains proteins within the plasma compartment. The blood and lymph circulations provide the conduits for transferring absorbed nutrients and water to the entire body (1).

The gastrointestinal system of mammals is supplied by three direct branches of the aorta: the celiac artery, and the superior and inferior mesenteric arteries (2). The celiac artery supplies blood flow to the stomach, liver, and spleen, while the superior mesenteric artery supplies the entire small intestine, proximal portions of the colon, and the pancreas. The inferior mesenteric artery delivers blood flow to the distal colon and rectum except the distal rectum, which is supplied by rectal arteries arising from the internal iliac arteries. Total blood flow through these arteries of the gastrointestinal tract is typically 20–25% of cardiac output in the unfed state (3). Along the mesenteric border of the intestine, arterial and venous branches form multiple arcades, anastomose with one another, and provide a pathway for collateral blood flow. The arcade gives rise to vasa recta, which branch to encircle the intestine and ultimately pierce the circular muscle (1).

The intraorgan distribution of blood flow within the tissue layers of the intestine is not uniform, neither in humans nor in different animal species (Table 3.1**)**. Blood flow appears to correspond to the functional importance of the tissue layer with the highest perfusion of the mucosa and submucosa (4). The major arterial vessels of the intestinal wall are located principally as an arterial plexus in the deep submucosa (5). From here vessels distribute further into arterioles and capillaries which form an extensive network just beneath the mucosal epithelium. Of the mucosal blood flow, approximately 60% perfuses the vessels that terminate as end loops and that supply the epithelial cells in the intestinal villi. The remaining 40% supplies flow to the crypts and goblet cells (4). The mucosal capillaries drain into mucosal venules, which in turn drain into collecting venules which join to form main venules and finally drain into the portal vein except the distal rectum, which drains into the internal iliac veins. Lymph vessels are closely associated with the arteries

**Table 3.1** Resting blood flow values and transmural distribution.

| Organ blood flow (ml/min/100 g) | Total wall | Mucosa–submucosa*† | Muscle–serosa† |
|---|---|---|---|
| Stomach | | 38–77 | |
| Small intestine | 8–77 | 7–103 | 5–38 |
| Large intestine | 8–44 | 9–55 | 10–34 |

*$H_2$ gas clearance. †Inert gas washout.

supplying the gastrointestinal tract, ultimately draining into the cysterna chyli and then into the systemic circulation through the thoracic duct into the left subclavian vein.

## Intrinsic vasoregulation

In response to various functional stimuli, blood flow in the gastrointestinal tract, which is normally maintained within narrow limits, is altered (Fig. 3.1). This ability to modulate blood perfusion to the demand of the tissue in specific situations has been attributed to intrinsic vasoregulatory systems. The myogenic, metabolic, and hormonal mechanisms are considered to be of the greatest physiological significance in the intrinsic vasoregulation of gastrointestinal blood flow.

### Reactive hyperemia

The term reactive hyperemia is used to describe the overshoot in blood flow that occurs after the release of arterial occlusion. After brief periods of arterial occlusion all regions of the gastrointestinal tract exhibit a reactive hyperemia (1). The magnitude and duration of the reactive hyperemic response are related to the duration of occlusion. After a 60-s occlusion, blood flow increases to approximately twice control values. The cause for the vascular response is thought to be an accumulation of metabolites, a deficiency of oxygen, or both. The ability of the small bowel to repay the oxygen debt incurred during vascular occlusions largely depends on which vessel is occluded, i.e. artery or vein. With arterial occlusions there is inadequate repayment of the oxygen debt, inasmuch as oxygen extraction is depressed during the postocclusion hyperemia. The magnitude of the oxygen deficit is proportional to the duration of arterial occlusion. In contrast, venous occlusions are associated with an overpayment of oxygen in the postocclusion period, the magnitude of which is related to the duration of occlusion. It is suggested that arterial occlusions depress, whereas venous occlusions enhance, oxidative metabolism in the small intestine (6). Increasing basal intestinal oxygen consumption by intraenteric placement of nutrients prolongs the reactive hyperemic response and increases the oxygen payback-to-debt ratio (7).

During the ischemic period that accompanies arterial occlusion, gastrointestinal motility increases, with a rise in intraluminal pressure that becomes more prominent with greater durations of occlusion (6). Relatively uniform increases in intestinal blood flow occur only for occlusion periods less than 60 s. Increasing the duration of arterial occlusion results in a predominant hyperemia in the muscularis layer, which has been attributed to the enhanced motility observed with longer-lasting occlusion.

### Postprandial hyperemia

The term postprandial hyperemia is used to describe the increase in blood flow that occurs after a meal. It can be regarded as a functional response that exists to maintain adequate blood flow for preservation of intestinal function and integrity during digestion and absorption. This hyperemia is a complex phenomenon mediated by neural, humoral, and paracrine elements. The ingestion of foodstuffs results in a dual hemodynamic change within the splanchnic circulation: (i) the initial response during the anticipation and ingestion of food and (ii) a subsequent response during the digestion and absorption of chyme from the intestinal lumen.

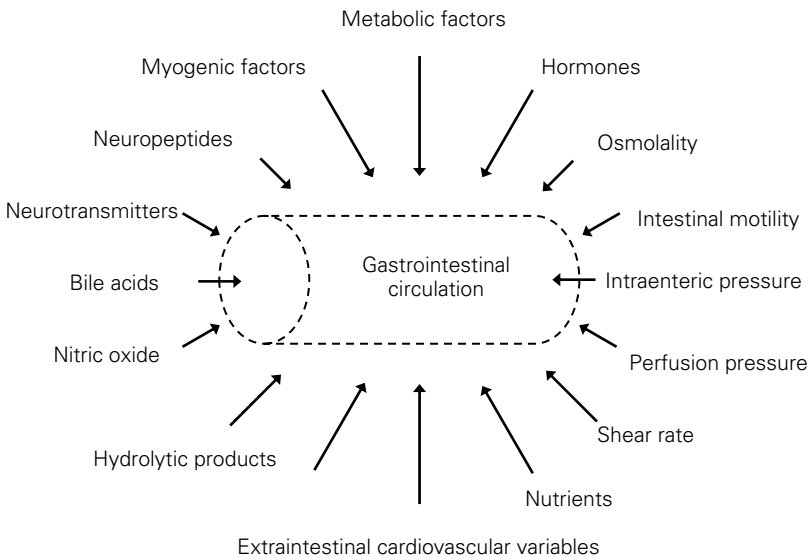


**Figure 3.1** Modulators of the gastrointestinal circulation.

The anticipatory/ingestion phase of digestion is mediated by the sympathetic nervous system and can be attenuated by adrenergic blocking agents (6). This phase is characterized by cardiovascular response such as increased cardiac output, elevated blood pressure and heart rate, and increased splanchnic and renal vascular resistance, with a variable response in skeletal and skin vascular resistance depending on the initial status of the individual (6,8). Within 5–30 min after meal ingestion, the extraintestinal cardiovascular variable returns to preprandial baseline values, whereas in the digestion/absorption phase the gastrointestinal blood flow increases with peak hyperemias achieved 30–90 min postprandially. Blood flow to the stomach and proximal bowel increases 30–90 min after ingestion of a meal, to the ileum after 45–120 min, whereas colonic blood flow tends to decrease transiently 30 min after a meal, which is probably due to tonic contractions elicited by the gastrocolic reflex (1). Blood flow in the superior mesenteric artery typically increases between 25% and 130% postprandially and may last 4–7 h depending on the nature of the meal (9). A smaller increase (10–60%) in blood flow is observed in isolated bowel segments in adult animals after intraluminal placement of digested food or nutrient solutions.

Considerable effort has been devoted to defining the luminal stimuli responsible for postprandial hyperemia. Mechanical stimulation of the mucosa, activation of intrinsic nerves, metabolic factors, release of vasoactive gastrointestinal hormones, changes in luminal pH, and a rise in luminal osmolality have all been implicated in postprandial hyperemia (1,6). Bile also appears to play an important role in postprandial hyperemia by rendering glucose and long-chain fatty acids vasoactive (10), by enhancing glucose-induced hyperemia, and by direct bile-induced hyperemia mediated by the bile acids, an assertion supported by the observation that cholestyramine abolishes the vasodilator effects of endogenous bile on ileal blood flow (11). The known hierarchy of nutrient inducers of postprandial hyperemia is led by lipids and fats in combination with bile salts. These are followed by glucose and other carbohydrates, with proteins, peptides, and amino acids as the least potent nutrient inducers.

Luminal placement of undigested food does not elicit a hyperemia, whereas digested food significantly increases blood flow, indicating that hydrolytic products of food digestion initiate the hyperemia. These hydrolytic products may be fragments, cleaved off protein molecules, which possess amino acid sequences similar to those found in vasoactive regulatory peptides normally produced in the intestinal mucosa (12). Although lipids produce the largest postprandial hyperemia, the vascular response elicited by protein and carbohydrate are not insignificant in that the three major dietary components of food appear to act synergistically on blood flow when placed in the bowel lumen (1).

The selectivity of postprandial hyperemia extends to the various layers of the gut wall. Differences in blood flow distribution exist among the mucosa, submucosa, and muscularis. Using radioactive labeled microspheres, Chou *et al*. (4) showed the local distribution of microspheres within the jejunum favoured the mucosa, which was also reported by Gallavan *et al*. (13). Postprandial gastric and colonic hyperemias are also attributed to increased mucosal flow (13). Thus, it appears that postprandial hyperemia is quite selective in its distribution to regions that are closely tied to digestive and absorptive processes, and that the mucosal region receives the largest share of the increase.

## Pressure flow autoregulation

Pressure flow autoregulation is the intrinsic ability of an organ to maintain a relatively constant blood flow despite imposed changes in perfusion pressure. The intensity of pressure flow autoregulation, which has been demonstrated in stomach, small and large bowel, increases during periods of enhanced functional activity. The metabolically active mucosa shows the greatest autoregulatory ability of all layers of the gut wall (14). Although blood flow is not perfectly regulated with an arterial pressure reduction from 100 to 50 mmHg, oxygen uptake remains relatively stable due to an increased oxygen extraction to compensate for the reduced blood flow (15). Additionally, autoregulation is enhanced by lack of oxygen (hypoxia, ischemia or increased metabolism). Pressure-flow autoregulation is absent in the neonatal intestine (16).

## Venous pressure elevation

Venous pressure elevation has proved to be a useful perturbation for determining whether metabolic or myogenic mechanisms are involved in local vasoregulation. The metabolic hypothesis predicts that acute venous hypertension causes vasodilation and increased capillary density as a result of reduced blood flow and vasodilator accumulation. According to the myogenic hypothesis, vascular resistance increases and capillary density decreases during venous pressure elevation because of a rise in intravascular pressure at the arteriolar and precapillary sphincter levels (1). In newborn animals, the intestinal vasculature dilates, rather than constricts, in response to venous hypertension, suggesting that metabolic factors are dominant in the hypermetabolic neonatal intestine (17). Findings consistent with a myogenic mechanism indicate rising vascular resistance in response to venous pressure elevation in the stomach, small and large bowel of adult animals (1).

In general, metabolic factors seem to exert a greater influence on precapillary sphincters in the stomach and colon, whereas myogenic factors dominate in the small intestine. As venous pressure is elevated, the percentage of total blood flow directed to the mucosa and submucosa is reduced, whereas the muscularis receives a larger fraction of the total blood flow. These observations indicate that the constriction of arteriolar and precapillary sphincter smooth muscles elicited by acute venous hypertension takes place in the mucosal and submucosal layers, and that the vasculature of the muscularis dilates in response to this stimulus.

## Mediators of intrinsic regulation

In accordance with the law of Laplace, resistance vessels should dilate when vascular transmural pressure is decreased and constrict when it is increased. Hence, the myogenic theory has been invoked to explain both pressure flow autoregulation and venous pressure elevation in the gastrointestinal tract. Since the intensity of all intrinsic vasoregulatory phenomena in the gastrointestinal tract is significantly influenced by the oxidative requirements of the tissue, metabolic factors have also received much attention (Table 3.2). In general, blood flow to the mucosa, submucosa, and muscular layers of the gastrointestinal tract is regulated by metabolic factors such as decreased $pO_2$, pH or osmolarity and increased $pCO_2$ or adenosine, which serve to maintain or increase blood flow to meet the tissue's need for oxygen and nutrient delivery and waste removal (18).

**Table 3.2** Potential vasoactive mediators of the gastrointestinal circulation.

| Vasoconstriction | Vasodilation |
|---|---|
| *Metabolic mediators* | |
| ↑$pO_2$ | ↓$pO_2$ |
| ↓$pCO_2$ | ↑$pCO_2$ |
| ↑pH | ↓pH |
| ↓Metabolites ($K^+$, lactate, adenosine, etc.) | ↑Metabolites |
| *Circulating humoral and paracrine mediators* | |
| Catecholamines (except liver and muscle) | Catecholamines (only liver and muscle) |
| Angiotensin II, vasopressin | Cholecystokinin, secretin, gastrin |
| Serotonin | Histamine, bradykinin |
| Platelet-activating factor (PAF) | Nitric oxide (NO) |
| Constrictor prostaglandins | Dilator prostaglandins |
| Endothelin-1 (vascular smooth muscle) | Endothelin-1 (endothelium) |
| *Neural mediators* | |
| ↑Sympathetic tone (adrenergic) | ↓Sympathetic tone |
| ↓Parasympathetic tone (cholinergic) | ↑Parasympathetic tone |
| Neuropeptid Y | Substance P |
| | Vasoactive intestinal polypeptide (VIP) |

Any condition that reduces oxygen delivery, increases oxygen demand, or both, will lead to a reduction in tissue oxygen tension and an accumulation of vasodilator metabolites in the immediate perivascular space. These changes relax arteriolar and precapillary sphincter smooth muscles, thereby increasing blood flow and recruiting more perfused capillaries, which stabilize capillary oxygen tension ($pO_2$). These mechanisms increase the surface area for oxygen exchange and decrease the capillary-to-cell diffusion distance. An ultimate effect of these changes is that cell $pO_2$ is maintained above the critical level at which oxygen availability limits energy metabolism (19). An inverse linear correlation exists between intestinal blood flow and the hematocrit, and a direct linear correlation between the arteriovenous oxygen difference and hematocrit in the stomach and intestine (1).

In addition to these metabolic factors, adenosine is a ubiquitous vasodilator produced in the gut that may contribute to nutrient-induced hyperemia (18). Most of the hormones and peptides produced in the gastrointestinal mucosa, such as cholecystokinin, secretin, gastrin, neurotensin, substance P, and vasoactive intestinal polypeptide (VIP), act as vasodilators when infused into the splanchnic circulation (Table 3.2). There is evidence that at least some of these products exert their effect via paracrine mechanisms (1). A variety of endogenous autocoids, e.g. serotonin, histamine, and prostaglandin, have also been implicated as local paracrine mediators in the regulation of intestinal blood flow. Although these substances are diverse in regard to structure and overall biological activity, they share an ability to dilate the gastrointestinal vasculature (6).

Nitric oxide (NO) is a potent endothelial cell-derived vasodilator that is synthesized from L-arginine by the enzyme NO synthase. NO produced by endothelial cells diffuses to adjacent vascular smooth muscle, where it binds to soluble guanylate cyclase, which in turn increases the production of cGMP, with subsequent relaxation of smooth muscle (1). NO appears to be a key mediator of mucosal blood flow responses both under basal conditions and in response to irritants (20) as well as a key regulator of intestinal motility, fluid balance, and electrolyte absorption (18). While NO has been implicated in maintaining mucosal integrity and perfusion in some pathological conditions, excessive NO production appears to injure the mucosa directly in other conditions (21). The nature of the contribution of NO to intrinsic regulation of gastrointestinal blood flow remains poorly understood. NO has been demonstrated to contribute to basal vascular tone inasmuch as inhibition of NO synthase decreases resting intestinal blood flow (1). There is a growing body of evidence that implicates NO as a mediator of the intestinal hyperemia associated with conditions as diverse as portal hypertension and central vagal stimulation (1). Therefore, modulation of tissue NO levels may offer po-

tential therapeutic benefits in maintaining and/or restoring gastrointestinal blood flow.

## Extrinsic vasoregulation

Blood flow within the gastrointestinal tract is also influenced by extrinsic neural and neurohumoral factors (Table 3.2). This control of the splanchnic vascular bed can be an important component in the overall reflex control of the circulation, especially during periods of stress. Blood vessels are innervated by both vasoconstrictor and vasodilator fibers, considered sympathetic and parasympathetic, respectively. On the other hand, the myenteric and submucous nerve plexuses have continuous capillary networks, which functionally connect them. Submucosal neurons are the primary vasomotor effectors and excitation of these neurons leads to vasodilation and therefore increased flow to the mucosa (22). The influence of sympathetic nerves on the gastrointestinal circulation overshadows that of the other determinants. Activation of additional receptors can either facilitate (e.g. nicotinic drugs) or reduce (e.g. muscarinic drugs) sympathetic neurotransmitter release (23). Norepinephrine causes redistribution of flow to and within the mucosa, with increased flow to the villi (hyperemia) and decreased flow to the crypts. The parasympathetic fibers travel predominantly with vagus nerves, all with acetylcholine as the primary transmitter (24).

The phenomenon in which stimulation of sympathetic nerves or intra-arterial infusion of norepinephrine produces an initial intense vasoconstriction with a decrease in blood flow, followed by a escape-like return of blood flow toward baseline levels despite continued nerve stimulation or norepinehrine infusion, is called autoregulatory escape (1). Three mechanisms commonly invoked to explain autoregulatory escape are redistribution of blood flow from the mucosa to submucosa, adaptation of adrenergic receptors to continued nerve stimulation, and accumulation of vasodilator substances. The latter is the most popular theory, suggesting that vasodilator substance accumulation in the vasoconstrictor phase leads to arteriolar dilation and consequent restoration of blood flow to normal (1).

The afferent nerves which serve as luminal sensors regulate local blood flow by releasing neuroendocrine substances. They are type C fibers that are activated by mechanical or thermal stimuli, ischemia, and hypoxia and bring about an increased local blood flow. This reflex vasodilation appears to be the result of activation of inhibitory synaptic nerves from sympathetic and myenteric ganglia and synaptic activity in submucosal neurons containing VIP (23). VIP has been proposed to be engaged as a neurotransmitter in the control of blood flow in all segments of the gastrointestinal tract. VIP innervation of vascular smooth muscle is found in all vessels of the gastrointestinal tract, including large conduit arteries in the mesentery with the arterial innervation being most dense in the mucosa.

Circulating vasoactive substances that affect gastrointestinal blood flow include adrenergic agents, vasopressin and angiotensin II. Norepinephrine, a predominantly α-adrenergic receptor stimulant, causes intestinal vasoconstriction, a decrease in capillary density and a reduction in oxygen uptake, whereas epinephrine can cause either α-receptor-mediated vasoconstriction at high doses or β-receptor-mediated vasodilation at low doses, as well as a variable response in oxygen uptake (25). Both vasopressin and angiotensin II are potent physiological vasoconstrictors that reduce blood flow and increase vascular resistance in all gastrointestinal organs. Vasopressin causes a decrease in capillary density and a reduction in oxygen uptake, in contrast to angiotensin II, which either reduces or does not affect splanchnic oxygen uptake (1). There appears to be cooperation between angiotensin II and the sympathetic nervous system that takes place at several different levels, namely that angiotensin II can induce pressure responses through (i) central activation of the sympathetic nervous system by catecholamine release from the adrenal medulla, (ii) nervous activation at the ganglionic level, and finally (iii) facilitation of the norepinephrine release from peripheral sympathetic nerve endings (24).

## Transcapillary fluid exchange

### Capillary structure

The fenestrated capillaries found in the mucosal layer of the gastrointestinal tract contain numerous fenestrae that often face and are in close proximity (2 μm) to the basal aspect of the mucosal epithelium. The fenestrae are circular openings of 200–300 Å radius in the capillary endothelium, with more than 60% of the fenestrae covered with a diaphragm. Open fenestrae are permeated by tracer molecules ranging in size from 25 to 150 Å radius, whereas the diaphragms are considered to account for the observation that tracer molecules of > 50 Å radius exit through only a fraction of the fenestral population. The greatest numbers of fenestrae occur in the tips of the villi and in the crypts and their frequency increases from arterial to venous ends of the capillaries (26).

### Lymphatics

The lymphatics originate as single elongate, blind-ended vessels with a diameter of approximately 20 μm and lying roughly 50 μm beneath the epithelial cells. These lymph vessels join a plexus of lymphatic capillaries in the glandular layer of the mucosa and finally drain in the submucosal network of collecting lymphatics. While these lymphatics largely contribute to the drainage of capillary filtrate throughout the gastrointestinal tract,

intestinal lymphatics also play a role in the removal of fluid entering the mucosal interstitium via electrolyte-coupled transport (27).

## Interstitium

The hydrostatic and oncotic pressures within the interstitium are determinants of transcapillary and lymphatic fluid fluxes. Thus, the spatial orientation of the interstitium, blood and lymph capillaries relative to the transporting epithelium is of considerable importance (28). The mucosal interstitial space is not homogeneous and can be considered as two functionally distinct compartments. The hydrostatic and oncotic pressures in the immediate vicinity of the subepithelial capillaries are different from those near the central lacteal. The juxtacapillary fluid compartment is a narrow space (2 μm) between the epithelial cell layer and the subepithelial capillaries and represents a small proportion of total mucosal fluid volume; hence, its protein concentration would tend to be very sensitive to changes in transepithelial fluid movement (29). Consequently, there may be larger changes in interstitial oncotic and hydrostatic pressures near the subepithelial capillaries than detected at the lacteal.

It is also important to recognize that tissues are not simply bathed in an aqueous solution but are surrounded and supported by a glycosaminoglycan gel that is the main component of the interstitial matrix. An important property of this glycosaminoglycan gel is that, although it can resist compression, it tends to fall apart when stretched. Since hyaluronic acid is anionic, there exist within the gel areas of negative charges. Dilution of the hyaluronic acid concentration by matrix hydration greatly increases the hydraulic conductivity, which is very low in the normally hydrated interstitium. For example, doubling of the interstitial volume would increase the hydraulic conductance more than 1000-fold. Matrix hydration can be increased as a result of either an enhanced capillary filtration rate or secondary net fluid absorption from the lumen. In contrast, active fluid secretion by gastrointestinal epithelium tends to dehydrate the interstitium. The compliance characteristics of the interstitium play a pivotal role in determining the rate of net fluid movement across capillary and lymphatic walls. At a normal or low interstitial fluid volume (approximately 25 ml/100 g), the compliance is also low (0.4 ml/mmHg/100 g). Consequently, small increments in intestinal fluid volume produce substantial increases in interstitial fluid pressure. When the interstitial fluid volume exceeds 30 ml/100 g, an abrupt change in interstitial compliance takes place (4.0 ml/mmHg/100 g). The reason for this sudden change is a reversible structural alteration in the interstitial matrix (i.e. disentanglement of hyaluronic acid chains and rupture of cross-links) that occurs at the inflection of the compliance curve. If the interstitial hydration is the result of enhanced net fluid absorption, the compliance increase is greater than that produced by increases in venous pressure, possibly because the congested microvasculature contributes to the stiffness of the tissue (30).

Another important property of the interstitium is the ability of hyaluronic acid within the matrix to exclude large molecules and behave like a net with a defined pore size (31). For example, albumin is excluded from approximately 40% of the total water space of the normal hydrated interstitium. However, as the interstitial fluid volume increases, the degree of albumin exclusion falls, matrix porosity increases (32), and the frictional resistance to the diffusion of macromolecules is reduced.

## The Starling forces

The direction and rate of fluid movement across capillaries in the gastrointestinal tract are directed by the hydrostatic and oncotic pressure gradients between the blood and interstitium. According to the Starling hypothesis, net capillary filtration rate ($J_{v,c}$) can be described by the formula

$$J_{v,c} = K_{f,c} [(P_c - P_t) - \sigma_d (\pi_c - \pi_t)]$$

where $J_{v,c}$ is the net capillary filtration (or absorption) rate, $K_{f,c}$ is the capillary filtration coefficient, $P_c$ is the capillary hydrostatic pressure, $P_t$ is the interstitial fluid pressure, $\sigma_d$ is the osmotic reflection coefficient, $\pi_c$ is the oncotic pressure in the capillary, and $\pi_t$ is the interstitial oncotic pressure (Fig. 3.2).

### *Net capillary filtration rate*

Total intestinal lymph flow provides an accurate estimate of net capillary filtration rate within a tissue. The major driving force for lymphatic filling and lymph flow is the interstitial lymphatic hydrostatic pressure gradient. Resting values for intestinal lymph flow range between 0.02 and 0.08 ml/min/100 g in cats and between 0.13 and 0.38 ml/min/100 g in rats. Presumably due to the interstitial compliance characteristics of intestinal interstitium, there is a positive, nonlinear relationship between interstitial hydrostatic pressure and lymph flow in the small intestine (30,33,34). Interstitial volume expansion is considered to be the origin of the increase in interstitial pressure required for enhanced lymphatic filling. Furthermore, lymph formation and flow are enhanced by intermittent decreases in lacteal pressure during contractions of the lymphatic wall and/or villus (26).

### *Capillary filtration coefficient*

The number of perfused capillaries as well as the size and number of pores in each capillary influence the capillary filtration coefficient, which is an expression of the hydraulic conductance of a capillary bed. A change in $K_{f,c}$ reflects

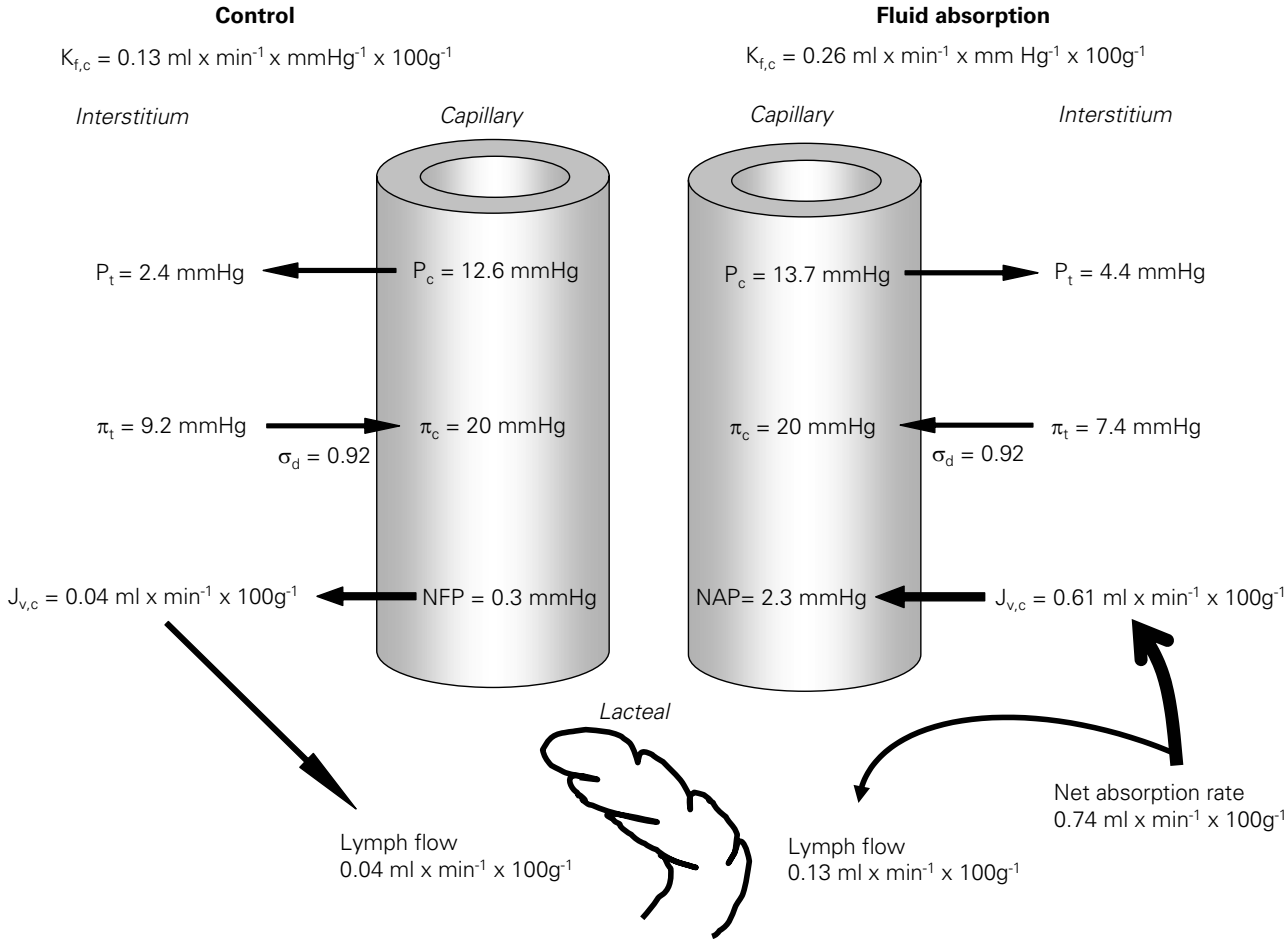


**Figure 3.2** Starling forces and capillary membrane parameters in the small intestine under control (nontransporting) and during fluid absorption. NFP, Net capillary filtration pressure; NAP, net capillary absorptive pressure.

an alteration in either the number of perfused microvessels or their permeability. The basal filtration coefficients vary by a factor of 5 among the organs in the alimentary tract. Organs with a relatively large muscle mass, such as the stomach, have the lowest filtration coefficient, whereas the liver, with its highly permeable sinusoids, has the highest. Capillary pressure changes lead to an alteration of $K_{f,c}$ due to capillary recruitment or derecruitment. In the small intestine an increase in portal pressure is generally associated with reductions in blood flow and filtration coefficient with an increased vascular resistance. The myogenic reduction, caused by constriction of the arteriolar resistance vessels and precapillary sphincters, protects the small intestine against edema formation following sudden elevations in venous pressure. In contrast, increased venous pressure leads to an elevated filtration coefficient in the colon and pancreas, suggesting a weaker myogenic control of precapillary sphincters in those organs. On the other hand, when blood flow is decreased by arterial pressure reduction an inverse relationship between $K_{f,c}$ and blood flow has been observed in the small intestine. This inverse relationship can be explained by myogenic relaxation of precapil-

**Table 3.3** Effects of physiological, pathological, and pharmacological conditions on intestinal capillary filtration coefficient ($K_{f,c}$)

| | |
|---|---|
| *Conditions or agents increasing $K_{f,c}$* | |
| Acetylcholine | Hyperthermia |
| Aminophylline | Hypoxia |
| Arterial hypotension | Isoproterenol |
| Bradykinin | Neostigmine |
| Cholecystokinin | Nitroglycerin |
| Cholera toxin | Phentolamine |
| Denervation | Propranolol |
| Epinephrine | Prostaglandin $E_1$ |
| Glucagon | Secretin |
| Glucose absorption | Serotonin |
| Histamine | Sodium nitroprusside |
| Hemorrhagic shock | Sodium nitrite |
| *Conditions or agents decreasing $K_{f,c}$* | |
| Acute arterial hypertension | Norepinephrine |
| Adenosine | Pentagastrin |
| Angiotensin II | Phenylephrine |
| Ergotamine | Portal hypertension |
| Hypothermia | Serotonin |
| Luminal distension | Sympathetic nerve stimulation |

lary sphincter muscle as vascular transmural pressure is reduced through lowering arterial pressure or by reduction of precapillary sphincter tone as a result of accumulation of metabolites as blood flow decreases. A variety of physiological conditions and pharmacological agents are known to alter the capillary filtration coefficient. As shown in Table 3.3, vasodilators generally increase while vasoconstrictors reduce $K_{f,c}$ (33). For example, during isoproterenol-induced vasodilation a direct relationship between $K_{f,c}$ and blood flow is observed in the small intestine resulting from progressive relaxation of both resistance and precapillary sphincter smooth muscle. There is a large body of evidence for a true increase in $K_{f,c}$ due to elevated capillary permeability produced by hemorrhagic shock (35), bradykinin (36), histamine (37), glucagon (38), and arterial hypoxemia (39). Even though small changes in capillary pore diameter should markedly influence filtration, since flow through cylindrical channels is proportional to the fourth power of the radius, intestinal filtration coefficient changes of more than 50% are rarely observed with agents or under conditions that dramatically alter the permeability of intestinal capillaries to macromolecules, such as histamine, bradykinin or ischemia. These findings may be explained by the fact that most of the hydraulic conductance across intestinal capillaries takes place through the 'small pores' (47 Å radius), whereas most of the permeability to macromolecules is through the relatively few 'large pores' (250 Å radius) (40).

### *Capillary pressure*

Microvascular pressure has been estimated in the small intestine of dogs, cats, and rats using venous occlusion and stop-flow isogravimetric techniques (26). As shown in micropuncture studies, the microvascular pressures in the villi are significantly lower than the microvascular pressures in the intestinal muscle layers, probably because of the very low resistance in mucosal venules. In addition to these techniques, capillary pressures and relative flows in various layers of the intestine have been used to calculate a weighted average microvascular pressure for the whole small intestine. The overall resting value for this microvascular pressure is approximately 16 mmHg at normal portal pressure, which is greater than the values reported for the liver (6 mmHg) and pancreas (13 mmHg). The different values among these organs presumably reflect variations in the resting precapillary ($R_a$)-to-postcapillary ($R_v$) resistance ratio and in venous pressure. The intestinal $R_a : R_v$ ratio (15 : 1) is higher than that in skeletal muscles (5 : 1), causing a lower intestinal capillary pressure compared with that in skeletal muscles (> 20 mmHg). Similar to their influence on $K_{f,c}$, vasodilators increase while vasoconstrictors decrease microvascular pressure, presumably resulting from the fact that most vasoactive drugs primarily exert their effect on precapillary resistance vessels (26,27).

During an increase of arterial pressure in the small intestine only 5–10% of the incremental change is transmitted to the capillaries. In contrast to this observation, an increase of venous pressure has more profound effects, as 60–70% of the incremental change is transmitted to the capillaries. Nearly all of an increment in venous pressure is transmitted back to the hepatic sinusoids and pancreatic capillaries. A rise of $R_a$ and a fall of $R_v$ dampen the increase in microvascular pressure produced by venous hypertension in the small intestine. The increased $R_a : R_v$ ratio due to a venous pressure elevation reduces the change in capillary pressure by 6.5 mmHg for an increase in venous pressure from 0 to 30 mmHg (27,34).

### *Interstitial fluid pressure*

Values for interstitial pressure in the small intestine ($P_t$) have been determined using micropuncture techniques and calculated using the Starling equation. The calculated values compare favorably with the indirect estimates obtained by Guyton's capsule technique. At normal levels of arterial and portal pressures, $P_t$ ranges from – 2.0 to 2.4 mmHg (34,41,42). When portal pressure exceeds 5 mmHg, intestinal interstitial fluid pressure is consistently positive. The micropuncture technique allows measurements of $P_t$ in a single layer of small intestine and yields a value of 0.5–1.0 mmHg for $P_t$ (in the mucosa of the rat small intestine) that increases during intestinal absorption and decreases during cholera toxin-induced intestinal secretion. $P_t$ increases in response to intra-arterial infusion of glucagon (38) or bradykinin (41) and decreases during local arterial hypotension (43). The value of $P_t$ in the liver (~ 6.0 mmHg) clearly exceeds that of most other organs. The relationship between hepatic $P_t$ and venous pressure is linear for venous pressures of 0–30 mmHg, so that approximately 65% of the elevation in hepatic venous pressure is transmitted to the interstitium (27).

### *Osmotic reflection coefficient*

The capillary osmotic reflection coefficient ($\sigma_d$) describes the percentage of the total oncotic pressure generated across the microvascular endothelium. Various physiological and pharmacological interventions alter $\sigma_d$. For example, it is well known that intestinal lymph flow and lymphatic protein flux increase after a meal (28,44). While intestinal vascular permeability is not altered during the absorption of glucose or electrolytes, fat absorption is associated with a pronounced reduction in $\sigma_d$ (from 0.92 to 0.70) (45). This amount of reduction in $\sigma_d$ could decrease the effective absorptive force due to the transcapillary oncotic pressure gradient by as much as 3.0 mmHg. A loss in the net absorptive force of this magnitude would dramatically reduce the effectiveness of capillaries in re-

moving absorbed fluid. If one assumes that an increased vascular permeability leads to a rise in $K_{f,c}$, it is possible that the resulting small absorptive force remains sufficient to drive fluid into the capillaries.

### *Transcapillary oncotic pressure gradient*

Based on the assumption that lymph provides a reasonable reflection of interstitial fluid composition, the transcapillary oncotic pressure gradient has been estimated from lymph and plasma using either an osmometer or equations that relate protein concentration to osmotic pressure. Several studies have shown that the basal oncotic pressure difference is similar for stomach, small intestine, pancreas, and colon, with the liver exhibiting a negligible gradient. At normal capillary permeability, in all splanchnic organs except the liver, the oncotic pressure difference increases when capillary pressure increases. Of interest is the finding that the calculated osmotic pressure difference in the liver of cirrhotic patients is 10 mmHg compared with 3 mmHg in normal subjects (46). This rise in the oncotic pressure gradient with chronic portal hypertension suggests that the liver sinusoids are converted from highly permeable vessels under normal conditions to vessels displaying selective solute restriction ($\sigma_d > 0$) when portal pressure is chronically elevated. Capillarization (deposition of collagen fibers in the space of Disse) has been invoked to explain this change in capillary permeability induced by portal hypertension.

## Interactions of capillary and interstitial forces during water absorption

While the Starling forces often receive attention in the context of pathological processes such as edema formation, these factors are more frequently involved in the day-to-day assimilation of ingested water through the gut mucosa (Fig. 3.2). The first compartment exposed to water absorbed in the small bowel is the mucosal interstitium. Accumulation of absorbed fluid in the interstitial space of the lamina propria initiates a series of physical changes within the matrix that ultimately facilitate the removal of absorbed fluid via lymph and blood microvessels. As much as a doubling of interstitial volume is predicted at water absorption rates in excess of 1.8 ml/min/100 g tissue. Several physiological consequences of this interstitial volume expansion occur during net fluid absorption in the intestine, such as an increased hydraulic conductivity of the interstitial matrix, a reduction in the ability of the interstitium to retard the diffusive and convective migration of solutes, an elevated interstitial hydrostatic pressure, and a decrease of interstitial oncotic pressure. Since mucopolysaccharides tend to immobilize interstitial fluid, the hydraulic conductivity of the normally hydrated interstitium is normally quite low. The interstitial hydraulic conductivity increases to approximately 200 times the nonabsorptive value at an absorption rate of 1.0 ml/min/100 g and rises 1000-fold at a rate of 2.0 ml/min/100 g. These profound changes in hydraulic conductivity allow small hydrostatic pressure gradients within the mucosal interstitium to move large amounts of fluid between the epithelia and microvessels. The interstitial volume expansion combined with fluid absorption also lead to a reduction in the extent of albumin exclusion within the intestinal interstitium. The diffusion rate of albumin is reduced by at least one-third its velocity in water because the volume or effective surface area for diffusion is limited by the exclusion effect and even more reduced as a result of the steric interaction between the solute and that portion of the matrix it is able to penetrate. A dramatic fall of albumin exclusion to < 10% occurs during high absorption rates, leading to a significantly enlarged area available for diffusive exchange. The most important physiological consequences of interstitial volume expansion associated with intestinal fluid absorption are the alterations of interstitial hydrostatic and oncotic pressures (Fig. 3.3). Because the interstitial hydrostatic pressure increases and the interstitial oncotic pressure decreases, the removal of absorbed fluid from the lamina propria is enhanced by the following mechanisms: opposing further capillary filtration and converting filtering capillaries to absorbing ones, and providing an increased hydrostatic pressure gradient for lymphatic filling (47). Even though lacteal pressure was measured over a narrow range of absorption rates, using a micropuncture technique and calculations of interstitial pressure from Starling force measurements, it appears that interstitial hydrostatic pressure rises significantly as absorption rate increases, presumably because of progressive interstitial volume expansion. Several studies provide evidence that interstitial (lymph) oncotic pressure is reduced by 2–7 mmHg (from a normal value of 10 mmHg) during fluid absorption. In addition, there is clear evidence that the magnitude of the reduction in oncotic pressure is dependent on the net fluid absorption rate (28). Another important point is that the elevated interstitial hydrostatic pressure produced by net fluid absorption leads to an increased rate of intestinal lymph formation. During net fluid absorption, lymph flow increases to as high as 0.45 ml/min/100 g (from normal values of 0.02–0.08 ml/min/100 g). Several factors contribute to the variability in the magnitude of increased lymph flow, including: tonicity of fluid placed into the lumen, portal vein pressure, intraenteric pressure, motility, and the use of 'contaminated' lymph from other tissues.

At absorption rates higher than 0.20 ml/min/100 g, not the lymph but the capillaries are the major routes for removal of absorbed fluid from the interstitium, with capillaries accounting for up to 85% of the total volume removed. The differential role of capillaries and lymphatics in removing absorbed fluid when the absorption

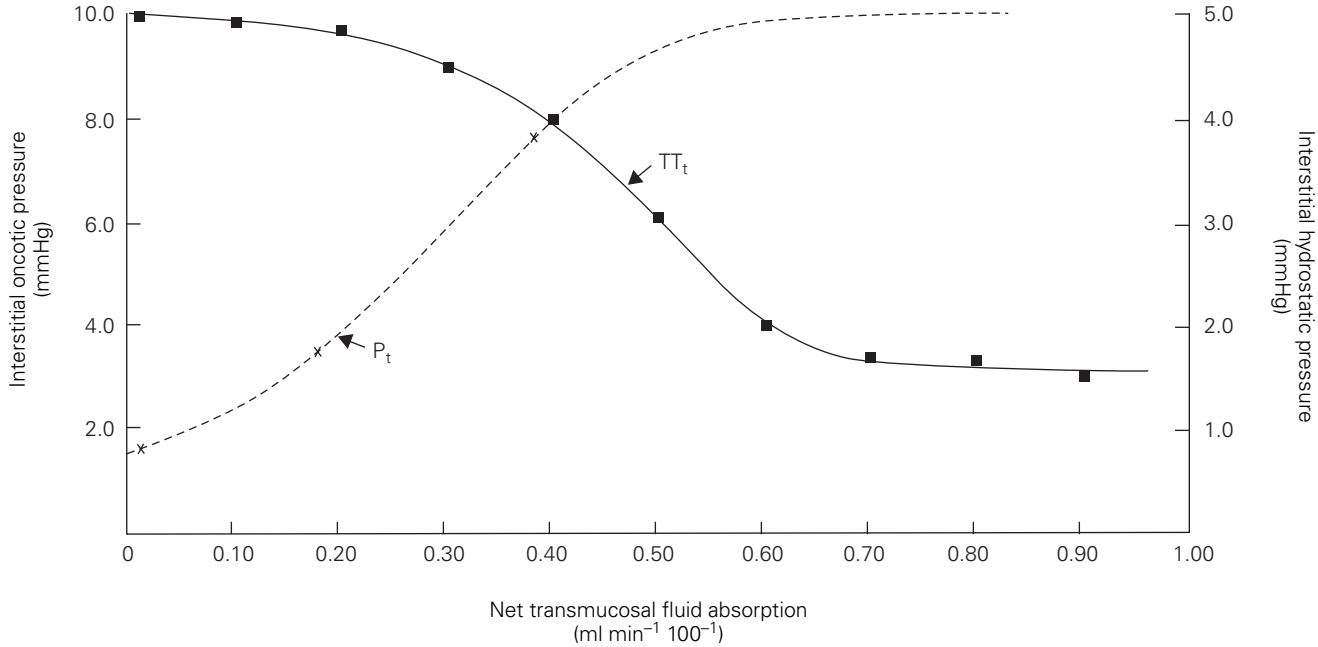


**Figure 3.3** Steady-state relations between interstitial hydrostatic ($P_t$) and oncotic ($\pi_t$) pressures and net fluid absorption rate. (From ref. 28, with permission.)

rate is altered is consistent with the concept of a change in interstitial compliance at low absorption rates. The absorption-induced changes in microvascular and interstitial forces modify the balance of pressures across intestinal capillaries to produce a net absorptive force of 2.3 mmHg. This force, linked to the increased capillary hydraulic conductance, drives 82% of the absorbed fluid into the capillaries. Lymph flow, enhanced by the rise in interstitial hydrostatic pressure, removes the remaining 18% of absorbed fluid from the mucosal interstitium (26,27).

## Summary and conclusions

The past three decades have witnessed a dramatic increase in our knowledge of the gastrointestinal circulation. While many of the anatomical and physiological characteristics of this vascular bed resemble those of other regional circulations, there are several features of the gastrointestinal circulation that are unique to it. These unique features are primarily geared towards regulating blood flow and capillary exchange to meet the needs imposed on the gastrointestinal tract during the assimilation of a meal. A number of potential mediators of the vascular responses to food and water ingestion have been identified, but the precise mechanisms that underlie these responses remain undefined. Future research will provide more insights into the physiology of the gastrointestinal circulation and should facilitate our understanding of the vascular dysfunction that accompanies acute and chronic diseases of the gastrointestinal tract and liver.

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# Part 2
# Factors Involved in the Pathogenesis of Renal Dysfunction and Ascites in Cirrhosis

# Chapter 4
# The Renin–Angiotensin–Aldosterone System in Cirrhosis

Mauro Bernardi and Marco Domenicali

## Essential physiology

The renin–angiotensin system (RAS) (Fig. 4.1) plays a fundamental role in the regulation of volume and composition of the body's extracellular fluids, and cardiovascular function. The most important site for renin synthesis, storage, and release is the juxtaglomerular apparatus, located in close contact with the afferent glomerular arteriole and macula densa of the distal convoluted tubule within the kidney. The classical RAS pathway (1) initiates with the proteolysis of the $\alpha_2$-globulin angiotensinogen by renin to form the decapeptide angiotensin I, which is converted by angiotensin converting enzyme (ACE) to the octapeptide angiotensin II (AII), the final effector of the system. AII is then cleaved by aminopeptidases to angiotensin III and, finally, IV. AII generation also results from alternative pathways, involving the tissue plasminogen activator and chymostatin-sensitive AII-generating enzyme (1).

Systemic RAS activation (1) follows renin cleavage of circulating angiotensinogen, mainly synthesised by the liver, and AII generation by ACE, mainly located in lung vasculature. The entire RAS pathway also resides in several tissues, including blood vessels, kidneys, heart, liver, and adrenal glands. Namely, within the kidney, proximal tubule cells express angiotensinogen, which is secreted in the tubular lumen, and present high ACE concentration on their brush border membrane. Tissue RAS activation leads to the local generation and effects of AII. Thus, RAS acts as both an endocrine and paracrine system (1).

Systemic RAS is mainly activated by reduced renal perfusion, as occurs in case of fluid loss, sodium depletion, arterial hypotension, and postural changes from supine to erect through: (i) stretch of the afferent glomerular arteriole, (ii) β-adrenergic drive to the juxtaglomerular apparatus and the muscle layer of glomerular afferent arterioles, and (iii) reduced tubular sodium and chloride delivery to the macula densa. Many other circulating and

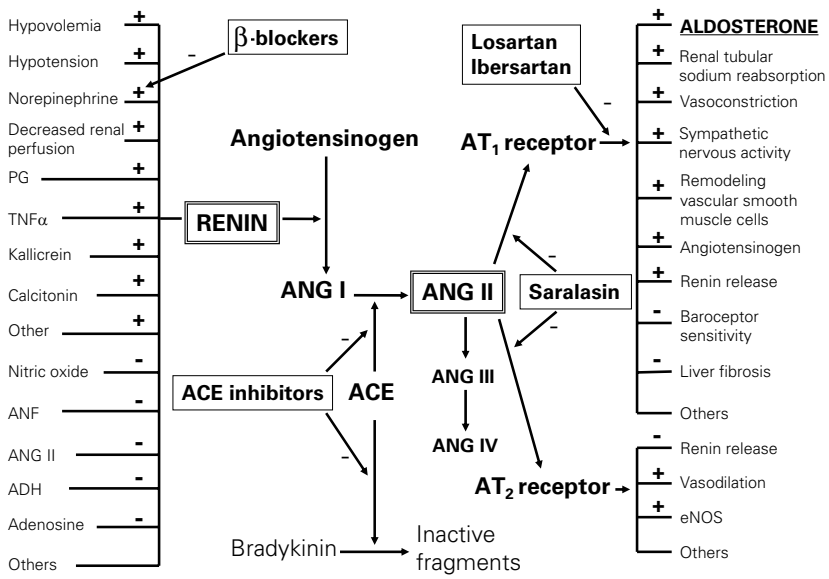


**Figure 4.1** Schematic representation of the renin–angiotensin–aldosterone system and its main modulating factors and effects. The sites of action of drugs inhibiting the system activity and mentioned in the text are also shown. ACE, Angiotensin converting enzyme; ADH, anti-diuretic hormone; ANF, atrial natriuretic factor; AII-III-IV, angiotensin II-III-IV; $AT_{1\text{-}2}$, angiotensin subtype 1-2 receptors; eNOS, endothelial constitutive nitric oxide synthase; PG, prostaglandins; TNF-α, tumor necrosis factor-alpha; +, stimulation; –, inhibition.

local factors influence renin release. Namely, AII, nitric oxide (NO), atrial natriuretic factor (ANF), vasopressin, and adenosine inhibit renin release, while prostaglandins, kallikrein, calcitonin, and tumor-necrosis factor-α (TNF-α) stimulate renin release (2).

Several substances, the description of which is beyond the scope of this chapter, regulate tissue RAS. We just mention that intrarenal RAS is also mainly regulated by the extracellular fluid volume (2).

Among others, the most important effects of AII are vasoconstriction, renal sodium retention, and adrenal gland cortex stimulation. Powerful vasoconstriction ensues stimulation of smooth-muscle cells and facilitation of norepinephrine release by sympathetic nerves. Renal vasculature is extremely sensitive to AII, as a subpressor dose reduces renal blood flow and increases glomerular efferent arteriolar resistance. This preserves glomerular filtration at the lower end of renal blood flow autoregulation. AII-stimulated sodium reabsorption occurs at the proximal convoluted tubule, by direct stimulation of the tubular epithelium and as a consequence of enhanced filtration fraction (3,4).

Two specific membrane receptors mediate AII actions (3,4): $AT_1$ receptor, identified in vessels, kidneys, adrenal glands, heart, and brain, and $AT_2$ receptor, present in vessel wall and several organs, including heart and kidneys. $AT_1$ receptor promotes, among other effects, vasoconstriction, aldosterone release, mesangial cell contraction, sodium reabsorption by the proximal tubule, sensitivity of the tubuloglomerular feedback mechanism, trophic effect on cardiomyocytes and vascular smooth-muscle cells, angiogenesis, and fibrogenesis. The effects mediated by $AT_2$ receptor are less defined, but evidence exists which supports its role in promoting vasodilation, activating endothelial NO synthase, tissue regeneration, and apoptosis. Both receptors regulate renin secretion.

Since AII represents the main stimulus for aldosterone synthesis and release by the glomerulosa cells (4,5), the factors regulating renin release also influence aldosterone secretion. Moreover, direct stimulation to adrenal glomerulosa cells results from severe hyponatremia, hyperkalemia, and NO, whereas ANF and dopamine cause inhibition.

The principal target site of aldosterone is the nephron cortical collecting duct and the adjacent convoluted tubule (5). Aldosterone binding to specific cytoplasmic receptors is followed by the translocation of hormone–receptor complex to nuclear acceptors, RNA transcription, and synthesis of specific proteins with prolonged half-life. These enhance sodium flux from the tubular lumen to the cell cytosol, and then to the extracellular fluid and peritubular capillaries. The latter step is achieved by increasing the $Na^+$-$K^+$ ATPase activity, and is coupled with potassium and hydrogen secretion in the tubular lumen.

As for RAS, tissue aldosterone production occurs in endothelial and vascular smooth-muscle cells, which express the aldosterone-synthase gene (5). The physiological importance of locally produced aldosterone, which is also regulated by AII and modifications in dietary sodium and potassium, is not clear.

Abnormalities in renal sodium handling and cardiovascular homeostasis commonly occur in cirrhosis: changes in the renin–angiotensin–aldosterone system (RAAS) and their consequences are therefore expected.

## The renin–angiotensin–aldosterone system in cirrhosis

Abnormalities in systemic RAS activity and aldosterone secretion in cirrhosis have been defined over half a century, while studies on tissue, mainly renal, RAAS have appeared only recently and are devoted to patients in the pre-ascitic stage of the disease.

### Pre-ascitic cirrhosis

With few exceptions, reduced plasma renin concentration or activity (PRA) has been documented by several studies in supine patients (6) (Fig. 4.2). Despite a preserved profile of daily fluctuations, both the mean daily level and afternoon surge were also depressed in recumbent patients (7). Conversely, either mildly increased or reduced PRA has been reported in the upright position (8–11). Values in the normal range were also reported during moderate physical exercise (10). Such variability probably results from different experimental conditions and sodium intake.

Systemic RAAS activity, however, may not reflect that of intrarenal RAAS. In fact, sodium retaining pre-ascitic rats with long-term bile duct ligation showed normal plasma renin concentration/activity, but increased renin mRNA and reduced angiotensinogen within the kidney (12). These findings, suggesting that intrarenal RAAS is activated earlier than the systemic pathway, have been confirmed in humans with pre-ascitic cirrhosis. In fact, lower-body negative pressure, which reduces central blood volume, pre-eminently enhanced renal renin and AII secretion rates (13). Moreover, despite baseline suppression of systemic RAAS, sodium overload induced by high sodium diet was reversed by the $AT_1$ receptor antagonist losartan administered at a dose not perturbing either renal or systemic hemodynamics (14).

Plasma aldosterone of pre-ascitic patients is in the low to normal range in the supine position (6) (Fig. 4.2), and normal (10,11) or barely increased (8,9) in the upright posture. An increased individual variability characterizes the rhythmicity of aldosterone secretion. Since the plasma cortisol circadian rhythm is preserved, such a

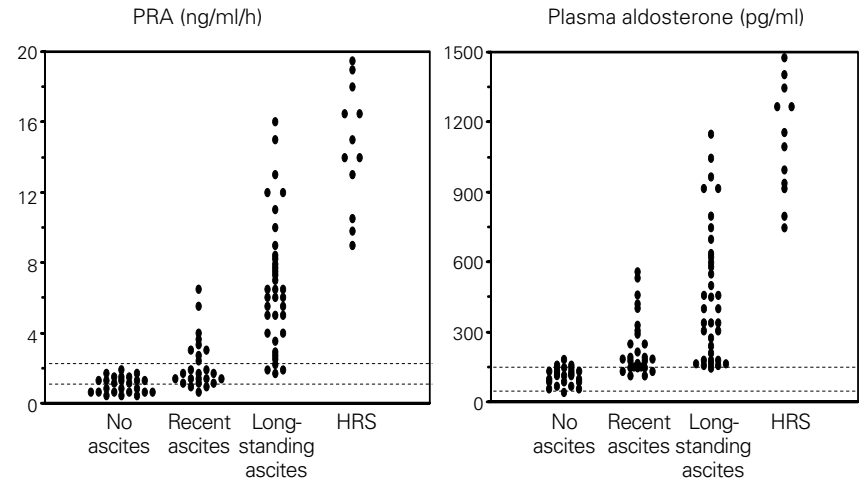


**Figure 4.2** Plasma renin activity (PRA) and plasma aldosterone concentration in different stages of cirrhosis. The measurements were done in supine patients receiving a diet providing 40 mmol/day of sodium. Normal values are those included between the dashed lines. No ascites, patients with pre-ascitic cirrhosis; recent ascites, patient developing ascites for the first time; HRS, hepatorenal syndrome.

chronobiological alteration cannot be attributed to a deregulated activity of the pituitary–adrenal axis (7).

### Cirrhosis with ascites

RAAS activity in cirrhotic patients with ascites varies through the different disease stages (Fig. 4.2); furthermore, it can be greatly affected by drugs such as diuretics. Up to 70% of patients developing ascites for the first time show normal or even reduced PRA and aldosterone while supine (6). Such patients show normal RAAS activity even when sitting and doing moderate physical exercise (10). As cirrhosis progresses, RAAS activation becomes more frequent and pronounced, so that it is the rule in severely decompensated patients (6), is not suppressed by central blood volume expansion (15,16), and is enhanced by upright posture (16).

In advanced cirrhosis, the circadian profiles of both PRA and plasma aldosterone are disrupted, with time-related values permanently increased throughout the day either in patients maintained supine for 24 h (7), or allowed to be mobile during daylight hours (17).

## Regulation of the renin–angiotensin–aldosterone system

### Pre-ascitic cirrhosis

PRA suppression in supine patients with pre-ascitic cirrhosis cannot be attributed to a defective hepatic synthesis of angiotensinogen, which could affect the *in vitro* generation of AI in the PRA assay. In fact, even when angiotensinogen synthesis is actually depressed, as in advanced cirrhosis (18), striking elevations of PRA are found. Therefore, the "low-renin" status of early cirrhosis reflects a reduced renin release, as confirmed by the determination of plasma renin concentration (6).

The main regulators of RAAS, such as arterial pressure, renal perfusion, plasma electrolyte concentrations, and adrenergic tone, are usually normal in supine pre-ascitic cirrhotic patients (7,9,10). Subtle changes in sympathoadrenergic activity are revealed by circadian evaluation (19), but should account for activation rather than a suppression of RAAS.

Studies on the effect of postural changes helped in clarifying the regulation of RAAS activity in pre-ascitic cirrhosis, by showing that PRA is only reduced in the supine position, while it is in the normal range during standing (9) (Fig. 4.3a). The existence of a plasma volume expansion in pre-ascitic cirrhosis is undisputed (20). Therefore, we proposed that during standing there is blood pooling in the venous splanchnic area, whereas on lying down translocation of blood towards the central circulation occurs with suppression of RAAS (21).

In conclusion, RAAS activity changes in pre-ascitic cirrhosis are largely determined by the volume status, and imply that the supine-induced redistribution of blood volume is hemodynamically effective, suppresses the RAAS, and leads to natriuresis (22,23). These findings also suggest that both systemic and intrarenal RAAS are suppressed. In contrast, normal PRA in the upright posture does not mean that intrarenal RAS is not activated. In fact, lower-body negative pressure, which promotes a blood volume shift away from the central circulatory area, as occurs with upright posture, leads to a pre-eminent activation of intrarenal RAS in pre-ascitic cirrhotic patients, but not in healthy subjects (13). Interestingly, both lower-body negative pressure and upright posture are associated with renal sodium retention (13,23).

How a slight reduction in central blood volume secondary to venous splanchnic pooling activates intrarenal RAAS remains elusive. Subtle intrarenal hemodynamic abnormalities have been described in cirrhosis with ascites and preserved renal blood flow (see below) (24), and

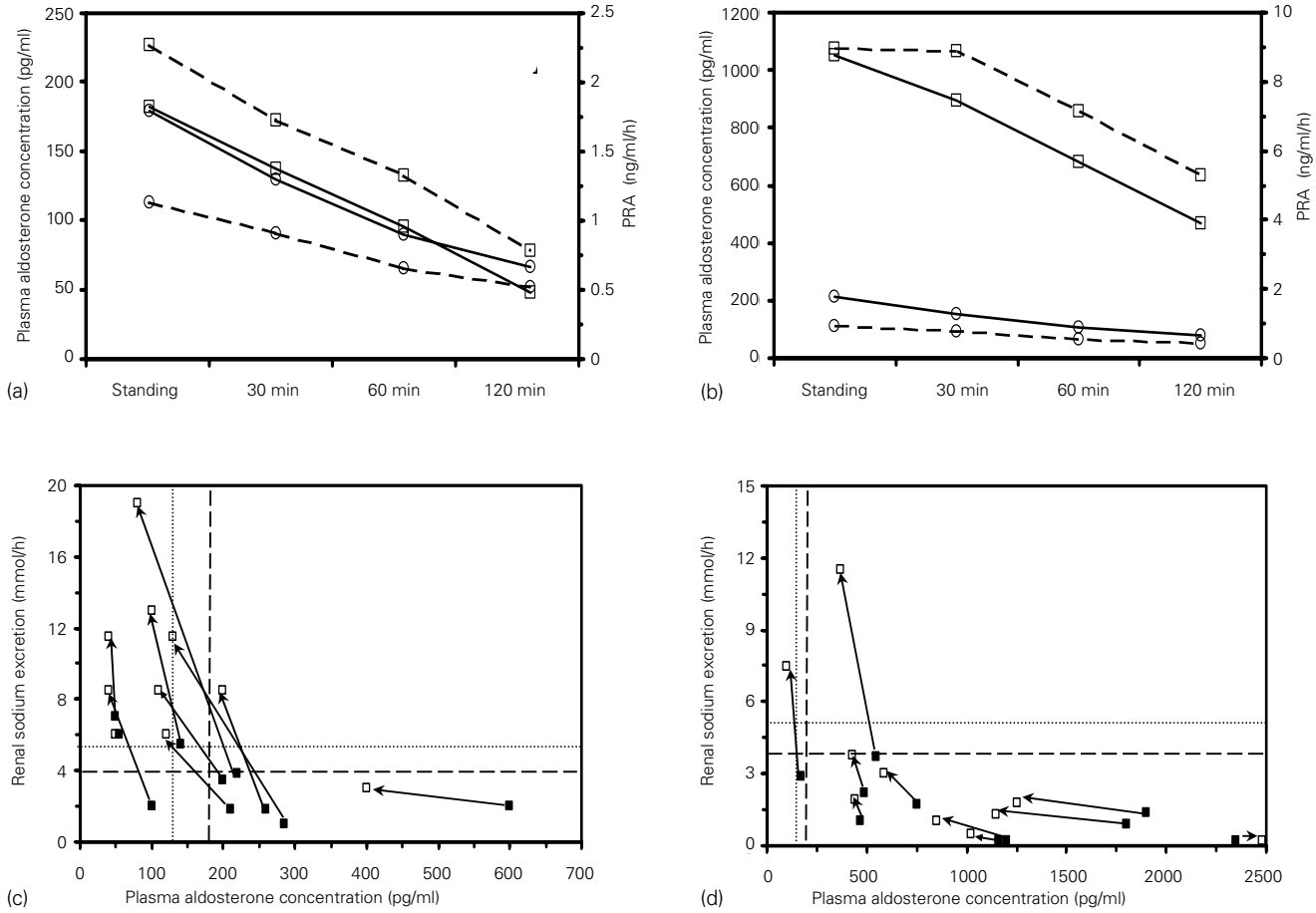


**Figure 4.3** Effect of postural change from upright to supine position on renin–angiotensin–aldosterone system and renal sodium excretion in cirrhotic patients in the pre-ascitic stage and with ascites receiving a diet providing 120 mmol/day of sodium. (a) Plasma renin activity (continuous line) and plasma aldosterone concentration (dashed line) in patients with pre-ascitic cirrhosis (□) and healthy control subjects (○). Values in the upright posture: standing; values in the supine posture: 30, 60, 120 min from lying down. (b) Plasma renin activity and plasma aldosterone concentration in patients with cirrhosis and ascites (□) and healthy control subjects. Other symbols and legends as in (a). (c) Correlations between plasma aldosterone concentration and hourly renal sodium excretion in patients with pre-ascitic cirrhosis in upright (■; $r_s = -0.79$; $P < 0.01$) and supine position (□; $r_s = -0.49$; $P > 0.05$). Supine values of plasma aldosterone concentration were the average of levels measured 30, 60 and 120 min after the assumption of the supine position. The maximal value of plasma aldosterone concentration and the minimal value of renal sodium excretion disclosed by controls in the upright (dashed lines) and supine positions (dotted lines) are also reported. (d) Correlations between plasma aldosterone concentration in patients with ascites in upright (■; $r_s = -0.73$) and supine position (□; $r_s = -0.89$, $P < 0.001$). Supine values of plasma aldosterone concentration were determined as in (c). The maximal value of plasma aldosterone concentration and the minimal value of renal sodium excretion disclosed by controls are indicated as in (c). In both (c) and (d) the supine-induced changes in renal sodium excretion were largely predicted based on the changes in plasma aldosterone concentration. (Data from references 9,16,23,44.)

may also occur at an earlier stage of the disease. More recently, Doppler ultrasound examination of patients with pre-ascitic cirrhosis showed a slight increase in resistance indices of intrarenal arteries (25).

The chronobiological approach has clearly indicated that RAS activity is a major determinant of aldosterone secretion in pre-ascitic cirrhosis (7). In fact, the mean daily levels of PRA and plasma aldosterone were closely correlated. It should be emphasized that a normal plasma aldosterone can coexist with a depressed PRA. Accordingly, by plotting these variables, the regression line slope in pre-ascitic cirrhotic patients was steeper than that in healthy subjects. This suggests that adrenal glomerulosa cells are sensitized to AII, as in sodium-depleted individuals (26) and long-term bile duct ligated dogs (27). An impaired hepatic degradation of aldosterone (28) may also contribute. In addition, the increased NO production reported in pre-ascitic patients (29) could reset aldosterone secretion at a higher level, as NO stimulates steroidogenesis in a dose-dependent manner (5).

Despite the putative adrenal cortex sensitization to AII, renin does not control the circadian rhythm of plasma aldosterone in pre-ascitic cirrhosis as in healthy subjects (7).

### Cirrhosis with ascites

Although the liver inactivates renin, a substantial reduction of its hepatic extraction has not been found in decompensated cirrhosis (6). Thus, RAS activation in this setting is attributable to enhanced renin secretion.

RAS activation in advanced cirrhosis results from different pathways acting in concert. Arterial pressure and renal blood flow or glomerular filtration rate are closely and inversely correlated with PRA (6). Moreover, upright posture induces an increase in PRA which is proportional to the standing-induced reduction in glomerular filtration rate (30). In patients with preserved renal perfusion, the blood flow through the outer cortical nephrons, which are mainly responsible for renin release, is reduced because of a relative redistribution towards juxtamedullary nephrons, and this is related to an increase in PRA (24). All these findings imply that renin release is stimulated through a stretch receptor-like mechanism in the glomerular afferent arteriole. In addition, reduced renal perfusion also leads to enhanced proximal tubular sodium reabsorption and, hence, reduced sodium delivery to the macula densa, with increased renin secretion from the juxtaglomerular apparatus. Direct stimulation also ensues from sympathoadrenergic activation, and β-blocking agents suppress PRA to an extent proportional to baseline PRA levels (24,31). Finally, abnormal productions of glucagon, growth hormone, parathyroid hormone, prostaglandins, thromboxanes, and TNF-α may contribute to RAS activation in advanced cirrhosis (6).

The fundamental mechanism activating RAS in advanced cirrhosis is represented by a reduced effective central blood volume with arterial vasodilation of the afferent arteriole (32). In fact, maneuvers improving effective arterial blood volume, such as head-out water immersion (8,15) and the assumption of supine posture (16), reduce, albeit do not normalize, PRA (Fig. 4.3b). The further enhancement of effective arterial blood volume obtained by combining head-out water immersion with norepinephrine infusion suppresses RAAS to a greater extent (15).

Secondary hyperaldosteronism in advanced cirrhosis results from both enhanced release and reduced clearance, the former mechanism being far more important (28). Suppression of renin release by different means (β-blockade, head-out water immersion, changes in posture, and plasma volume expansion) consistently reduce plasma aldosterone (6) (Fig. 4.3b). In contrast to what occurs in pre-ascitic patients and healthy subjects, a longitudinal correlation between time-related PRA and plasma aldosterone has been reported in patients with ascites (7). Therefore, in decompensated cirrhosis, RAS not only governs the degree, but also the circadian rhythmicity, of aldosterone secretion. Such a pivotal role is favored by adrenal sensitization to AII, which appears to be more pronounced in patients with ascites compared with pre-ascitic patients (7). Additional stimulating factors for aldosterone secretion can be hyponatremia and hyperkalemia.

## Renal sodium retention

Renal sodium retention is a cardinal manifestation of cirrhosis, as it is responsible for ascites formation, which is the most frequent complication of the disease (33).

### Pre-ascitic cirrhosis

It has been clearly demonstrated that renal sodium retention precedes ascites formation. In animal models of cirrhosis, a positive sodium balance develops 1–2 weeks before the development of ascites (34,35), leading to plasma volume expansion. The latter finding has been confirmed in human cirrhosis (20), and indisputably means that renal sodium retention also occurs in pre-ascitic patients. The demonstration of such an abnormality was somewhat hampered by the results of seminal studies performed in patients under prolonged (24 h) bed rest, which failed to reveal an increased renal sodium reabsorption. Actually, hypernatriuresis exceeding by three- to five-fold the dietary sodium intake was found (22,36). Considering that posture greatly influences renal sodium handling, it was finally shown that patients with pre-ascitic cirrhosis exhibited renal sodium retention while standing (23) (Fig. 4.3c).

Renal sodium retention in pre-ascitic cirrhosis may result from many efferent mechanisms, including RAS and sympathetic nervous system activation, and renal hemodynamic abnormalities. Renal perfusion and glomerular filtration rate are usually preserved in both experimental and human pre-ascitic cirrhosis (37), pointing to factors promoting an exaggerated tubular reabsorption. Although a subtle activation of the sympathoadrenergic system (19) may play a role, this does not appear to prevail. In fact, in patients with pre-ascitic cirrhosis, lower-body negative pressure produced renal sodium retention without increasing renal sympathoadrenergic activity (13), and $AT_1$ receptor blockade by losartan resolved upright posture-induced renal sodium retention, despite the rise in plasma norepinephrine being preserved (38).

Several findings support the contention that RAS is a major determinant of pre-ascitic renal sodium retention. In dogs with dimethylnitrosamine-induced cirrhosis developing pre-ascitic positive sodium balance, proximal tubular sodium reabsorption was unaltered (34), suggesting that sodium retention mainly occurs at the

distal nephron, the site of action of aldosterone. However, plasma aldosterone is not increased in this model. Contrariwise, pre-ascitic renal sodium retention in rats with $CCl_4$-induced cirrhosis was temporally related with increasing renal aldosterone excretion, and was prevented by the aldosterone antagonist spironolactone (35). In upright pre-ascitic cirrhotic patients, renal sodium retention was associated with a borderline elevation in plasma aldosterone (Fig. 4.3a), reduced urine sodium/potassium ratio (23), and increased sodium reabsorption by the distal nephron (39). It should be noted that slight increases in plasma aldosterone can induce a substantial reduction of sodium excretion, as the relationship between these variables is hyperbolic. Moreover, supine-induced natriuresis, a phenomenon largely linked to ANF release due to the heightened venous return (9), is also closely related to plasma aldosterone reduction (Fig. 4.3c) and an increase in urine sodium/potassium ratio (22,23). Further evidence for the importance of aldosterone in promoting renal sodium retention in pre-ascitic cirrhosis is provided by the direct correlation between plasma aldosterone and blood volume (20), which is lowered by spironolactone (40).

Pre-ascitic renal sodium retention could also be induced by other mechanisms. In fact, low-dose losartan, which selectively antagonizes the effects of intrarenal AII without perturbing renal and systemic hemodynamics, blunts the standing-induced renal sodium retention (38). This suggests that intrarenal AII accumulation would play a role by acting at the proximal convoluted tubule. In fact, sodium retention occurs at both the proximal and distal nephron, the former being reverted by losartan.

The combined action of AII and aldosterone may partly account for the finding that, for any given value of plasma aldosterone concentration, patients with pre-ascitic cirrhosis excreted about two-thirds of the amount of urinary sodium excreted by healthy subjects (20), a feature suggesting a tubular sensitization to the hormone (Fig. 4.4). In fact, the proximal action of AII reduces distal sodium delivery, so that the site of aldosterone action would be exposed to a decrease in delivery. This can explain why normal plasma aldosterone concentrations are correlated with reduced renal sodium excretion (23), plasma volume is reduced by spironolactone (40), and selective $AT_1$ receptor blockade also induces natriuresis (38).

## Cirrhosis with ascites

Many studies in cirrhotic patients with ascites have shown increased renal excretion or plasma levels of aldosterone and a close inverse relationship between aldosterone and renal sodium excretion (6). This was fully confirmed by circadian studies (36). Some reports, however, questioned the importance of aldosterone. It should be underlined that, in advanced cirrhosis, the role of aldosterone may

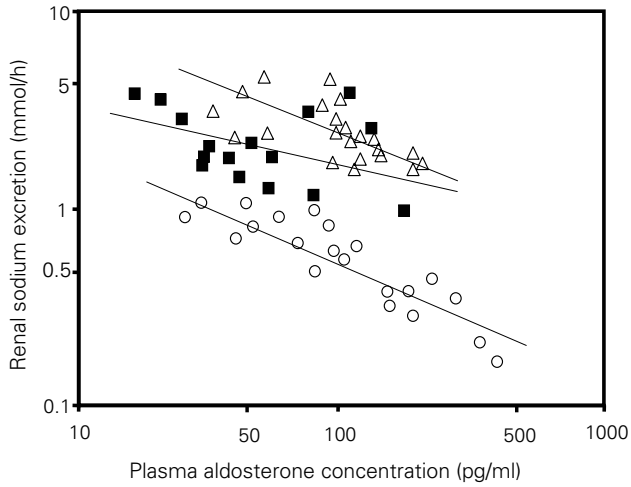


**Figure 4.4** Correlations between supine values of plasma aldosterone concentration and hourly renal sodium excretion in healthy subjects (△; $r = -78$; $P < 0.001$), patients with pre-ascitic cirrhosis (■; $r = -59$; $P < 0.05$), and patients with cirrhosis developing ascites for the first time (○; $r = -94$; $P < 0.001$) receiving 40 mmol/day of sodium. The regression line is progressively and significantly down-shifted in patients with cirrhosis (without and with ascites) compared with controls. (Data from reference 20.)

be disguised by factors, such as poor renal perfusion, enhanced AII production, and increased sympathoadrenergic activity, which promote sodium reabsorption in the proximal tubule. As a result, both proximal and distal sodium reabsorption are increased. The relative contribution of sodium extraction by the distal nephron prevails in patients with preserved glomerular filtration rate, while proximal tubular reabsorption prevails when renal perfusion is impaired (41). This explains why the suppression of aldosterone synthesis by aminoglutethimide (42) fails to resolve renal sodium retention. Similarly, ACE inhibitors (6) do not increase natriuresis, as their administration reduces the glomerular filtration rate by inducing arterial hypotension or, in any case, decreasing post-glomerular resistance (see below). Finally, an apparent discrepancy between changes in plasma aldosterone and renal sodium excretion can reflect their hyperbolic relationship, so that, when hyperaldosteronism is severe, an even striking reduction of plasma aldosterone would barely increase sodium excretion (Fig. 4.3d). As a result, expansion of central blood volume by head-out water immersion (43) or the assumption of the supine posture (44) only provokes natriuresis if plasma aldosterone decreases below a critical threshold value.

Plasma aldosterone inversely correlates with renal sodium excretion even in those patients accumulating ascites for the first time and showing plasma aldosterone within the normal range. Aldosterone is likely to maintain its pathogenic role due to tubular sensitization, which is even more marked in cirrhosis with ascites than

in pre-ascitic cirrhosis. Namely, for any given value of plasma aldosterone, the amount of sodium excreted by ascitic patients with preserved renal perfusion is about one-quarter of that eliminated by healthy subjects (20) (Fig. 4.4). Chronobiological studies confirmed this finding by showing that aldosterone effectiveness in these patients was four-fold greater than in healthy subjects throughout the day (36). This abnormality still awaits explanation, but the simultaneous presence of other sodium-retaining substances/mechanisms is probably involved. Among these, it is likely that AII is implicated, as supported by the inverse correlation between PRA and lithium clearance, a reliable marker of sodium delivery to the distal nephron. Interestingly, there was such a finding in patients with ascites and increased PRA, but not in healthy subjects (45).

The crucial importance of aldosterone on the clinical ground is witnessed by the effectiveness of anti-mineralocorticoid drugs (46,47). Interestingly, unopposed hyperaldosteronism can even extinguish the effects of the potent loop diuretics (46). Adequate antagonism of aldosterone requires the administration of spironolactone at dosages proportional to plasma concentration of the hormone (48). Obviously, when proximal sodium reabsorption becomes prevalent, spironolactone alone cannot promote natriuresis (49). This is the rule when there is a severe reduction in renal perfusion.

## Renal perfusion abnormalities

Reduced effective arterial blood volume frequently impairs renal perfusion and glomerular filtration rate in advanced cirrhosis, eventually leading to renal failure.

As reported above, plasma renin concentration/activity are elevated in advanced cirrhosis and inversely correlate with renal plasma flow or glomerular filtration rate (37). Given the potent vasoconstrictor activity of AII, to which renal vasculature is highly sensitive, and the intrarenal AII generation is present, it is tempting to think that the RAS is involved in the pathogenesis of renal vasoconstriction. Abnormalities in blood flow distribution within the renal cortex, with relative ischemia of outer cortical nephrons, could also be related to RAS activation, as PRA correlates inversely with outer cortical nephron blood flow (24).

These findings, however, do not clarify whether RAS activation is the cause or effect of renal ischemia. In fact, poor perfusion pressure enhances renin release by reducing the stretch of the afferent glomerular arteriole and sodium delivery to the macula densa. Infusion of AII and inhibition of the RAS by different means have contributed to elucidate this item.

AII infusion to healthy subjects reduces renal perfusion without affecting glomerular filtration rate. In cirrhotic patients, pressor doses of AII induce similar changes, even though renal plasma flow is only moderately reduced (50). This suggests that AII predominantly increases post-glomerular resistance, and is therefore supportive of renal function in advanced cirrhosis. ACE inhibition by captopril in patients with ascites usually induced a substantial reduction in glomerular filtration rate (6), which is largely attributable to a concomitant reduction in arterial pressure (see below). As this effect is associated with a decrease in renal vascular resistance, allowing renal plasma flow to remain steady or even increase, it appears that RAS inhibition greatly impairs post-glomerular resistance. Such a deleterious effect is also seen with a low dose of captopril, which does not alter arterial pressure (51). β-Blockers inhibit renin release, but their administration to patients with advanced cirrhosis is not followed by untoward effects on renal function (24,31,52), as unopposed α-adrenergic drive ultimately leads to an increase in peripheral vascular resistance. β-Blockade does not revert the altered intrarenal blood flow distribution reported above (24), suggesting that this abnormality is the cause, rather than the consequence of, RAS activation. As a whole, these data suggest that RAS activation in advanced cirrhosis is crucial to preserve arterial pressure and maintain glomerular filtration rate at the highest possible levels for the actual renal perfusion pressure.

The potential adverse effect of RAS activation on renal perfusion is probably prevented by an increased intrarenal synthesis of prostaglandins and other vasodilators. In fact, patients with elevated PRA and fairly preserved renal perfusion showed an enhanced renal excretion of $PGE_2$, $PGF_{2\alpha}$, and kallikrein (52). In fact, nonsteroidal anti-inflammatory agents, which inhibit prostaglandin synthesis, can precipitate functional renal failure in patients with advanced cirrhosis (52).

An imbalance between vasodilator and vasoconstrictor factors, including AII, is likely to be involved in the pathogenesis of hepatorenal syndrome (HRS), characterized by a striking intrarenal arterial vasoconstriction (see Chapter 28). Indeed, cirrhotic patients with HRS have the highest levels of plasma renin concentration/activity (6) (Fig. 4.2); moreover, elevated PRA independently predicts the development of HRS (53). These findings are probably related to severe systemic hemodynamic abnormalities leading to a striking reduction in effective arterial blood volume. Interestingly, renal prostaglandin and kallikrein excretion in HRS are reduced compared with cirrhosis with ascites and preserved renal perfusion (52), suggesting that the exhaustion of prostaglandin and, possibly, the synthesis of other vasodilating factors precipitate the syndrome. In this context, RAS activation is likely to be pathogenetically relevant.

In conclusion, RAS activation in advanced cirrhosis probably plays a compensatory role to preserve renal function through its effects on arterial pressure and

post-glomerular resistance. However, a striking activation of the RAS, especially when not counterbalanced by local vasodilators, may favor intrarenal vasoconstriction.

## Cardiovascular abnormalities

Patients with advanced cirrhosis present a hyperdynamic circulatory syndrome, characterized by high cardiac output and low peripheral vascular resistance due to arterial vasodilation (see Chapter 11). Arterial vasodilation leads to a reduction in effective arterial blood volume, which activates vasoconstrictor systems aimed at counterbalancing the reduced vasomotor tone. These include RAS, as suggested by the inverse correlations between either arterial pressure or peripheral vascular resistance and PRA reported in several studies (6). Arterial pressure is often reduced to an extent that would be insufficient to stimulate renin release through a stretch receptor-like mechanism, but it should be considered that actual arterial pressure results from the combined effects of vasodilating substances and compensatory vasoconstrictor systems.

A reduced vascular response to AII distinctively characterizes advanced cirrhosis in both experimental models and humans. Seminal studies showed that AII administration to cirrhotic patients with ascites yielded a reduced pressor effect compared with that observed in healthy subjects, irrespective of the mode of administration (boluses, short-term or long-term infusions) (6). Consistent results were obtained in subsequent studies evaluating the pressor response (54) or vascular reactivity in the forearm (55). In one study, however, changes in forearm blood flow under AII infusion did not differ between cirrhotic patients and controls (56). As the patients enrolled had a less advanced disease with respect to the previously cited studies, vascular hyporeactivity to AII probably parallels the severity of cirrhosis.

Reduced vascular reactivity to AII has been confirmed in experimental cirrhosis, both *in vivo* and *in vitro*. These studies can also help in elucidating the mechanisms underlying this abnormality. In rats with cirrhosis showing a reduced pressor response to AII, an increased number but a reduced affinity of AII receptors in the mesenteric artery was documented, suggesting a post-receptor signaling defect (57). Vascular hyporeactivity to AII is largely mediated by the endothelium, as endothelium denudation of aortic rings isolated from cirrhotic rats restores AII-induced contractility (58) (Fig. 4.5). Endothelial cells synthesize a number of vasodilating substances with paracrine action, which counteract AII effects (59). Enhanced NO production is likely to play a major role, as AII-induced vascular contractility is restored by NO synthase inhibition with Nω-nitro-L-arginine (58). The mesenteric bed shows the greatest impairment in AII-induced contractility (60), a finding coherent with the contention that vasodilation mostly occurs in this arterial district both in experimental models and cirrhotic patients (see Chapter 12).

ANF may represent another factor interfering with RAS activity, as it counteracts AII action and inhibits renin release, and its plasma levels are often increased in cirrhosis (see Chapter 6). In patients with ascites, maneuvers provoking a sudden increase in cardiac output and plasma ANF, such as head-out water immersion (61) and the assumption of the supine position (16), lead to a significant, albeit transient, arterial pressure reduction. This implies an exaggerate decline in peripheral vascular resistance. Interestingly, plasma ANF rise is inversely correlated with both peripheral vascular resistance and PRA (16). In addition to the ANF vasodilating effect, arterial pressure reduction may reflect the inhibition of renin release by ANF.

Despite impaired vascular responsiveness to AII, RAS is crucial to counteract vasorelaxation. In fact, ACE inhib-

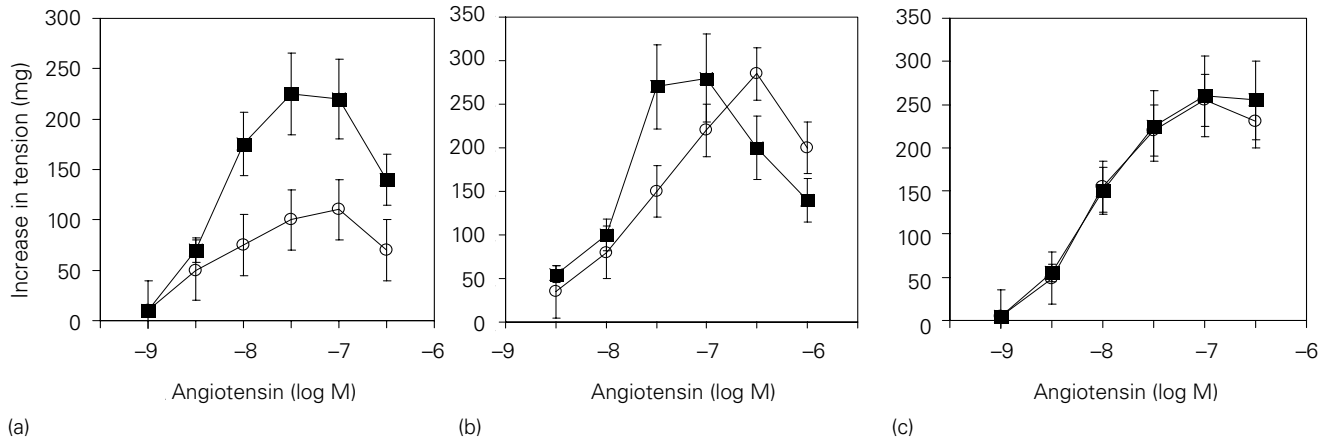


**Figure 4.5** Reduced vascular contractility to angiotensin II in rats with experimental cirrhosis (○) is reverted by endothelial denudation and nitric oxide synthase inhibition. Control rats, ■. Curves of dose–response to angiotensin II in aortic rings: (a), intact; (b), endothelium-denuded; (c), intact pretreated with Nω-nitro-L-arginine. (Data from reference 59.)

itors and AII antagonists, even at dosages which do not alter arterial pressure in healthy subjects, induce hypotension in patients with ascites (6,62), the magnitude of which is roughly proportional to the baseline PRA. These findings caution against the administration of such drugs in patients with hyperdynamic circulatory syndrome and a remarkable activation of RAS, especially when given together with low sodium diet and diuretics.

Tissue RAS, in addition to controlling the vascular tone, is also involved in regulating inflammatory and reparative processes by promoting cytokine production, inflammatory cell adhesion, chemotaxis, and macrophage activation. As a result, growth of fibroblasts, synthesis of types I and III fibrillar collagens, and angiogenesis are stimulated (3). These effects become important in disease processes, such as heart failure. In fact, there is evidence that adverse vascular remodeling by fibrous tissue induced by coronary heart disease or hypertension is largely and independently promoted by tissue RAS (63).

Cardiac contractile dysfunction has been documented in cirrhosis, which has been termed "cirrhotic cardiomyopathy" (see Chapter 15). Although major pathological changes are not usually evident, cardiomyocyte edema and mild fibrosis associated with ventricular hypertrophy have been reported. Whether chronic activation of tissue RAS is involved in cirrhotic cardiomyopathy is not known at present. However, it is interesting to note that the aldosterone antagonist canrenone minimized the abnormal cardiac response to a postural change presented by pre-ascitic cirrhotic patients under baseline conditions (64).

## Hepatic fibrosis, angiogenesis, and portal hypertension

Fibrogenesis is a key event leading to cirrhosis. It follows hepatic stellate cell (HSC) activation and transformation into a myofibroblastic phenotype, which releases various cytokines, such as transforming growth factor-$\beta_1$ (TGF-$\beta_1$), and matrix proteins favoring type I collagen formation (65). RAS activation is likely to be important in these processes.

In bile-duct ligated rats, liver expression of $AT_1$ receptor and ACE mRNA is increased (66), and AII stimulated TGF-ß mRNA synthesis by rat HSC *in vitro* (67). Moreover, ACE inhibition (67) and $AT_1$ receptor blockade (68) significantly reduced liver fibrosis in different rat models. Chronic hyperaldosteronism may also be important, as aldosterone promotes heart fibrosis through still undefined mechanism(s), but possibly favoring sodium entry into fibroblasts (63). Interestingly, the anti-mineralocorticoid canrenone inhibits proliferation and motility induced by platelet-derived growth factor in human HSC *in vitro* (69). However, it appears that these effects are independent of the drug aldosterone antagonism.

RAS can also contribute to portal hypertension by increasing intrahepatic vascular resistance, as AII induces HSC contraction, thus increasing sinusoidal resistance (70). RAS inhibition, therefore, can be expected to lower portal pressure. Arroyo *et al.* (71) found that the infusion of the AII competitive analog saralasin to patients with ascites led to a ≈ 20% reduction in wedge hepatic venous pressure, which was proportional to a concomitant drop in arterial pressure. This may suggest that portal pressure decrease simply mirrored systemic hemodynamic changes. However, as hepatic blood flow remained steady, a reduction in post-sinusoidal vascular resistance was proposed. More recently, several studies (62,72) evaluated the effect of $AT_1$ receptor antagonists losartan and irbesartan in cirrhotic patients. The results were not entirely consistent, as losartan induced a striking drop in hepatic venous pressure gradient (HVPG) by ≈ 45% in one study, while it failed to influence portal pressure significantly in another. Irbesartan induced a mild, clinically nonsignificant, HVPG reduction by ≈ 12%. Moreover, the negative impact of RAS inhibition on arterial pressure and glomerular filtration rate (see above), which occurred in two studies, may prevent the use of these drugs in advanced cirrhosis.

Finally, RAS activation can increase portal pressure through aldosterone-dependent blood volume expansion (20), which contributes to the portal hypertension. In fact, spironolactone administration reduces portal pressure (40,73). Interestingly, the combined administration of spironolactone and nadolol to cirrhotic patients without ascites reduced the incidence of variceal bleeding and ascites (74).

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# Chapter 5
# The Sympathetic Nervous System in Cirrhosis

Francis J. Dudley and Murray D. Esler

## Introduction

The sympathetic nervous system (SNS) occupies a position of central importance in the maintenance of the body's homeostasis. Cardiovascular, renal, gastrointestinal, reproductive, metabolic, and thermoregulatory control are, at least in part, regulated through this division of the autonomic nervous system. The SNS is also a major determinant of the mammalian response to stress (1).

SNS dysfunction has been implicated in the pathophysiology of many disorders including the autonomic insufficiency syndromes, essential hypertension, cardiac failure, coronary artery spasm, mitral valve prolapse, and Raynaud's syndrome (2). Studies performed over the last two decades have also indicated that, in cirrhosis, changes in SNS activity occur that attempt to maintain the integrity of the systemic circulation and contribute to the pathophysiology of two of the more devastating complications of chronic liver disease: portal hypertension and ascites formation.

## Assessment of sympathetic nervous system activity

A diverse range of tests are available for the assessment of SNS activity (Table 5.1) and are considered complementary rather than competitive as they measure different aspects of SNS function (3). It is, however, important to recognize their limitations when evaluating reports of SNS activity under differing physiological and pathological conditions.

### Total sympathetic nervous system activity

#### *Plasma norepinephrine concentration*

The recognition in 1957 that the spillover of norepinephrine (NE) into the venous drainage of an organ was proportional to the rate of electrical stimulation of its sympathetic innervation and the development of isotopic methods for determining the concentration of catecholamines in plasma led to the use of arterial NE measurements as a helpful guide to overall SNS activity (4–6), despite a number of well-recognized caveats (7–12).

#### *Norepinephrine spillover rate*

Interpretation of plasma NE levels must be tempered by the recognition that they are dependent not only on sympathetic tone and NE release but also on the rates of removal of the neurotransmitter from the plasma (Fig. 5.1) (7–12). Changes in plasma clearance can have a profound effect on blood levels and if ignored could adversely affect data interpretation (13). The problem of changes in plasma clearance can be overcome by using radiotracer techniques which allow one to adjust for clearance and thereby derive a more accurate measure of overall sympathetic activity (7).

### Regional sympathetic nervous system activity

Regional differences may exist in the response of the SNS to different stimuli (3,14–19) with sympathetic outflow being activated in some vascular beds while it is unchanged or inhibited in others. As a result, arterial plasma NE measurements are of limited value as an index of regional sympathetic activity. Alternative methodology has been developed to address this problem.

**Table 5.1** Assessment of sympathetic nervous system (SNS) activity.

| |
|---|
| Total SNS activity |
| Plasma norepinephrine concentration (NE) |
| Norepinephrine spillover rate |
| Regional SNS activity |
| Clinical microneurography |
| Regional arteriovenous plasma (NE) |
| Regional NE spillover rates |
| Power spectral analysis of circulatory rhythms |
| Pharmacological autonomic blockade |

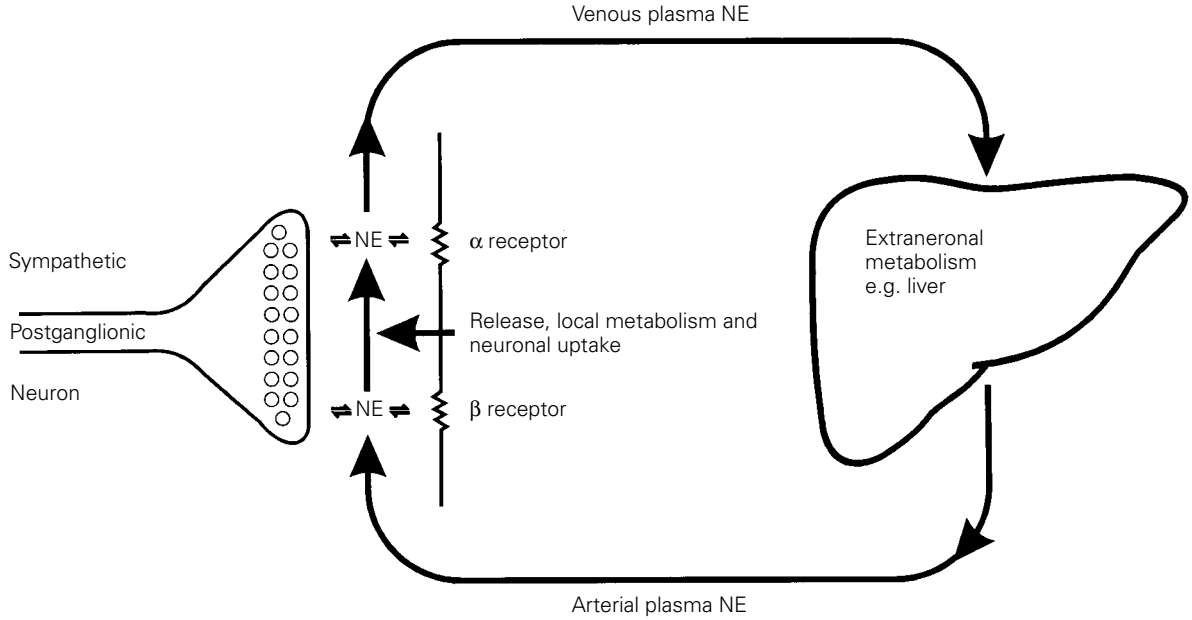


**Figure 5.1** Norepinephrine (NE) release and metabolism in man.

### *Clinical microneurography*

This technique measures sympathetic nerve firing rates to skeletal muscle and skin by electrophysiological recording. The technique involves the insertion of fine tungsten electrodes through the skin and the positioning of the electrode tip in sympathetic fibers of, usually, the common peroneal or median nerves. Multifiber recordings of nerve activity are generated and are directly proportional to regional SNS activity (20,21).

In clinical research microneurographic methods for studying sympathetic nerve firing rates can only be applied using easily accessible nerves to skeletal muscle and skin: access to the sympathetic nerve supply of internal organs is limited. The use of this methodology is therefore effectively restricted to the upper and lower limbs.

### *Regional plasma norepinephrine levels and spillover rate*

Regional differences in arteriovenous plasma NE levels provide an assessment of local SNS activity but interpretation may be hampered by variations in local clearance. The use of radiotracer techniques combined with plasma NE measurements in arterial blood and the venous drainage of a particular organ allows assessment of that organ's release of NE into the circulation, eliminating any confounding effects that could arise as a result of changes in local NE clearance (19,22–24).

A possible limitation of the isotope dilution methodology for the determination of regional NE spillover is the release of isotopically labeled NE back into the plasma (Table 5.2) after its neuronal uptake (tracer recycling). Recycling must be insignificant if the isotope dilution methodology is to reflect regional SNS activity accurately. This prerequisite appears to have been satisfactorily established (25).

It should be emphasized that these methods measure the arteriovenous gradient of plasma NE and its clearance and NE spillover is calculated and is inferred to reflect NE release and sympathetic activity. A variety of other factors, however, influence the rate at which NE released into the interstitial space diffuses into plasma (16,26,27). Released NE is subject to several possible fates: re-uptake into sympathetic nerves, O-methylation after uptake into extraneuronal cells, and diffusion into plasma. The rate of overflow is determined not only by the rate of NE release (sympathetic nerve activity) but also by the activity of the competing clearance

**Table 5.2** Factors determining regional norepinephrine (NE) spillover to plasma.

| |
|---|
| Neuronal release of NE |
| Nerve firing rate |
| Nerve density |
| Organ mass |
| Neuronal NE uptake and metabolism |
| Sympathetic cleft width |
| Local extraneuronal uptake and metabolism |
| Capacity for O-methylation |
| Diffusion into circulation |
| Capillary permeability to NE |
| Blood flow |

Adapted from Esler *et al.* (16).

mechanisms of uptake, metabolism and diffusion into the circulation. Diffusion into plasma will also be dependent on regional blood flow (26) and capillary permeability to NE (27).

A further limitation of both microneurography and plasma NE levels and spillover is the recognition that the effect of activation of the SNS is also dependent on receptor characteristics and tissue responsiveness to catecholamines.

#### *Power spectral analysis of circulatory rhythms*

Independent high-frequency and low-frequency rhythmic influences on heart rate (RR interval) and arterial pressure can be identified by this technique and have been used as a non-invasive tool to obtain a quantitative assessment of autonomic activity which can differentiate between sympathetic and parasympathetic activity (28–30). However, low-frequency heart rate and arterial pressure variability are only partly determined by the intrinsic centrally controlled oscillation of the activity of sympathetic nerves and, as such, changes in the power spectral analysis may not always be solely due to changes in sympathetic activity.

### Pharmacological autonomic blockade

Pharmacological blockade of the SNS can provide semiquantitative information on both systemic and regional SNS activity (31–33).

The measured changes of power spectral analysis and pharmacological blockade will be dependent not only on neural activity but also on changes in end organ responsiveness.

## The sympathetic nervous system and cardiovascular homeostasis

### In health

The SNS has a central role in maintaining cardiovascular homeostasis (1). This control system consists of: peripheral receptors responsive to changes in cardiovascular stability; afferent, central, and efferent integrated pathways; and neurohumoral effectors that ultimately modulate vascular tone (34).

Baroreceptors are an integral part of the regulatory system involved in the maintenance of circulatory stability (35). High-pressure arterial baroreceptors such as those found in the carotid sinus and aortic arch are present within the arterial vascular tree and will respond to changes in arterial pressure which, in turn, is dependent upon both cardiac output and peripheral vascular resistance (34). Low-pressure cardiopulmonary baroreceptors respond to changes in cardiac preload which is dependent on venous return to the heart and central blood volume (36).

The sensation by the low-pressure cardiopulmonary or high-pressure arterial baroreceptors of a decrease in cardiac preload (37) or arterial pressure (38) leads to a decrease in the tonic inhibitory traffic in the afferent glossopharyngeal pathways to the central nervous system, leading to an increase in efferent sympathetic activity which will increase arterial vascular tone and activate other neurohumoral systems to help preserve cardiovascular stability (38). The peripheral effects of this increased sympathetic activity include: constriction of the arterial resistance vessels, increasing peripheral vascular resistance; stimulation of renin release from the kidney, leading to the activation of the renin–angiotensin–aldosterone system (RAAS); and positive chronotropic and inotropic effects on the heart. The resultant increase in angiotensin II production will further increase the tone of the arterial resistance vessels. SNS activity is also important in controlling the non-osmotic release of arginine vasopressin (AVP) (38). Adrenergic pathways running from the glossopharyngeal baroreceptor nuclei to the supraoptic and paraventricular nuclei in the hypothalamus terminate on neurons in these nuclei that synthesize AVP (39–41). Electrical activity of the supraoptic neurons correlates with AVP release and is altered by systemic blood pressure changes in the appropriate direction to also incriminate AVP in blood pressure control (40).

Thus, a decrease in cardiac preload or arterial pressure inhibits baroreceptor activity and results in a simultaneous activation of the vasoconstrictor systems: the SNS, the RAAS, and the non-osmotic release of AVP. The activation of the three systems is interdependent and has additive and synergistic effects on vascular smooth muscle cells (42). The immediate response to this integrated system involves the vascular effects of the three hormones, but maintenance of the response is assisted by AVP-induced water retention, aldosterone-mediated sodium retention, and an SNS and angiotensin II-mediated increase in the proximal renal tubular reabsorption of sodium (38).

### In cirrhosis

Activation of the SNS is commonly present in patients with liver disease. The presence of increased sympathetic nerve firing rates in cirrhosis was inferred initially from the higher heart rate and cardiac output that are commonly present in these patients (43). Biochemical methods have subsequently demonstrated the presence of an elevated concentration of the sympathetic neurotransmitter NE in plasma (9,44–46) and increased rates of NE spillover to plasma for the body as a whole (Fig. 5.2) (8,47–50) and from individual organs and vascular beds such as the heart (51), the kidneys (8,9,49), and the

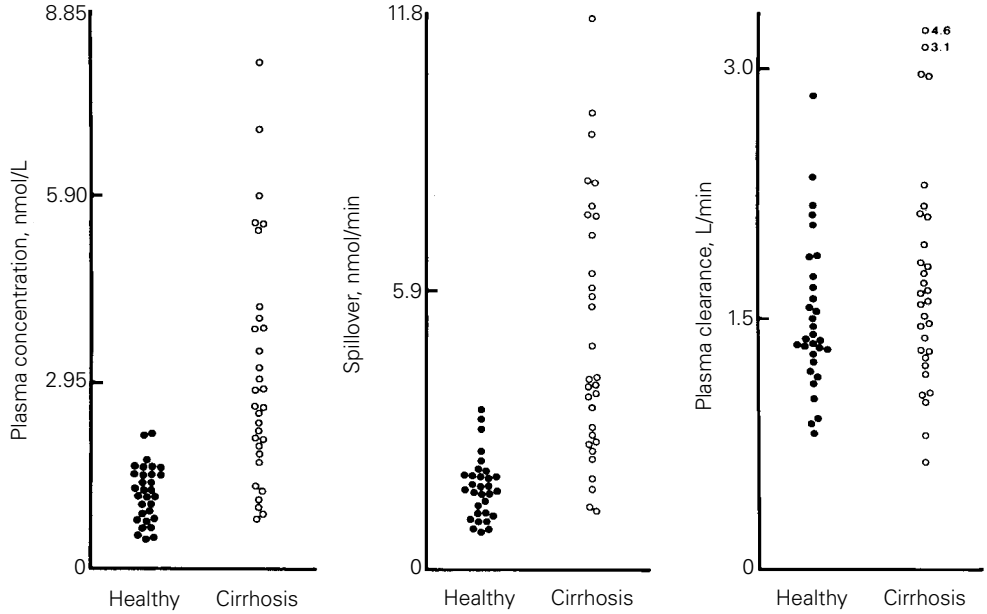


**Figure 5.2** Total plasma norepinephrine (NE) kinetics in healthy persons and patients with cirrhosis. The arterial plasma NE concentration (left) and the overall rate of spillover of NE to plasma (middle) were higher in patients with cirrhosis ($P < 0.001$) but NE plasma clearance (right) was similar in the two groups. (Reproduced with permission, Esler *et al*. (54).)

hepatomesenteric circulation (Fig. 5.3) (47,51,52). The magnitude of SNS activity in different vascular beds in a patient with cirrhosis can vary, suggesting that there may not be a uniform increase in SNS activity (53). NE spillover from the pulmonary vasculature in cirrhotic patients is similar to controls despite evidence of increased SNS activity in other vascular beds (Fig. 5.3) (54).

In general there is a direct relationship between SNS activity and the severity of the underlying liver disease (45,49,54,55). SNS activity is directly related to Child–Pugh score (Fig. 5.4) (54) and portal venous pressure (Fig. 5.5) (50,56) and is most pronounced in patients with ascites (9,45,48,57) and the hepatorenal syndrome (44,56–58). Studies of the diurnal changes in the urinary

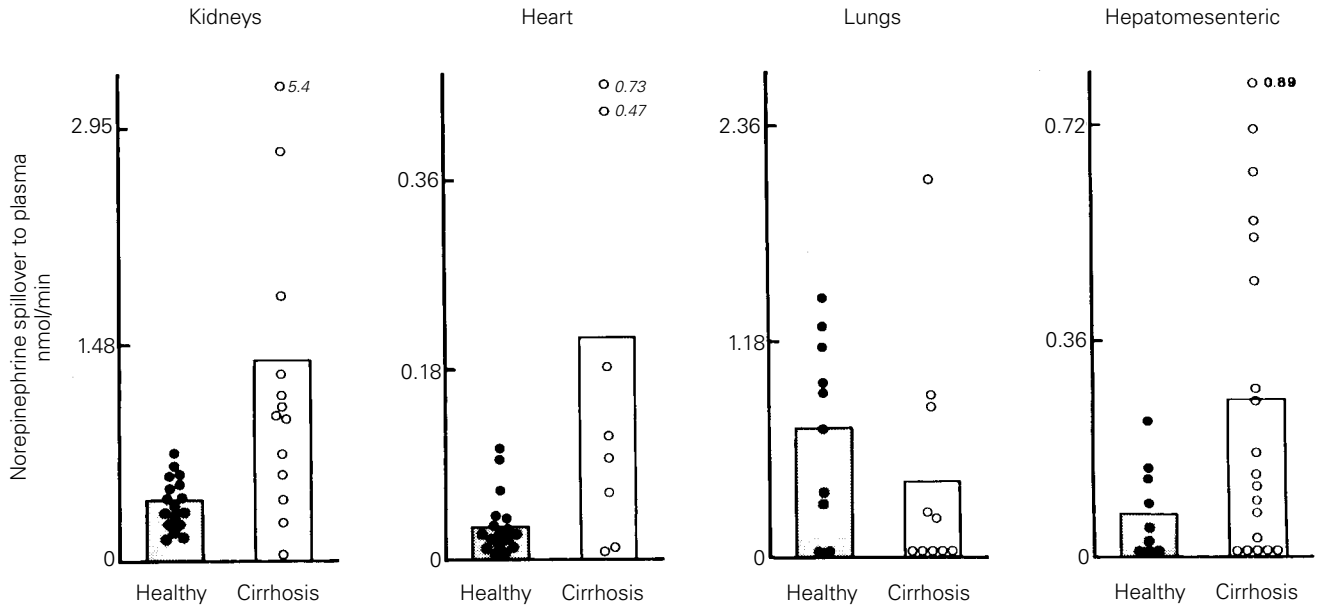


**Figure 5.3** Rates of regional norepinephrine (NE) overflow to plasma. NE spillover into the hepatomesenteric circulation ($P = 0.039$) and from the kidneys ($P = 0.001$) and heart ($P = 0.024$) was higher in patients with cirrhosis than in healthy persons, in contrast to that from the lungs which was slightly lower in the patients ($P = 0.065$). (Reproduced with permission, Esler *et al*. (54).)

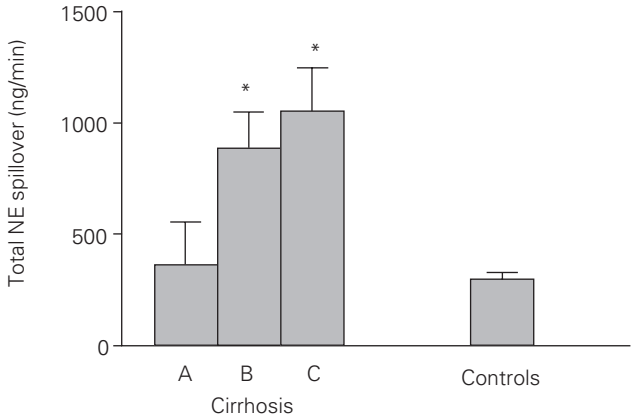


**Figure 5.4** The relation of the severity of the liver disease as assessed by Child–Pugh grade to total norepinephrine (NE) spillover in cirrhotic patients: $P = 0.012$—Spearman rank correlation applied using the numerical scale of Pugh grading. (Adapted from Esler *et al*. (54).)

excretion of NE and its metabolites have indicated that, even in patients with well-compensated cirrhosis in whom overall SNS activity is similar to healthy controls, there are subtle changes in the sympathetic control of cardiovascular homeostasis (59). The normal diurnal rhythm of adrenergic activity is lost and the sympathetic control of heart rate and arterial pressure altered. The relationship between the severity of liver disease and SNS activity is strongly supported by the finding that plasma NE is an independent predictor of survival in patients with cirrhosis (60,61).

SNS activity in cirrhosis is governed by the activity of both the high-pressure arterial and low-pressure cardiopulmonary baroreceptors.

### *Arterial baroreceptor activity*

A decrease in peripheral vascular resistance is clinically evident early in the course of chronic liver disease due to a decrease in the tone of arterial resistance vessels and the opening of arteriovenous shunts in almost all vascular beds, including the skin, muscle, lungs, splanchnic circulation, and brain. These vascular changes contribute to the development of hypoxia, portal hypertension, and cerebral edema (62). The decreased peripheral vascular resistance is manifest clinically by a hyperdynamic circulatory state with a resting tachycardia, a bounding pulse, wide pulse pressure, and a warm periphery with capillary pulsation (63). There is a tendency toward arterial hypotension despite coexisting increases in cardiac output (64,65) and plasma volume (66). The total peripheral vascular resistance decreases as the liver disease progresses and ascites develops (67) and is consistent with the presence of an endogenous vasodilator

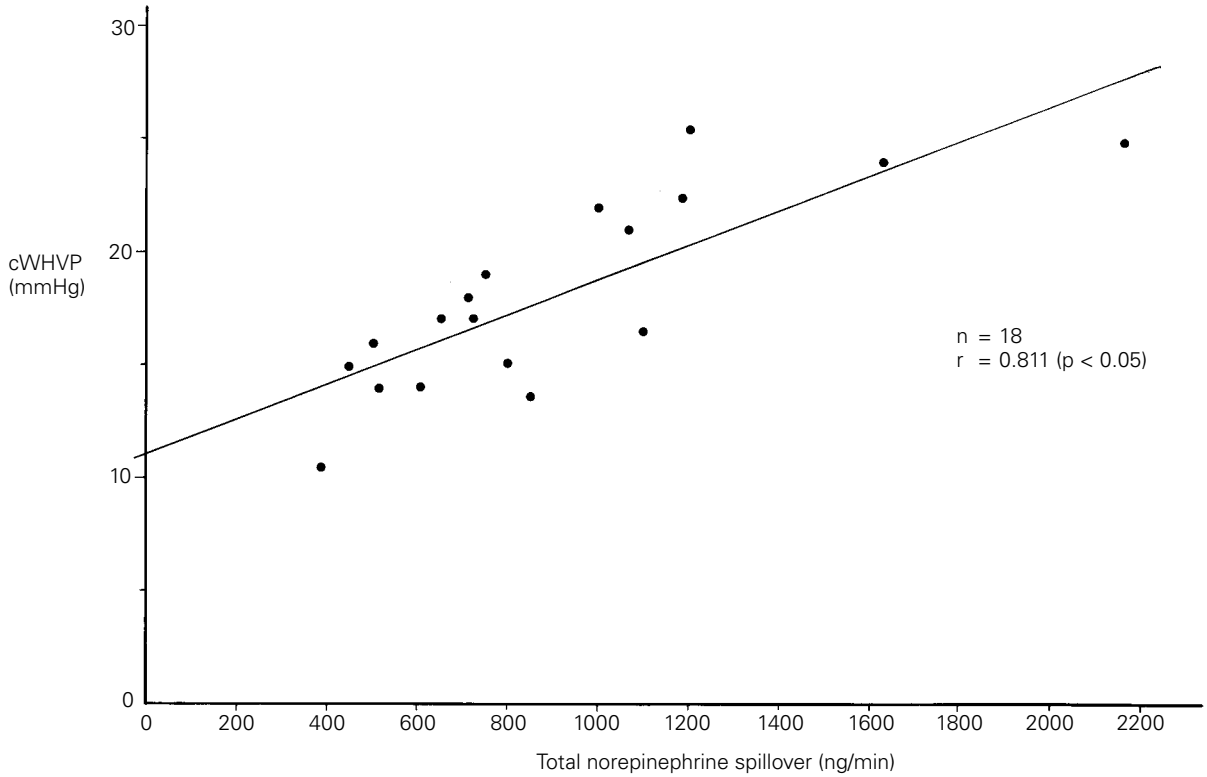


**Figure 5.5** The relation between corrected wedged hepatic vein pressure (CWHVP) and total norepinephrine (NE) spillover in cirrhotic patients. Portal pressure correlated directly with overall sympathetic nervous activity ($r = 0.811$; $p < 0.05$).

produced by, or not inactivated by, the diseased liver. The magnitude of the vasodilation that is evident in different regional vascular beds varies as cirrhosis develops and is complicated by the onset of ascites and the hepatorenal syndrome. Splanchnic vasodilation persists and increases while other vascular beds (renal, cerebral, peripheral) respond to activation of homeostatic neurohumoral control systems, such as the SNS, with an increase in vascular resistance (67–69). The resultant decrease in renal blood flow and glomerular filtration rate contributes to the development of ascites and the hepatorenal syndrome.

The SNS may be activated in cirrhosis as a compensatory response, initiated by changes in arterial baroreceptor activity, to maintain systemic hemodynamic homeostasis in the presence of peripheral vasodilation (38,70). Chronic SNS stimulation is well documented to accompany other forms of longstanding arteriolar vasodilation such as that produced by vasodilator antihypertensive drugs including hydralazine (71,72). There is also an inverse correlation between peripheral vascular resistance and SNS activity in cirrhosis (Fig. 5.6), implying that vasodilation may be responsible for the sympathetic activation (54). Finally, inhibition of sympathetic tone with clonidine results in a significant fall in mean arterial pressure due to a decrease in both cardiac output and peripheral vascular resistance (54), again consistent with the concept that activation of the SNS in these patients is a compensatory mechanism to maintain circulatory homeostasis particularly within the arterial vascular tree.

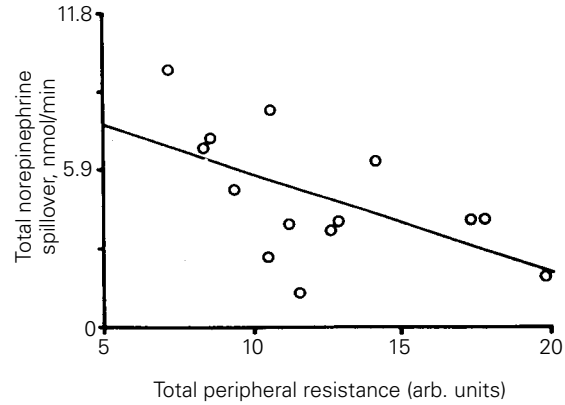


**Figure 5.6** The relation between the total peripheral vascular resistance and overall sympathetic nervous activity. Total norepinephrine (NE) plasma spillover correlated inversely with calculated total peripheral vascular resistance ($r = -0.58$; $P = 0.031$). (Reproduced with permission, Esler *et al*. (54).)

### *Cardiopulmonary baroreceptor activity*

It is also possible that changes in cardiac preload will be recognized by low-pressure baroreceptors and modulate SNS activity in patients with cirrhosis, and some studies have demonstrated an inverse relationship between measures of central blood volume and plasma NE in patients with cirrhosis. Right and left atrial pressures and pulmonary arterial pressure are usually normal in patients with cirrhosis, as are plasma concentrations of atrial natriuretic factor (ANF), an indirect indicator of central filling (73–76). In fact, as ascites develops, plasma ANF levels tend to increase slightly, suggesting an expanded central blood volume. The activity of the low-pressure cardiopulmonary baroreceptors would therefore be expected to be normal or increased in cirrhosis and lead to an increase in the tonic inhibitory neural activity to the controlling centers in the brain and result in decreased efferent sympathetic outflow to the periphery.

However, the low-pressure cardiopulmonary baroreceptors present in the lungs, the walls of the atria and ventricles, and at the junctions of the vena cavae and pulmonary veins with the atria are responsible, at least in part, for the altered responsiveness of the systemic circulation to changes in posture (77–80) and to physical exercise (81,82) in patients with cirrhosis. In the presence of portal hypertension, patients with chronic liver disease develop an expanded plasma volume, which increases as the disease progresses (66). In the erect position this expanded plasma volume is largely sequestered in the splanchnic venous bed (77) and the lower limbs and results in a decreased cardiac preload, a decrease in cardiac output, and an increase in sympathetic efferent activity. On assuming the supine position there is a disproportionate increase in venous return and cardiac preload and this can result in an increased cardiac output, decreased efferent sympathetic activity, and a decrease in systemic vascular resistance and thereby contribute to the development of the hyperdynamic circulatory state that is clinically evident in many of these patients (77–80). Similar changes in cardiac preload are probably also responsible for the hemodynamic response to a number of therapeutic interventions that are occasionally used to manage patients with decompensated chronic liver disease. An increase in cardiac preload occurs as a result of head-out water immersion (83), following the insertion of transjugular intrahepatic portosystemic (84–86) or peritoneovenous shunts (87–89) and occasionally after therapeutic paracentesis with intravascular volume replacement in patients with tense ascites (90,91). All these maneuvers can result in an increase in cardiac output, decreased efferent SNS activity, and a decrease in systemic vascular resistance.

The sensitivity of both the low-pressure cardiopulmonary and high-pressure arterial baroreceptors to changes in cardiac preload and arterial pressure, respectively, appears normal in patients with cirrhosis and the overall sympathetic activity in the individual patient will reflect the balance of tonic inhibitory activity in the afferent neural pathways to the central nervous system from both types of baroreceptor.

## The sympathetic nervous system and the liver

### In health

The sympathetic nerve supply to the hepatobiliary system originates from the celiac ganglion and enters the liver via the porta hepatis and two intercommunicating plexuses. The anterior plexus surrounds the hepatic artery and the posterior plexus encircles the portal vein and bile duct (92,93). Within the liver the nerve fibers supply the biliary system, the parenchymal cells, and the hepatic arterial and portal venous beds (92,93). The fiber types are predominantly monoaminergic and peptidergic, but a variety of putative transmitter substances have been identified (94). Nerve terminals lie in close proximity to smooth muscle cells, fibroblasts, biliary epithelial cells, sinusoidal endothelial cells, and parenchymal cells (92,93,95,96) and have the potential to modulate the tone of the intrahepatic portal vascular bed (97,98). Stimulation of hepatic sympathetic nerves causes hepatic arterial constriction and an increase in intrahepatic vascular resistance. Portal blood flow does not change and there is a rise in portal pressure (99,100). Stimulation of sympathetic nerves induces a graded constriction of terminal portal venules and sinusoids, presumably by contraction of perisinusoidal stellate cells (101). Hepatic sympathetic stimulation also has an effect on hepatic metabolism and bile formation. It increases glycogenolysis and glucose release from the liver but decreases urea formation, ketone body formation, xenobiotic metabolism and oxygen consumption (102–105). Sympathetic stimulation also results in a decrease in bile flow and bile acid secretion (102,105). Afferent sympathetic fibers arising in the liver may be responsive to osmo, ionic, baro, metabolic, and nociceptive stimuli (106).

### In cirrhosis

Chronic liver disease is associated with striking changes in the density and distribution of the sympathetic nerves. Nerve fibers are increased in the portal areas and fibrous septa but are decreased in regenerative nodules (107).

The hepatic microcirculation also undergoes substantial change as a result of liver injury and the development of cirrhosis. Hepatic sinusoids become capillarized with the loss of fenestrae and the formation of a basement membrane (108) and hepatic stellate cells develop a contractile phenotype being transformed into myofibroblasts (109–113). Both the size of endothelial fenestrae and the tone of the myofibroblasts are sensitive to sympathetic tone (114) and other pressors (115,116) and an increase in SNS activity will lead to an increase in intrahepatic vascular resistance and restrict access of plasma to the space of Disse. Hepatomesenteric SNS activity is increased in cirrhosis and contributes to the pathogenesis of the increased intrahepatic resistance and to the development of portal hypertension (50,54). It is possible that increased hepatic SNS activity could also limit flow-dependent clearance. The increase in intrahepatic vascular resistance in response to sympathetic stimulation would be expected to be greater than normal in patients with cirrhosis because of the increased density of sympathetic nerves and activated stellate cells (111) and this is supported by the increased sensitivity of the hepatic microcirculation to (i) norepinephrine in animal models of cirrhosis (117) and (ii) the central inhibition of the SNS by the $\alpha_2$ agonist clonidine in human subjects with cirrhosis (54).

Norepinephrine is also considered to possess a potent anti-apoptotic effect on hepatocytes and has been shown to enhance hepatocyte growth kinetics and possibly promote the development of regenerating nodules (118).

## The sympathetic nervous system and the kidney

### In health

There is an extensive network of sympathetic nerve fibers supplying the kidney with a high density of nerve terminals concentrated on the afferent and efferent glomerular arterioles, the thick ascending limb of the loop of Henle, the proximal and distal convoluted renal tubules, and the juxtaglomerular complex (119). At a low level of renal stimulation, which is subthreshold for renal vasoconstriction, sympathetic activity has both antinatriuretic and antidiuretic effects. It also leads to stimulation of renin secretion by the juxtaglomerular complex (120,121). In the normal situation basal renal sympathetic nerve activity is too low to influence renal hemodynamics but is sufficient to alter renin secretion and the renal tubular reabsorption of sodium and water (120).

### In cirrhosis

The SNS is thought to play an important role in the pathogenesis of ascites formation, and in the development of the hepatorenal syndrome in patients with chronic liver disease (56,58,122). Renal efferent sympathetic activity is often increased, particularly in the presence of decompensated disease (45,49,123–125), and results in an increase in both afferent and efferent arteriolar resistance and a decrease in the glomerular ultrafiltration coefficient $K_f$ (119). The latter is due in part to a decrease in glomerular capillary surface area. There is also a fall in the glomerular hydrostatic pressure gradient which is largely responsible for the decrease in glomerular filtration rate (GFR) and indicates that the effect on renal vascular resistance is greater in the afferent than in the efferent arterioles (119).

At moderate levels of sympathetic activity the fall in renal plasma flow is greater than the fall in GFR, indicating that there has been vasoconstriction of both the afferent and efferent arterioles and the filtration fraction is increased. However, when there is a marked increase of sympathetic nervous system activity, as in the hepatorenal syndrome, GFR and renal plasma flow fall in parallel, indicating that afferent arteriolar constriction predominates. Inhibition of the increased sympathetic neural outflow to the kidneys in patients with cirrhosis (54) leads to a fall in renal vascular resistance but renal blood flow is unchanged due to a concomitant fall in mean arterial pressure. GFR increases significantly, again indicating that the vascular effects of the increased renal sympathetic activity in cirrhosis are largely due to a preferential increase in afferent arteriolar tone.

The SNS also has an effect on the tubular handling of sodium,an increase in α receptor tone resulting in an increased proximal tubular reabsorption of sodium, and an increase in β receptor tone resulting in increased renin secretion (126). That this may be important in the pathogenesis of sodium retention in cirrhosis is supported by the findings in animal studies which demonstrate that renal denervation improves the ability to excrete a saline load and improves the attenuated diuretic and natriuretic response to atrial natriuretic peptide (127,128). However, the role of an activated SNS in the pathogenesis of the avid renal sodium retention evident in cirrhosis remains unclear. Although there is a negative correlation between plasma norepinephrine and urinary sodium in advanced cirrhosis (45), suppression of plasma norepinephrine levels by head-out water immersion does not correlate with the associated increase in urinary sodium excretion (129,130). Additionally, a chronobiological study identified no correlation between urinary norepinephrine and urinary sodium excretion in cirrhotics with ascites (59). Finally, although inhibition of SNS activity by clonidine results in a significant increase in urinary volume and free water clearance, urinary sodium excretion falls slightly (54).

There is considerable evidence supporting the presence of a hepatorenal baroreceptor reflex (131–136) which is responsive to changes in sinusoidal pressure, an increase resulting in changes in hepatic afferent nerve activity and an increase in cardiopulmonary and renal sympathetic activity. This reflex arc may be important in the genesis of the renal hemodynamic changes and the impaired renal tubular handling of sodium and water associated with cirrhosis.

In summary, current studies in patients with advanced liver disease indicate that the associated increase in SNS activity has a major role in the pathogenesis of the increased renal vascular resistance, acting preferentially at the level of the afferent arteriole and impairing both renal blood flow and GFR. The role of the activated SNS in the pathogenesis of the associated renal tubular sodium retention remains unclear.

## Pharmacological inhibition of sympathetic nervous system activity in cirrhosis

### Nonselective β-adrenergic blockade

Nonselective β-adrenergic blockade partially reverses the hyperdynamic circulatory state in patients with portal hypertension. The changes in systemic hemodynamics are largely predictable, are dose related and reflect plasma propranolol levels (137,138). There is a consistent 20–30% fall in cardiac output but minimal change in mean arterial pressure as the fall in cardiac output is balanced by a 20–30% increase is systemic vascular resistance (139,140). These changes are achieved at plasma levels that result in a decrease in heart rate by 15–20%.

The effects of nonselective β blockade on the splanchnic circulation are, however, largely unpredictable (139,141–143). Although the doses used to treat portal hypertension result in a reproducible and consistent 30–35% fall in portal (144–148) and azygos blood flow (139,149), the decrease in portal venous pressure is much more variable and averages 10–15% (139,149). This is largely due to an increase in resistance to outflow from the portal venous bed (150) at the level of the portosystemic collaterals as there tends to be a greater fall in variceal pressure than in portal pressure (151). Overall hepatic perfusion is minimally affected (139,149).

In summary, the portal hypotensive effect of nonselective β blockade depends on two competing drug-induced changes. A decrease in portal blood flow is contributed to by both $\beta_1$ blockade decreasing cardiac output (139,149,152) and $\beta_2$ blockade increasing splanchnic arterial resistance (144–146,153). This tends to lower portal pressure but the effect is at least partly balanced by an increase in portal vascular resistance, mainly at the level of the portosystemic collaterals. As a result, the portal hypotensive effect of nonselective β blockade is greater and more predictable in those patients without evidence of extensive portosystemic collaterals (154). The portal hypotensive effect of nonselective β blockade cannot be predicted by basal clinical, laboratory or hemodynamic criteria, the systemic hemodynamic response to blockade or by plasma propranolol levels (137,155). The portal hypotensive effect has important therapeutic implications, as the risk of bleeding is dependent on the change in portal pressure being significantly less with a fall of ≥ 20% (156–158) or to ≤ 12 mmHg (144).

Nonselective β adrenergic blockade has also been shown to decrease gastric mucosal blood flow by approximately 10% (159,160), to prevent meal-induced increases in portal blood flow (161,162), and it has the therapeutic

potential to limit the development of portal hypertension and portosystemic shunts (163–165).

Importantly, nonselective β blockade has been shown to have no adverse effects on renal (166–168) and cerebral perfusion and function (169,170).

## Inhibition of efferent sympathetic neural activity

The effects of acute inhibition of sympathetic neural activity with the $\alpha_2$ agonist clonidine effectively reduces total sympathetic activity as well as that to regional vascular beds such as the kidneys and the hepatomesenteric circulation (Fig. 5.7) (54). The effects on the systemic circulation include a significant decrease in mean arterial pressure due to both a decrease in cardiac output and systemic vascular resistance (Fig. 5.8) (54).

Sympathetic inhibition to the kidney results in a fall in renal vascular resistance but renal blood flow remains unchanged because of the concomitant fall in perfusion pressure (54). Renal vascular resistance largely depends on afferent and efferent arteriolar tone. The GFR rises significantly after clonidine administration, implying that the decrease in renal vascular resistance must have been caused by a preferential decrease in afferent arteriolar tone allowing glomerular filtration pressure and rate to increase. If proportional falls had occurred in afferent and efferent arteriolar tone, glomerular filtration pressure and rate would have fallen because of the lower mean arterial pressure. These findings support the view that the increased renal vascular resistance and impaired GFR often evident in patients with cirrhosis are, at least in part, due to the modulation of afferent arteriolar tone by the sympathetic nervous system.

Because of the increased sodium filtered load seen after inhibition of sympathetic activity by clonidine and experimental evidence supporting the direct neural facilitation of renal tubular sodium reabsorption (171), an increase in urine volume and sodium excretion would be expected following clonidine. Substantial increases occur in urine volume and solute-free water clearance but urinary sodium excretion actually falls slightly, suggesting that the SNS is not a major factor contributing to the pathogenesis of sodium retention in cirrhosis.

Portal pressure falls after clonidine administration but total hepatic blood flow is not diminished despite a fall in cardiac output and blood pressure (Fig. 5.8) (50,54). This suggests that the fall in portal pressure must have been due, at least in part, to a reduction in neurally regulated post-sinusoidal vascular resistance. A special feature of the hepatomesenteric sympathetic inhibition with clonidine compared with such inhibition in other vascular beds is its extreme sensitivity (Fig. 5.9), being almost complete with an intravenous dose of 1 μg/kg (54). This finding implies that clonidine may have differential effects on different regional sympathetic outflows. Clonidine may act on the central autonomic neuronal pool projecting to the gut and the liver at a lower dose than is required for the drug to act on nuclei projecting to some other sites. In this context it may be relevant that certain hypothalamic nuclei exert a differentiated and to some extent specific excitatory influence on the sympathetic nervous regulation of the hepatic circulation, sparing the systemic circulation (172). Alternatively, it is possible that an increased sensitivity of the

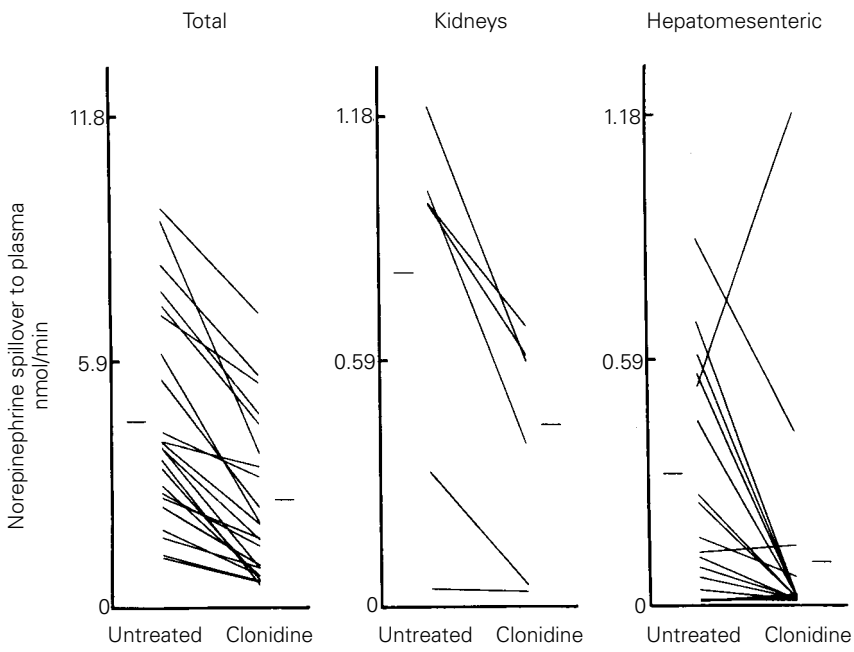


**Figure 5.7** Effect of intravenous clonidine 2.5 μg/kg on norepinephrine (NE) spillover to plasma from the body as a whole ($P < 0.001$), the kidneys ($P = 0.018$), and the gut and liver ($P = 0.009$). (Reproduced with permission, Esler *et al.* (54).)

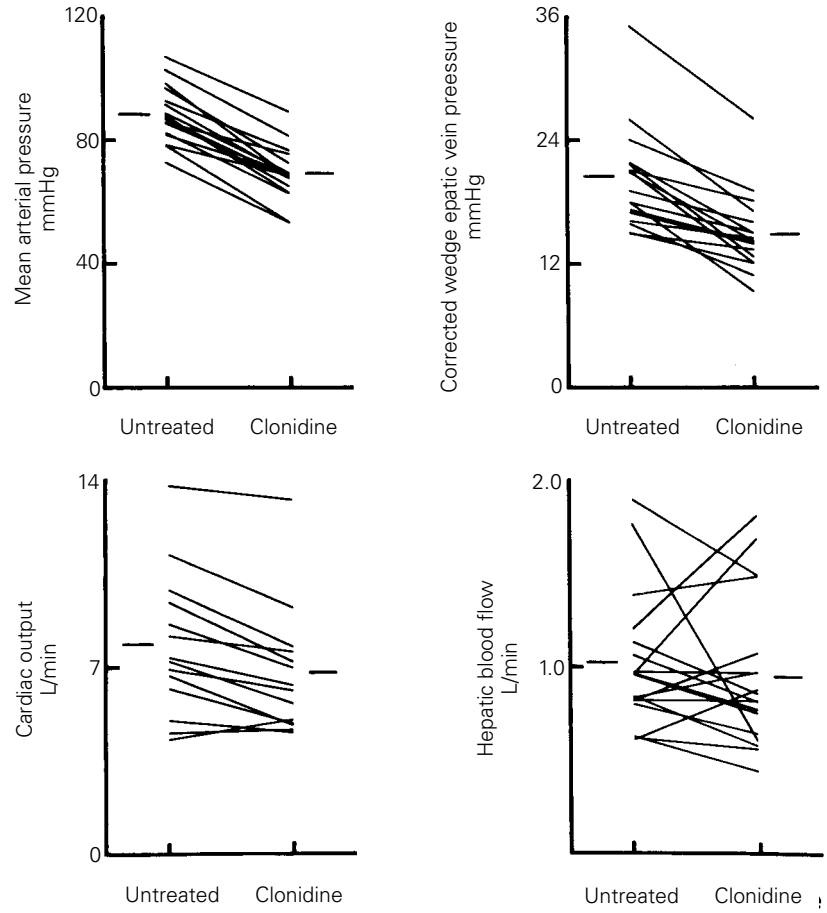


**Figure 5.8** Effect of intravenous clonidine, 2.5 μg/kg on the systemic and hepatomesenteric circulation. Mean arterial pressure ($P = 0.0001$), corrected wedged hepatic vein pressure ($P = 0.0003$), and cardiac output ($P = 0.002$) were all reduced significantly but hepatic blood flow was unchanged. (Reproduced with permission, Esler *et al*. (54).)

hepatic microcirculation to norepinephrine in cirrhotics (114,117), due to increased innervation or a higher density of activated stellate cells (111), could be responsible. The clinical relevance is that it may prove possible with further testing to find a therapeutic window for clonidine dosage that would favorably modify portal hypertension in cirrhosis without adversely affecting the reflex systemic cardiovascular adjustments to hypotension that accompany blood loss from esophageal variceal hemorrhage.

## α-Adrenergic receptor sensitivity in cirrhosis

### Peripheral arterial microcirculation

Vascular smooth muscle tone is maintained by a complex interplay of both endothelial dependent and independent factors (173). These factors have the potential to both contract and relax vascular smooth muscle and the resultant resistance to flow through the vessel is dependent on the overall balance of these competing stimuli. In cirrhosis there is an increased synthesis of the vasodilator nitric oxide by endothelial cells and it has been suggested that this synthesis has been activated by increased levels of circulating substances such as endotoxin and tumor necrosis factor-α (174). In these circumstances it is likely that vascular tone would be maintained initially by the compensatory production of local vasoconstrictors such as endothelin and later by activation of systemic neurohumoral pressor control systems such as the SNS. A change in local vascular resistance would not become evident unless the compensatory mechanisms were unable to counter the local effects of the postulated vasodilators. Both the vasodilator and the activation of local and systemic compensatory mechanisms would be expected to alter vascular smooth muscle cells at either a receptor or post-receptor level so that they will become more resistant to infused pressors such as norepinephrine (175–177).

Animal models of cirrhosis have shown normal or increased vascular sensitivity to infused vasoconstrictors (178), whereas the entire range of responsiveness to infused vasoconstrictors has been shown in whole body studies in cirrhotics (179–181). The differences in reactivity were often only apparent in cirrhotics with more severe disease.

Many *in vivo* studies assessing the sensitivity of the peripheral circulation to norepinephrine in cirrhotics have

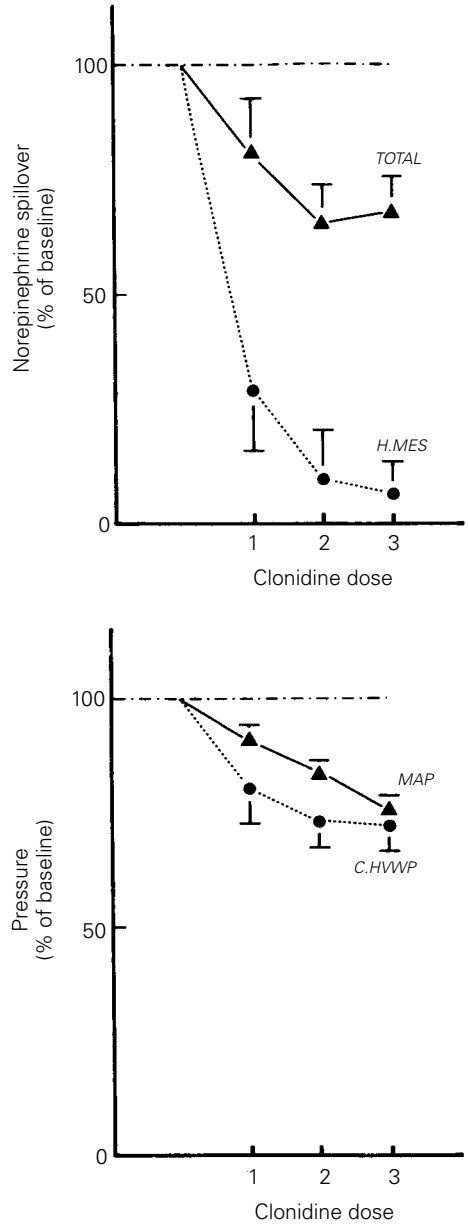


**Figure 5.9** Effect of intravenous clonidine at cumulative doses of 1, 2, and 3 μg/kg on total and hepatomesenteric norepinephrine (NE) spillover rates and on arterial and corrected wedged hepatic vein pressures in patients with cirrhosis. Sympathetic nervous system (SNS) activity was more sensitive to inhibition by clonidine in the hepatomesenteric than in the systemic circulation ($P < 0.001$). Similarly, portal pressure was more sensitive to reduction by clonidine than was arterial pressure ($P = 0.21$). (Reproduced with permission, Esler *et al*. (54).)

used the intravenous infusion of norepinephrine in doses sufficient to alter systemic blood pressure (179–181). This has meant that it has been difficult to separate clearly peripheral from central effects because of the stimulation of baroreceptor-mediated homeostatic reflexes, potential cardiac inotropic effects of norepinephrine, suppression of endogenous neurohumoral pressor activity, and altered disposition and clearance of the infused pressors. This has made interpretation of the data difficult. By studying the isolated forearm circulation *in vivo* during the infusion of subpressor doses of norepinephrine many of these problems can be avoided. However, such studies have also produced variable results, possibly due to methodological differences, reporting either reduced or unaltered vasoconstrictor responses to norepinephrine in cirrhosis (182,183).

It is possible that changes in the local control of vascular tone in patients with liver disease could be responsible for the impaired response of the peripheral circulation to norepinephrine. Nitric oxide (NO) is one of the endothelial-derived vasodilators that has been implicated in the pathogenesis of the reduced vascular resistance in portal hypertension (184–186). Increased synthesis of NO is probably largely responsible for the observed impaired vascular responsiveness to pressors, including norepinephrine, as the abnormality can be partially corrected by the inhibition of nitric oxide synthase activity (187–191).

## Hepatic microcirculation

In contrast to the systemic circulation, the hepatic microcirculation demonstrates an increased sensitivity to pressors, including norepinephrine (114,117,192). The increased hepatomesenteric sympathetic activity that is evident in many patients with cirrhosis (54) may therefore contribute to the pathogenesis of the associated increase in intrahepatic resistance. An increase in the density of activated stellate cells may contribute to the increased sensitivity to NE (111). The development of cirrhosis is also associated with a decreased synthesis of NO by the endothelial cells within the liver (193–195) and it is likely that changes in the endothelial dependent and independent control of vascular tone within the hepatic sinusoids is another important factor contributing to the documented increased sensitivity to α-adrenergic stimulation.

## Mesenteric arterial microcirculation

A decrease in the resistance of the mesenteric arterial vascular bed is evident in all forms of portal hypertension and the resultant increase in flow into the portal venous bed contributes to the maintenance of the portal hypertensive state (196,197). Studies in cirrhotic and noncirrhotic animal models of portal hypertension have demonstrated that the resistance vessels in the mesenteric arterial bed are hyporesponsive to pressors including $\alpha_1$-adrenergic agonists (187–190,198). The impaired responsiveness is greater in animals with decompensated liver disease and is largely corrected by inhibition of NO production or removal of the endothelium (189,190). It is likely that an

increased synthesis of endothelial vasodilators, predominantly NO (191) and possibly prostaglandins (199,200), is responsible for the impaired response to α-adrenergic stimulation.

### Cerebral microcirculation

Power spectral analysis of RR interval and arterial pressure allows a quantitative assessment of autonomic activity and differentiation between sympathetic and parasympathetic drive. This methodology has been used to demonstrate that the autonomic response to passive tilting is impaired in patients with cirrhosis and ascites (176) and that, despite adequate baroreceptor function, there is an impaired response of the cerebral resistance microcirculation to sympathetic activation (201).

## β-Adrenergic sensitivity in cirrhosis

Cirrhotic cardiomyopathy refers to a syndrome where patients with cirrhosis exhibit impaired cardiac contractility (202), particularly in response to exercise (203,204) or a change in posture (205). A number of factors have been implicated in contributing to the development of impaired cardiac function in these patients, including changes to the physiochemical properties of the cell membrane of cardiac muscle (206), neurohumoral changes associated with cirrhosis (90,207–209), and ventricular overload induced by the hyperdynamic circulation (210). More importantly, patients with cirrhosis have been shown to have an attenuated cardiovascular response to catecholamines. The cardiac β-adrenergic receptor and its post-receptor signal transduction pathway are of critical importance in modulating myocardial contraction (211) and *in vitro* and *in vivo* studies in animal models of cirrhosis have demonstrated a decreased receptor density (177,206,212), possibly due to downregulation secondary to high plasma NE levels (213–215), without changes in binding affinity (206,212) as well as an impaired β-adrenergic receptor signal transduction pathway (216,217). It is likely that this insensitivity to β-adrenergic stimulation is a critical factor in the development of cirrhotic cardiomyopathy (202).

The functional role of β-adrenergic receptors in the control of vascular tone remains controversial, but activation of the receptors does stimulate the endogenous release of NO (218,219). Studies of β-adrenergic receptor function in the peripheral and splanchnic vasculature in cirrhosis or portal hypertension are, however, limited. $\beta_2$-adrenergic relaxation of the mesenteric veins of animals with portal vein stenosis is impaired and is possibly the result of the defective neuronal release of NO (220). The fact that the tone of the mesenteric venous bed can be modulated by β-adrenergic activity is of obvious clinical relevance with the widespread use of nonselective β blockade in the treatment of portal hypertension. Any resultant increase in portosystemic collateral venous resistance has the potential to limit the portal hypotensive effect of the medication.

## Conclusions

The SNS is of central importance in the maintenance of cardiovascular homeostasis in patients with cirrhosis and can contribute to the pathogenesis of (i) the renal vascular and tubular changes that characterize patients with decompensated cirrhosis, ascites, and the hepatorenal syndrome and (ii) the increased hepatic vascular resistance that contributes to the development of portal hypertension.

A change in end-organ responsiveness to NE also contributes to the pathogenesis of the cardiomyopathy, peripheral vasodilation, and increased intrahepatic vascular resistance that are often evident in patients with cirrhosis.

Finally the pharmacological modulation of SNS activity is of proven value in the management of portal hypertension and of potential value in the treatment of ascites and the hepatorenal syndrome.

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# Chapter 6
# Atrial Natriuretic Peptide and other Natriuretic Factors in Cirrhosis

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## Natriuretic peptides

Natriuretic peptides (NPs) are structurally related but genetically distinct peptides that contribute to the regulation of circulatory and body fluid homeostasis in health and disease (1–6). All NPs contain a 17-amino acid ring closed by a disulfide bond between two cysteine residues, that is critical for their biological activities.

Atrial natriuretic peptide (ANP) is a 28-amino acid peptide mainly synthesized by cardiac atria (1–3,6). ANP gene encodes for the 151-amino acid prepro-ANP. Following cleavage of a signal sequence, the 126-amino acid pro-ANP is stored in electron-dense secretory granules. On release, pro-ANP is cleaved into a C-terminal mature peptide ($ANP_{99–126}$) and a N-terminal ($ANP_{1–98}$) portion, further processed to form $ANP_{1–30}$ (long-acting natriuretic peptide, LANP), $ANP_{31–67}$ (vessel dilator, VD) and $ANP_{79–98}$ (kaliuretic peptide, KP), that circulate into the bloodstream and have renal and/or vasorelaxant actions. The main stimulus leading to ANP release is atrial stretch, not atrial (transmural) pressure (6): plasma ANP is thus related to atrial volume, increases in response to high sodium diet, head-down tilt, head-out water immersion (HWI), and saline infusion, and decreases on standing, sodium restriction, or administration of diuretics. ANP is therefore a reliable index of the fullness of the intrathoracic vascular compartment. ANP release is also stimulated by tachycardia, glucocorticoids, thyroid hormones, angiotensin II, vasopressin (AVP), endothelin-1, and norepinephrine. In healthy subjects, plasma ANP ranges between 30 and 50 pg/ml, increases with age, and has a half-life of 2–4 min. Plasma ANP is elevated in renal and, even more, in congestive heart failure (CHF), correlates directly with the severity of the disease, and can exceed normal values by more than 100-fold. Within the kidney, a different pro-ANP post-translational process leads to the synthesis of urodilatin ($ANP_{95–126}$) by cortical collecting tubule cells. Urodilatin is released into the tubular lumen, to be excreted into urine, and is also in the bloodstream where its concentration is 9–12 pg/ml. In healthy humans, urinary urodilatin parallels sodium excretion (UNaV) and increases following saline administration (7).

Brain natriuretic peptide (BNP) is a 32-amino acid peptide produced by ventricular myocardium in response to myocyte stretch. BNP is synthesized in bursts and is constitutively released as the active hormone ($BNP_{77–108}$) and an N-terminal, inactive fragment ($NT\text{-}proBNP_{1–76}$). So, in contrast to ANP, which is regulated at the level of release from storage granules, BNP regulation occurs during gene expression (6). In healthy humans, plasma BNP concentration ranges between 20 and 30 pg/ml, is slightly higher in women, and increases with age. In patients with CHF, BNP markedly increases, exceeding ANP concentration in more severe cases. BNP and NT-proBNP correlate with functional status and outcome in patients with CHF, and are of major value for emergency diagnosis of CHF and rapid screening of left ventricular dysfunction. BNP and NT-proBNP also correlate with left ventricular dilation, remodeling, and dysfunction, as well as with outcome in patients with myocardial infarction. Plasma BNP levels are also elevated, but to a lesser extent, in patients with diastolic dysfunction, left ventricular hypertrophy, and right ventricular disorders (6).

C-type natriuretic peptide (CNP) is a 22-amino acid peptide analogous to ANP and BNP. It has the 17-amino acid ring structure, but lacks the C-terminal tail. Outside the central nervous system, CNP is mainly located in the vascular endothelium, where it acts as a paracrine factor, reducing vascular tone and inhibiting smooth muscle cell proliferation in response to vascular injury. Production and/or release of CNP is stimulated by cytokines [transforming growth factor-β, tumor necrosis factor-α, interleukin (IL)-1α, IL-1β, and others]. CNP circulates at very low levels (3–5 pg/ml) and is excreted into urine.

Dendroaspis natriuretic peptide (DNP) is a 38-amino acid peptide isolated from the venom of the green mamba. DNP-like immunoreactivity is detectable in human plasma, and increases in CHF (8). However, whether DNP is indeed an endogenous hormone in humans remains to be established (9).

Natriuretic peptides act by binding to specific, high-affinity receptors (NPRs). Two functional classes of receptors have been described: the biological receptors (NPR-A and -B), and NPR-C. NPR-A and B have different affinities for NPs (NPR-A: ANP $\geq$ BNP > CNP; NPR-B: CNP > ANP > BNP). They are linked to the guanylcyclase (GC)-dependent signaling cascade and mediate many of the cardiovascular and renal effects of NPs. So, any increase in plasma ANP and/or BNP is associated with increments in plasma and urinary guanylate 3′5′-cyclic monophosphate (cGMP). cGMP, however, is also the second messenger of nitric oxide, that is increased in cirrhosis (10). Indeed, in one study (11), no correlation was observed between ANP and cGMP in cirrhosis. NPR-A is most abundant in large blood vessels, while NPR-B predominates in the brain. Both receptor types are also present in the kidney.

NPR-C interacts with NPs in the order ANP > CNP $\geq$ BNP and acts to remove NPs from circulation (12). NPs are also inactivated by cleavage by neutral endopeptidase (NEP), located on the surface of endothelial and smooth muscle cells, myocytes, renal tubular cells, and fibroblasts. Elimination of endogenous NPs takes place mainly in the lungs, liver, and kidneys.

Following interaction with NPR-A, NPs elicit a spectrum of renal, hemodynamic, and endocrine actions, all of which serve to reduce cardiac preload and afterload, thereby improving cardiac performance. In the kidney, NPs have marked diuretic and natriuretic effects, and increase urinary phosphate, calcium, magnesium, and chloride. The natriuretic effect of NPs is mainly due to the inhibition of sodium reabsorption in the inner medullary collecting duct, an effect that results from coordinated inhibition of apical sodium channel and basolateral $Na^+$,$K^+$,ATPase. Other renal effects of NPs include: an increase in glomerular filtration rate (GFR), due to dilation of preglomerular and constriction of postglomerular arterioles; impairment of the glomerulotubular balance; inhibition of sodium reabsorption in the proximal tubule; and inhibition of AVP-mediated water reabsorption. These latter effects, however, occur at pathophysiological or even pharmacological levels. NPs also inhibit the release of renin, the production of endothelin, and the synthesis and secretion of aldosterone and AVP, and counteract the sodium-retaining effects of angiotensin II and norepinephrine.

NPs relax vascular smooth muscle cells, causing vasodilation of both arteries and veins, especially when precontracted by vasoconstricting agents, thus reducing ventricular preload and afterload and also arterial pressure, at least under some circumstances. Interestingly, ANP-induced hypotension usually does not preclude the natriuretic response. NPs also increase capillary permeability, with shifting of intravascular fluid to the interstitial compartment, and reduce sympathetic tone. More recently, NPs were found to have antimitogenic effect on endothelial and vascular smooth muscle cells, and myocytes.

The physiology and pathophysiology of ANP were first investigated by evaluating the effects of exogenous ANP. The recent availability of NEP inhibitors (that enhance the plasma levels of endogenous NPs), NPR antagonists (that block the biological effects of endogenous NPs), and transgenic animals with alterations in the synthesis of NPs, NPRs or their signaling mechanisms, has added new insight to our knowledge of the role of NPs in the regulation of cardiovascular and body fluid homeostasis. HS-142-1, an NP antagonist, impairs the natriuretic response to an acute volume load in normal rats. In rats with CHF, it reduces UNaV, increases renal vascular resistance, plasma renin activity (PRA), plasma aldosterone (PAC), and catecholamines, and blunts the natriuretic response to exogenous ANP. Transgenic mice overexpressing the ANP gene manifest life-long hypotension, whereas ANP gene knockout mice have salt-sensitive hypertension (13). Mice with NPR-A gene deletion have an impaired natriuretic and vasorelaxant response to exogenous ANP and increments in arterial pressure proportional to the loss of NPR-A alleles. These data confirm the major role of ANP in the overall regulation of fluid balance and cardiovascular homeostasis.

Intravenous urodilatin elicits biological effects quite similar to those of ANP through interaction with NPR-A, and is inactivated by NPR-C and NEP. However, the most likely "physiological" function of urodilatin is that of an intrarenal, paracrine regulator of sodium and water homeostasis. This hypothesis is consistent with the observation that ANP stimulates NPR-A and inhibits sodium reabsorption when applied to the luminal surface of the distal tubule (7).

BNP has similar affinity for NPR-A and exerts the same biological activities as ANP. In addition, it has direct lusitropic (relaxant) properties in the myocardium. The effects of BNP seem to be dose-dependent. In our experience (14), "physiological" levels of BNP affect only distal sodium reabsorption, whereas "pathophysiological" doses are required to modify systemic and renal hemodynamics (15). Transgenic mice overexpressing the BNP gene are hypotensive, whereas BNP gene knockout mice have high plasma ANP and normal arterial pressure, but show multifocal fibrotic lesions in the ventricles, suggesting that BNP has antifibrotic activity in the heart (16).

C-type natriuretic peptide interacts with NPR-B and perhaps with other, GC-independent receptor(s), since the vasorelaxant and hypotensive effects of CNP infusion are not abolished in GC-deficient mice (17). In rats, CNP is less natriuretic than ANP, but has a more marked hypotensive effect. However, infusion of CNP to healthy subjects, raising its plasma levels up to 10-fold those observed in disease states, has no effects on plasma and

urinary cGMP, renal sodium handling or cardiovascular homeostasis, suggesting that this compound is a paracrine, rather than an endocrine factor (18). CNP gene knockout mice show severe dwarfism and early death, suggesting that CNP may regulate endochondral ossification (19).

## Natriuretic peptides in cirrhosis

Data on NPs in cirrhosis refer mainly to ANP, the most extensively studied compound. It should be pointed out that several data on ANP also apply to BNP, that has a similar behavior to ANP (see later) and exerts its biological activities by interacting with the same receptor/NPR-A).

### *Atrial natriuretic peptide*

A deficient production of endogenous natriuretic factor(s) has traditionally been considered as a possible mechanism for sodium retention in cirrhosis (20). After the discovery of ANP, several investigations were therefore conducted to test this hypothesis. However, these studies clearly indicate that ascitic patients, with or without renal failure, have higher (usually two-to-four times) than normal plasma ANP levels (Fig. 6.1) (21–30). Sodium retention of cirrhosis cannot therefore be attributed to a deficiency of ANP.

Data on compensated cirrhotics are somewhat conflicting, with studies reporting either normal (21,27–29) or increased (25,31–34) ANP. This discrepancy is not surprising. In fact, patients with compensated cirrhosis and portal hypertension are rather heterogeneous with respect to the presence and degree of alteration of systemic hemodynamics and sodium handling (35), the latter ranging from intolerance to a normal sodium diet to the ability to escape to mineralocorticoids (36).

Due to the substantial contribution of the liver to ANP clearance (1–3), an impaired removal of ANP might account for the high plasma ANP levels in cirrhosis with ascites. To address this point, Ginès *et al*. (23) measured ANP in plasma samples from the coronary sinus, right atrium, pulmonary artery, hepatic vein, and femoral vein in decompensated cirrhotics and healthy subjects undergoing right heart catheterization. Patients had significantly higher plasma ANP levels in every vascular territory studied. The estimated cardiac release of ANP was eight times greater in patients than in controls (Fig. 6.2), while peripheral and splanchnic extraction of ANP was similar in both groups, indicating that the elevated levels of ANP are due to an enhanced cardiac release.

The main mechanism stimulating the release of ANP is atrial stretching (1–4). It is conceivable that the same mechanism could be operative also in cirrhosis. Patients with decompensated cirrhosis have high blood volume and cardiac output (CO) (35), suggesting that the volume of blood circulating through the cardiac chambers is increased. Left atrial volume is also elevated and correlates with plasma ANP (25). Further evidence supporting this contention comes from evaluation of the effect of volume expansion on plasma ANP and the relationship between ANP and systemic hemodynamics. The former investigations showed that ANP release in response to volume expansion is not impaired in cirrhosis. So, plasma ANP increases in response to high sodium intake (33,36), infusion of saline (37), administration of mineralocorticoids (38), HWI (39–41), and peritoneovenous shunting (24,42). The second group of investigations disclosed significant correlations between plasma ANP and blood volume (25,29), mean pulmonary artery pressure (43), left atrial volume (25), and CO (29). Furthermore, changes in plasma ANP paralleled those of systemic hemodynamics dur-

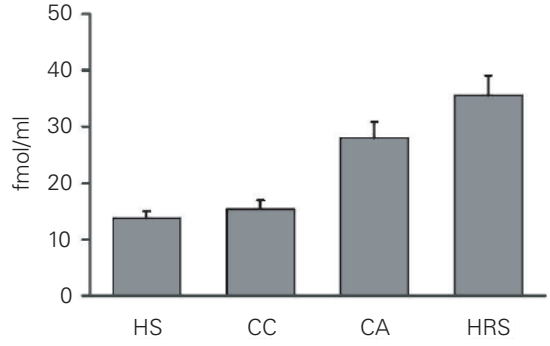


**Figure 6.1** Plasma levels (mean ± SE) of atrial natriuretic peptide in healthy subjects (HS) and patients with compensated cirrhosis (CC), cirrhosis with ascites (CA) and cirrhosis with ascites and hepatorenal syndrome (HRS). ANOVA F = 7.37, $P < 0.001$; HS vs. CA and HRS: $P < 0.01$; CC vs. CA and HRS : $P < 0.01$. Data from La Villa *et al*. Plasma levels of brain natriuretic peptide in patients with cirrhosis. Hepatology 1992; 16: 156–61.

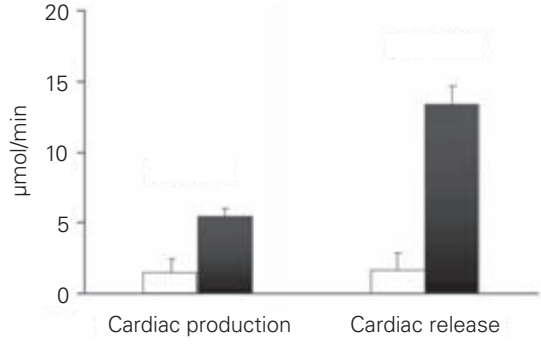


**Figure 6.2** Estimated cardiac production and release of immunoreactive atrial natriuretic peptide (mean ± SE) in 11 healthy subjects (empty bars) and 11 cirrhotic patients with ascites. Data from Ginès *et al*. Atrial natriuretic factor in cirrhosis with ascites: plasma levels, cardiac release and splanchnic extraction. Hepatology 1988; 8: 636–42.

ing postural changes (30,31). Henriksen *et al*. (44) showed that although cirrhotic patients have high CO, the mean transit time of blood in the central area is very short, so that the amount of blood residing in this compartment at a particular moment is actually reduced. However, mean transit time of blood appears to be reduced in right chamber-pulmonary artery and lung vascular beds, but not beyond cardiopulmonary circulation (45). Indeed, blood entering the left atrium at each cardiac cycle, the main determinant of atrial stretching and ANP release, must be equal to stroke volume, and stroke volume is increased in supine cirrhotics (46).

Other possibilities deserve consideration. Patients with ascites usually have elevated levels of angiotensin II and norepinephrine (35). However, the possibility that these factors stimulate ANP release is unlikely. In fact, volume expansion induces a dissociation between plasma ANP, which increases, and the circulating levels of renin and norepinephrine, that in contrast decrease (30,31,37,39–41). A more exciting hypothesis is that plasma ANP might be related to the so-called "cirrhotic cardiomyopathy" (47). The finding of an increased ventricular ANP gene expression in cirrhotic rats (48) is in keeping with this contention. However, in the only clinical study investigating this possibility, no relationship was found between the plasma levels of N-terminal ANP and either structural alterations of the heart or diastolic dysfunction (49).

The high plasma ANP levels of cirrhosis usually occur in the setting of sodium retention and ascites. In addition, plasma volume expansion is usually associated with greater than normal increase in plasma ANP and reduced or absent natriuresis (35,38,39–41), suggesting that cirrhotic patients may have an abnormal natriuretic response to ANP (50). This contention appeared consistent with the results of studies evaluating the renal effects of exogenous ANP. Salerno *et al.* (51) administered an ANP bolus to 14 patients with variable degrees of sodium retention. In patients without or with moderate sodium retention, ANP had similar effects as in healthy subjects. In contrast, the natriuretic response to ANP was attenuated or absent in patients with avid sodium retention and high baseline PRA and PAC. Arterial pressure fell after ANP injection in both cirrhotics and controls, the drop being greater in cirrhotic patients. Laffi *et al*. (52) evaluated renal hemodynamics, diuresis, natriuresis, PRA, and PAC in 15 ascitic patients before, during, and after ANP administration. ANP induced similar increments in plasma and urinary cGMP in all patients, but the natriuretic response was quite different from one patient to another. Five patients (responders) showed a remarkable increase in UNaV ($\geq$ 200 μmol/min), six patients (nonresponders) had no changes in UNaV and four had an intermediate response. Nonresponders had higher baseline PRA and plasma ANP and lower solute-free water clearance than responders. During ANP infusion, changes in UNaV correlated with those of renal hemodynamics. Responders showed parallel increases in renal plasma flow (RPF), GFR and sodium excretion (UNaV), whereas nonresponders had reductions in RPF and GFR. Arterial pressure decreased to a similar extent in all patients, but nonresponders more marked increments in PRA and heart rate. This and similar investigations indicate that the natriuretic response to exogenous ANP is highly variable in cirrhosis, being reduced or absent in patients with avid sodium retention and marked activation of the main sodium-retaining systems (41,43,53). In these investigations, ANP was usually given at pharmacological doses, raising plasma ANP to 200–1000 pmol/l, a point that should be kept in mind when these data are applied to the pathophysiology of ANP in cirrhosis. In fact, pharmacological doses of ANP usually induce a reduction in blood pressure. In susceptible patients, ANP-induced hypotension may lead to further activation of the renin–angiotensin–aldosterone (RAAS) and sympathetic nervous systems (SNS) and impair renal hemodynamics and GFR, contributing to the blunted natriuretic response. These adverse effects are more likely to occur in patients with more marked alterations of systemic and renal hemodynamics and activation of vasoconstricting and sodium-retaining factors (52).

Among the several mechanisms that might explain why the renal response to ANP is attenuated in cirrhotic patients with ascites (54), some can be ruled out with confidence. ANP from cirrhotic patients has the same chromatographic pattern and affinity for NPR-A as synthetic human $ANP_{99–126}$, indicating that there is no dysregulation in the process of synthesis and maturation of ANP in cirrhosis (55). Correction of ANP-induced hypotension did not improve the blunted natriuretic response to ANP (53).

Cirrhotic patients with high plasma ANP levels have elevated plasma and/or urinary levels of cGMP (24,38,40,41). Furthermore, cGMP showed appropriate increments in response to exogenous ANP (42,53), indicating that the agonist-receptor signaling mechanism that involves cGMP as a second messenger is not impaired in cirrhosis. Recently, however, Angeli *et al*. (56) found increased activity of cGMP phosphodiesterase and reduced content of cGMP in renal tissue of rats with experimental cirrhosis and ascites, a phenomenon that could contribute to the renal resistance to NPs. To our knowledge, the possibility that alterations in the signal transduction beyond cGMP generation may modify the response to ANP has never been investigated.

Patients with blunted natriuretic response to ANP usually have higher PRA, PAC, and plasma norepinephrine compared with values in responders (39–41,52,53). In these patients, UNaV before and during HWI correlated directly with the ANP/PAC ratio (57), and inversely with sympathetic nerve activity (58). Furthermore, suppression of antinatriuretic factors in experimental cirrhosis

restores the natriuretic effectiveness of ANP (59). Thus, overactivity of antinatriuretic factors is of major importance in determining the renal resistance to ANP in cirrhosis. Antinatriuretic factors can act by either reducing distal sodium delivery (angiotensin II and renal sympathetic nerves) or increasing sodium reabsorption in the distal tubule (aldosterone). Abraham *et al*. (60) administered mannitol and ANP plus mannitol to 12 ascitic patients who had renal resistance to ANP alone. Mannitol did not modify UNaV in any patient, but increased distal sodium delivery in six of them. In these latter patients, ANP plus mannitol induced a five-fold increase in UNaV, providing evidence for the first of the above contentions. On the other hand, a role for hyperaldosteronism in the renal resistance to ANP in cirrhosis is suggested by the observation that bolus injection of ANP increases urinary potassium in cirrhotic patients with blunted natriuresis but not in healthy subjects (51).

Several lines of evidence indicate that endogenous NPs activating NPR-A are involved in the regulation of sodium excretion in cirrhosis, despite their apparently reduced natriuretic effectiveness. In patients with compensated cirrhosis, the increased cardiac release of ANP contributes, together with suppression of sodium-retaining factors, to re-establish sodium balance during long-term administration of a high sodium diet (61) or mineralocorticoids (38). Salerno *et al*. (26) measured plasma ANP and sodium excretion in 24 cirrhotic patients with tense ascites who were not taking diuretics. Plasma ANP was elevated in all patients and correlated with UNaV, despite avid sodium retention, suggesting that ANP stimulates sodium excretion in cirrhosis, although being unable to counterbalance sodium-retaining forces. Furthermore, in cirrhotic patients submitted to HWI both basal and stimulated natriuresis correlated with the ANP/PAC ratio (57). Finally, Angeli *et al*. (28) measured plasma ANP in 44 patients with ascites, classified into three groups according to their response to a standardized stepped-care medical treatment. Group 1 patients underwent natriuresis in response to bed rest and low sodium diet, group 2 responded to spironolactone, whereas group 3 patients did not respond. Patients, as a whole, had higher plasma ANP than control subjects. However, plasma ANP progressively fell from group 1 to group 3 patients, whereas PRA progressively increased. Sodium excretion correlated with the logarithm of ANP/PRA and ANP/PAC. All these data are consistent with the above contention.

Dussaule *et al*. (62) gave sinorphan, a NEP inhibitor, to 11 cirrhotic patients with ascites. Sinorphan induced a 1.8 times increase in plasma ANP, increments in plasma and urinary cGMP, a two-fold increase in UNaV, and a reduction in PAC. Interpretation of these data as supporting a role for endogenous NPs in the regulation of UNaV in cirrhosis, however, requires caution, since several other hormones and autacoids involved in the regulation of sodium handling are substrates for NEP, including angiotensin II, bradykinin, and endothelin.

Angeli *et al*. (63) administered HS-142-1, a specific antagonist of GC-NPRs, to rats with experimental cirrhosis and ascites. Blockade of NPRs did not influence systemic hemodynamics, but markedly affected renal function, inducing significant reductions in RPF, GFR, diuresis, and UNaV. HS-142-1 also induced a two-fold increase in PRA and PAC. These results provide the best evidence available so far that endogenous NPs play a major role in the maintenance of renal function and sodium excretion in cirrhosis.

Warner *et al*. (54) raised the possibility that ANP could exacerbate arterial vasodilation in cirrhosis. As previously reported, ANP reduces blood pressure in cirrhotic patients when used in pharmacological amounts (51–53). Bernardi *et al*. (30,31) evaluated systemic hemodynamics and plasma ANP in cirrhotic patients in the standing and supine positions. Standing patients had the same hemodynamic pattern as healthy subjects; on assumption of the supine position, they developed a hyperdynamic circulation, with high CO, reduced peripheral vascular resistance (PVR), and an increase in plasma ANP, raising the possibility that this hormone might be involved in the decline of PVR observed during recumbence. Increments in plasma ANP to the levels usually observed in ascitic cirrhosis do not modify blood pressure or PVR in healthy humans (1–4), but cirrhotics have marked vasomotor instability and may be more susceptible to ANP-induced vasodilation. Indeed, we found that administration of low-dose ANP to patients with compensated cirrhosis, to increase plasma ANP to the same range of ascitic patients, reduced left ventricular filling volume, stroke volume, CO, and arterial pressure, and increased PRA and plasma norepinephrine (64). On the other hand, no hemodynamic changes were observed in response to inhibition of NEP in cirrhotic humans (62) or blockade of NPRs in cirrhotic rats (63). Whether ANP contributes to the hemodynamic derangement of cirrhosis remains to be established.

In conclusion, patients with cirrhosis and portal hypertension eventually develop alterations of systemic hemodynamics, with high plasma volume and CO and low PVR, renal function, with sodium retention and ascites, activation of the RAAS and SNS, and non-osmotic release of AVP (35,65). Even if direct measurements of "central volume" indicates effective hypovolemia (43,45), the amount of blood that fills cardiac atria and ventricles appears to be increased in cirrhotic patients with high ANP levels. This phenomenon probably stimulates ANP release, leading to enhanced plasma levels of this NP. This finding, by itself, is consistent with both the overflow (66) and the peripheral arterial vasodilation (65) hypotheses of ascites formation, but only the latter explains the apparently paradoxical increment in both natriuretic and antinatriuretic factors. According to this hypothesis, arterial vasodilation, mainly

occurring in the splanchnic circulation, is the initial event in the pathogenesis of sodium retention, since it leads to effective hypovolemia and baroreceptor-mediated activation of the main sodium-retaining systems. The increased venous return that follows arterial vasodilation would stimulate the release of ANP. The high circulating levels of ANP (and BNP, the other NP acting through NPR-A) contribute to the maintenance of renal function and sodium excretion, which at any time appears to be determined by the balance between vasodilator/natriuretic and vasoconstrictor/antinatriuretic factors. The role of ANP in the hemodynamic derangement of cirrhosis, if any, remains to be established.

### *Other pro-ANP derivatives*

Plasma N-terminal ANP concentration was normal in compensated and increased in decompensated cirrhosis (49). Circulating levels of LANP and KP, the other measured pro-ANP derivatives (see above), were elevated in cirrhotic patients, and increased more in patients than in controls during HWI (67,68). After HWI, plasma ANP returned to baseline levels within 30 min, while LANP and KP were elevated for at least 1 h, suggesting different clearance mechanisms.

### *Urodilatin*

The urinary excretion rate of urodilatin was within the normal range in both compensated and decompensated cirrhotic patients, suggesting that urodilatin has an independent regulation from that of ANP or BNP in cirrhosis (69). Infusion of urodilatin to compensated and ascitic cirrhotic patients improved UNaV by enhancing fluid delivery to the distal tubule and inhibiting sodium reabsorption in this segment of the nephron (70).

### *B type natriuretic peptide*

Like ANP, plasma BNP is about four-fold higher in cirrhotic patients with ascites, with or without renal failure compared with levels in healthy controls (Fig. 6.3) (27), and correlated with the degree of hepatic and renal impairment, PRA, PAC, and ANP, while it was normal (27,49) or elevated (34) in compensated cirrhotics. Wong *et al*. (49) evaluated the relationship between plasma BNP and "cirrhotic cardiomyopathy" in 36 patients (21 with alcoholic cirrhosis). Both pre-ascitic and ascitic patients had significantly increased interventricular septal thickness and left atrial size, prolonged isovolumetric relaxation time, increased deceleration time, and a trend towards a reduction in the E/A ratio, all signs of diastolic dysfunction. Systolic function was normal. Plasma BNP significantly correlated with septal thickness, deceleration time, and left ventricular end-diastolic diameter (Fig. 6.4), suggesting that the increased plasma BNP in cirrhosis could be related to cardiomyopathy. This contention received further support by a recent study by Henriksen *et al*. (71), who measured pro-BNP and BNP in 51 patients with alcoholic cirrhosis (21 with ascites). Both pro-BNP and BNP were higher in patients than in healthy controls, and were related to parameters reflecting the severity of cirrhosis (Child score, serum albumin, coagulation factors II, VII, and X) and to markers of cardiac dysfunction (QT interval, heart rate, plasma volume), but not to indicators of the hyperdynamic circulation. Hepatic disposal of pro-BNP and BNP was not impaired in cirrhosis. Taken together, these data indicate that the elevated plasma levels of BNP in cirrhosis are due to enhanced ventricular production of this NP and reflect the presence of cardiac dysfunction. Further studies, however, are needed to establish whether plasma BNP could be a reliable marker of the presence and degree of heart dysfunction in cirrhosis, also in patients with non-alcoholic cirrhosis.

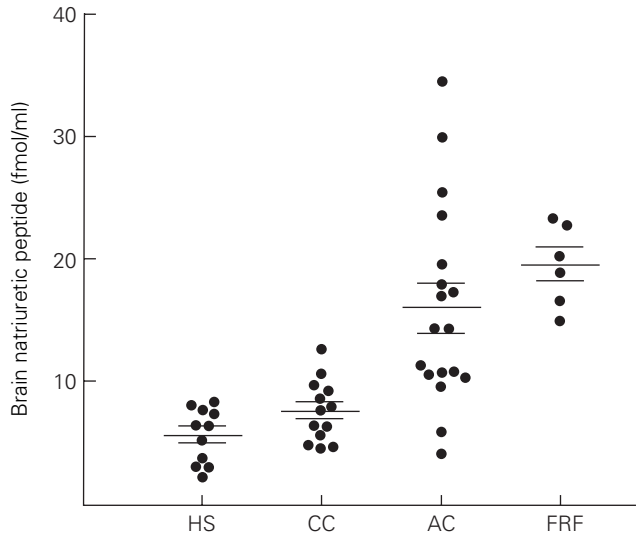


**Figure 6.3** Individual plasma levels of brain natriuretic peptide in healthy subjects (HS) and patients with compensated cirrhosis (CC), ascitic cirrhosis (AS), and functional renal failure (FRF). ANOVA: F = 15.04, $P < 0.001$; HS vs. AC and FRF: $P < 0.01$; CC vs. AC and FRF: $P < 0.01$. (Reproduced wit permission from La Villa *et al*. Plasma levels of brain natriuretic peptide in patients with cirrhosis. Hepatology 1992; 16: 156–61.)

In cirrhotic patients with ascites, administration of low-dose BNP induced increments in plasma and urine cGMP, but did not modify renal hemodynamic or UNaV (72).

### *C type natriuretic peptide*

Gulberg *et al*. (73) measured plasma CNP in healthy subjects and cirrhotic patients with normal and impaired renal function and found significantly lower levels in

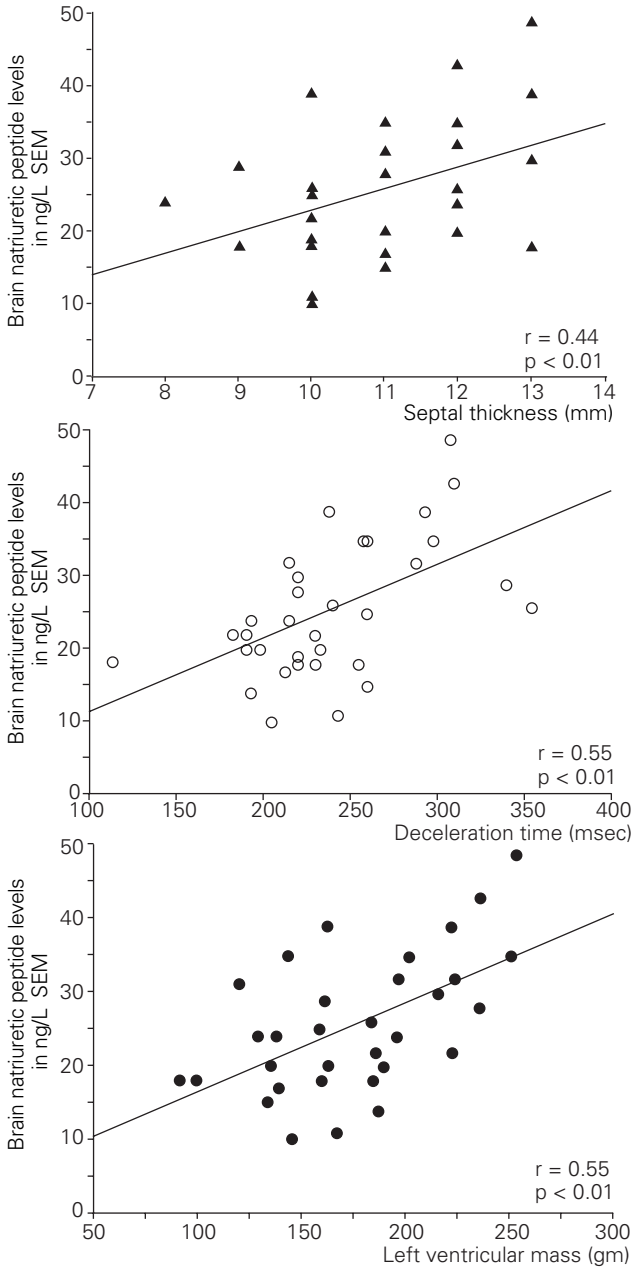


**Figure 6.4** Correlations between BNP levels and interventricular thickness (top), deceleration time (middle), and left ventricular mass (bottom) in ascitic patients and the subgroup of pre-ascitic patients with high BNP levels. (Reproduced with permission from Wong F *et al*. Brain natriuretic peptide: is it a predictor of cardiomyopathy in cirrhosis? Clin Sci 2001; 101: 621–8, © the Biochemical Society and the Medical Research Society.)

patients than in controls. In contrast, urinary CNP was two- to three-fold higher in patients with renal impairment than in the other groups. Finally, in patients with refractory ascites or the hepatorenal syndrome treated with ornipressin or insertion of a transjugular intrahepatic porto-systemic shunt (TIPS), urinary CNP decreased three- to four-fold while UNaV increased. The same group of investigators later found an inverse relationship between plasma CNP and arterial compliance, which is increased in cirrhosis (74). According to these investigators, a downregulation of CNP occurs in cirrhosis, which may compensate for the persistent vasodilation.

Administration of pharmacological doses of CNP to cirrhotic rats with ascites significantly decreased portal pressure and PVR and increased CO, but had no effect on UNaV, probably because of activation of antinatriuretic factors secondary to systemic vasodilation (75). In the same experimental model, administration of $ANP_{4-23}$, a ring-deleted analog of ANP that selectively activates NPR-B, induced splanchnic vasoconstriction and reduced portal pressure, without affecting UNaV, despite a concomitant increase in plasma ANP (76).

### *Dendroaspis natriuretic peptide*

DNP-like immunoreactivity is significantly elevated in ascitic patients and even more in those with refractory ascites (77), and correlates directly with PRA, bilirubin and endothelin, and inversely with albumin, prothrombin time, and serum sodium, while no correlations are found between DNP and arterial pressure, renal function, UNaV, or cGMP.

## Natriuretic hormone

Virtually all animal cells have high K+ and low Na+ relative to the external medium. These ionic gradients are generated and maintained by Na+,K+-ATPase (the sodium pump), a membrane enzyme that catalyzes the active transport of Na+ and K+ across cell membranes. Within the kidney, Na+,K+-ATPase plays a major role in sodium reabsorption by actively pumping Na+, which enters tubular cells at the luminal surface, across basolateral membrane to the peritubular interstitium. In cardiac myocytes, Na+,K+-ATPase influences the availability of cytosolic Ca2+ and contraction. With a similar mechanism, Na+,K+-ATPase influences the tone of vascular smooth muscle cells. Na+,K+-ATPase consists of α, β, and γ subunits. The former is a transmembrane protein that contains the binding site for ATP and a specific binding site for cardiac glycosides. The evolutionary persistence of an ouabain-binding site on Na+,K+-ATPase may be due to the requirement of a specific conformation of the enzyme for successful ion translocation. An alternative, more exciting hypothesis is that an endogenous compound(s) with digitalis-like properties would exist in mammalians that regulates Na+,K+-ATPase activity by binding to a specific receptor on the α subunit of the enzyme.

In 1961, De Wardener *et al*. (78) provided evidence of the existence of a "natriuretic hormone" (NH) by showing that volume expansion causes the appearance

in the blood of a factor that increases sodium excretion when transferred to a euvolemic animal. Further studies, although unsuccessful in characterizing the responsible compound(s), led to the demonstration that the postsalt fraction after Sephadex chromatography of plasma and urine from humans and animals contains a substance(s) of low molecular weight (< 1000 Da) that increases sodium excretion in experimental animals without affecting renal hemodynamics or GFR. The natriuretic response to this extract has an immediate onset, peaks after 40–60 min and lasts 2 h. Other activities of this fraction include inhibition of ouabain binding and $Na^+,K^+$-ATPase activity *in vitro*, inhibition of the short-circuit current in toad bladder ("antinatriferic" activity), inhibition of sodium transport in isolated renal tubules, and cross-reactivity with antidigoxin antibodies. Finally, the natriuretic factor(s) contained in plasma and urinary extracts raises blood pressure when administered to experimental animals (79). All biological activities of these extracts increase during HWI, infusion of saline, high sodium intake, administration of mineralocorticoids, and renal failure (79). Although indirect, available evidence is therefore sufficient to support the contention that volume expansion stimulates the release into circulation of an endogenous digitalis-like substance(s) with natriuretic and vasoconstricting activities, the so-called NH, which contributes to the maintenance of cardiovascular homeostasis and sodium balance by regulating the activity of renal and vascular $Na^+,K^+$-ATPase. NH is thought to play an important role in the pathogenesis of hypertension and the mechanisms of adaptation to reduced renal mass in chronic renal failure (78,79).

Further investigations in this field led to the more or less complete characterization of several mammalian $Na^+,K^+$-ATPase inhibitors, including the cardenolides, such as digoxin and a steroidal isomer of plant ouabain [ouabain-like factor or endogenous ouabain (EO)], the bufodienolides (bufalin and marinobufogenin) (79–81), and other unrelated substances, suggesting the existence of different $Na^+,K^+$-ATPase inhibitors (82). Evidence for endogenous synthesis has been provided for EO and bufadienolide-like factors (82). In particular, EO, first detected in human plasma by Hamlyn *et al*. (83) and later isolated from both adrenals and the hypothalamus (82), is the most interesting among these substances since it has most of the biochemical and biological properties of the putative NH, including the ability to increase UNaV and blood pressure in experimental animals. Measurements of EO in physiological conditions and disease states associated with alterations of blood pressure and fluid balance, such as high sodium diet, essential hypertension, congestive heart failure, and primary hyperaldosteronism, gave results consistent with the above possibility. It has to be kept in mind that, although it is usual to consider that EO is a $Na^+,K^+$-ATPase inhibitor, at nanomolar concentrations it actually stimulates the pump and acts therefore as a sodium-retaining factor (81,82).

### *Natriuretic hormone in cirrhosis*

None of the substances supposed to be the NH have ever been measured in patients with cirrhosis. Available information on NH in cirrhosis is therefore based on measurements of one or more biological activities thought to be related with the presence of NH in the postsalt fraction of plasma or urinary extracts after Sephadex chromatography.

In keeping with the concept that a deficient production of NH could play an important role in the pathogenesis of sodium retention in cirrhosis (20), initial investigations, performed with bioassay methods, showed a reduced NH activity in plasma and urinary extracts from cirrhotic patients with or without ascites (84–86). Subsequent data, however, failed to confirm these reports. Nanji and Greenway (87) found a higher concentration of digoxin-

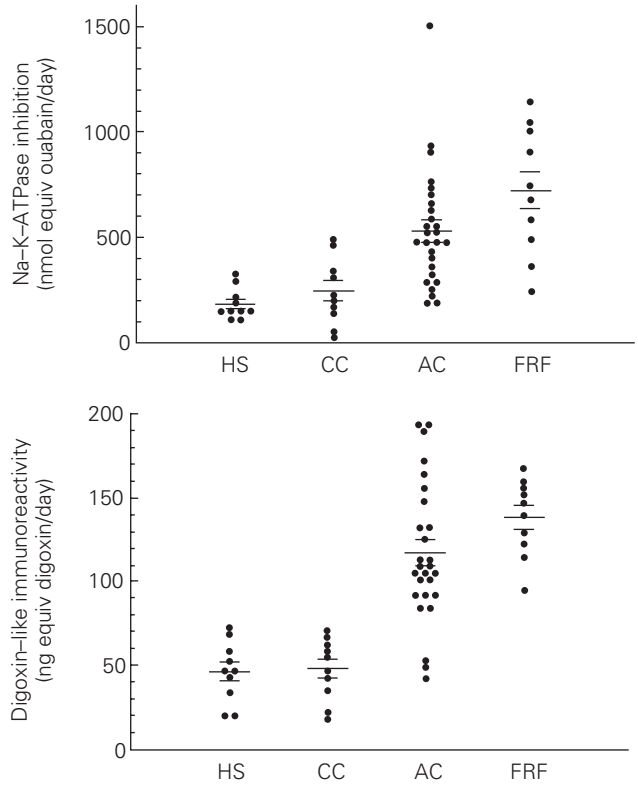


**Figure 6.5** Individual values of Na-K-ATPase inhibition (top) and digoxin-like immunoreactivity (bottom) found in the urine of healthy subjects (HS) and patients with compensated cirrhosis (CC), ascitic cirrhosis (AC), and functional renal failure (FRF). ANOVA: Na-K-ATPase inhibition: F = 11.33, $P < 0.001$; HS vs. AC and FRF: $P < 0.01$; CC vs. AC and FRF: $P < 0.01$; digoxin-like immunoreactivity: F = 20.30, $P < 0.001$; HS vs. AC and FRF: $P < 0.01$; CC vs. AC and FRF: $P < 0.01$. (Reproduced with permission from La Villa *et al*. Natriuretic hormone activity in the urine of cirrhotic patients. Hepatology 1990; 12:467–75.)

like immunoreactivity (DLI) in cirrhotic patients with ascites and edema than in those with no evidence of fluid overload. Yang *et al*. (88) measured DLI in plasma and urine of healthy subjects and a large series of cirrhotic patients. DLI was moderately increased in patients with compensated cirrhosis, and remarkably elevated in those with ascites, with and without renal failure.

To better assess the role of NH in the pathogenesis of sodium retention in cirrhosis, La Villa *et al*. (89) obtained urinary extracts by gel chromatography from 10 healthy subjects, 10 cirrhotic patients without ascites, and 37 patients with ascites (10 with renal failure) and simultaneously evaluated in each urinary extract three activities related to NH, namely inhibition of $Na^+$,$K^+$-ATPase, DLI, and the natriuretic response of assay rats. All these activities were significantly increased in patients with ascites, with and without renal failure, when compared with healthy subjects and compensated cirrhotics (Fig. 6.5). In the whole series of patients included in the study, $Na^+$,$K^+$-ATPase inhibition significantly correlated with DLI, and both parameters with the degree of hepatic and renal impairment, PRA, PAC, and plasma norepinephrine. The finding of elevated NH activities in the setting of sodium retention and ascites suggests that these patients may have a renal resistance to the natriuretic effect of NH. Indeed, Asbert *et al*. (90) showed that urinary extracts from cirrhotic patients are natriuretic in control rats, but not in cirrhotic rats with ascites.

The only firm conclusion that can be drawn from these data is that sodium retention of cirrhosis cannot be due to a deficiency of the so-called NH. A better understanding of the role of NH in the pathophysiology of ascites in cirrhosis requires better characterization of structure, biosynthesis, origin, mechanism(s) of release, mechanism(s) of action, as well as availability of antagonist(s) of this still putative hormone.

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# Chapter 7
# Arachidonic Acid Metabolites and the Kidney in Cirrhosis

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## Introduction

Arachidonic acid metabolites or eicosanoids encompass a group of compounds formed by either stereospecific or nonspecific oxidation of arachidonic acid. The eicosanoids comprise a biologically potent group of compounds, many of which may have profound effects on renal function. They include the leukotrienes, prostaglandins, thromboxanes, isoprostanes, endocannabinoids, hydroxyeicosatetraenoic acids (HETEs), hydroperoxyeicosatrienoic acids (HPETEs), platelet-activating factor (PAF), epoxytrienoic acids (EETs), and lipoxins. Arachidonic acid is a 20-carbon unsaturated fatty acid containing four double bonds (C20:4). It is formed by elongation and desaturation of its precursor, linoleic acid (C18:2), and is an important constituent of cell membranes, in which it is esterified within phospholipids, principally in the 29 position. In this form it is a prime target for the hormonally sensitive release of arachidonic acid and conversion to eicosanoids. Phospholipase $A_2$ (EC 3.1.1.4) splits arachidonate from the 29 position of phospholipids and is the main mechanism of release in many cell types including the macrophage. Phospholipase C (EC 3.1.4.3) results in the formation of diacylglycerol, which may release arachidonic acid directly (diacylglycerol lipase) or may be phosphorylated by diacylglycerol kinase and then act as a substrate for phosphatidate specific phospholipase $A_2$. Thus, arachidonic acid may also be released in the phosphatidylinositol response. Activation of both phospholipase $A_2$ and C can be initiated by a rise in intracellular calcium. Liberated arachidonic acid can be either re-esterified into phospholipids by acyl-CoA transferase or metabolized into the various eicosanoids by means of the cyclooxygenase or lipoxygenase pathways (Fig. 7.1). More recent studies have shown that a novel class of prostanoids (the $F_2$-isoprostanes) may be formed through a non-enzymatic free-radical-catalyzed lipid peroxidation of arachidonic acid on phospholipids *in situ*.

Eicosanoids can affect renal function in several ways. They may have direct effects on tubular function, altering both salt and water reabsorption. They may affect systemic hemodynamics directly and indirectly. One of the most important actions in the kidney involves their effects on renal blood flow. Eicosanoids may cause renal vasodilation or constriction, and at the finer level may directly alter glomerular function by modulating the glomerular capillary ultrafiltration coefficient ($K_f$). Approximately 90% of the renal blood supply circulates through the cortex, and the majority of the remainder supplies the outer medulla, with about 1% being supplied to the inner medulla. The proportion of blood that is filtered to form glomerular filtrate is the filtration fraction, and is the derivative of glomerular filtration rate/effective renal plasma flow (GFR/ERPF) with a typical value of 15–25%. Since plasma is the source of glomerular filtrate, it is not surprising that glomerular plasma flow is the most important determinant of GFR. The pressure gradient from capillary lumen to Bowman's capsule is primarily dependent on perfusion pressure, i.e. the difference between mean arterial pressure and renal venous pressure. GFR is also dependent on oncotic pressure in the renal afferent arteriole. Since afferent arteriolar oncotic pressure opposes glomerular filtration, low oncotic pressures, as frequently observed in liver disease, are normally expected to increase GFR. This is offset by two factors: (i) reduced oncotic pressure increases renal vascular resistance, and (ii) low oncotic pressure reduces the glomerular capillary ultrafiltration coefficient ($K_f$), which is the rate of formation of fluid by a single glomerulus per mmHg of effective filtration pressure. It is a function of the surface area of the glomerulus and hydraulic conductivity (i.e. permeability) of the glomerular capillary wall (1). The glomerulus is not a fixed structure. It is invaginated by mesangial cells that express actin and are contractile in an analogous way to the hepatic stellate cell. Thus, *in vivo* microscopy studies have shown that mesangial cells will contract, causing a reduction in the surface area of the glomerulus, thus decreasing $K_f$ and thus the filtration fraction (2) (Fig. 7.2). Thus, the filtration fraction may be affected by changes in afferent and efferent arteriolar tone as well as changes in $K_f$. As will become apparent, changes in vascular tone at the pre- and

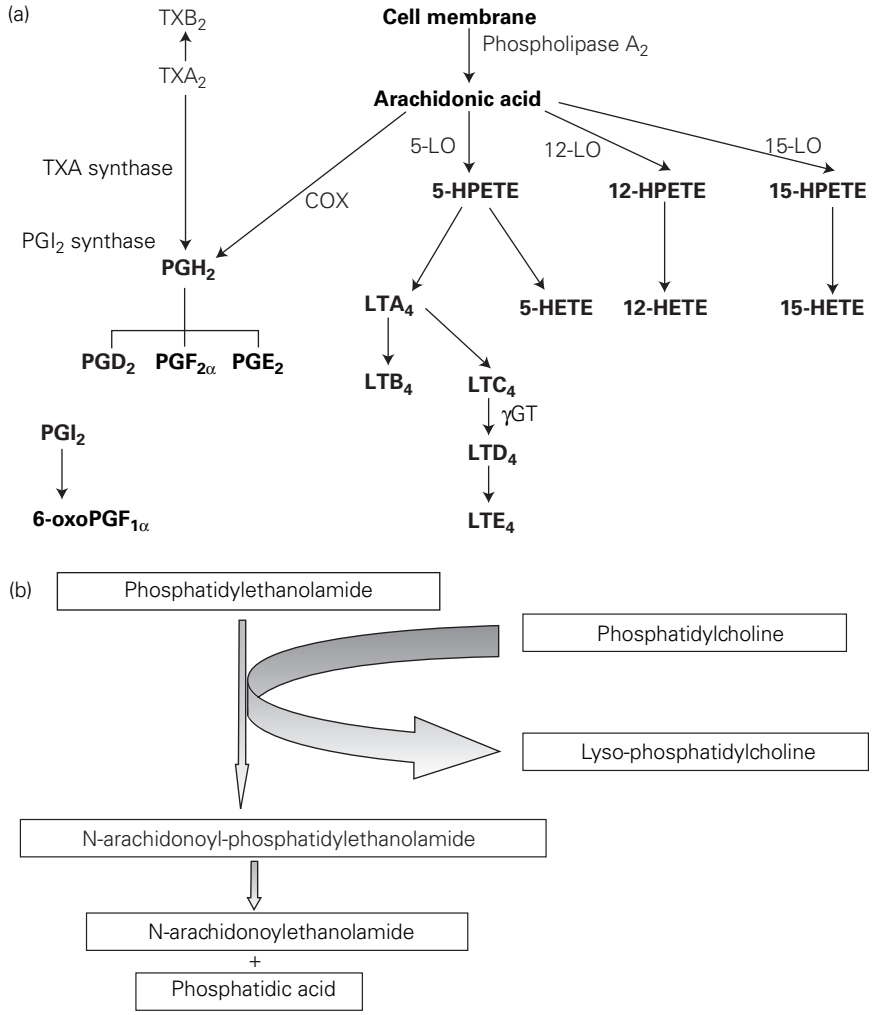



**Figure 7.1** (a) Pathways of eicosanoid synthesis. (b) Synthesis of anandamide. Arachidonic acid can be incorporated into a variety of biologically active lipid mediators, including leukotrienes, prostaglandins, isoprostanes, and HETEs. COX, Cyclooxygenase; γGT, gamma-glutamyltransferase; LO, lipoxygenase; TX, thromboxane; PG, prostaglandin; LT, leukotrienes; HPETE, hydroperoxyeicosatetranoic acid; HETE, eicosatetranoic acid. TxA2, thromboxane A2; AVP, arginine vasopressin; ANGII, angiotensin II; PGE2, prostaglandin E2; GFR, glomerular filtration rate.

postglomerular arteriole levels are critically regulated by eicosanoids, with many causing contraction of mesangial cells and thus decreasing the surface area of glomeruli. Conversely, some prostanoids decrease the glomerular responses to other vasoactive peptides.

## Initial studies on prostaglandins in liver disease

Following the discovery of prostaglandins about 30 years ago, it was not long before the role of prostaglandins in the renal abnormalities in cirrhosis was studied. The first investigation in 1974 by Arieff and Chidsey studied the effect of infused $PGA_1$, a renal vasodilator, on renal function in three groups of cirrhotic patients subdivided on the basis of their ERPF (3). They observed that renal function could be correlated with severity of liver disease and that $PGA_1$ increased ERPF, GFR, and sodium excretion only in patients with reasonably well-preserved renal

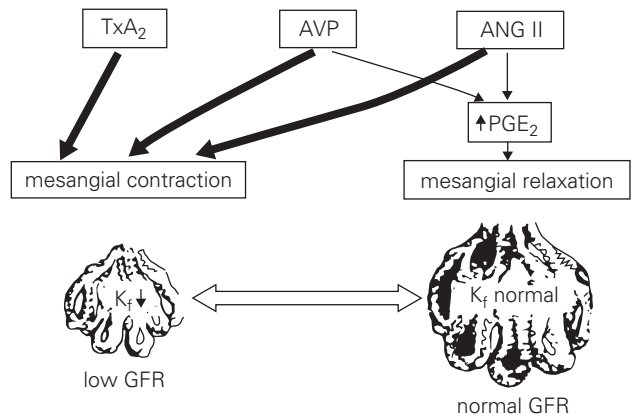



**Figure 7.2** Several vasoactive peptides and eicosanoids can cause contraction of the mesangial cell, with decreases in the surface area of the glomerulus and the glomerular capillary ultrafiltration coefficient ($K_f$). (Reproduced with permission from Scharschmidt *et al*. Arachidonate metabolites and the control of renal function. Fed Proc 1983; 42:3058–63.)

function. The next study, by Boyer and Reynolds, published initially in abstract form in 1976 and then as a full paper in 1979, showed that administration of indomethacin caused a decrease in ERPF and creatinine clearance (4,5). Moreover the decrease in GFR was greatest in those patients with ascites (Fig. 7.3). Infusion of $PGA_1$ restored renal function in patients given indomethacin, increasing creatinine clearance from 47 to 63 ml/min, and ERPF from 355 to 580 ml/min in the seven patients in whom indomethacin caused decreases in GFR and ERPF. A further observation from these studies was that this effect was associated with a decrease in the filtration fraction (from 14% to 11%). That is, the amount of glomerular filtration per milliliter of renal blood flow decreased. Filtration fraction depends on efferent arteriolar tone as well as the glomerular capillary ultrafiltration coefficient ($K_f$) as modulated by contraction of mesangial cells. Indomethacin was also associated with a 60% decrease in plasma renin activity, which was restored by infusion of $PGA_1$. At this time there were simultaneous publications by Zipser and colleagues, who, prompted by the initial observations published by Boyer, showed that cirrhotic patients with ascites had a 10-fold increase in urinary $PGE_2$ excretion rate, and that indomethacin caused a marked reduction in creatinine clearance (from 72 to 32 ml/min) (6,7). Furthermore, plasma renin and aldosterone levels decreased following administration of indomethacin, and the pressor response to angiotensin II in cirrhotic patients was normalized by indomethacin. These two fundamental studies opened the doors to the study of prostaglandins and related compounds in cirrhosis. The important observation that nonsteroidal anti-inflammatory drugs (NSAIDs) cause renal impairment in cirrhotic patients was of clear practical importance, but moreover indicated that renal prostaglandins are important in the maintenance of renal function in cirrhotic patients with ascites. This resulted in a multitude of studies in which the effects of different prostanoids and other eicosanoids on renal function were studied. Eicosanoids can affect renal function directly and indirectly, and these effects are summarized in Table 7.1.

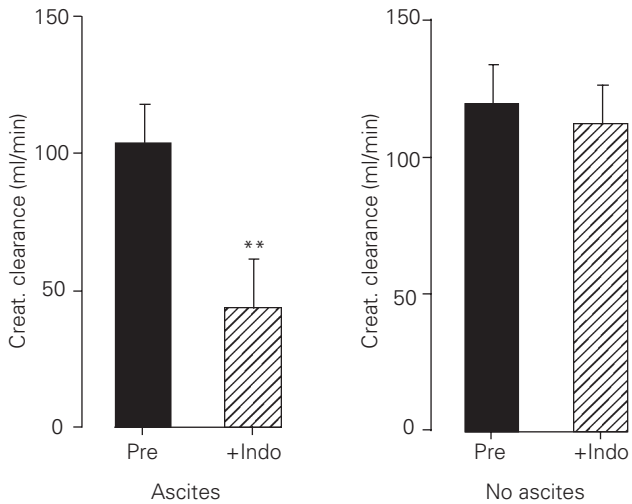



**Figure 7.3** Administration of indomethacin to patients with ascites caused a prompt reduction of creatinine clearance. In contrast, indomethacin had no effect on creatinine clearance in patients without ascites. ** $P < 0.05$. (Adapted from Boyer *et al*. Effect of indomethacin and prostaglandin A1 on renal function and plasma renin activity in alcoholic liver disease. Gastroenterology 1979; 77:215–22.)

## Cyclooxygenase

There are many studies on the role of prostaglandins in renal homeostasis, and prostaglandins have a pivotal role in the regulation or preservation of renal function in patients with liver disease. Prostaglandin synthesis is partly dependent on the differential regulation of the two isoforms of cyclooxygenase (COX; prostaglandin $G_2/H_2$ synthase, COX1 and COX2). In the kidney COX1 is expressed primarily in collecting duct, and COX2 is predominantly expressed in the macula densa, and nearby cells in the cortical thick ascending limb (8). COX2 is induced by a variety of stimuli including cytokines and endotoxin (9).

Zhonghua Qi *et al*. also showed distinct and opposing effects of COX1 and COX2 products on the systemic blood pressure response to angiotensin II (AII) in mice (10). Infusion of AII (150 pmol/kg/min) caused a prompt increase in mean arterial pressure (MAP), followed by a gradual decline. The mechanism of the spontaneous fall in blood pressure could relate to de-sensitization of the AII receptors (11), or the synthesis of compensatory vasodilators, such as nitric oxide (NO), bradykinin, or vasodilatory prostanoids (12,13). In the presence of a COX2 inhibitor (SC58236), the initial increase in blood pressure (BP) induced by AII is enhanced, and the subsequent spontaneous decrease of BP markedly attenuated. These data suggest that in control mice the constrictor effects of AII are counteracted by augmented production of COX2-derived vasodilators, so that medullary blood flow remains unchanged.

AII selectively stimulated $PGE_2$ and $PGI_2$ production in the renal medulla via COX2 in interstitial cells, which produces a renal medullary vasodilator action. Renal

**Table 7.1** Effects of eicosanoids on renal function.

- Renal vasodilation
- Renal vasoconstriction
- Contraction or relaxation of mesangial cells, and modulation of glomerular capillary ultrafiltration coefficient ($K_f$)
- Permeability of collecting ducts to water
- Sodium reabsorption in the tubules
- Indirectly through systemic effects (e.g. vasodilation) or modulation of other mediators
- Indirectly by altering gene transcription or enzyme activity

medullary blood flow has been intimately linked to the control of systemic blood pressure and renal sodium excretion, and correlates with renal salt excretion: in fact acute infusion of a COX2 inhibitor causes a marked decrease in urine volume and sodium excretion.

In contrast to COX2, COX1 is present in the collecting duct, a major site of salt and water reabsorption; however, acute infusion of a COX1 inhibitor did not affect medullary blood flow, nor did it affect renal sodium or water excretion, or modify AII-induced diuresis or natriuresis. Zhonghua Qi *et al*. suggest that reduced renal medullary blood flow could contribute to salt retention (10).

These findings have many clinical implications. Thus, administration of a NSAID to patients with decreased effective arterial blood volume, such as occurs in decompensated cirrhosis, will induce a significant decrease in renal function (14–16), since COX1-derived prostaglandins ($PGE_2$ and prostacyclin) are involved in the maintenance of renal function in cirrhosis and ascites (17). The role of COX1 in the maintenance of renal function is also highlighted by the observation that inhibition of COX1 by a selective COX1 inhibitor (SC-560) causes adverse renal hemodynamic changes, while the administration of an inhibitor of COX2 (celecoxib) had little effect (17). This is despite the observation that COX2 is upregulated in the kidney in rats with cirrhosis and ascites in response to decreased effective arterial blood volume.

The action of loop diuretics is known to depend on renal prostaglandins (18), and can be inhibited by NSAIDs. Prostaglandins also play a role in the modulation of action of antidiuretic hormone (ADH), a hormone important in renal water transport. Thus, $PGE_2$ inhibits the hydro-osmotic effect of ADH, whereas NSAIDs enhance the tubular effect of ADH (19).

### Renal eicosanoids

Glomeruli comprise endothelial cells, epithelial cells, mesangial cells, and glomerular macrophages. Whole rat or rabbit glomeruli generate $PGE_2$, $PGI_2$, $PGF_2$, and $TXA_2$, with their dominance in that order, whereas human glomeruli synthesize $PGI_2$ predominantly (20). Cultured rat and human mesangial cells produce $PGE_2$, but in humans can also produce $PGF_2$ and $PGI_2$. The form of prostanoid produced by the epithelial cells includes $PGE_2$ or $PGI_2$. Endothelial prostanoids are as yet undefined, but prostacyclin is likely to predominate. Leukotrienes are 5-lipoxygenase products synthesized by cells of the mononuclear cell lineage including infiltrating inflammatory cells and glomerular macrophages, but a transcellular metabolism of leukocyte-generated $LTA_4$ probably exists in glomeruli (21). Infusion of $LTC_4$ into the renal artery causes renal vasoconstriction (22), an effect that may be mediated by $LTD_4$, which acts preferentially on post-glomerular arteriolar resistance vessels and depresses $K_f$ and thus GFR (23). The principal cysteinyl leukotriene binding to the kidney is $LTD_4$ on high-affinity receptors on the mesangial cell with a $K_d$ of approximately 10 nM (24). It causes contraction of mesangial cells and in the rat glomerulus inhibits atrial natriuretic peptide (ANP)-induced cGMP synthesis (25). However, basal synthesis of cysteinyl leukotrienes is likely to be low, since the normal excretion rate of $LTE_4$ is 5 ng/h (26). Isolated pig kidneys synthesize cysteinyl leukotrienes in the absence of infused cells, but the sources of the LTs are unknown (27). Human and rat glomeruli generate both 12-HETE and 15-HETE, by the action of 12- and 15-lipoxygenase, and 12-lipoxygenase activity is exhibited by mesangial cells (28). 12-HETE has other actions in the adrenal gland relevant to cirrhosis since it is involved in AII-mediated secretion of aldosterone (29).

It is now well established that various eicosanoids can affect both salt and water transport through direct actions on the epithelial cells lining the tubules. Proximal tubules produce only 1–5% as much cyclooxygenase products as do the collecting ducts (20). Prostaglandins clearly have some effect on tubular function since the thick ascending loop of Henle contains $PGE_2$ receptors in high density within the medulla. In this site, $PGE_2$ is believed to inhibit sodium chloride reabsorption. The predominant eicosanoids produced in the proximal tubules are metabolites of the cytochrome P-450 pathway. The renal cytochrome P-450 system catalyzes the enzymatic conversion of arachidonic acid to the EETs (5,6- or 8,9- or 11,12- or 14,15-epoxides or the HETEs). These may have important effects on tubular function. 20-HETE has been singled out for particular study by McGiff's group in the USA and will be discussed in more detail later. Leukotrienes have no known action on renal tubules.

## Prostaglandin $E_2$

The renal actions of prostaglandin $E_2$ are summarized in Table 7.2. Quantitatively, prostaglandin $E_2$ is the predominant prostanoid formed in the kidney (20). This has been demonstrated by direct measurement of prostaglandin production by isolated renal segments, as well as by several studies demonstrating that urinary excretion of $PGE_2$ is markedly higher than that of other prostanoids (6,7,30). In the rat and rabbit, it comprises about 60% of all prostanoids formed by the glomerulus, but in humans

**Table 7.2** Renal actions of prostaglandin $E_2$.

- Major prostanoid made in the kidney
- Stimulates release of renin from juxtaglomerular apparatus
- Direct effect on glomeruli, and antagonizes response to pressor hormones
- Antagonizes antidiuretic action of antidiuretic hormone
- Renal vasodilator

and pigs, prostacyclin appears to be the predominant glomerular prostanoid (20). In the juxtaglomerular region, $PGE_2$ as well as $PGI_2$ stimulate the release of renin (31). The collecting tubules are the predominant source of prostanoids, mainly $PGE_2$ (95%). Medullary collecting ducts produce more $PGE_2$ than cortical collecting tubules. The predominant actions are vasodilation and modulation of water excretion. Four $PGE_2$ receptors have been identified and cloned. These receptors are designated $EP_1$ through $EP_4$. Using *in situ* hybridization techniques, Breyer and colleagues have localized the EP receptors in the human kidney (32,33). The phosphatidyl-inositol-hydrolysis response-coupled $EP_1$ receptor was highly expressed in the cortical, outer medullary, and inner medullary collecting ducts. The Gi-coupled $EP_3$ receptor was primarily expressed in the cortical and outer medullary collecting ducts as well as in the medullary thick ascending limb. However, the $EP_3$ receptor was absent from the inner medullary collecting duct. The Gs-coupled $EP_4$ receptor was highly expressed in the glomerulus. No $EP_2$ receptors were identified in the human kidney (32,33). $PGE_2$ binding has also been characterized in isolated rat glomeruli, and the response of rat mesangial cells to $PGE_2$ is indicative of receptors. Treatment with indomethacin increases receptor density in the inner medulla.

In the medullary thick ascending limb (TALH), $PGE_2$ inhibits sodium chloride reabsorption both directly and as mediated by ADH. However, the cortical segment of the TALH exhibits fewer receptors for $PGE_2$, and there is less evidence to implicate any functional relevance in this site. Inhibition of COX augments sodium reabsorption in the loop of Henle and blocks inhibition of sodium reabsorption by furosemide. However, it is not clear whether these are direct effects or effects indirectly mediated by decreases in glomerular plasma flow. As noted, the collecting tubules are the predominant source of prostanoids, mainly $PGE_2$ (95%). Medullary collecting ducts produce more $PGE_2$ than cortical collecting tubules. Infusion of $PGE_2$ or $PGI_2$ into the renal artery causes natriuresis (20). This may comprise both a hemodynamic response and a direct tubular effect. In the collecting system, the predominant effect of $PGE_2$ appears to involve water transport. $PGE_2$ stimulates hydraulic conductivity in the cortical collecting duct, and inhibits the response to ADH (34). This is secondary to the effect of $PGE_2$ on cAMP synthesis, both stimulating cAMP synthesis directly and suppressing it in response to ADH. Inhibition of COX impairs water excretion in cirrhosis (35). Although ADH stimulates $PGE_2$ synthesis, it appears that the concentration required far exceeds those that affect water metabolism.

After it was established that NSAIDs cause renal impairment, there followed many studies applying the then recently developed radioimmunoassays for the determination of urinary prostaglandins in cirrhosis and the hepatorenal syndrome. In the first study measuring urinary $PGE_2$ excretion in cirrhosis, Zia and colleagues demonstrated that urinary $PGE_2$ was increased in three conditions associated with high renin levels—namely, Bartter's syndrome, renovascular hypertension, and cirrhosis with ascites (6). Several studies have since confirmed that urinary $PGE_2$ excretion is increased in patients with cirrhosis and ascites. In the original studies on the urinary origin of prostaglandins, it was shown that relatively little infused $PGE_2$ appeared in the urine, suggesting that the kidney was the most likely source. Infusion of AII into both human volunteers and the renal arteries of dogs caused significant increases in urinary $PGE_2$ excretion (36). Zusman and Keiser had previously shown that addition of AII, as well as bradykinin or ADH, increased synthesis of $PGE_2$ by renomedullary interstitial cells (37). Further studies investigated the effect of the loop diuretic furosemide, which causes a marked increase in urinary volume and sodium excretion. Infusion of furosemide increases renal cortical blood flow and renal synthesis of $PGE_2$ (38). Planas *et al*. studied the effect of furosemide on renal function and the effect of lysine acetylsalicylic acid on furosemide-induced renal functional changes (39). They observed that acute injection of furosemide increased both ERPF and GFR and that lysine acetylsalicylic acid decreased ERPF and GFR. Lysine acetylsalicylic acid blunted the response to furosemide and, conversely, furosemide protected against the adverse effects of lysine acetylsalicylic acid. In view of the latter observation, it is not clear whether or not furosemide improves renal function by increasing $PGE_2$ synthesis, because, if inhibition of renal $PGE_2$ synthesis was complete with lysine acetylsalicylic acid, then furosemide must be acting by a different mechanism, otherwise it would not have preserved renal function. A subsequent study by Quiroga *et al*. demonstrated that 30% of patients with cirrhosis and ascites actually exhibited decreased GFR following furosemide infusion, and that this was associated with a fall in urinary excretion of 6-oxo-$PGF_1$ (40). This is discussed further in the section on prostacyclin.

A second but important issue is the effect of prostaglandins on water metabolism. The collecting duct is the ultimate regulator of renal salt and water excretion. Balance between intake and renal excretion of salt and water is fine-tuned by several hormones targeted to the collecting duct. Secretion of ADH from the posterior pituitary gland increases water permeability of the apical membrane of the collecting duct cell, resulting in increased water reabsorption (this is reviewed in Chapter 25). ADH acts on the $V_2$ receptor, which is linked to a G protein, causing stimulation of adenyl cyclase on the plasma membrane, increasing intracellular cAMP levels. There are several counterregulatory mechanisms. Prostaglandin $E_2$ plays a critical physiological and pathophysiological role in counteracting the effects of ADH, since $PGE_2$ produc-

tion itself is stimulated by ADH, and inhibition of COX augments ADH antidiuresis (41). Grantham and Orloff have shown that $PGE_1$ increases osmotic water permeability, but when added to tubules pretreated with ADH, $PGE_1$ blunted osmotic water flow (42). Likewise, $PGE_2$ increases osmotic water flow in rabbit cortical collecting ducts, but blunts the action of ADH. This effect seems to be secondary to inhibition of cAMP generation by ADH. $PGE_2$ decreases cAMP production in microdissected rat or rabbit cortical collecting ducts. It appears that $PGE_2$ inhibits cAMP production at low concentrations and yet has a stimulatory effect at high concentrations (43). The observation that this effect of $PGE_2$ is pertussis sensitive suggests that $PGE_2$ inhibits ADH-stimulated cAMP production by a G protein mechanism. However, $PGE_2$ can clearly stimulate inositol triphosphate turnover, with formation of diacylglycerols and, presumably, activation of protein kinase C. Breakdown of $PIP_2$ (phosphatidyl inositol biphosphate) may be a biochemical signal mediating the inhibitory effects of $PGE_2$ on ADH-stimulated osmotic water flow. Experiments by Breyer have indicated that $PGE_2$-mediated inhibition of ADH occurs at a pre-cAMP step, since $PGE_2$ has no effect on 8-chlorophenylthio-cAMP-mediated water transport (43). Exposure of cortical collecting ducts to pertussis toxin followed by ADH and then $PGE_2$ demonstrated that pertussis blocks $PGE_2$ inhibition of ADH-stimulated water transport. $PGE_2$ also acts through other pathways in that it induces a depolarization of the luminal collecting duct membrane in a pertussis-toxin-insensitive pathway. $PGE_2$ causes a prompt increase in cytosolic calcium in rabbit cortical collecting duct. Addition of the protein kinase C inhibitor staurosporine markedly attenuates the inhibitory effect of $PGE_2$ on cAMP-analog-induced osmotic water permeability and augments the effect of $PGE_2$ alone on water permeability. Thus, staurosporine blocks the inhibitory pathway and reveals a dominant stimulatory effect of $PGE_2$ on osmotic water permeability. It appears that when $PGE_2$ is applied to the tubule after exposure to ADH, it interferes with a post-cAMP pathway, whereas when administered before ADH it interferes with cAMP generation but not with cAMP-generated water flow. To date there are no published studies on the interaction of $PGE_2$ with the aquaporin system of water channels.

In normal animals, the excretion rate of $PGE_2$ is directly proportional to urinary flow rate. This is clearly relevant to studies in cirrhosis, since urinary $PGE_2$ excretion is highest in patients with ascites, in whom the urinary flow rate is decreased. Because infusion of $PGE_2$ does not contribute to urinary levels, one can conclude that renal synthesis must be increased. In patients with impaired solute-free water clearance, in which urinary $PGE_2$ excretion is decreased, marked decreases in urinary volumes and GFR are observed (41). When water-retaining cirrhotic patients are challenged with water to increase their urine output to the level observed in the normal controls under water deprivation conditions (0.6 ml/min), a urinary $PGE_2$ excretion comparable to that of normals (0.28 vs. 0.18 ng/min) is observed (40). Nevertheless, patients with mildly impaired solute-free water clearance exhibit a much greater increase of $PGE_2$ excretion than normal subjects. The observation that urinary $PGE_2$ excretion has a strong correlation with GFR may indicate decreased renal synthesis of $PGE_2$, or decreased excretion secondary to impaired urine flow rates and renal blood flow (30).

Prostaglandin $E_2$ comprises more than 95% of prostanoids produced in the collecting ducts, the major site of water control. Administration of indomethacin to patients with ascites, who have increased renal $PGE_2$ production, decreases GFR and urine output. Approximately 25% of patients with cirrhosis and ascites have severely impaired solute-free water clearance, and the majority have mild impairment of solute-free water clearance in response to water loading. A study by Reznick *et al*. showed that, following volume loading by insertion of a peritoneovenous shunt (PVS), ADH levels are suppressed, and yet solute-free water clearance is impaired, suggesting that factors other than ADH are involved (44). Cirrhotic patients with ascites generally show increased urinary $PGE_2$ excretion. However, if these patients are subdivided into those with negative solute-free water clearance, in whom GFR is decreased to about 43 ml/min, and those with positive solute-free water clearance (GFR < 87 ml/min), urinary $PGE_2$ excretion is lower in the former group (0.12 ng/min) compared with controls (0.26 ng/min) or those with ascites and a positive solute-free water clearance (0.6 ng/min). Moreover, following water loading, controls exhibited increased urinary $PGE_2$, from 0.26 to 1.65 ng/min, as did cirrhotic patients with intact water clearance (0.6 to 3.9 ng/min). However, patients with impaired water clearance could only increase urinary $PGE_2$ from 0.12 to 0.28 ng/min. These data demonstrate that water loading increases urinary $PGE_2$ excretion and that, in turn, increased $PGE_2$ production may decrease water reabsorption in the collecting tubules. This study also demonstrated that administration of lysine acetylsalicylic acid caused a decrease in solute-free water clearance in all those cirrhotics with ascites who had a positive free water clearance (4.8 to 0.6 ml/min; normals, 12 ml/min) (26). With respect to sodium metabolism, administration of misoprostol, a $PGE_1$ analog, to patients with cirrhosis had a tendency to decrease urinary sodium excretion but had no effect on renal dysfunction induced by indomethacin (45). Whether this is a direct effect or is secondary to vasodilation is not known.

## Prostacyclin

Prostacyclin is a renal vasodilator. Apart from its well-known formation by vascular endothelial cells, it is produced primarily in the renal cortex (20). In the renal

cortex, it is made by glomerular endothelial cells, being the major prostanoid formed by human glomeruli, which produce lesser amounts of $PGE_2$ or thromboxane. In the rat glomerulus, $PGE_2$ synthesis exceeds that of prostacyclin or thromboxane synthesis by a factor of 2 to 3. The glomerulus is composed of endothelial, mesangial, and epithelial cells, as well as bone-marrow-derived glomerular macrophages located in the mesangium (46). Rat mesangial cells produce $PGE_2$, but with substantial amounts of prostacyclin and thromboxane (47). Glomerular epithelial cells synthesize lesser amounts of prostanoids than mesangial cells produce. Addition of AII, PAF, or ADH to cultured rat mesangial cells causes the formation of $PGE_2$, but no other eicosanoids. Human mesangial cells make both prostacyclin and $PGE_2$ following stimulation with AII, PAF, or ADH (48). AII also augments both $PGE_2$ and prostacyclin synthesis by whole glomeruli (49), which modify the actions of this hormone on the kidney (50). These prostanoids act on mesangial cells, increasing intracellular cAMP. Studies measuring glomerular planar surface area have shown that glomeruli contract in response to AII to 80% of their surface area (2). This decreases the surface area available for filtration and thus the $K_f$. Inhibition of prostaglandin synthesis by NSAIDs augments the response to AII, causing a 7% decrease in surface area alone, and a shift in the dose–response curve to AII, suggesting that endogenous prostaglandins limit fluctuations of $K_f$ induced by vasoactive peptides, thus preserving the filtration fraction. Similarly, increasing prostaglandin synthesis by addition of arachidonic acid inhibits the response to AII. The effect of infused prostaglandins is confounded by the fact that both prostacyclin and $PGE_2$ cause release of renin, and thus release of AII by juxtaglomerular cells. Further, addition of converting enzyme inhibitors to whole glomeruli increases both prostacyclin and $PGE_2$ synthesis (51). When the effects of AII are blocked by antagonists, both $PGE_2$ and prostacyclin are purely vasodilatory, and have no effect on $K_f$. Infusion of AII after inhibition of COX causes reduction of ERPF, GFR, and $K_f$ (52). Thus, endogenous prostaglandins are important for the preservation of renal function in states with an activated renin–angiotensin system. Models in which COX inhibition causes a reduction in RBF can be reversed by preventing AII synthesis by captopril (53).

Experiments in the dog (54) have shown that there is a six- to 10-fold increase in the formation of both $PGE_2$ and prostacyclin in the kidney following an acute increase in renal venous pressure (RVP) from 5 to 40 mmHg, but no increase in thromboxane release. Renal blood flow was maintained despite the increased RVP, but treatment with indomethacin caused a prompt reduction of RBF and GFR. The changes in RVP are akin to those observed in cirrhotic subjects with and without ascites. A study by Mullane and Gliedman has shown a marked increase in RVP in cirrhotics, presumably as a consequence of altered structure in the caudate lobe, through which the inferior vena cava passes (55). The presence of ascites increases RVP further, so that in the presence of tense ascites the intra-abdominal (and therefore renal) pressure may be as high as 30 mmHg. This may partly explain the close association of a markedly increased renal synthesis of $PGE_2$ and prostacyclin with the presence of ascites.

With regard to the kidney in cirrhosis, it is difficult to be certain how much of the effect of COX inhibitors can be ascribed to prostacyclin and how much to $PGE_2$. Whereas urinary $PGE_2$ excretion reflects renal synthesis, the same does not necessarily hold true for either 6-oxo-$PGF_1$ (a stable prostacyclin hydrolysis product) or $TXB_2$ (a stable thromboxane hydrolysis product). Infusion studies using radiolabeled prostacyclin or $TXB_2$ have shown that 6% of infused radioactivity appears in the urine (56,57). Although this may seem to be a small percentage, the overall size of the vascular endothelium (one of the largest "organs" in the body) makes such a contribution very significant. Prostacyclin is the main COX product of blood vessels. The major systemic metabolite is 2,3-dinor-6-oxo-$PGF_1$. Approximately 14% of infused 6-oxo-$PGF_1$ is excreted in the urine unchanged (58), 21% as 2,3-dinor-6-oxo-$PGF_1$, and 20% as 2,3-dinor-6,15-diketo-13,14-dihydro-20-carboxyl-$PGF_1$. Organic acids such as $PGE_2$ may be secreted actively. However, experiments by Rosenkranz *et al*. showed no effect of probenecid on the urinary excretion of 6-oxo-$PGF_1$, suggesting that this compound is not handled by the organic acid pathway (59).

Guarner *et al*. first suggested that endogenous prostacyclin may be responsible for the hyperdynamic circulation observed in cirrhosis. In their study they observed increased urinary excretion of the 2,3-dinor metabolite of prostacyclin in cirrhotics with and without ascites as well as in those with the hepatorenal syndrome (HRS) (60). These data are consistent with increased vascular synthesis of prostacyclin in cirrhosis. This paper implied that systemic prostacyclin synthesis was similar in the three cirrhotic groups, when assessed by urinary 2,3-dinor-6-oxo-$PGF_1$ expressed as concentration/mg creatinine. Such an analysis assumes that renal function has no effect on the urinary excretion rate of 6-oxo-$PGF_1$. Although this is probably true for $PGE_2$, there seems to be no basis for assuming that it is true for other renal eicosanoids synthesized in the glomerulus or proximal to the collecting ducts. Indeed, studies by others had shown a correlation between urinary 6-oxo-$PGF_1$ excretion and GFR (30). In a follow-up study, Moore and colleagues observed that the urinary excretion rates of both 6-oxo-$PGF_1$ and its 2,3-dinor metabolite (as well as thromboxane and its dinor metabolite) were markedly elevated in patients with severe liver failure, and decreased in parallel to creatinine clearance as patients developed renal failure (61) (Fig. 7.4). This study demonstrated that, when corrected for GFR, production of both prostacyclin and thrombox-

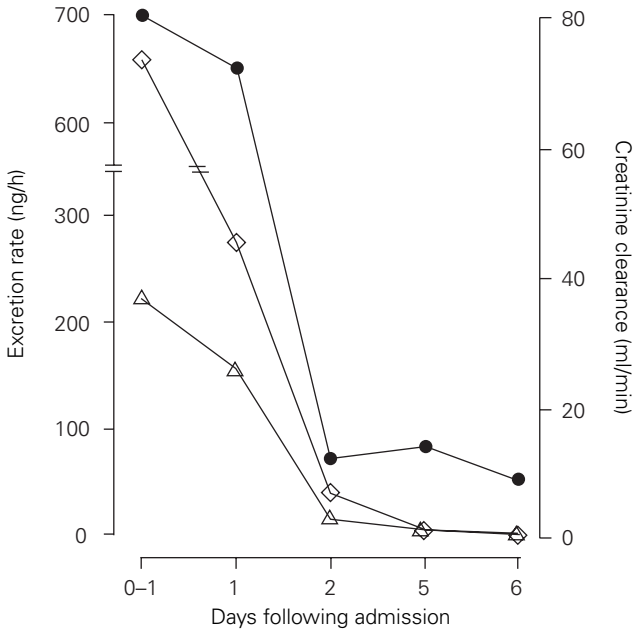


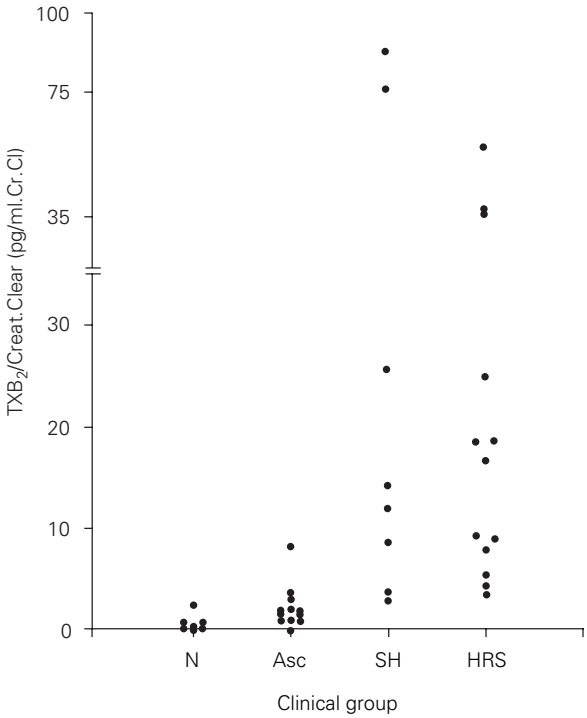

**Figure 7.4** Urinary excretion rates of thromboxane $B_2$ and 6-oxo-$PGF_1$ were followed serially in patients with alcoholic hepatitis during the development of the hepatorenal syndrome. Urinary excretion rates of both degradation products of thromboxane $A_2$ (open triangles) and prostacyclin (open diamonds) were markedly elevated (normal levels, 2–6 ng/h) and fell in parallel with the fall in creatinine clearance (closed circles). This strongly suggests that the excretion rates of these compounds should be corrected for creatinine clearance. (Adapted from Moore *et al*. Systemic and renal production of thromboxane $A_2$ and prostacyclin in decompensated liver disease and hepatorenal syndrome. Gastroenterology 1991; 100:1069–77.)

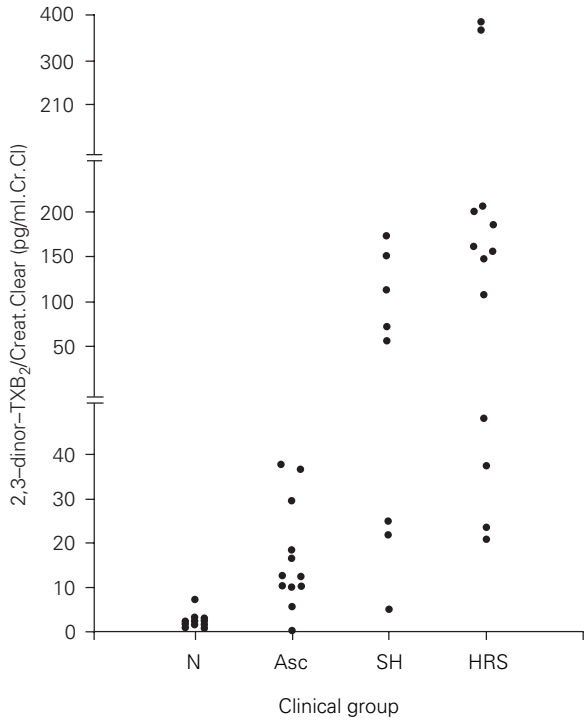
ane was directly proportional to severity of liver disease, rather than to renal function (Fig. 7.5). The magnitude of increased urinary excretion of systemic prostacyclin metabolites is well below that predicted from infusion of a vasodilatory dose of prostacyclin. Based on this, it was suggested by Moore *et al*. that the amount of prostacyclin formed is unlikely to be sufficient to cause vasodilation (61). In retrospect, this is probably incorrect. The vasodilating effect of prostacyclin is probably localized to the vascular bed where it is synthesized, and such calculations are therefore likely to be invalid. Moreover, studies by Zipser *et al*. and Bruix *et al*. have shown that administration of NSAIDs causes a modest increase in splanchnic resistance and restores vascular responsiveness to infused AII (7,62) (Fig. 7.6). Although the latter observation could conceivably be attributable to an effect on the renal vascular resistance, the data of Bruix *et al*. make it much more likely that this is a direct vascular effect, mediated through inhibition of prostacyclin synthesis.

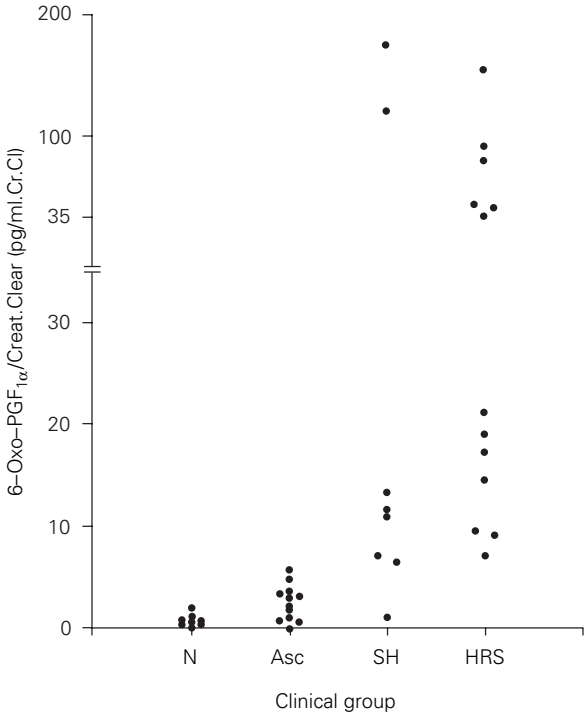
The production of prostacyclin by the vascular bed can be stimulated by catecholamines, renin, ADH, and endotoxin, and has been observed in models of noncirrhotic portal hypertension (63–65). Interestingly, Guarner *et al*. have also reported that intestinal decontamination with antibiotics caused a modest decrease in urinary excretion of 2,3-dinor-6-oxo-$PGF_1$ (66). Cirrhotic patients have increased levels of endotoxin in hepatic, portal, and arterial blood, which are thought to be secondary to impaired reticuloendothelial function, and these endotoxin levels may stimulate phospholipases and prostaglandin synthesis. This author is aware of no studies on the effects of inhibiting the other hormone systems on systemic prostacyclin production. The observation that both systemic prostacyclin and thromboxane production are increased suggests that there is increased platelet–endothelial cell interaction, which itself may be enhanced during endotoxemia (61).

Studies by Ros *et al*. have investigated the interaction between NO and prostaglandin synthesis in the carbon tetrachloride model of cirrhosis (67). This model has a three-fold elevation of urinary 6-oxo-$PGF_1$ excretion, and urinary sodium excretion is 20% of the normal level, but GFR is preserved. Although this investigation was titled as a study on prostacyclin, the only reference to this compound was in its measurement in the urine. Since $PGE_2$ is the major prostanoid made by rat kidney, it is likely that most of the effects of inhibition of COX described below are attributable primarily to inhibition of $PGE_2$ synthesis. Inhibition of NO synthesis increased mean arterial pressure in the cirrhotic animals from 88 to 102 mmHg, and in controls from 116 to 123 mmHg. Renal vascular resistance doubled in the control animals, and GFR remained unchanged. In the cirrhotic animals, however, renal vascular resistance (higher than controls: $6.76 \pm 0.6$ vs. $4.76 \pm 0.7$ mmHg/min/ml) showed no change, but GFR increased from 2.4 to 3.3 ml/min. Urine sodium excretion increased in each group by a factor of 2 to 3. Inhibition of prostaglandin synthesis had no effect on mean arterial pressure in either group and yet increased renal vascular resistance from 5.3 to 8.5 mmHg/min/ml in those with cirrhosis. This was accompanied by a fall in renal plasma flow but no change in GFR. In other words, inhibition of prostaglandin synthesis alone caused a decrease in renal blood flow, but GFR was preserved by an increase in the filtration fraction. Conversely, inhibition of NO synthesis caused the GFR to increase in the cirrhotic animals, despite no change in renal blood flow, indicative that this also caused an increase in filtration fraction. Inhibition of both parameters was additive. Mean arterial pressure increased in both controls and cirrhotic animals, and this was accompanied by a 60% fall in renal plasma flow, but with GFR unchanged. The effect of each treatment on the filtration fraction is shown in Table 7.3.

The most striking aspect of these data is the effect of each agent in increasing the filtration fraction. The filtration fraction is affected by changes in efferent arteriolar



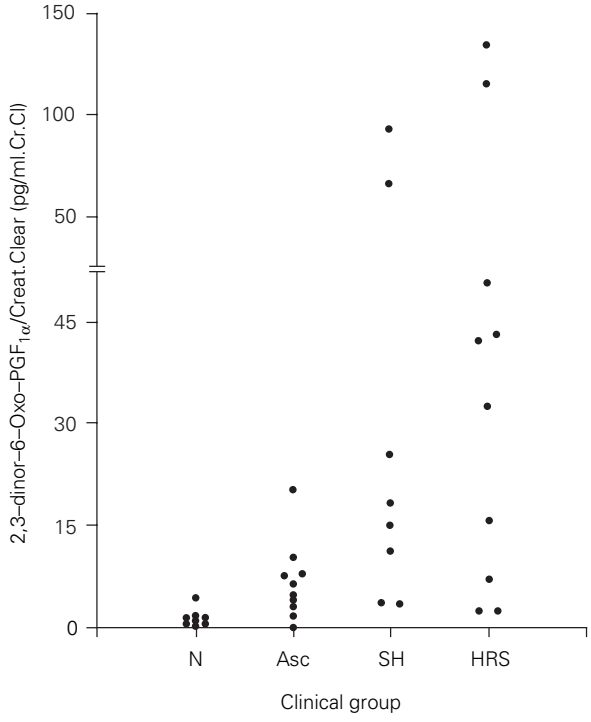




**Figure 7.5** Urinary excretion of thromboxane $B_2$, 6-oxo-$PGF_1$, and their dinor metabolites in patients with ascites (Asc), severe liver failure but preserved renal function (SH), patients with the hepatorenal syndrome (HRS), and normal controls (N). There was a positive correlation of these levels with the severity of liver disease as assessed by plasma bilirubin or coagulopathy. (Adapted from Moore *et al*. Systemic and renal production of thromboxane $A_2$ and prostacyclin in decompensated liver disease and hepatorenal syndrome. Gastroenterology 1991; 100:1069–77.)

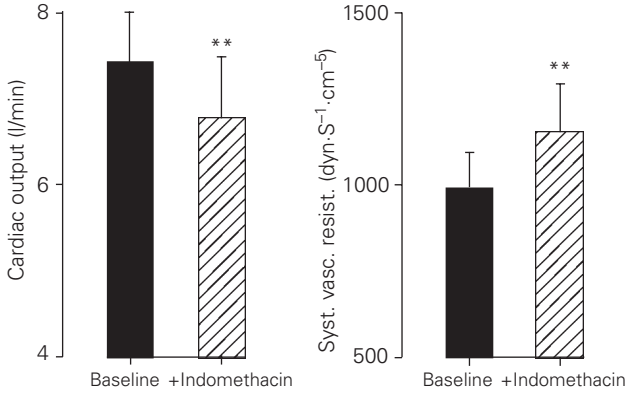



**Figure 7.6** Administration of indomethacin to patients with cirrhosis caused a decrease in cardiac output and an increase in systemic vascular resistance. These data suggest that endothelial prostacyclin production is important in the development of a hyperdynamic circulation. (Adapted from Bruix *et al*. Effects of prostaglandin inhibition on systemic and hepatic hemodynamics in patients with cirrhosis of the liver. Gastroenterology 1985; 88:430–5.)

tone and in factors that affect glomerular plasma flow and the $K_f$. What is most surprising is that this is in contrast to predictions based on the known effects of $PGE_2$, prostacyclin, or NO in inhibiting mesangial cell contraction and thus acting as counterregulatory mechanisms against factors tending to decrease $K_f$ and thus GFR. Indeed, these differences in the cirrhotic rat's response to NSAIDs, in contrast to those observed in humans, highlight the differences between different animal species and human liver diseases.

Although a story emerged in the 1980s suggesting that an imbalance between the synthesis of the vasodilatory prostanoids ($PGE_2$ and prostacyclin) and the renal vasoconstrictor thromboxane was causative in the pathogenesis of the hepatorenal syndrome (68), a critical look at the data does not support such a notion

**Table 7.3** Effects of inhibition of nitric oxide synthase (NOS) and/or cyclooxygenase (COX) on filtration fraction in rats with carbon tetrachloride-induced cirrhosis with ascites.

| | Filtration fraction | |
|---|---|---|
| | Normal rats | Cirrhotic rats |
| Inhibition of NOS | | |
| Before | 22% | 20% |
| After | 38% | 28% |
| Inhibition of COX | | |
| Before | 22% | 20% |
| After | 26% | 33% |
| Inhibition of NOS and COX | | |
| Before | 22% | 20% |
| After | 58% | 66% |

From Ros *et al*. Role of nitric oxide and prostacyclin in the control of renal perfusion in experimental cirrhosis. Hepatology 1995; 22:915–20.

(69). Although the current data support the idea that renal $PGE_2$ synthesis is decreased in the hepatorenal syndrome, prostacyclin production is increased (61). An immunohistochemical study of antiprostacyclin synthase antibodies by Govindarajan *et al*. has shown that in normal kidneys there is intense staining in cortical and medullary peritubular capillaries in the adjacent renal interstitial cells and in the glomerular mesangial region. The immunochemical staining pattern was unchanged for prostacyclin synthase in patients with the hepatorenal syndrome (70). In contrast, there was a marked diminution of staining for COX (termed PG endoperoxide synthase in the paper) in the patients with the hepatorenal syndrome. Whether this phenomenon is causally associated with the pathogenesis of the hepatorenal syndrome or reflects a staining artefact produced by prolonged reductions of renal perfusion is not known.

An interesting observation has emerged from studies on the effect of furosemide on renal function and prostacyclin and $PGE_2$ synthesis. Administration of an i.v. bolus of furosemide increases urine output and urinary excretion of $PGE_2$. On examining the effects of furosemide on GFR in patients with cirrhosis, one finds that some patients exhibit an increase and some a decrease in creatinine clearance. Despite the increase in urine volume, it was observed that in the group in which GFR increased (from 99 to 129 ml/min, $n = 15$) there was an associated increase in the urinary excretion of 6-oxo-$PGF_1$ (from 478 to 1034 pg/min), and in the group with an associated decrease in GFR (from 102 to 71 ml/min, $n = 6$) there was a corresponding fall in urinary 6-oxo-$PGF_1$ excretion (from 1032 to 548 pg/min) (40). Moreover, there was a strong correlation between the change in 6-oxo-$PGF_1$ excretion rate and the change of GFR. What is clear from these data is that basal urinary 6-oxo-$PGF_1$ and $PGE_2$ excretion are highest in those who exhibit a fall in GFR following injection of furosemide. Previous studies have suggested that the group with the highest $PGE_2$ excretion had relatively intact solute-free water clearance (41). Subsequent studies have demonstrated that urinary 6-oxo-$PGF_1$ should be corrected for creatinine clearance (Cr.Cl) (61). On re-examining the effect of furosemide on corrected 6-oxo-$PGF_1$ excretion in this study, it is observed that following infusion of furosemide the urinary excretion of 6-oxo-$PGF_1$ is 8 pg/ml Cr.Cl in the GFR-positive responders and 7.8 pg/ml Cr.Cl in the GFR-negative responders, compared with basal levels of 4.8 and 10.1 pg/ml Cr.Cl in the two groups, respectively (40). These data could be interpreted to suggest that renal prostacyclin and $PGE_2$ synthesis are increased in the group most susceptible to the adverse effects of furosemide, and that these are compensatory mechanisms. Further studies are needed to determine the effect of prostacyclin on renal function in cirrhosis.

## Thromboxane $A_2$

Thromboxane $A_2$ was first described in 1975 by Hamberg *et al*. (71). It is an unstable prostanoid formed by thromboxane synthase, an enzyme similar to cytochrome P-450, and rapidly undergoes hydrolysis to $TXB_2$, with a half-life of 1–2 s. It acts on one or more specific cell surface G-protein-coupled receptors (20). At present, only one receptor has been identified and cloned (72). However, it is likely that more than one receptor exists, because there are high- and low-affinity binding sites in different tissues, and other agonists such as the $F_2$-isoprostanes appear to act on a closely allied but separate group of receptors. Recent studies in rat and murine kidneys by immunostaining or *in situ* hybridization have localized thromboxane receptors in glomeruli, arterial walls, luminal membranes of the medullary thick ascending limb of Henle's loop, and both the luminal and basolateral membranes of the distal convoluted tubules and the basolateral membranes of the collecting tubules (73–77). In the glomeruli, the receptor appears to be localized mainly along the lumina of the glomerular capillary loops. Parietal epithelial cells of the Bowman's capsule, podocytes, and mesangial cells also demonstrate immunostaining for the receptor. These observations are supported in a study by Asano *et al*. showing that $TXA_2$ receptor mRNA was abundantly expressed in the glomerulus, followed by the distal convoluted tubule as well as the medullary thick ascending limb of Henle's loop and outer medullary collecting duct (76).

In the kidney, infusion of analogs of thromboxane such as U46619 causes marked renal vasoconstriction and a fall in GFR (78). This is presumably a direct effect on preglomerular vessels and the glomeruli. Exposure of mesangial cells to U46619 causes contraction and a decrease in the surface areas of the glomeruli (79). This in turn decreases the $K_f$ and thus the filtration fraction. Infusions of $TXB_2$ into human volunteers have demonstrated that it has a half-life of 7 min, and 5.3% was converted and excreted as 2,3-dinor-$TXB_2$ (80). A study by Zipser and Martin showed that, following infusion of $TXB_2$ into the brachial vein and renal artery, 2% and 14% are excreted unmetabolized, respectively (57). Although the fractional excretion of unchanged $TXB_2$ is small, patients with thromboembolic disease have increased urinary concentrations of $TXB_2$, suggesting that systemically formed $TXB_2$ may contribute significantly to the overall urinary $TXB_2$ profile (81).

The role of thromboxane in the pathogenesis of the hepatorenal syndrome was first proposed by Kronborg and Zipser (68,82,83). They measured urinary excretion of $TXB_2$ in patients with cirrhosis and in those with alcoholic hepatitis who were developing hepatorenal syndrome. Whereas the urinary excretion rate of $PGE_2$ was low in patients developing hepatorenal syndrome and high in those with cirrhosis, urinary $TXB_2$ concentrations were high in those with hepatorenal syndrome and low in those with cirrhosis. This paper described their data in terms of urinary concentrations, because the urinary concentration of $TXB_2$ was unaffected by water deprivation and low urinary flow rates in normal controls (84). Based on the presentation of data showing that development of renal failure in liver disease causes a reduction in the excretion of $PGE_2$ and an increase in $TXB_2$ concentrations, a story emerged of an imbalance of vasodilators ($PGE_2$) and vasoconstrictors (thromboxane). Other studies, however, took the more reasoned approach of quantifying the rate of formation of thromboxane by the urinary excretion rate of either $TXB_2$ or 2,3-dinor-$TXB_2$ (30,61). These studies then found that urinary excretion of $TXB_2$ was in fact low or similar to that in patients with decompensated liver disease but relatively well-preserved renal function. However, since patients with thromboembolic disease have increased urinary $TXB_2$ excretion (81), the contribution of nonrenal $TXB_2$ to urinary levels needs consideration, and the control population should be matched as far as possible for severity of liver disease to the target population (i.e. those with hepatorenal syndrome). Serial study of patients in the developing phase of hepatorenal syndrome demonstrated that urinary excretion rates of both 6-oxo-$PGF_1$ and $TXB_2$ as well as their 2,3-dinor metabolites were markedly elevated in patients prior to the development of hepatorenal syndrome, and that their urinary excretion rates fell in parallel with the creatinine clearance (61) (see Fig. 7.4). These data could be interpreted as showing that renal synthesis of both thromboxane and prostacyclin fell as patients developed hepatorenal syndrome. However, the most likely explanation has to be that urinary excretion rates of these prostanoids are GFR dependent, and thus urinary excretion rates should be corrected for GFR. When this is done, a very different pattern emerges, that is, urinary 6-oxo-$PGF_1$ and $TXB_2$ are both markedly elevated in proportion to the severity of liver disease and appear to be independent of renal function (see Fig. 7.5). These data do not, of course, exclude a significant contribution from renal thromboxane to the renal abnormalities that occur in liver disease, but they do not support a close association of urinary $TXB_2$ and the development of hepatorenal syndrome. A study using the thromboxane inhibitor dazoxiben in patients with hepatorenal syndrome was disappointing (85), and although often quoted as evidence against the role of thromboxane in the renal abnormalities, this study in fact neither proved nor disproved anything. The dosage of dazoxiben was too low for effective inhibition of renal synthesis of thromboxane, and the use of synthase inhibitors is confounded by the potential for PG endoperoxides to be directed down other pathways, such as to form $PGD_2$, which can also act on the thromboxane receptor and cause renal vasoconstriction. The most important data have emerged from the studies of Laffi,

Pinzani, and Gentilini (86–88), who studied the effects of both a thromboxane synthase inhibitor and a thromboxane receptor antagonist on water handling in cirrhotic patients. They observed that administration of OKY 046, a thromboxane synthase inhibitor, to non-azotemic cirrhotic patients with ascites and avid sodium retention increased GFR by 20%, whereas renal blood flow was unchanged. In other words, the filtration fraction increased, presumably as a consequence of intrarenal thromboxane on mesangial cells. Further studies showed that when administered with furosemide, OKY 046 enhanced the increase in furosemide-induced solute-free water clearance by a factor of 4 (from 1 to 4 ml/min) and increased the natriuretic response to furosemide in this population. These studies also observed that furosemide not only increased urinary $PGE_2$ excretion, as observed by others, but also increased GFR as well as urinary 6-oxo-$PGF_1$ and $TXB_2$ excretion. This group followed up these studies with an investigation of a thromboxane receptor antagonist, which should not be complicated by diversion of endoperoxides into the $PGD_2$ pathway. This study involved assessment of the effects of thromboxane blockade during a water diuresis. In cirrhotics with ascites, thromboxane receptor blockade increased solute-free water clearance from 1.7 to 3.1 ml/min, but had no effect on urinary electrolyte excretion or renal function. There was a small increase in renal plasma flow. Thus, intrarenal thromboxane synthesis seems to cause a modest decrease in GFR in cirrhotics with ascites and to limit the diuretic response to furosemide or water loading (86).

## Cysteinyl leukotrienes

The cysteinyl leukotrienes were initially termed slow reacting substance of anaphylaxis (SRS-A). It is now established that the cysteinyl leukotrienes comprise leukotrienes $C_4$, $D_4$, and $E_4$ ($LTC_4$, $LTD_4$, and $LTE_4$), which are formed by the action of 5-lipoxygenase (5-LO) on arachidonic acid to form 5-HPETE and $LTA_4$, which in turn is converted to $LTB_4$, a potent chemoattractant to neutrophils, or to $LTC_4$ and thence to $LTD_4$. Of the cysteinyl leukotrienes, $LTD_4$ is the most active on a molar basis, and much of the action of $LTC_4$ may be related to the conversion of $LTC_4$ to $LTD_4$. The pathway is shown in Fig. 7.7. $LTD_4$ is inactivated by metabolism to $LTE_4$, which has insignificant biological activity and is excreted unchanged in the urine or bile or undergoes more extensive ω- or β-oxidation to the 2,3-dinor and tetranor metabolites (26,89–91).

These compounds elicit arteriolar vasoconstriction in the cardiac, pulmonary, and renal vascular beds, as well as augmentation of vascular permeability to macromolecules in postcapillary venules. The direct effects of infusion of $LTC_4$ were first reported by Badr and colleagues (22). Infusion of $LTC_4$ into the rat produced a significant elevation of blood pressure with reductions in cardiac output and renal blood flow. These effects were abolished by a leukotriene receptor antagonist, but not by saralasin (an AII antagonist) or a COX inhibitor. Subsequently, Gulbins *et al.* showed that $LTD_4$ and $LTE_4$ could cause contraction of preglomerular vessels in the rat kidney with a 50% reduction of GFR (92). A study by Barnett *et al.* showed that both $LTC_4$ and $LTD_4$ along with ADH, AII, and PAF, decreased the surface area of isolated rat glomeruli by approximately 10–15% (93). Whereas both PAF and AII caused a seven- to 10-fold increase in $PGE_2$ synthesis, this was not observed when the glomeruli were incubated with $LTC_4$ or $LTD_4$. A subsequent study by Badr and colleagues demonstrated binding of $LTD_4$ to cultured glomerular mesangial cells (24). Binding was found to be stereoselective, specific, saturable, and reversible. High- and low-affinity binding sites were identified. $LTD_4$ was found to initiate a phosphatidylinositol response and the formation of $IP_3$. The question as to whether the kidney could synthesize leukotrienes was for a long time complicated by the nature of many studies in which blood was allowed to circulate through the kidney either *in vivo* or *in vitro*. Blood cells can make $LTA_4$ through the 5-lipoxygenase (5-LO) pathway, and this may be involved in a transcellular transfer and metabolism by other cell types that contain $LTC_4$ (21). However, perfusion of the isolated pig kidney by a blood-free perfusate, with oxygenation by FC43, a perfluorinated oxygen carrier, by Moore and colleagues has shown unequivocal increased synthesis of cysteinyl leukotrienes following injection of the calcium ionophore A23187 (27). Acute injection of lipopolysaccharide had no effect over a period of 1–2 h of perfusion. Moreover, this study demonstrated extensive metabolism of $LTE_4$ by the kidney to form both ω- and β-oxidation products. Whether tissue-resident macrophages in the glomeruli were responsible for the stimulated increase in urinary $LTE_4$ is not known, but transcellular transfer of $LTA_4$ was effectively excluded during these experiments.

Several studies have now shown increased plasma concentration of atrial natriuretic peptide (ANP), the action of which is to increase salt excretion and to increase GFR through a cGMP-dependent pathway. Both ANP and the NO donor, nitroprusside, increase glomerular cGMP, and this can be significantly and dose-dependently inhibited by $LTD_4$ (25). Following the demonstration by Orning *et al.* that the major pathway for elimination of cysteinyl leukotrienes in humans involved the kidney and hepatobiliary systems (89), it was suggested by Keppler *et al.* that leukotrienes may be involved in the pathogenesis of hepatorenal syndrome (94). Two studies, one by Moore *et al.* and the other by Huber *et al.*, demonstrated increased urinary excretion of cysteinyl leukotrienes by patients developing hepatorenal syndrome (26,95,96) (Fig. 7.8). Urinary excretion of $LTE_4$ is

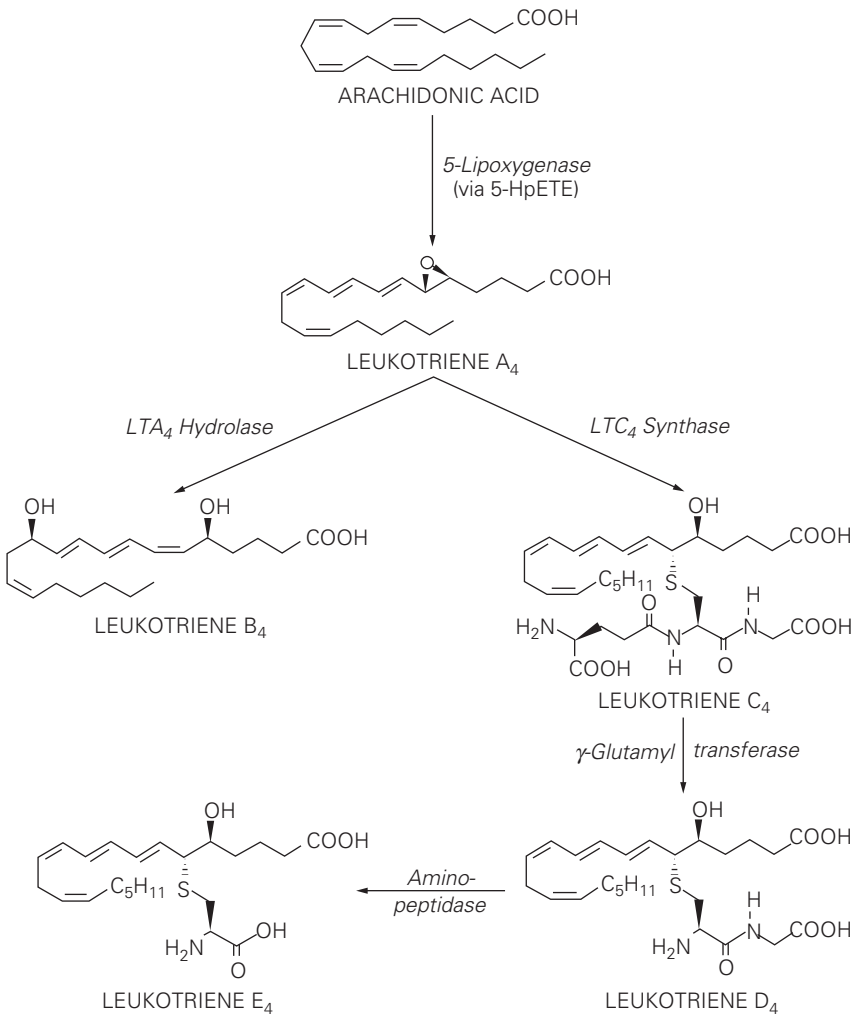



**Figure 7.7** Formation and breakdown of the cysteinyl leukotrienes.

GFR dependent, and increased synthesis was confirmed by infusion of radiolabeled $LTE_4$ into a patient with hepatorenal syndrome together with measurement of unchanged urinary radiolabeled $LTE_4$ and endogenous $LTE_4$ in the same subject (26). Huber *et al.* also noted that patients with hepatorenal syndrome had increased urinary levels of N-acetyl-$LTE_4$, and suggested that this may be a more specific marker of renal synthesis (95). Further studies by Keppler's group confirmed the previous observations (26) that urinary excretion of $LTE_4$ is increased in patients with biliary obstruction and decreased after biliary drainage (97). Although the latter observation was quoted as supporting a diversion of biliary excretion through renal pathways (which must occur), it does not exclude increased synthesis secondary to endotoxemia, which was relieved by biliary drainage. A recent study by Titos *et al.* (98) showed that there is over-expression of 5-LO mRNA in cirrhotic rat liver; in contrast, liver from control animals had low expression of 5-LO mRNA, while hepatic concentrations of $LTC_4$synthase were similar in both groups. Moreover, they showed that cultured rat hepatocytes with different degrees of purity showed different expression of 5-LO mRNA: in highly purified hepatocytes there is no evidence of 5-LO mRNA expression. A transcellular metabolism is suggested to be involved in the formation of cysteinyl leukotrienes in rat hepatocytes. This involves the synthesis of $LTA_4$ by an intermediate cell, uptake by hepatocytes, and then further metabolism to the respective cysteinyl leukotriene. Titos *et al.* confirmed what had been known for some time, namely that 5-LO is limited to cells of the myeloid series, and is only present in Kupffer cells in cirrhotic liver, and these cells may release $LTA_4$, thus hepatocytes rich in $LTC_4$ synthase transform $LTA_4$into cysteinyl leukotrienes (99,100). The studies of Titos *et al.* and Graupera *et al.* support the concept that hepatocytes have an important role in the formation of cysteinyl leukotrienes and the regulation of intrahepatic vascular tone. One of the major potential targets for hepatic derived cysteinyl leukotrienes includes the hepatic stellate cell, in which expression of actin is upregulated in cirrhosis, and which can contract

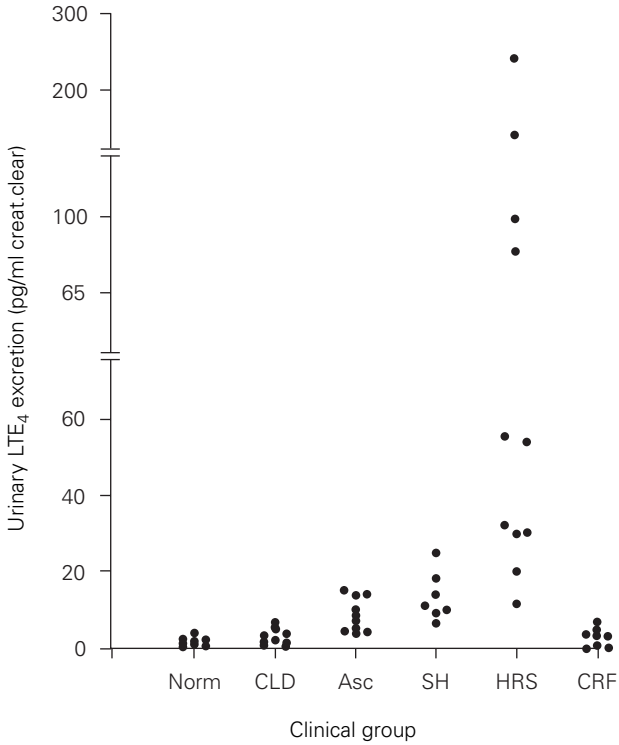



**Figure 7.8** Urinary excretion of leukotriene $E_4$, the metabolic product of $LTC_4$ and $LTD_4$, was markedly elevated in patients with the hepatorenal syndrome (HRS). Control groups included normals, compensated liver disease (CLD), ascites (Asc), severe hepatic failure (SH), and chronic renal failure (CRF). (Adapted from Moore *et al*. Increased production of cysteinyl leukotrienes in hepatorenal syndrome. J Hepatol 1990; 11:263–71.)

in response to $LTC_4$ or $LTD_4$ (98). A role for cysteinyl leukotrienes in the pathogenesis of portal hypertension is supported by the observation that inhibition of 5-LO decreases portal pressure (this may involve the type 3 receptor, since antagonists at types 1 and 2 receptors do not decrease portal pressure) (99), and hepatic leukotrienes may therefore have an indirect role on renal function through hepatorenal reflexes.

## Cytochrome P-450 arachidonate products

Oxidation of arachidonic acid (AA) by a variety of cytochrome P450 (CYP) enzymes leads to the formation of HETEs (hydroxyeicosatetraenoic acids) and EETs (epoxyeicosatrienoic acids), the latter of which are hydrolyzed to dihydroxyeicosatrienoic acids (DHTs) by cytosolic epoxide hydrolase. CYP-AA metabolites differ from prostaglandins in that they are stored in tissue lipids and have a primary intracellular locus of action. One of the most important CYP-AA metabolites relevant to cirrhosis is the ω-hydroxylase product 20-HETE (101). 20-HETE affects movement of ions, constricts blood vessels, is involved in tubuloglomerular feedback and renal autoregulation, and is mitogenic (102–105). Increased synthesis of 20-HETE is a major factor elevating blood pressure in the spontaneously hypertensive rat (106). It is predominantly formed in the renal arterioles with significant production in the renal medulla (104). In the preglomerular arterioles, it causes vasoconstriction in a non-COX-dependent manner by inhibiting potassium channels causing vasoconstriction (104). 20-HETE, together with 19-HETE, the function of which is unknown, is the principal CYP product in the medullary thick ascending limb, and is also formed in the proximal tubules (107). The medullary thick ascending limb is crucial to the regulation of extracellular volume. The thick ascending limb is the target for loop diuretics such as furosemide. Both 19- and 20-HETE affect $Na^+$-$K^+$-ATPase activity and are vasoactive. Their vasoactivity is partially dependent on COX activity, which can metabolize 20-HETE to vasoactive compounds (105). 20-HETE exerts a furosemide-like effect on medullary thick ascending limb cells, and inhibits the ATP-sensitive potassium channel in the apical membrane of the rat medullary thick ascending limb, and thus potassium recycling, an essential requirement for the activity of the Na-K-2Cl-cotransporter.

Both 20-HETE and 19-HETE are released from the rabbit or rat kidney in response to AII or endothelin-1, respectively, but ADH and bradykinin have no effect on their formation. In humans, 20-HETE is excreted in the urine as the glucuronide (108). A study by Sacerdoti *et al.* has shown that urinary 20-HETE excretion is increased five-fold above controls in patients with ascites, and two-fold above patients with cirrhosis alone (101) (Fig. 7.9). Moreover, Sacerdoti demonstrated that urinary 20-HETE excretion rate was highest in those with the lowest renal blood flow (101,109). Whether glucuronidation of the HETEs occurs in the kidney, or whether it occurs predominantly in the liver, thus reflecting an extrarenal site of synthesis, is unknown. The mechanism of increasing 20-HETE synthesis is unclear, but prime candidates include AII as well as endothelin-1. Recent studies have shown that endothelin-1 stimulates a four-fold increase in urinary 20-HETE efflux from the rat kidney (105), and that endothelin-1 levels are increased in both cirrhotics and patients with hepatorenal syndrome (110).

## Isoprostanes

The isoprostanes were first described in 1990, and are formed by peroxidation of arachidonic acid (111) (Fig. 7.10). They are formed as esterified prostanoids following oxidation of arachidonyl-containing phospholipids (112). Measurement of plasma, urinary, or tissue $F_2$-isoprostanes is now considered to be the gold standard for the assessment of lipid peroxidation *in vivo* (113,114). More

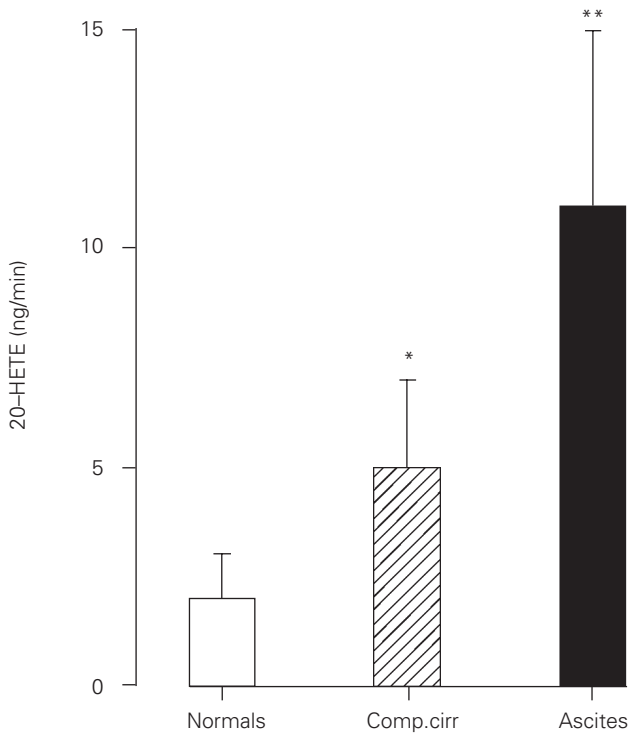



**Figure 7.9** Urinary 20-HETE is markedly increased in patients with ascites, compared with controls and compensated cirrhotics. 20-HETE is excreted as a glucuronide into the urine. It is involved in renal autoregulation and blood pressure control. Comp. Cirr, Compensated cirrhosis. (Adapted from Sacerdoti *et al*. Eicosanoid excretion in hepatic cirrhosis: predominance of 20-HETE. J Clin Invest 1997; 100:1264–70.)

importantly, isoprostanes also have potent biological activity causing intense renal vasoconstriction when infused into the rat (111,115). They act on a thromboxane-like receptor, with their renal actions being antagonized by thromboxane receptor antagonists. However, various data indicate that they may interact with a receptor separate from the conventional thromboxane receptor, since transfection experiments can confer binding of thromboxane analogs, but not the $F_2$-isoprostane 8-iso-$PGF_2$ (116). Plasma concentrations of the $F_2$-isoprostanes are increased in liver disease, particularly so in hepatorenal syndrome (117). More recently, both E/D ring isoprostanes have been described as well as isothromboxanes (118,119). These results have enormous implications for assays of prostanoids employing immunoassay, because some antisera will detect, with high cross-reactivity, similar isomeric forms (120). At present, the role of $F_2$-isoprostanes or related compounds in the etiology of renal dysfunction or salt and water retention in cirrhosis is unknown (121,122).

## Platelet-activating factor

Originally described in 1972 by Benveniste, PAF (or PAF-acether) has been characterized as 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine. It causes platelet aggregation and vasoconstriction when administered intravenously. At high dosages it increases tissue permeability and causes cardiac depression and shock. It is produced and degraded by a wide variety of cell types. Specific PAF receptors have been identified, and antagonists have been developed. PAF has been described in plasma and in urine. There are currently few data on PAF in cirrhosis, principally because it is present in very small amounts in biological fluids and PAF is extremely difficult to measure accurately, with many methodological pitfalls. Plante and colleagues have shown that infusion of PAF into the renal arteries of dogs causes a marked reduction of urinary sodium excretion, with no change in GFR or RBF (123). These effects could be blocked by the antagonist BN 52021. Studies of cirrhotic patients by Caramelo *et al.* showed that decompensated cirrhotics exhibited an eight-fold elevation of plasma PAF activity compared with controls, and a four-fold elevation in those with compensated cirrhosis (124). Serum PAF-acetylhydrolase activity was similar in all groups. Moreover, Caramelo *et al*. suggested that PAF is implicated as an important mediator of hyperdynamic circulation associated with liver cirrhosis (124). Recently, a study by Yang Y *et al*. (125) showed that concentrations of PAF, present in Kupffer cells in cirrhotic rat liver, were increased under basal conditions and increased further upon stimulation by endothelin-1 (ET-1) (126,127). Cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) have been implicated in the augmentation of PAF synthesis (128).

## Kallikrein–kinin system

The kallikrein–kinin system is a vasodilatory system of peptides produced in a variety of tissues including the kidney. Initially, kinins are synthesized as internal sequences within several larger $B_2$-microglobulins known as kininogens. They are released by the action of an endopeptidase of the trypsin type found in blood, tissue, and urine. These kinin-releasing endopeptidases are termed kallikreins. The kallikreins normally exist in an inactive form, becoming activated by tissue injury, sepsis, and hypoxia (129). One of the most potent kinins released is bradykinin. It causes formation of NO and vasodilation. Endothelial factors that are neither prostanoids nor NO seem to account for the major components of the vasodilatory responses to bradykinin. One of these, endothelial-derived hyperpolarizing factor, is postulated to be a labile arachidonic acid metabolite. In the isolated rat kidney, following inhibition of both COX and nitric oxide synthase, one can assess the effect of bradykinin, which stimulates phospholipases and nitric oxide synthase. Inhibition of COX and nitric oxide synthase decreases the vasodilatory response to bradykinin by 15% and 40%, re-

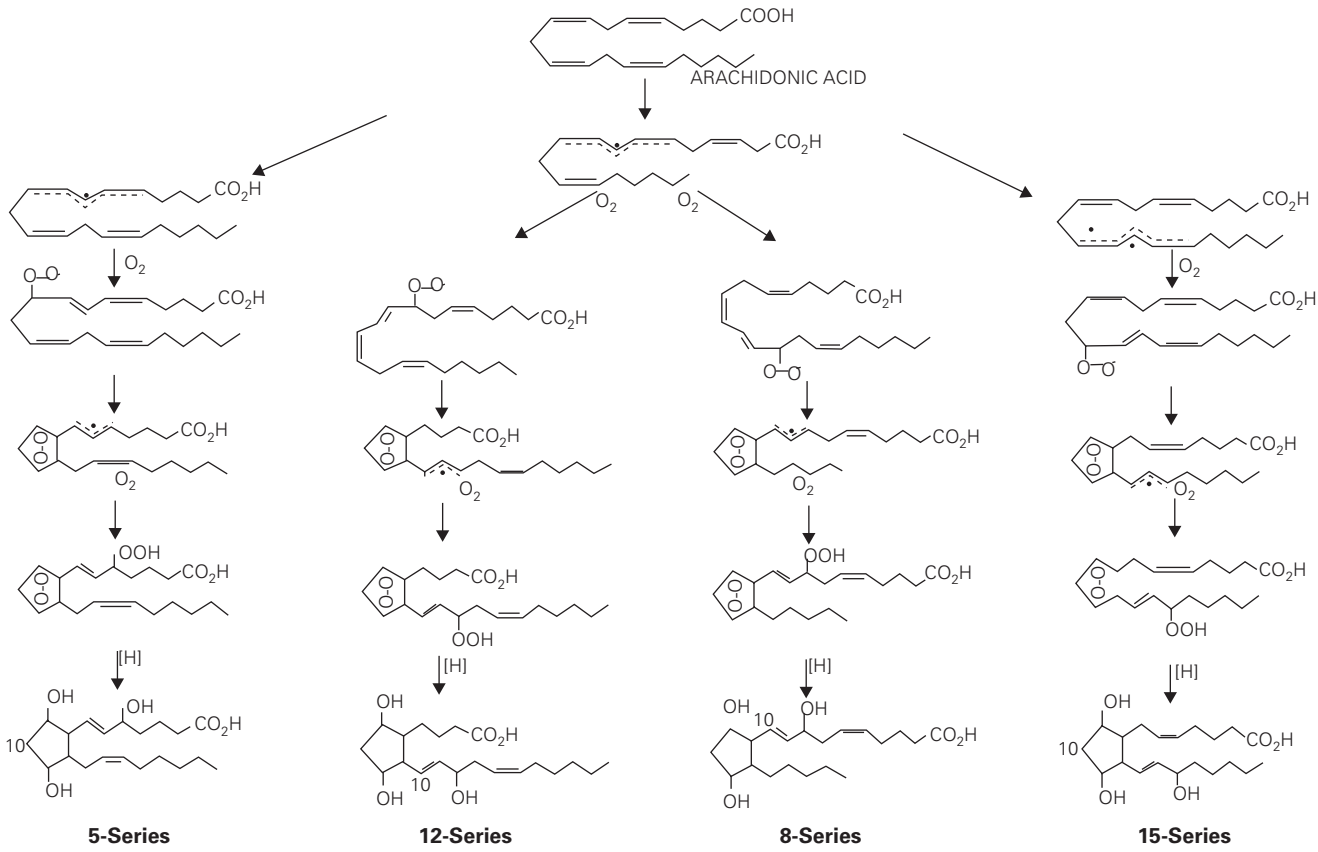



**Figure 7.10** Pathway for the formation of $F_2$-isoprostanes. These biologically active prostanoids are formed by non-enzymatic oxidation of arachidonic acid. Unlike conventional prostanoids, they may be formed as esterified complexes on phospholipids and subsequently released as preformed prostaglandins into the circulation.

spectively. The third component, comprising 45% of the vasodilatory activity, is greatly reduced by CYP inhibitors. Bradykinin also increases capillary permeability and causes formation and release of TNF and superoxide by macrophages and endothelial cells, respectively. Bradykinin is broken down by an aminopeptidase and angiotensin converting enzyme in the lung, with an equally large capacity of the liver to degrade this kinin, mainly through the aminopeptidase system. Infusion of intrarenal bradykinin (a nonapeptide) causes increases in both salt and water excretion. It is not, however, associated with an increase in urinary kinin secretion (130). This suggests that filtered kinins are rapidly degraded by kininase II in the proximal tubules within the kidney. This further suggests that urinary kinins are derived from the kidney. Renal kallikreins are formed in the renal cortex, with little activity in the medulla. Stop-flow studies have shown that renal kallikrein is secreted into the tubular lumen in the distal tubules, and immunohistochemical studies have colocalized it to the enzyme renin, located in the wall of the glomerular afferent arteriole. Infusion of AII or a volume challenge increases urinary kallikrein activity, and spironolactone, which antagonizes the renal actions of aldosterone, decreases urinary levels (130).

The plasma kallikrein–kinin system is activated in cirrhosis. The effects of the serine protease inhibitor, aprotinin, which is known to bind plasma kallikrein, were investigated by MacGilchrist *et al.* in cirrhotic patients (131). Infusion of aprotinin caused acute increases in urinary sodium excretion, renal plasma flow, and GFR. Systemic hemodynamics was essentially unaffected. The effects were quite dramatic, with GFR increasing from 66 to 103 ml/min (134). With respect to urinary/renal kallikrein activity, a study by Pérez-Ayuso and colleagues has shown increased urinary kallikrein activity in cirrhotics with ascites compared with controls (132). Activity was, however, decreased in those developing hepatorenal syndrome. Whether this reflects decreased renal synthesis or decreased urinary excretion in those with renal failure is unknown. At present, there are few studies on the role of the renal kallikrein–kinin system in cirrhosis. It is almost as if interest has dwindled with the emergence of new and exciting mediators, and further studies are required in this interesting area.

## Endocannabinoids

Recent studies have highlighted the importance of

anandamide, an endocannabinoid, in the pathogenesis of vasodilation in cirrhosis (133). Anandamide, otherwise known as arachidonoyl ethanolamide, acts on the CB1 receptor present in vascular tissue, as well as the central nervous system and in certain peripheral tissues including pituitary gland, immune cells, reproductive tissues, gastrointestinal tissues, sympathetic ganglia, heart, lung, urinary bladder and adrenal gland, and on CB2 receptors expressed mainly by immune cells, particularly B cells and natural killer cells (134).

These two endocannabinoid receptors have been cloned (135,136). Endogenous agonists for these receptors include arachidonoyl ethanolamide (anandamide), 2-arachidonyl glycerol, and 2-arachidonyl glyceryl ether (noladin ether). Anandamide behaves as a partial cannabinoid receptor agonist with marginally greater CB1 than CB2 affinity. The pharmacological properties of 2-arachidonyl glycerol and 2-arachidonyl glyceryl ether have been less well characterized. Anandamide and 2-arachidonyl glycerol may both serve as neurotransmitters or neuromodulators, and are probably synthesized by neurons. Once released, they are rapidly removed from the extracellular space by a membrane transport process. Once within the cell, anandamide is thought to be hydrolyzed to arachidonic acid and ethanolamine by the microsomal enzyme, fatty acid amide hydrolase (FAAH); 2-arachidonyl glycerol can also be hydrolyzed enzymatically, both by FAAH and by other hydrolases that are yet to be characterized.

Recent studies suggest that the vascular cannabinoid CB1 receptor is involved in the pathogenesis of vasodilation in cirrhosis. Using the $CCl_4$-treated rat model of cirrhosis, Batkai and colleagues showed that i.v. injection of SR141716A, a selective CB1 cannabinoid receptor antagonist, caused a sustained increase in systolic blood pressure in cirrhotic rats, but had no effect in control animals (Fig. 7.11) (133). A similar response was also observed in rats with biliary cirrhosis (133). Injection of anandamide causes a triphasic blood pressure response in normal rats, in that there is a sharp transient decrease in blood pressure that lasts 5–10 s (phase I), a short-lasting (30–60 s) pressor response (phase II), followed by further decrease in blood pressure which lasts up to 6 min (phase III). The prolonged depressor phase is known to be CB1 receptor-mediated. In rats with biliary cirrhosis, the first two non-specific phases of the effect of anandamide were present but the subsequent hypotensive component was absent. A similar trend was observed in rats with $CCl_4$-induced cirrhosis (133). These studies also showed that injection of a monocyte extract isolated from the blood of cirrhotic rats or patients with cirrhosis causes CB1-receptor-mediated hypotension when injected into normal rats (133).

A study of Ros *et al*. confirmed the finding of Batkai S *et al*. and emphasizes that the cardiovascular responses we observed in cirrhotic rats are probably mediated by

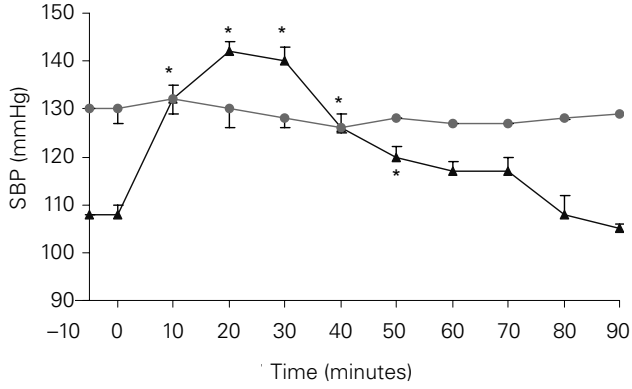



**Figure 7.11** The effect of CB-receptor blockade on cardiovascular parameters in rats with $CCl_4$-induced cirrhosis. Systolic blood pressure (SBP) was monitored in conscious cirrhosis and control rats, SR141716A (3 mg/kg) was injected intravenously at 0 min. Data represented as means ± SE; $n = 6$, control; 7, cirrhotic. *$P < 0.05$ compared with corresponding baseline value.

circulating blood cells, mainly monocytes, acting via CB1 cannabinoid receptors (137). The same study also investigated the relationship between NO and anandamide. The use of a NO synthesis inhibitor did not prevent the hypotension mediated by anandamide (137); this is in contrast to results from Deutsch *et al*. (138), who showed that anandamide stimulated renal endothelial cells to release NO. A similar conclusion has been reported by Fimiani and colleagues (139). The role of anandamide in vasodilation, cardiac and renal function will prove to be very interesting, and there is much work to be done in the future at dissecting out the pathways involved.

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# Chapter 8
# Nitric Oxide and Systemic and Renal Hemodynamic Disturbances in Cirrhosis

Manuel Morales-Ruiz and Wladimiro Jiménez

## General concepts in nitric oxide biology

Much of the chemistry of nitric oxide (NO) is characterized by the fact that it is a radical species. This special property confers to NO the potential to interact in cells with heme-containing proteins, thiols, oxygen and other species with unpaired electrons such as superoxide ($O_2^-$). For example, the best-known target of NO is the soluble guanylate cyclase (sGC). The interaction between NO and the heme group of sGC stimulates the enzymatic catalysis of this enzyme resulting in increased intracellular 3′,5′-cyclic guanylate monophosphate (cGMP) levels that ultimately signal relaxation (1).

NO is endogenously produced in several cells types through nitric oxide synthases (NOS) which catalyze L-arginine and oxygen into L-citrulline and NO. Three isoforms of NOS have so far been cloned and characterized: neuronal NOS (nNOS or NOS1) (2), inducible NOS (iNOS or NOS2) (3,4), and endothelial NOS (eNOS, or NOS3) (5,6). nNOS produces NO in low concentrations (nM) and its enzymatic activation is dependent on the $Ca^{2+}$/calmodulin pathway. Under physiological conditions, nNOS is involved in the relaxation of sphincters (gut, bladder, urethra), penile erection, neurotransmission, and memory formation, while high levels of NO following stimulation of N-methyl-D-aspartate (NMDA) receptors may induce neurotoxicity (7–9). By contrast, iNOS expression is mainly induced by proinflammatory stimuli such as bacterial lipopolysaccharide, and/or inflammatory cytokines [tumor necrosis factor-α, interleukin-1 and/or interferon (IFN)-γ] and its enzymatic activation is unaffected by the intracellular concentration of $Ca^{2+}$. iNOS was originally discovered in macrophages and was immediately appreciated as an important component of the antimicrobial and the inflammatory activity, despite the fact that it can be harmful and cause tissue damage in the host (10). Of the three isoforms, eNOS is the most sensitive to the intracellular concentration of $Ca^{2+}$. Studies using pharmacological inhibition of eNOS have demonstrated that NO production in the endothelium is important to maintain vascular tone and inhibit leukocyte and platelet adherence (11). In addition, eNOS gene disruption resulted in fertile and viable eNOS-/- with a hypertensive phenotype, increase in smooth muscle cell growth in response to vascular injury, predisposition to form neointimal proliferation, and a poor response to growth factor-stimulated angiogenesis (12).

## Nitric oxide and the pathogenesis of systemic circulatory dysfunction in cirrhosis

Evidence supporting a role for NO in the pathogenesis of arterial vasodilation in cirrhosis includes early investigations from our laboratory demonstrating that cirrhotic rats with ascites show higher pressor responsiveness to the administration of increasing doses of $N^G$-nitro-L-arginine (L-NNA) than control animals (Fig. 8.1) (13). In agreement with this study, the normalization of NO production in cirrhotic rats (measured as the aortic

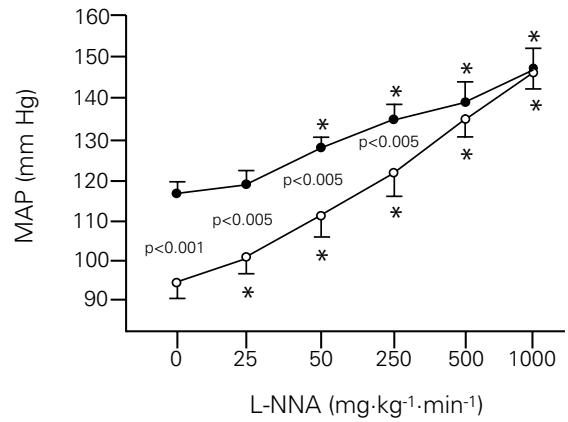


**Figure 8.1** Pressor effect of $N^G$-nitro-L-arginine (L-NNA) in cirrhotic rats with ascites. Mean arterial pressure (MAP) under basal conditions and after administration of sustained, increasing dose of L-NNA in cirrhotic (empty circles) and control (full circles) rats. $P < 0.01$ vs. baseline values. Reprinted with permission (13).

concentration of cGMP) by the administration of low doses of $N^G$-nitro-L-arginine methyl ester (L-NAME) was associated with the normalization of systemic hemodynamics and the improvement of renal sodium and water excretion in cirrhotic rats with ascites (14). This increased NO production was also directly assessed by measuring cGMP as a biological marker of NO bioactivity (15,16). In the first of these articles, Ros *et al.* (15) described that the cGMP accumulation measured in a coincubation assay was higher in arterial vessels of cirrhotic rats with ascites than in vessels of control rats. Moreover, they observed that denudation of endothelium reversed these differences. Similar results were obtained by Niederberger and colleagues with the additional contribution that the higher levels of cGMP were obtained in cirrhotic rats with ascites compared with rats with portal hypertension induced by portal vein ligation (PVL), which also presented elevated NOS activity (17).

Impaired pressor response to endogenous vasoconstrictors is a common finding in advanced liver disease. A number of groups investigating this phenomenon in different models of cirrhosis or portal hypertension have claimed a role for NO. Castro *et al.* (18) showed that NOS, but not cyclooxygenase activity, is the mechanism which accounts for the hyporesponsiveness to angiotensin II shown by isolated aortic rings of cirrhotic animals. Furthermore, the same group has reported that aortic rings from cirrhotic rats with ascites present an enhanced response to acetylcholine compared with control animals (16). In addition, Sievers *et al.* showed a partial restoration of the pressor effect of vasoconstrictors in a different vascular territory (the splanchnic vasculature) of cirrhotic rats with ascites in response to NOS inhibition (19). Similar evidence has also been described in mesenteric preparations of portal hypertensive rats induced by partial portal vein ligation (20) and in cirrhotic rats *in vivo* (21,22).

The NOS isoform responsible for NO hyperproduction in cirrhosis has also been investigated. Two studies observed an increased mRNA abundance of eNOS and iNOS in arterial vessels of cirrhotic rats treated with $CCl_4$. However, although eNOS protein was correspondingly increased and activated in the cirrhotic condition, no iNOS protein expression was found in these animals (23,24). Moreover, Xu *et al.* showed evidence of an increased expression of nNOS in aorta of cirrhotic animals in the same cirrhosis model (25). Paralleling the results obtained in $CCl_4$-induced cirrhosis rats, PVL animals showed higher eNOS protein levels and enhanced eNOS activity in arterial vessels, but not iNOS protein expression (26–28). Results derived from the bile duct ligation (BDL) model of cirrhosis are more controversial. While Liu *et al.* are in agreement with eNOS being the main enzymatic source of NO (29), Tazi *et al.* detected iNOS protein in aorta of cirrhotic animals supporting a potential role of both eNOS (which they proposed to be upregulated by shear stress) and iNOS (30). In addition, there is only one study (31) simultaneously addressing whether a differential expression of the eNOS and iNOS isoforms occurs in the different organs of rats with portal hypertension, with and without cirrhosis. Stumm *et al.* (31) observed that eNOS was expressed mainly in endothelial cells in all organs studied and that iNOS expression was only present in thymus and gut but not in the arterial vessels of the three experimental models mentioned above.

Collectively, these data provide a framework for the key role of NO in the hyperdynamic circulatory state; however, an important question still remains to be answered. What is the temporal relationship between the onset of the hyperdynamic circulation and the activation of NO production in cirrhosis? As seen in the above-mentioned studies, splanchnic and systemic hemodynamics are, to a great extent, corrected by the inhibition of NO synthesis. However, since these studies were performed after the onset of the hyperdynamic circulatory state, it is difficult to extrapolate whether NO is the cause or the consequence of this abnormality. This problem has recently been addressed by two independent groups. Iwakiri and colleagues (32) found that PVL rats maintain their hyperkinetic circulation in spite of being genetically engineered to lack the eNOS and iNOS genes (32). These results, however, were not confirmed by Theodorakis *et al.*, who demonstrated that deletion of the eNOS rather than the iNOS gene preferentially protects PVL rats from portal hypertension and increased abdominal aortic blood flow (33). Clearly, more research is needed to define whether increased NO precedes or is a consequence of the hyperdynamic circulation in cirrhosis.

Evidence of increased NO production has also been obtained in human cirrhosis. For example, plasma (34,35), portal (36–38) and hepatic vein (37) or exhaled breath (39,40) concentration of nitrites and nitrates (NOx) are elevated in cirrhotic patients compared with controls. Moreover, NOS activity has been reported in neutrophils and monocytes of cirrhotic patients with ascites (41,42) and iNOS protein has been detected in peritoneal macrophages of cirrhotic patients but not healthy subjects (43), consistent with similar results obtained in cirrhotic rats (44). Several groups have investigated the impact of NO inhibition in the hyperdynamic circulation of cirrhotic patients. Campillo *et al.* showed that the inhibition of NO synthesis in the forearm arterial bed of patients with advanced cirrhosis corrected arterial hyporesponsiveness to vasoconstrictors (45). Other studies, aimed at investigating the effects of the systemic administration of $N^G$-monomethyl-L-arginine (L-NMMA) in cirrhotic patients, showed a statistical association between NOS inhibitor treatment and an increase in arterial pressure (46,47).

Most studies assessing the contributory role of NO to the circulatory dysfunction in cirrhosis have focused on

the vasodilator properties of this substance. Recent data, however, indicate that eNOS activity also affects vascular remodeling in cirrhotic rats (48). Fernández-Varo *et al*. demonstrated that conductive vessels of cirrhotic rats undergo an intense process of vascular remodeling, characterized by a decrease in the thickness and the total area of the vascular wall (Fig. 8.2), which is corrected by long-term inhibition of NOS activity. In addition, this pharmacological treatment resulted in higher arterial pressure and peripheral resistance and lower arterial compliance (which is a measure of the elasticity of the arterial system) than cirrhotic rats receiving vehicle. These results indicate that active vasodilation and vascular remodeling may be part of a complex multifactorial process that plays a major role in the hyperdynamic circulatory syndrome occurring in cirrhosis.

## Pathophysiological role of nitric oxide in the cirrhotic liver

NO has emerged as an important regulator of hepatic vascular tone under physiological conditions (49–52). Therefore, it is also likely to have important implications in the pathogenesis of the increased intrahepatic vascular resistance in portal hypertension. In this regard, there are studies showing that NO production and eNOS protein activity are decreased in perfused cirrhotic livers from $CCl_4$-treated rats and isolated endothelial cells from $CCl_4$-treated rats and BDL rats, respectively (53,54). In addition, Sarela *et al.* have shown that the activity of intrahepatic calcium-dependent NOS was lower in cirrhotic patients compared with noncirrhotic subjects (36). In the study performed by Rockey *et al.* (54), equal levels of mRNA and protein eNOS were associated with a significant decrease in sinusoidal eNOS activation in cirrhotic animals, suggesting a post-translational control of eNOS activity. In this context, it has recently been shown that enhanced expression and interaction of caveolin-1 with eNOS may contribute to impaired NO production and reduced NOS activity in livers from $CCl_4$-treated and BDL rats (55–57). Similar to these findings, Yokomory and colleagues demonstrated by immunohistochemistry and Western blot that liver specimens from cirrhotic patients presented an over-expression of caveolin-1 (58). However, although the authors presented data of eNOS and caveolin-1 colocalization, the *in vivo* interaction of these two proteins still needs to be demonstrated in human cirrhosis. Another important mechanism leading to impaired eNOS activation has recently been described in $CCl_4$-induced cirrhosis rats (59). The major finding of this report is the impaired Akt activation in cirrhotic livers and the subsequent decrease in the incorporation of phosphate groups into serine 1176 in the rat eNOS sequence, which is one of the important steps in the process of eNOS activation (60). The *in vivo* experiments performed in the same study showed that the i.v. administration of an adenoviral vector carrying a constitutively active mutant of Akt to cirrhotic rats increased eNOS phosphorylation and enhanced intrahepatic release of NO, as estimated by the hepatic content of cGMP. Furthermore, restoring eNOS activity by myr-Akt gene delivery normalizes portal pressure, decreases superior mesenteric blood flow and ameliorates arterial hypotension in cirrhotic rats (Fig. 8.3). From the preceding discussion, it can be predicted, therefore, that Akt-dependent eNOS phosphorylation may be an important mechanism in the control of intrahepatic tone. Thus, pharmacological activation of Akt remains an area of further investigation in cirrhosis.

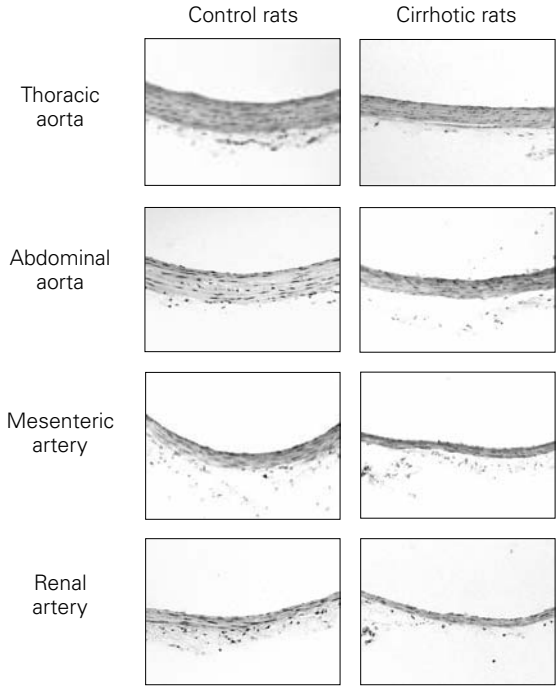


**Figure 8.2** Vascular remodeling in cirrhosis. Photomicrographs of representative cross-sections of thoracic and abdominal aorta, and mesenteric and renal arteries from a control rat and a cirrhotic rat with ascites. Note the marked reduction of the wall thickness and the diminution in the number of nuclei in the cirrhotic vessel (hematoxylin and eosin staining; original magnifications, x 200). Reprinted with permission (48).

## Pulmonary circulation and nitric oxide in cirrhosis

Enhanced production of NO in the lung of cirrhotic patients with hypoxemia has been demonstrated in a number of investigations assessing NO concentration in exhaled air (39,40,61–63). There is substantial evidence obtained from isolated perfused and ventilated lungs that the mixed exhaled NO level reflects the rate of pulmonary vascular production (64). In addition, liver transplantation (65) results in a decreased respiratory

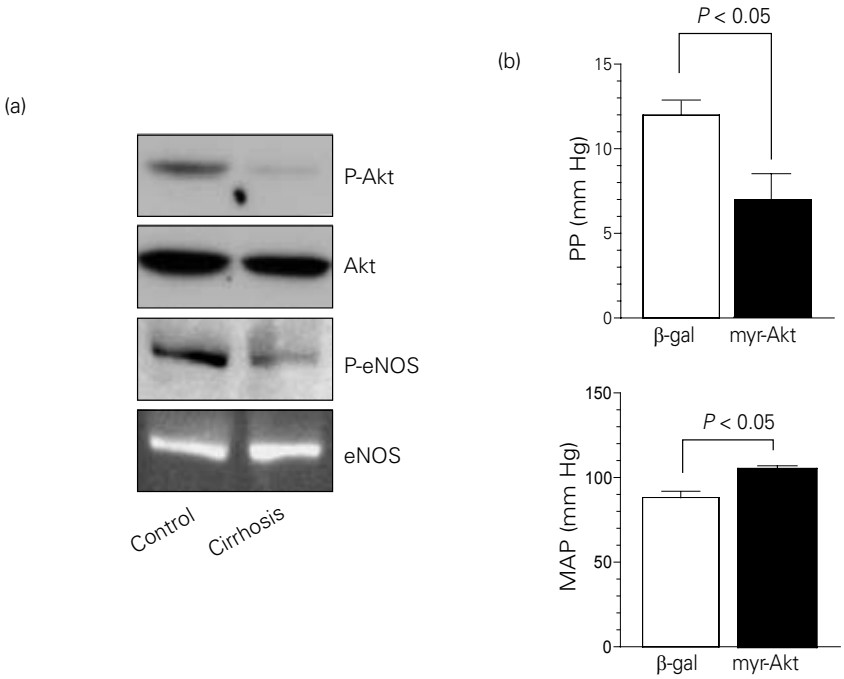


**Figure 8.3** myr-Akt transduction of cirrhotic livers reduces portal pressure and increases mean arterial pressure. (a) Liver tissue from control rats and cirrhotic rats were subjected to immunoblot analysis with anti-phospho-Akt, anti-total Akt, anti-phospho-eNOS, or anti-total eNOS specific antibodies. Representative Western blot analyses showed impaired Akt and eNOS activation in the liver of cirrhotic rats. (b) Transduction of myr-Akt into the liver of cirrhotic animals produced a 40% decrease in portal pressure (PP) and a significant increase in mean arterial pressure (MAP) compared with β-galactosidase-transduced cirrhotic rats. Reprinted with permission (59).

NO level which is significantly related to oxygenation improvement in cirrhotic patients.

Experimentally, the role of NO in the pathogenesis of the hepatopulmonary syndrome has been investigated in BDL rats (66). These animals develop intrapulmonary vasodilation associated with increased circulating levels of NO and blunted pressor response to phenylephrine in isolated pulmonary artery rings. This impaired vasoconstrictor response in pulmonary arterial vessels is reversed by NOS activity inhibition (66), thus pointing to NO as an important mediator of the pulmonary vasodilation observed in BDL rats with hepatopulmonary syndrome. However, recent studies conducted in isolated lungs, instead of isolated vessels, of BDL rats did not confirm these findings. The blunted hypoxic pulmonary vasoconstriction characteristic of these animals was not reversed by either NOS activity inhibition with L-NNA or sGC activity inhibition with 1*H*-[1,2,4]oxadiazolol[4,3-α]quinoxaline-1-one (ODQ) (67).

Conflicting results have also been obtained regarding the NOS isoform responsible for the increased NO production in the lungs of BDL rats. Fallon *et al.* (66) found a strong relationship between intrapulmonary shunting and lung eNOS protein overexpression in rats with biliary cirrhosis. In this study no differences were detected in pulmonary iNOS protein expression between cirrhotic and control animals. Increased expression of eNOS in BDL rats with pulmonary vasodilation has been also described by Carter *et al.* (67), although two other investigations did not find any differences in either pulmonary mRNA messenger or protein eNOS expression (68,69). In contrast, it has been shown that the increased exhaled NO in BDL rats is mainly related to increased production by pulmonary intravascular macrophages expressing iNOS, together with a moderate increase in pulmonary expression of eNOS (70).

## The heart and nitric oxide in cirrhosis

The heart is another functionally compromised organ in cirrhotic patients, but whether this is a consequence of arterial vasodilation or there is a specific cirrhotic cardiomyopathy has been the subject of extensive discussion (71). Cardiac function abnormalities in cirrhosis are not apparent. Cirrhotic patients display a reduction in right ventricular volume, which is probably secondary to the reduced venous return from the systemic circulation and, in addition, they have increased left ventricular preload and volume, which is an indication of left ventricular dysfunction (72,73). This abnormality may remain latent because of low vascular resistance or, in other words, reduced afterload. The existence of an abnormal ventricular behavior can, however, be unveiled during exercise, since it has been demonstrated that left ventricular end-diastolic pressure increases and the stroke index and the left ventricular ejection fraction decrease when challenging these patients with physiological or pharmacological stress (74,75). Moreover, cardiac structural abnormalities, including increased left ventricle thickness, have also been described (76).

Early investigations by Van Obbergh *et al.* (77) in BDL rats showed that isolated working heart preparations of

these animals present decreased coronary pressure and contractility in comparison with control rats. Moreover, nonspecific NOS activity inhibition with L-NMMA significantly increased these values in cirrhotic but not in control rats. This study is, therefore, the first experimental indication suggesting a role for NO in the blunted ventricular contractility frequently found in cirrhosis. In line with these results, Liu *et al*. (78) more recently observed that L-NAME treatment restores the depressed isolated left ventricular papillary muscle contractile response to isoprenaline in BDL rats, without any significant effect in control animals. This abnormal ventricular muscle contractile response was associated with increased ventricular iNOS, but not eNOS mRNA and protein and soluble cGMP content. This investigation, therefore, demonstrated that activation of the inducible NO metabolic pathway occurs in biliary cirrhotic rats and supports the concept that increased NO production might play a role in the pathogenesis of cirrhotic cardiomyopathy. However, whether this is a specific characteristic of hearts from cirrhotic BDL rats or it is a general phenomenon also occurring in other experimental models of cirrhosis and in the heart of patients with advanced liver disease still remains to be elucidated.

## Renal dysfunction and nitric oxide in cirrhosis

Advanced liver disease is accompanied by a cohort of renal function abnormalities, including enhanced tubular sodium and water reabsorption, impaired glomerular function and marked renal vasoconstriction (79). The intensity of these disturbances varies widely from patient to patient and they are closely related to the severity of the liver disease. They are, however, related to the origin of ascites formation and, eventually, to the development of the hepatorenal syndrome, which is a major cause of death in cirrhosis (79).

The results of numerous functional investigations have demonstrated that NO is an important mediator of the renal excretory and hemodynamic alterations in cirrhosis (80,81). In fact, the renal excretion of nitrates and nitrites is increased in cirrhotic rats (82) and acute NOS activity inhibition ameliorates renal excretory function in these animals (13,80,82). Chronic inhibition of NOS activity also improves sodium and water excretion (25,83). These effects are mediated by the increase in blood pressure resulting from the inhibition of systemic vascular NOS but also by a direct intrarenal effect, since the administration of NOS inhibitors to cirrhotic rats at doses not affecting arterial pressure also ameliorates diuresis and natriuresis (80,84). Taken together these findings strongly suggest that increased synthesis of NO occurs in the kidneys of rats with experimental cirrhosis. However, the studies aimed at characterizing the NOS isoforms involved in this phenomenon are more controversial. Although early work by Kanwar *et al*. (85) showed decreased $Ca^{2+}$-dependent and -independent renal NOS activity in BDL rats, most recent investigations show increased iNOS mRNA and/or protein expression in the kidneys of rats with biliary cirrhosis (86,87). In contrast, investigations performed in $CCl_4$-induced cirrhosis and ascites rats have clearly demonstrated that the renal tissue of these animals overexpresses eNOS mRNA in comparison with control rats. Moreover, higher eNOS protein abundance was found in cirrhotic than in control kidneys, which was mainly located by immunological staining in the endothelial lining of the renal arterioles (88). These findings are in keeping with functional investigations by Garcia-Estañ *et al*. (89) showing that acetylcholine, a NO-dependent vasodilator acting through the eNOS pathway, induces a higher renal vasodilator response in cirrhotic rats with ascites than in control animals.

The improvement in renal excretory function observed in most studies inhibiting NOS activity in cirrhotic rats laid the rationale to assess whether NO inhibition may improve renal function in human cirrhotics. In a randomized, placebo-controlled, crossover study, La Villa *et al*. (46) examined the hemodynamic renal and neurohormonal effects of the acute inhibition of NOS activity in a group of seven patients with compensated cirrhosis. L-NMMA administration significantly increased blood pressure, systemic vascular resistance, renal blood flow, glomerular filtration rate, and sodium excretion. The authors attributed the favorable effects on renal function produced by L-NMMA to the fact that NOS inhibition improved arterial blood volume, thereby ameliorating renal perfusion and sodium excretion. More recently, however, Thiesson *et al*. (47) failed to confirm these findings. Although in this study L-NMMA administration increased blood pressure and dose-dependently decreased renin and angiotensin II, sodium and water excretion did not improve, probably because of a significant decrease in renal blood flow. Therefore, additional clinical studies should be carried out to define whether NOS inhibitors might be useful in the treatment of ascites in cirrhosis.

## Spontaneous bacterial peritonitis, circulatory failure and nitric oxide in cirrhosis

Spontaneous bacterial peritonitis (SBP) is a common complication of patients with advanced liver disease (90,91). In spite of the efficacy of the current antibiotic therapy in resolving peritoneal infection, the mortality rate in cirrhotic patients with SBP still remains high. The pathogenesis of this phenomenon is unclear, although it seems to be related to the development of an extreme arterial vasodilation resulting in oliguria, marked sodium retention, pronounced overactivity of the renin–angio-

tensin and sympathetic nervous systems, and renal failure (92).

There are several studies indicating that NO is produced in the peritoneal cavity of cirrhotic patients. Actually, ascites contains significant quantities of nitrates and nitrites (35), being the highest levels found in patients with SBP (93). Bories *et al.* (94) also described that, different from what is observed in patients with other infections, NO concentration in ascites is higher than that found in serum of cirrhotics with SBP at the onset of bacterial infection. In addition, they suggested that SBP leads to a long-lasting local production of NO. In agreement with these investigations, studies performed in our laboratory (43) showed higher concentrations of nitrates and nitrites in ascitic fluid of patients with SBP than in those without bacterial infection. Moreover, we also found that peritoneal macrophages from patients with cirrhosis and ascites when cultured *in vitro* release significant amounts of $NO^-_2$, while cells from non-infected cirrhotics do not. Furthermore, peritoneal macrophages from cirrhotic patients with SBP, which were immediately harvested after therapeutic paracentesis, express iNOS mRNA and protein, while those obtained from ascites of patients without peritonitis do not (Fig. 8.4). Expression of the iNOS enzyme in peritoneal macrophages of patients with SBP has also been observed by Bories *et al.* (95), who also proposed that IFN-γ plays a determinant role in upregulating NO production, particularly under conditions of infection. Therefore, it is conceivable that peritoneal macrophages producing large amounts of NO at the site of infection may contribute to the accentuation of the splanchnic vasodilation in cirrhotic patients with SBP. However, since experimental studies have shown that the intraperitoneal administration of a selective iNOS inhibitor to cirrhotic rats with ascites provokes bacterial peritonitis (44), it is also possible that the induction of iNOS contributes to the control of SBP or its associated pathology in human cirrhosis. The two possibilities are not mutually exclusive.

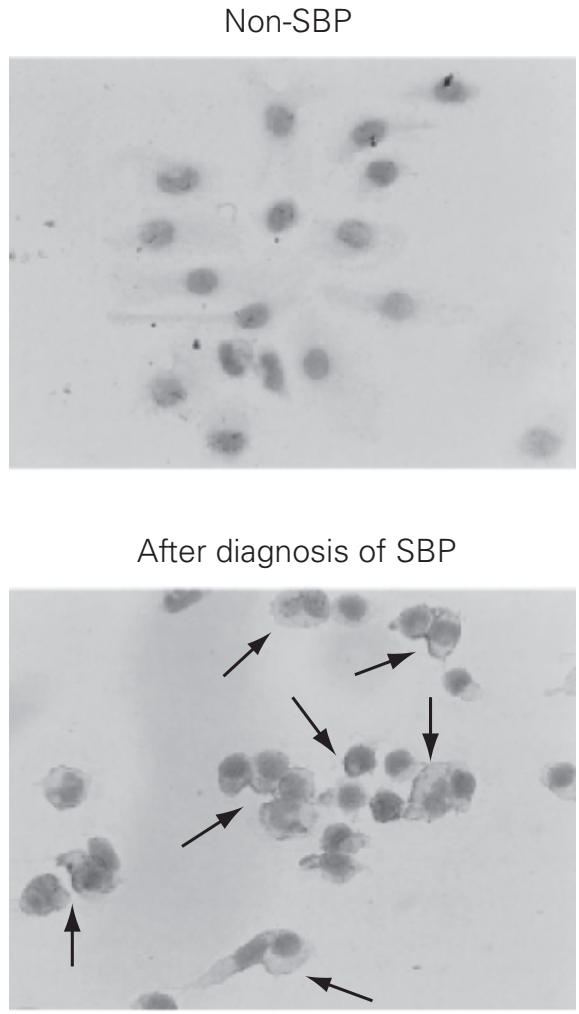


**Figure 8.4** Immunocytochemical localization of iNOS protein in human peritoneal macrophages. Cells were obtained by therapeutic paracentesis, centrifuged, and seeded on slides. Adherent cells were fixed and stained for iNOS using a specific monoclonal antibody. From ref. (43), reprinted with permission.

## New therapeutic strategies for cirrhosis based on tissue specific nitric oxide availability

Experimental models of cirrhosis and portal hypertension have improved our understanding of the pathophysiological role of NO (Table 8.1). There is now a strong body of evidence supporting the concept of increased NOS activity in the systemic and splanchnic areas of both cirrhotic patients and rats with experimental cirrhosis and/or portal hypertension. These results raise the possibility that the use of specific NOS inhibitors could be explored as treatment for the hyperdynamic state in cirrhosis. However, as seen in other sections, in addition to inducing vasodilation, NO also possesses other biological functions, one of the most relevant being its bactericidal activity (44). Thus, the attempts to inhibit any NOS isoform must be carefully considered. This situation substantially differs from the defective eNOS activation described in cirrhotic livers where the restoration of intrahepatic eNOS activity may be associated with undesirable side effects such as the aggravation of systemic vasodilation, as observed with the use of conventional NO-donors (96). Several pharmacological and gene therapy approaches have been followed to specifically deliver NO to this organ. These new therapies include the use of liver-targeted NO donors, such as the NO-releasing derivative of ursodeoxycholic acid NCX-1000 (97), and the hepatic gene transfer of nNOS (98), eNOS (99) or a constitutively active

**Table 8.1** Overview of the pathophysiological role of tissue-specific NO production in cirrhosis.

| Tissue/organ | NO production | Mechanism | Pathophysiological consequences |
|---|---|---|---|
| Splanchnic and systemic vasculature | Elevated | Increased eNOS activity by Akt phosphorylation, shear stress and Hsp90 interaction<br>Increased eNOS protein expression | Vasodilation<br>Increase in splanchnic blood flow<br>Vascular remodeling<br>Angiogenesis? |
| Liver | Decreased | Impaired eNOS activation due to increased eNOS–caveolin-1 association and impaired Akt activity | Increase in intrahepatic resistance |
| Lung | Elevated | Macrophage expression of iNOS<br>Pulmonary overexpression of eNOS | Pulmonary vasodilation |
| Heart | Elevated | iNOS expression | Decreased coronary pressure and contractility<br>Abnormal ventricular contractile response |
| Kidney | Elevated | iNOS and/or eNOS protein expression | Decreased renal blood flow, glomerular filtration rate and impaired excretory function |
| Peritoneal cavity | Elevated | Increased iNOS expression in peritoneal macrophages | Microbicidal activity<br>Splanchnic vasodilation? |

mutant of Akt (59). Although all these methods have successfully reduced portal pressure in experimental models of cirrhosis, the relevance of these new therapeutic approaches in cirrhotic patients remains to be determined. Moreover, some conflictive issues should be solved before the promising translation of any of these treatments to patients. For example, in contrast to the results obtained in the transduction of a wild-type form of eNOS, BDL rats transduced with a mutant form of eNOS that mimics phosphorylated eNOS did not display reduced portal pressure (55). These findings are difficult to reconcile and raise the need for agreement between different methods of transgene delivery, type of viral vectors and the dose to be used. Indeed, studies of the precise molecular mechanisms involved in the efficiency of viral uptake or viral cell targeting in each experimental model of cirrhosis would be desirable.

Further research should provide a solid basis for therapeutic approaches to specifically supplement NO or activate eNOS in cirrhotic livers and suppress splanchnic and systemic NO overproduction, without affecting the immunological response to bacterial infection. We predict that the combination of all these theoretical concepts in the design of new therapeutic approaches for the treatment of cirrhosis will produce superior results to those obtainable with the consideration of only a single pathophysiological role of NO in cirrhosis.

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# Chapter 9
# Endothelin and Systemic, Renal, and Hepatic Hemodynamic Disturbances in Cirrhosis

Veit Gülberg and Alexander L. Gerbes

## Introduction

Hemodynamic alterations in cirrhosis, namely the hyperdynamic circulatory state, were detected more than 50 years ago (1). However, it took several decades until the current concept of the role of circulatory abnormalities in the pathogenesis of ascites formation and renal dysfunction in liver disease was elaborated (2). A scholarly review of the key features of these hemodynamic alterations and their consequences is provided in Part 3 of this book. Briefly, generalized hyperdynamic circulation is seen in the splanchnic and systemic vascular beds in cirrhosis. The pathophysiological basis for the hyperdynamic circulation is controversial; however, the initiating event appears to be the development of systemic vasodilation (3): vasodilation activates neurohumoral systems of sodium retention and renal vasoconstriction and subsequently leads to an increase in intravascular volume. These pathophysiological events manifest clinically as increased cardiac output, reduced mean arterial pressure, and reduced systemic vascular resistance, considered to precipitate ascites formation and hepatorenal syndrome (HRS) (4).

Since peripheral vasodilation is considered the primary event in circulatory dysfunction of cirrhosis, the role of the vascular endothelium in regulation of hemodynamics in cirrhosis has gained much attention throughout recent years. Indeed, endothelial dysfunction can be observed in chronic liver disease, comprising enhanced vascular permeability (5), increased release of endothelium-derived vasoactive substances such as nitric oxide (6), hyporeactivity to endogenous vasoconstrictors (7,8), and increased vascular compliance (9). Therefore, endothelin (ET), an endothelium-derived vasoactive peptide, attracted attention as a possible contributor to systemic, renal, and hepatic hemodynamic disturbances in cirrhosis. ET isopeptides and their receptors are widely expressed in a variety of tissues such as vessels, heart, lung, kidney, central nervous system (10), and the liver (11,12). Besides their vasoactive properties, namely vasoconstriction and vasodilation, these peptides exert numerous biological activities such as bronchoconstriction and hormone release, increase inotropy (13), and stimulate mitogenesis of fibroblasts (14) and vascular smooth muscle cells (15).

The present chapter summarizes in brief the biology of ETs and ET receptors, reviews the vascular effects of ETs and delineates the potential role of ETs in systemic, renal, and hepatic hemodynamics in cirrhosis of the liver.

## Endothelin genes, isopeptides, and receptors

In 1988, a vasoconstrictor substance was isolated by Yanagisawa *et al.* from the pig arterial endothelial cell culture and called ET-l (16). It is a peptide composed of 21 amino acids with two disulfide bonds between amino acids l and 15 and 3 and 11. The loss of these bonds leads to the reduction of biological activity. ET is processed by cleaving 164 amino acids from the 203-amino-acid pre-pro-endothelin by means of specific endopeptidases, resulting in big endothelin (39 amino acids). Big endothelin is subsequently converted to ET by an endothelin-converting enzyme. In man, the ET gene encoding 212-amino-acid prepro-ET is localized on chromosome 6 and consists of 5 exons and 4 introns. In subsequent studies further endothelin isomers, referred to as endothelin-2 (ET-2) and endothelin-3 (ET-3), were identified (17). ET-2 is very similar to ET-1, while ET-3 differs from ET-1 at six out of 21 positions. These isomers are encoded by three independent genes. They differ in their chemical structure and potency of smooth muscle contracting effect (17). All endothelin isomers (ET-l, ET-2, ET-3) demonstrate structural similarity to toxic venom (sarafotoxins) produced by scorpions and snakes. ET-1 is produced mainly by endothelial cells, vascular smooth muscle cells, and, to a lesser extent, by astrocytes and neurons in the central nervous system, Sertoli cells, mesangium, and hepatocytes. ET-2 is mainly produced within the kidney and intestine, whereas the highest levels of ET-3 are found in the brain, where it is probably involved in neuronal function regulation. The factors stimulating endothelin production by endothelial cells include mechanical

stimulation of the endothelium (shear stress), thrombin, calcium ions, vasoactive hormones, cytokines, growth factors, endotoxins, and low-density and high-density lipoproteins. The substances inhibiting endothelin synthesis comprise nitric oxide (NO), cGMP, atrial natriuretic peptide (ANP), prostacyclin ($PGI_2$), and bradykinin.

## Regulation of endothelin gene expression

The human ET-1 gene contains five exons, four introns, and 5′- and 3′-flanking regions, and spans approximately 6.8 kb of DNA. Each of the five exons encodes a portion of prepro ET-1. The primary transcription start site in endothelial cells was mapped by S 1 nuclease protection to a position 98 base pairs downstream from the CAAT box and 31 base pairs from the TATAA box (17). Several characteristic regulatory elements are found within the ET-1 gene: motifs of the consensus binding sequence for the transcription factor nuclear factor-1 (18), four copies of the acute-phase reaction regulatory element, and sequences highly homologous to the consensus AP-1/Jun-binding site (17,19). The presence of an AP-1/Jun-binding site in the 5′-flanking region could explain the rapid induction of ET-1 mRNA following treatment of endothelial cells with phorbol ester. The regulation of expression of ET-1 mRNA in cultured endothelial cells has been studied extensively. Increased mRNA levels have been observed after treatment of the cells with growth factors and cytokines such as thrombin (20), transforming growth factor (TGF)-β (21), tumor necrosis factor (TNF)-α (22), interleukin (IL)-1 (23), and insulin, or with vasoactive substances (24,25) such as norepinephrine, angiotensin II (AII), vasopressin, bradykinin, and oxidized or acetylated low-density lipoprotein (LDL). The increase in expression of ET-1 mRNA induced by thrombin, AII, and vasopressin has been shown to be promoted by an increase in intracellular $Ca^{2+}$ concentration and activation of protein kinase C (PKC) via GTP-binding protein following stimulation of their receptors (20,26). Although no shear stress responsive element was detected in the ET-1 gene, mRNA expression and ET production in endothelial cells are regulated by fluid shear stress: high shear stress sharply decreases mRNA levels (27,28), whereas low shear stress increases ET-1 mRNA expression (29). Pulsatile stretch also causes enhanced production of ET-1 in endothelial cells (30). Shear stress appears to regulate ET-1 gene transcription via an upstream *cis* element by a distinct mechanism not dependent on PKC or cAMP pathways (31). In contrast, the expression of ET-1 mRNA is inhibited by nitric oxide, prostacyclin, and ANP, presumably via cGMP-mediated inhibition of phosphatidylinositide metabolism (32). Heparin also decreases ET-1 mRNA expression via inhibition of PKC (33). An important feature of ET-1 mRNA, which is conserved among different species, is the presence of two AUUUA sequences in the 3′-untranslated region. These sequences are known to mediate selective mRNA degradation and probably account for the short half-life of the message.

## Endothelin receptors and signal transduction

The existence of two distinct ET receptors was suspected from the observation that ET-1 is more potent than ET-3 in inducing vasoconstriction while both peptides elicit equipotent vasodilatory effects. This was confirmed by the identification and cloning of the $ET_A$ receptor (vasoconstrictor) and the $ET_B$ receptor (vasodilator), which belong to the superfamily of G protein-coupled receptors (34,35). In addition, a third receptor, $ET_C$, has been cloned whose function and distribution are largely unknown (36). So far, the latter has only been found in *Xenopus laevis* and no evidence of the expression of this receptor exists in vertebrates.

These receptors exhibit distinct selectivity for ET isopeptides. The $ET_A$ receptor binds ET-1 and ET-2 with a higher affinity than ET-3 and the $ET_B$ receptor displays similar affinities for all three isopeptides, while the $ET_C$ receptor is selectively activated by ET-3 (34,35). Like ET isopeptides, distribution of $ET_A$ and $ET_B$ receptors is wide and overlapping (35). In the last couple of years, the increasing availability of specific agonists and antagonists of $ET_A$ and $ET_B$ receptors has enabled discrimination between $ET_A$ and $ET_B$ functions in a given tissue. In addition, heterogeneity of $ET_B$ receptors has recently been postulated, and a current hypothesis holds the existence of two putative $ET_B$ receptor subtypes, $ET_{Bl}$ and $ET_{B2}$, which differ in their selectivity towards some agonists and antagonists and appear to elicit specific biological effects (37). Accordingly, $ET_A$ and putative $ET_{B2}$ receptors may induce contraction of vascular smooth muscle cells, while putative $ET_{Bl}$ receptors of endothelial cells promote vasodilation through production of NO or prostacyclin (37). It is now clear that the biological effects of ET are mediated by activation of diverse transduction pathways, which probably contribute to the isopeptides' diversity of the responses triggered by ET (35). Binding of ET to both $ET_A$ and $ET_B$ receptors leads to an increase in cytosolic $Ca^{2+}$ that may involve at least three mechanisms: an initial release of $Ca^{2+}$ from intracellular stores with resultant production of inositol phosphate 3 (IP3) by activated phospholipase C, followed by a rapid influx of extracellular calcium through calcium channels and an inhibition of the active extrusion of $Ca^{2+}$ from the cell (38). Regulation of cyclic AMP levels by ET is not universal and displays cell specificity. Stimulation of $ET_A$ receptors increases cAMP in vascular smooth muscle cells and rat anterior pituitary gland (39,40), while $ET_B$ receptors are associated with an inhibition of the adenylyl cyclase system in rat kidney epithelial cells, rat inner medullary collecting duct and bovine endothelial cells (40–42). Opposite observations have also been reported, with ET binding leading to inhibition of adenylyl cyclase in rat brain cap-

illary endothelial cells and rat myometrium (43,44), and $ET_B$ stimulation resulting in increased synthesis of cAMP in iris sphincter of smooth muscle cells (45). Activation of phospholipase A2 by ET constitutes an additional transduction pathway of the peptide in specific cell types and has been demonstrated following stimulation of either $ET_A$ (46,47) or $ET_B$ receptors (46,48), resulting in the production of arachidonic acid, with subsequent release of various eicosanoids, such as prostaglandins, leukotrienes, and thromboxanes. Calcium-dependent stimulation of constitutive NO synthase occurs following activation of $ET_B$ receptors in endothelial cells and mesangial cells, and leads to the generation of NO (49,50). Finally, it has been shown that ET may activate membrane-associated proteins such as ras, Grb2, or $pp60^{src}$ that are known to initiate phosphorylation cascades leading to early gene expression and cell proliferation (51,52).

## Vascular activity of endothelins

Endothelins, especially ET-1 which binds to both $ET_A$ and $ET_B$ receptors with similar affinity, exert a dual vascular activity (Fig. 9.1). At pharmacological doses ET-1 induces a strong and long-lasting vasoconstriction after initial vasodilation (16). The *in vivo* hemodynamic responses to intravenously injected ET-1 are complex, depending upon the vascular bed, and include both direct vasoconstrictor and indirect effects, such as endothelium-mediated vasodilation, and reflex mediated responses. For instance, ET-1 produces a significant decrease in cardiac output irrespective of possessing a direct positive inotropic action (53). This might result from a decrease in venous return due to severe vasoconstriction of large veins and cardiac ischemia due to extreme coronary vasoconstriction. ET-1 exerts vasoconstrictor and vasodilator actions through stimulation of $ET_A$ receptors in vascular smooth muscle and $ET_B$ receptors in endothelial cells, respectively, in principle. However, $ET_B$ receptors also contribute to vasoconstriction in some blood vessels, e.g. mesenteric (54) and coronary (55) vasculature. Further, the relative importance of ET receptor subtypes in vascular tissues differs among species and among vascular beds even in the same animal. For example, the renal vasoconstriction by ET-1 is mediated by a mixed population of $ET_A$ and $ET_B$ receptors in rats (56) but mostly by $ET_A$ receptors in dogs (57) and humans (58). Similarly, constrictions of pulmonary artery are mainly mediated by $ET_B$ receptors in rats (59) and rabbits (60), whereas $ET_A$ receptors predominate in guinea-pig (61) and human blood vessels (62).

Since ET-1 is endogenously synthesized and secreted in adjacent vascular endothelial cells or epithelial cells, it seems reasonable that ET-1 is a physiological regulator of vascular and/or other smooth muscle tone. Surprisingly, heterozygous knockout of the ET-1 gene results in moderately increased blood pressure in mice (63). A similar elevation of blood pressure was obtained in mice with heterozygous disruption of the $ET_B$ receptor (64,65) and in wild-type mice after treatment with BQ-788, an $ET_B$-specific receptor antagonist (64). Thus, the $ET_B$ receptor seems dynamically involved in the maintenance of systemic blood pressure in animals (66) and total peripheral vascular resistance in humans (67). All these studies suggest an important role for $ET_B$ receptors in the negative feedback control of the endogenously

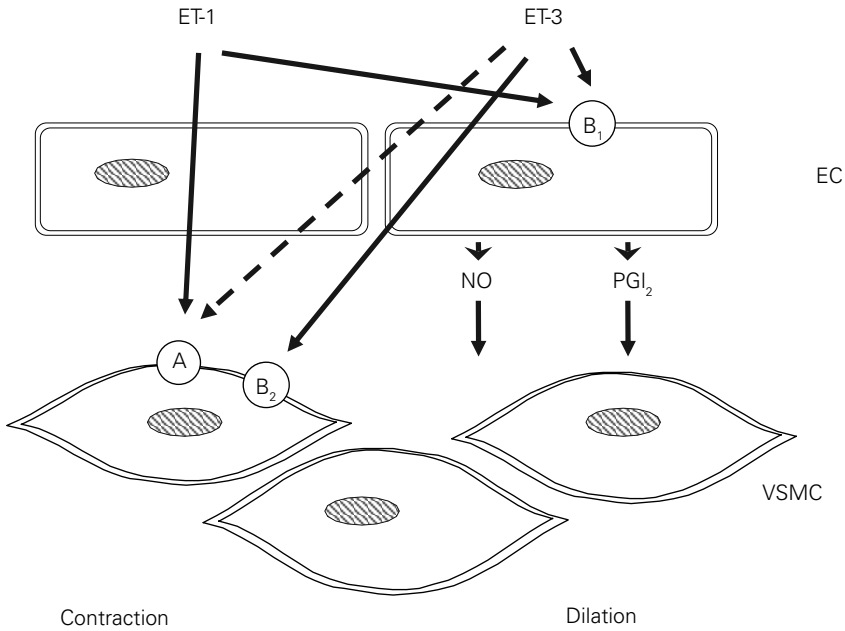


**Figure 9.1** Schematic view of the autocrine and paracrine vascular activities of endothelins: endothelin-1 (ET-1) binds with similar affinity to both $ET_A$ (A) and $ET_{B1}/ET_{B2}$ (B1/B2) receptors. Endothelin-3 (ET-3) has considerably lower affinity towards $ET_A$ receptors but binds to $ET_B$ receptors with similar affinity as ET-1. Activation of ET receptors on vascular smooth muscle cells (VSMC) induces cell contraction, while stimulation of ET receptors on endothelial cells (EC) leads to release of vasodilating mediators such as nitric oxide (NO) or prostacyclin ($PGI_2$). (Adapted from Gülberg V. Endothelin-1 und Endothelin-3 in der Pathophysiologie hämodynamischer Veränderungen bei Leberzirrhose. Doctoral thesis at the Faculty of Medicine, Ludwig-Maximilians University, Munich 1996.)

produced vasoconstrictor ET-1, both in animal models and in humans.

## Endothelin in liver disease

### Endothelin plasma concentrations in cirrhosis

Patients with advanced cirrhosis have elevated plasma ET-1 and ET-3 concentrations (68–71). ET levels are particularly high in those with HRS (68) or portopulmonary hypertension (72). Moreover, plasma ET-l concentrations correlate positively with the severity of liver disease as measured by Child–Pugh score (70,73,74), the hepatic blood flow as determined by the D-sorbitol clearance and the hepatic venous pressure gradient (75). Inverse correlations between ET plasma concentrations have been reported with the functional liver cell mass measured by the galactose elimination capacity (73) and with creatinine clearance (68,71,76), respectively. It has therefore been suggested that ET-1 release may be upregulated in cirrhosis as a compensatory mechanism to maintain arterial pressure (68). However, maneuvers that increase blood volume such as head-out water immersion or volume expansion using saline and albumin infusions failed to decrease circulating ET-1 concentrations in cirrhotic patients (71,77). In addition, changes in posture failed to induce significant changes of ET-1 concentrations in cirrhotic patients with or without ascites (78,79), while only ET-3 increased in patients without ascites when assuming the upright position (78).

### Endothelin production sites in cirrhosis

As mentioned above, elevated arterial and venous plasma concentrations of ET-1 and ET-3 have been described. Interestingly, there is an increased hepatosplanchnic release of ET in these patients (73). Indeed, in the liver of cirrhotic rats increased concentrations of ET-1 peptide (80,81) and, moreover, an increased receptor density have been described (80). As demonstrated by Leivas *et al*. (82), this results from enhanced transcription of the ET-1 gene in the cirrhotic rat liver while the content of ET-1 mRNA does not differ from control animals in other territories such as aorta, lung, and kidney. In addition, the expression of the transcripts of ET-1 (11), as well as

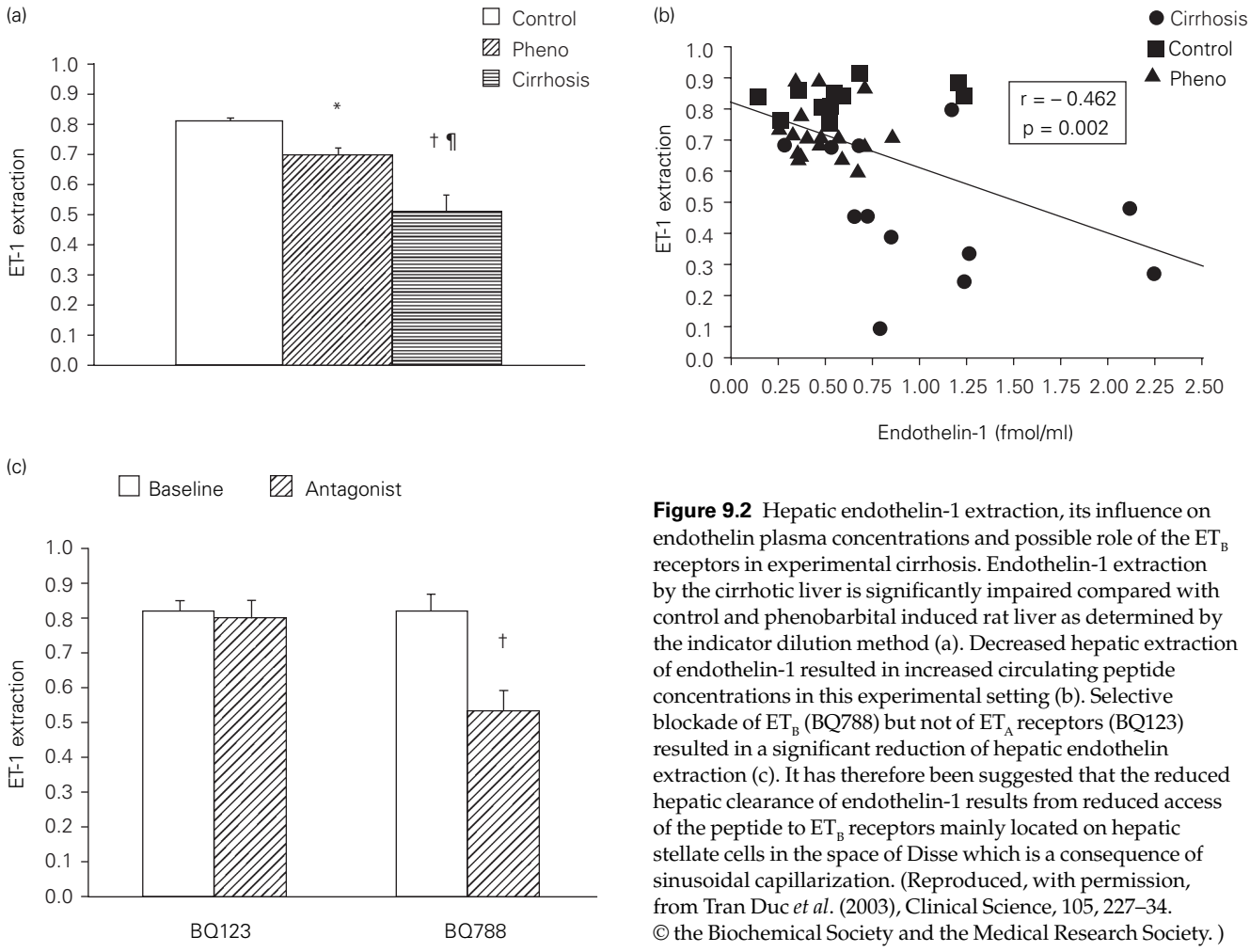


**Figure 9.2** Hepatic endothelin-1 extraction, its influence on endothelin plasma concentrations and possible role of the $ET_B$ receptors in experimental cirrhosis. Endothelin-1 extraction by the cirrhotic liver is significantly impaired compared with control and phenobarbital induced rat liver as determined by the indicator dilution method (a). Decreased hepatic extraction of endothelin-1 resulted in increased circulating peptide concentrations in this experimental setting (b). Selective blockade of $ET_B$ (BQ788) but not of $ET_A$ receptors (BQ123) resulted in a significant reduction of hepatic endothelin extraction (c). It has therefore been suggested that the reduced hepatic clearance of endothelin-1 results from reduced access of the peptide to $ET_B$ receptors mainly located on hepatic stellate cells in the space of Disse which is a consequence of sinusoidal capillarization. (Reproduced, with permission, from Tran Duc *et al*. (2003), Clinical Science, 105, 227–34. © the Biochemical Society and the Medical Research Society. )

$ET_A$ receptor and $ET_B$ receptor mRNA, is significantly enhanced in liver samples of cirrhotic patients (83, Fig. 9.2). In addition, it has recently been shown that cirrhosis reduces hepatic ET-1 clearance, probably by capillarization of hepatic sinusoids and reduced access to $ET_B$ receptors (84, Fig. 9.3).

## Role of endothelin in the pathogenesis of portal hypertension

Expression of ET-1 in isolated liver cells was detected mainly in sinusoidal endothelial cells and stellate cells and to a lesser extent in Kupffer cells. Following liver injury by bile duct ligation, ET-1 mRNA increased mainly in stellate cells and to a lesser extent in endothelial cells (85). These data support the finding of ET-1 overexpression in stellate cells and sinusoidal endothelial cells of human cirrhotic liver (11). Hepatic stellate cells are located in the space of Disse and surround the sinusoidal capillary composed of endothelial cells and Kupffer cells. This anatomical location suggests a role for stellate cells in the regulation of the diameter of liver sinusoids and thus of portal pressure, possibly by endocrine or paracrine effects. ET receptors have been found mainly in stellate cells and sinusoidal endothelial cells, but also in Kupffer cells and hepatocytes (86–88). As shown recently, the ET-induced decrease in the hepatic microvascular blood flow is predominantly mediated by the contraction of stellate cells (87–91). Furthermore, a recent study suggests an effect of ET on hepatic microvascular exchange in cirrhosis, possibly by affecting the fenestration of sinusoidal endothelial cells (92). This might be another mechanism contributing to the decrease of hepatic function in cirrhosis.

ET causes an increase of portal pressure *in vivo* as well as in isolated perfused liver (93,94). In cirrhotic liver, but not in controls, portal pressure decreases upon administration of an ET-receptor antagonist (95). These data suggest a role of ET in modulating portal pressure, particularly in portal hypertension. In liver injury or cirrhosis as well as with ischemia-reperfusion injury, there is Kupffer cell activation as well as an increase in portal pressure. Interestingly, the activation of Kupffer cells seems to contribute to the increase of portal pressure. This is partly mediated

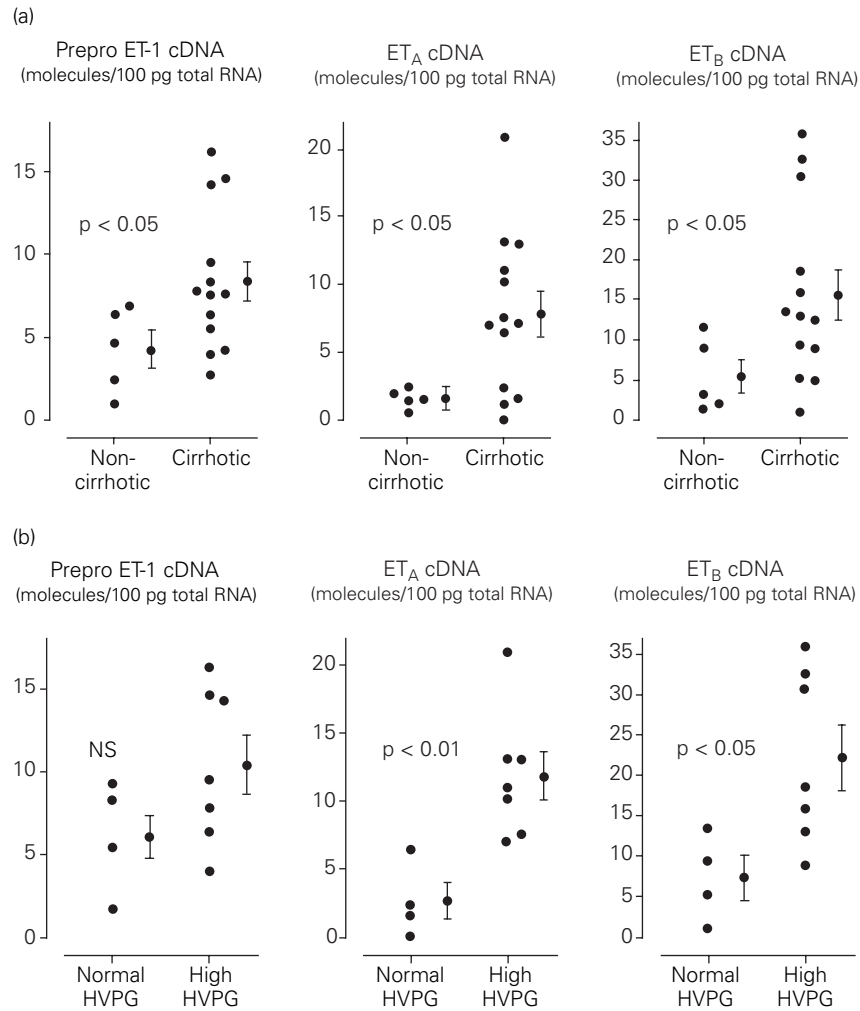


**Figure 9.3** Hepatic expression of endothelin-1 and its receptors in specimen from human livers. Quantitative polymerase chain reaction demonstrated significantly increased gene expression of ET-1 and its receptors in human cirrhotic livers compared with specimen from healthy controls (a). When comparing cirrhotic patients with normal and increased hepatic venous pressure gradients the most striking difference was observed for $ET_A$ receptor expression, followed by $ET_B$ expression, while the difference in prepro ET-1 expression did not reach statistical difference (b). (Reproduced with permission from Leivas *et al.*, J Vasc Res 1998; 35:186–93. Courtesy of S. Karger AG, Basel.)

by ET, as was recently shown in isolated perfused rat liver (96). Therapeutic interventions to ameliorate portal hypertension in experimental cirrhosis probably require blockade of $ET_A$ and $ET_B$ receptors (97). However, effectiveness of this approach is still controversial (98).

## Contribution of endothelins to systemic hemodynamic alterations

As mentioned above, maneuvers that increase blood volume such as head-out water immersion or volume expansion using saline and albumin infusions failed to decrease circulating ET-1 concentrations in cirrhotic patients (71,77). These observations as well as the lack of effect of chronic ET antagonism on systemic hemodynamics in cirrhotic rats (82) raised doubt about the relevance of ET in the regulation of arterial pressure in cirrhosis. However, recently it has been demonstrated that the response to regional infusion of ET-1 is blunted even in patients with compensated cirrhosis and normal systemic hemodynamics (99): blockade of the $ET_A$ receptor resulted in a higher increase of forearm blood flow in these patients compared with that in healthy controls, whereas $ET_B$ antagonism resulted in similar vasoconstriction. Simultaneous blockade of nitric oxide synthase completely abolished the effect of $ET_B$ antagonism in healthy controls, while it was only attenuated in patients with compensated cirrhosis (100). In contrast to these observations, ET-1 infusion induced vasodilation in patients with end-stage cirrhosis at concentrations that caused vasoconstriction both in healthy controls and in patients after orthotopic liver transplantation (101). In decompensated cirrhosis, unlike in patients with well-compensated cirrhosis, $ET_A$ receptor antagonists induced a similar increase of forearm blood flow as in healthy controls. Taking into account that ET-1 is mainly released in a direction to act on adjacent vascular smooth muscle cells and endothelial cells, a significant disturbance of the endothelin system may be assumed already in compensated cirrhosis with almost normal circulating ET plasma concentrations. It is suggested that with deteriorating liver disease the blunted vascular response to ET-1 results in vasodilation and that normal tonic vascular activity to ET can be restored after liver transplantation.

## Role of endothelins in renal dysfunction

In the last decade, a large body of evidence has accumulated indicating that locally produced vasoactive substances, including ET-1, play an important role in the regulation of kidney hemodynamics and its excretory function. The renal medulla is an important site of generation of ET-1 and actually contains the highest concentrations of immunoreactive ET-1 in the body (102,103). Likewise, remarkable amounts of ET-1 are detectable in most renal cell types along the nephron, where they act in proximity to their production site. ET-1 affects three aspects of renal function: (i) hemodynamics of the kidney, (ii) tubular handling of electrolytes and water, and (iii) proliferation and mitogenesis of certain renal cell types such as mesangial cells and vascular smooth muscle cells.

The renal vasculature is highly sensitive to ET-1 activity compared with other vascular beds. ET-1 is the most potent renal vasoconstrictor agent known. Systemic infusion of ET markedly decreases renal blood flow (RBF) as a result of a profound and sustained increase in renal vascular resistance. The sustained renal vasoconstriction results from constriction of the glomerular afferent and efferent arterioles as well as the arcuate and interlobular arterioles. The sustained renal vasoconstriction is often preceded by a transient vasodilatory response, possibly due to $ET_B$ receptor-mediated release of NO (102,104). Glomerular filtration rate (GFR) is also decreased in response to systemic infusion of ET. Similarly, short-term infusion of ET-1 into the renal artery decreases RBF, GFR, and urinary flow rate (102). Long-term infusion of ET-1 into conscious dogs resulted in increased renal vascular resistance and decreased renal perfusion/filtration (102,105). In most clearance studies, the adverse hemodynamic effects of ET-1 were associated with decreased sodium excretion, which was attributed to the high doses of ET-1. A systemic infusion of ET-1 at high doses resulted in a profound antidiuretic and antinatriuretic response, apparently secondary to the decreased RBF and GFR. However, when ET-1 was administered at low doses it induced diuretic and natriuretic effects (102).

In this context, accumulating evidence suggests that the stimulatory effects of ET-1 on water and sodium excretion are mediated through $ET_B$ receptors. First, pretreatment of rats with a highly selective $ET_B$ antagonist significantly abolishes the diuretic and natriuretic responses induced by big ET-1 (106). This finding is in line with several reports that demonstrated high abundance of $ET_B$ receptors in the renal medullary collecting duct epithelium, the main inhibitory site of ET-1 action on sodium and water reabsorption. Second, activation of $ET_B$ receptors stimulates the release of NO, which plays a crucial role in the regulation of renal hemodynamics and excretory function (107). Indeed, inhibition of NO production coupled to $ET_B$ activation has been shown to reduce sodium excretion and suppress the pressure-natriuresis response and the diuretic and natriuretic responses induced by big ET-1. Moreover, it has been shown that ET-1 acts through $ET_B$ receptors to induce transient medullary vasodilation, which may contribute to the diuretic/natriuretic actions of locally produced ET-1 in the renal medulla (107).

Since the renal ET system may contribute to the body fluid volume and electrolyte balance, it has been suggested that perturbation in this system may also add to renal dysfunction in cirrhosis and HRS (108). Several

observations seem to support this contention: thus the highest circulating concentrations of ET-1 and ET-3 have been reported in patients with HRS (68). In addition, the inverse correlation between indicators of renal function and circulating ET concentrations are in line with this assumption (68,71,75,76,78). To date, however, there is no evidence for enhanced renal synthesis of ET in HRS and studies investigating urinary excretion of ET provide conflicting results (109,110). Remarkably, in a pilot study including three patients with HRS, the selective $ET_A$ receptor antagonist BQ-123 improved GFR without affecting systemic hemodynamics (111).

## Conclusion

In conclusion, there is increasing evidence for a contribution of endothelin to systemic, renal, and hepatic hemodynamic disturbances in cirrhosis. While elevated plasma concentrations of ETs may merely represent the tip of the iceberg in decompensated cirrhosis, vascular hyporesponsiveness to the vasoconstrictive activity can already be observed in well-compensated cirrhotic patients. Several investigations using either selective or nonselective ET receptor antagonists suggest a role of the ETs and ET receptors in systemic vasodilation, portal hypertension, and renal vasoconstriction. However, due to the complex derangement of ET receptors, depending on the vascular territory, it has not been classified whether selective $ET_A$ or $ET_B$ antagonists and nonselective antagonists, respectively, will provide an effective therapeutic approach to the hemodynamic alterations underlying ascites formation and hepatorenal syndrome in patients with cirrhosis.

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# Chapter 10
# Carbon Monoxide and the Heme Oxygenase System in Cirrhosis

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## Heme oxygenase and its products

The term "heme oxygenase" is used to describe a family of structurally related, microsomal proteins that are responsible for the physiological degradation of heme (1–3). In the presence of oxygen and NADPH, these heme oxygenase proteins work in combination with NADPH-ferrihemoprotein reductase (NADPH-cytochrome P450 reductase) to catalyze the enzymatic conversion of heme into carbon monoxide (CO), biliverdin, and ferrous iron (Fig. 10.1) (4). The alpha-meso bridge in the heme macrocycle is the specific target of heme oxygenase. It is this carbon atom that is released as CO, and heme oxygenase is the sole source of CO derived from the physiological breakdown of heme. CO and the two other heme-derived products, biliverdin and ferrous iron, have been shown to have important cellular functions, which are summarized in Fig. 10.1 and in more detail below. New functions of CO continue to be uncovered, and, doubtless, we still have much to learn about this simple yet powerful molecule.

In mammals, three isoforms of heme oxygenase, called HO-1, HO-2, and HO-3, have been described (5–7), all products of separate genes. Some important characteristics of these three isoforms are presented in Table 10.1.

HO-1, which was first described nearly 35 years ago (4), is highly inducible by a number of conditions, as shown in Table 10.2. Although some have speculated that heme induces HO-1 by increasing oxidative stress on cells, several earlier studies indicated that, at least in hepatocytes, heme-dependent upregulation of HO-1 did not occur by a stress pathway, nor in a manner like that of transition metals (2,14,23,56). Thus, for example, stressful perturbations, such as arsenical- or hydrogen peroxide-induced oxidative stress, heat shock, or cobalt ions led to HO-1 upregulation by activating mitogen-activated protein kinase pathways (especially JNK and ERK), whereas heme had no effect (14). Then, too, antioxidants and thiol donors decreased stress-dependent HO-1 induction but had no effect on heme induction (17,56). In addition, cobalt protoporphyrin, which cannot activate oxygen to more damaging reactive oxygen intermediates (ROIs), is nevertheless a highly potent and efficacious inducer of HO-1 (24,25). Recently, Ogawa *et al*. have proposed a novel mechanism by which HO-1 is induced by its substrate heme (57). These, and subsequent

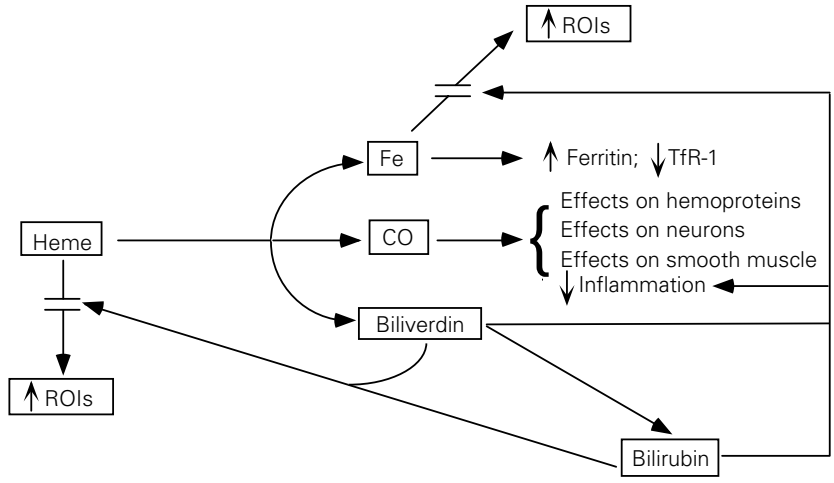


**Figure 10.1** The products of the heme oxygenase reaction and some of their important functions. (Adapted with permission from Ref. (3).) CO, Carbon monoxide; Fe, iron; ROIs, reactive oxygen intermediates; TfR, transferrin receptor.

**Table 10.1** Characteristics of the isoforms of heme oxygenase.

| | Isoforms | | |
|---|---|---|---|
| Characteristic | HO-1 | HO-2 | HO-3 |
| Major function | Catalytic heme degradation and formation of CO, BV, iron | Catalytic heme degradation and formation of CO, BV, iron | Heme sensing/binding protein (?) |
| Inducibility | Highly inducible by many conditions (see Table 10.2) | Constitutive | Uninducible |
| Tissue localization | Ubiquitous, highest in spleen, liver, kidneys | Mainly brain and testes | Ubiquitous, highest in liver, prostate, kidneys |
| Molecular mass of the protein (kDa) | ~ 32–33 | ~ 36 | ~ 32–33 |
| mRNA (number of transcripts/transcript size) | 1 of ~ 1.8 kb (1550 bp) | 2 of ~ 1.3, ~ 1.7 kb (1627, 1636 bp) | *1 of ~ 2.4 kb (~ 2225 bp) |
| $V_{max}$ (nmol BV/mg prot/min) | 56.7 (†580 ± 44) | 4.0 | 0.4 |
| Apparent $Km_{heme}$ (μM) | 0.24 (†3.8 ± 0.5) | 0.67 | ND |

CO, carbon monoxide; bp, Base pairs; BV, biliverdin; kDa, kilodalton; Km, Michaelis constant; ND, not determined; $V_{max}$, maximal velocity. *mRNA transcripts identified in rat (7) †Values for the chick form of the enzyme (8). Other values are for the rat form (9).

(58) results strongly suggest that the heme-mediated expression of HO-1 mRNA is controlled by a heme-binding transcription factor called BACH1. Under conditions of low intracellular heme concentrations, BACH1 forms a heterodimer with small Maf proteins. This heterodimer binds to heme responsive element(s) in the promoter of the HO-1 gene, which represses the expression of HO-1 mRNA. However, when heme concentrations increase, BACH1 binds to this excess heme, and loses its ability to repress HO-1 gene expression.

HO-1 is also strongly induced by heat shock in some systems, and indeed has been called heat shock protein

**Table 10.2** Some conditions that induce heme oxygenase-1.

| Conditions | References |
|---|---|
| Aging (in brain) | Iijima *et al.*, 1999 (10) |
| Angiogenesis/Vascular endothelial growth factor (VEGF) | Fernández and Bonkovsky, 2003 (11); Jozkowiz *et al.*, 2003 (12) |
| Arsenicals—arsenite; phenylarsine oxide | Keyse and Tyrrell, 1989 (13); Elbirt *et al.*, 1998 (14); Lu *et al.*, 2000 (15); Shan *et al.*, 1999 (16); Gildemeister *et al.*, 2001 (17) |
| Ets-family proteins | Deramaudt *et al.*, 1999 (18) |
| Fulminant hepatic failure | Dong *et al.*, 2002 (19) |
| Heat shock | Shibahara *et al.*, 1987 (20); Gabis *et al.* 1996 (21); Elbirt *et al.*, 1998 (14); Yamagami *et al.* 2003 (22) |
| Heme | Lincoln *et al.*, 1988 (23); Gabis *et al.*, 1996 (21), Shan *et al.*, 2000, 2002 (24,25) |
| Hemoglobin, myoglobin | Nath *et al.*, 2000 (26) |
| Hepatic cirrhosis | Wei *et al.*, 2003 (27) |
| Hydrogen peroxide | Keyse and Tyrell, 1989 (13); Elbirt *et al.*, 1998 (14); Fauconneau *et al.*, 2002 (28) |
| Hypoxia, hyperoxia | Lee *et al.*, 2000 (29); Kacimi *et al.*, 2000 (30); Ohlmann *et al.*, 2003 (31); Dennery *et al.*, 2003 (32) |
| Inflammatory cytokines | Fukuda and Sassa, 1993 (33); Strandell *et al.*, 1995 (34) |
| Ischemia/reperfusion | Tachini *et al.*, 1993 (35); Amersi *et al.*, 1999 (36); Mayer *et al.*, 2003 (37) |
| Lipopolysaccharide (LPS) | Camhi *et al.*, 1995 (38); Wiesel *et al.*, 2000 (39) |
| Other metalloporphyrins | Llesuy *et al.*, 1994 (40); Cable *et al.*, 1994 (41); Shan *et al.*, 2000, 2002 (24,25) |
| Phorbol esters | Alam *et al.*, 1995 (42); Lu *et al.*, 2000 (15) |
| Portal hypertension (in splanchnic organs) | Fernández and Bonkovsky, 1999, 2001 (43,44) |
| Prostaglandins | Rossi and Santoro, 1995 (45); Koizumi *et al.*, 1995 (46); Lee *et al.*, 2003 (47); Li *et al.*, 2003 (48) |
| Systemic arterial hypertension | Sabaawy *et al.*, 2001 (49); Ono *et al.* 2003 (50) |
| Thiol scavengers (GSH depletion) | Oguro *et al.*, 1996 (51) |
| Tobacco smoke | Müller and Gebel, 1994 (52); Pinot *et al.*, 1997 (53) |
| Transition metals | Kikuchi and Yoshida, 1983 (54); Lincoln *et al.*, 1988 (23); |
| Ultraviolet light (UV) | Keyse and Tyrrell, 1989 (13); Vile *et al.*, 1994 (55) |

32 (HSP32), reflecting its molecular weight of 32 kDa (13). The human HO-1 gene, known as HMOX1, is located on chromosome 22 and consists of 5 exons and 4 introns (59). Of the three isoforms, HO-1 is the most catalytically active (See Table 10.1 for $V_{max}$ and Km values).

Yachie *et al*. described a patient with an inborn deficiency of HO-1 (60). The patient was 26 months old when he first presented with recurrent fever, a generalized erythematous rash, growth retardation, and marked hepatomegaly. Analysis of the patient's HO-1 gene indicated a loss of exon 2 from the maternal allele and a two-nucleotide deletion in exon 3 of the paternal allele. Both parents were phenotypically normal. A lymphoblastoid cell line derived from the patient was overly sensitive to heme-induced cell injury. Unfortunately, the patient died at age 6 years. Autopsy showed increased iron levels in the liver and kidney, and amyloid deposits in the liver and adrenal glands (61). Overall, this patient showed a more severe involvement of endothelial cells and the reticuloendothelial system than was seen in mice with a targeted disruption of the HO-1 gene, where disruption of iron metabolism is the predominant finding (62,63). These HO-1 knockout mice were highly sensitive to hepatic injury by iron, implying an important role of this enzyme in iron homeostasis, as well as its important metabolic and cytoprotective roles.

In contrast to the highly inducible HO-1, HO-2 appears to be a constitutive enzyme that is found mostly in the brain and testes (Table 10.1). The human HO-2 gene, known as HMOX2, is located on chromosome 16, and also consists of 5 exons and 4 introns (59). Although no human examples of HO-2 deficiency have been reported, HO-2 knockout mice have been described (64). These mice exhibited normal hippocampal long-term potentiation, but showed less responsiveness to electrical stimulation, consistent with a role for CO, generated by HO-2, in the modulation of nonadrenergic, noncholinergic relaxation of myenteric ganglia. These HO-2 knockout mice also exhibited decreased ejaculatory muscle reflex activity, suggesting that CO plays a role in male reproductive behavior (65). When exposed to hyperoxia, these mice showed increased toxicity, probably due to increased pulmonary levels of iron (66). CO generated by HO-2 has been shown to be an endogenous hyperpolarizing factor in the gastrointestinal tract, as evidenced by the findings that HO-2 knockout mice had depolarized smooth muscle cells and the membrane potential gradient in the gut was abolished (67). Thus, CO probably plays an important role in normal gut function.

A third isoform of heme oxygenase, referred to as HO-3, has been described (7). A cDNA, isolated from rat brain, corresponded to a new protein that was closely related to HO-2 (approximately 90% amino acid sequence similarity). When expressed in *Escherichia coli*, the HO-3 protein did not cross-react with polyclonal antibodies to HO-1 or HO-2. It was a relatively poor catalyst for heme degradation (see $V_{max}$ and Km values in Table 10.1) and produced spectra consistent with being a hemoprotein. In a subsequent study, HO-3 was found to be mainly expressed in rat hippocampus, cerebellum, and cortex (68). HO-3 was also expressed in primary cultures of rat type 1 astrocytes. The precise function of HO-3 is unclear, but, given the relatively low ability of this protein to catalyze the degradation of heme, it is likely that it is involved in heme sensing or heme binding. A BLAST search (in January 2005) of the rat HO-3 amino acid sequence showed that its closest match was to mouse HO-2, but no sequences designated as HO-3 were found for any species besides the rat.

The role of heme oxygenase is to catalytically convert heme into equimolar amounts of CO, biliverdin, and ferrous iron, but this function can be viewed from two distinct, but related, perspectives (69). The first view is to consider heme oxygenase as one of the means for controlling the intracellular concentrations of heme, specifically the small, and rapidly turning over, "regulatory" heme pool that is in dynamic equilibrium with the heme that is incorporated into hemoproteins. This regulation is done in coordination with 5-aminolevulinate synthase (ALAS), the rate-controlling enzyme of heme synthesis. When the rate of synthesis of heme is balanced with the rate of degradation of heme (controlled by heme oxygenase), the size of the "regulatory" heme pool is maintained. If the intracellular concentration of heme increases, then HO-1 is induced and ALAS is repressed, until the level of heme returns to normal. The removal of excess heme from the cell is a critical process, because elevated levels of heme are potentially very toxic to cells. However, as described above, induction of HO-1 occurs at lower, relatively nontoxic concentrations of heme.

A second way to view the function of heme oxygenase is from the point of view of generating physiologically important products, in this case, CO, biliverdin, and ferrous iron. CO has structural and biological properties that are similar to nitric oxide (NO), which is now known to be central to a number of signaling pathways and functions (70). Both CO and NO can activate guanylate cyclase, and thus increase the levels of cyclic GMP, resulting in relaxation of smooth muscle (71). When this relaxation occurs in blood vessels, vasodilation results with a corresponding lowering of blood pressure. Relaxation of smooth muscle in the gastrointestinal tract causes relaxation of sphincters and decreased peristaltic activity. However, NO is 50–100 times more powerful as a guanylate cyclase activator than is CO. Thus, when both NO and CO productions are intact, NO effects predominate. CO also has effects that are independent of guanylate cyclase, including its ability to bind tightly to hemoproteins. For example, the binding of CO to a cytochrome P450-dependent epoxygenase results in its inhibition, with a subsequent decrease in the contractility of bile canaliculi (72). CO also affects the central nervous system, causing reductions in systemic blood pressure and production of neuronal

adaptation (73) and retrograde potentiation (74), and the enteric nervous system, as already described.

Biliverdin is a potent antioxidant, as is bilirubin, which is made from biliverdin in most mammals by the action of biliverdin reductase (75,76). The concentrations of these two products of heme degradation are usually sufficiently high to act as defenses against oxidative stress-induced injury to mammalian tissues. Their sites of action, however, are probably determined by their solubilities. Because unconjugated bilirubin is quite soluble in lipids, it is most effective in biological membranes. Biliverdin is more water soluble and thus more likely to inhibit oxidative damage in more aqueous environments.

In contrast to the antioxidant effects of biliverdin and bilirubin, the release of ferrous iron by the heme oxygenase-mediated degradation of heme might result in increased oxidative stress to the cells and tissues, because this iron can potentially act to catalyze Fenton-type chemistry with the production of highly toxic reactive oxygen species (77–80). The increase in intracellular iron also acts to modulate the expression levels of a number of mRNA species, including the divalent cation transporter (DCT-1), ferroportin (IREG), ferritin, transferrin receptor-1, and the erythroid form of ALAS. Such changes, specifically the upregulation of ferritin and the downregulation of transferrin-1 receptors, tend to limit any further increase in iron concentrations, and thus to restrict any further damage due to iron toxicity.

## Heme oxygenase in hepatic cirrhosis and portal hypertension

In the last few years, evidence has accumulated showing that the HO enzymes and their by-products are important players in the pathophysiology of cirrhosis and portal hypertension. For example, increased hepatic HO activity and/or expression of HO-1 mRNA and protein have recently been described in experimental (27) and human hepatic cirrhosis (81,82). Recent data, derived from our studies, indicate that the levels of both the mRNA and protein expression of the inducible HO-1 isoform are increased in the liver and splanchnic organs from rats with portal hypertension due to portal vein constriction, compared with sham-operated control animals (Fig. 10.2) (43). In contrast, the constitutive HO-2 isoform is expressed at similar levels in the liver and splanchnic organs from both experimental groups (Fig. 10.2) (43). In addition, the observed increase in HO-1 protein levels results in a higher HO enzymatic activity in splanchnic organs from portal hypertensive rats (Fig. 10.3) (44). In

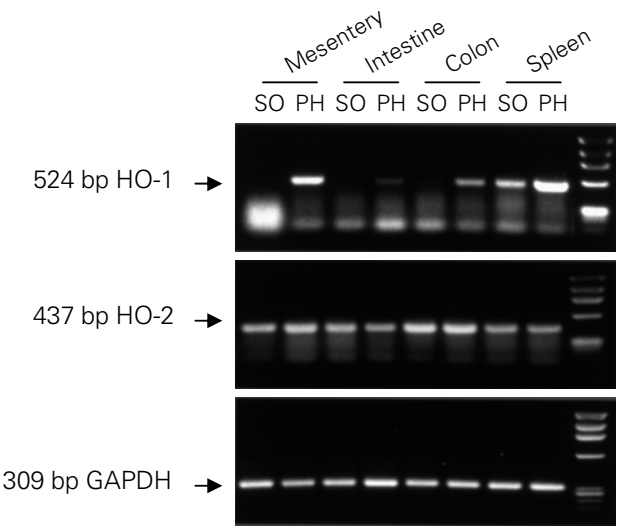


**Figure 10.2** Expression of HO-1 and HO-2 mRNAs in splanchnic organs from portal hypertensive (PH) rats and sham-operated (SO) control animals. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression is shown as a control. Representative of four reverse transcription-polymerase chain reaction (RT-PCR) analyses. (Reproduced with permission from ref. (43).)

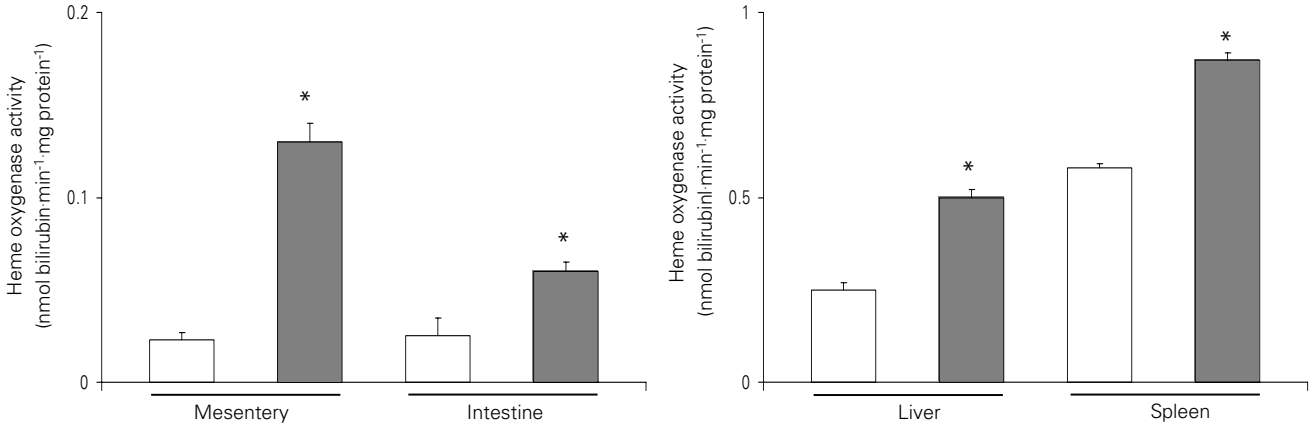


**Figure 10.3** HO enzymatic activity (nmol bilirubin/min/mg protein) in microsomal fractions obtained from the mesentery, intestine, liver, and spleen of portal hypertensive rats (hatched bars) and sham-operated control animals (open bars). Data are shown as mean ± SEM of four to five assays. *$P < 0.05$ vs. sham-operated rats. (Reproduced with permission from ref. (44).)

rats subjected to bile duct ligation (83), there was a progressively increased expression of HO-1 in aorta and mesenteric arteries, compared with sham operated rats. This increase in HO-1 expression coincided with the progression from acute cholestatic liver injury (week 1) to fully developed cirrhosis with intense systemic arterial vasodilation (week 4). Again, no change in the expression of HO-2 was observed. HO-1 over-expression has also been found in the lungs of portal hypertensive rats (84), and in the liver (27) and cardiac ventricles (85) from rats with secondary biliary cirrhosis. In addition, recently it was shown that cirrhotic patients have an increased production of CO, a product of the HO reaction, which increases further in the presence of a severe bacterial infection, such as spontaneous bacterial peritonitis (86).

Taken together, these results suggest the importance of HO-1 in the pathophysiology of portal hypertension and shed light on new avenues for further investigations and development of new therapeutic applications. The precise mechanisms whereby HO-1 gene expression is upregulated in portal hypertension and liver cirrhosis are still unknown. As already described, HO-1 is transcriptionally activated by numerous physical or chemical factors (Table 10.2), some of which may be increased during portal hypertension, including cytokines, endotoxin, and shear stress. In addition, the pathophysiological significance of HO-1 induction in portal hypertension is not yet fully understood. Some of the potential roles of HO-1 in portal hypertension and liver injury are discussed below.

## Heme oxygenase and splanchnic arteriolar vasodilation

The portal hypertensive syndrome is characterized by increased portal blood flow, which is the result of a marked arteriolar vasodilation in splanchnic organs draining into the portal vein (87). Although the mechanisms underlying this splanchnic vasodilation are not fully understood, previous studies have suggested that this could be due, at least in part, to an overproduction of endogenous vasodilators, and to a decreased vascular reactivity to vasoconstrictors. Several lines of experimental evidence indicate that the vasodilator NO (70) plays an important role in the pathogenesis of these hemodynamic disturbances ((88–92) and see Chapter 8).

CO, a product of HO activity, shares many properties with NO. Both CO and NO are involved in the homeostatic control of cardiovascular tone and in the modulation of blood vessel function, including their relaxation and inhibition of platelet aggregation (93). As described above, both NO and CO enhance the activity of soluble guanylate cyclase, leading to a several-fold increase in the production of cyclic guanosine monophosphate (cGMP), although the potency of CO in this regard is about 50–100 times lower than that of NO (94). Other mechanisms of action of CO, independent of cGMP, are also possible, including stimulation of calcium-activated potassium ($K_{Ca}$) channels with subsequent hyperpolarization of smooth muscle cells, inhibition of a cytochrome P450-dependent monooxygenase system, and/or inhibition of endothelin release from endothelial cells (94).

Given the similarities between the chemical structures and biological actions of CO and NO, it is likely that both vasoactive systems contribute to the circulatory abnormalities of portal hypertension. In this regard, we have recently demonstrated that HO-1 plays a role in modulating mesenteric vascular reactivity in portal hypertensive rats (44). Thus, in perfused mesenteric vascular beds, in the absence of NO synthase inhibition, the HO inhibitor zinc mesoporphyrin (ZnMP) had no effect on hyporeactivity to the vasoconstrictor potassium chloride (a depolarizing agent) (Fig. 10.4). Vascular hyporeactivity to potassium chloride was only partially corrected by a NO synthase inhibitor (L-NAME), but it was completely reversed by a combination of ZnMP and L-NAME. In addition, the magnitude of the effect of simultaneous perfusion with ZnMP plus L-NAME on pressure response to potassium chloride was significantly higher than the addition of the effects of ZnMP and L-NAME when perfused alone. This finding could suggest the existence of a synergistic regulatory interaction between the HO and NO synthase systems in mediating the splanchnic hyporeactivity to potassium chloride in portal hypertensive rats. Because ZnMP had no effects in normal animals, the consequences of HO inhibition on vascular reactivity were selective for portal hypertensive rats. When we assessed pressure responses to the vasoconstrictor methoxamine (an $\alpha_1$-adrenergic agonist), we found that the hyporeactivity to methoxamine was unaffected by HO inhibition, but it was completely overcome by NO synthase blockade (44). The effect of simultaneous inhibition of both HO and NO synthase on pressure response to methoxamine was not significantly different from that obtained after blockade of only NO synthase, indicating that HO plays little, if any, role in modulating vasoconstrictive responses to methoxamine in mesenteric vessels of portal hypertensive rats. Taken together, these findings indicate that the mesenteric hyporeactivity to $\alpha_1$-adrenergic stimulation in portal hypertensive rats is mediated mainly by NO synthase, whereas membrane depolarization is synergistically regulated by both NO synthase and HO. These studies also suggest that alterations in the vasoconstriction stimulated by both membrane depolarization and $\alpha_1$-adrenergic activation contribute to the decreased vascular reactivity in portal hypertension. Abnormal depolarizations have been previously observed under some pathological conditions, such as arterial hypertension (95), and hypoxia (96).

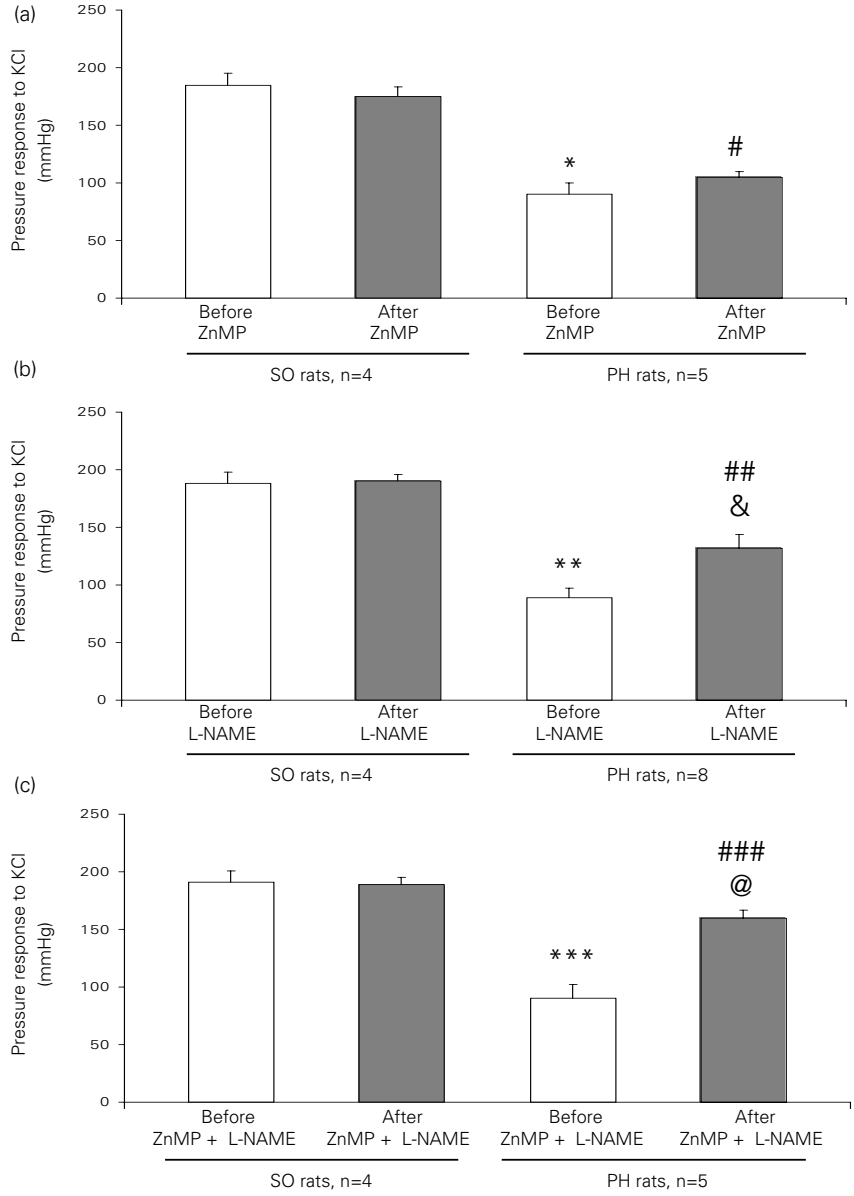


**Figure 10.4** Pressure response to potassium chloride (KCl; mmHg) in mesenteric vascular beds of portal hypertensive (PH) rats and sham-operated (SO) control animals, before (open bars) and after (hatched bars) perfusion with 10 μM ZnMP alone (a), 100 μM L-NAME alone (b), or 10 μM ZnMP plus 100 μM L-NAME (c). Data are shown as mean ± SEM. $^{*}P < 0.01$ vs. SO rats before ZnMP; $^{\#}P < 0.01$ vs. SO rats after ZnMP; $^{**}P < 0.01$ vs. SO rats before L-NAME; $^{\#\#}P < 0.05$ vs. SO rats after L-NAME; $^{\&}P < 0.05$ vs. PH rats before L-NAME; $^{***}P < 0.01$ vs. SO rats before ZnMP plus L-NAME; $^{@}P < 0.01$ vs. PH rats before ZnMP plus L-NAME; $^{\#\#\#}P < 0.05$ vs. PH rats after L-NAME. (Reproduced with permission from Ref. (44).) L-NAME, Nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase; PH, portal hypertensive; SO, sham operated; ZnMP, zinc mesoporphyrin, an inhibitor of heme oxygenase.

It should be noted that depolarizing agents cause contraction of vascular smooth muscle cells by activation of voltage-dependent calcium channels, resulting in calcium entry to the cytoplasm from the extracellular space (97). $\alpha_1$-Adrenergic agonists induce vasoconstriction by generation of inositol triphosphate and calcium release from the endoplasmic reticulum (97). In mesenteric vessels, this $\alpha_1$-adrenergic-stimulated vasoconstriction is independent of changes in membrane potential (98). Therefore, it is conceivable that there might be distinct mechanisms underlying the hyporeactivity to vasoconstrictors in portal hypertension.

The role of HO in modulating mesenteric vascular reactivity in portal hypertension could be related to the fact that this enzymatic system is responsible for the endogenous production of the powerful vasodilator, CO (94,99). Because, as already mentioned, NO is a more potent activator of soluble guanylate cyclase than CO (94), when NO synthase and HO are both active, guanylate cyclase activation is mainly caused by NO. Therefore, it is not surprising that in our studies the effect of HO inhibition on vascular tone was detectable only after inhibition of NO synthase (44). Because CO-induced smooth muscle cell relaxation occurs only if CO is produced close to its target (i.e. soluble guanylate cyclase) (94), it is likely that the upregulated HO-1 involved in modulation of mesenteric vascular reactivity in portal hypertension is located within arterial walls (i.e. in the endothelium or smooth muscle or both) (43).

Rats treated with carbon tetrachloride to produce a chemically-induced cirrhosis had decreased perfusion pressure and lessened response of the superior mesenter-

ic vein to several vasoconstrictors (KCl, phenylephrine, and endothelin-1), compared with untreated rats (100). Treatment of the cirrhotic rats with tin mesoporphyrin (to inhibit HO activity) increased perfusion pressure, and partially restored the constrictor responses to KCl, phenylephrine, and endothelin-1. Stepwise inhibition of CO and NO production resulted in a successive increase in response to vasoconstriction, suggesting that both CO and NO regulate vascular tone in the superior mesenteric vein. In contrast to previous studies (43,44), the level of HO-2 protein was increased, whereas the level of HO-1 protein was unchanged. Rats transfected with an adenovirus containing the human HO-1 gene showed reduced perfusion pressure and lower vasoconstrictor response to phenylephrine. These results support the idea that HO is a critical component in the mechanisms that control splanchnic resistance in carbon tetrachloride-induced cirrhosis in rats.

## Heme oxygenase and increased intrahepatic vascular resistance

Vascular resistance is increased in the cirrhotic liver. This increase formerly was thought to be a fixed, mechanical consequence of the distortion of the hepatic vascular architecture caused by fibrosis, scarring, and nodule formation (87). However, there is increasing evidence that the vascular resistance of the cirrhotic liver can be modified by vasoactive agents, which modulate the responsiveness of several contractile elements located within the hepatic vascular bed, both at the sinusoidal level and at extrasinusoidal sites (101). In this regard, it has long been recognized that the liver's vasculature includes vessels with abundant vascular smooth muscle cells, which are therefore contractile (e.g. small portal venules in portal areas). In addition, hepatic stellate cells are located in the perisinusoidal space of Disse, in close contact with hepatocytes, endothelial cells, and Kupffer cells. These nonparenchymal cells contain contractile proteins, and have the ability to relax in response to locally produced vasodilators, such as NO, and to contract in response to vasoconstrictors, such as endothelin (101). An imbalance between these vasoactive molecules may result in a sustained contraction of stellate cells, and subsequent constriction of sinusoids, which could be partly responsible for the increased hepatic vascular resistance in cirrhosis. In this regard, it has been shown that, in the injured liver, endothelin-1 production is increased (101), and NO synthesis is reduced (92).

Physiologically, in normal livers, HO is also involved in modulation of hepatic sinusoidal tone and portal venous regulation (72). These effects are thought to be secondary to the generation of the vasodilator CO from the constitutive HO-2 isoform, activation of soluble guanylate cyclase, and subsequent production of cGMP, similar to that of NO. HO-2 is expressed mainly in parenchymal cells, but also in Kupffer cells and hepatic stellate cells (43). In portal hypertension and cirrhosis, the expression and enzymatic activity of HO-1 are increased significantly in the liver, specifically in hepatocytes and Kupffer cells (43,44,81,82). Under these pathological situations, CO overproduction due to HO-1 induction could be necessary to improve sinusoidal perfusion and to protect liver homeostasis (72). The increased expression of HO-1 in the cirrhotic liver may also represent a refined intracellular system to control NO synthase activity, because increased HO-1 activity may limit the availability of heme, both a substrate for HO and an important cofactor for NO synthase (93). In addition, HO-1 has been shown to be protective in several models of liver injury, including hepatic porphyria (102), acetaminophen-induced hepatitis (103), hepatic ischemia-reperfusion injury (36), hemorrhagic shock (104), endotoxin-mediated hepatic dysfunction (105), and liver transplantation (69,106).

## Heme oxygenase and oxidative stress

HO-1 is a stress-inducible enzyme, which can be expressed and upregulated in virtually all cells facing contact with noxious stimuli. Hence, HO-1 induction can be regarded as a general response to oxidant stress. First, HO enzymatically breaks down heme, thereby mitigating the hazardous cellular effects of this pro-oxidant (2). Second, biliverdin and its reduced product bilirubin are both potent free radical scavengers with antioxidant and anti-inflammatory properties (75,76). Third, iron released from heme enhances the synthesis of ferritin, which additionally has antioxidant capabilities (107). Fourth, HOs, or at least HO-1, prevent free iron accumulation in the cells, not only indirectly by stimulating ferritin production, but also directly by extruding iron outside the cell (108). In this activity, HO-1 cooperates with a recently identified Fe-ATP pump (109). An indication for a role of HO-1 in iron extrusion is the accumulation of iron in the cells of HO-1 knockout mice (62,63), or in the liver and kidney of a boy who lacked a functional HO-1 gene (60). Accordingly, gene transfer of HO-1 to cells derived from HO-1 knockout animals restored the cells' capability to control their cellular iron level (108). In cells of the cardiovascular system, CO inhibits inflammatory response, influencing synthesis of cytokines, and cell proliferation, and preventing cell apoptosis. Those effects are mediated through both cGMP-dependent and cGMP-independent mechanisms (110,111).

## Heme oxygenase and angiogenesis

Recent data indicate the involvement of HO-1 in the regulation of angiogenesis. Thus, over-expression of

HO activity by HO-1 gene transfer into endothelial cells causes a significant increase in cell proliferation and capillary formation (112). In addition, HO-1 activity modulates the production of the potent angiogenic factor vascular endothelial growth factor (VEGF) (12,113). More recently, we have demonstrated, using an *in vivo* model of angiogenesis in chick chorioallantoic membranes, that VEGF increases the expression of HO-1 protein by a mechanism dependent on an increase in cytosolic calcium levels and activation of protein kinase C (11). Moreover, we have shown that HO-1 activity is involved in the signaling pathway(s) by which VEGF stimulates *in vivo* angiogenesis in chorioallantoic membranes, because this effect is markedly attenuated by the HO inhibitor ZnMP (11).

Previous studies had found evidence of increased angiogenesis and VEGF over-expression in the abdominal cavities of portal hypertensive rats and cirrhotic patients (114–119). The pathophysiological significance of this neovascularization is not well understood, but it could be involved in the formation of portosystemic collateral circulation and/or in the increased splanchnic blood flow during portal hypertension (87). Therefore, the possibility exists that HO-1 over-expression could play a role modulating angiogenic processes in portal hypertension. This is a new and fascinating area for investigation, which, by incorporating advances in angiogenesis and anti-angiogenic factors, may lead to novel approaches for therapy of portal hypertension.

## Summary

In summary, HOs are a family of heme-degrading enzymes with critical and primordial roles in protecting against oxidative and other stress and against excessive and inappropriate levels of intracellular heme. In addition, the products of the HO reaction, namely, CO, biliverdin (bilirubin), and iron have numerous and important physiological functions. Emerging evidence points to the importance of HO and these products in the pathophysiology of cirrhosis and portal hypertension, probably chiefly serving to abrogate adverse hemodynamic effects. Indeed, evidence continues to accumulate that upregulation of and/or gene therapy with HO-1 may decrease ischemia-reperfusion injury, not only in the liver, but also in other parenchymal organs. Seen in this light, HO and its products deserve additional study and will one day probably find a place in our therapeutic armamentarium.

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# Part 3
# Systemic and Splanchnic Hemodynamic Abnormalities in Cirrhosis

# Chapter 11
# The Systemic Circulation in Cirrhosis

Søren Møller and Jens Henriksen

## Introduction

Apart from obvious signs of portal hypertension and chronic liver dysfunction, patients with cirrhosis often present clinically with signs of circulatory dysfunction. Among these signs are palmar erythema and reddish skin are signs of vasodilation, raised and bounding pulse, and a low systemic blood pressure. Increased cardiac output in patients with cirrhosis was already described 50 years ago by Kowalski and Abelmann (1) and a hyperdynamic circulation is today a well-characterized element in the clinical appearance of these patients (2). The hyperdynamic syndrome comprises an increase in the heart rate, cardiac output, and plasma volume, and a decrease in the systemic vascular resistance with a low normal or decreased arterial blood pressure (3–7). Within the last two decades, documentation has been produced that a number of neurohumoral homeostatic systems, like the renin–angiotensin–aldosterone (RAAS) and sympathetic nervous systems (SNS), and the hypothalamic/neuropituitary release of vasopressin are highly activated in the majority of patients with cirrhosis (8–11). Over the last decade, intensive research has revealed that the nitric oxide (NO) and endothelin systems are implicated in the hemodynamic derangement found in cirrhosis (12–17). In addition, experimental and clinical evidence suggests that a cardiac dysfunction is present in cirrhotic patients and it appears that a latent cirrhotic cardiomyopathy may play a role in the circulatory disturbances in cirrhosis (18,19). The last 10 years have also shown that the retention of fluid and the formation of ascites are closely linked to the derangement in systemic hemodynamics, and it is apparent that the abnormal distribution of blood flow and volume is important for kidney dysfunction and sodium and water retention (10,11,20,21).

This chapter reviews some basic elements of the systemic circulation in cirrhosis in relation to recent investigations of hemodynamic alterations in different vascular areas and bioactive substances with potential effects on the physiology and pathophysiology of circulatory homeostasis and dysregulation in cirrhosis.

## The hyperdynamic circulation in cirrhosis

A hyperdynamic circulation is often absent in early stages of compensated cirrhosis and mild portal hypertension, but as the disease progresses from the portal hypertensive, pre-ascitic stage to the decompensated, portal hypertensive, ascitic stage, there is an overall direct relation between the severity of the cirrhosis, for instance, as reflected by the Child score, and the degree of hyperdynamic circulation (3,22–24) (Fig. 11.1). In clinical practice, however, the pattern may be more complex and some patients with early cirrhosis may exhibit a hyperdynamic circulatory state and a few patients with decompensated cirrhosis and considerable fluid retention may present a relatively normal circulation. It is noteworthy that the circulation is hyperdynamic only in the supine position, and the cardiac output in particular is not significantly elevated in most cirrhotic patients in the upright position (25,26). Moreover, pharmacological treatment, for example with β-blockers, may attenuate the hyperdynamic circulatory state. But on the whole, there is a direct relation between the degree of systemic circulatory derangement and the progression of the liver disease.

### Pathophysiology of arteriolar vasodilation

There is both experimental and clinical support for the presence of an excess of circulating vasodilator(s). Cross-perfusion studies have demonstrated that vasodilation is inducible by transferral of blood from portal hypertensive animals (27). In humans, peripheral vasodilation may be accomplished either by overproduction of circulating vasodilators or by vasodilators of intestinal or systemic origin that escape degradation in the diseased liver or bypass the liver through portosystemic collaterals (28). A predominantly splanchnic vasodilation precedes renal sodium and water retention and plasma volume expansion, which correlates with activated counterregulatory vasoconstrictor systems (7,28). Schrier and coworkers in 1988 proposed the so-called "peripheral arterial vasodilation hypothesis" (29). According to this theory, primary

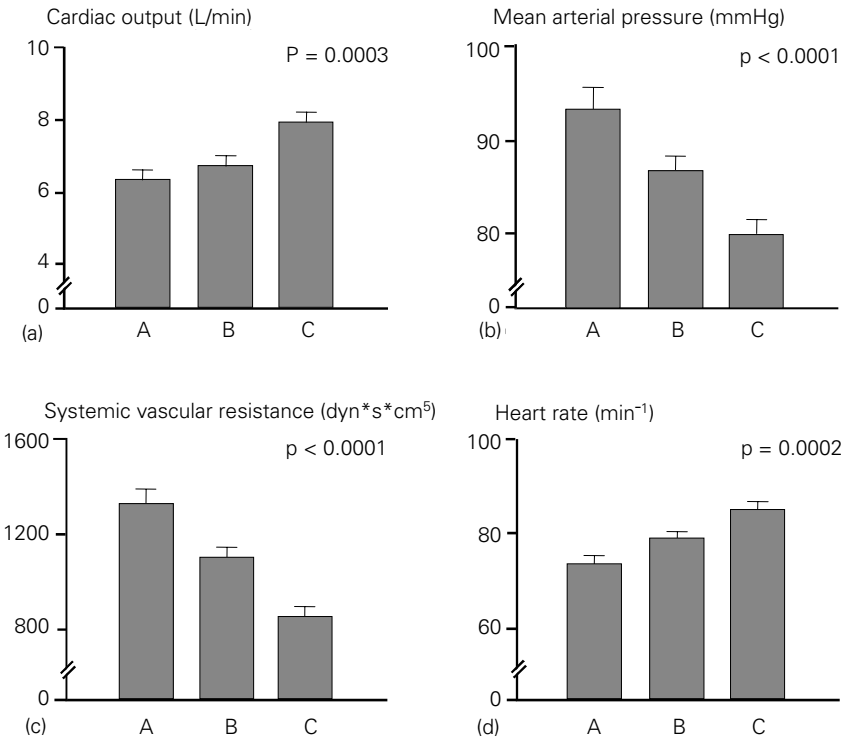


**Figure 11.1** Values of cardiac output, mean arterial blood pressure, systemic vascular resistance, and heart rate in 151 patients with cirrhosis: Child class A: $n = 44$; Child class B: $n = 61$; Child class C: $n = 46$. The increase in cardiac output and heart rate is most pronounced in patients with advanced disease. The mean arterial blood pressure and systemic vascular resistance are lowest in patients with advanced cirrhosis. Data from ref. (24).

splanchnic arteriolar vasodilation leads to arterial underfilling with reduction of the systemic overall vascular resistance and arterial blood pressure. A reduced effective blood volume, which is the part of the blood volume where baroreceptors are located, leads to activation of vasoconstrictor systems and secondary sodium-water retention (7,21,29,30). Thus, most of the hemodynamic changes seen in cirrhosis can be explained by this theory (Fig. 11.2).

## Circulating vasodilators

A number of candidates have been identified as listed in Table 11.1. In recent years, research has focused especially on NO, calcitonin gene-related peptide (CGRP), and adrenomedullin, and therefore these substances will be discussed in detail below.

Other substances with vasodilating properties that have been implicated are natriuretic peptides, tumor necrosis factor (TNF)-α), interleukins, substance P, and, recently, endocannabinoids (15,31–37).

### *Nitric oxide*

NO is a gaseous molecule with potent vasodilating properties which is synthesised from L-arginine by NO synthase (NOS) in the vascular endothelium (38). Three isoforms have been identified: inducible NOS (iNOS), constitutive endothelial NOS (ecNOS), and neuronal NOS (ncNOS) (15,39). iNOS is synthesized in various cells, such as macrophages, smooth muscle cells, hepatic stellate cells, and hepatocytes stimulated by inflammatory cytokines (15). eNOS is produced in response to different stimuli, such as shear stress, increases in blood flow, and endogenous vasodilators such as bradykinin, endotoxins, TNF-α, and probably adrenomedullin (15,38). In portal hypertension, there seems to be a diminished release of NO from sinusoidal endothelial cells in the cirrhotic liver (39,40) and experimental gene transfer of eNOS to cirrhotic livers has been shown to decrease portal pressure (41). In the systemic circulation, however, there is evidence of eNOS upregulation, which is probably related to shear stress (31,38,42). Exhaled air from cirrhotic patients contains higher NO levels than that of controls and correlates with the severity of disease and degree of hyperdynamic circulation (43,44). In 1991 Vallance and Moncada proposed that NO is implicated in the peripheral vasodilation in cirrhosis (12). This hypothesis has been substantially supported by the finding of amelioration of the hyperdynamic circulation after NO inhibition. Thus, in animal models and cirrhotic patients, blockade of NO formation significantly increases arterial blood pressure and decreases plasma volume and sodium retention (45–48). The results of some studies, however, have failed to support a major role of NO for the vasodilation in cirrhosis (49,50). Taken together, however, there is a large body of evidence that the NO production in the systemic circulation is increased in patients with cirrhosis and plays a major role in the arteriolar and splanchnic vasodilation and vascular hyporeactivity (51) (see below).

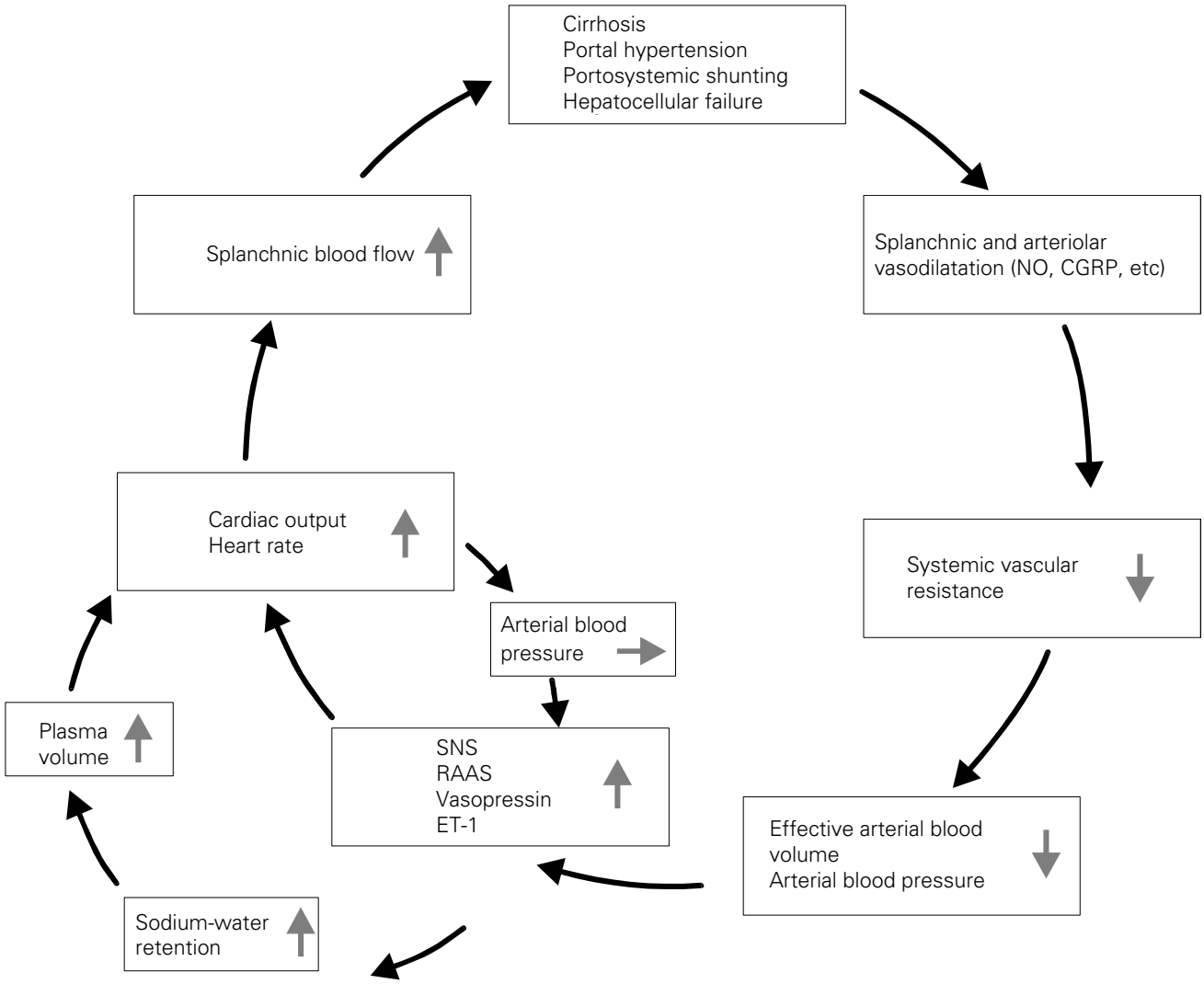


**Figure 11.2** Pathophysiology of the splanchnic and peripheral arteriolar vasodilation and systemic hemodynamic changes in cirrhosis. Endogenous vasodilators may escape hepatic degradation, owing to portosystemic shunting and/or hepatocellular damage, and induce vasodilation preferentially in the splanchnic vascular area. Reduced systemic vascular resistance leads to a reduced effective arterial blood volume and hence activation of vasoconstrictor systems. The hemodynamic and clinical consequences are increases in cardiac output, heart rate, and plasma volume and decreased renal blood flow, low arterial blood pressure, and fluid and water retention. The development of the hyperdynamic circulation may increase portal inflow and further aggravate the portal pressure in a vicious cycle. SNS, Sympathetic nervous system; RAAS, renin–angiotensin–aldosterone system; ET-1, endothelin-1; NO, nitric oxide; CGRP, calcitonin gene-related peptide.

The NO production seems to precede development of the hyperdynamic circulation, but the initiating stimuli are still not clarified (15).

### *Calcitonin gene-related peptide*

CGRP, a 37-amino-acid neuropeptide with a neurotransmitter function, is on a molar basis the most powerful vasodilating peptide known (52). It has been reported to be elevated in cirrhosis and to increase with the severity of the liver disease, with the highest concentrations seen in patients with ascites and the hepatorenal syndrome (52,53). However, the part this powerful vasodilator plays in central hemodynamic alterations in cirrhosis and portal hypertension is unknown. Møller *et al.* reported CGRP to be highly elevated and directly correlated to the cardiac output and negatively to the systemic vascular resistance (54); these findings were further substantiated in other patients in whom a covariation of CGRP with central hemodynamics was found (32). Henriksen *et al.* have recently demonstrated a significant relationship between CGRP and arterial compliance, an index of the mechanical state of the arterial system (55,56). A high value in arterial compliance, as assessed by the ratio

**Table 11.1** Vasodilatory and vasoconstricting forces involved in disturbed hemodynamics in cirrhosis.

| |
|---|
| Vasodilator factors |
| Adenosine |
| Adrenomedullin |
| Atrial natriuretic peptide (ANP) |
| Bradykinin |
| Brain natriuretic peptide (BNP) |
| Calcitonin gene-related peptide (CGRP) |
| Carbon monoxide (CO) |
| Endocannabinoids |
| Endothelin-3 (ET-3) |
| Endotoxin |
| Enkephalins |
| Glucagon |
| Histamine |
| Interleukins |
| Natriuretic peptide of type C (CNP) |
| Nitric oxide (NO) |
| Prostacyclin ($PGI_2$) |
| Substance P |
| Tumor necrosis factor-α (TNF-α) |
| Vasoactive intestinal polypeptide (VIP) |
| Vasoconstrictor factors |
| Angiotensin II |
| Endothelin-1 (ET-1) |
| Epinephrine and norepinephrine |
| NeuropeptideY |
| Renin–angiotensin–aldosterone system (RAAS) |
| Sympathetic nervous system (SNS) |
| Vasopressin (ADH) |

between the stroke volume and the pulse pressure, was related to high circulating CGRP, which further supports the hypothesis that CGRP is implicated in the systemic vasodilation and hence the hyperdynamic circulation in cirrhosis. The results of experimental studies with CGRP antagonists in portal hypertensive animals also favor a role for CGRP in modulating vasodilation in portal hypertension, but the final definition of its role in the hemodynamic alterations in cirrhosis must await human studies with specific antagonists (33,57).

### *Adrenomedullin*

Adrenomedullin is a vasodilating peptide of 52 amino acids with sequence similarity to CGRP (58). It is primarily released from the adrenal medulla and induces relaxation of smooth muscle cells (58). Injection of adrenomedullin in rats induces pronounced vasorelaxation, probably by the release of NO, and leads to a decrease in the systemic vascular resistance and arterial blood pressure (58). Various authors have reported increased circulating concentrations of adrenomedullin with relationship to the severity of liver disease in patients with cirrhosis (34,59,60). The circulating levels of adrenomedullin seem to be higher in decompensated patients with cirrhosis and correlate with the activity of pressor substances, such as endothelin, renin, vasopressin, and catecholamines (34,59,61). After liver transplantation, circulating adrenomedullin levels remain increased and may have a function in volume regulation (62). However, the potential role played by this very potent NO-generating agent in the hyperdynamic circulation and abnormal volume distribution in cirrhosis needs further research.

## Resistance to pressor hormones

It has been known for several years that patients with cirrhosis are hyporesponsive to the pressor effects of potent vasopressors such as norepinephrine, angiotensin II (AII), and vasopressin (63–65). There may be an upward shift in the pressor concentration giving 50% effect as maximal effect and reduced maximal effect (66,67). This may be brought about by a change in receptor affinity, a decrease in the numbers of receptors, and a variety of post-receptor defects (66,68). Helmy *et al.* have reported hyporesponsiveness to AII and endothelin-1, chiefly because of enhanced NO generation (51,69). However, specific inhibition of these systems indicates that they contribute to the maintenance of basal vascular tone in cirrhosis (17,70). At present, the sustained systemic vasodilation, in spite of high activation of all vasoconstrictor systems, is most likely to be related to changes in receptor affinity, downregulation of receptors, and post-receptor defects, but future research should disclose further the molecular pathophysiology.

## Neurohumoral counterregulation

As described above and in Fig. 11.2, the splanchnic arterial vasodilation leads to activation of a number of vasoactive systems of importance to the circulatory homeostasis and reactivity, autonomic function, and sodium-water retention in cirrhosis (10,11).

Numerous studies have shown marked activation of SNS, RAAS, the endothelin system, and increased release of vasopressin (see Table 11.1 and Part 2 of this book) (10,11). Recently, the use of specific inhibitors such as captopril, losartan, propranolol, and endothelin-antagonists in patients with cirrhosis has improved our knowledge of the importance of these systems in the counterregulatory process (17,69–72). The pronounced activation of these systems indicates a decreased effective arterial blood volume and emphasizes the importance of the size of the central and arterial blood volume (73,74).

Substantial evidence supports peripheral and splanchnic vasodilation as a key feature in the imbalance between vasoconstricting and vasodilating forces in cirrhosis (69,75–77).

## Hemodynamics of specific vascular beds

The overall systemic vascular resistance is decreased in patients with cirrhosis (23). However, a closer look at single individual organs and tissues may reveal areas of overperfusion, normal perfusion, and hypoperfusion (see Table 11.2) (15,78). Thus, in advanced cirrhosis, for example, renal blood flow is low and a major part of the increased cardiac output passes through the hepatosplanchnic inlet to be returned through portosystemic collaterals (7,79).

Modern technology used to assess regional perfusion has shown that the circulation in most of the vascular territories is disturbed in cirrhosis, and the circulatory abnormalities are appropriately characterized as a universal dyscirculatory or hyperkinetic syndrome (80,81). The hemodynamic changes in specific vascular beds in cirrhosis are summarized in Table 11.2. In the following section, the hemodynamics of the major vascular beds is discussed.

**Table 11.2** Hemodynamic changes in specific vascular beds in cirrhosis.

| |
|---|
| Systemic circulation |
| Plasma volume ↑ |
| Total blood volume ↑ |
| Noncentral blood volume ↑ |
| Central and arterial blood volume ↓ (→) |
| Cardiac output ↑ |
| Arterial blood pressure ↓ (→) |
| Heart rate ↑ |
| Systemic vascular resistance ↓ |
| Heart |
| Left atrial volume ↑ |
| Left ventricular volume → (↑) |
| Right atrial volume → ↑ ↓ |
| Right ventricular volume → ↑ ↓ |
| Right atrial pressure → ↑ |
| Right ventricular end diastolic pressure → |
| Pulmonary artery pressure → ↑ |
| Pulmonary capillary wedge pressure → |
| Left ventricular end diastolic pressure → |
| Hepatic and splanchnic circulation |
| Hepatic blood flow ↓ → (↑) |
| Hepatic venous pressure gradient ↑ |
| Postsinusoidal resistance ↑ |
| Renal circulation |
| Renal blood flow ↓ |
| Glomerular filtration rate ↓ → |
| Pulmonary circulation |
| Pulmonary blood flow ↑ |
| Pulmonary vascular resistance ↓ (↑) |
| Cerebral circulation |
| Cerebral blood flow ↓ → (↑) |
| Cutaneous and skeletal muscle circulation |
| Skeletal muscular blood flow ↑ → ↓ |
| Cutaneous blood flow ↑ → ↓ |

### The hepatic and splanchnic circulation

From a hemodynamic point of view, the level of portal pressure is determined by the hepatic vascular resistance and portal inflow. Factors that determine the hepatic vascular resistance include both structural and dynamic components (75). Among the structural components are histological characteristics such as steatosis, fibrosis, and regenerative nodules. Dynamic structures include cells with contractile properties such as hepatic stellate cells, myofibroblasts, and smooth muscle cells (82). Portal venous inflow is mainly determined by the degree of splanchnic vasodilation. In healthy subjects, the hepatic blood flow equals the splanchnic blood flow, but patients with portal hypertension have a substantial portosystemic collateral circulation and an increased mesenteric inflow of up to several liters per minute has been reported (82,83). Thus, a large part of the increased cardiac output is returned through portosystemic collaterals. In man, collateral blood flow through the azygos vein has been determined by the constant infusion thermodilution technique (84). The azygos blood flow is especially important, as the azygos vein drains esophageal varices and an increase in azygos flow is associated with an increased risk of variceal bleeding (85). In addition, there may be portopulmonary collaterals and mesenteric arteriolar dilations, which are not co-determined by this technique (86,87). The increased splanchnic blood flow can be reduced pharmacologically by β-blockers, nitrates, octreotide, terlipressin, and infusion of these drugs may in some patients in part reverse the hyperkinetic mesenteric circulation (82,88).

As mentioned above and outlined in Chapter 8, there seems to be a defective sinusoidal eNOS-derived production of NO (15). In addition, recent investigations of endogenous vasoactive substances have focused especially on endothelin-1, AII, catecholamines, and leukotrienes in the increased hepatic-sinusoidal resistance (6,89,90). The hemodynamic imbalance with a predominant sinusoidal constriction may contribute significantly to the development of portal hypertension and be an important target for treatment.

The role of the increased portal-sinusoidal and mesenteric capillary pressure in the genesis and perpetuation of ascites is considered in Chapters 12 and 14.

### The renal circulation

Renal hypoperfusion in cirrhosis and its role in sodium-water retention are discussed elsewhere in this book (see Chapters 27). However, some features of systemic hemo-

dynamic alterations are important for the low renal blood flow and renal dysfunction in cirrhosis. Decreased mean arterial blood pressure and increased renal venous hydrostatic pressure, especially in patients with ascites, will reduce the effective renal perfusion pressure (91). Activation of RAAS may contribute to the decreased renal perfusion, but the system may also have more complex regulatory effects within the kidney (7,8). Studies have shown that vasopressin does not change the renal perfusion substantially (92,93). Norepinephrine, epinephrine, and endothelin-1 are powerful renal vasoconstrictors and probably important elements in the renal hypoperfusion and sodium-water retention in advanced cirrhosis (92,94,95). Local vasodilators, like prostaglandins, are most likely operative in order to compensate, at least in part, the progressive renal vasoconstriction seen in advanced cirrhosis (96,97). Nicholls and coworkers and Lenz and coworkers have shown that normalization of the low arterial blood pressure in decompensated cirrhosis, either by infusion of norepinephrine or by vasopressin, increases the renal perfusion and sodium excretion in some patients (98,99). As Gentillini *et al.* reported, patients with compensated cirrhosis and arterial hypertension seem to have a lesser degree of renal dysfunction than do arterial hypotensive patients (26). Infusion of pressor doses of AII to decompensated patients has also been found to increase renal perfusion and normalize arterial blood pressure (7,92). Accordingly, a combination of prolonged administration of ornipressin or terlipressin and albumin infusion has recently been reported to reverse the hepatorenal syndrome (100,101). Forrest *et al.* (102) administered low dose theophylline and observed an increase in renal venous blood flow of more than 50%, which suggests that adenosine plays a role in the renal vasoconstriction in cirrhosis. A major problem with intensive diuretic treatment and effective treatment of portal hypertension is the adverse effects on the systemic hemodynamics, as deranged systemic hemodynamics will in itself reduce renal function. Treatment with α-adrenergic blocking agents and, potentially, with endothelin-1 blockers should reverse the renal vasoconstriction, but their effect on arterial blood pressure may overrule beneficial local renal effects (95,103). Gerbes *et al.* recently reported the results of a multicenter study of a vasopressin receptor antagonist (VPA-985) on hyponatremia in patients with cirrhosis (104). They found that vasopressin receptor blockade improved serum sodium without causing further deterioration in the renal function or the arterial blood pressure. This type of drug may thus have a role in the management of these patients.

## The pulmonary circulation

Pulmonary vascular resistance is most often decreased in cirrhosis (105). Analysis of the pulmonary circulation in relation to lung function may be obscured by the presence of cardiac dysfunction or the result of heavy smoking, which is commonly the case in patients with alcoholic cirrhosis (106). Thus, there may be considerable chronic obstructive lung disease in addition to hepatic dysfunction. But independently of smoking status, these patients have a compromised lung function with reduced transfer factor and ventilation/perfusion abnormalities (54,86,106,107). It has now been documented that areas with a high perfusion rate in relation to alveolar ventilation are present in a substantial number of patients (54,106). Besides the abnormal ventilation/perfusion ratio and the presence of regular pulmonary arteriovenous shunts, portopulmonary shunts have also been described (86,105,106,108).

The condition with reduced transfer factor, abnormal ventilation/perfusion ratio or shunts, low arterial oxygen saturation, and pulmonary hyperdynamics is termed the hepatopulmonary syndrome (86,109,110). Fallon *et al.* have recently reported increased pulmonary vascular endothelial NOS and increased expression of endothelin-receptors and NO-dependent pulmonary vasodilation in experimental models (111,112), which is supported by clinical studies showing increased NO in the exhaled air of cirrhotic patients (44,113). The hepatopulmonary syndrome has been reversed by successful orthotopic liver transplantation in some patients (86,114) and recently by insertion of a transjugular intrahepatic portosystemic shunt (TIPS) (115). The frequency of the hepatopulmonary syndrome in patients with cirrhosis is not yet established. Different reports have given different frequencies of reduced arterial oxygen saturation in cirrhotic patients varying from about 10% to as high as 70% (24,116,117).

Thus, it may be concluded that a hyperkinetic condition with reduced pulmonary vascular resistance is present in cirrhotic patients without chronic obstructive lung disease, but, when these patients do develop chronic obstructive lung disease, pulmonary hypertension with increased pulmonary vascular resistance may follow, as in other patients with chronic pulmonary dysfunction.

## The cerebral circulation

Although the last decade has seen a considerable increase in our understanding of cerebral perfusion, most studies have been directed towards patients with cerebral ischemia, hypertension, stroke, and related conditions. Only a few studies with conflicting results have been carried out in cirrhosis. Cerebral perfusion seems to be normal in patients without encephalopathy (118,119), reduced in patients with early encephalopathy, but both reduced and increased perfusion with cerebral edema have been reported in comatose cirrhotic patients (120). In patients with fulminant hepatic failure, autoregulation is absent and therefore cerebral blood flow changes with alterations in arterial blood pressure (121). Autoregula-

tion of the cerebral blood flow is generally preserved in patients with cirrhosis, but it may be impaired in patients with the advanced disease (119,121). In some patients, however, regional cerebral blood flow may be impaired and the abnormalities may reverse after liver transplantation (122,123). Acute changes in cerebral blood flow have also been reported in relation to insertion of TIPS (124). The regulation of the cerebral circulation in patients with cirrhosis is complex and should be made the topic of much future research.

### The peripheral circulation

The cutaneous and muscular blood flow may be increased in patients with cirrhosis (125). Palmar erythema, spider nevi, and potatory face were early recognized as clinical signs of cutaneous hyperperfusion. These types of circulatory abnormalities illustrate the capillary hyperperfusion and the presence of arteriovenous fistulae. Muscular blood flow is reported to be increased, normal, and reduced in patients with cirrhosis (126–128). Evaluation of limb blood flow (brachial artery, femoral artery) by color Doppler and spectral Doppler has failed to disclose a clear hyperdynamic perfusion of the limbs (126,127). Moreover, estimates of skin blood flow by nuclear medicine techniques have shown normal capillary skin blood flow in cirrhotic patients (129) and Doppler measurements of the forearm blood flow seem to be the same in patients with cirrhosis and controls (49,130). Venous occlusion plethysmography of the arm and thigh may give a combination of cutaneous and muscular blood flow (125) but this method has also given identical baseline values in patients and controls (51,65). The cutaneous and muscular circulations in cirrhosis are, however, important topics for new research.

At present, it can be concluded that cardiac output is increased in patients with cirrhosis and their systemic vascular beds are hyperperfused, normoperfused, and hypoperfused, but the exact distribution of the increased cardiac output to the different organs, tissues, and types of vessels remains to be clarified. The blood flow pattern will probably change as the disease advances through the preportal hypertensive pre-ascitic, portal hypertensive pre-ascitic, portal hypertensive ascitic stages, to the full-blown hepatorenal syndrome.

## Circulatory dysfunction in cirrhosis

Numerous observations favor the presence of a surplus of circulating vasodilators combined with a potential resistance to pressor substances and an autonomic nervous dysfunction as a major cause of the general circulatory dysfunction and vascular hyporeactivity (21,28,29,74,77, 131,132). Moreover, these pathophysiological mechanisms may be involved in cardiac dysfunction and impaired homeostasis of the arterial blood pressure and blood volume. Each of these aspects is discussed in this section.

### Background of vascular hyporeactivity

The pathophysiological background of the hyporeactivity of the vascular system in cirrhosis is still under debate. The reduction in systemic vascular resistance and the decreased responsiveness may be associated either with an inability of the vessels to respond to constrictor influences or with the presence of vasodilators. Various studies have shown impaired responses to circulatory challenges, such as pressor stimuli, as a cause of the vascular hyporesponsiveness (67,133–135). Data from experimental studies have shown decreased reactivity to potent vasoconstrictors like AII (51,136,137) and catecholamines (63,135). The reduced responsiveness could be explained either by a decrease in α-adrenergic receptor sensitivity or by α-adrenergic responsiveness and both could play an additional role in the altered vascular reactivity (64,138). As mentioned above, research in recent years has paid attention primarily to potent vasodilators such as NO, CGRP, and adrenomedullin (15,56,61). Several animal experiments and human studies have provided ample support for the role of these vasodilating substances in the hyporeactivity in cirrhosis (45,47,48,69,139–141), whereas others have reported opposite findings (64,130). Arterial compliance, assessed as the stroke volume relative to the pulse pressure, is an index of the stiffness of the arterial system (142), and it reflects both the composition of the arterial wall (collagen and elastic fibers, deposits, etc.) and the dynamics due to alterations in the smooth vascular tone. Arterial compliance is increased in cirrhosis (26,55) with significant relations to CGRP, but not to vasoconstrictor systems such as the SNS or the endothelin system (55,56,143). The results of these studies suggest that the vascular hyporeactivity in cirrhosis expressed by the increased arterial compliance is closely associated with the vasodilatory derangement in cirrhosis. NO may also affect vascular remodeling in cirrhosis. Thus, Fernández-Varo *et al.* recently demonstrated reduced wall thickness and wall area in cirrhotic rats (144). After NO-blockade, wall thickness and wall area increased and arterial compliance decreased, which suggest that NO is involved both in static and in dynamic vascular changes (144). Although the role played by NO is still under discussion, an increasing number of studies point to this gas as a major candidate in decreased vascular contractility/vasodilation which is consistent with the "peripheral arterial vasodilation hypothesis" (Fig. 11.2) (51,135).

### Autonomic dysfunction

Evidence of autonomic defects in patients with cirrhosis has emerged from various studies on hemodynamic

responses to standard cardiovascular reflex tests, such as Valsalva ratio, heart rate variability, and isometric exercise (145–149). Most studies on this issue have found a high prevalence of autonomic dysfunction in cirrhosis with associations to liver dysfunction and survival (72,149). The results of Mohamed *et al.* suggest that the autonomic dysfunction is temporary, arises as a consequence of liver dysfunction, and may be reversible after liver transplantation (150). Whereas most studies have focused on defects in the SNS, recent investigations have emphasized the importance of a vagal impairment for sodium and fluid retention (72,145,147). Sympathetic responses to dynamic exercise seem to be normal in patients with cirrhosis, but those to isometric exercise are clearly impaired (133,151). Similarly, blood pressure responses to orthostasis are impaired, probably because of a blunted baroreflex function (148,152,153). Abnormal cardiovascular responses to pharmacological stimulations with AII, norepinephrine, and vasopressin in terms of impaired responses in blood flow and blood pressure have been reported in patients with cirrhosis (134,154,155). Dillon *et al.* (72) have described the correction of autonomic dysfunction in cirrhosis by captopril, which indicates that vagal dysfunction in cirrhosis is partly caused by a neuromodulation by AII. Involvement of the RAAS is also supported by data from Villa *et al.*, who recently reported that canrenone, an aldosterone antagonist, normalized cardiac responses to postural changes in compensated cirrhotic patients (153).

At present the pathophysiological basis for the autonomic dysfunction is unknown, but could take place within the central nervous system, through damage to the peripheral nerves or changes in neurotransmission in terms of a post-receptor defect as an explanation of the vascular hyporeactivity. From the amount of available data, a multifactorial etiology to the hyporesponsiveness in cirrhosis seems most likely (Fig. 11.3).

## Homeostasis of the arterial blood pressure

The level of the arterial blood pressure depends on the cardiac output and the systemic vascular resistance. The latter is determined by the tone and relaxation of the smooth muscle cells in the small arteries and arterioles, which are governed by complex local and central neurohumoral regulation (142,156). SNS, RAAS, and vasopressin are all involved, but the role of the endothelin system in the maintenance of the arterial blood pressure in cirrhosis is still under discussion, as a relation to arterial pressure has been demonstrated only in some studies (16,157,158). Potent vasodilators such as NO, CGRP, histamine, bradykinin, and serotonin have been implicated in the blood pressure regulation (159). Guevara *et al.* reported a significant inverse relation of the potent vasodilator adrenomedullin to arterial blood pressure and endothelin in patients with cirrhosis, which suggests that these two potent vasoactive systems play a role in blood pressure regulation in cirrhosis (34). NOS blockade results in higher arterial blood pres-

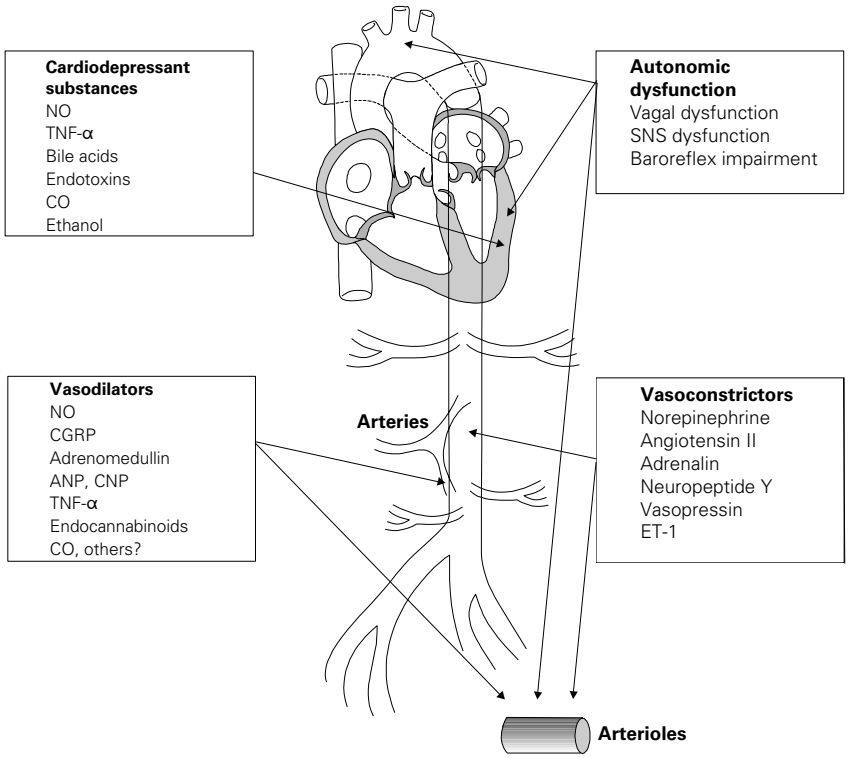


**Figure 11.3** The causes of vascular hyporeactivity in cirrhosis may originate in the central nervous system, the autonomic nervous system, from local mediators or within the smooth muscle cell/heart muscle cell. An autonomic dysfunction may act at cardiac, arterial, and arteriolar levels. Vasodilators and vasoconstrictors may act variably at cardiac, arterial, and arteriolar levels. At the smooth cellular (arteriolar) level, hyporeactivity could be caused by increased concentrations of vasodilators (NO, nitric oxide; CGRP, calcitonin gene-related peptide; ANP, atrial natriuretic peptide; CNP, c-natriuretic peptide; TNF-α, tumor necrosis factor-α; endocannabinoids; CO, carbon monoxide) and/or decreased sensitivity to vasoconstrictors (ET-1, endothelin-1); SNS, sympathetic nervous system.

sure in cirrhotic rats (144), and recently, Ros *et al.* found that inhibition of the endocannabinoid CB1 receptor increased arterial blood pressure in experimental cirrhosis and that anandamide from the monocytes of cirrhotic rats may contribute to arterial hypotension in cirrhotic animals. However, the significance for human cirrhosis awaits further studies (37).

The circulatory outcome of the decreased systemic vascular resistance in cirrhosis is an increase in heart rate (HR), cardiac output, and expanded plasma volume. The arterial blood pressure is kept low normal, depending on the state of the disease, as a circulatory compromise between vasodilating and counterregulatory vasoconstricting forces, local factors, and Starling forces (10,29,73,156) (Fig. 11.2). Previous studies have shown a relation between the degree of arterial hypotension in cirrhosis and the severity of hepatic dysfunction, signs of decompensation, and survival (3,10,160).

The arterial blood pressure possesses a circadian variation, but is maintained within its normal range by an arterial negative feedback baroreceptor reflex (161). In most studies, values of arterial blood pressure in cirrhosis are reported as single measurements in the resting, supine, and awake patient. Møller *et al.* (5) reported the results of 24-h determinations in cirrhotic patients. During the day, the systolic, diastolic, and mean arterial blood pressures were substantially reduced compared with those of age- and sex-matched controls, whereas at night the values were unexpectedly normal. Consequently, the drop from daytime to night-time and the rise from night-time to daytime showed lower values than those of the controls. It is known from other diseases, such as uremia and different types of heart failure, that the circulation of patients classified as "nondippers" is abnormally regulated (162). The fact that at night the patients had a combination of normal blood pressure and increased heart rate suggests abnormal regulation of the circulation also in cirrhosis. Prolonged rest in the supine position (as during sleep) would be expected to lessen the abnormal distribution of the blood volume and improve the ability to maintain a normal "sleeping" arterial blood pressure, only at the cost of an increased heart rate and cardiac output. The upright position during the day or shifting from the supine to the upright position may further aggravate central hypovolemia, and normal arterial blood pressure cannot be maintained even when the heart rate and cardiac output are increased. The negative correlation of the arterial blood pressure to the Child score during the day and at night confirms that the hemodynamic derangement is related to the severity of the liver disease. These results were later confirmed in other patients with significant relation to splanchnic hemodynamics (163).

The above-mentioned results suggest that the systemic hypotension, the abnormal diurnal variation in arterial blood pressure, and the marked activation of neurohumoral systems contribute to the sodium and fluid retention and the abnormal regulation and distribution of the circulating medium in patients with cirrhosis.

## Homeostasis of blood volumes

Most investigators agree that in cirrhosis with advanced decompensation the central and arterial blood volume is decreased (73,74,164,165). There is, however, still some controversy about the size of the central and arterial blood volume in the pre-ascitic stage (73,74,164,166). The powerful pressor and sodium-water retaining systems are activated in cirrhosis. In most cases there seems to be a progressive increase from normal values to slightly increased values in preportal hypertensive patients to a further increase in portal hypertensive pre-ascitic patients to highly increased values in ascitic patients and patients with the hepatorenal syndrome (8). However, mechanisms and time frames may differ from one patient to another and in accordance with the etiology of the cirrhosis (167). This is topic for important future research.

Recent research indicates that patients with early cirrhosis are able to expand their central and arterial blood volume in response to plasma volume expansion (166,168). Patients with advanced cirrhosis are unable to do this. Both categories of patients, however, respond to plasma volume expansion with a further decrease in systemic vascular resistance and no change in arterial blood pressure (166,168). We recently reported that, although patients with advanced disease are unable to expand their central blood volume, renin activity falls significantly, which indicates that albumin infusion affects the effective arterial blood volume (168). These results also agree with those of investigations into central volume expansion on changing from the upright to the supine position (164,169).

The increased cardiac output and plasma volume in cirrhosis should be considered secondary to the activation of neurohumoral mechanisms consequent to arterial vasodilation, low arterial blood pressure, and reduced central and arterial blood volume. However, a non-volume-dependent activation of the sympathetic nervous system through hepatic reflexes, owing to portal hypertension, should be considered (170). This has been documented in animal experiments and there are indications of the presence of such a reflex in man (170). Although the relative importance of non-volume-dependent sympathetic activation and volume/arterial pressure-dependent activation of sympathetic nervous activity and other neurohumoral systems has not yet been finally settled, the latter is probably the far most important quantitatively.

## Cardiac dysfunction

Cardiac output is increased in cirrhosis primarily be-

cause of the increased heart rate, but the stroke volume may also be raised in some patients (1,171,172). Circulation times are rapid even in the presence of cardiac enlargement. The cardiac output may be increased as a compensatory response consequent to the peripheral vasodilation, the presence of portosystemic shunts, and arteriovenous shunts. We recently reported that the red blood cell cardiac output is normal in cirrhosis, and the low blood viscosity in addition to vasodilation and expanded blood volume are major determinants of the hyperdynamic circulation (173).

At first glance, cardiac function in patients with cirrhosis may appear normal at rest, but after physical or pharmacological strain, cardiac contractility seems impaired and has thus given the clinical entity the name "cirrhotic cardiomyopathy", which is different from alcoholic heart muscle disease (18,19,174). The mechanisms underlying cirrhotic cardiomyopathy are considered in Chapter 15. Patients with cirrhosis may have abnormal heart volumes, pressures, and cardiac function, but determination of the myocardial mass and heart volumes has given different results, depending on the methods used (175,176). Most studies of patients with cirrhosis have shown the myocardial mass within the normal range (172,177). However, some authors have reported an increased left ventricular mass with left eccentric hypertrophy (174,178). Some studies have shown a normal left ventricular end-diastolic volume and an enlarged left atrium (175,176,179). In others, increased end-diastolic and end-systolic volumes of the left ventricle have been found (148,172). With respect to the right heart, reduced, normal, and increased atrial and ventricular volumes have all been reported (172,179,180).

In most studies of cirrhotic patients, the right ventricular pressure (RVP), pulmonary artery pressure (PAP), and left atrial or pulmonary capillary wedge pressure (PCWP) are in the upper normal limit, but within the normal range during rest (Table 11.2). Right atrial pressure (RAP) is often slightly raised in cirrhosis, especially in decompensated patients, and paracentesis has been shown to lower the RAP, PAP, and PCWP (181), which indicates that the transmural pressures may be normal. A persistently increased RAP may raise a suspicion of right ventricular dysfunction.

Physical and pharmacological strain may affect cardiac pressures. Thus, after exercise the left ventricular end-diastolic pressure increases, but the cardiac stroke index and left ventricular ejection fraction fall during exercise, which indicates an abnormal ventricular response to an increase in ventricular filling pressure (19). Pharmacologically increased left ventricular afterload leads to an increase in PCWP without changing cardiac output (182). A similar pattern is seen after insertion of TIPS, but the increased cardiac pressures tend to normalize with time (183,184), and after infusion of plasma expanders (185). Infusion of a plasma protein solution, however, increases cardiac output, as well as the RAP, PAP, and PCWP, whereas infusion of packed red blood cells may not change these variables (185).

In the resting cirrhotic patient, the work of the heart is reduced, and cardiac failure may become manifest only under strain. The left ventricular ejection fraction (LVEF), i.e. the stroke volume relative to the left ventricular end-diastolic volume, has been reported to be normal at rest in some studies (186,187), increased in others (148,172,174), and reduced in one study of patients with ascites (180). The maximal aerobic exercise capacity and heart rate are lower in most patients with cirrhosis than in normal subjects (187). After exercise, the LVEF increases significantly less in cirrhotic patients than in controls (182). The reduced cardiac performance is probably caused by a combination of blunted heart rate response to exercise, reduced myocardial contractility, and profound wasting of skeletal muscle with impaired oxygen extraction (187,188). Normalization of the low systemic vascular resistance with vasoconstrictors results in a rise in the left atrial and left ventricular filling pressures (19). Hence, attempts to normalize the reduced cardiac afterload seem to unmask a latent ventricular failure, which appears to be resistant to inotropic drugs (18). The expanded blood volume in advanced cirrhosis increases cardiac preload leading to a persistent increase in cardiac output, which may overload the heart with an impaired cardiac contractility as the outcome (25,172,189). The potential effects of changes in preload in patients with cirrhosis are illustrated in Fig. 11.4.

The morphological basis for cirrhotic cardiomyopathy is cardiac hypertrophy, patchy fibrosis, subendothelial edema, and increased heart weight (18). These changes may affect the stiffness of the myocardial wall and result in impaired left ventricular filling and diastolic dysfunction. Determinants of diastolic dysfunction include impaired left ventricular diastolic filling despite a high stroke volume and delayed early diastolic transmitral filling (171). Studies of ventricular diastolic filling in cirrhosis support the presence of a subclinical myocardial disease with diastolic dysfunction, which, in ascitic patients, improves after paracentesis and worsens after TIPS (171,174,180,184). In addition, peptides that reflect ventricular dysfunction [brain natriuretic peptide (BNP) and proBNP] are increased in a substantial number of patients with cirrhosis (190).

## The hyperkinetic syndrome

Complications to cirrhosis relating to the hyperdynamic circulation and the liver are part of a multi-organ failure and may affect the prognosis of the patient significantly. The function of the central circulation in cirrhosis is disturbed with an increased cardiac output and heart rate

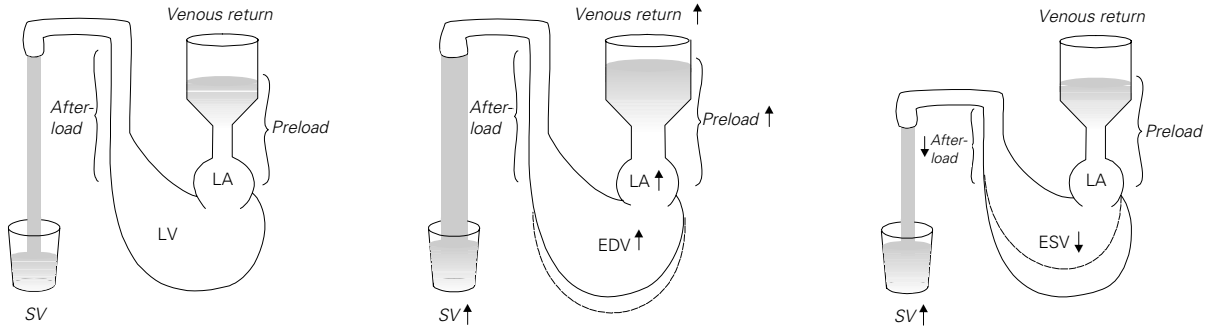


**Figure 11.4** Simplified illustration of the effects on circulation and filling of the heart chambers of increased preload and decreased afterload. (A) Normal loading. (B) Increased preload. Increased venous return leads to increased left atrial (LA) and left ventricular end-diastolic volumes (EDV), and increased stroke volume (SV) and cardiac output. (C) Decreased afterload. Reduced systemic vascular resistance leads to reduced ventricular end-systolic volume (ESV) and increased stroke volume and cardiac output. The hyperdynamic circulation in cirrhosis with increased sympathetic nervous activity may be a combination of B and C. From ref. (182).

and a decreased central and arterial blood volume. Cardiac pressures and performance, the systolic, and diastolic functions are clearly impaired with relation to the degree of liver dysfunction. The impaired cardiac contractility termed cirrhotic cardiomyopathy is different from that seen in alcoholic heart muscle disease. The cirrhotic heart is overloaded with a high-output failure and at the same time hyperdynamic and dysfunctional, and strain may unmask a latent heart failure. In addition, the circulation and function of a variety of organs are disturbed, including the lungs, kidneys, brain, and peripheral tissues (Fig. 11.5). This aspect may be important to consider in the clinical handling of the patient and in the assessment of prognosis (85). At present, no specific treatment can be recommended and the only radical treatment option is liver transplantation.

## Conclusion

The hyperdynamic circulation in cirrhosis is related to the degree of portal hypertension and liver failure and is more pronounced in patients with fluid retention than in those without. Peripheral and splanchnic vasodilation in relation to portal hypertension with portosystemic collaterals is probably responsible for the abnormal blood volume distribution with reduced "effective arterial blood volume" in the central and arterial parts of the circulation with activation of baroreceptor and volume receptor reflexes as the outcome. Cardiac function may be impaired owing to the presence of a cirrhotic cardiomyopathy. The enhanced systemic vasodilation and counterregulatory overactivity of vasoconstrictor systems seem to play a major role in the development of a multi-organ failure

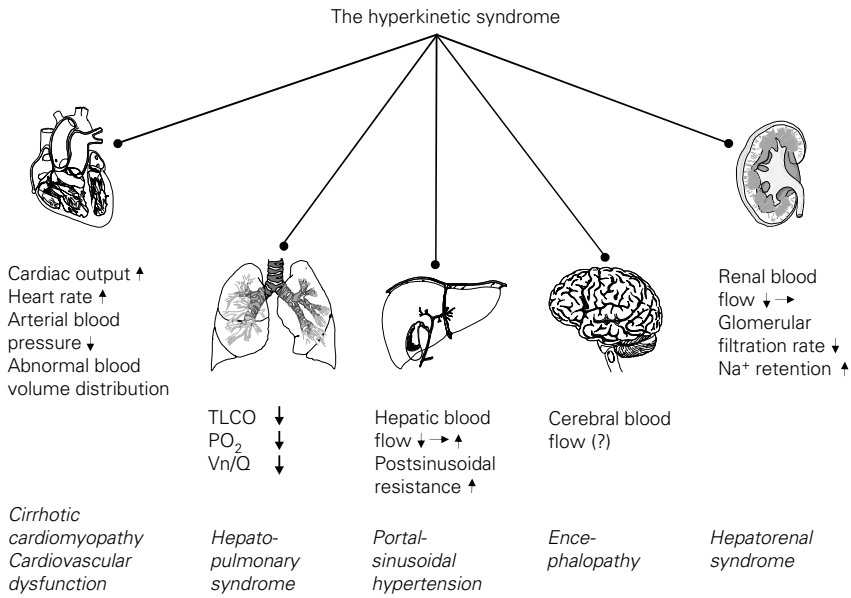


**Figure 11.5** Hemodynamic consequences of the hyperdynamic circulation in cirrhosis. The circulation is affected in multiple organs, including the heart, lungs, liver, kidneys, and possibly the brain, and the hyperdynamic syndrome refers to universal hemodynamic alterations. Flow in muscles and skin is poorly investigated in cirrhosis. $T_{LCO}$, carbon dioxide diffusing capacity; $PO_2$, arterial oxygen saturation.

in cirrhosis with impaired function and perfusion of kidneys, lungs, brain, skin, and muscles. Although major questions remain unsolved, the circulatory and neuroendocrine derangements play important roles in the clinical aggravation, renal disorders with sodium-water retention, hepatopulmonary function and ventilation, and circulatory reactivity. At the moment, liver transplantation is the only radical treatment, but studies of specific neuroendocrine agonists and antagonist show promising results and these agents have a potential for future pharmacotherapy of the derangement of circulatory function in cirrhosis.

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# Chapter 12
# The Splanchnic Circulation in Cirrhosis

Jaime Bosch and Juan Carlos García-Pagán

## Introduction

Cirrhosis of the liver is accompanied by profound disturbances in the splanchnic hemodynamics. These are not limited to the intrahepatic circulation, but involve also the splanchnic and systemic vascular beds. These hemodynamic disturbances are responsible for the development of portal hypertension. This syndrome appears in the majority of patients with cirrhosis and is responsible for the most frequent and severe clinical complications of cirrhosis: massive gastrointestinal bleeding from gastroesophageal varices and other portal hypertension related lesions, ascites and renal dysfunction, and spontaneous bacterial peritonitis or systemic infections. Because of the combined impact of these complications, portal hypertension represents the main cause of death and of liver transplantation in patients with cirrhosis.

This chapter reviews the mechanism and consequences of the circulatory disturbances associated with cirrhosis, and aims to provide a rational basis for the treatment of portal hypertension and its complications.

## Hemodynamic disturbances in the splanchnic circulation in cirrhosis

As in any vascular system, the pressure gradient along the portal venous system is the result of the product of portal blood flow and the vascular resistance that opposes that flow. According to Ohm's law, this relationship is defined by the equation:

$$\Delta P = Q \times R$$

in which $\Delta P$ is the portal pressure gradient, Q is blood flow within the portal venous system (that in portal hypertension includes also the portal-systemic collaterals), and R is the vascular resistance of the portal venous system, which in turn represents the sum of the serial resistance of the portal vein and the hepatic vascular bed, and of the parallel resistance of the collaterals. Factors influencing vascular resistance are interrelated by Poiselle's law in the equation:

$$R = 8uL/pr^4$$

in which u is the coefficient of blood viscosity, L is the length of the vessel and r its radius. It follows that changes in the radius of the vessels is the main factor influencing vascular resistance, and are therefore, of major influence in the pathophysiology of portal hypertension (1). The relationship between the above-mentioned parameters is completely applicable to flow through rigid tubes. However, in the vascular system other factors contribute to modulate resistance. Thus, contrary to what happens in rigid tubes, as pressure increases, elastic blood vessels will distend, offering less resistance to flow. This relationship between changes in the volume of a blood vessel for a given change in transmural pressure is known as compliance.

Portal pressure may increase because of an increase in portal blood flow, an increase in vascular resistance, or by a combination of both.

### Splanchnic arteriolar vasodilation in cirrhosis

An increased portal venous inflow is characteristically observed in advanced stages of portal hypertension (2,3). Increased portal venous inflow is the result of a marked arteriolar vasodilation in splanchnic organs draining into the portal vein. Since the portal pressure gradient is determined by the product of portal-collateral blood flow and the resistance in the portal venous system, it follows that the increased portal inflow maintains and aggravates the portal hypertensive syndrome (3). Thus, in portal hypertension a vascular territory—the portal venous system—simultaneously exhibits increased blood flow and outflow vascular resistance (4,5).

Increased portal blood inflow is likely to represent a multifactorial phenomenon, which may involve neurogenic, humoral and local mechanisms (6). An increased amount of circulating or paracrine vasodilators and

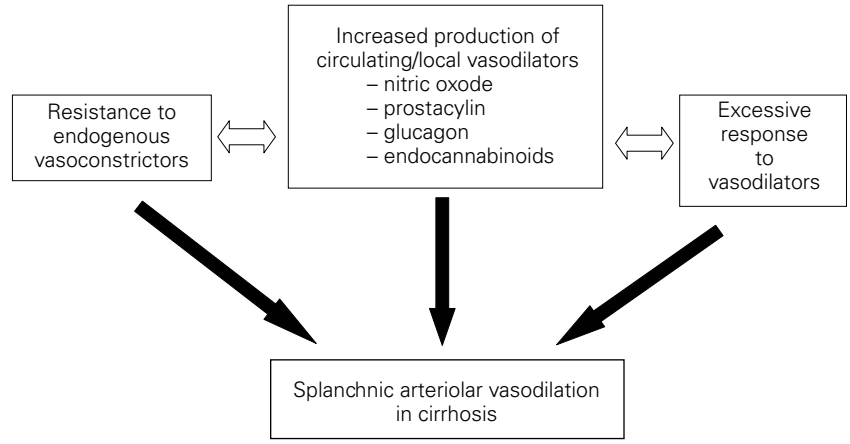


**Figure 12.1** Mechanisms involved in the pathophysiology of the splanchnic vasodilation of cirrhosis.

a vascular hyporesponsiveness to vasoconstrictors is thought to contribute to the systemic and splanchnic vasodilation observed in portal hypertension. In addition, an enhanced response of the mesenteric vascular bed to endothelium-dependent vasodilators may also be involved (Fig. 12.1).

Several studies have focused on the potential role of circulating vasodilators. Cross-circulation studies between portal hypertensive and normal animals have demonstrated the development of splanchnic vasodilation in the recipient, indicating that transferable humoral factors are implicated in the pathogenesis of the splanchnic hyperemia (7). Many candidate substances have been proposed, mainly vasodilators of splanchnic origin that undergo hepatic metabolism and may accumulate in the systemic circulation because of reduced hepatic uptake due to liver disease and/or portal-systemic shunting (7). More recently, the role that paracrine vasodilators, such as nitric oxide (NO), play in the pathophysiology of splanchnic vasodilation have gained most of the attention.

## Circulating vasodilators

Glucagon is probably the humoral vasodilator for which there is more evidence for a significant role in promoting splanchnic hyperemia in portal hypertension. Glucagon is a 29-amino-acid peptide derived from the sequential cleavage of a 160-amino-acid precursor preproglucagon, which is secreted by pancreatic α-cells and the oxyntic mucosa of the stomach and primarily inactivated by the liver.

Many studies have demonstrated that plasma glucagon levels are elevated in patients with cirrhosis as well as in experimental models of portal hypertension. Hyperglucagonism results, in part, from decreased hepatic clearance of glucagon, but more importantly from an increased secretion of glucagon by pancreatic α-cells (8). Support for a role of glucagon in modulating the splanchnic blood flow derives from physiological studies showing that in rats with experimental portal hypertension, the normalization of circulating glucagon levels by means of glucagon antibodies or by somatostatin infusion partially reverses the increased splanchnic blood flow, a response that can be specifically blocked by preventing the fall in circulating glucagon by a concomitant glucagon infusion (Fig. 12.2) (9–10). Conversely, other studies showed that increasing the circulating glucagon levels of normal rats to values similar to those observed in portal hypertension cause a significant increase in splanchnic blood flow. On the basis of these studies it has been suggested that hyperglucagonism may account for approximately 30–40% of the splanchnic vasodilation

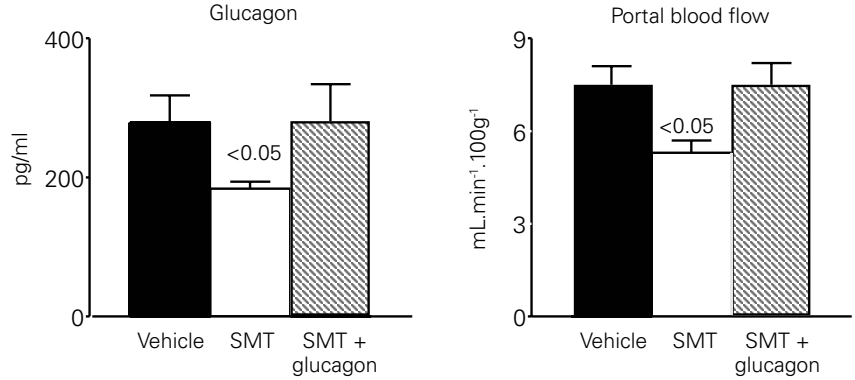


**Figure 12.2** Plasma glucagon and portal blood flow in portal vein ligated rats receiving vehicle, somatostatin (SMT) or SMT + glucagon.

of chronic portal hypertension. Glucagon may promote vasodilation by a dual mechanism: relaxing the vascular smooth muscle, and decreasing the sensitivity to endogenous vasoconstrictors, such as norepinephrine, angiotensin II and vasopressin, that have vasoactive effects mediated by the activation of adenyl cyclase. The role of glucagon in splanchnic hyperemia in portal hypertension provided a rationale for the use of somatostatin and its synthetic analogs in the treatment of portal hypertension. However, some studies showed that in experimental portal hypertension, splanchnic vasodilation is not necessarily associated with hyperglucagonism, raising doubts on the relevance of the hyperglucagonism in the pathophysiology of portal hypertension (11).

Several other circulating vasodilators have also been involved in the pathogenesis of the splanchnic vasodilation. Bile acids are increased in portal hypertension and have vasodilatory properties. However, data in the literature are controversial (12,13) and their role in mediating hyperdynamic circulation is not well defined. Likewise, the role that the capsaicin–calcitonin gene-related peptide (CGRP) vasodilatory pathway plays in the systemic and splanchnic vasodilation of portal hypertension is controversial (14–16). Indeed, permanent ablation of sensory neurons by neonatal capsaicin treatment prevented in one study (15) the expected development of the hyperkinetic circulation when rats were induced to develop portal hypertension. This was not observed in another study despite confirming almost a complete depletion of CGRP (14). More recently, treatment of cirrhotic rats with a specific antagonist of CGRP promoted a significant increase in the systemic and splanchnic vascular resistance. These effects were significantly greater in cirrhotic than in control rats, suggesting a role of CGRP in the development of the hyperkinetic circulation (16).

### *Endocannabinoids*

Recent data suggest a role for endocannabinoids in the hyperdynamic circulation of portal hypertension (17,18). Increased levels of the endogenous cannabinoid anandamide have been found in the monocyte fraction of blood from cirrhotic humans and rats and an increased expression of the cannabinoid CB1 receptors was found in human hepatic endothelial cells (17). In addition, CB1 receptor blockade reduced portal blood flow and pressure and increased arterial pressure in cirrhotic rats (17,18). The mechanism of action is not well understood. It has been suggested that it could be due, at least in part, to an increased NO production, mediated by the activation of endothelial CB1 receptors (17). However, the data are not conclusive (18).

Other candidates, including neuropeptides, adenosine, endotoxin and a variety of vasodilatory gastrointestinal hormones, have also been studied. However, available evidence is scarce for most of them.

## Local paracrine vasodilators

Most investigators now agree that local paracrine/autocrine vasodilators, mainly NO, but also carbon monoxide (CO) and prostacyclin, play a major role in the pathogenesis of the circulatory abnormalities associated with chronic portal hypertension.

### *Nitric oxide*

Experimental studies using specific NO inhibitors have shown that NO is involved in the regulation of splanchnic and systemic hemodynamics in portal hypertensive animals as well as normal animals (19). The administration of specific NO antagonists causes splanchnic and systemic vasoconstriction. This vasoconstrictive effect is significantly greater in portal hypertensive than in control animals, suggesting that an excessive production of NO may be responsible, at least in part, for the vasodilation observed in portal hypertension (19,20). In addition, NO inhibition has been shown to correct the characteristic vascular hyporesponsiveness to vasoconstrictors that is present in portal hypertension, which is thought to contribute to the systemic and splanchnic vasodilation (Fig. 12.3) (21–24). The finding that patients with cirrhosis have increased serum and urinary concentrations of nitrite and nitrate products of NO oxidation (NOx) also supports a role for NO in the genesis of the circulatory disturbances of portal hypertension (25). Portal venous NOx levels are higher than levels in blood from peripheral veins (26,27), suggesting that there is an enhanced release of NO from the splanchnic bed. This was confirmed by *in vitro* studies on the splanchnic vasculature from portal hypertensive rats (28) where an overproduction of NO was clearly demonstrated. Interestingly, NO production correlated with the degree of shear stress and with the magnitude of the baseline vasodilation and impaired vasoconstrictor response (28). However, other studies suggest that, in addition to NO, other factors may also be involved in the hyporesponsiveness to vasoconstrictors (29,30).

The increased production of NO is due both to an increased expression and activity of eNOS (endothelial nitric oxide synthase) (28,30–34). Factors likely to activate eNOS include shear stress and circulating vasoactive factors such as endothelin, angiotensin II, vasopressin, and norepinephrine. As a consequence, it was postulated that this increased production of NO might be more an adaptive response to the increased splanchnic blood flow (and therefore shear stress) than its cause. However, recent studies have demonstrated that NO overproduction by eNOS precedes the development of the

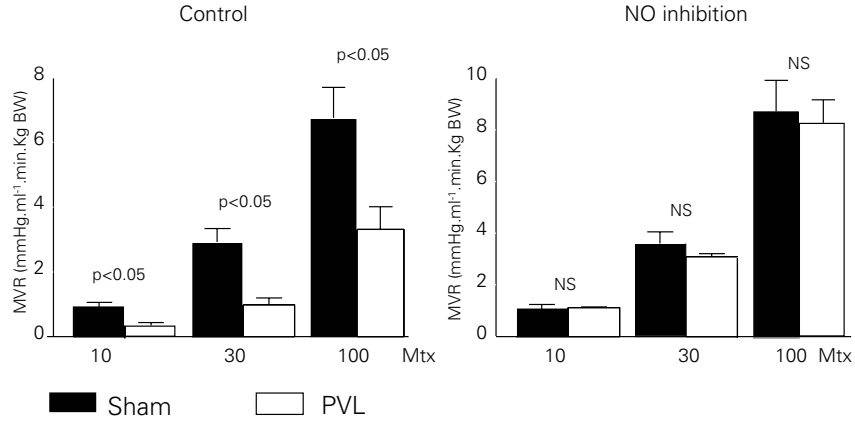


**Figure 12.3** Mesenteric artery hyporesponsiveness to methoxamine in partial portal vein ligated rats (PVL) is corrected by NO inhibition. MVR, Mesenteric vascular resistance; MTX, methoxamine.

hyperdynamic circulation of portal hypertensive rats (35). The posttranslational regulation of eNOS in portal hypertension has been further evidenced by recent studies in the partial portal vein ligated model of portal hypertension, showing that upregulation of eNOS catalytic activity, rather than eNOS overexpression, is the initial event that induces NO overproduction in the splanchnic circulation (36). Indeed, eNOS phosphorylation by AKT seems to be the mechanism of the initial upregulation of eNOS activity and NO-mediated hyporesponsiveness to vasoconstrictors (36). Later on, other mechanisms for an increased production of NO become important. Among these, it has been demonstrated than an enhanced signaling of the molecular chaperone heat shock protein 90 (HSP90) contributes to the excessive eNOS activity observed in the mesenteric territory of the portal hypertensive rats (37,38). In more advanced stages, such as those in cirrhotic rats with ascites, it has been recently shown that bacterial translocation is associated with a further increase in eNOS activity in the mesenteric artery, probably as a consequence of the increased levels of tetrahydrobiopterin that follow the increase in tumor necrosis factor (TNF)-α promoted by bacterial translocation (30). Tetrahydrobiopterin is a known cofactor and enhancer of eNOS activity (39). A role for TNF mediating the activation of NO synthesis is also supported by studies showing that the injection of anti-TNF antibodies attenuates the hyperkinetic circulation and decreases portal pressure in rats with prehepatic portal hypertension induced by partial portal vein ligation (40,41).

Chronic NO inhibition precludes the development of systemic vasodilation and delays, but does not prevent, the development of splanchnic vasodilation (42), suggesting that NO plays a major role in the pathogenesis of the hyperdynamic circulation, but other factors may also be involved. This concept has been recently confirmed by studies performed in mice with targeted disruption of the gene(s) encoding eNOS and iNOS, showing that these animals are still able to develop the hyperdynamic circulation associated with portal hypertension (43).

### *Prostacyclin*

Prostacyclin is an endogenous cyclooxygenase (COX)-derived vasodilator prostanoid produced by vascular endothelial cells that causes vascular smooth muscle relaxation by activating adenylate cyclase and augmenting the intracellular level of cyclic AMP (44). Several studies have supported a role for prostaglandins in the hyperdynamic circulation of portal hypertension (45,46). Thus, it has been shown that patients with cirrhosis have increased systemic levels of prostacyclin (47) and prostacyclin has also been found to be increased in the portal vein (46) and aorta (48) of portal-hypertensive rats. In addition, the inhibition of prostaglandin biosynthesis by indomethacin reduces the hyperdynamic circulation and portal pressure in patients with cirrhosis and portal hypertension (49), as well as in experimental models of portal hypertension (45,50), and attenuates the vascular hyporesponsiveness to vasoconstrictors of the mesenteric vascular bed (45,51). Moreover, it has also been shown that indomethacin administration was also able to reduce the increased gastric blood flow observed in portal-hypertensive rats but not in sham operated controls, which suggests that prostaglandins are mediators of the increased gastric blood flow observed in portal hypertension (52). Thus, there is evidence suggesting that prostaglandins contribute to the hemodynamic abnormalities of portal hypertension.

Two different isoforms of COX are involved in the biosynthesis of prostacyclin (44,53,54): COX-1 and COX-2. COX-1 is constitutively expressed but it can also be stimulated by factors similar to those that stimulate the constitutive isoform of NO synthase (eNOS) (53,55). COX-2 can also be found constitutively expressed in some tissues including the liver (56,57) and in the mesenteric

vascular bed (54). COX-2 is an inducible isoform of cyclooxygenase that, similarly to the inducible isoform of NOS, is usually expressed or overexpressed after stimulation with proinflammatory agents (54). Recent studies have suggested that both isoenzymes COX-1 and COX-2 are involved in the increased prostacyclin production of the mesenteric vascular bed of portal vein-ligated rats which contributes to a decreased contraction to norepinephrine (48). Indeed, an upregulation of COX-1 and COX-2 expression was found in the mesenteric vascular bed and aorta of partial portal vein ligated rats (48). Either selective COX-1 or selective COX-2 inhibition reversed the hyporeactivity to norepinephrine and the increased endothelial-dependent vasodilation to acetylcholine. However, the effect of selective COX-2 inhibition was significantly greater than that evoked by selective COX-1 inhibition (48). This differential effect of COX-1 and COX-2 selective blockade suggests that COX-2 mainly produces vasodilatory products (i.e. $PGI_2$), while COX-1 generates both vasodilatory and vasoconstrictor prostanoids (48). Removal of the functional endothelium also corrected the hyporesponse to norepinephrine of the mesenteric vascular bed of portal hypertensive rats. In this situation, COX blockade does not modify the response. Altogether, these data suggest that the generation of endothelial vasodilator prostanoids, in a major part from COX-2, is, at least in part, responsible for the increased mesenteric blood flow in portal hypertension (48).

In addition to hyporesponsiveness to vasoconstrictors in portal hypertension, several studies have shown an enhanced response to endothelium-dependent vasodilators in $CCl_4$ cirrhotic rats (58), partial portal vein ligated rats (59), and in human cirrhosis (60), further suggesting an increased synthesis of NO.

Experimental studies clearly show that when one of the vasoactive mediators, such as NO biosynthesis (42) or prostaglandins (61), are chronically inhibited, other vasoactive pathways may be enhanced, preventing the correction of splanchnic vasodilation. This suggests that there is an interrelationship between these vasoactive systems, which are coupled to cause the splanchnic vasodilation. Furthermore, it appears that none of these vasoactive factors is the only factor responsible for the splanchnic vasodilation present in portal hypertension, which is likely to be multifactorial in origin.

## Portosystemic collateral circulation

The development of portal-collateral circulation is one of the main complications of portal hypertension. Increased portal pressure is the main factor leading to the formation of portosystemic collaterals that divert portal blood to the systemic circulation, bypassing the liver. Formation of collaterals is a complex process involving the opening, dilatation, and hypertrophy of pre-existing vascular channels (1). Recently, VEGF-driven active angiogenesis has been shown to modulate collateral formation (62). Also, *in vivo* arterial angiogenesis suggested that angiogenesis is increased in portal hypertensive rats, a situation that can be partially inhibited by NO antagonists (63).

Portal-collateral circulation itself may play a major role modulating portal hypertension. In advanced portal hypertension the collateral circulation may carry over 90% of the blood entering the portal system (1), and in these circumstances it is obvious that the vascular resistance of these vessels may markedly influence the overall resistance to portal blood flow and, therefore, portal pressure. These vessels have a substantial amount of smooth muscle, and may thus exhibit active changes in diameter promoted by vasoactive substances. The resistance of the collaterals, although lower than that of the cirrhotic liver, is higher than that of a normal liver. The elements that modulate collateral resistance are not well known. Studies performed in isolated perfused portosystemic collateral bed suggest that NO may modulate portal-collateral vascular resistance (64). These vessels are hypersensitive to serotonin (65), which markedly increases their vascular resistance, while this can be decreased by β-adrenergic stimulation (64). Further evidence on the active modulation of resistance in portal-collateral vessels has been reported in studies in intact portal hypertensive animals, in which the administration of selective 5-HT2 receptor blockers causes a significant decrease in portal pressure without modifying the systemic hemodynamics and portal inflow, suggesting that portal-collateral resistance is responsible for part of the increase in portal pressure (66). Therefore, active changes in collateral resistance may influence portal pressure.

Besides endogenous stimuli, the portal-collateral resistance increases significantly by several pharmacological agents, especially by splanchnic vasoconstrictors. This is important because some of these substances, such as non-selective β-blockers, vasopressin and its derivatives, are used to reduce portal pressure. The increase in portal-collateral resistance brought about by these agents attenuates their portal pressure reducing effect (10,67). Another circumstance in which active changes in portal-collateral resistance appear to play a major role modulating the changes in portal pressure is the paradoxical increase of portal pressure observed in portal hypertensive animals after blood volume restitution following hemorrhage (68).

Gastroesophageal varices are the most clinically threatening portal-systemic collaterals, its rupture being one of the major causes of death of cirrhotic patients. It is well known that for varices to develop the portal pressure gradient must increase above 10 mmHg (1). Variceal bleeding is rarely, if ever, observed if the pressure gradient is

less than 12 mmHg (1). Therefore, this threshold gradient defines clinically significant portal hypertension.

### Increased intrahepatic vascular resistance

Increased resistance to portal blood flow is the primary factor in the pathophysiology of portal hypertension and may occur at any site within the portal venous system. In cirrhosis, increased intrahepatic vascular resistance is the consequence of the distortion of the liver vascular architecture caused by fibrosis, scarring and nodule formation. Careful pathological studies have suggested that thrombosis of medium and small portal and hepatic veins may contribute to the progression of cirrhosis and portal hypertension (69). In addition, the active contraction of different contractile cell types, in response to several agonists, in the liver promotes a further increase in the intrahepatic resistance (70). It has been claimed that this dynamic and reversible component may represent up to 40% of the increased intrahepatic vascular resistance in cirrhosis.

Contractile elements influencing the hepatic vascular bed include vascular smooth muscle cells of the intrahepatic vasculature (i.e. small portal venules in portal areas) (71), activated hepatic stellate cells (HSCs) (pericyte cells located in the perisinusoidal space of Disse with extensions that wrap around the sinusoids and reduces its caliber after contraction) (72,73), and hepatic myofibroblasts that may compress the regenerating nodules or venous shunts within the fibrous septa.

An increased production of vasoconstrictors and an exaggerated response of the hepatic vascular bed to them, as well as an insufficient release of vasodilators together with an insufficient response to vasodilators of the hepatic vascular bed, are the mechanisms that have been implicated in the pathogenesis of the dynamic component of the increased intrahepatic resistance of the cirrhotic liver. An extensive review on the mechanisms of increased intrahepatic vascular resistance can be found in the following chapter (Chapter 14).

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# Chapter 13
# Physiology of Hepatic Circulation in Cirrhosis

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## Introduction

The understanding of the biological mechanisms involved in the pathogenesis of portal hypertension is essential for the development of rational therapies for this devastating complication of chronic liver diseases. Ascites, gastroesophageal varices, gastropathy, and portosystemic encephalopathy are all consequences of this important hemodynamic dysfunction. Increase in the intrahepatic vascular resistance is a primary event in the development of portal hypertension. Although anatomical abnormalities are the main cause of the increased vascular resistance in cirrhotic livers, an enhanced intrahepatic vascular tone has been demonstrated in cirrhotic patients (1,2) and cirrhotic rats (3). This chapter is an overview of both anatomical and functional mechanisms that lead to the development of an increased intrahepatic vascular resistance in cirrhosis.

## Anatomical mechanisms

It is extremely important to recognize that blood flow entering the portal system is not regulated by the liver needs but by changes in vascular resistance at the level of the splanchnic arterioles. Consequently, the liver does not regulate its own blood flow. In the presence of postprandial splanchnic hyperemia, for example, the liver has to accommodate a large increase in portal blood flow. In normal conditions, the intrahepatic circulation is a low-resistance, high-capacitance vascular system that is able to adapt to a wide range of portal blood flows keeping the portal pressure in its normal low levels between 4 and 8 mmHg (4–7).

Different anatomical lesions have been implicated in the development of an increased intrahepatic resistance in chronic liver diseases. Fibrosis, hepatocyte enlargement, sinusoidal collapse, defenestration of endothelial sinusoidal cells, development of basement membrane in the space of Disse, and regenerative nodule formation, individually or in groups, impair the mechanisms that regulate the intrahepatic vascular resistance to portal blood flow in order to maintain a normal portal pressure.

Liver fibrosis represents the hepatic tissue repair process that results from an imbalance between production and degradation of connective tissue in response to a chronic injury (8). It is a multifunctional process that involves different liver cells, cytokines, chemokines, and growth factors (9). Although activated hepatic stellate cells are believed to represent the principal fibroblastic cell type involved in liver fibrogenesis, fibroblast cells (a different liver cell population) present within the portal field or around central veins also play an important role in scar formation (10).

The distribution of extracellular matrix within the liver lobule depends on the nature of the pathogen and may change as liver disease develops (11). For example, portal hypertension in hepatic schistosomiasis results from a granulomatous reaction to parasite eggs in the portal venules causing periportal fibrosis (12). However, in late schistosomiasis, an elevated wedge hepatic venous pressure gradient may be observed, reflecting the presence of an increased sinusoidal vascular resistance due to deposition of collagen in the space of Disse (perisinusoidal fibrosis) and consequent sinusoidal narrowing (13). Correlation and lack of correlation between portal hypertension and different types of liver fibrosis have been reported (14–19).

In advanced cirrhosis, collagen in the space of Disse (perisinusoidal fibrosis) may resemble a basement membrane and serve as a barrier hindering exchange processes between the sinusoidal blood and the hepatocytes. In fibrotic livers from carbon tetrachloride-treated rats, sinusoidal collagenization and a reduced number of fenestrae decreases the diffusion space for large molecules such as albumin. However, small molecules such as sucrose and water are still able to diffuse into the extravascular space in fibrotic livers (20). The transition from the normal permeable perisinusoidal space to a partially impermeable collagenous membrane associated to endothelial defenestration has been described as capillarization of the

sinusoid (21). This process may be an intermediary step towards sinusoidal venulization, which would represent a defense mechanism to maintain the blood flow in the cirrhotic liver (22). With capillarization, the thickened space of Disse causes narrowing of the sinusoids and results in increased vascular resistance (23,24).

Endothelial fenestration is an important determinant of transinusoidal exchange and also plays a role in modulating intrahepatic resistance. Endotoxemia, commonly observed in cirrhotic patients, can by itself produce a reversible and significant decrease in the porosity of the sinusoidal endothelium (25).

Although antifibrogenic therapy has been expanding rapidly (9), hepatocyte enlargement remains the only anatomical factor that can be effectively ameliorated resulting in reduction of portal hypertension. Abstinence from alcohol may resolve portal hypertension (26) along with hepatomegaly, liver cell enlargement, and steatosis. Shibayama *et al*. have studied the sinusoidal microcirculatory effects of liver cell enlargement in rats with cirrhosis induced by a diet deficient in choline (27). In this model, sinusoidal stenosis is caused by liver cell enlargement secondary to fat accumulation, and is correlated with increased sinusoidal vascular resistance, portal hypertension, and development of ascites. With a normal diet, hepatocyte size normalizes with disappearance of sinusoidal stenoses and marked decrease in vascular resistance. Similar conclusions were reported using a carbon tetrachloride-induced cirrhotic model, in which the increase in sinusoidal resistance was correlated with the encroachment of enlarged hepatocytes on the sinusoidal space (28). In an additional study, septal fibrosis in the rat liver was shown to have a significant impact on portal pressure only in the presence of deformation of the sinusoids induced by hepatocyte enlargement (29).

However, in addition to hepatocyte enlargement, inflammatory processes related to alcohol, choline-deficient diet, and other agents may also be involved in the development of portal hypertension by causing intrahepatic circulatory dysfunction. Withdrawal of these conditions would reduce portal hypertension not only by decreasing hepatocyte enlargement but also by reducing the functional component determined by inflammation.

It seems that a hepatocyte enlargement of at least 50% is necessary to increase the intrahepatic vascular resistance. The liver could not compensate for bigger hepatocyte enlargements due to the restriction of the hepatic cytoskeleton resulting in portal pressure increase (30). In fact, no correlation between portal hypertension and a 45% enlargement of hepatocyte was observed (31). However, in a very peculiar study, high fat diet-fed ducks did not develop portal hypertension although they experienced a 350% increase in the ratio liver weight/body weight due to fatty infiltration (32). Although the relationship between hepatocyte enlargement and the development of portal hypertension has been extensively studied (26–35), it is still not completely understood.

Portal hypertension is frequently observed in acute liver failure and acute hepatitis. In these conditions, increase in portal pressure is probably caused by sinusoidal obstruction secondary to dropout of hepatocytes and, consequently, sinusoidal collapse. There is a direct correlation between degree of reticulin collapse and portal pressure in acute liver failure (36). In addition, severity of acute hepatitis, hepatic venous pressure gradient, and fractional area of sinusoidal collapse also demonstrate close correlation (37).

## Functional mechanisms

Bhathal *et al*. have shown that, in carbon tetrachloride-treated cirrhotic livers, the total vascular resistance increases in more than 110% and that about 25% of the increase can be reversed by vasodilator substances (3). Interestingly, the magnitude of the vasodilation seemed to be similar for different vasodilators and was almost negligible in normal livers. Therefore, the microvascular bed in cirrhotic livers has an enhanced intrinsic vascular tone that can be pharmacologically reversed. This is particularly interesting if we consider that, in portal hypertensive cirrhotic patients, a 20% decrease in hepatic vein pressure gradient is associated with a significantly lower risk of variceal bleeding (38–40).

Although different factors such as circulating vasoconstrictor substances may interfere in the intrahepatic vascular tone, the increased vascular tone observed in cirrhotic livers seems to be determined mainly by an imbalance between the production of vasodilators (41–43) and vasoconstrictors (44,45) in intrahepatic vessels. Although different hepatic cells are able to produce vasoactive substances, endothelial cells are mainly responsible for the intrinsic intrahepatic vascular tone control. Interposed between the lumen and the contractile elements of the vessel wall, endothelial cells react to different intraluminal stimuli, releasing vasoconstrictor and vasodilator substances to adjust the vascular tone to a particular situation (46). However, in cirrhotic livers, this endothelial function is impaired (41,42,45), allowing the development of an increased intrahepatic vascular tone.

### Contractile elements of the intrahepatic circulation

Portal venules and hepatic venules contain limited amounts of smooth muscle in their walls but nevertheless are contractile and respond to pharmacological agents (47). Endothelial cells, Kupffer cells, and stellate cells contain filaments, tubules, and contractile proteins that suggest the presence of contractile activity in these cells (48). While both endothelial and Kupffer cells are

responsive to a variety of pharmacological vasoactive agents and seem to regulate the sinusoidal blood flow by contracting or bulging into the sinusoid (47), the participation of normal stellate cells in the sinusoidal blood flow control is still a matter of debate (47–50). However, activated hepatic stellate cells (HSCs) present in cirrhotic livers are more likely to have a constrictive effect on sinusoids (44).

Located in the space of Disse, HSCs are believed to function as pericytes controlling the sinusoidal diameter (51–53). The contractile properties of HSCs have been demonstrated in normal livers (49,53,54) and, with great enhancement, after liver injury (55) and the consequent activation of this cell type. Activated HSCs express the cytoskeletal α smooth muscle actin gene, characteristic of vascular smooth muscle, whose amount seems to correlate with their capacity to contract (55,56). HSCs respond to several vasoactive substances, such as nitric oxide (NO), endothelin, carbon monoxide (CO), prostaglandin $F_{2\alpha}$, thrombin, angiotensin II, thromboxane $A_2$, substance P, and arginine vasopressin (53,55,57–60). Hence, status of HSC activation and ratio of vasoconstrictive/vasodilatory agents present in the hepatic microcirculation can affect the vascular resistance in cirrhotic liver.

## Decreased production of vasodilators in cirrhotic livers

### *Nitric oxide*

NO plays a central role in the intrinsic vascular tone control in different vascular beds (61–63) including the intrahepatic circulation (64,65). Most of the information about the role of NO in the intrinsic intrahepatic vascular tone control comes from studies in which rat liver perfusion was used as an experimental model. Mittal *et al*. have shown that, after incubation in the presence of the NO synthase blocker Nω-nitro-L-arginine (NNA), the perfused liver developed a hyper-responsiveness to norepinephrine (64), suggesting that NO modulates the vascular tone in normal livers. Later, this result was confirmed using different vasoconstrictors such as angiotensin II, endothelin-1 (author's unpublished data), and methoxamine (specific $\alpha_1$-adrenergic agonist) (66) or vasoconstrictive doses of bradykinin (65). Additional studies confirmed the role of NO in the normal intrahepatic vascular tone control. The hepatic vasculature is able to relax in response to acetylcholine (a NO-dependent vasodilator) (41) and produce NO in response to shear stress (41,67), phenomena that are inhibited by NO synthase inhibitors.

As mentioned before, an increased vascular tone is a significant component of the increased intrahepatic vascular resistance in cirrhosis. In studies performed in the isolated perfused cirrhotic liver, the greatest reduction in portal pressure by vasodilator compounds was observed after infusion of the NO donor sodium nitroprusside (3). Although this was indirect evidence of a deficient production of NO in cirrhotic livers, more recent studies specifically focused on this abnormality. Both acetylcholine-induced vasodilation and shear stress-induced NO production were shown to be severely impaired in cirrhotic livers (41,43).

It has been shown that, although the expression of endothelial NO synthase in carbon tetrachloride-treated cirrhotic livers is similar to the expression in normal livers, the activity of this enzyme is significantly reduced in this experimental model of cirrhosis. This deficient activity is associated with increased protein levels of caveolin -1 and an increased binding of eNOS to caveolin-1, probably preventing the binding of the enzyme to calmodulin and its subsequent activation (43). Later, this mechanism of NO production impairment was observed also in bile duct ligated rats, a model of liver fibrosis with cholestasis (68).

Recently, we have developed a modified rat liver perfusion model in which not only the perfusion pressure but also the sinusoidal pressure is measured during the experiment (66). Using this experimental model, we observed that, in normal livers, NO modulates the vascular tone in the presinusoidal, sinusoidal, and post-sinusoidal areas. In addition, we observed that a deficient NO production was a major factor causing the increased intrahepatic vascular tone in cirrhosis (Fig. 13.1). Deficit in NO production was present in both the sinusoidal and post-sinusoidal areas but not in the presinusoidal, where NO production is normal. Actually, indirect evidence indicated that the presinusoidal vascular resistance was decreased in these livers (66).

Since a deficient production of NO is involved in the development of the increased intrahepatic vascular resistance in cirrhosis, replenishment of the liver vasculature with this vasodilator is a target for treating portal hypertension in this condition. Nitrates have been used with success for treating different cardiovascular conditions. However, for different reasons, nitrates are not effective in treating portal hypertension in cirrhotic patients (69). We studied the vasodilation induced by nitroglycerin (NTG, a nitrate) and S-nitroso-N-acetylpenicillamine (SNAP) in normal and cirrhotic rat livers (Fig. 13.2) (70). Different from the spontaneous NO donor SNAP, NTG requires bioactivation to cause its vasodilatory effect (71,72). We observed that NTG caused less vasodilation in cirrhotic livers than in normal livers, indicating that its bioactivation is impaired in this condition (70). This may explain, at least in part, the poor response to nitrates among cirrhotic patients.

In cirrhotic livers, SNAP caused more vasodilation than NTG. However, the vasodilation induced by SNAP was still less in cirrhotic livers that in normal livers. This

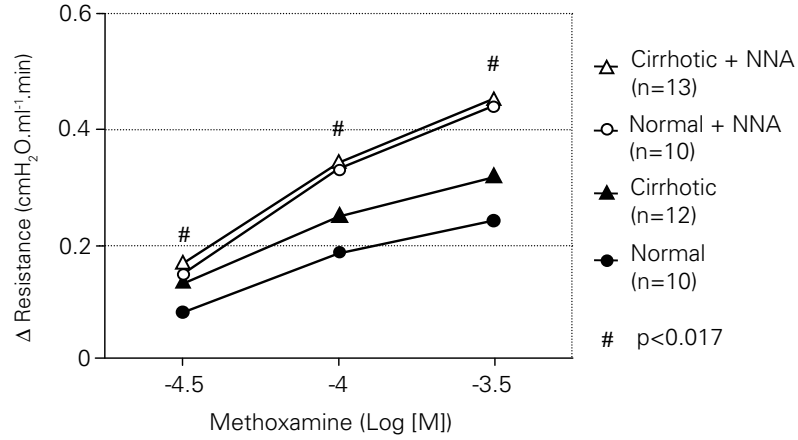


**Figure 13.1** In the absence of the nitric oxide synthase Nω-nitro-L-arginine (NNA), the vasoconstrictive response to methoxamine in cirrhotic livers was higher ($P = 0.025$) than in normal livers. NNA increased the response to methoxamine in normal ($P < 0.001$) and cirrhotic livers ($P = 0.001$) to the same level ($P = 0.686$). These results indicate that deficient production of NO is a major factor causing the increased response to vasoconstrictors observed in cirrhotic livers. (Reproduced with permission from Loureiro-Silva *et al.*, 2003 (66).)

indicated that, in addition to a decreased NO production, a decreased response to NO occurs in cirrhotic livers. This decreased response could be caused by: (i) increased NO inactivation by superoxide anion, a product of oxidative stress; (ii) dysfunction of the cGMP cascade, an enzymatic system that mediates the NO-induced vasorelaxation; and (iii) dysfunction of cGMP-independent mechanisms of NO-induced vasorelaxation.

Physical impairment of NO diffusion through structurally changed vessel walls was previously thought to be involved in the impaired vascular response to NO in cirrhotic livers. The intrahepatic vascular permeability, particularly to high-molecular-weight molecules such as albumin (MW 66 000), is decreased in cirrhotic livers (20,73). However, small molecules such as water and sucrose (MW 342.3) have a normal diffusion from the intravascular to the extravascular space in carbon tetrachloride-treated cirrhotic livers (20), which practically rules out anatomical factors as cause of the impaired diffusion of NO (MW 30). An altered redox status, however, may impose an important spatial range constraint to NO because of its high reactivity (74).

Portal hypertension in cirrhosis is frequently associated with splanchnic and systemic overproduction of NO, which is a key factor in the development of a hyperdynamic circulatory state (75–77). Liver-specific NO donors would replenish the intrahepatic circulation with NO, reducing the intrahepatic vascular resistance without causing additional impairment in the systemic circulation. NCX-1000, a ursodeoxycholic derivative, was designed to deliver NO exclusively to the liver. In cirrhotic rats, this compound was able to reduce significantly the hyper-responsiveness of the intrahepatic circulation to methoxamine during rat liver perfusion and, *in vivo*, abolish the portal pressure increase induced by increase in portal blood flow. These beneficial hemodynamic effects of NCX-1000 were associated with an increased cGMP concentration in cirrhotic liver tissue (78).

NO influences vascular homeostasis not only by modulating the vascular tone. NO inhibits platelet aggregation, adhesion, and activation, thus keeping platelets in a resting stage. Consequently, decreased bioavailability of NO by injury to the endothelium may be accompanied

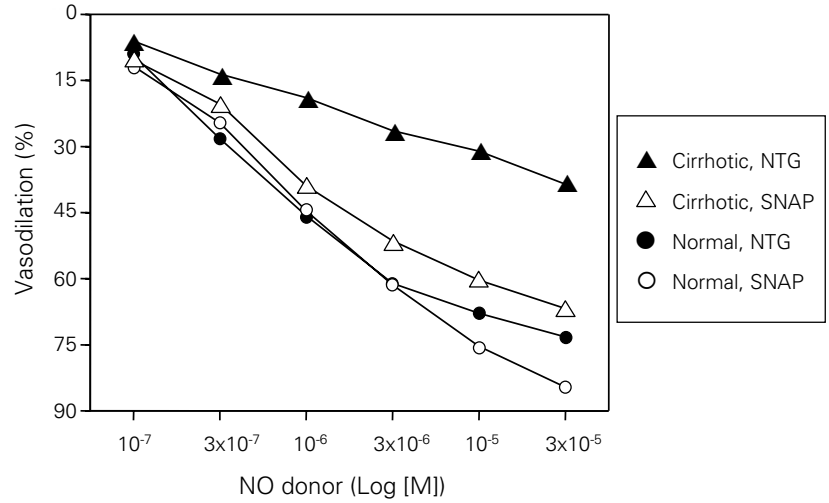


**Figure 13.2** Vasorelaxation induced by the nitric oxide (NO) donors nitroglycerin (NTG) and S-nitroso-N-acetylpenicillamine (SNAP) in normal and cirrhotic rat livers after pre-constriction with methoxamine ($10^{-4}$ M) during rat liver perfusion. SNAP and NTG caused similar vasorelaxant responses in normal livers ($P = 0.430$); in cirrhotic livers, SNAP produced a greater relaxation than NTG ($P < 0.001$). Both SNAP ($P < 0.001$) and NTG ($P = 0.002$) induced a greater vasorelaxation in normal livers than in cirrhotic livers. Comparison: repeated measures ANOVA. (Reproduced with permission from Dudenhoefer *et al.*, 1999 (70).)

by increased thrombogenesis (79). In fact, thrombosis of portal and hepatic veins of medium and large size is frequently observed in liver cirrhosis, which may contribute to the progression of portal hypertension (80). Moreover, NO maintains the anti-adhesive nature of the endothelium by suppression of specific adhesion molecules leading to inhibition of leukocyte and monocyte adhesion (81,82), which is a known key event in the development of atherosclerosis and thus endothelial dysfunction (83–84).

Finally, NO scavenges free oxygen radicals and directly interferes with enzyme systems producing oxygen radicals (85,86), thereby protecting the endothelium from oxidative stress. Indeed, NO inhibition has been demonstrated to increase lipid peroxidation induced by carbon tetrachloride (87). Taken together, the vasodilatory, antiatherogenic, and anti-thrombotic properties of NO imply that the endothelial dysfunction is one of the main factors causing increased intrahepatic vascular resistance in cirrhosis.

### *Carbon monoxide*

CO is a product of degradation of iron-protoporphyrin IX by the enzyme heme-oxygenase (HO). Like NO, CO activates soluble guanylyl cyclase inducing several regulation processes, including vasorelaxation (88). HO exists in three isoforms: the inducible HO-1, the constitutive HO-2 (88), and HO-3 (89). HO-1, which is identical to heat shock protein 32, is induced by different stimuli such as cytokines, heavy metals, and oxidants. The HO substrate iron-protoporphyrin can also upregulate the inducible isoform (88). The liver is one of the major organs in which heme molecules are degraded by HO. While the inducible isoform HO-1 is observed predominantly in Kupffer cells, the constitutive isoform HO-2 is present in parenchymal cells but not in Kupffer cells. HO-2 in parenchymal cells appears to play a major role in regulation of microvascular tone in physiological conditions (90). However, induction of HO-1 also increases CO generation, resulting in vascular resistance decrease in perfused rat livers (91). Cyclic GMP-independent CO-induced vasorelaxation, a mechanism mediated by inhibition of cytochrome P450, was observed in lipopolysaccharide-treated rat livers (92). In normal or pathological conditions, the decrease in hepatic vascular resistance occurs through CO release, resulting in sinusoidal relaxation. Although both isoforms were undetectable in HSCs (90), the participation of these cells in the CO-mediated sinusoidal relaxation has been suggested (56).

Fernandez and Bonkovsky (93) have studied HO isoform expression in partial portal vein-ligated (portal hypertensive) and sham-operated rats. Using reverse transcription-polymerase chain reaction and Western blot analysis to assess the expression of HO mRNA and protein, the authors observed that HO-1 expression in hepatocytes and splanchnic organs was significantly higher in portal vein-ligated animals than in sham-operated controls, and that HO-2 was expressed in all liver cell types and splanchnic organs of animals from both groups.

HO-1 activity in liver tissue from cirrhotic patients is higher than in normal liver tissue (94), suggesting that the CO production in cirrhotic livers is not impaired. Therefore, a hypothetical decreased release of CO is not involved in the development of the increased intrahepatic vascular tone in cirrhosis. On the contrary, it has been suggested that an increased CO production could be involved in the decreased production of NO in cirrhotic livers (94).

### *Prostacyclin*

Prostacyclin ($PGI_2$) is a prostaglandin with a potent vasodilator effect. In the liver, $PGI_2$ is produced by endothelial cells and vascular smooth muscle cells. It causes vasorelaxation by activating adenyl cyclase and, consequently, increasing cAMP production. The same stimulatory factors that are involved in endothelial NO production also promote the release of $PGI_2$ (95). During rat liver perfusion, Mittal and collaborators observed that indomethacin (a prostaglandin synthesis inhibitor) increases the intrahepatic vascular response to norepinephrine only in the presence of NO production inhibition, indicating that $PGI_2$plays a role in the intrahepatic vascular tone control that is secondary to the role of NO (64).

There is little information about the production of $PGI_2$ in cirrhotic livers. Studying the role of eicosanoids on the vascular tone control in cirrhotic rats, Graupera *et al*. reported that, after vasoconstriction induced by methoxamine ($\alpha_1$-adrenergic agonist), normal and cirrhotic livers produced similar amounts of 6-keto prostaglandin $F_{1\alpha}$ (the end metabolite of $PGI_2$) (45). This finding indicates not only that the normal liver produces $PGI_2$ in response to a hemodynamic challenge, but also that this production is preserved in cirrhotic livers. However, similarly to the increased production of CO, it does not seem to be enough to compensate for the decreased production of NO in cirrhotic livers.

## Increased production of vasoconstrictors in cirrhotic livers

### *Endothelins*

Endothelins (ETs) constitute a family of 21-amino-acid peptides with potent vasoconstrictive effect occurring in at least four isoforms, named ET-1, -2, -3, and β. Different cell types, including sinusoidal endothelial cells

and HSCs, synthesize ETs (96). As major inducers of HSC contractility, ETs have emerged as possible mediators of the increased vascular tone observed in cirrhotic livers (53,59,97). ET-1 is the most extensively studied of these peptides since it is, in equimolar terms, the most potent vasoconstrictor known so far (98). Two types of G-protein-coupled receptors, $ET_a$ and $ET_b$, mediate the vascular response to ETs (99). Both receptors are present on vascular smooth muscle cells and produce a sustained vasoconstriction (100). Endothelial cells express only $ET_b$ receptors, which bind ET-1 with lower affinity than $ET_a$ receptors and cause vasodilation by promoting the release of NO and prostacyclin (100). ET receptors are expressed in the liver mainly on HSCs and sinusoidal endothelial cells (SECs), but also to a lesser extent on Kupffer cells and hepatocytes (97,101).

In normal liver, ET-1 causes an increase in portal pressure *in vivo* as well as in isolated perfused liver, suggesting a contributory role of ET-1 in modulating hepatic vascular resistance (49,102–104). However, the site of action is controversial. Several reports suggest mainly a sinusoidal effect being mediated predominantly by the contraction of HSC (53,56,58). On the other hand, Kaneda *et al*. have located the point of maximal contraction in response to ET-1 at the distal segment of preterminal portal venules and thus, a presinusoidal level (105). Unpublished data from our laboratory suggest that, in normal perfused rat livers, ET-1 causes similar presinusoidal, sinusoidal, and post-sinusoidal vascular resistance increases. However, neither acute nor chronic blockade of endothelin receptors has shown significant effects on portal pressure in normal rats (106–108). This suggests a minor role for ET-1 in physiological modulation of the intrahepatic vascular tone.

In liver cirrhosis, arterial and venous plasma concentrations of ET-1 (and ET-3) are elevated and a hepatosplanchnic release of ETs, which seems to correlate with sinusoidal pressure, has been reported (109–112). Although this may be related to the abnormal vasodilatory state observed in systemic and splanchnic vascular beds, gene expression of ET-1 is largely enhanced in cirrhotic liver. Activated HSC and SEC seem to be the major sites of ET-1 synthesis (44,111,113). Additionally, an increased ET-receptor density (114) and an increased ET-receptor gene expression (113) are found in liver tissue of cirrhotic rats and patients, respectively. The degree of activation and ET-induced contractility of isolated murine HSCs increase proportionally to the progression of liver injury (55). However, ET-1-induced sinusoidal constriction is enhanced in the ethanol-induced fatty liver (115) but not in the cirrhotic liver, where ET-1 effects on hepatic hemodynamics have been reported to be blunted (55,111). Although short-term administration of nonselective ($ET_a$ and $ET_b$) ET receptor blockers were reported to reduce portal hypertension in experimental cirrhosis (106–108), chronic blockade of ET receptors failed to prevent the development of portal hypertension and hyperdynamic circulation in cirrhotic rats (116), rendering the exact role of ETs for the pathogenesis of portal hypertension in liver cirrhosis open to further investigation.

Interestingly, NO donors or endogenous NO oppose or even completely abolish the vasoconstrictive effect of ET-1 in perfused liver or activated HSCs in culture (49,58,117,118). Additionally, it has been demonstrated that NO has a blocking effect on ET generation (119). Therefore, NO production in the microcirculatory unit of the liver is of crucial importance to counterbalance at least the enhanced contractility of HSCs to different stimuli.

### *Eicosanoids*

The families of prostaglandins, leukotrienes, and thromboxanes are called eicosanoids. They are derived from 20-carbon essential fatty acids that contain three, four, or five double bonds: 8,11,14-eicosatrienoic acid (dihomo-γ-linolenic acid), 5,8,11,14- eicosatetraenoic acid (arachidonic acid), and 5,8,11,14,17-eicosapentaenoic acid. In humans, arachidonate is the most abundant precursor. To produce prostaglandins and thromboxane, arachidonic acid is first metabolized by cyclooxygenases, key enzymes in the inflammatory process (120).

An excessive production of vasoconstrictor eicosanoids, mainly thromboxane, was reported to be associated to the hyper-responsiveness of cirrhotic livers to the $\alpha_1$-adrenergic agonist methoxamine (45). Although an increased basal (not stimulated) production has not been reported, this study indicates that eicosanoids may play a role in the increased intrahepatic vascular tone observed in cirrhotic livers.

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# Chapter 14
# Alterations of Hepatic and Splanchnic Microvascular Exchange in Cirrhosis: Local Factors in the Formation of Ascites

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## Introduction

Filtration of fluid from microcirculatory vessels is a normal physiological process that aids the exchange of substances between plasma and tissue (1–3). Normal fluid homeostasis is controlled by refined mechanisms that include fluid intake, renal handling of water and salt, hemodynamic and oncotic forces, dynamic shifts in proteins between the various compartments of the body, and neurohumoral regulation. The balance between intravascular and extravascular fluid is governed by the Starling forces, i.e. capillary and interstitial fluid hydrostatic pressure, plasma and interstitial fluid oncotic pressure, microvascular permeability/hydraulic conductivity, and lymphatic drainage (2–5).

Endothelial cells of continuous capillaries have small pores (paracellular clefts), 0.8–2.0 nm in diameter, that allow passage of water and small solutes. Larger pores, 20–30 nm in diameter, allow leakage of proteins with diameters approaching 10–15 nm. The gastrointestinal tract has fenestrated capillaries with basement membrane. Sinusoids with collections of fenestrae measuring 100 nm in diameter and no basement membranes are found almost entirely in the liver and spleen (6,7). The microcirculation in the liver consists of two vascular beds: the hepatic sinusoids and the peribiliary capillary plexus. The latter gets its blood from the hepatic artery, whereas the sinusoids receive their supply from a mixture of portal venous and hepatic arterial blood, as well as from the outflow of the peribiliary capillary plexus.

Surplus capillary filtrate from the liver and gastrointestinal tract drains via capsular and hilar spaces through hepatic and intestinal lymphatics into the thoracic duct (8,9). The peritoneal space is chiefly drained through lymphatics on the abdominal side of the diaphragm to the right lymphatic duct (2,10,11). If there is an imbalance between filtrative forces and those which drain the peritoneal space, surplus fluid appears and sequestration of fluid into the peritoneal cavity leads to the formation of ascites.

In heart, kidney, and liver disease, fluid retention with the formation of edema is a characteristic feature in the progressive stage, and in cirrhosis the accumulation of fluid in the peritoneal cavity occurs in a substantial number of patients with advanced disease (12). Ascites (Greek *askos* = "leather bag," "wineskin") was formerly considered to be a local peritoneal edema (13). However, the pathogenesis of hepatic ascites is much more complex than that of peripheral edema. Since Lieberman *et al*. proposed the "overflow theory" of ascites formation (14) and Schrier *et al*. put forward the "peripheral arterial vasodilatation theory" (15), much effort has been devoted to the systemic factors in the hepatic formation of ascites (kidney function, blood volume expansion, hyperdynamic circulation, etc.). However, the last decades have seen a considerable advance in the understanding of microvascular dynamics and pathophysiology of the interstitium (2). This progress in knowledge has been achieved mainly by investigations with electron microscopy, studies with indicators of different molecular sizes, and advances in the understanding of the dynamics of lymphatics and peritoneal dialysis.

This chapter describes the factors that affect the filtration rate of fluid from the hepatosplanchnic microcirculation. Some cornerstones and recent advances in the understanding of trans-sinusoidal and transperitoneal fluid exchange in the normal state and in cirrhosis are considered.

## The normal blood–lymph barrier

The normal hepatosplanchnic vascular bed is a low-pressure system with a portal venous pressure of about 8–10 mmHg, a small pressure gradient of 2–5 mmHg across the sinusoids of the liver, and with a small drop in pressure from the intestinal capillaries to the portal vein (16).

From a structural point of view, the normal blood–lymph barrier consists of the capillary endothelial cell, a basement membrane to which the endothelial cells are attached, occasionally by pericytes, which wrap around the capillary (in the gastrointestinal tract and peritoneal membrane, but not in hepatic or splenic capillaries), connective tissue spaces, and end with the terminal lymphatics (2,6,17–20). The microcirculation in the normal liver has discontinuous capillaries (sinusoids) without a basement membrane (17,21–23). The fenestrae in the sinusoidal lining allow almost unrestricted passage of large and small proteins in the same proportion (bulk flow) (18,24). In the normal condition, the area of the fenestrae occupies about 10% of the capillary wall (25). The porous nature of the sinusoids and the architecture of the liver leave an impression of a microvasculature designed for rapid transport of large amounts of fluid, solutes, and metabolites (Fig. 14.1). The exchange of material is either diffusive (i.e. governed by differences in concentration) or filtrative/convective (i.e. governed by sieving and fluid volume flow, owing to differences in pressure) (7). Small molecules (sodium, chloride, glucose, lactate, etc.) and gases (oxygen, carbon dioxide) are transported through the entire capillary wall almost entirely by simple diffusion or facilitated by carriers (7). In the liver, this transport rate is large and almost solely limited by the hepatic plasma flow rate (26). The transport of large molecules (proteins) is in the main filtrative/convective, but, at very low rates of lymph flow, diffusion may be perceptible. Permselectivity, i.e. preferential transport of smaller molecules owing to restriction of larger molecules in the fenestrae, is only present for very large proteins (24,27). Studies by Goresky with albumin [mol. wt 69 000, Stokes–Einstein (S-E) diameter 7 nm] have shown that the sinusoidal–perisinusoidal communication is normally free, i.e. limited only by plasma flow (26). This is well understood from our present knowledge of the morphology of the liver sinusoidal lining. Electron microscopic studies confirm that restriction (permselectivity) can be present only for very large molecules with an S-E diameter above approximately 20 nm (18,22,28). Thus, in the normal liver there is close agreement between results obtained by morphometric and by physiological methods. The perisinusoidal space (space of Disse) may therefore be regarded as a paravascular part of the plasma volume. [This concept is further stressed by the fact that the conventionally determined plasma volume (indicator dilution technique) includes this paravascular space in the liver.]

The transport of fluid and solutes from the perisinusoidal space into portal connective tissue and subcapsular spaces is not clearly defined from a morphological point of view (6,8,18,29). However, most physiological studies with molecules of different sizes indicate no appreciable permselectivity here, except at very low rates of lymph flow (24,26). The transport from portal and subcapsular interstitium to terminal lymphatics is considered to take place by bulk carriage (18,30). Owing to the large size of the fenestrae in the liver sinusoids and the bulk flow of fluid with a high lymphatic content of protein, no oncotic pressure gradient exists across the sinusoids or the liver blood–lymph barrier (30), and it is possible that liver tissue is the barrier limiting the movement of plasma proteins from blood to lymph.

**Figure 14.1** Transmission electron micrograph of sinusoidal lining (bottom), perisinusoidal space (space of Disse) (middle), and microvili from a hepatocyte (top) in normal liver. About 10% of the area of the capillary wall has fenestrae with a diameter of about 100 nm and no basement membrane. This allows almost unrestricted passage of water, solutes, and proteins.

An exception is the peribiliary capillary plexus, which has continuous type capillaries with a much lower permeability than the normal liver sinusoids (23). Filtration of fluid with a low protein content from the peribiliary capillary plexus is the reason why the normal liver lymph–plasma protein ratio is not 1.00, but rather 0.95

(30,31). The significance of the peribiliary capillary plexus in normal fluid dynamics is probably small and will not be considered further in this review.

The microcirculation in the gastrointestinal tract has a fenestrated capillary wall with a basement membrane that is relatively impermeable to macromolecules, but very permeable to water and smaller molecules (19,32,33). Therefore, only part of the plasma oncotic pressure is exerted across the microvascular endothelium. Published values of the transmicrovascular oncotic pressure gradient for the stomach, small intestine, and colon range from 11.5 to 13.0 mmHg (34). Intestinal capillaries show permselectivity with characteristic sieving (35). The intestinal lymph–plasma protein ratio is normally relatively low (35,36) in accordance with the relatively tight capillary membrane here. Normally, fluid from the gastrointestinal interstitium is drained to intestinal lymphatics and further into the thoracic duct, and fluid, solutes, and proteins are only to a very limited extent transported into the peritoneal space.

The blood capillaries in the peritoneal membrane are the continuous type with a basement membrane. Over the last decade a number of studies on transperitoneal dynamics have been performed in uremic patients undergoing peritoneal dialysis (37–40). The essentials of these studies are that both diffusive and filtrative transport participate in the overall transperitoneal dynamics. The peritoneal clearance of low molecular substances, such as glucose and creatinine, is in the order of 5–10 ml/min. Whereas fluid and low molecular solutes exhibit a major direct transperitoneal transport, proteins and other high molecular substances are in the main transported back into the bloodstream via the subdiaphragmatic lymphatics (2,40–44).

The Starling equation describes the transcapillary dynamics (19,20):

$$J_v = K_f [(P_{mv} - P_t) - \sigma(\pi_p - \pi_t)]$$

where $J_v$ is the filtration rate, $K_f$ the filtration coefficient, $P_{mv}$ the microvascular hydrostatic pressure, $P_t$ the tissue hydrostatic pressure, $\sigma$ the osmotic reflection coefficient, $\pi_p$ the microvascular (plasma) oncotic pressure, and $\pi_t$ the tissue oncotic pressure. The filtration coefficient ($K_f$) is a measure of the hydraulic conductance of the microvascular barrier between plasma and tissue. $K_f$ is determined by two major factors: water permeability and the surface area available for exchange. The reflection coefficient ($\sigma$) describes the fraction of the effective oncotic pressure generated across the microvasculature. Impermeable proteins have a $\sigma$ value of 1, since they generate 100% of their osmotic pressure difference. Conversely, small freely permeable proteins ($\sigma = 0$) do not generate any osmotic pressure difference. Values of $\sigma$ indicate that 92%, 85%, and 78% of the total plasma oncotic pressure gradient is generated across the microcirculatory barrier in the small intestine, colon, and stomach, respectively, but that 0% is generated in the liver (31,35,45,46). In microcirculatory beds in which $\sigma$ for protein is > 0, the oncotic pressure gradient across the microvascular barrier is an important determinant of fluid dynamics. For example, a fall in $\pi_p$, caused by a reduction in the concentration of plasma proteins, can disturb the Starling forces and lead to an accumulation of interstitial fluid in amounts too large to be cleared by the lymphatics.

## Circulatory and microvascular changes in chronic liver disease

Portal-sinusoidal hypertension is an essential feature in the hepatic ascites syndrome. Various theories have been put forward as to its genesis (15,47–49). A decrease in the overall vascular cross-sectional area, an increase in vascular resistance located post-sinusoidally or close to the outlet of the sinusoids, and an increased mesenteric inflow are important elements (47). The hydrostatic pressure in the sinusoids is increased in cirrhosis. Because the hepatic and splanchnic microcirculations are in series, the events occurring in the liver are transmitted upstream to the splanchnic organs in the form of increased pressure. A small presinusoidal component has been described in some patients with alcoholic liver disease, but most investigators agree on the concept that sinusoidal and portal pressure are increased to the same degree in cirrhosis. Thus, the elements in the Starling equation favor an outward movement of fluid in portal-sinusoidal hypertension (8,30).

The sinusoidal lining is altered in cirrhosis (18,50,51). Most studies indicate a tightening of the sinusoidal wall. During the progression of cirrhosis, the sinusoids of the liver become transformed into less permeable capillaries, and some sinusoids develop a continuous, defenestrated endothelium, with development of a basement membrane and even pericytes. This transformation of the capillary barrier directly affects the rate at which fluid and protein filter from the plasma into the surrounding tissue, as described by the Starling equation. The so-called capillarization with appearance of a basement membrane and collagenization of the perisinusoidal space was first described by Schaffner and Popper in 1963 (50), and later studies on morphology have confirmed these observations (28). These results, combined with newer investigations on protein kinetics, indicate a reduction in the number of fenestrae rather than in their size (18,52,53). This implies that the sinusoidal permselectivity remains small in cirrhosis, and consequently the effective oncotic pressure gradient across the sinusoids is also small (9). However, the tightening of the sinusoidal wall and collagenization of the perisinusoidal space decrease the hydraulic conductivity, but these alterations in the liver blood–lymph barrier only represent a limited counterweight against the increased sinusoidal

pressure. Tracer kinetic studies have shown a substantially increased blood-to-lymph transport of water, solutes, and proteins with increased trans-sinusoidal filtration and highly elevated hepatic lymph flow (53,54). Direct measurements on surgically exposed lymph vessels in cirrhosis have thus shown an enlarged thoracic duct with a lymph flow 5–15 times higher than normal values (2.5–3.5 l/24 h) (9,55). Whereas the lymph drainage keeps pace with the enhanced filtration, the spillover of fluid into the peritoneal cavity is minimal and the patient will be free of ascites (9,18), but when the trans-sinusoidal filtration exceeds the transport capacity of the lymph vessels, surplus fluid will pass into the peritoneal space. The rate of transport into the peritoneal space is, however, relatively small (< 5%) compared with that of lymph entering directly into the thoracic duct (18,42,43,54).

Tracer kinetic studies with protein indicators of different molecular sizes have indicated that most of the intraperitoneal protein comes from the liver via trans-sinusoidal filtration (18,24,30,56). Concentrations of small and large proteins in proportion to their level in plasma as well as equal relative transport rates suggest bulk carriage, i.e. flow through openings without restriction (18,24,27,30,52). The liver (and spleen) is the only location that can provide this type of transport. In contrast, earlier studies by Witte *et al*. have concluded that in advanced cirrhosis, ascitic fluid is predominantly generated by the intestines (55). This may be true for the water and nonprotein components of the ascitic fluid, but later protein kinetic studies have shown that trans-sinusoidal filtration is the main origin of ascitic fluid protein (7,24,27,52).

Figure 14.2 illustrates different forms of transport kinetics from plasma to ascitic fluid, and Table 14.1 summarizes clearance rates from plasma to ascitic fluid of substances with different molecular weights.

In patients with cirrhosis, increases in hydrostatic pressure in the hepatic and splanchnic microcirculations constitute a major factor in the formation of ascites. Increased intravascular hydrostatic pressure also increases the amount of filtering surface area of the microcirculation through two major mechanisms. First, increased pressure promotes the opening of nonflowing capillaries, which can then contribute to fluid filtration. Second, a mechanism that might not be readily apparent from Starling's equation is an increase in the length of filtering vs. absorbing sections of capillary (19).

In cirrhosis, there is a significant vasodilation of lymphatics in the intestine and an increase in the number of mesenteric lymphatic vessels. The lymph–plasma ratio of proteins in the intestine is reduced from 0.60 to 0.18. Thus, oncotic pressure in the interstitium is decreased to an even greater extent than in plasma. Considering that permeability to plasma proteins in the intestine stays relatively constant during cirrhosis, it is evident that the lymphatic dilution of protein is the result of greatly enhanced capillary filtration.

The gastrointestinal transvascular filtration in portal hypertension is dominated by a protein-poor filtrate, which will equilibrate with the protein-rich fluid coming from trans-sinusoidal filtration (55). On the assumption that lymph provides an accurate reflection of the contents of interstitial fluid, the oncotic pressure gradient across the microvascular barrier can be estimated from lymph and plasma either by using an osmometer or by measuring the protein concentration and subsequently applying equations that relate protein concentration to osmotic

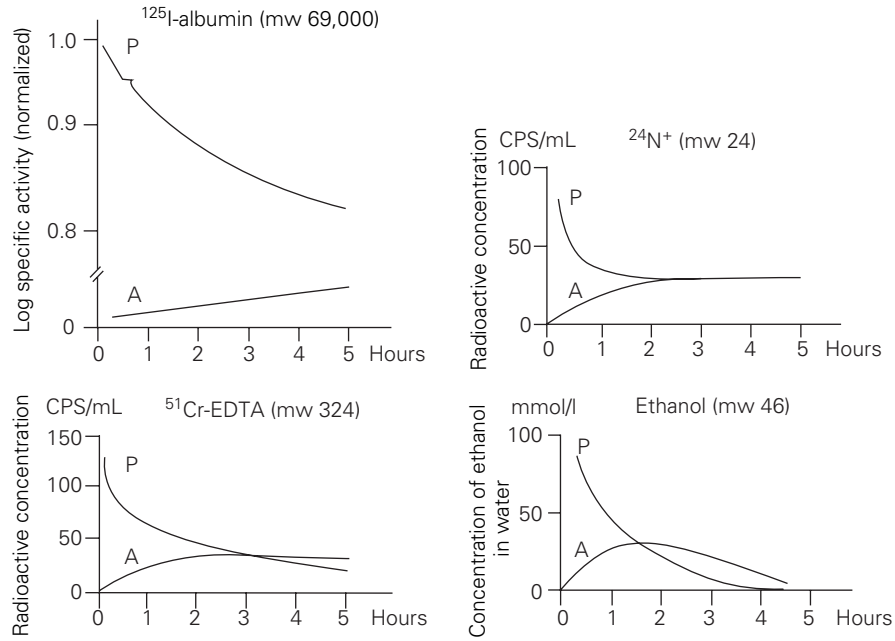


**Figure 14.2** Radioactive and chemical concentrations in plasma (P) and ascitic fluid (A) after i.v. injection of substances with different molecular weights. Transport rates can be determined from these curves. A net transport from plasma into ascitic fluid takes place within the first 1–3 h of i.v. injection. Moreover, it is characteristic that when the plasma curve crosses the ascitic fluid curve the slope of the ascitic concentration–time curve is close to 0, indicating no net transport. The deviation in plasma albumin activity around 40 min after injection is due to lymphatic recirculation of tracer.

**Table 14.1** Clearance from plasma to ascitic fluid of high and low molecular substances in patients with cirrhosis.

| | IgG | Albumin | $^{51}$Cr-EDTA | $^{24}$Na+ | Ethanol |
|---|---|---|---|---|---|
| Molecular weight | 150 000 | 69 000 | 342 | 24 | 46 |
| Mean clearance (ml/min) | 0.2 | 0.2 | 26 | 44 | 51 |
| Range | (0.04–0.6) | (0.04–0.6) | (19–32) | (36–57) | |
| Ratio | 0.95[a] | | 0.59[b] | | 1.16[c] |

[a]The immunoglobulin G (IgG)/albumin clearance ratio (0.95) is significantly above the ratio between the free diffusion coefficients (0.6, $P < 0.01$), but not significantly different from 1.0, which indicates that the transport is filtrative-convective.
[b]The $^{51}$Cr-EDTA/$^{24}$Na+ clearance ratio (0.59) is significantly above the ratio between the free diffusion coefficients (0.39, $P < 0.01$), but also significantly below 1.0 ($P < 0.01$), which indicates that transport is a combination of diffusion and filtration. The latter is about 10 ml/min.
[c]The ethanol/$^{24}$Na+ clearance ratio (1.16) is above the ratio between the free diffusion coefficients (0.81) and above 1.0, which indicates that the surface area for the transport of ethanol is larger than that for $^{24}$Na+. (Earlier studies on labeled water have showed erroneously high values, because this technique included diffusion of water in water.)

pressure. Basal filtration coefficients may vary by a factor of 20 among the splanchnic organs and liver (19,33,35,45). These variations may represent true differences in microvascular permeability or the exchange surface area, or may contain some technical artefacts of the evaluations of $K_f$. $\sigma$ is approximately 0.8–0.9, whether the portal hypertension is acute or chronic. Intestinal capillary permeability does not appear to be substantially increased by either acute portal hypertension or cirrhosis (35).

## Transport from the peritoneal cavity to the bloodstream

The dynamics of transperitoneal transport has recently been studied extensively in uremic patients on peritoneal dialysis (4,38–40,56,57). In many ways, the transport kinetics in these patients is similar to that of patients with cirrhosis. In cirrhosis, ascitic fluid is always equilibrated with the surrounding interstitial tissue and plasma, which gives it the same osmolality as that of the plasma (58,59). This is because of the effect of the small solute crystalloid osmotic pressure. As the Donnan effect across the peritoneal membrane is small, no substantial electric potential difference exists across this membrane. It should, however, be kept in mind that different substances are transported from ascitic fluid back into plasma by different routes and by different mechanisms (18).

### Transport of highly soluble substances with low molecular weight

An example is ethanol (mol. wt 46) which crosses all biological membranes easily (7). The transport rate is fast (transperitoneal clearance is about 20–50 ml/min), and a relatively slow equilibration is seen only in patients with a very large ascitic volume (60). Transport takes place through the entire peritoneal membrane. Diffusion is by far the dominating transport process (see Table 14.1).

### Transport of low molecular extracellular substances

These are plasma electrolytes, creatinine, glucose, $^{51}$Cr-EDTA, etc. These substances have a transperitoneal clearance of 5-45 ml/min (58,61–64). They are transported by diffusion, as well as by filtration (7). The main transport takes place across the entire peritoneal surface area. As the concentration of a number of these endogenous substances is relatively stable over time, a significant net transport takes place only during accumulation and removal of ascitic fluid (net reabsorption, peritoneovenous shunting, or paracentesis).

### Transport of high molecular substances

As mentioned above, the ascitic origin of most of these substances (albumin, transferrin, immunoglobulins, lipoproteins, etc.) is the result of trans-sinusoidal filtration. The magnitude and mechanisms of protein transport from ascitic fluid back into the plasma have been studied by introducing different protein tracers into the ascitic fluid, followed by blood and ascitic fluid sampling (10,18,24,30,37,39,43,65,66). Occurrence of a protein tracer in plasma after a substantial time lag, an almost linear rise in plasma concentration (Fig. 14.3), and a similar rate of transport of smaller and larger protein indicators suggest bulk flow transport through tubes (42,56,57). This is consistent with animal experiments, which have shown that the peritoneal cavity is mainly drained by lymphatics on the abdominal side of the diaphragm (39,42,44). The transport rate in the right lymphatic duct is relatively small (42). It should, however, be stressed that some protein kinetic studies, especially those performed in uremic patients and in animals, have indicated that there is also a significant protein transport directly into the interstitial space of the peritoneal membrane (37,39,40). This agrees with recent kinetic results showing that some of the protein loss in patients on chronic ambulatory peritoneal di-

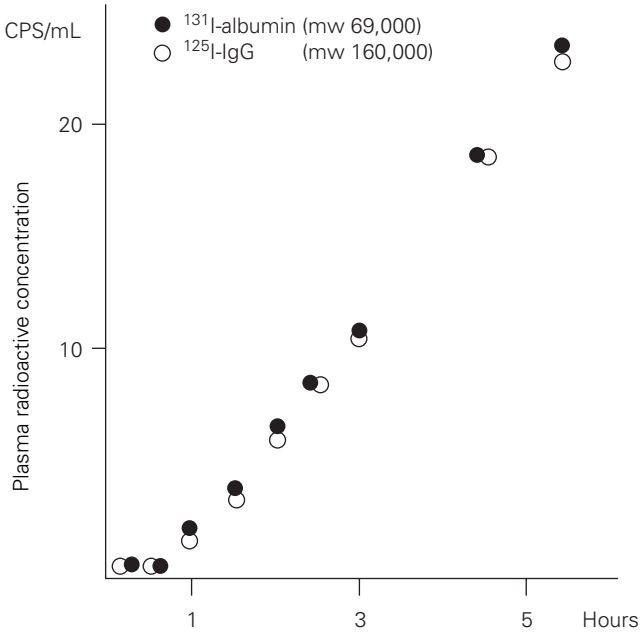


**Figure 14.3** Time–activity curves in plasma after intraperitoneal injection of radiolabeled albumin and immunoglobulin G. The plasma activity curve shows a characteristic delay of 0.3–2 h before it rises in an almost straight line, with the same velocity of small and large molecular proteins, indicating transport through tubes (lymphatics).

alysis may come from the interstitial space next to the peritoneal membrane (57). Whether this holds true in patients with cirrhosis is not known at present. In cirrhosis most results indicate that the main transport of ascitic fluid protein takes place through the lymphatic route (42,56,57). However, the overall transport rate of ascitic fluid protein back into the plasma is relatively low (clearance about 0.05–0.2 ml/min, see Fig. 14.4). The capacity of this transport of ascitic fluid protein back into plasma is the rate-limiting step for steady ascitic fluid reabsorption (see below).

The porosity of the capillary membrane is currently under debate. Two-pore and three-pore models have been proposed (67,68). The latter includes facilitated transport of water by specific transport proteins (aquaporin) and possibly also an albumin transporter (albondin) (69,70). However, the importance of these specific transport mechanisms has not been established in the peritoneal cavity, but their quantitative role is most likely to be minor (69,71).

## Dynamics of local elements in the formation and therapy of hepatic ascites

The presence of protein in the peritoneal space is an important requirement for fluid sequestration at this site. Reabsorption of isotonic protein-free fluid is complete and relatively quick, owing to the effect of the plasma oncotic pressure (58,62,72). When protein is present in the peritoneal space (as in cirrhosis) fluid and solute movements take place in the different areas, as described above, and ascitic fluid is formed. The protein concentration in cirrhotic ascitic fluid is much lower than that in plasma because of the hydrostatic/oncotic equilibration over the large surface area of the gastrointestinal tract (24,61,73,74). Accordingly, the oncotic pressure in ascitic fluid is close to that of the intestinal lymphatics (55). As intestinal blood capillaries are relatively impermeable to protein, increased gastrointestinal capillary pressure produces a filtrate low in protein concentration (74).

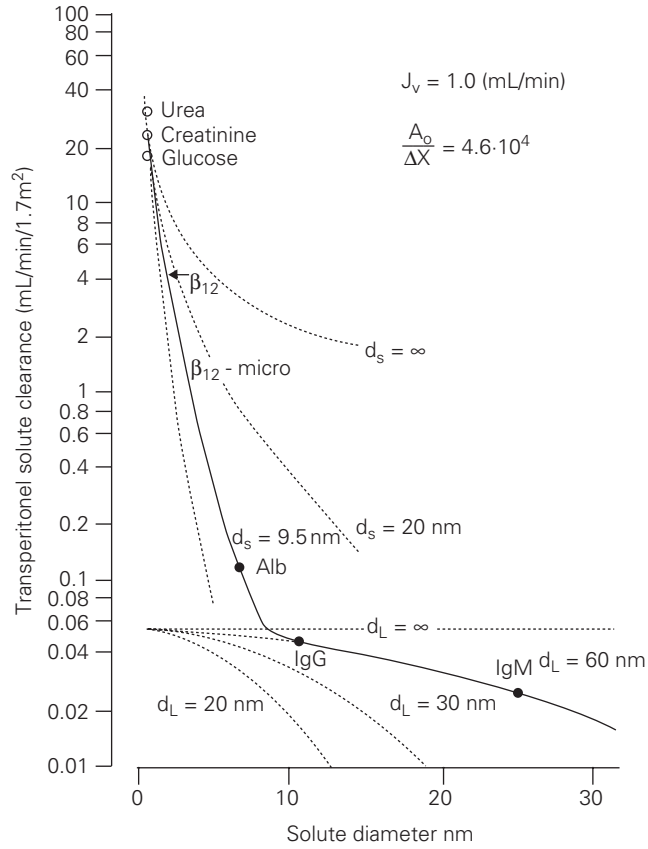


**Figure 14.4** Transperitoneal solute clearance vs. molecular radius of solute. The solid line is simulation for a two-pore system with a small pore diameter ($d_s$) = 9.4 nm and a large pore diameter ($d_s$) = 60 nm. Moreover, the presence of an unrestricted pore area over pore length ($A_o/\Delta_x$) of $4.6 \times 10^4$ cm and a filtration rate ($J_V$) of 1.0 ml/min are assumed. Simulation of transperitoneal solute clearances for different values of small pore diameters and large pore diameters are illustrated by dotted lines. It is seen that these values of low molecular and high molecular substances obtained in patients with uremia are close to the transport rates in cirrhotic patients with ascites. Data from Rippe and Stelin (56). Modified and reproduced with the kind permission of the authors.

Thus, ascitic fluid may be considered to be a mixture of protein-rich fluid from trans-sinusoidal filtration and protein-poor fluid from transcapillary gastrointestinal filtration, the mixing ratio being governed by hydrostatic/oncotic forces (3). Consequently, the oncotic pressure of the ascitic fluid will decrease, but the effective oncotic pressure gradient (i.e. plasma minus ascitic fluid oncotic pressure) will increase and thereby counteract the filtrative force of the elevated portal-intestinal capillary pressure (the so-called protein "wash-down" effect) (3,61). The effective oncotic pressure gradient thus plays a role in the dynamics of transperitoneal/transintestinal fluid, but the size of the oncotic pressure gradient is governed by the magnitude of the transmural hydrostatic pressure, i.e. the portal pressure (16,18,75). Hence, the effective oncotic pressure gradient may be looked upon as a mirror image of the portal venous pressure.

Owing to the low capacity of ascitic fluid-to-plasma transport of proteins, this transport becomes the rate-limiting step in the amelioration of ascites (76). During intensive diuretic treatment, plasma oncotic pressure may increase and portal pressure may be reduced (plasma volume contraction), which results in a decreased rate of ascites formation and increased transperitoneal transport of water and crystalloids back into plasma (77,78). However, the effective oncotic pressure gradient cannot become higher than the portal pressure, which stresses the presence of protein in the peritoneal cavity as a crucial element in fluid accumulation here (61).

Transport rates to and from the peritoneal space are not only of academic interest, they are also important for therapy. Thus, for the reasons mentioned above, mobilization of fluid by diuretic treatment should be adjusted to the rate of inflow into the peritoneal cavity/rate of protein transport back into plasma (59,61,62,72).

The slow peritoneal lymph drainage can be accelerated by surgical implantation of an artificial "megalymphatic," i.e. a peritoneovenous shunt (79,80). The astonishing ameliorating effect of this therapy on ascites illustrates the importance of efficient drainage from the peritoneal cavity. However, numerous complications and side effects render the therapy of limited clinical value (79,80).

Another important way of removing ascitic fluid and peritoneal protein is by paracentesis (81). A number of controlled clinical studies have proved the efficacy of this treatment (for overview, see 82). Owing to the transport dynamics described above, paracentesis, particularly repeated paracentesis, should never be performed without giving the patients a plasma expander, as this procedure will otherwise lead to a reduction in the effective plasma volume and vasodilation with postparacentesis circulatory dysfunction with potential major adverse effects (82–84).

## Effect of vasodilators/constrictors on microvascular fluid dynamics

Acute portal hypertension alone is not enough to cause severe edema or ascites, owing to "protein wash-down" and lack of peritoneal protein. However, splanchnic arteriolar vasodilation may play a crucial role in the formation of ascites, transmitting arterial pressure directly through the arterioles with decreased resistance to the filtering capillaries (85–87), which can deliver fluid, when the liver supplies protein by trans-sinusoidal filtration. The autonomic nervous system and modulators with paracrine and autocrine activity (e.g. serotonin, nitric oxide, endothelins, substance P, vasoactive intestinal polypeptide, calcitonin gene-related peptide) may influence the microvascular fluid exchange (85–94). This may be brought about by several mechanisms: recruitment of capillaries, altered balance between pre- and postcapillary vascular tonus, change in microvascular permeability, and altered receptor/transporter status. Unlike several microvascular beds, the sinusoids of the liver probably do not exhibit recruitment either in the normal or in the pathological state (7,95). Available evidence indicates that all sinusoids are open, although there may be a change in the contribution of portal venous/hepatic artery flow and some outflow regulation in different parts of the liver (96).

The sympathetic and parasympathetic nervous systems modulate the vascular tone in the splanchnic area and in the liver. This holds true in the normal state and in patients with cirrhosis (97–99). There are indications that increased sympathetic nervous activity may raise portal venous pressure and α- and β-adrenoceptor blockers reduce portal and sinusoidal pressure (100–102). In this way, autonomic tone may contribute to trans-sinusoidal and transcapillary gastrointestinal filtration. The role played by neurogenic vasodilators, such as the calcitonin gene-related peptide and substance P for transvascular dynamics, is not known.

Contractile elements in the lining of the sinusoidal fenestrae have been described in animal experiments and *in vitro* growth of sinusoidal endothelial and stellate cells has shown a modulating effect of different vasoactive substances (103–105). Consequently, neurohumoral modulators may, at least in part, participate in the control of local trans-sinusoidal exchange. Capillary permeability increases in the presence of bradykinin and glucagons. Consequently, the leakage of plasma proteins caused by bradykinin and glucagon may reverse the lymphatic dilution of protein observed under the increased capillary filtration. The role of these mechanisms in pathophysiology is at present merely speculative, but it opens up the possibility of pharmacological intervention.

The universal vasodilator, nitric oxide, and the endothelial vasoconstrictors (endothelins) play an im-

portant role in the systemic and splanchnic circulatory changes in cirrhosis (85–89,94,105,106). Their role in the local transvascular fluid dynamics in cirrhosis is, however, at present unclear.

## Conclusion

Recent investigations of local factors in the genesis and perpetuation of hepatic ascites indicate that most findings are explained by an imbalance between local transvascular filtration and lymph drainage. According to this concept, the amount of ascitic fluid is governed by increased trans-sinusoidal filtration of protein-rich fluid (consequent on portal-sinusoidal hypertension) with additional gastrointestinal transcapillary filtration of protein-poor fluid and transperitoneal hydrostatic/oncotic equilibration on the one hand and lymphatic drainage (fluid, proteins) and direct transperitoneal transport (water, crystalloids) on the other (Fig. 14.5). Although the lymph drainage is increased in absolute terms, it may be relatively inadequate to remove surplus trans-sinusoidal filtrate and protein, which consequently accumulate in the peritoneal space where a secondary equilibration takes place across the gastrointestinal capillaries and peritoneal membrane with formation of ascites as the outcome. Available evidence indicates that microvascular permeability may be decreased in this condition, owing to sinusoidal capillarization, and possibly also in the peritoneal membrane.

Local factors in the genesis, perpetuation, and amelioration of ascites are important for several aspects of therapy and should be taken into account whenever the pathophysiology and therapy of the hepatic ascites syndrome are being considered. Owing to its complex structural and pathophysiological changes, ascitic fluid cannot be seen as a simple edema, and the Starling forces must be analyzed in detail, together with the circulatory changes in the systemic circulation and the kidney.

The influence of vascular modulators, such as nitric oxide and the endothelins, on capillary pressure and microvascular permeability/hydraulic conductivity is at present not clear, but it is likely that the balance between

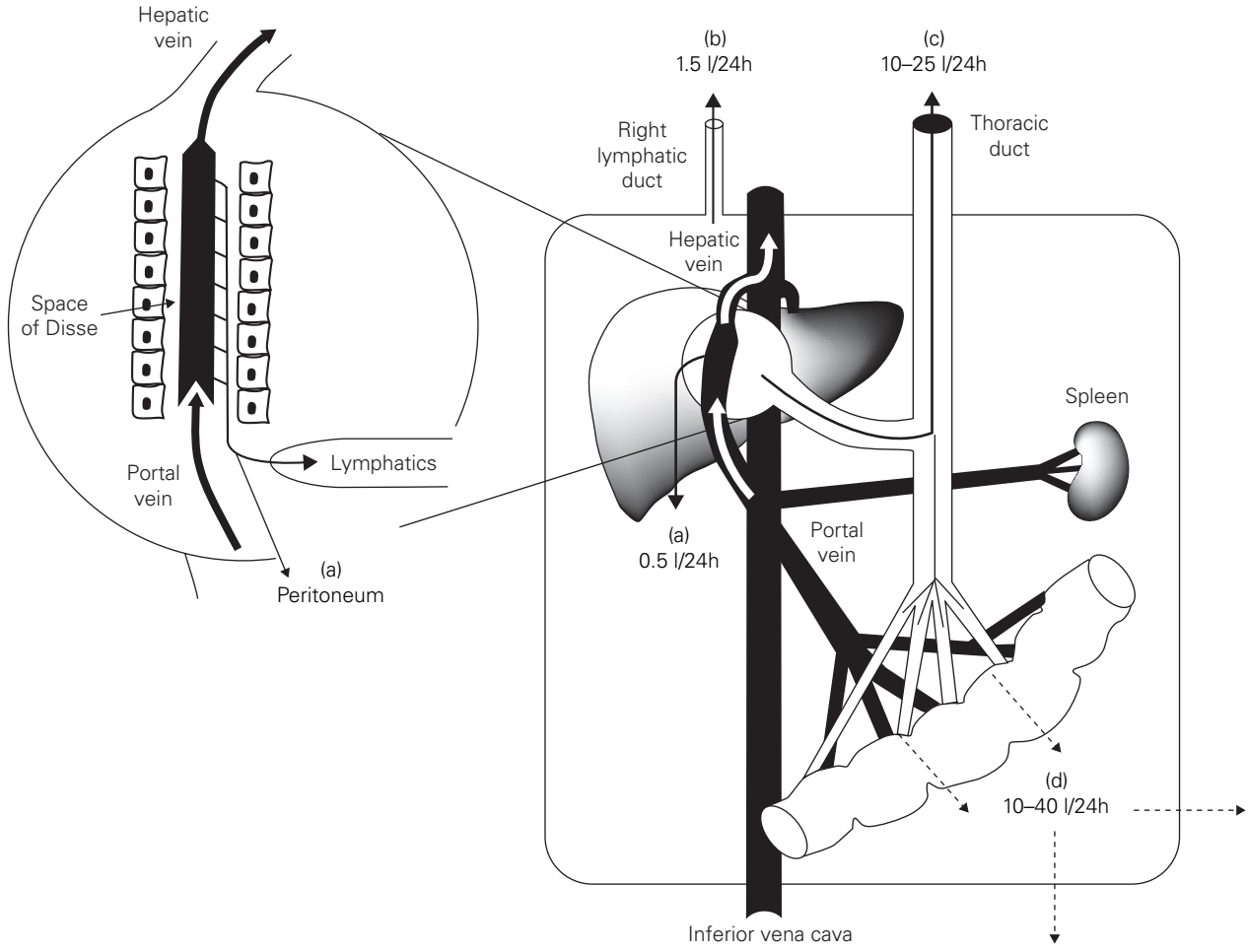


**Figure 14.5** Summary of transperitoneal fluid dynamics in cirrhosis. The size of the trans-sinusoidal (a), peritoneal lymphatic (b), thoracic duct (c), and transintestinal-transperitoneal (d) fluid transport is indicated.

pre- and postcapillary vascular tone may be regulated by these modulators. Their potential role in therapy belongs to the future.

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# Chapter 15
# The Heart in Cirrhosis

Hongqun Liu and Samuel S. Lee

## Historical background

In 1884, M. Fluckiger reported the presence of cyanosis and clubbing in a 37-year-old woman with cirrhosis (1). This was one of the earliest publications noting significant circulatory changes in cirrhotic patients. Seven decades later, with the development of more sophisticated methods for measuring cardiac output and blood pressure, Kowalski and Abelmann first systematically investigated the cardiovascular abnormalities in cirrhosis (2). They determined that the circulation is hyperdynamic, manifesting as increased cardiac output at rest, combined with decreased peripheral vascular resistance. After the exclusion of anxiety, anemia, thiamine deficiency and other possible factors, they concluded that the cardiovascular changes were due to the peripheral vasodilation resulting from overproduction of vasodilator material by the cirrhotic liver. Besides the vasodilation, prolongation of the electrocardiographic QT interval was also observed in some patients. Over the next few years, subsequent investigators confirmed the observation of vasodilation in cirrhosis (2–6). For example, Murray and colleagues found that the increased cardiac output is related to liver function, and unrelated to the etiology of cirrhosis, anemia, or nutritional deficiencies (2).

In view of the well-documented increase in resting cardiac output, it is perhaps not surprising that the heart itself remained unstudied until 1969. In that year, Gould *et al*. examined cardiac function under physiological challenge in patients with alcoholic cirrhosis (7). These authors found that exercise significantly increased mean pulmonary artery pressure and left ventricular end-diastolic pressure—the latter indicating increased ventricular diastolic filling. Despite the increased diastolic filling, in the majority of the subjects the stroke work index remained unchanged or even fell—a highly abnormal result. In another study of patients with alcoholic cirrhosis, Regan *et al*. infused angiotensin, and found that a significant rise in ventricular end-diastolic pressure occurred while stroke output failed to increase—again a subnormal response (8). Limas and colleagues, also studying patients with alcoholic cirrhosis, documented abnormal ventricular responses to cardiovascular stimuli, and also observed the failure of cardiac glycosides to improve contractility (9). Numerous subsequent studies in the 1970s and 1980s confirmed blunted ventricular responsiveness to physiological and pharmacological challenges in patients with liver disease, and all assumed that this was due to alcohol, i.e. reflective of mild alcoholic cardiomyopathy (reviewed in 10).

A paradigm shift occurred in the mid to late 1980s when a few studies showed that animal models, and later patients, with non-alcoholic cirrhosis also showed blunted cardiac response to stimuli. Caramelo and colleagues in 1986 reported that volume infusion into rats with $CCl_4$-induced cirrhosis depressed cardiac output by half (11). This and other studies thus led to the realization that this syndrome is not due to alcohol, but to cirrhosis *per se*, and the term "cirrhotic cardiomyopathy" was coined (10,12,13). The hallmark of this syndrome is baseline hyperkinetic circulation with an attenuated cardiac response to stress. Cirrhotic cardiomyopathy may become unmasked by diverse interventions including pharmacological stress, exercise, and procedures ranging from insertion of transjugular intrahepatic portosystemic stent-shunts (TIPS) to liver transplantation (12–15). Over the past decade, this syndrome has transformed from an academic curiosity to a clinically significant entity that may complicate or affect the prognosis of several other conditions (summarized in Table 15.1). This chapter will summarize all aspects of cirrhotic cardiomyopathy, and other issues of the heart in cirrhosis.

## Clinical descriptions of cirrhotic cardiomyopathy

### Systolic contractile function

The pioneering study of Gould and his co-workers (7) found that under exercise stress, indices of left ventricular contractility such as the stroke index, mean systolic ejection rate, stroke-work and stroke-power either showed a blunted increase or actually declined. Another study on cardiac response to exercise in cirrhotic patients was

**Table 15.1** Possible clinical syndromes associated with the heart in cirrhosis.

| Organ or tissue | Clinical syndrome or problem | Role of cardiac dysfunction or problem | Comments | Reference |
|---|---|---|---|---|
| Heart, endocardium | Infective endocarditis | Increased prevalence | 0.34% vs. 0.1% of noncirrhotics in autopsy series | Snyder 1977 (16) |
| Heart, pericardium | Pericardial effusions | Increased prevalence | Small effusions almost never clinically significant | Numerous case reports |
| Liver | TIPS insertion | Aggravate diastolic dysfunction; precipitate overt LVF | Usually transient; related to increased preload. LVF in 12% post-TIPS* | Huonker 1999 (25)<br>Merli 2002 (26)<br>*Gines 2002 (27) |
| Liver | Transplantation | Precipitate overt LVF; worsen outcomes | Usually transient. 7–15% deaths post-transplantation due to cardiac causes | Sampathkumar 1998 (71)<br>Nasraway 1995 (66)<br>Rayes 1995 (65) |
| Kidney | Hepatorenal syndrome in SBP | Precipitate hepatorenal syndrome? | Patients with HRS after SBP have inadequate LV function | Ruiz del Arbol 2003 (75) |
| Kidney | Salt/water retention | CCM—involved in pathogenesis? | Inadequate pump function decreases effective circulating volume? | Wong 1999 (74) |
| Whole body, brain | Reduced quality of life; fatigue | CCM—involved in pathogenesis? | Fatigue, quality of life unrelated to CCM in one study to date | Girgrah 2003 (83) |

CCM, Cirrhotic cardiomyopathy; TIPS, transjugular intrahepatic portosystemic stent; LVF, left ventricular failure; HRS, hepatorenal syndrome; SBP, spontaneous bacterial peritonitis.

carried out by Kelbaek and colleagues (17), who used echocardiography and radionuclide angiocardiography to investigate cardiac function in patients with alcoholic cirrhosis. This study found that, with exercise, the cardiac performance was impaired. Furthermore, echocardiography demonstrated that left atrial dimensions in the cirrhotic patients were significantly larger than in controls. Echocardiography also showed that ventricular wall compliance in cirrhotic patients was reduced. Radionuclide angiocardiography demonstrated that at baseline, the left ventricular ejection fraction in cirrhotic patients and control subjects was not significantly different. However, when the subjects were exposed to graded exercise, ejection fraction in cirrhotic patients increased by only 6% compared with a 14% increment in the control group.

Other indices to evaluate exercise-induced left ventricular (LV) dysfunction are cardiac output and heart rate. Grose and colleagues (18) reported that during maximal exercise, cardiac output increases > 300% in healthy control subjects, compared with < 100% increase in cirrhotic patients. In normal subjects, the cardiac output increase is due to a progressive linear augmentation of heart rate and an enhancement of stroke volume. The latter is produced by increased left ventricular ejection fraction and decreased end-systolic volume. In cirrhotic patients, the exercise capacity was greatly reduced. There was a large reduction in peak heart rate response, i.e. chronotropic incompetence. Patients with severe chronotropic incompetence demonstrated the greatest degree of impaired exercise tolerance. In those patients, there was no increase in left ventricular ejection fraction and no decrease in end-systolic volume, indicating that they were unable to compensate for chronotropic incompetence by enhancing systolic contractility.

Posture change provides another challenge for the cirrhotic heart. Changing posture from supine to standing normally decreases left ventricular end-diastolic volume. Bernardi and colleagues reported that, upon standing, healthy volunteers maintain cardiovascular homeostasis by increasing ejection fraction and heart rate, whereas in cirrhotic patients, stroke volume and cardiac output decrease despite marked increments in heart rate (19). This altered response to posture change in cirrhotic patients is further evidence of impaired myocardial contractility.

The cirrhotic heart also responds abnormally to pharmacological stress. Infusion of vasopressors to normalize the peripheral vasodilation of cirrhosis often unmasks an attenuated myocardial response. In one of the earliest such studies, angiotensin infusion in doses sufficient to normalize the peripheral vascular resistance in patients with alcoholic cirrhosis produced clear evidence of myocardial dysfunction (9). Specifically, the pulmonary capillary wedged pressure (a reflection of left ventricular end-diastolic pressure) approximately doubled, while cardiac output did not change—a highly aberrant response. Using echocardiography to assess contractility, these authors also found that the short-acting cardiac glycoside ouabain did not affect contractile function in cirrhosis, while the same dosage of ouabain in normal volunteers resulted in significant shortening of total electromechanical systole, pre-ejection period, and left ventricular pre-ejection time, that is, cardioacceleration. Since this study, many others have demonstrated similar

blunted ventricular responsiveness to drug challenges (reviewed in 13,20).

## Diastolic function

The most important determinants of left ventricular diastolic function include ventricular relaxation, passive properties (stiffness), and transmitral pressure gradient. Using two-dimensional Doppler and pulsed and color Doppler echocardiography examination, Finucci and colleagues (21) showed that in cirrhotic patients, A-wave velocity was increased, E-wave velocity unchanged, and E/A ratio, a measure of the degree of diastolic relaxation, was significantly decreased. The deceleration time was prolonged, which indicates that transmitral pressure gradient was decreased, while the left ventricular end-diastolic volume was increased. These results therefore indicate impairment of diastolic relaxant function in cirrhotic patients. Pozzi *et al*. demonstrated a similar diastolic dysfunction in cirrhotic patients, irrespective of the postviral or alcoholic cause of liver disease (22). Another study by Wong *et al*. also demonstrated that the isovolumic relaxation time is significantly prolonged in cirrhotic patients compared with controls (23), which means the passive properties (elastic compliance) of the left ventricle are decreased. Left atrial diameter was significantly increased in both pre-ascitic and ascitic patients compared with controls. Decreased E/A ratio consistent with other studies was also observed. The authors speculated that the etiology of the ventricular stiffness might be multifactorial, including myocardial hypertrophy, angiotensin II, endothelin, cytokines, or increased sympathetic output with α-adrenergic stimulation (23).

Over the past two decades, TIPS have become popular to treat recurrent variceal bleeding or resistant ascites. This procedure shunts a significant portal venous volume to the right side of the heart, and thus, by increasing preload, stresses the heart. Not surprisingly, several studies have documented abnormal ventricular contractile function following TIPS insertion (24–26). In particular, diastolic dysfunction appears to worsen with the increased preload stress. There are now several case reports of acute left ventricular failure (LVF) following TIPS, and in a large multicenter controlled trial comparing TIPS with large volume paracentesis to treat ascites, 12% of the TIPS group vs. none of the paracentesis group developed overt congestive heart failure (27).

## Structural/histological changes

Cardiac structural and histological changes in cirrhosis were first noticed five decades ago (12). These early postmortem investigations in patients with alcoholic cirrhosis demonstrated myocardial hypertrophy and ultrastructural changes including myocyte edema, fibrosis, exudation, nuclear vacuolation, and unusual pigmentation. More recently, echocardiography shows that in cirrhotic patients, the right and left atrial diameters, and the LV posterior wall + interventricular septum thickness are significantly increased compared with controls (22). Another echocardiographic study reported that the interventricular septal thickness is significantly increased in both pre-ascitic and ascitic patients compared with controls (23). LV relative wall thickness (defined as LV wall thickness + septal thickness relative to the internal dimensions of the LV) is also significantly greater in ascitic patients but not pre-ascitic patients (23).

## Electrophysiological changes

Following the original report of prolonged QT interval by Kowalski and Abelmann, sporadic reports of increased frequency of cardiac arrhythmias in cirrhosis appeared over the next few decades (reviewed in 10,13). These arrhythmias included atrial fibrillation, ectopic atrial and ventricular beats, and ventricular arrhythmias. However, the vast majority of these studies were done in patients with alcoholic cirrhosis, and the contribution of alcohol to these syndromes remained uncertain. For example, the "holiday heart" syndrome of acute atrial fibrillation induced by heavy alcohol intake during holidays is well known. In 1993, Kempler and colleagues demonstrated that prolonged QT interval is related to the degree of autonomic dysfunction in patients with primary biliary cirrhosis (28). Several further reports of prolonged QT interval followed in the next decade, and conclusively showed that this electrophysiological abnormality is found in cirrhosis of all causes (reviewed in 13). Moreover, the prevalence of QT prolongation appears to increase in patients with more severe liver failure (29), and also appears to be reversible after liver transplantation (30,31). Moreover, Henriksen *et al*. documented that in patients with a prolonged QTc interval, the time interval between the electrocardiographic and actual mechanical onset of systole is substantially longer than in patients with a normal QTc interval (32). The clinical significance of these repolarization changes is yet unclear. Prolonged QT interval has been associated, in certain noncirrhotic cardiac conditions, with a particular type of ventricular tachycardia, the "torsade de pointes" arrhythmia. However, in the absence of a known inducer of torsade de pointes, such as hypokalemia, this particular arrhythmia has not been described in cirrhotic patients.

The underlying mechanism of the QT prolongation also remains incompletely elucidated. Animal studies have suggested some interesting possibilities. Moreau and colleagues (33) documented abnormal function of $K^+$ and $Ca^{2+}$ channels in peripheral vascular myocytes isolated from cirrhotic or portal-hypertensive rats. In ventricular myocytes from cirrhotic rats, Ward *et al*. demonstrated a substantial decrease in two types of $K^+$ currents, both

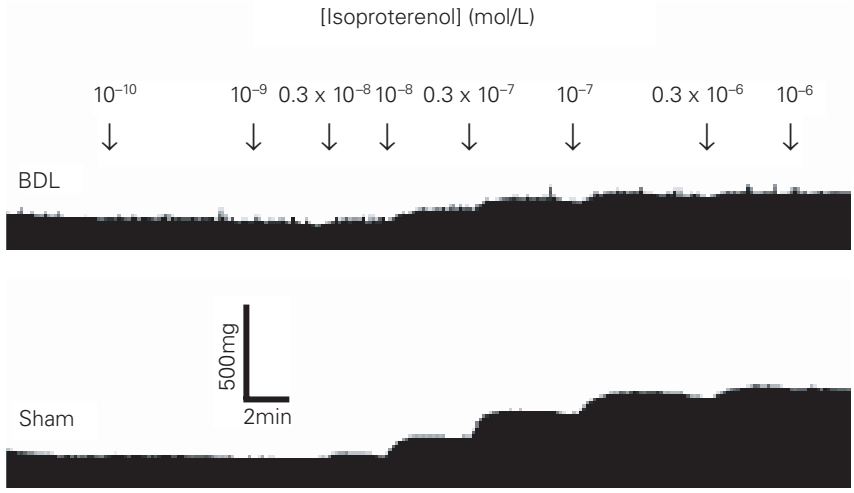


**Figure 15.1** Representative tracings of isoproterenol dose–response in isolated left ventricular papillary muscles from bile duct-ligated (BDL) cirrhotic rat and sham-operated control (Sham). The muscles were electrically stimulated at 1 Hz. Note blunted responses to isoproterenol in the cirrhotic papillary muscles. (Reprinted from *Journal of Hepatology*, 26, Ma, Lee and Meddings, 'Effects of altered cardiac membrane fluidity on β-adrenergic receptor signalling in rats with cirrhotic cardiomyopathy', 904–12., Copyright 1997, with permission from EASL.)

the $Ca^{2+}$-independent transient outward $K^+$ current, $I_t$, and the sustained delayed rectifier current ($I_{sus}$) (34). The global effect of these $K^+$ channel abnormalities on repolarization would be to prolong the action potential and, therefore, the QT interval.

## Pathogenic mechanisms of cirrhotic cardiomyopathy

A widely used animal model of experimental biliary cirrhosis is the rat with chronic (> 3 weeks) bile duct ligation (BDL). This model displays many of the characteristics of human cirrhosis including portal hypertension with portosystemic collaterals, ascites, jaundice, muscle wasting, portal hypertensive gastropathy, and cardiovascular disturbances including hyperdynamic circulation, hepatopulmonary syndrome, and cirrhotic cardiomyopathy. The presence of cirrhotic cardiomyopathy in this model is confirmed by severely blunted contractile responsiveness to the α-adrenergic agonist isoproterenol (Fig. 15.1), and impaired ability to generate the stimulatory second messenger cAMP (Fig. 15.2). We and other centers have therefore used this model extensively in mechanistic studies of the heart in cirrhosis, detailed in this section.

### Membrane lipid biochemical/biophysical changes

All mammalian cell plasma membranes are composed of a lipid bilayer. In these membranes, the ability of the lipid and protein moieties to move in various ways such as lateral diffusion, rotation, and wobbling, can be quantified by measuring the movement signals generated by fluorescent lipid probes inserted in the membrane. The biophysical term for this movement ability is membrane fluidity (35). Since many membrane proteins including virtually all receptors undergo some conformational change when occupied by the appropriate ligand, it is reasonable to believe that normal membrane fluidity would be necessary for proper function of membrane-bound receptors or proteins. Over the past three decades, this has been abundantly demonstrated in many systems such as β-adrenoceptors in turkey erythrocytes (36). Many factors determine membrane fluidity, chief among them the lipid composition of the bilayer. In particular, the ratio of membrane cholesterol to phospholipid content appears to be a critical determinant of fluidity, with higher ratios becoming less fluid. In the cirrhotic BDL rat

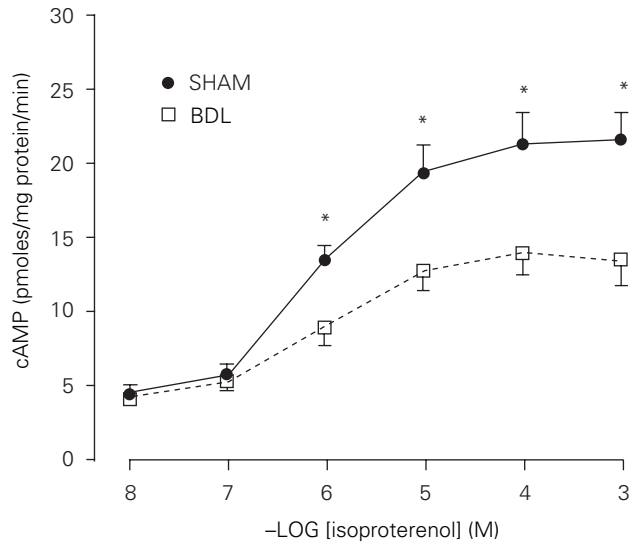


**Figure 15.2** Isoproterenol-stimulated cAMP generation in cardiomyocyte plasma membranes from bile duct-ligated cirrhotic (BDL) rats and sham-operated controls. *Significantly different from corresponding BDL value, $P < 0.05$. (Reproduced from Ma Z *et al*. Am J Physiol 1994; 267: G87–G93.)

model, we showed that the fluidity of cardiac sarcolemmal plasma membranes is decreased, associated with an increase in the membrane cholesterol content and thus the cholesterol/phospholipid ratio. Furthermore, restoration of the normal values for membrane fluidity *in vitro* in cardiomyocyte membranes from cirrhotic rats also results in normalization of isoproterenol-stimulated cAMP generation (Fig. 15.3) (37), thus suggesting that normal membrane fluidity is needed for proper function of the β-adrenergic signaling pathway. Further elucidation of the α-adrenergic system in the cirrhotic heart is described in the next subsection.

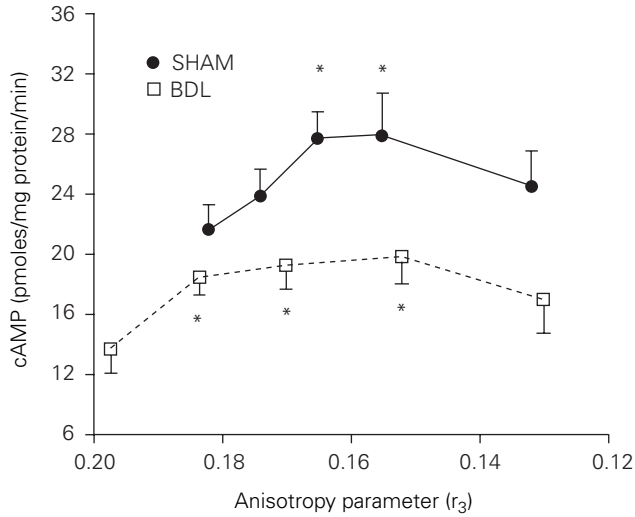


**Figure 15.3** Effects of the fluidizing fatty acid analog $A_2C$ on cardiac plasma membrane fluidity in BDL cirrhotic and sham-operated rats. Fluidity is represented by the anisotropy parameter $r_s$; larger numbers indicate decreased fluidity. The baseline cirrhotic value of $r_s$ is significantly different from the baseline control membrane, and restoration of normal $r_s$ values significantly increases cAMP generation. *Significantly different from baseline value. (Reprinted from *Gastroenterology*, 121, Ward, Liu and Lee, 'Altered cellular calcium regulatory systems in a rat model of cirrhotic cardiomyopathy', 1209–18, Copyright 2001, with permission from American Gastroenterological Association.)

## β-Adrenergic receptor function

Because the β-adrenergic receptor system is the main determinant of ventricular contractility (Fig. 15.4), it would be reasonable to examine closely this system in the cirrhotic heart. However, obtaining antemortem heart tissue from patients with cirrhosis is ethically unfeasible, so studies were generally done in animal models of cirrhosis. The only β-adrenoceptor study done in human cirrhosis was the seminal work of Gerbes and colleagues, who examined lymphocyte $\beta_2$-adrenoceptors in patients with severe cirrhosis (38). It is known that, in general, lymphocyte $\alpha_2$-adrenoceptors mirror the status of cardiac β-receptors (which are predominantly $\beta_1$-adrenoceptors). Given these considerations, these investigators found that lymphocyte β-adrenoceptor density is significantly lower in patients with decompensated cirrhosis, i.e. severe ascites.

In the BDL rat model, we have extensively studied adrenergic receptor function in cirrhotic cardiomyopathy. Cardiomyocyte sarcolemmal plasma membrane β-adrenoceptor density is significantly decreased compared with controls (39), although the binding affinity of β-adrenoceptors to the ligand isoproterenol is unchanged (39,40). Occupation of the receptor by an appropriate ligand and induces a conformational change that activates the membrane-bound heterotrimeric G-protein (the stimulatory Gs-subunit), and subsequently adenylate cyclase, to produce its second messenger, cAMP (Fig. 15.4). The cAMP then phosphorylates several intracellular proteins that lead to calcium release to stimulate actin–myosin cross-linking to produce cell contraction. We showed that the β-adrenoceptor signal transduction pathway is impaired at several different levels, including membrane

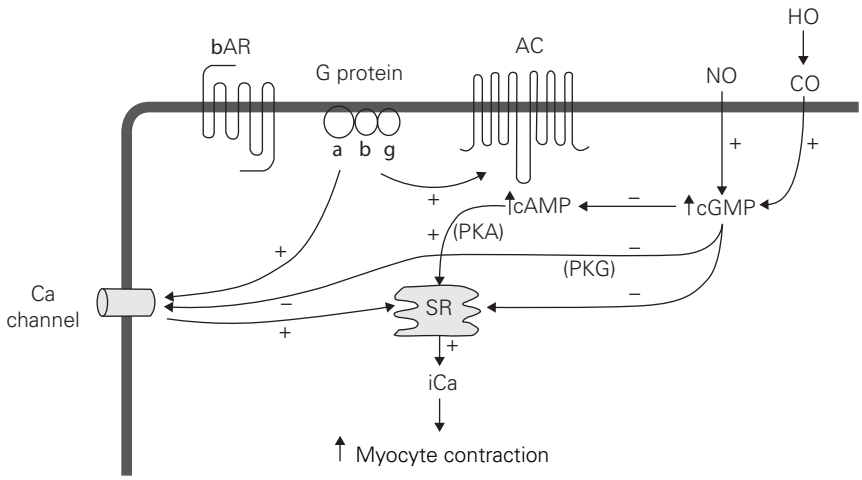


**Figure 15.4** Schema of regulatory influences on cardiomyocyte contractility. βAR, β-adrenergic receptor; AC, adenylate cyclase; NO, nitric oxide; HO, heme oxygenase; CO, carbon monoxide; PKA, protein kinase A; PKG, protein kinase G; SR, sarcoplasmic reticulum; iCa, intracellular calcium. + denotes stimulatory influence; – denotes inhibitory influence. (Reproduced from Garcia Estan J *et al*., Clin Sci 2002; 102:213–22.)

content and function of the stimulatory Gs-protein (37), uncoupling of the receptor–ligand complex from G-protein (40), and impaired activity of the adenylyl cyclase enzyme itself (37).

Because ventricular contractility is dependent on the interplay of stimulatory β-adrenergic and inhibitory muscarinic cholinergic receptors, contractile impairment may result from cardiac muscarinic M2 receptor overactivity. However, in the cirrhotic heart, muscarinic receptor characteristics including receptor density and binding affinity are unchanged, although the overall muscarinic function is attenuated (41). The M2 system transduces its negative inotropic effect via stimulation of the inhibitory Gi-protein, and content of this protein is reduced in cardiomyocyte plasma membranes (37). Therefore, it is likely that the blunted muscarinic function is due to the marked decrease in its Gi-protein-linked signal transduction, and we believe that this overall attenuation of the muscarinic system is compensatory, in response to the impaired β-adrenergic stimulatory system.

## Calcium dynamics

The intracellular movement of free calcium is central to myocyte contraction, as this ion is the ultimate determinant of actin–myosin cross-linking. Calcium can either enter the cell through membrane voltage-gated or ligand-operated calcium channels, or be released from the sarcoplasmic reticulum (SR) in response to various signals such as ryanodine or caffeine [thus the SR calcium release mechanism is termed the ryanodine receptor (RyR)]. Recent investigation of the cell calcium dynamics showed that the elicited peak inward current of the myocardial membrane L-type calcium channel ($Ica_{,L}$) in BDL myocytes is significantly less than that in controls (Fig. 15.5) (42). Indeed, at all membrane potentials, $Ica_{,L}$ current densities from cirrhotic cardiomyocytes are consistently decreased compared with controls, and the magnitude of isoproterenol-induced increment in $Ica_{,L}$ is also attenuated in cirrhotic cardiomyocytes. Moreover, the L-type calcium channel protein expression is decreased in BDL ventricles compared with sham controls.

As for intracellular calcium kinetics, ryanodine receptor binding experiments showed no difference between BDL and controls; nor were differences found in mRNA transcription and protein expression of the key proteins involved in SR calcium release, the ryanodine receptor and sarcoplasmic reticulum $Ca^{2+}$-pump ATPase (SERCA2) (42). These results demonstrate that the abnormalities of the calcium delivery system lie in the cardiomyocyte plasma membrane, whereas the intracellular calcium-regulatory systems are intact. Forskolin, a direct stimulator of adenylate cyclase, proportionally increases $Ica_{,L}$ in BDL and controls to a similar degree

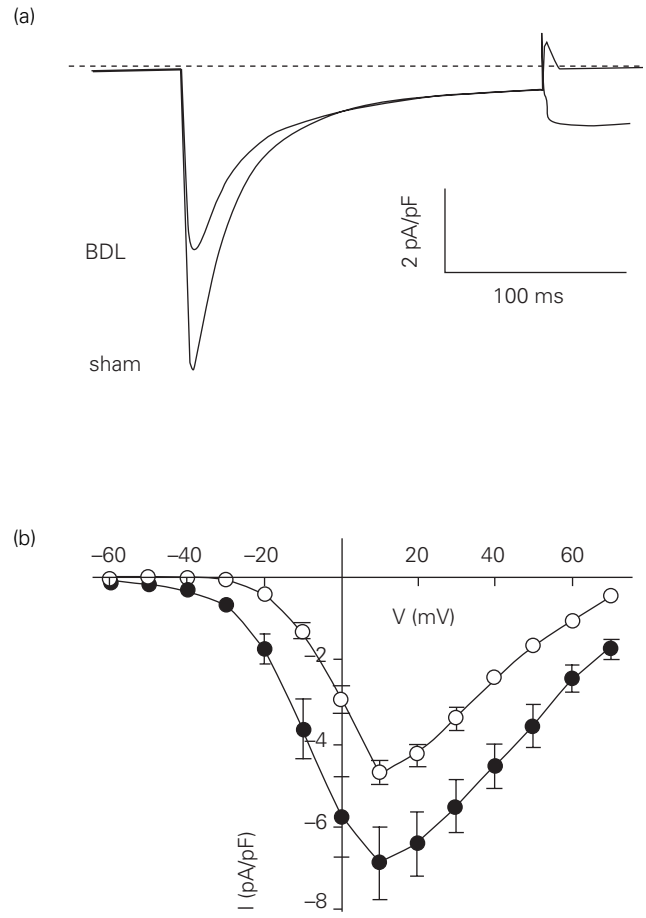


**Figure 15.5** L-type $Ca^{2+}$ currents from ventricular myocytes of BDL cirrhotic and sham-control rats. (a) Representative current density from BDL and sham myocytes, showing decrease in BDL. (b) Current–voltage relationships in the entire group of BDL ($n$ = 19; open circles) and sham-control ($n$ = 16; closed circles) myocytes, showing significant decrease in the BDL group. (Reproduced from Ward C *et al.*, Gastroenterology 2001; 121:1209–18.)

(42). This suggests that the defect in calcium kinetics in the plasma membrane is upstream to the adenylate cyclase enzyme.

## Gases acting via cGMP

Nitric oxide (NO) and carbon monoxide (CO) are ubiquitous gases that transduce their signals mainly by stimulating guanylate cyclase to produce the second messenger cGMP. NO plays an important role in the general regulation of myocardial function (43). Several studies have demonstrated that NO inhibits aspects of β-adrenergic cardiac stimulation (44–46). The mechanism by which NO inhibits the β-adrenergic system remains incompletely clarified, but studies have suggested the following possibilities: (i) direct inhibition of adenylate cyclase (47), (ii) decreasing extracellular $Ca^{2+}$ influx in L-type Ca

channels by cGMP-dependent mechanisms or by membrane hyperpolarization secondary to cGMP-dependent activation of $Ca^{2+}$-dependent $K^+$ channels (48), or (iii) inhibition of intracellular $Ca^{2+}$ release by the SR ryanodine receptor (49).

It is now clear that NO is overproduced in cirrhosis (reviewed in 50), with significantly increased serum levels of the end-products of NO metabolism, nitrates/nitrites in cirrhotic patients (51) and animal models (52). The augmented NO results from the activation of both the endothelial-constitutive NO synthase (eNOS) and inducible (iNOS) isoforms. The former is upregulated by the shear stress produced by the hyperdynamic circulation, and the latter probably due to the increased levels of cytokines such as interleukins and tumor necrosis factor-α.

The possible role of NO in the genesis of cirrhotic cardiodepression has recently been reviewed (53). In the isolated working heart of BDL rats, Van Obbergh *et al*. (54) administered the NOS inhibitor NG-monomethyl-L-arginine and observed increased systolic pressure and the peak rate of rise of left ventricular pressure (dP/dt), whereas no significant effects were seen in hearts from control animals. We found an increased iNOS mRNA transcription and protein expression in the ventricles of BDL-cirrhotic rats, while eNOS mRNA and protein were unchanged (55). The iNOS was localized to predominantly cardiomyocytes by immunohistochemistry. In control hearts, the NO donor S-nitroso-acetyl-penicillamine depressed papillary muscle contractility. Finally, the NOS inhibitor L-NAME reversed the decreased isolated papillary muscle contractility in cirrhotic rats (Fig. 15.6). These results indicate that NO produced by iNOS upregulation may be involved in cardiodepression in the cirrhotic rat.

CO is produced by the enzyme heme oxygenase (HO), which exists in two isoforms, inducible (HO-1, also known as heat shock protein-32) and constitutive (HO-2). HO catalyzes the oxidation of heme to CO, iron, and biliverdin. Although some studies also implicate CO as a negative inotropic agent in pathophysiological states, the evidence for such a role is not nearly as conclusive as that for NO. In an experimental canine model of right-sided congestive heart failure, HO-1 gene transcription is increased in the right ventricle, but not in the left (56). We reported that HO-1 mRNA transcription and protein expression are increased in cirrhotic ventricles compared with sham-operated controls (57). The ventricular cGMP content in BDL rats is significantly increased compared with controls; this increase is abrogated by administration of the HO inhibitor, zinc protoporphyrin IX (ZnPP). ZnPP also reverses the decreased papillary muscle contractility in cirrhotic rats, but has no significant effect in muscles from control animals. Although these results suggest a possible pathogenic role of the HO–CO–cGMP pathway in cirrhotic cardiomyopathy, several questions remain to be clarified. In particular, the interaction of the NO and CO systems (in general, CO is a much weaker stimulator of guanylate cyclase than NO), the exact nature of which is still incompletely defined, must be elucidated before accepting the conclusion that the HO–CO system is involved in cirrhotic cardiomyopathy.

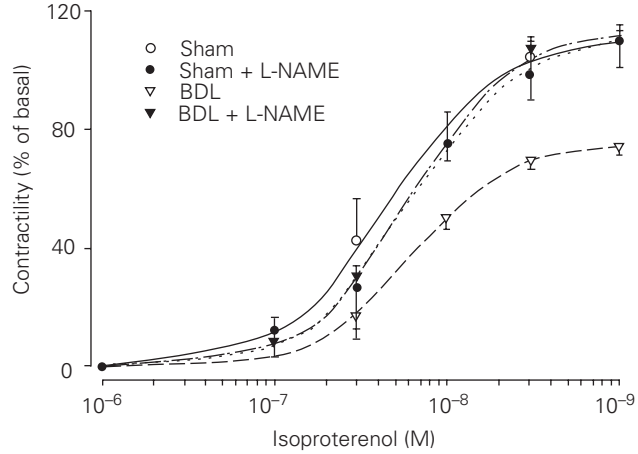


**Figure 15.6** Effect of the NO synthase inhibitor L-NAME on isolated papillary muscle contractility. The maximal response (Rmax) of the BDL cirrhotic muscles is significantly decreased; the other curves are not significantly different from each other. (Reprinted from *Gastroenterology*, 118, Ward, Liu and Lee, 'Contribution of nitric oxide to the pathogenesis of cirrhotic cardiomyopathy in bile duct-ligated rats', 937–44, Copyright 2000, with permission from American Gastroenterological Association.)

## Coronary atherosclerosis in cirrhosis

Longstanding clinical dogma held that ischemic heart disease is rare in patients with cirrhosis. In part, this was probably based on the premise that because the liver controls lipid metabolism and cholesterol biosynthesis, liver dysfunction and/or failure should be associated with a lower incidence of coronary artery disease (CAD). Previous studies have suggested that in the early to middle stages of the disease, very-low-density lipoprotein levels are normal while cardioprotective high-density lipoprotein levels are high. In addition, the peripheral vasodilation that accompanies cirrhosis reduces arterial blood pressure, thus decreasing another major atherosclerosis risk factor. On the other hand, diabetes mellitus is an independent risk factor for coronary atherosclerosis, and Type 2 diabetes is relatively common with patients with cirrhosis (58).

Coronary angiography showed a 30% prevalence of moderate to severe CAD in patients older than 50 undergoing evaluation for orthotopic liver transplantation (59); amongst these patients with CAD, about 1/3 had clinically unsuspected disease only identified by angiography. However, other studies have reported a lower prevalence: Plotkin and colleagues noted only 5%

of patients undergoing transplant assessment had CAD (60). An accurate prevalence of CAD in cirrhosis may be difficult to ascertain because many patients with severe CAD are not even referred for transplant assessment, so series from tertiary transplant centers suffer from referral bias. A recent study showed that in the BDL rat, total cholesterol level and low-density (LDL) cholesterol were significantly increased whereas high-density (HDL) cholesterols were significantly lower compared with those in sham controls (61). The weight of evidence therefore suggests that cirrhosis is not protective against the development of CAD.

## Liver transplantation and cardiac function

Candidates for liver transplantation suffer from severe end-stage liver failure. These patients generally show the greatest extent of cardiovascular abnormalities, including more pronounced hyperdynamic circulation. Whether the degree of cirrhotic cardiomyopathy also correlates with advancing stages of liver failure remains unclear, although indirect evidence suggests that this is indeed the case. For example, the prevalence of electrocardiographic QT prolongation worsens with increasing liver failure (62).

Issues surrounding cirrhotic cardiomyopathy and liver transplantation have been recently reviewed (63). Experience in liver transplant centers over the past three decades has underscored the hazards of ignoring the heart in patients with end-stage liver failure. Given the tremendous physical and cardiovascular stress imposed by this lengthy and complicated surgery, it is not surprising that latent cardiac dysfunction may become overt. Moreover, the increased peripheral resistance seen after successful liver transplantation adds significantly to the ventricular workload by increasing afterload. A study found that 3 days after liver transplantation, systemic vascular resistance increased by 28%, and cardiac output decreased from 8.8 ± 0.7 l/min to 6.5 ± 0.5 l/min (64).

Even though most patients with overt heart disease such as CAD or congestive heart failure are excluded from liver transplantation, postoperative heart failure remains a significant problem. Cardiac dysfunction contributes to 7–15% of deaths after liver transplantation (65,66); thus in some centers, this is the third most important cause of death after rejection and infection. Indeed, although overt or severe ventricular failure is still uncommon after liver transplantation, mild or subclinical cardiac dysfunction is surprisingly common in the immediate or later postoperative period. A study reported overt heart failure in only 2% of post-transplant patients during the first postoperative week, but 56% showed radiographic evidence of pulmonary edema at some point during the postoperative period (67). Two other studies have reported a high prevalence of LV contractile dysfunction noted up to several months post-transplantation, which the authors ascribed to cardiotoxicity of tacrolimus or cyclosporin (68,69). However, an accompanying editorial suggested instead cirrhotic cardiomyopathy as an alternative explanation (70).

Whether systolic or diastolic dysfunction, like QT prolongation, improves after liver transplantation is not yet completely clear. However, small series suggest that contractility does indeed improve after transplantation. Sampathkumar *et al*. reported early overt LV failure in seven of 754 patients following transplantation, but by 15 months of follow-up, all had resolved, with no recurrence of LVF (71). Similarly, Park *et al*. also found some improvement of hemodynamics in a predominantly pediatric population examined before and after transplantation (72).

## Cirrhotic cardiomyopathy: a role in renal dysfunction?

Given that cardiodepression exists in cirrhosis, is there a relationship between myocardial dysfunction, peripheral vascular changes and sodium retention in cirrhosis? Currently a popular theory to explain the pathogenesis of sodium/water retention leading to ascites is the "primary peripheral vasodilatation" hypothesis (73). This theory suggests that a primary peripheral vasodilation decreases the effective circulating blood volume, thus inducing the kidneys to retain salt and water in an effort to maintain adequate volume status. A large amount of experimental work has attempted to support this theory, much of it based on NO as the primary determinant of peripheral vasodilation, but virtually all focused on the peripheral circulation (reviewed elsewhere in this book; see Chapters 8 and 16). In conceptual terms, the effective circulating volume depends on three factors: the actual blood/plasma volume, the tone of the vessels, and the pump that propels the fluid. In cirrhosis, it has been well documented that the actual blood and plasma volume are significantly expanded, so the problem does not lie with this factor. It then becomes clear that no matter what the state of the peripheral vessels, adequate pumping function is absolutely necessary to maintain an effective circulating volume. Therefore, cardiac contractility is a primary determinant of the effective circulating volume, and it becomes surprising that virtually no attention has been paid to the heart in this issue, at least until very recently.

Wong and colleagues found that LV size at end-diastole is decreased and end-diastolic volume parallels the change in LV size in ascitic cirrhotic patients, but not in normal controls and pre-ascitic cirrhosis (74). A reduced cardiac preload seems paradoxical in the presence of an increased total central blood volume in the ascitic cir-

rhotic patients. However, these authors suggested that the central blood volume is preferentially distributed to the dilated pulmonary circulation and, coupled with decreased LV compliance, would reduce the LV preload enough to induce the kidneys to retain sodium and water. This study, although suggestive, does not offer direct experimental confirmation of a possible heart–kidney link in cirrhotic sodium retention.

More recently, Ruiz del Arbol and his Spanish colleagues studied 23 patients admitted with spontaneous bacterial peritonitis (75). During the hospital course, despite infection resolution, eight patients subsequently developed hepatorenal syndrome, whereas 15 had unimpaired renal function. Cardiac output and arterial pressure in the latter group remained unchanged after infection resolution, whereas in the former group these parameters decreased significantly with infection resolution. Moreover, the baseline cardiac output in the renal-impaired group at admission was already significantly lower than the renal-unimpaired group. Interestingly, systemic vascular resistance, an index of peripheral vascular tone, remained unchanged in the group that developed hepatorenal syndrome, indicating that the decreased cardiac output directly led to reduced renal perfusion. These interesting results are discussed in detail in the accompanying editorial (76), but, in brief, strongly suggest that inadequate LV contractility is an important determinant of renal dysfunction associated with spontaneous bacterial peritonitis.

One additional point bears mention. If, indeed, NO overactivity is the primary mechanism underlying the peripheral vasodilation, and also cardiodepression as detailed previously, this dual action to aggravate renal sodium/water retention might explain the ineffectiveness of any compensatory diuretic and natriuretic mechanisms in advanced cirrhosis.

## Management of cirrhotic cardiomyopathy

At present, because no definitive diagnostic criteria for cirrhotic cardiomyopathy exist, clinical management of patients who may have this syndrome is uncertain. Obviously, it is difficult to make definitive recommendations for treatment when even clear identification and diagnosis of such patients remain unclear. Recent reviews have summarized empirical treatment recommendations (13,77). In the lack of accepted "gold standards" of diagnosis, the clinician must maintain a high level of suspicion for the presence of the syndrome, especially in situations that significantly stress the cardiovascular system such as surgery, hemorrhage, infection, and vasoactive drug administration. As underscored in the first sections of this chapter, static, unstressed cardiac tests such as resting echocardiography may not detect this syndrome. However, the exact type of physiological or pharmacological challenge to unmask the disorder remains to be clarified. The good news is that attempts at a classification scheme that includes specific diagnostic criteria are currently under way, so these criteria should be available in 2005.

If a significant cardiac stress such as liver transplantation, major surgery, or TIPS induces overt ventricular failure, standard treatment measures for noncirrhotic heart failure should be instituted. As mentioned above, sodium retention and cardiac dysfunction may become a positive-feedback cycle, so interrupting this cycle might arrest the deterioration of or even improve cardiac function. Such measures include bed rest, salt restriction, diuretics, afterload reduction, and attempts to improve hypoxemia. Correcting hypoxemia in cirrhosis may be extremely difficult as it is likely to result from hepatopulmonary syndrome (78) with intrapulmonary shunting or ventilation/perfusion mismatch that does not respond to simple measures such as inhalational oxygen supplementation. Recommendations about inotropic-stimulating drugs in cirrhosis are currently based almost exclusively on animal studies, and the applicability to the human condition remains unclear. However, given the multiple defects in the cardiac β-adrenergic system described earlier, we believe that patients will probably not benefit from β-agonists such as dobutamine and isoproterenol. This contention is supported by studies in cirrhotic patients, showing blunted cardiovascular responses to acute doses of dobutamine (79) and isoproterenol (80,81). Phosphodiesterase inhibitors that inhibit cAMP degradation, such as amrinone or milrinone, would be theoretically attractive because most of the β-adrenergic signaling defects are upstream of adenylate cyclase. Unfortunately, the only available study with either of these two drugs is not promising. Orii and colleagues administered amrinone to cirrhotic patients undergoing partial hepatectomy for hepatocellular carcinoma, to try to decrease hepatic ischemia-reperfusion injury (82). This drug did not significantly change cardiac output or arterial pressure in these patients.

Cardiac glycosides such as digitalis are useful in some forms of noncirrhotic ventricular failure. In the only human study to date, the short-acting cardiac glycoside, ouabain, did not affect cardiac contractility in a small group of patients with alcoholic cirrhosis and relatively severe cardiomyopathy (9). Conclusions based on that study should be interpreted cautiously, as many patients may have had alcoholic cardiomyopathy.

In general, it would appear that besides nonspecific supportive measures, the best way to treat cirrhotic cardiomyopathy in the long run is to improve liver function. Liver transplantation notwithstanding, ways of improving liver function are relatively limited at present, such as eliminating/inactiving viral hepatitis C or B, maintain-

ing alcohol abstinence in those with alcoholic cirrhosis, and reducing inflammation in chronic inflammatory cirrhoses such as autoimmune hepatitis. Other than these measures, specific treatments for cirrhotic cardiomyopathy await the development of accepted diagnostic criteria and focused clinical trials.

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# Part 4
# Ascites and Sodium Retention in Cirrhosis

# Chapter 16
# Pathogenesis of Sodium Retention in Cirrhosis: the Arterial Vasodilation Hypothesis of Ascites Formation

Patricia Fernández-Llama, Pere Ginès, and Robert W. Schrier

## Introduction

The kidney plays a central role in the pathogenesis of a number of complications commonly seen in patients with cirrhosis (1,2). During the natural course of cirrhosis, a progressive impairment in kidney function occurs and the kidneys are no longer able to maintain the extracellular fluid volume within normal limits (3). This is mainly due to an abnormally increased tubular sodium reabsorption, which leads to an inability to adjust the amount of sodium excreted by the kidneys to the amount of sodium ingested with the diet. The clinical consequences of sodium retention are an increase in total extracellular fluid volume that results in the accumulation of fluid within the peritoneal cavity as well as in the interstitial tissue. Sodium retention is often associated with an impaired ability to eliminate free water, which may lead to dilutional hyponatremia due to a disproportionate increase in total body water relative to the total sodium content (4,5). As the disease progresses, a vasoconstriction of the renal circulation may develop, which causes renal hypoperfusion and reduced glomerular filtration rate (GFR) and, eventually, renal failure (the so-called hepatorenal syndrome) (6). All these abnormalities of renal function contribute significantly to the high morbidity and mortality characteristic of cirrhosis (7). (See Chapter 21.)

The mechanisms leading to sodium retention and ascites and edema formation in cirrhosis are not completely understood. A number of factors are known to play an important role, including several extrarenal and intrarenal vasoactive and sodium-retaining systems; disturbances in the systemic circulation characterized by arterial vasodilation located mainly in the splanchnic circulation, which leads to reduced total systemic vascular resistance, increased cardiac output, and arterial hypotension; and the diseased liver causing sinusoidal portal hypertension (1–3) (see Chapters 11, 12, 13 and 14). The understanding of the pathogenesis of sodium retention in cirrhosis is essential to the design of treatment strategies to decrease morbidity and improve survival of patients with this disease. The first part of this chapter describes the general features, nephron sites, and clinical consequences of sodium retention of cirrhosis. Detailed information about specific factors responsible for sodium retention in cirrhosis may be found elsewhere in this book (see Chapters 4, 5 and 6). The second part of the chapter describes the pathogenesis of ascites formation in cirrhosis as explained by the arterial vasodilation hypothesis, which links hemodynamic abnormalities present in patients with cirrhosis with the development of sodium retention.

## Sodium retention in cirrhosis

### General features

Sodium is the most prevalent osmotically active cation in the extracellular fluid. The total content of sodium in the body determines the size of the extracellular fluid volume in such a way that any increase in total sodium content is associated with increased extracellular fluid volume, while any decrease in total sodium content determines a reduction of extracellular fluid volume (see Chapter 1) (8,9). To keep the extracellular fluid volume constant, the organism has developed very precise mechanisms to adapt the excretion of sodium to the intake of sodium, thus maintaining a neutral sodium balance. Because nonrenal sodium excretion is minimal, the regulation of sodium excretion takes places exclusively in the kidneys. Under normal circumstances, an increase in sodium intake is followed by an increase in the renal excretion of sodium. Conversely, a decrease in sodium intake is associated with renal sodium retention. The adjustment of sodium excretion to changes in sodium intake takes place over several days and involves both sensor mechanisms that recognize alterations in extracellular fluid volume and effector mechanisms that modulate renal sodium excretion (9–11).

Since the pioneering studies by Farnsworth and Krakusin (12) and Eisenmenger *et al*. (13), it has been well established that sodium retention is the most common abnormality of kidney function in patients with cirrhosis and ascites and plays a fundamental role in the formation of ascites and edema. As in other sodium-retaining states, the total amount of sodium retained by cirrhotic patients, and the subsequent gain of extracellular fluid, depends on the balance between sodium intake and sodium excretion. If the amount of sodium excreted in the urine is lower than that ingested, patients accumulate extracellular fluid as ascites and/or edema. By contrast, if the amount of sodium excreted in the urine is greater than that ingested, patients lose extracellular fluid and ascites and/or edema decrease. Therefore, both sodium ingestion and excretion are very important factors in the accumulation of ascites and should be monitored in patients with ascites. The important role of sodium retention in the pathogenesis of ascites formation is supported by the fact that ascites can disappear in some patients by just reducing sodium intake or by increasing urinary sodium excretion with the administration of diuretics (13,14). Although no studies have been performed assessing the chronological relationship between sodium retention and the formation of ascites in patients with cirrhosis, investigations in experimental animals have provided conclusive evidence indicating that sodium retention precedes ascites formation, further emphasizing the important role of this abnormality of renal function in the pathogenesis of ascites in cirrhosis (15–18). Moreover, as discussed in more detail later, the pharmacological increase of sodium reabsorption caused by the administration of mineralocorticoids or nonsteroidal anti-inflammatory drugs (NSAIDs) may induce the development of ascites in patients in the pre-ascitic stage of cirrhosis (19–22).

The intensity of sodium retention in cirrhosis with ascites varies considerably from patient to patient. Some patients have only a moderate impairment in sodium excretion with relatively preserved urinary sodium concentration, whereas sodium excretion is markedly impaired in others, who have very low or even undetectable urine sodium (1,2) (Fig. 16.1). The proportion of patients with marked sodium retention depends on the population of cirrhotic patients considered. Most patients who require hospitalization for the treatment of ascites have marked sodium retention, as they excrete less than 10 mEq/day of sodium (23). As could be anticipated, sodium retention is particularly intense in patients with refractory ascites (24–26). By contrast, when a population of cirrhotic patients with mild or moderate ascites is considered, the proportion of patients with marked sodium retention is low and most patients excrete > 10 mEq/day spontaneously (i.e. without diuretic therapy). The response to diuretics is usually better in patients with moderate sodium retention than in those with marked sodium retention (14,27). It is important to emphasize that the increased sodium retention in cirrhosis should not be viewed as a fixed and irreversible disorder. Rather, the intensity of sodium retention usually changes during the course of cirrhosis. In some patients with marked sodium retention, sodium excretion may improve spontaneously over time. This occurs frequently in patients with alcoholic cirrhosis who abstain from alcohol after their first episode of ascites. Conversely, patients with moderate sodium retention may show a progression toward avid sodium retention. Although no studies have been reported assessing sequential changes in sodium excretion for prolonged periods of time in cirrhotic patients with ascites, it is the clinical experience that in most patients the intensity of sodium retention tends to increase with time.

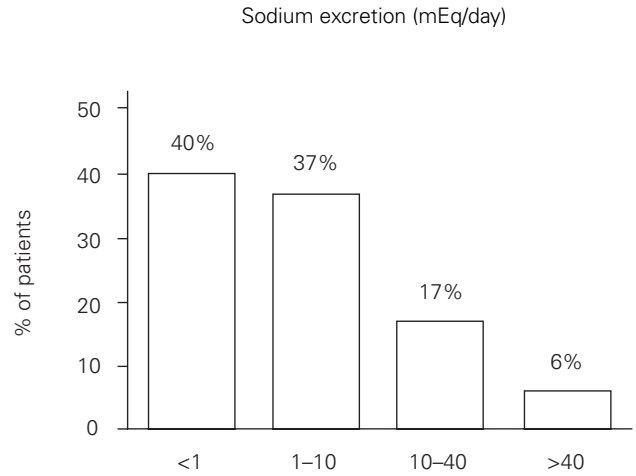


**Figure 16.1** Urinary sodium excretion in a series of 216 cirrhotic patients hospitalized for the treatment of an episode of ascites. All patients were studied after a minimum of 5 days on a 50 mEq/day sodium diet and without diuretic therapy. Values in healthy subjects studied under the same conditions are 40–60 mEq/day. (Reproduced by permission from Ginès P, Fernández-Esparrach G, Arroyo V, Rodés J. Pathogenesis of ascites in cirrhosis. Semin Liver Dis 1997; 17:175–89.)

Patients with cirrhosis in the pre-ascitic stage (without a past history of ascites or edema) do not exhibit overt sodium retention but may have subtle abnormalities in renal sodium handling (19,20,28–43) (Table 16.1). The finding of increased blood volume in these patients strongly supports the existence of sodium retention sufficient to expand the intravascular volume but without causing ascites or edema (29–31). Pre-ascitic cirrhotic patients come into sodium balance as long as their sodium intake is maintained within normal limits. However, some patients may be unable to handle a sodium load and develop ascites and/or edema in conditions of high sodium intake or when intravenous saline solutions are given (28,32,33). This abnormal renal sodium handling of some pre-ascitic patients is also evidenced by the lack of escape to the sodium-retaining effect of mineralocorti-

**Table 16.1** Evidence for abnormal renal sodium handling in patients with cirrhosis in the pre-ascitic stage.

| Feature | References |
|---|---|
| Increased blood volume | 33–35 |
| Inability to excrete an acute or chronic sodium load | 25,32,36,37,42,45,46 |
| Lack of escape to mineralocorticoids | 24,25 |
| Reduced sodium excretion in upright posture | 38 |
| Increased sodium excretion during recumbency | 39 |
| Increased atrial natriuretic peptide levels | 44,45,47 |

coids (19,20). Healthy subjects treated with mineralocorticoids for several days show an early phase characterized by sodium retention that results in increased extracellular fluid volume and plasma expansion, followed by increased sodium excretion with return of extracellular fluid volume and plasma volume to normal values despite the persistent administration of mineralocorticoids. This escape phenomenon is aimed at preventing a persistent sodium retention and subsequent development of edema and is due to the suppression of sodium-retaining mechanisms together with activation of natriuretic mechanisms. Approximately one-quarter of pre-ascitic cirrhotic patients do not show this escape phenomenon and develop marked sodium retention with formation of ascites and edema when treated with mineralocorticoids (20). Finally, it has been shown that patients in the pre-ascitic stage under a normal sodium diet retain sodium while they are in upright posture, whereas they show an exaggerated natriuresis, compared with healthy subjects, during bed rest (34,35). It has been suggested that this increased natriuresis during recumbency is responsible for the maintenance of sodium balance and may help prevent the formation of ascites or edema that would occur as a consequence of sodium retention that takes place during standing.

These subtle abnormalities in renal sodium handling that present in compensated pre-ascitic cirrhotic patients and are responsible for the increased blood volume are probably a homeostatic mechanism to compensate for the increased vascular capacitance of the splanchnic vascular bed in cirrhosis due to arterial vasodilation. This interpretation is supported by the observation that a pharmacologically induced vasodilation is followed by sodium retention and increased plasma volume and ascites and edema formation in most pre-ascitic cirrhotic patients (44,45). It is also supported by the finding that patients who develop ascites or edema while on a high-sodium diet or who fail to escape to the sodium-retaining effect of mineralocorticoids are those with more marked abnormalities in systemic hemodynamics, as indicated by higher cardiac output and lower total systemic vascular resistance (20). An alternative interpretation suggests that abnormal sodium handling in pre-ascitic cirrhotic patients is related to the degree of liver dysfunction (33,38).

## Sodium transport in renal tubules

Between 90 and 99% of filtered sodium is reabsorbed along the renal tubules. Approximately 60–70% of the filtered load of sodium and water is absorbed in the proximal tubules. The remaining 30–40% is delivered to the thick ascending limb, where as much as 20–30% of delivered sodium is absorbed in the absence of water reabsorption. The distal convoluted tubule and the collecting duct are each responsible for 5–10% of sodium reabsorption. While the so-called distal nephrons (i.e. distal convoluted tubule and collecting duct) reabsorb a small percentage of the sodium that is filtered by the glomerulus, this amount is sufficient to develop important pathological conditions. It has been clearly documented that an increase or decrease in sodium reabsorption at this part of the nephron can induce hypertension or hypotension, respectively (46).

Renal tubule sodium absorption is mediated by membrane proteins named sodium transporters and channels (Fig. 16.2). In the proximal tubule there are two main proteins that facilitate sodium transport in the apical or luminal plasma membrane, namely the sodium hydrogen exchanger isoform 3 (NHE-3) and the sodium phosphate cotransporter type 2 (NaPi-2) (47). In the thick ascending limb of the loop of Henle apical sodium transport is mediated mainly by the sodium two-chloride potassium cotransporter (NKCC2/BSC1) (47). Loop diuretics such as furosemide or bumetanide inhibit this apical sodium transporter in the loop of Henle and therefore produce natriuresis. Apical sodium transport in the distal tubule is facilitated by the sodium chloride cotransporter also called thiazide sensitive cotransporter (NCC/TSC) (47). Thiazide diuretics produce natriuresis by inhibiting the NCC/TSC protein. Finally, in the connecting tubules and collecting ducts, sodium transport in the apical membrane is mediated by the epithelial sodium channel (ENaC) (47). This channel is a heteromeric protein formed by three subunits known as $\alpha$, $\beta$, and $\gamma$ subunits. The sodium reabsorption in the collecting duct can be blocked by amiloride and triamterene. These potassium-sparing diuretics block the epithelial sodium channel. Spironolactone, a specific aldosterone antagonist, is also considered a potassium-sparing diuretic, although it has a different mechanism of action. It has been demonstrated that NCC/TSC and ENaC are both regulated by aldosterone (48,49). The

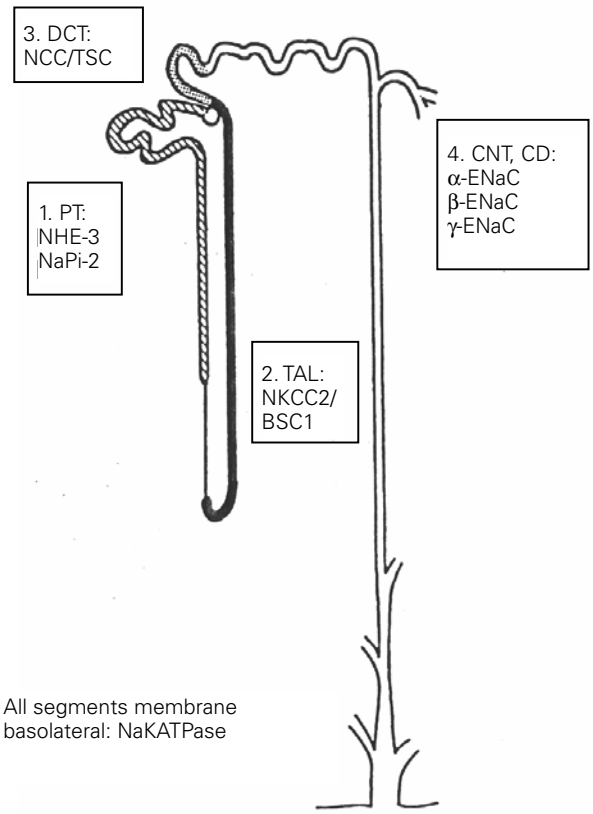


**Figure 16.2** Sodium transporter distribution along the nephron. PT, Proximal tubule; NH-3,sodium hydrogen exchanger isoform 3; NaPi-2, sodium phosphate cotransporter type 2; TAL, thick ascending limb; NKCC2/BSC1, sodium two-chloride potassium cotransporter; DCT, distal convolutes tubule; NCC/TSC, thiazide sensitive cotransporter; CNT, connecting tubule; CD, collecting duct; EnaC, epithelial sodium channel.

transport of sodium in the basolateral plasma membrane along the renal tubules is mediated by the sodium potassium ATPase (NaKATPase) (47).

## Nephron sites of sodium retention in cirrhosis

Glomerular filtration rate is normal or only moderately reduced in most patients with cirrhosis and ascites; therefore, the retention of sodium in this condition is mainly due to an increase in tubular sodium reabsorption (50). The tubular site where sodium retention occurs in cirrhosis has been investigated extensively, both in animal models and patients, using different approaches. The results of these studies show discrepant findings. Micropuncture techniques have been applied to study sodium reabsorption in experimental models of cirrhosis and ascites. These studies showed that sodium retention is markedly increased in the proximal convoluted tubule (51,52). The micropuncture technique allows the assessment of the amount of sodium inside the renal tubule at different nephron segments and, therefore, estimates sodium reabsorption along the renal tubule. This technique, however, has some limitations because it only allows exploration of the cortical segments of the superficial nephrons. It has been postulated that in cirrhosis there is a redistribution of renal plasma flow from the superficial to the juxtamedullary nephrons (53). Therefore this technique may not provide precise information on segmental sodium reabsorption.

Another method used in humans to investigate the nephron site of sodium retention is based on the measurement of free water clearance after a water load. The generation of free water occurs in the loop of Henle by the reabsorption of sodium chloride without a concomitant reabsorption of water. Free water clearance, therefore, depends on the amount of sodium delivery to this segment and on the plasma levels of the antidiuretic hormone (ADH). In normal conditions, the water load suppresses ADH and free water clearance depends largely on sodium delivery beyond the proximal tubule. Patients with cirrhosis and ascites showed a reduction in maximum diluting ability, urine flow rate, and free water clearance, indicating the existence of an increased proximal fractional reabsorption of sodium (54). The main disadvantage of measurement of free water clearance in decompensated cirrhosis is the existence of a non-osmotic hypersecretion of ADH. Because of this, free water clearance mainly reflects water reabsorption in the collecting tubules due to the action of this hormone. In addition, even in the complete absence of ADH, some water is reabsorbed in the collecting duct.

Another method that has been extensively used to study the nephron site of sodium retention in cirrhosis, particularly in humans, is lithium clearance. Lithium is not bound to plasma proteins and is freely filtered by the glomerulus in the same way as sodium, potassium, and others ions are filtered. Lithium is reabsorbed in the proximal tubule in a proportion similar to that of sodium and water, but is neither reabsorbed nor secreted in the distal convoluted tubule and collecting duct. Because the fraction of filtered lithium excreted in the urine is probably close to that delivered from the proximal tubule to the loop of Henle, the clearance of lithium is equal to the flow of tubular fluid leaving the proximal tubule and entering the loop of Henle (55). Thus, lithium clearance has evolved as a measure of the ability of the proximal and distal tubule to reabsorb sodium and water. This technique has been used for many years and nowadays some researchers are still using it. The lithium clearance method can be applied to patients with cirrhosis with or without ascites, and can be performed under different conditions of sodium intake. Lithium clearance studies in liver cirrhosis have provided interesting results. The majority of the studies done in cirrhotic patients with ascites showed that fractional lithium clearance is reduced, pointing to an increase in proximal sodium reabsorption (56,57). Interestingly, fractional distal sodium reabsorption is also increased, especially in those

patients whose sodium balance remained positive. When lithium clearance studies were performed in cirrhotic patients without ascites an increase in distal fractional sodium reabsorption was also observed under conditions of normal sodium diet (58) or low-sodium diet (100 mmol/day) (59). Although lithium clearance has provided interesting data regarding intrarenal sodium reabsorption, it is still an indirect measurement of intrarenal sodium handling. In addition, substantial reabsorption of lithium in the loop of Henle and in distal and collecting tubules has been reported, particularly in conditions of effective hypovolemia (60–62).

Other studies have used the natriuretic and kaliuretic response to diuretic administration in cirrhotic patients to estimate where the sodium is reabsorbed in the renal tubule (63). It has been observed that after the administration of furosemide, patients with diuretic-resistant ascites have a decrease in fractional sodium excretion compared with cirrhotic patients with diuretic-responsive ascites; however, kaliuresis was similar in both groups, which suggests that the proximal tubule is the site of enhanced sodium reabsorption in these patients with diuretic-resistant ascites. Clinical studies using spironolactone to block the mineralocorticoid receptor indicate that this agent induces natriuresis in a large proportion of cirrhotic patients with ascites without renal failure; the results point to a major role of increased sodium reabsorption in the distal sites of the nephron in these patients (27,64–66). Therefore, despite numerous studies in cirrhosis, this subject is still not fully understood due to limitations of the methodologies used so far.

Over the past decade, most of the sodium transporters and channels located in the renal tubule have been cloned and sequenced. This has allowed the possibility of obtaining polyclonal antibodies against these proteins and the development of a new approach for the analysis of renal sodium transport in physiological and pathological conditions (67,68). This approach, known as the targeted proteomic approach, is based on the assumption that sodium reabsorption by the different nephron segments parallels the amount of the specific transporters and channels in the luminal membrane of tubular cells, and offers various advantages over the techniques used so far (69–72). This approach allows exploring the entire tubule of both superficial and deep nephrons. A study where proteomic analysis was applied to investigate sodium retention in an experimental model of cirrhosis with ascites induced by $CCl_4$ has recently been reported (73). The study shows different changes along the renal tubule sodium transporters. First, increased abundance of the sodium chloride cotransporter of the distal tubule (NCC/TSC) and the epithelial sodium channel of the collecting duct (ENaC), both of which have been shown to be aldosterone-induced transporters (48,49); second, an increased abundance of the sodium potassium two-chloride cotransporter of the thick ascending limb (NKCC/BSC1); and third, a decreased abundance of the proximal sodium transporters, namely sodium hydrogen exchanger type 3 (NHE-3) and sodium phosphate cotransporter isoform 2 (NaPi-2). Therefore, these data do not support the existence of increased sodium reabsorption in the proximal tubule in $CCl_4$ cirrhotic rats with ascites. In contrast, they point to an important role for hyperaldosteronism and a possible role of the thick ascending limb of the loop of Henle in the pathogenesis of sodium retention and ascites formation in cirrhosis. These findings are in agreement with results from a previous study showing marked increases in renal NKCC2 in another rat model of liver cirrhosis (common bile duct ligation) (74). This result, together with the finding that cirrhotic rats have a greater diuretic and natriuretic response to furosemide compared with that of control rats, points to a role of the thick ascending limb of Henle's loop in the renal sodium retention in rats with secondary biliary liver cirrhosis (75). A proteomic profile of renal transporters and channels has also been used to study the water excretion defect in an experimental model of liver cirrhosis (76). It is important to note that this method assesses the abundance of each renal tubule transporter protein as an estimation of the degree of sodium reabsorption in the different nephron segments.

An extensive concept exists that sodium retention predominantly occurs in the collecting tubules in the early stages after the onset of ascites, and in the proximal tubules in the final phases of the disease. This proteomic approach has therefore provided interesting results and points to other parts of the nephron, apart from the proximal and distal tubule, as an important site of the nephron involved in sodium retention in cirrhosis.

## Clinical consequences

Because sodium is retained together with water iso-osmotically in the kidney, sodium retention is associated with fluid retention, leading to expansion of extracellular fluid volume and increased amount of fluid in the interstitial tissue. In some patients with cirrhosis, the total extracellular fluid volume may increase up to 40 l (compared with the normal average 14 l in a 70-kg healthy adult), which represents an approximate cumulative gain of 3380 mEq of sodium (26 l of excess times 130 mEq/l). In most patients with cirrhosis, sodium retention is manifested by the development of ascites. The most common clinical symptom of ascites is discomfort due to abdominal swelling. In cases with marked accumulation of fluid, physical activity and respiratory function may be impaired. Other clinical consequences related to the presence of ascites are the appearance of abdominal wall hernias and the spontaneous infection of ascitic fluid (also known as spontaneous bacterial peritonitis; see Chapters 34 and 35). Both complications, especially the latter,

contribute markedly to the high morbidity and mortality associated with the presence of ascites.

Accumulation of fluid in the subcutaneous tissue, as edema, is also common in patients with cirrhosis and sodium retention and in most cases occurs concomitantly with the existence of ascites. Edema is most commonly observed in the lower extremities, but generalized edema may occur as well. Mild or moderate edema may decrease or even disappear during bed rest and reappear during the daytime, reflecting an increased natriuresis in the supine position compared with the upright position (35). Both hypoalbuminemia and increased venous pressure in the inferior vena cava due either to constriction of the vena cava within the liver or increased intra-abdominal pressure caused by ascites contribute to the high incidence of leg edema in cirrhotic patients with ascites.

Sodium retention in cirrhosis may also be manifested by pleural and/or pericardial effusions. Clinically significant pleural effusions occur in up to 10% of patients with cirrhosis (77–79). In most cases the effusion is mild or moderate, more frequent on the right side, and associated with the presence of ascites. Left-sided effusions are not uncommon, but usually occur in patients who have right-sided effusions as well. Occasionally, large right pleural effusions may exist in the absence of ascites and constitute the main manifestation of sodium retention (78,80). These cases usually recur after therapy and are due to the existence of anatomical defects in the diaphragm that cause a communication between the peritoneal and pleural cavities. The gradient between the positive intra-abdominal pressure and the negative intrathoracic pressure explains the passage of all fluid formed in the peritoneal cavity to the pleural cavity. Although less commonly than ascitic fluid, pleural fluid may also become infected spontaneously, a condition known as spontaneous bacterial empyema (81,82). Finally, between one- and two-thirds of cirrhotic patients with ascites also have mild or moderate pericardial effusions, as demonstrated by echocardiography (83,84), that disappear after the elimination of ascites. These pericardial effusions in patients with cirrhosis are not associated with clinical symptoms.

### Assessment of sodium excretion in clinical practice

The assessment of the urinary excretion of sodium is useful in the clinical management of patients with cirrhosis and ascites as it allows the physician to quantify precisely the intensity of sodium retention. Urine must be collected under conditions of fixed and controlled sodium intake (usually a low-sodium diet of approximately 80 mEq/day during the previous 5–7 days), as sodium intake may influence sodium excretion. The amount of sodium ingested does not affect the excretion of sodium in patients with marked sodium retention, who have very low urine sodium concentrations regardless of the amount of sodium taken with the diet, but may affect sodium excretion in patients with mild or moderate sodium retention. Diuretics should not be given during the 5–7-day period prior to urine collection to avoid a pharmacological increase in sodium excretion. This period of time is particularly important in patients receiving spironolactone or other aldosterone antagonists that have a very prolonged half-life. Finally, although the measurement of sodium concentration in a spot of urine may provide a rough estimate of sodium excretion, the assessment of sodium excretion in a 24-h period is preferable because it is more representative of sodium excretion throughout the day and takes into account the urine output. Sodium excretion is calculated as follows: sodium excretion (mEq/day) = urine sodium (mEq/l) × urine volume (l/day).

In clinical practice, sodium excretion should be measured under the conditions stated above in patients with first-onset ascites or when there are signs suggestive of a progressive sodium retention (i.e. marked increase in ascites or edema despite compliance with the sodium-restricted diet and diuretic therapy). On the other hand, the measurement of sodium excretion in patients under diuretic therapy is very useful to monitor the response to treatment.

Because the amount of extracellular fluid volume retained as ascites or edema depends on the balance between sodium output and sodium intake, the measurement of sodium excretion is of major importance in decisions concerning the dietary management of cirrhotic patients with ascites. Furthermore, sodium excretion is one of the best predictors of the response to diuretic treatment. Therefore, the measurement of urine sodium concentration is very helpful in establishing the therapeutic schedule in cirrhotic patients with ascites. Patients with marked sodium retention in whom a positive sodium balance is anticipated despite a restriction in sodium intake should be started on moderately high doses of aldosterone antagonists (e.g. spironolactone 100–200 mg/day) alone or in association with loop diuretics (e.g. furosemide 20–40 mg/day). Conversely, patients with moderate sodium restriction would be likely to respond to low doses of aldosterone antagonists (e.g. spironolactone 50–100 mg/day) without loop diuretics. Finally, the severity of sodium retention also provides prognostic information in patients with ascites. Patients with a baseline urine sodium concentration < 10 mEq/day have a median survival time of only 1.5 years compared with 4.5 years in patients with urine sodium concentrations > 10 mEq/day (Fig. 16.3) (see Chapter 21) (85–87).

## Pathogenesis of ascites formation in cirrhosis

Sodium retention in cirrhosis occurs in the setting of marked alterations in the systemic circulation characterized mainly by a reduction in total systemic vascular resistance and increased cardiac output (1–3) (see Chapters

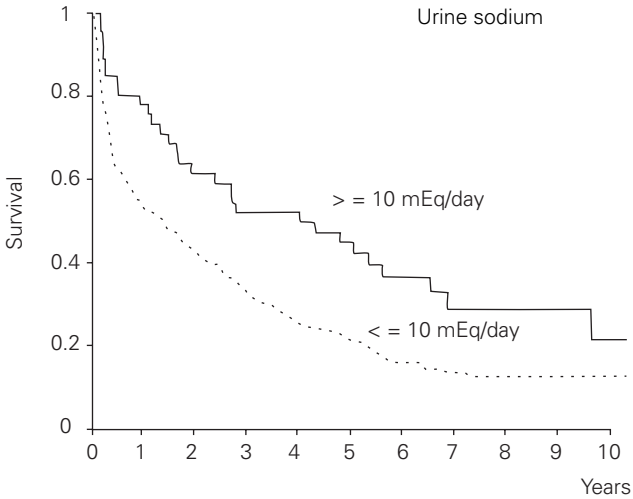


**Figure 16.3** Long-term survival according to glomerular filtration rate in a series of 204 patients with cirrhosis admitted to the hospital for the treatment of ascites. (Reproduced from Schrier RW, ed. *Diseases of the Kidney and Urinary Tract*, 7th edn. Philadelphia: Lippincott, Williams and Wilkins, 2000.)

11 and 12). A key question on the pathogenesis of ascites is how renal function abnormalities are linked to these circulatory changes. One possible explanation is that the reduction in total systemic vascular resistance and increased cardiac output represent an adaptive response of the systemic circulation to the expansion of the extracellular fluid (ECF) volume and plasma volume secondary to renal sodium retention. Alternatively, the disturbances in systemic hemodynamics may represent the primary factor responsible for abnormalities in renal function. Although controversy between these two different interpretations still exists, most data obtained from clinical studies support the latter explanation. Another important question is why in cirrhosis the fluid retained by the kidneys is localized predominantly in the peritoneal cavity as ascites rather than in the interstitial tissue. This part of the chapter reviews the current knowledge on these important issues.

## Regulation of extracellular fluid volume and formation of edema: the concept of effective arterial blood volume and arterial underfilling

Two major mechanisms have been proposed to account for sodium retention in major edematous states: a primary abnormality in the kidney and a secondary response of the kidney to a disturbed systemic circulation (Fig. 16.4) (88). In the first type, known as primary edema, there is a primary defect in renal sodium excretion that results in expansion of the ECF volume and subsequent formation of edema. The increase in plasma volume leads to high cardiac output, arterial hypertension, suppression of vasoconstrictor and antinatriuretic systems (renin–angiotensin–aldosterone system; sympathetic nervous system), and activation of natriuretic systems (natriuretic peptides) as a homeostatic response to prevent the excessive increase of ECF volume. However, these changes are not operative because the impaired kidneys are unresponsive to variations in humoral factors. In the second type, known as secondary edema, sodium retention occurs because of a primary disturbance in the systemic circulation resulting in the activation of antinatriuretic mechanisms that remain persistently activated despite the progressive expansion of the ECF volume.

The precise regulation of the ECF volume by the kidneys requires the existence of sensor mechanisms strategically located within the cardiopulmonary and arterial circula-

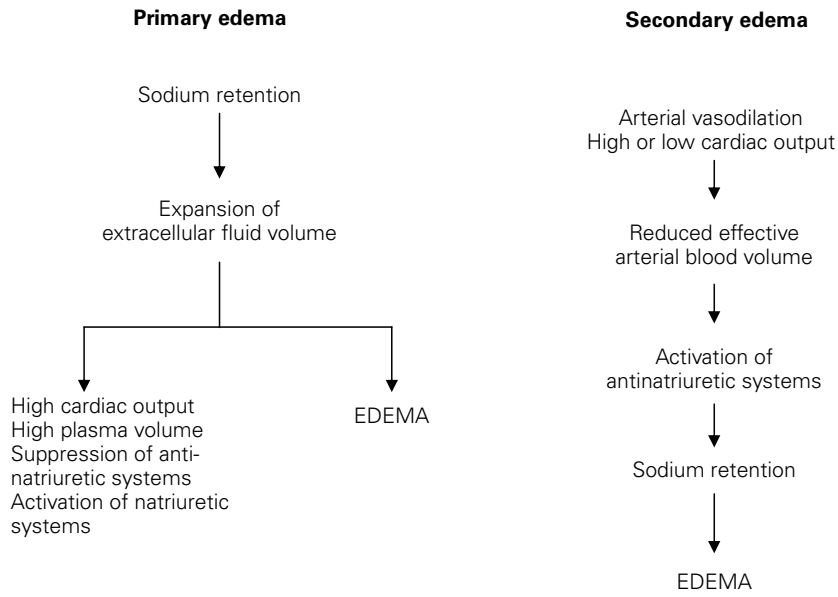


**Figure 16.4** Proposed pathogenic mechanisms of edema in major edematous states: primary vs. secondary edema. (Reproduced from Arroyo V, Ginès P, Rodés J, Schrier RW, *Ascites and Renal Dysfunction in Liver Disease*, 1st edn, Oxford: Blackwell, 1999; 412.)

tions capable of sensing changes in volume or pressure (88–94). Receptors located in cardiac atria and pulmonary arteries detect changes in central blood volume through atrial or pulmonary artery distension, whereas receptors located in the aorta, carotid sinus, and juxtaglomerular apparatus regulate the pressure of the arterial circulation. Both types of receptors send afferent impulses that travel along the glossopharyngeal and vagus nerves to the nucleus tractus solitarius in the medulla and hypothalamus, resulting in inhibitory influences on the main vasoconstrictor and antinatriuretic systems. The cardiac atria also have sensor mechanisms that involve the release of atrial natriuretic peptide in response to increases in atrial transmural pressure. Receptors also exist within the hepatic circulation and central nervous system, but their physiological significance remains to be elucidated.

Observations made in subjects with arteriovenous fistulas suggest that arterial receptors are more important than cardiopulmonary receptors in the regulation of ECF volume (88,95,96). In this condition, the shunting of blood from the arterial tree to the venous compartment gives rise to sodium retention in association with a hyperdynamic circulation including an increased cardiac output, reduced systemic vascular resistance, and increased venous return to the heart. Closure of the fistulas, which induces stimulation of arterial receptors, results in prompt natriuresis and a decrease in central venous pressure that would by itself be associated with renal sodium and water retention. Conversely, the restoration of fistula patency results in sodium retention despite a rise in the central venous filling pressure, which by itself would stimulate cardiopulmonary receptors and induce renal sodium excretion. Moreover, under several pathological conditions, sodium retention persists despite the presence of expanded ECF volume and total blood volume, which indicates that it is not the size of the ECF volume or total blood volume that regulates sodium excretion. These apparent contradictions led to the introduction of the term "effective arterial blood volume" (EABV), which implies that not all body fluid compartments are equally efficient in sensing changes in body fluid volume (88–91). It is likely that the body fluid compartment that modulates renal sodium excretion and ECF volume is the arterial circulation and that the main determinants of the fullness of the arterial circulation are cardiac output and systemic vascular resistance (97,98). It is for this reason that we have used the term "arterial underfilling" for a decrease in EABV. Under normal circumstances, EABV is well correlated with ECF volume. However, this correlation is lost in major edematous states because EABV remains contracted despite progressive expansion of total ECF volume.

## Ascites as primary edema: the overflow theory

The existence of a primary renal sodium retention in cirrhosis with ascites was proposed in an attempt to explain the paradox of coexistence of sodium retention and increased plasma volume in patients with ascites (99,100). According to this theory, the expansion of plasma volume would result in increased cardiac index and reduced systemic vascular resistance as circulatory mechanisms of adaptation to the excess of intravascular volume. The existence of portal hypertension and circulating hypervolemia would lead to "overflow" of fluid within the peritoneal cavity. It has been proposed that the primary signal for sodium retention would arise from the liver, as a consequence of either intrahepatic portal hypertension, by means of hepatic low-pressure baroreceptors, or liver failure, by means of decreased hepatic clearance of a sodium-retaining factor or reduced hepatic synthesis of a natriuretic factor (101–108). A strong body of evidence has been accumulated over the last two decades indicating that the hemodynamic pattern of cirrhotic patients with ascites does not correspond to that predicted by the overflow theory because the arterial vascular compartment is not overfilled, as arterial pressure is low in most patients despite the increased plasma volume and cardiac index. Moreover, there is marked overactivity of vasoconstrictor mechanisms, which would be suppressed if there were overfilling in the systemic arterial circulation (1,2) (reviewed in Chapter 11).

Because of the increasing evidence against the existence of vascular overfilling in cirrhosis with ascites, the overflow theory has been redefined recently to explain changes that occur in the pre-ascitic stage of cirrhosis. Proponents of this theory suggest that in the pre-ascitic stage of cirrhosis, subtle sodium retention leading to plasma volume expansion would have two components: one related to the circulatory changes occurring in the splanchnic circulation aimed at maintaining the EABV, and one related to the existence of intrahepatic portal hypertension (109,110). Although the discussion of circulatory and renal abnormalities in pre-ascitic cirrhosis is beyond the scope of this chapter, recent studies in patients with cirrhosis without ascites indicate that the existence of arterial vasodilation is of crucial importance in the development of sodium retention and ascites formation in this condition. In fact, pre-ascitic cirrhotic patients with sinusoidal portal hypertension treated with mineralocorticoids exhibited impaired mineralocorticoid escape and developed ascites only when marked arterial vasodilation was present (20). Moreover, a significant proportion of pre-ascitic cirrhotic patients developed sodium retention and ascites and edema when treated with the vasodilator drugs prazosin or carvedilol, α-adrenergic blockers (44,45). It is important to note that the development of sodium retention in these studies was not related to the degree of portal hypertension or liver failure. In fact, in patients receiving α-adrenergic blockers, sodium retention occurred despite a marked reduction in portal pressure and improvement in liver perfusion (44,45).

## Ascites as secondary edema: from the classic theory of ascites to the arterial vasodilation theory

The traditional concept of ascites formation in cirrhosis (111,112) considers that the key event in ascites formation in cirrhosis is a "backward" increase in hydrostatic pressure in the hepatic and splanchnic circulations owing to the increased resistance to portal flow. This would cause disruption of the Starling equilibrium and increased filtration of fluid into the interstitial space. Initially, this capillary hyperfiltration is compensated by an increased lymphatic flow that returns the fluid to the systemic circulation by way of the thoracic duct. However, as portal hypertension increases, the lymphatic system is not able to drain the excess interstitial fluid, which then accumulates in the peritoneal cavity as ascites. Loss of fluid from the intravascular compartment results in true hypovolemia, which is then sensed by cardiopulmonary and arterial receptors, resulting in compensatory renal sodium retention. The retained fluid cannot adequately fill the intravascular compartment and suppress the sodium-retaining signals to the kidney, because fluid is continuously leaking into the peritoneal cavity, thus creating a vicious cycle. In cases involving extreme hypovolemia, renal vasoconstriction develops, leading to the hepatorenal syndrome. This underfill hypothesis is similar to the backward theory of edema formation in heart failure, which suggests that sodium retention and formation of edema are secondary to the disruption of Starling equilibrium in the microcirculation owing to the backward increase in capillary hydrostatic pressure (113). This classical underfilling theory of ascites formation, however, does not correspond to the systemic hemodynamic abnormalities associated with cirrhosis. If this theory were correct, changes in systemic circulation would consist of reductions in plasma volume and cardiac index and an increase in systemic vascular resistance. However, findings in patients with cirrhosis and ascites are exactly the opposite, with increased plasma volume and cardiac index and reduced systemic vascular resistance (1–3,114) (see Chapters 11 and 12).

These traditional backward theories of edema formation in cirrhosis and heart failure have been replaced by new theories that fit more precisely with the modern concepts of regulation of ECF. As previously discussed, a reduction in EABV or arterial underfilling seems to be the main determinant of sodium retention in major edematous states (88–91,97). Arterial vasodilation would be the triggering factor for sodium retention in cirrhosis, whereas a reduction in cardiac output would be the triggering factor in heart failure. The arterial vasodilation theory considers that the reduction in EABV in cirrhosis with ascites is not attributable to true hypovolemia, as proposed by the classical underfilling theory, but rather to a disproportionate enlargement of the arterial tree secondary to arterial vasodilation (Fig. 16.5) (3,115). According to this theory, portal hypertension is the initial

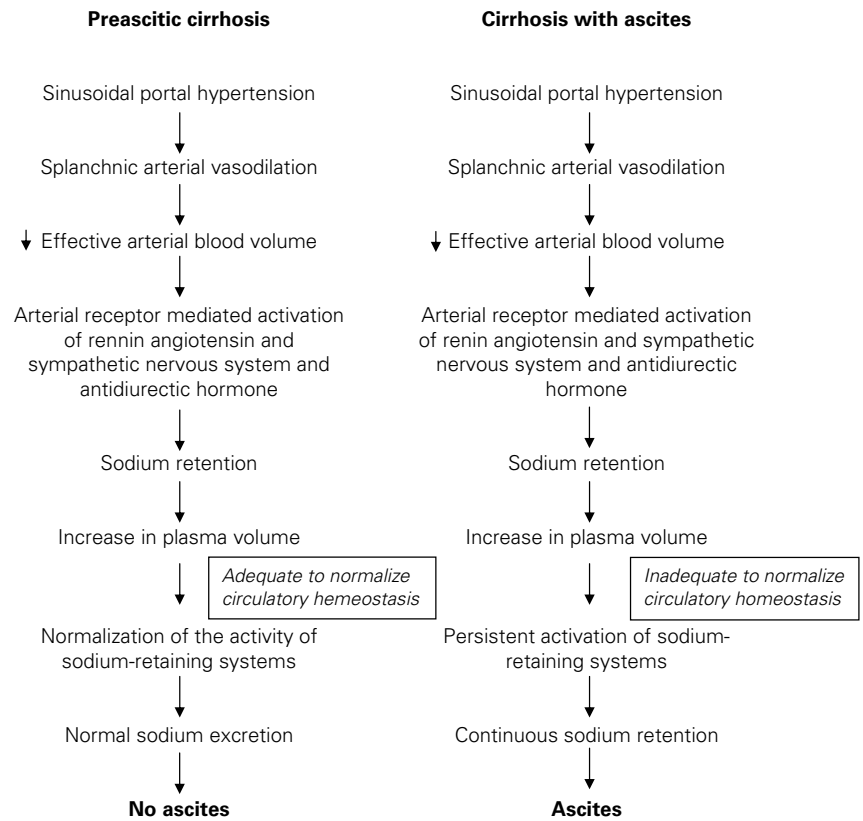


**Figure 16.5** Hemodynamic, neurohumoral, and renal function abnormalities in cirrhosis without and with ascites according to the arterial vasodilation theory. (Reproduced from Arroyo V, Ginès P, Rodés J, Schrier RW, *Ascites and Renal Dysfunction in Liver Disease,* 1st edn, Oxford: Blackwell, 1999; 414–15.)

event with resultant splanchnic arteriolar vasodilation causing underfilling of the arterial circulation. The arterial receptors then sense the arterial underfilling and stimulate the sympathetic nervous system and the renin–angiotensin–aldosterone system and cause non-osmotic hypersecretion of antidiuretic hormone. Renal sodium and water retention are the final consequences of this compensatory response to arterial underfilling. In the early stages of cirrhosis, when splanchnic arteriolar vasodilation is moderate and the lymphatic system is able to return the increased lymph production to the systemic circulation, the EABV is stabilized by transient periods of sodium retention. The fluid retained by the kidneys increases plasma volume and suppresses the signals stimulating the antinatriuretic systems, and sodium retention terminates. Therefore, no ascites or edema is formed at this early stage, and the relationship between EABV and ECF volume is maintained. As liver disease progresses, splanchnic arterial vasodilation increases, thus resulting in a more intense arterial underfilling and more marked sodium and water retention. At this time, the EABV can no longer be maintained by the increased plasma volume, probably because the retained fluid leaks from the splanchnic circulation into the peritoneal cavity as ascites

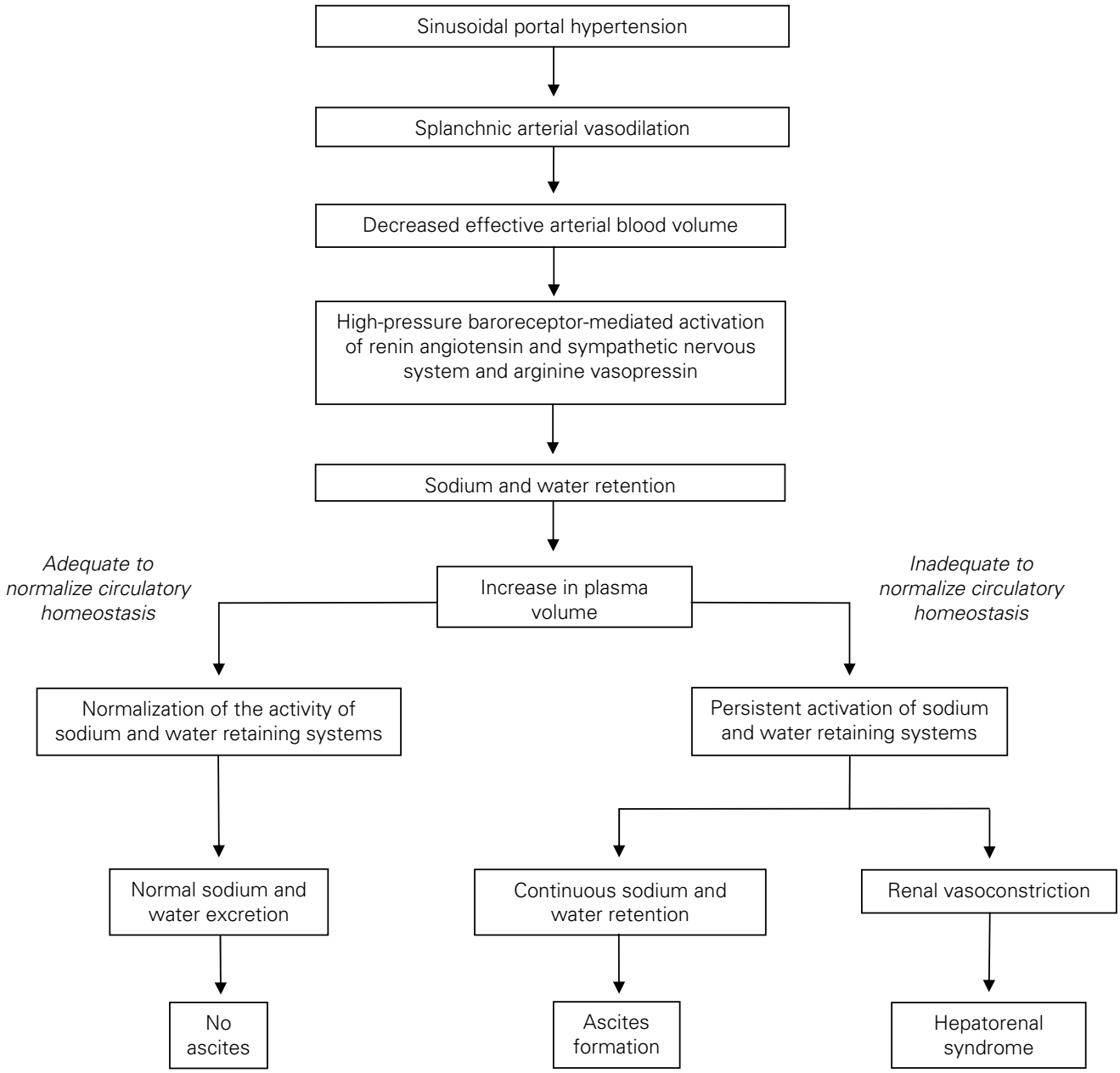


**Figure 16.6** Pathogenesis of functional renal abnormalities and ascites formation in cirrhosis according to the arterial vasodilation hypothesis. (Reproduced from Schrier RW, ed. *Diseases of the Kidney and Urinary Tract*, 7th edn. Philadelphia: Lippincott, Williams and Wilkins, 2000.)

and/or from the systemic circulation to the interstitial tissue as edema. A persistent stimulation of vasoconstrictor systems occurs in an attempt to maintain EABV. The activation of these systems perpetuates renal sodium and water retention, which accumulates as ascites. The correlation between EABV and ECF volume is no longer maintained, because EABV remains contracted despite progressive expansion of ECF volume. The hepatorenal syndrome probably represents the most extreme manifestation of the reduction in EABV (3,115) (Fig. 16.6). Studies in experimental models of portal hypertension aimed at investigating the chronological relationship between abnormalities in the systemic circulation and sodium retention indicate that arterial vasodilation with reduced systemic vascular resistance precedes sodium retention and subsequent plasma volume expansion (116,117).

The arterial vasodilation theory provides a reasonable explanation not only for the circulatory changes and activation of antinatriuretic systems observed in cirrhosis with ascites, but also for the preferential location of retained fluid in the peritoneal cavity. The existence of splanchnic arterial vasodilation causes a "forward" increase in splanchnic capillary pressure that enhances the effects of portal hypertension on the filtration coefficient in splanchnic capillaries, which facilitates the formation of ascites (118) (see Chapters 3 and 14 for a detailed discussion).

## Conclusion

In recent years, much progress has been made in the understanding of factors involved in the pathogenesis of ascites and renal dysfunction in cirrhosis. It is currently believed that ascites and renal dysfunction are the final adverse consequences of overactivity of several vasoconstrictor and sodium-retaining mechanisms. The link between the diseased liver and activation of these vasoconstrictor mechanisms is not completely known, but much evidence indicates that it consists of a circulatory dysfunction that affects mainly the arterial circulation and is characterized by an inability to maintain a normal effective arterial blood volume. Efforts should now be focused on the evaluation of factors responsible for the circulatory dysfunction of cirrhosis. Research on the pathogenesis of this circulatory dysfunction may yield clues that will help in the design of more pathophysiologically oriented therapeutic approaches to the management of ascites and the renal functional abnormalities of cirrhosis.

## Acknowledgements

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# Chapter 17
# Experimental Models of Cirrhosis and Ascites

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The availability of animal models is crucial for the study of human diseases. This is especially true for those diseases with a clear metabolic and/or nonhereditary component, such as liver cirrhosis. Consequently, it is not surprising that our current understanding of the mechanisms underlying liver cirrhosis and the pathophysiology of the associated complications is, to a great extent, based on experimental models of liver disease. Although several common models of experimentally induced liver disease have been developed, carbon tetrachloride ($CCl_4$)-induced cirrhosis in rats is, by and large, the most popular model, mainly because of its availability, versatility, and reproducibility. In this chapter, we discuss the advantages and disadvantages of the rat $CCl_4$-induced model compared with other models of cirrhosis, and provide an overview and an update of the progress achieved in the study of renal dysfunction and formation of ascites in these animals.

## Animal models of liver cirrhosis

The species used as animal models of liver disease are, in order of frequency, rats, mice, rabbits, and dogs. According to the etiology, the experimental models of liver disease can be classified as cholestatic, nutritional, alcoholic, immunological, and toxic (Table 17.1). This chapter will only address the cholestatic, nutritional, and toxic models because they have been the most useful in defining the pathophysiology of liver cirrhosis. Immunological models such as subcutaneous injection of *Schistosoma mansoni* and repeated administration of concanavalin A to mice, or alcoholic models such as continuous intragastric infusion of ethanol into rats are not discussed because cirrhosis is either not well established in these models or it develops over a long term. These animal models are reviewed elsewhere (1,2).

### Cholestatic cirrhosis

#### *Biliary cirrhosis*

Bile duct ligation (BDL) has been used as an experimental model of cirrhosis in the rat, rabbit, dog, and monkey (3,4). The technique is quite simple and involves the double ligation of the extrapancreatic common bile duct close to the liver hilus with a silk thread and excision of the bile duct between the two knots. Following this procedure, biliary cirrhosis is usually fully developed 4 weeks postoperatively. From the histological point of view, this model is characterized by significant infiltration of connective tissue in the portal zone and enhanced proliferation of bile duct epithelial cells and hepatocytes (5). The hemodynamic disturbances developed by BDL animals include sinusoidal portal hypertension and a hyperdynamic state characterized by increased portal pressure and cardiac output and decreased systemic and splanchnic vascular resistance with a highly variable degree of portosystemic shunting (4,5). BDL rats have also been shown to develop ascites and renal impairment (6). The main drawbacks of this model are its relatively high mortality and the species differences in the response to biliary obstruction.

**Table 17.1** Classification of animal models of liver disease according to the etiology.

| Cholestatic | Nutritional | Hepatotoxic | Immunological | Alcoholic |
|---|---|---|---|---|
| Bile duct ligation | Choline-methionine-deficient diets | Dimethylnitrosamine<br>Thioacetamide<br>D-galactosamine<br>Carbon tetrachloride | *Schistosoma mansoni*<br>Concanavalin A | Ethanol-containing diets |

## Nutritionally induced hepatic cirrhosis

### *Choline/methionine-deficient diets*

Rats and mice that have been fed with choline/methionine-deficient diets develop centrolobular or central-portal fibrosis and cirrhosis after 12–24 weeks of treatment (7). Liver injury induced by low-choline and low-methionine diets is probably secondary to the severe depletion of hepatic anti-oxidant mechanisms, such as reduced gluthathione and S-adenosylmethionine, which leads to hepatic inflammation and subsequently to fibrosis and cirrhosis. Rats fed with choline/methionine-deficient diets display signs of portal hypertension, ascites, and collateral blood circulation (8), but in general hemodynamic and renal studies have been somehow overlooked in this model. By contrast, this experimental model has frequently been used for studies of liver carcinogenesis. The varied responses among the animals, the distinct species variations in the susceptibility to choline deficiency, and the difficulty in studying the histopathological stages are considered the main disadvantages of this model.

## Hepatotoxin-induced cirrhosis

### *Dimethylnitrosamine-induced cirrhosis*

Dimethylnitrosamine (DMNS) is a common hepatic carcinogenic agent that is metabolized by the microsomes to an active methylating moiety which causes hepatocellular injury by means of covalent binding and methylation of nucleic acids (DNA adducts) and proteins in liver cells (9). The DMNS model has been used in rats and dogs and DMNS has been administered either by feeding or intraperitoneal injections (10,11). After 3–4 weeks of DMNS administration, animals become cirrhotic and develop portal hypertension, sodium retention and accumulate ascites (10,11). Unlike other models, DMNS does not cause steatosis in hepatocytes. An important feature of this model is that cirrhosis appears to be stable or progressive for several months after discontinuation of DMNS administration. The main disadvantage is that DMNS is highly toxic and is a potent carcinogenic agent.

### *Thioacetamide-induced cirrhosis*

Administration of thioacetamide (TAA) (0.03% in tap water) over a period of 2 or 3 months results in a characteristic lesion in rat livers which corresponds to patterns of macronodular cirrhosis (12,13). The exact mechanism by which TAA induces liver cirrhosis is unknown, but TAA is a potent oxidant agent that produces liver inflammation, fibrosis, and cirrhosis secondary to the induction of hepatic oxidative stress. TAA-induced cirrhotic rats exhibit portal hypertension and ascites and these animals constitute the animal model that most closely reproduces the changes in amino acid metabolism and the development of encephalopathy seen in human cirrhosis (12–16). Unfortunately, little is known about the hemodynamic and renal features of TAA-induced cirrhosis.

### *D-galactosamine-induced cirrhosis*

D-galactosamine (GalN) is a hepatotoxin that induces liver damage by depleting uridine triphosphate and other uridine nucleotides, thus diminishing RNA and protein synthesis. Liver injury is usually produced by repetitive injection of GalN (at least three times per week) into the peritoneum of mice, rats, and dogs in which cirrhosis is fully established after 7–12 weeks of administration of the hepatotoxin (17,18). GalN induces progressive liver dysfunction accompanied by the development of portal hypertension, a hyperdynamic circulatory state (i.e. increased cardiac output and decreased peripheral vascular resistance), a significant decrease in renal blood flow, and the accumulation of ascites (17,18). Nevertheless, rather than being an animal model useful for the study of renal dysfunction and ascites formation, the GalN model is considered to resemble the acute liver injury seen in human hepatitis.

### *$CCl_4$-induced cirrhosis*

$CCl_4$ is, at large, the most widely used hepatotoxin for induction of liver cirrhosis in laboratory animals. $CCl_4$-induced liver damage is mainly produced by the trichloromethyl radical ($^{\bullet}CCl_3$), a reactive metabolite that is originated during the oxidative metabolism of $CCl_4$ by members of the cytochrome P450 family (19,20) (Fig. 17.1). The reactive species $^{\bullet}CCl_3$ elicits production of reactive oxygen intermediates and causes peroxidative degradation of the polyunsaturated fatty acids of the cellular membrane phospholipids (20,21). Thus, lipid peroxidation of the hepatic tissue is considered to be responsible for hepatocellular damage and enhanced production of connective tissue in the $CCl_4$ cirrhosis model. Several compounds, including phenobarbital, acetone, and ethanol, which induce the activity of the microsomal cytochrome P450, are able to potentiate the hepatotoxicity of the $CCl_4$ metabolites and accelerate the formation of cirrhosis (19,20) (Fig. 17.1). Since the microsomal enzyme oxidizing system is closely modulated by oxygen, which inhibits $CCl_4$ metabolism (19,20), hypoxic conditions are required for ensuring $CCl_4$ toxicity. Therefore, hepatocellular injury and necrosis are not uniformly distributed within the liver lobule and are confined to areas where oxygen tension is low, such as the centrolobular region (19,20). Interestingly, the phenobarbital-inducible cytochrome P450 isoform, the most important $CCl_4$-metabo-

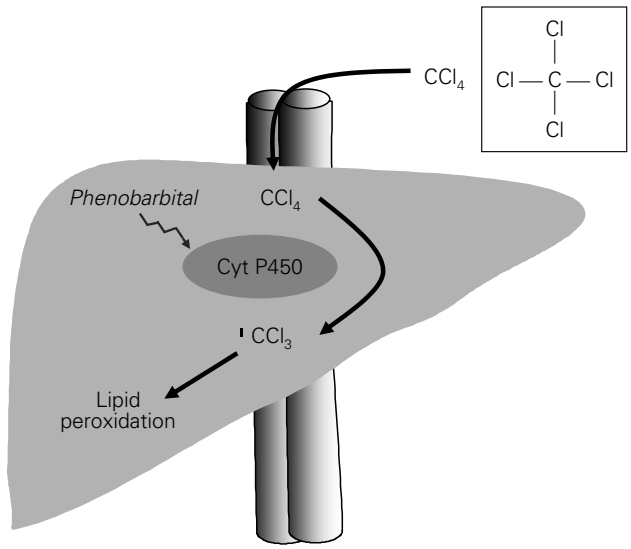


**Figure 17.1** Mechanism for carbon tetrachloride ($CCl_4$) hepatotoxicity. Transformation of $CCl_4$ to the $\bullet CCl_3$ free radical by hepatic microsomal cytochrome P450 (Cyt P450) is essential for the induction of lipid peroxidation and hepatic injury by this hepatotoxin. Previous exposure to phenobarbital, a potent inducer of Cyt P450, increases $CCl_4$ injury.

lizing enzyme in the rat liver, is predominantly localized in the centrolobular region (20).

The first investigation characterizing the main features of $CCl_4$-induced cirrhosis was performed by Cameron and Karunaratne in 1936 (22). Following this initial study, $CCl_4$ has been extensively used to induce cirrhosis in rats, mice, pigs, guinea pigs, hamsters, baboons, and dogs (23,24). Since $CCl_4$ can be absorbed through the lungs and the gastrointestinal tract, the hepatotoxin can be given by different routes including subcutaneous, intramuscular, or intraperitoneal injections, gastric gavage or inhalation in a closed chamber (25–31). Therefore, theoretically the combination of different animal species (seven) with the different routes of administration (five) results in thirty-five different ways to induce experimental cirrhosis by $CCl_4$. In practice, however, most experimental studies are performed in rats and mice in which $CCl_4$ is administered either by inhalation or injection.

To induce cirrhosis in rats by $CCl_4$ injection, doses ranging between 0.5 and 1 ml/kg, administered at time intervals of 3–5 days, are required. The hepatotoxin is given emulsified with mineral oil (v/v) and animals may or may not be previously submitted to microsomal enzyme induction. Subcutaneous injections yield low mortality rates of about 5% but cirrhosis develops only after 20 weeks or more (25). Intramuscular (26) or intraperitoneal (27) injections reduce this period from 5 to 10 weeks, especially if phenobarbital is given throughout the cirrhosis induction program. However, these routes of $CCl_4$ administration can cause inflammation and necrosis of the subcutaneous or muscular tissue which are difficult and laborious to cure, and may increase mortality by 50% or more. The dose of $CCl_4$ and the time interval to produce cirrhosis depends on the method employed, but in general, cirrhosis appears more rapidly when $CCl_4$ is given in higher doses at short intervals. The time required to produce cirrhosis varies widely from animal to animal. While some rats show a fully developed cirrhosis 10 weeks after treatment with $CCl_4$ and phenobarbital, others exhibit only mild histological changes. Proctor and Chatamra (28) were able to obtain a more homogeneous response by adjusting each dose of $CCl_4$ to the individual loss of body weight. These authors gave $CCl_4$ orally, via a nasogastric tube, to rats pretreated with phenobarbital and 75% of the animals developed ascites within a period of 8–10 weeks of treatment. However, oral administration of $CCl_4$, especially in repetitive doses, results in higher mortality rates and less consistent induction of liver damage (28).

In our laboratory, cirrhosis is induced in male rats weighing 160–180 g by repeated inhalation of $CCl_4$ in a closed chamber (24,29), using the method of McLean and McLean (30) modified by López-Novoa *et al.* (31,32). To shorten the time period required to induce cirrhosis, phenobarbital is given together with drinking water (0.3 g/l) starting 1 week prior to and throughout the cirrhosis induction program. The size of the $CCl_4$ inhalation chamber is $70 \times 25 \times 30$ cm and five to seven rats are included in each inhalation session. Air from a high-pressure unit is passed via a flowmeter (1 l/min), bubbling through a flask containing $CCl_4$ into the closed chamber. Animals are exposed to $CCl_4$ twice weekly (usually Monday and Friday) starting with 0.5 min of bubble air and 0.5 min of the gas atmosphere. The dose is increased to 1 min of bubble air and 1 min of gas atmosphere on the fourth inhalation session. Afterwards, the dosage is increased in a 1-min-step fashion every three sessions until 5 min of bubble air and 5 min in gas atmosphere are reached. Since high doses of $CCl_4$ have anesthetic properties, animals need to be continuously checked to prevent respiratory arrest. Therefore, it is highly recommended to build the inhalation chamber with transparent materials such as glass or methacrylate. Following this procedure, severe fibrosis and cirrhosis can be obtained at week 6 after starting the inhalation sessions and these lesions are almost constantly present at the 10th week. Ascites usually develops after week 10, although the initial episodes of ascites are often transient, occurring during the first or second day following $CCl_4$ inhalation and disappearing spontaneously thereafter. In most of the cases, it is necessary to maintain $CCl_4$ administration to generate massive ascites. The mortality rate prior to the occurrence of ascites (8–10 weeks) is 15% but increases to around 25% in rats with permanent ascites (12–16 weeks) (24,29–35). It must be taken into consideration that $CCl_4$ is potentially

toxic for the investigator. This risk can be avoided by performing the inhalation session in a fume hood. In our experience, inhalation of $CCl_4$ in a closed chamber is a safe, highly reproducible, and relatively predictable method to induce cirrhosis in the rat.

In the $CCl_4$-induced cirrhosis model, apparent hepatocellular injury with centrilobular necrosis and steatosis and significant inflammation constitute the main histological features during early stages of intoxication (23,24,33). During chronic administration, from 6 to 9 weeks of $CCl_4$ intoxication, the major features are hepatocyte necrosis, inflammatory infiltration, hepatocyte regeneration, and perivenular and periportal deposition of connective tissue. Continued hepatocyte regeneration and active fibrogenesis combined with reticulin collapse and capillarization result in marked architectural distortion, which finally leads to micronodular cirrhosis (23,24,36). In order to avoid recovery of the damaged tissue, the time interval between the administration of each dose of $CCl_4$ should not be too long (23,24). It is generally recommended to give two doses of $CCl_4$ per week. In any event, the evolution of liver disease in rats chronically intoxicated with $CCl_4$ evolves in three phases: (i) an initial stage of acute liver injury, with focal liver cell necrosis, acidophilic degeneration, and steatosis; (ii) a pre-cirrhotic stage, characterized by marked centrilobular and sinusoidal fibrosis, with formation of thin fibrous septa which begin to dissect the liver lobules, but without nodular regeneration; and (iii) a stage of well-established cirrhosis, in which the combination of nodular regeneration of liver cell plates surrounded by thick connective tissue septa with proliferating bile ducts define a picture of a monolobular (micronodular) cirrhosis. Macroscopically, the liver appears enlarged, firm, brownish, with a finely nodular surface. In advanced stages, however, the liver may be smaller than normal, while its surface becomes grossly nodular. Fibrosis and cirrhosis may be associated with variable degrees of fatty degeneration and liver cell necrosis, which are more prominent the first few days after $CCl_4$ administration. Stages (i) and (ii) are reversible after stopping $CCl_4$, but not when cirrhosis is well established. Although cirrhosis usually remains unaltered for weeks, there is some reabsorption of collagen, which results in a thinner fibrous septa in the advanced stages of cirrhosis.

The $CCl_4$-induced model in rats has been extensively used in the study of the pathophysiology of liver cirrhosis, mainly because it allows, as discussed before, different routes of administration of the hepatoxin and presents a high reproducibility and relatively consistent histological and pathophysiological features. Nevertheless, some objections have been raised against the $CCl_4$-induced cirrhosis model. First, it has been argued that $CCl_4$ can injure organs other than the liver, particularly the kidneys. After analyzing the broad literature in this field, one can positively affirm that the number of extrahepatic lesions caused by $CCl_4$ intoxication is minimal (2). In this regard, no evidence of renal tubular damage has been observed under microscopic examination in rats with $CCl_4$-induced cirrhosis and ascites (31,32). Moreover, the urinary excretion of N-acetyl-β-d-glucosaminidase, a sensitive marker of tubular necrosis, does not increase during the cirrhosis induction program (37). Second, the use of phenobarbital has been contended as a nonselective enzymatic inductor for the inhalation protocol. In this regard, chronic administration of low doses of $CCl_4$ combined with alcohol has been proposed as a reasonable alternative model of portal hypertension (38), although a complete evaluation of this model is necessary. Finally, $CCl_4$-induced cirrhosis is a limited model that is not suitable for post-hepatitis or alcoholic cirrhosis. Moreover, this model does not satisfactorily develop hepatic encephalopathy and other abnormalities in nitrogen metabolism observed in human disease.

## Intrahepatic, splanchnic, and systemic hemodynamic derangements in rats with $CCl_4$-induced cirrhosis

Rats with $CCl_4$-induced cirrhosis present both intrahepatic and extrahepatic circulatory abnormalities that closely reproduce those observed in human cirrhosis (39–42). The most relevant intrahepatic hemodynamic changes in these animals are increased hepatic vascular resistance resulting in portal hypertension and impaired sinusoidal exchange, which represents a major determinant of liver failure in cirrhosis. The chronic increase in portal pressure promotes the opening of porto-systemic collaterals that divert blood from the portal vein to the systemic circulation, thus bypassing the liver. The ensuing portal-systemic shunting is one of the major consequences of the portal hypertension syndrome. This alteration is fully present in the $CCl_4$-induced cirrhotic rat, although its incidence varies from animal to animal. Mean values of portal-systemic shunting range from 31 ± 13% in a group of rats studied by Vorobioff *et al.* (half had ascites) (26) to 68 ± 11% in a series of rats with cirrhosis and ascites studied in our laboratory (29). Portal hypertension in these animals is, to a great extent, maintained by a hyperdynamic portal venous inflow secondary to a generalized vasodilation within the splanchnic vascular bed (26,40).

Rats with $CCl_4$-induced cirrhosis exhibit an important systemic hemodynamic derangement characterized by arterial hypotension, low peripheral resistance, high cardiac index, and increased blood volume (26,43,44). Although the pathogenesis of these systemic hemodynamic alterations in cirrhosis is not completely understood, it is probably related to the presence of portal hypertension, splanchnic arteriolar vasodilation, and portal-systemic shunting. Early cross-perfusion experiments in $CCl_4$-in-

duced cirrhotic rats demonstrated the existence of one or more endogenous vasodilator factors that could account, at least in part, for the hyperkinetic condition of the cardio-circulatory system in these animals (45). Since then, many substances have been proposed as potential candidates, including prostacyclin, adenosine, bile acids, neurotensin, substance P, vasoactive intestinal peptide, secretin, gastrin, cholecystokinin, calcitonin gene-related peptide, and glucagon (46–50), but definitive proof of their involvement is still lacking. Although new systems, such as the endogenous cannabinoid anandamide (51,52), have been implicated in the pathogenesis of arterial vasodilation, the compound that has received the most attention is nitric oxide (NO). NO is a powerful vasodilator that is locally produced by the actions of NO synthase (NOS) which catalyzes the conversion of L-arginine into L-citrulline with NO as the gaseous byproduct (53 and reviewed in 54). In mammals, NOS exists in three different isoforms: neuronal (NOS1), inducible (NOS2) and endothelial constitutive (NOS3) (54). The original studies linking NO to the pathogenesis of arterial vasodilation in cirrhosis were obtained in rats with $CCl_4$-induced cirrhosis in the early 1990s (55). Further evidence indicating that NO participates in the pathogenesis of arterial vasodilation derives from studies showing that chronic administration of orally active NO synthesis inhibitors to cirrhotic rats with ascites at a dosage that normalizes the activity of NOS in the arterial wall is associated with normalization of systemic hemodynamic disturbances, suppression of vasoconstrictor systems, and marked improvement in sodium and water excretion (56,57 and reviewed in 58). Increased NO activity has also been reported as the mechanism underlying the impaired pressor responsiveness to vasoconstrictors frequently seen in decompensated cirrhosis (59,60). Indeed, NOS3 expression, NO production, and NO-dependent vasorelaxation have been found to be increased in arterial vessels from cirrhotic rats with ascites (61–63). A recent investigation also points to an overactivity of NOS3 as the potential mechanism for vascular remodeling occurring in large conductive vessels of $CCl_4$-cirrhotic rats (64). The increased activity of NO in the systemic vasculature of rats with experimental cirrhosis cannot be considered a generalized phenomenon, because NO activity may change from one vascular bed to another. For instance, NOS3 is increased in the splanchnic, systemic, and renal circulation but decreased in the intrahepatic circulation where it is believed to play a critical role in the pathogenesis of sinusoidal portal hypertension in cirrhosis (65,66).

## Renal dysfunction in rats with $CCl_4$-induced cirrhosis

The experimental model of $CCl_4$-induced cirrhosis in rats enables the execution of longitudinal and time-course studies. For this reason, this model has proven to be very useful in investigating the chronological relationship between hemodynamic derangements and the appearance of renal disorders in cirrhosis. This has been especially true for delineating the sequence of events leading to sodium and water retention and the formation of ascites in cirrhosis (29–32,35,67,68). According to these studies, the progression of portal hypertension in cirrhosis leads to a marked vasodilation in the splanchnic and systemic arterial vascular beds, resulting in decreased arterial blood pressure and the homeostatic activation of the endogenous vasoactive systems [the renin–angiotensin–aldosterone system (RAAS) and sympathetic nervous system (SNS), and antidiuretic hormone (ADH)]. In their attempt to return arterial pressure to normal levels, these endogenous vasoconstrictor systems induce marked renal sodium and water retention which constitutes a critical step for the accumulation of fluid in the peritoneal cavity (i.e. formation of ascites). Because glomerular filtration rate is unchanged in $CCl_4$-induced cirrhotic rats, the predominant mechanism of sodium and water retention in the kidneys of these animals is the presence of an increased tubular reabsorption. In the following pages there is a brief description of the neurohumoral systems and factors involved in the regulation of sodium and water renal handling and their role in the pathogenesis of renal dysfunction in experimental cirrhosis.

### Renin–angiotensin–aldosterone system

The RAAS is one of the most extensively investigated endogenous vasoactive systems. The RAAS is activated in most patients with cirrhosis, ascites, and marked sodium retention who also present hyperaldosteronism (69). The role of aldosterone in the pathogenesis of sodium retention in cirrhosis was first addressed in our laboratory by using the $CCl_4$-cirrhotic rat model (29). These animals showed a close chronological and quantitative relationship between the activation of the RAAS, as estimated by the urinary excretion of aldosterone ($U_{Ald}V$), and the onset of sodium retention (Fig. 17.2). Sodium excretion, sodium balance and $U_{Ald}V$ were measured daily throughout the cirrhosis-induction protocol. During the first weeks of the study and prior to sodium retention, rats treated with $CCl_4$ showed a similar $U_{Ald}V$ as that of the control animals. However, within the week during which sodium retention started in cirrhotic rats, $U_{Ald}V$ was significantly higher compared with control animals. $U_{Ald}V$ in cirrhotic rats particularly increased during the week that ascites was first detected, when sodium retention was more intense and sodium balance more positive (Fig. 17.2). To assess the effect of aldosterone inhibition on renal sodium retention, a group of cirrhotic rats received, from the beginning of the $CCl_4$ treatment, a daily subcutaneous injection of spironolactone. None of the

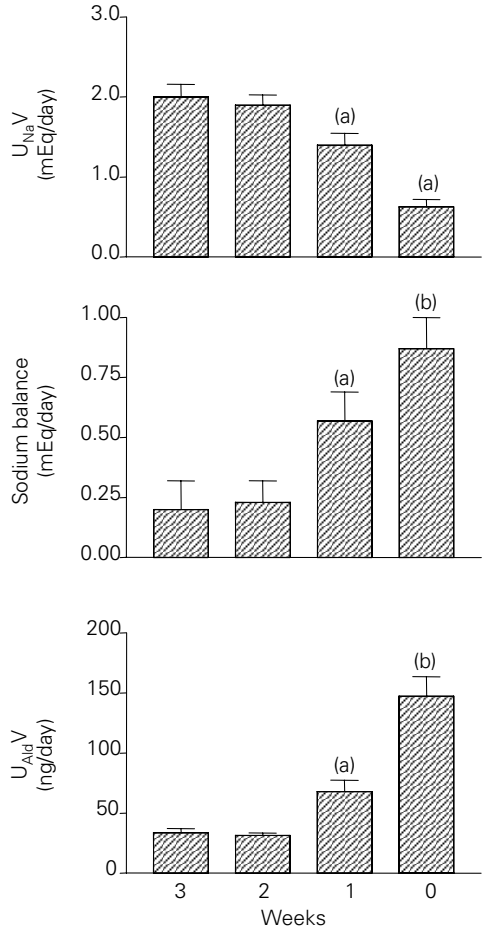


**Figure 17.2** Temporal relationship between urinary sodium excretion ($U_{Na}V$), sodium balance, and urinary excretion of aldosterone ($U_{Ald}V$) in rats with $CCl_4$-induced cirrhosis. Week 0 represents the week in which ascites was first detected. Weeks 3, 2, and 1 represent the three consecutive weeks before the appearance of ascites. (a) $P < 0.05$ and (b) $P < 0.01$ for weeks 3 and 2. Reprinted by permission of the publisher from Jiménez *et al.* (29).

cirrhotic animals treated with spironolactone developed ascites within 13 weeks of inhaling $CCl_4$. By contrast, all animals in which the tubular effect of aldosterone was not blocked developed ascites.

The mechanisms underlying the activation of the RAAS in cirrhosis are probably related to the systemic circulatory disturbances present in this disease. This contention was evaluated by assessing the temporal relationship between the onset of hyperaldosteronism and sodium retention and the decrease in arterial pressure in spontaneously hypertensive rats submitted to the $CCl_4$ program of cirrhosis induction (67). In these animals, the onset of hyperaldosteronism and sodium retention was chronologically related to a significant decrease in arterial pressure, with arterial hypertension disappearing in all animals when sodium retention became evident (67).

## Sympathetic nervous system

The SNS is another important mechanism involved in the regulation of renal function. Given that stimulation of renal nerves decreases renal blood flow and the glomerular filtration rate and promotes sodium reabsorption in the proximal tubule, loop of Henle, and distal nephron, the SNS is thought to play an important role in the pathogenesis of sodium retention and ascites formation in cirrhosis. In fact, rats with $CCl_4$-induced cirrhosis have increased circulating levels of norepinephrine when sodium retention is present (70), but not when the sodium balance is normal (71). In addition, cirrhotic rats with ascites submitted to acute bilateral surgical renal denervation exhibit a normal renal ability to excrete a sodium overload (72).

## Antidiuretic hormone

Patients with cirrhosis and ascites are unable to eliminate solute-free water in response to a water overload (73). These patients present dilutional hyponatremia and hypo-osmolality as a consequence of renal water retention. Although new topics of research, such as dysregulation of renal water channels (i.e. aquaporins) (74), are constantly emerging as relevant factors involved in the impairment of the renal diluting ability in cirrhosis**,** the presence of a non-osmotic hypersecretion of ADH is, by far, considered to be the key event in this renal disorder (73). Most of the data supporting this contention have been obtained in the experimental model of $CCl_4$-induced cirrhosis in rats.

Linas *et al.* (75) were the first to demonstrate that cirrhotic rats with ascites exhibit a decreased ability to excrete free water associated with elevated plasma levels of ADH. These authors also showed that this impairment was not present in Brattleboro rats (rats with congenital diabetes insipidus) induced to cirrhosis, suggesting that ADH is a key factor in the pathogenesis of renal water retention in cirrhosis. Subsequently, a longitudinal study performed by Camps *et al.* (35) established the existence of a close chronological relationship between the hypersecretion of ADH and the impairment of solute-free water excretion in rats with $CCl_4$-induced cirrhosis. A conclusive proof of the role of ADH in the pathogenesis of this renal disorder in cirrhosis was obtained by showing that renal water metabolism in rats with $CCl_4$-induced cirrhosis, ascites, and impaired free water excretion was normalized following the administration of a $V_2$-ADH receptor antagonist (76). Unfortunately, this selective $V_2$-ADH analog disclosed agonistic activity in humans (77).

The initial attempts to develop aquaretic drugs (i.e. drugs that increase solute-free water excretion) rapidly evolved into the development of two families of drugs: the nonpeptide $V_2$-ADH receptor antagonists and the

κ-opioid agonists. The first class of drugs inhibits the tubular effect of ADH whereas the second reduces ADH secretion by the neurohypophysis. Some of these novel drugs have been tested in the $CCl_4$-experimental model before their use in clinical trials. The nonpeptide $V_2$-ADH receptor antagonist, OPC-31260, was found to increase solute-free water excretion in cirrhotic rats with ascites and dilutional hyponatremia (78). In these animals, a more prolonged aquaretic effect was obtained with another nonpeptide $V_2$ receptor antagonist, SR-121463A (79). Moreover, a dual nonpeptide $V_{1a}/V_2$-ADH antagonist (conivaptan) of recent development has proven to be therapeutically useful for the treatment of water retention in rats with cirrhosis and ascites without affecting arterial pressure (80). On the other hand, the acute subcutaneous administration of the κ-opioid agonist, RU-51599 (niravoline), to rats with cirrhosis, ascites, and impaired solute-free water excretion resulted in a significant decrease in ADH plasma levels and the normalization of renal water metabolism, with the aquaretic effect of this compound being comparable to that of nonpeptide $V_2$-ADH antagonists (78,81) (Fig. 17.3). Taken together, these findings raise the possibility that within the next few years patients with decompensated cirrhosis, ascites, and dilutional hyponatremia will be managed with diuretics in combination with a class of such aquaretic drugs (82).

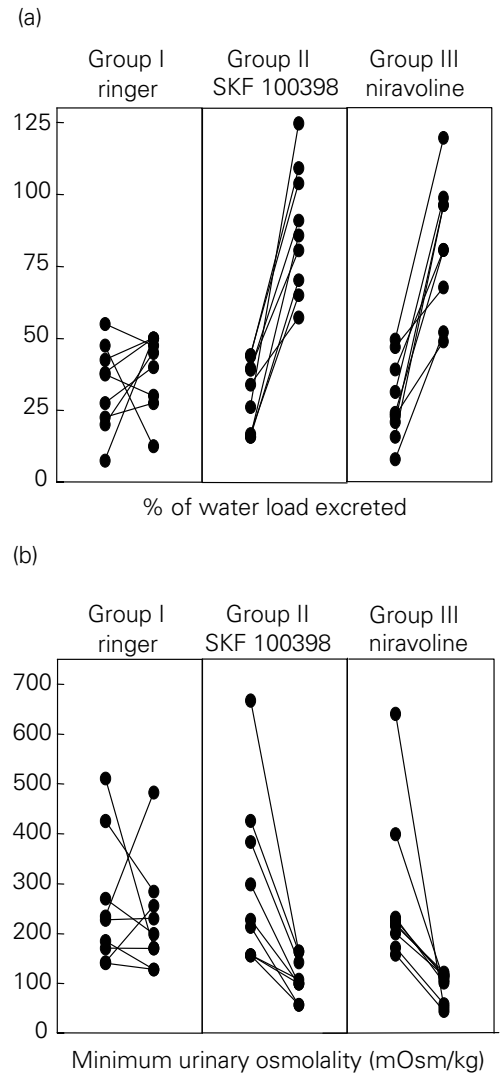


**Figure 17.3** Individual values of percentage of the water load excreted (a) and minimum urinary osmolality (b) before and following a subcutaneous injection of either Ringer´s solution, $d(CH_2)_5Tyr(Et)$-VAVP (SKF 100398, $V_2$-ADH receptor antagonist), or RU-51599 (niravoline, κ-opioid agonist) to rats with cirrhosis, ascites, and impaired water excretion. Reprinted by permission of the publisher from Bosch-Marcé *et al.* (81).

## Endogenous natriuretic systems

Endogenous natriuretic peptides (ENP) are a family of at least four different substances that inhibit renal tubular sodium reabsorption: atrial natriuretic peptide (ANP) (83), brain natriuretic peptide (BNP) (84), C-type natriuretic peptide (CNP) (85), and urodilatin (86). ANP and BNP are mainly synthesized in the heart, CNP is synthesized in the central nervous system and in vascular endothelium, whereas urodilatin is found exclusively in the kidney (83–86). All these peptides decrease mean arterial pressure and all, except CNP, promote diuresis and natriuresis in healthy subjects and euvolemic animals.

Early investigations in patients with cirrhosis revealed the existence of normal or increased plasma levels of immunoreactive ANP and BNP despite having marked sodium retention (87–91). This paradoxical observation has been extensively investigated in the experimental model of $CCl_4$-induced cirrhosis in rats. By giving pharmacological doses of ANP to rats with $CCl_4$-induced cirrhosis, Olivera *et al.* (92) and López *et al.* (93) demonstrated renal resistance to the diuretic and natriuretic effects of ANP in these animals. More recently, Angeli *et al.* reported that the administration of a selective ENP receptor antagonist to rats with $CCl_4$-induced cirrhosis and ascites leads to a marked impairment in renal plasma flow, glomerular filtration rate, urine volume, and urinary excretion of sodium (94). These renal effects were associated with an activation of the RAAS without changes in systemic hemodynamics (94). These results strongly suggest that ENP counteract the antinatriuretic and renal vasoconstrictor effects of the endogenous vasoactive systems in cirrhosis.

## Prostaglandins

By antagonizing the vascular and tubular effects of the endogenous vasoconstrictor systems, prostaglandins (PGs) are involved in the maintenance of renal perfusion and the modulation of sodium and ADH-mediated water reabsorption (95). Cirrhosis with ascites probably represents the medical condition in which the compensatory role

of PGs in the maintenance of renal function is most evident (95–97). In fact, the administration of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, ibuprofen, meclofenamate, and naproxen, to patients with cirrhosis and ascites produces an acute and reversible impairment in renal hemodynamics, sodium excretion, and solute-free water clearance (96,97). PG inhibition with NSAIDs also dramatically decreases the natriuretic response to loop diuretics (i.e. furosemide and spironolactone) in patients with cirrhosis and ascites (98). The critical role of PGs in the maintenance of renal function can also be appreciated in rats with cirrhosis and ascites. In cirrhotic rats, there is a close temporal relationship between sodium retention, RAAS activation, and the urinary excretion of the stable prostacyclin metabolite, 6-keto-$PGF_{1\alpha}$ (68). Moreover, administration of NSAIDs to rats with $CCl_4$-induced cirrhosis is associated with a significant impairment in renal perfusion and glomerular filtration rate (99,100). The clinical consequence of these findings is that cirrhotic patients cannot be treated with NSAIDs because of the high risk of developing worsening ascites, dilutional hyponatremia, and renal failure.

Cyclooxygenase (COX) is the key enzyme in the formation of PGs from arachidonate and is the major therapeutic target for NSAIDs (101). Two isoforms of COX, designated COX-1 and COX-2, have been identified (reviewed in 102). Conventional NSAIDs inhibit both COX-1 and COX-2 and this feature accounts not only for the therapeutic actions but also for the unwanted side-effects of these drugs. The discovery of the two isoforms of COX has led to the development of novel compounds that, unlike conventional NSAIDs, selectively inhibit COX-2 (103). These novel compounds have a clinical efficacy comparable to that of conventional NSAIDs because they effectively inhibit inflammation but display less unwanted side-effects because they spare physiological PG production (103). Therefore, these novel anti-inflammatory compounds are of great interest in conditions such as cirrhosis with ascites, in which renal function is critically dependent on PGs. Recent studies in our laboratory performed in the $CCl_4$ experimental model of cirrhosis indicate that despite having increased renal COX-2 expression, renal function in cirrhotic rats appears to be mainly dependent on PGs derived from COX-1 (104,105) (Fig. 17.4). Therefore, since selective COX-2 inhibitors such as celecoxib did not compromise renal function in these animals, these novel compounds are likely to be the anti-inflammatory drugs of choice not only in patients with chronic liver diseases but also in circumstances that are more susceptible to NSAID-induced renal failure, such as congestive heart failure and nephrotic syndrome.

## Ascites formation in rats with $CCl_4$-induced cirrhosis

Ascites is the accumulation of fluid into the peritoneal cavity and is one of the fundamental signs of decompensated liver disease. Rats submitted to the $CCl_4$ inhalation protocol develop ascites when the disease is quite advanced, liver failure is prominent, and the hemodynamic abnormalities are well established (24,29,32,39). Rats usually exhibit the fibrotic precirrhotic stage and develop portal hypertension within 4–6 weeks of $CCl_4$ treatment, although systemic hemodynamic disturbances are not fully evident until cirrhosis is present, commonly within 8–10 weeks of $CCl_4$ treatment (24,29,32,39). After 10–12 weeks of hepatotoxin administration, rats start to retain sodium and accumulate fluid in the peritoneal cavity.

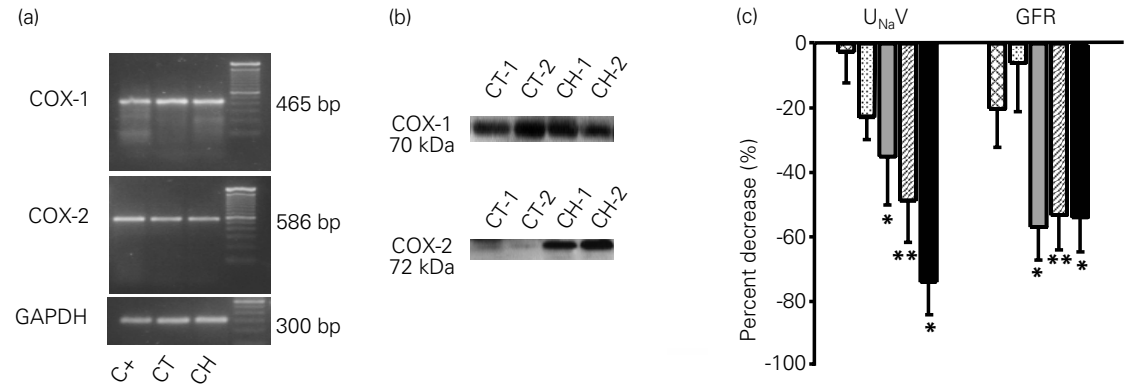


**Figure 17.4** Analysis of mRNA (a) and protein (b) expression of COX-1 and COX-2 in renal tissue isolated from control (CT) and cirrhotic rats with ascites (CH). (c) Effects of COX inhibition on renal function in rats with cirrhosis and ascites. Urinary sodium excretion ($U_{Na}V$) and glomerular filtration rate (GFR) were determined before and 60 min after administration of selective COX-2 inhibitors [SC-236 (crossed bars) and celecoxib (dotted bars)], nonselective COX inhibitors [6-MNA (gray bars) and ketorolac (dashed bars)], and a selective COX-1 inhibitor [SC-560 (solid bars)] to cirrhotic rats. *$P < 0.05$ and **$P < 0.01$ vs. baseline conditions. Reprinted by permission of the publisher from Bosch-Marcé *et al*. (104) and López-Parra *et al*. (105).

Ascites in cirrhosis has low total protein and lipid content. This feature is the parameter most widely used to distinguish transudative ascites resulting from portal hypertension (i.e. cirrhosis) from exudates such as those caused by neoplastic peritoneal lesions. Ascites caused by cirrhosis tends to have a high serum-to-ascites albumin gradient, or, in other words, low albumin concentration in the ascitic fluid. Free fatty acids, triglycerides, phospholipids, and cholesterol are also consistently present in the ascites of cirrhotic animals (106). Cytological examination of ascites reveals a stable population of macrophages (107). Low cell counts of polymorphonuclear leukocytes, usually < 150 cells/ml, are also present in cirrhotic ascitic fluid (108). These inflammatory cells produce relevant amounts of arachidonic acid-derived products (leukotriene $B_4$, lipoxin $A_4$, thromboxane $A_2$, $PGE_2$, and prostacyclin) and several proinflammatory cytokines such as interleukin-1 and -6 and tumor necrosis factor-α (109–111). Recent findings by Morales-Ruiz *et al.* (112) indicate that peritoneal macrophages of cirrhotic rats are also an important source of NO, which plays a major role in the host defense response against bacterial infection in these animals. The suitability of $CCl_4$-induced cirrhotic rats as an experimental model to assess the mechanisms underlying the pathogenesis of bacterial translocation and spontaneous bacterial peritonitis is addressed later in this book (see Chapter 33). In any event, since most of the products and mediators released by cells present in the ascitic fluid carry potent biological properties (113), ascites should no longer be considered as just an inert fluid and the consequence of renal sodium and water retention.

## Concluding remarks

As reviewed in this chapter, experimental models of cirrhosis and, in particular, $CCl_4$-induced cirrhotic rats are a very useful tool to investigate the pathophysiology of hemodynamic disorders, renal dysfunction, and ascites formation seen in human cirrhosis. Specifically, rats induced to cirrhosis by inhalation of $CCl_4$ exhibit most of the features observed in human cirrhosis, including widespread hepatic fibrosis and nodule formation accompanied by marked intrahepatic, splanchnic, and systemic hemodynamic alterations, abnormal vascular reactivity, hypervolemia, renal sodium and water retention, and accumulation of ascites in the peritoneal cavity. In addition, $CCl_4$-induced cirrhotic rats display a hyperactivity of the endogenous vasoactive systems (RAAS, SNS, and ADH) and a marked imbalance in the biosynthesis of endogenous natriuretic substances (ANP and BNP) and COX-derived products (PGs).

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# Chapter 18
# Medical Treatment of Ascites in Cirrhosis

Paolo Angeli and Angelo Gatta

Ascites, or the accumulation of fluid in the peritoneal cavity, is a common complication of end-stage cirrhosis, which accounts for over 75% of cases presenting with ascites. A diagnostic paracentesis is essential in all patients investigated for ascites prior to any therapy to exclude other causes of ascites and to exclude the possibility of spontaneous bacterial peritonitis (1). The ascitic fluid should be examined by microscopy, as well as for the determination of albumin or protein content, and cultured by direct inoculation into blood culture bottles. An ascitic neutrophil count of ≥ 250 PMN/mm$^3$ is diagnostic of spontaneous bacterial peritonitis (2). In patients with suspected malignant or pancreatic ascites, ascites cytology or amylase should be examined. The use of ascitic protein in the differential diagnosis and the consequent division of ascites into transudates (ascites protein < 25 g/l) and exudates (ascites protein ≥ 25 g/l) often leads to a misinterpretation of the causes of ascites. In fact, a low ascites protein is rare in patients with cardiac ascites (3), while it occurs in 30% of patients with tuberculous peritonitis and 20% of patients with malignancy (4). The difference between serum albumin and ascitic fluid albumin concentration (named serum–ascites albumin gradient) is more sensitive and specific than ascites protein to distinguish ascites due to portal hypertension (gradient ≥ 11 g/l) from ascites secondary to other pathogenic factors (gradient < 11 g/l).

Ascites is associated with a worsening of the prognosis in patients with cirrhosis. The probability of survival at 1 and 5 years after the appearance of ascites has been estimated at 50% and 20%, respectively (5). Even if the negative prognostic value of ascites in patients with cirrhosis is without doubt an epiphenomenon of the progression of liver disease (6), ascites *per se* may be the cause of certain complications in these patients such as spontaneous bacterial peritonitis (7). In addition, the discomfort and other unwanted side-effects (8) associated with the accumulation of ascitic fluid may strongly affect the quality of life in patients with chronic liver disease. These observations represent the rationale for the treatment of ascites in these patients.

## Bed rest and low sodium diet

The aim of medical treatment for ascites in patients with cirrhosis is to mobilize the intra-abdominal fluid by inducing a negative sodium balance. The achievement of a negative sodium balance can be pursued by reducing the sodium intake and/or enhancing renal sodium excretion by means of diuretics. In approximately 10–20% of cirrhotics with ascites, this can be obtained simply with bed rest and reducing dietary sodium intake (9–12). Spontaneous mobilization of ascites (spontaneous diuresis) is more likely to occur in patients without any previous history of ascites (10). Several lines of evidence suggest that in patients with cirrhosis and ascites, the upright position is associated with a striking activation of antinatriuretic factors such as the renin–angiotensin–aldosterone system and the sympathetic nervous system (13). As a result, baseline urinary sodium excretion as well as the natriuretic response to diuretics may be reduced by the upright position (14). Bed rest is probably of no real benefit in patients with preserved renal function; however, it may be beneficial in those with impaired renal function and/or a poor response to diuretics.

Low-sodium diet is used in the attempt to revert or alleviate positive sodium balance and improve diuretic drug effectiveness. It is widely used and almost universally recommended. However, such a strong agreement is not backed by the results of controlled clinical trials. Five studies have compared the efficacy of different dietary regimes (13,15–18). When dietary sodium restriction (22 mmol/day) was compared with unrestricted diet, dietary sodium restriction was not associated with a significant benefit of response rate to medical treatment, diuretic drug dosage, and costs (hospitalization time) (16–18), although the time for complete disappearance of ascites was significantly shorter (17,18). No statistically significant differences in survival between patients receiving restricted or unrestricted diet were found, although the survival of patients with previous gastrointestinal bleeding was better in the low-sodium diet group (18). In another study, the use of sodium-restricted diet

(22 mmol/day) was associated with a higher incidence of diuretic-induced renal failure and hyponatremia compared with an unrestricted diet (14). Finally, a controlled and slightly reduced sodium intake (120 mmol/day) has proved to be equivalent to a low-sodium diet (50 mmol/day) in terms of rate of response, need for diuretics, and even spontaneous diuresis (13).

The main criticism of these studies is that they probably suffer from noncompliance with the diet, although compliance was monitored by measurement of urinary sodium excretion (13). As a result, it is the current opinion that in cirrhotic patients who need diuretics to achieve a negative sodium balance, dietary salt intake should be moderately restricted unless there is a spontaneous improvement of the renal ability to excrete sodium. Recently, the International Ascites Club has suggested that dietary salt should be moderately restricted to 5.2 g/day (90 mmol) and should be continued unless there is a normalization of the renal ability to excrete sodium (19). Patient education is crucial for the maintenance of dietary sodium restriction, particularly following hospital discharge. A more severe reduction in dietary sodium content is considered unnecessary and even potentially detrimental (15) in the conventional therapy of ascites. In particular, severe sodium-restricted diets are unpalatable, leading to poor patient compliance and impaired nutritional status. While severe sodium restriction may not be advisable in most patients with cirrhosis and ascites (20,21), it may be considered in selected cases. However, considering the negative prognostic value of malnutrition (22), an adequate caloric diet should have priority over a marked sodium restriction in undernourished patients, even in those cases of diuretic-resistant ascites. The use of salt substitutes that contain potassium is contraindicated in the management in patients with cirrhosis, as they may induced hyperkalemia.

The medical treatment of ascites in cirrhotic patients should include both salt restriction and diuretics simultaneously, since a positive response to diet alone is unpredictable, rare, and in any case too slow.

## Diuretics

Loop diuretics, in particular furosemide, and potassium-sparing diuretics, in particular aldosterone antagonists, are the drugs most commonly used in the treatment of ascites in patients with cirrhosis (23,24). Other diuretics such as thiazide diuretics and metolazone, which inhibit sodium reabsorption in the cortical diluting segment or actetazolamide, an inhibitor of carbonic anhydrase activity in the renal proximal tubule, are sometimes considered only for treatment of ascites resistant to high doses of both an aldosterone antagonist and a loop diuretic (25). All diuretics inhibit the absorption of $Na^+$, $Cl^-$ and water by their interaction with luminal uptake mechanisms. Diuretics are kidney specific, not because they interfere with proteins specific for the kidney but because, with the only exception of aldosterone antagonists, they are secreted into the lumen of the tubule. This secretion, together with the water absorption along the tubule, contributes to the high free concentrations of diuretics in the tubule lumen. Furosemide and thiazides are taken up across the basolateral membrane by an anion exchanger while amiloride and triamterene are taken up by a cation exchanger. The exit step into the lumen is less clear but may involve ATP-driven pumps (26).

### Loop diuretics

Loop diuretics are organic acids that reach the renal tubular lumen by being actively secreted via the organic acid transport pathway of the straight segment of the proximal tubule (27). Once in the luminal compartment, loop diuretics are carried to the thick ascending limb of the loop of Henle where their primary action is to block the $Na^+$-$K^+$-$2Cl^-$ transporter across the luminal membrane of the epithelial cells (28,29). This transporter usually carries one $Na^+$ ion, one $K^+$ ion, and two $Cl^-$ ions, but net NaCl transport occurs because the bulk of $K^+$ recycles across the luminal membrane through $K^+$ channels. The $K^+$ recycling across the luminal membrane contributes to a transepithelial voltage along the thick ascending limb of the loop of Henle that is oriented with the lumen positive. This voltage drives reabsorption of one additional $Na^+$ ion through the paracellular pathway for each $Na^+$ ion absorbed transcellularly. The $Na^+$-$K^+$-$2Cl^-$ pathway appears to possess four ion-binding sites. Three of the sites must be occupied with $Na^+$, $K^+$, and $Cl^-$ before a second $Cl^-$ ion can bind to the carrier (30). Loop diuretics, such as furosemide and bumetanide, block the action of the $Na^+$-$K^+$-$2Cl^-$ pathway directly, competing with $Cl^-$ for the binding at the second anion site of the transport protein (31). In addition, torasemide may act through some metabolites by interfering with the $Cl^-$ channels in the basolateral membrane, thus preventing the passive exit of $Cl^-$ ions from the cells (32). Finally, observations that treatment with several different nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the natriuretic response to furosemide lead to speculation that renal prostaglandins might be involved in the tubular effects of loop diuretics (33). Access of the loop diuretic to the organic acid secretory site should also be determined mainly by the amount of blood flowing to the proximal nephron (34). Changes in the renal blood flow might then influence the amount of the drug reaching the tubular lumen. The avidity of the transport system for the loop diuretics, however, appears to be so great that considerable decrements in renal blood flow must occur before access to the transport sites becomes limited (35). The dose–response curves for loop diuretics relating the diuretic excretion rate to sodium

excretion follow a sigmoidal pattern. When a threshold level is exceeded, the natriuretic response increases in parallel with the rate of diuretic excretion until a maximum natriuresis is achieved. The plateau of the curve implies that there is a point at which additional drug becomes superfluous. Once the maximum is reached, a further increase in the natriuretic effect could be gained by giving multiple doses rather than a larger single dose. In healthy subjects, maximum natriuresis occurs with 40 mg of furosemide or 1 mg of bumetanide (36).

In normal subjects, all loop diuretics are absorbed quickly, with peak concentration attained within 0.5–2 h. The bioavailability of bumetanide and torasemide is approximately 80%, while that of furosemide ranges from 40% to 60%. The elimination half-life of loop diuretics is approximately 1–2 h. The onset of effect occurs within 30–60 min after an oral administration, peaking at 30–120 min and returning to baseline values within another 2–3 h. The pharmacokinetics of loop diuretics in patients with cirrhosis resembles those in normal subjects. A decrease in protein binding of loop diuretics associated with consequent slight effects on volume distribution and on half-life has been observed in cirrhotic patients with hypoalbuminemia when compared with healthy subjects (37). On the other hand, the elimination half-life of torasemide, which is the only loop diuretic to be extensively metabolized in the liver, was found to be 4.8 h in cirrhotic patients (32).

Two major groups of potassium-sparing diuretics or collecting duct diuretic drugs are available. Sodium-channel blockers, namely amiloride and triamterene, act from the luminal surface to inhibit $Na^+$ uptake by principal epithelial cells of the collecting duct. The actual $Na^+$ channel, also called epithelial $Na^+$ channel (ENaC), is probably made up of four subunits and its function is closely controlled by aldosterone. The ENaC is blocked with high affinity by amiloride and triamterene and is downregulated by aldosterone antagonists (38). As a result of their action on $Na^+$ reabsorption, the magnitude of the transepithelial voltage, oriented in the lumen negative direction, declines and $K^+$ secretion is inhibited (28,39,40). Amiloride and triamterene are both basic compounds and therefore are secreted into the urine by the organic base pathway. Limited data are available on the pharmacokinetics of these drugs. Approximately 30–90% of the ingested drug is absorbed from the gastrointestinal tract. The elimination half-life of the drug ranges from 6 to 10 h. Most of the amiloride is excreted unchanged into the urine; thus, a prolonged half-life may be expected in azotemic patients (41,42). Triamterene is quickly absorbed from the gastrointestinal tract. The bioavailability after an oral dose ranges from 30 to 70%. Approximately 80% of the absorbed drug is converted in the liver into a major active metabolite. The elimination half-life of the parent drug and its principal metabolite ranges from 3.3 to 5.5 h and from 2.1 to 5.3 h, respectively. In patients with liver disease the half-life of the native drug may be prolonged up to 13 h without any change in the excretion rate of the major active metabolite. By contrast, in azotemic patients the elimination half-life of the active metabolite is markedly increased without any change in the clearance of the parent drug. Thus, triamterene seems very complex to use in patients with liver or renal failure (43,44).

The second class of collecting duct diuretics is represented by the competitive aldosterone antagonists, which prevent aldosterone from stimulating $Na^+$ reabsorption and $K^+$ secretion (45). Aldosterone stimulates renal $Na^+$ reabsorption by increasing both the permeability of the luminal membrane of principal cells to $Na^+$ and the activity of the $Na^+/K^+$ ATPase pump in the basolateral membrane (46,47). The action of aldosterone involves interaction with a cytosolic receptor located in the blood side of the cell followed by a secondary interaction with a receptor located in the nucleus. Subsequently, RNA-mediated protein synthesis produces an aldosterone-induced protein, which is responsible for the changes in electrolyte transport. Spironolactone and other aldosterone antagonists competitively inhibit the binding of aldosterone to the specific receptor protein in the cytoplasm, thus blocking the synthesis of the aldosterone-induced protein (36,48). The relatively long half-life of this protein accounts for the lag of 2–4 days between the start and the end of the administration of these drugs and the onset or the disappearance of their natriuretic effect (49). Spironolactone is rapidly and almost completely absorbed from the gastrointestinal tract. It undergoes extensive metabolism in man, leading to numerous biologically active compounds. The antialdosteronic activity of this drug has been traditionally attributed to canrenone, which was thought to be the major spironolactone metabolite. However, recent studies have shown that it accounts for only 10–25% of the antimineralocorticoid activity of the drug and that other metabolites, in particular 7α-thiomethylspironolactone, appear to be quantitatively the major metabolites in serum (50). It should also be pointed out that, since spironolactone and other aldosterone antagonists act in the basolateral side of the tubular cells, their activity does not depend on the filtration or tubular secretion of the drugs but on their plasma levels. Spironolactone metabolites are tightly bound to plasma proteins, from which they are released slowly to the target organs (i.e. the kidney). The half-life of canrenone in healthy subjects has been estimated to range between 10 and 35 h after single or multiple doses of the drug, respectively. Controversial data exist on the pharmacokinetics of spironolactone in cirrhotic patients. Normal (51) or impaired (52) metabolism and excretion of spironolactone and its metabolites has been reported. Once daily or alternate day administration of aldosterone antagonists is advised in clinical practice.

## Diuretic treatment of uncomplicated ascites

Ascites may be complicated by several conditions, such as spontaneous bacterial peritonitis and hepatorenal syndrome. As a consequence, uncomplicated ascites is defined by the existence of a normal renal function and an ascitic neutrophil count of < 250 $mm^3$. The use of diuretics in uncomplicated ascites is considered the treatment of choice for patients with grade 2 or moderate ascites which is characterized by moderate symmetrical distension of abdomen. Grade 1 or mild ascites, which is detectable by ultrasound examination, should not be treated, while diuretics should follow paracentesis in the treatment of grade 3 or large ascites (Table 18.1). The following observations will mainly deal with the management of uncomplicated ascites (19).

A rational approach to the diuretic therapy of ascites in patients with cirrhosis must take into account the marked alterations of renal sodium handling which occur in this clinical condition. In healthy subjects more than 99% of the sodium filtered each day is reabsorbed along the renal tubule. Almost two-thirds of filtered sodium are reabsorbed in the proximal tubule, 25–40% along the loop of Henle, 5–10% along the distal convoluted tubule, and only 2–5% along the collecting duct. These data coupled with the lack of activation of the renin–angiotensin–aldosterone system might explain the higher natriuretic potency of loop diuretics compared with distal diuretics under normal conditions. The increase of sodium excretion following the administration of a high dose of furosemide or spironolactone may account for up to 30% and 2% of the filtered sodium, respectively (28). Renal sodium retention is a well-known feature in patients with cirrhosis and ascites. Considerable evidence demonstrates that it is attributable primarily to increased tubular sodium reabsorption rather than to a decrease of filtered sodium load, but the nephron sites which are involved still represent a controversial point (53). Studies which have evaluated renal sodium handling by clearance techniques during maximum water diuresis have emphasized alternatively proximal, diluting, or distal tubular sites (54–56). A major role of a tubular site beyond the proximal tubule has been suggested in studies using phosphate clearance (57,58). By means of lithium clearance, an index of the fluid delivery to the distal tubule (59), studies of some authors (62) and our studies too (60,61) have shown an increased sodium reabsorption in the proximal tubule in cirrhotic patients with ascites compared with control subjects (60–62). Similar findings were obtained in cirrhotic rats with ascites by means of lithium clearance (63). Distal sodium reabsorption, when evaluated as a percent of the distal delivery of sodium, was also higher in the cirrhotic patients. The proximal tubule seems to be quantitatively the primary site in renal sodium retention in cirrhosis with ascites, while the distal tubule reabsorbs almost all the sodium delivered, defining the final level of sodium into the urine (61). The mediators of the enhanced proximal tubular reabsorption of sodium have not been elucidated completely, while the increased reabsorption of sodium along the distal tubule is related in most cirrhotic patients with ascites to hyperaldosteronism (53). In this scenario, it is not surprising to observe that the administration of aldosterone antagonist to non-azotemic cirrhotics with ascites is followed by a good natriuretic response in most patients, while the administration of standard doses of a loop diuretic is less effective. In the only randomized study comparing spironolactone (150–300 mg/day) with furosemide (80–160 mg/day) in non-azotemic cirrhotic patients with ascites, spironolactone was significantly more effective than furosemide (64). Alternative explanations may involve alterations of the pharmacokinetics of furosemide in these patients and an altered expression or activity of the sodium-potassium-chloride transporter in the loop of Henle (29). The bioavailability of furosemide has been reported to be normal in non-azotemic cirrhotics with ascites (37,65), while alterations in the distribution volume of the drug due to the diffusion of the drug into ascites can account for only 8% of the administered dose (66). Finally, although there is a study showing an impaired tubular secretion of furosemide into the urine (67), other published reports found no alterations in the delivery of the drug to its site of action (68–72). So far no data exist on possible changes in the expression or in the activity of the sodium-potassium-chloride transporter in the loop of Henle in patients with cirrhosis (29). Thus, if a reduced delivery of furosemide into the urine may play a role in the resistance to its natriuretic effect in some patients, the most likely mechanism of resistance to furosemide is pharmacodynamic in nature. Likewise, a pharmacodynamic explanation appears to be reasonable in justifying the higher effectiveness of aldosterone

**Table 18.1** Treatments of uncomplicated ascites proposed by the International Ascites Club.

| Grades of ascites | Treatments |
|---|---|
| Grade 1 or mild ascites which is only detectable by ultrasound examination | No treatment |
| Grade 2 or moderate ascites which is manifest by moderate symmetrical distension of abdomen | Low-sodium diet and diuretics |
| Grade 3 or large ascites with marked abdominal distension | Total paracentesis plus low-sodium diet and diuretics |

antagonists compared with other collecting duct diuretics in the treatment of ascites in non-azotemic cirrhotic patients. The only randomized study comparing potassium canrenoate (150–500 mg/day) with amiloride (20–60 mg/day) showed that potassium canrenoate was significantly more effective than amiloride in these patients (73).

The most rational treatment of cirrhotic patients with ascites appears to be the administration of an aldosterone antagonist. A stepwise sequential therapy with increasing oral doses of an aldosterone antagonist (up to 400 mg/day) may be effective in mobilizing ascites in 60–80% of non-azotemic cirrhotic patients with ascites who do not respond to bed rest and dietary sodium restriction (11,12,74). The effective dosage of aldosterone antagonists depends on plasma aldosterone levels (75). Patients with moderately increased plasma levels require low doses of these drugs (100–150 mg/day), whereas patients with marked hyperaldosteronism may require as much as 200–400 mg/day. A further increase of the dosage up to 500–600 mg/day is of limited usefulness (11,12). Spironolactone should be given once per day with food in order to facilitate its intestinal absorption. Since transtubular potassium gradient (TTKG = urine potassium · plasma potassium$^{-1}$/urine osmolality · plasma osmolality$^{-1}$) is a good indicator of aldosterone bioactivity, it may be used as a guide to the proper use of aldosterone antagonists. A value of TTKG < 3.0 is indicative of a complete blockade of aldosterone activity (76). Due to pharmacokinetic and pharmacodynamic characteristics, the effect of aldosterone antagonists is not observed prior to 48 h from the start of the treatment. As a consequence, the dosage of these drugs may be increased every 3–5 days. Amiloride may represent an alternative to aldosterone antagonists in patients with little or no activation of the renin–angiotensin–aldosterone system (73). If no natriuresis occurs with the maximum dosage of an aldosterone antagonist, furosemide or another loop diuretic should be added at increasing oral doses in a stepwise fashion up to 160 mg/day (or equivalent doses of another loop diuretic). Higher doses are not justified by any changes in loop diuretics disposition in cirrhotic subjects and thus are not advisable. Although no studies have addressed the question of whether loop diuretics should be given once daily or more frequently, a good strategy involves administering the effective individual dose twice daily. Pharmacokinetic data and daily dosages of the diuretics which are commonly prescribed in cirrhotic patients with ascites are reported in Table 18.2.

Large-volume paracentesis can be considered today the treatment of choice of tense ascites, thus reducing the number of patients in which a rapid increase of diuresis by means of diuretics is necessary. Nevertheless, the onset of diuresis following a stepwise medical treatment of ascites may require too much time. Even in the absence of controlled clinical trials, a therapeutic regimen currently used in many centers for the treatment of ascites in cirrhotic patients involves combining *ab initio* aldosterone antagonist and furosemide (19,20,23). A therapeutic schedule begins with the administration of 40 mg/day of furosemide and 100–200 mg/day of an aldosterone antagonist. If there is no response within 4–5 days of treatment, the next step is to increase the dosages to 80 mg/day of furosemide and 200–300 mg/day of aldoster-

**Table 18.2** Pharmacokinetic data and daily dosages of diuretics commonly prescribed in cirrhotic patients with ascites.

| Diuretic drugs | Usual daily dosage, mg | Percentage of intestinal absorption | Elimination half-life, h |
|---|---|---|---|
| Proximal diuretics | | | |
| Acetazolamide | 250–1000 | Not available | 13† |
| Loop diuretics | | | |
| Furosemide | 20–160 | 40–100 | 1.4 |
| Bumetanide | 0.5–10 | 95 | 2.3 |
| Ethacrynic acid | 50–400 | Close to 100† | 0.5–1† |
| Torasemide | 10–40 | 80–90 | 4.8 |
| Distal convoluted tubule diuretics | | | |
| Hydrochlorothiazide | 25–100 | 65–74† | 5–15† |
| Metolazone | 2.5–10 | 64† | 48† |
| Collecting duct diuretics | | | |
| Group 1 (antialdosteronic drugs) | | | |
| Spironolactone | 100–400 | 70† | 126* |
| Potassium canrenoate | 100–400 | Close to 100† | 57.8 |
| Canrenone | 100–400 | Close to 100† | 57.8 |
| Group 2 (others) | | | |
| Amiloride | 5–20 | 30–90† | 6–10† |
| Triamterene | 50–300 | 30–70† | 13* |

†Data obtained only in healthy subjects; *data relative to the parent drug.

one antagonist, eventually peaking at 160 mg/day and 400 mg/day, respectively (19,20,23). The simultaneous administration of an aldosterone antagonist and a loop diuretic may offer three advantages. First, it leads to an earlier onset of diuresis. Second, it may reduce the incidence of hyperkalemia that is frequently observed when distal diuretics are given alone. Third, it may increase the efficacy of aldosterone antagonist by increasing the delivery of sodium to the distal tubule (11). In a Spanish multicenter prospective observational study, the most frequent diuretic schedule on admission to hospital was the combination of spironolactone and furosemide at a dose of 100 mg and 40 mg, respectively (77). Nevertheless, it has been recently observed that spironolactone alone requires less dose adjustment than spironolactone associated with furosemide (Fig. 18.1). As a consequence, it would be more suitable for starting the treatment of ascites on an outpatient basis (78).

The mobilization of ascites in either sequential or combined diuretic therapy should be monitored by daily weighing of the patient. An insufficient response to therapy is defined by a weight loss of < 1 kg in the first week or 2 kg every week thereafter until ascites is adequately controlled. The safe upper limit of the rate of weight loss is contentious, but most experts agree that the rate of weight loss should not exceed 500 g per day in patients without peripheral edema or 1 kg per day when edema is present (19,79). Following mobilization of ascites obtained either by means of diuretic therapy or paracentesis, diuretics should be adjusted to maintain patients with minimal or no ascites, thus avoiding diuretic-induced complications. Many patients continue to require a dietary sodium restriction and combined diuretic therapy in order to avoid the reaccumulation of ascites. Other patients, however, can be maintained without ascites with a moderate dietary sodium restriction and low doses of aldosterone antagonist (50–200 mg/day). In a placebo-controlled study, the oral administration of low doses of spironolactone was shown to be effective in preventing ascites recurrence in patients treated with paracentesis without any further increase in the prevalence of paracentesis-induced circulatory dysfunction (80). Finally, some patients may recover their ability to excrete sodium over a period of months and may be free of ascites on a normal sodium diet without diuretic therapy. Factors which can potentially affect the success rate of medical treatment of ascites in non-azotemic cirrhotic patients have not been investigated in a large number of patients (81). Low values of fractional sodium excretion, creatinine clearance, lithium clearance, and high values of plasma renin activity and plasma aldosterone concentration were found in patients who did not respond to the medical therapy of ascites compared with responders (11,12,25). In particular, creatinine clearance and plasma aldosterone concentration proved to be independent predictive factors of the response to the treatment. A low filtered sodium load as well as an increased proximal sodium reabsorption reduce the delivery of sodium to the distal tubule and thus to the sites of action of loop diuretics and collecting duct diuretics. When the delivery of sodium to the distal tubule is reduced a refractoriness to aldosterone antagonists will appear which cannot always be overcome by adding a loop diuretic (11). With respect to the degree of activation of the renin–angiotensin–aldosterone system, the finding of a markedly increased plasma renin activity and plasma aldosterone concentration in nonresponders to aldosterone antagonists, when compared with responders, probably reflects different degrees of reduction in the effective arterial blood volume in these patients (82,83). In clinical practice, most cirrhotic patients with diuretic-resistant ascites have hepatorenal syndrome, a syndrome characterized by the spontaneous development of increased levels of serum urea and serum creatinine concentration (20). In this respect it is important to stress that renal failure is greatly underestimated on the basis of serum creatinine levels and even on creatinine clearance values in cirrhotic patients (84). It should also be stressed that the conventional diuretic therapy of ascites should not be initiated in these patients until their renal function is stabilized. General recommendations for the rational medical treatment of ascites in patients with cirrhosis are summarized in Table 18.3.

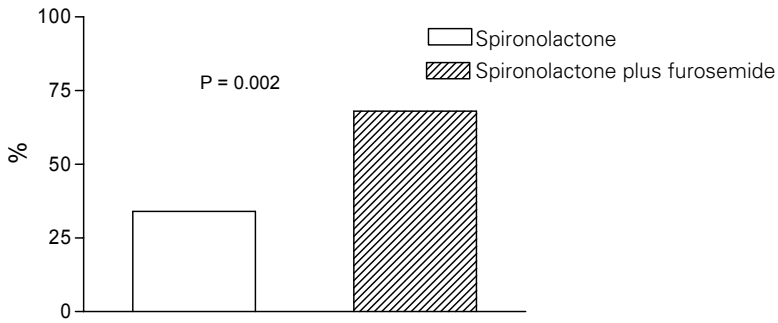


**Figure 18.1** Need to reduce the diuretic dosage due to an excessive response in patients treated with spironolactone alone and in those receiving spironolactone plus furosemide. Data from Santos J, Planas R, Pardo A *et al.* Spironolactone alone or in combination with furosemide in the treatment of moderate ascites in nonazotemic cirrhosis. A randomized comparative study of efficacy and safety. (Reproduced with permission from J Hepatol 2003; 39:187–92.)

**Table 18.3** Five general recommendations for the rational medical therapy of moderate uncomplicated ascites in patients with cirrhosis.

- Most of these patients can be managed in the outpatient setting. Start with low-sodium diet (80 mmol/day) and antialdosteronic drug (100–200 mg/day), monitoring body weight daily. Low doses of furosemide (20–40 mg/day) may be added in case of poor response to antialdosteronic drug.
- The goal of treatment should be to achieve a weight loss of 300–500 g/day in patients without peripheral edema and 1 kg/day in patients with peripheral edema. In most patients this therapeutic schedule is sufficient to mobilize ascites. Patients should be instructed to reduce the dose of diuretics if a greater loss of weight occurs.
- In nonresponders the dose of diuretics should be increased stepwise every 3–5 days to a maximum of 400 mg/day of antialdosteronic drug and 160 mg of furosemide.
- Once ascites has been reduced sodium restriction should be maintained while the diuretic dosage should be reduced.
- Patients not responsive to maximal diuretic doses or those who develop serious complications under diuretic treatment should be checked for refractory ascites. First, the compliance with low-sodium diet should be checked by determination of urine sodium excretion in patients who did not respond to maximal diuretic doses.

## Refractory ascites

Medical treatment based on low-sodium diet, aldosterone antagonists and loop diuretics achieves an elevated response rate, up to 90% of patients (11,12). However, this data could be true in controlled clinical trials, where more severely ill patients are not included. In clinical practice, failure of medical treatment is far more likely to occur. In 1996 the International Ascites Club defined "refractory ascites" as ascites that cannot be mobilized or the early recurrence of which cannot be satisfactorily prevented by medical therapy. Two types were identified as diuretic-resistant ascites and diuretic-intractable ascites (85). The diagnostic criteria have been recently revised and are shown in Table 18.4 (19). It has been proposed that a natriuresis < 50 mEq over 8 h after the intravenous administration of 80 mg of furosemide may identify patients with refractory ascites (86). The mechanism whereby ascites is resistant to diuretics in cirrhotic patients with hepatorenal syndrome is probably related to alterations in both the pharmacodynamics and the pharmacokinetics of diuretics (87). The delivery of sodium to the sites where loop diuretics and aldosterone antagonists inhibit sodium reabsorption may be markedly reduced in these patients as a result of both a decreased glomerular filtration rate and an enhanced proximal sodium reabsorption. These alterations of renal sodium handling reflect a more marked activation of antinatriuretic factors over that of natriuretic factors (82,87). The access of loop diuretics to the organic acid secretory site and aldosterone antagonist to the aldosterone receptors mainly depends upon the renal blood flow, which is impaired in patients with hepatorenal syndrome. In addition, severe hypoalbuminemia may reduce the delivery of furosemide to the kidney in cirrhotic patients with ascites (35,87).

Before defining ascites as diuretic resistant, several factors which may interfere with the action of diuretics should be excluded (24). Inadequate dietary sodium restriction is the leading cause of the spurious diuretic-resistant ascites and should be suspected in any patient whose ascites does not decrease despite a good natriuretic response. The initial step in the evaluation of a possible diuretic-resistant ascites involves measuring urinary sodium excretion. Inadequate physical activity, bacterial infections, portal vein thrombosis, and hepatocellular carcinoma are all conditions that in our experience may be related to the development of diuretic resistance in cirrhotic patients with ascites. Finally, the simultaneous administration of NSAIDs may be another reason for diuretic resistance, since the natriuretic response to furosemide and spironolactone is reduced when these agents are given to these patients (88–90). The first-line treatment option for cirrhotic patients with true diuretic-resistant ascites is repeated total paracentesis. After para-

**Table 18.4** Diagnostic criteria of refractory ascites proposed by the International Ascites Club.

**1** Duration of treatment: patients should be on intensive diuretic treatment (aldosterone antagonist 400 mg/day and furosemide 160 mg/day) for at least 1 week and on a low-sodium diet (90 mmol/day).

**2** Lack of response: mean weight loss < 0.8 kg over 4 days and urinary sodium output less than sodium intake.

**3** Early ascites recurrence: reappearance of grade 2 or 3 ascites within 4 weeks of initial mobilization.

**4** Diuretic-induced complications:

- Hepatic encephalopathy is the development of encephalopathy in the absence of other precipitating factors.
- Diuretic induced-renal failure is an increase of serum creatinine by > 100% to a value > 2 mg/dl in patients responding to treatment.
- Diuretic induced-hyponatremia is defined as a decrease of serum sodium by > 10 mmol/l to a serum sodium < 125 mmol/l.
- Diuretic-induced hypokalemia is defined as a decrease in serum potassium to < 3.5 mmol/l.
- Diuretic-induced hyperkalemia is defined as an increase in serum potassium > 5.5 mmol/l.

centesis patients should receive diuretics as tolerated in order to reduce the frequency of repeated paracentesis. Diuretics should be stopped if complications occur or if they are not effective (urine sodium < 30 mmol/day). Transjugular intrahepatic portosystemic shunt (TIPS) or peritoneovenous shunt (PVS) should be considered in patients requiring very frequent paracentesis (19). In a very small number of cases, paracentesis, TIPS or PSV may not be performed because of contraindications, thus some attempts to potentiate the effectiveness of the diuretic therapy may be undertaken (91). Whether intravenous use of furosemide or the shift to other loop diuretic can be advantageous has not been assessed in controlled studies. In pilot studies the oral administration of torasemide (20 mg) has been shown to induce a good degree of natriuresis in five patients with a poor response to furosemide (80 mg) (92). The effectiveness of torasemide in these patients may be related to either a larger amount of circulating parent drug due to a reduced liver metabolism (93), or to the basolateral component of torasemide action on the thick ascending limb of Henle's loop (32). Nevertheless, the long-term efficacy of this approach requires further verification. Another approach involves adding a third diuretic such as a distal convoluted tubule diuretic (hydrochlorothiazide or metolazone). Metolazone's usefulness has been proven in three patients who failed to respond to high doses of furosemide alone (94). Recently, we observed that the addition of metolazone (2.5–10 mg once per day) to an aldosterone antagonist and a loop diuretic was also effective in four out of five cirrhotic patients with true diuretic-resistant ascites. In all of these patients the treatment had to be discontinued after a few days due to the onset of diuretic-induced adverse effects (P. Angeli, unpublished data). The mechanism of this synergistic effect is still unknown. It has been proposed that a distal convoluted tubule diuretic may counteract increased sodium reabsorption in the distal tubule due to the adaptive processes that develop during chronic loop diuretic administration. Proximal diuretics and mannitol have been shown to act synergistically with loop diuretics and aldosterone antagonist (95–97), probably by increasing the delivery of sodium to the distal tubule, but there is limited experience in their chronic use, particularly in patients with cirrhosis. Other strategies for the treatment of true diuretic-resistant ascites in cirrhotic patients are suitable only in restricted situations. In a controlled clinical study it was observed that repeated infusions of human albumin solutions improved the efficacy of the conventional medical therapy of ascites. This supports the view that a reduction of effective circulating arterial volume may inhibit the response to diuretics in some patients (98). Accordingly, the weekly use of 100 ml of 20% human albumin solution in cirrhotic patients on diuretic treatment for ascites reduced the values of plasma renin activity considered to be the most sensible index of a reduced effective circulating arterial volume (99). These results suggest that human albumin solutions may be useful in cirrhotic patients with diuretic-resistant ascites. The use of albumin is particularly effective when the plasma level of albumin is reduced to such an extent to contribute to ascites formation and/or to reduce the delivery of furosemide to the kidney or when signs of markedly reduced effective circulating arterial volume (arterial hypotension and/or hepatorenal syndrome) are present.

## Complications of diuretic therapy

The use of diuretics in cirrhotic patients with ascites is associated with several complications, among which renal failure, hepatic encephalopathy, electrolyte and acid-base disorders, gynecomastia, and muscle cramps are the most common. Some of these complications (i.e. muscle cramps) may also occur in cirrhotic patients who have never received diuretics, and thus the impact of diuretics on their prevalence is difficult to define. The prevalence of diuretic-induced complications in cirrhotic patients ranges between 20 and 40% depending upon the types and doses of diuretics used and the clinical features of the patients included. In a study by Sherlock *et al*., in non-azotemic cirrhotic patients with ascites the prevalence of renal failure, hyponatremia, and hepatic encephalopathy was 34, 41, and 2%, respectively (100). Similarly, in 100 patients treated with spironolactone alone or spironolactone plus furosemide, the incidence of these complications in the whole series was 24, 22, and 27%, respectively (101). More recently, the prevalence of diuretic-induced adverse effects in non-azotemic cirrhotic patients with ascites who were treated by the conventional stepped care medical treatment was lower than 20% and no case of renal failure or hepatic encephalopathy was observed (11,12). Diuretic-induced renal failure is due to intravascular volume depletion. It often occurs as a result of a too aggressive diuretic therapy of ascites. Following the administration of a diuretic there is a fall in the plasma volume that is equal to the fluid lost in the urine. The plasma volume is then restored by fluid present in the peritoneal cavity (ascites) or in the interstitium (edema). Ascites reabsorption, the net passage of ascitic fluid to the intravascular compartment of extracellular fluid, is a rate-limited process which averages 300–500 ml/day ranging from 100 to 1000 ml/day. If diuretic therapy causes a loss of fluid above 300–500 ml/day, a reduction of intravascular volume may occur with a consequent prerenal azotemia. Reabsorption of the peripheral edema is not rate-limited and it can be mobilized at a rate equal to any reasonable rate of diuresis (102). Thus, the presence of peripheral edema makes a more rapid fluid loss possible. Diuretic-induced renal failure is usually mild and reversible after the withdrawal of diuretic therapy. Despite anecdotal

reports of hepatorenal syndrome developing in patients who had received diuretics, in a controlled trial involving cirrhotic patients with ascites, its prevalence was the same despite diuretic therapy (103). Diuretics have been traditionally considered among the potential precipitating factors of hepatic encephalopathy in patients with cirrhosis (104). The most accepted mechanism is an increase in the plasma ammonia level due to increased renal ammonia production following diuretic-induced hypokalemic alkalosis. This renal effect of hypokalemia appears to be mediated by a transcellular cation exchange, in which $K^+$ leaves the cell and electroneutrality is maintained in part by the movement of extracellular $H^+$ into the cell. The ensuing intracellular acidosis is a potent stimulus for ammonia production by the renal tubular cells (105,106). Recent studies have shown that some diuretics may also impair the urea cycle, leading to a reduced hepatic transformation of ammonia into urea (107). Hypokalemia occurs frequently in cirrhotic patients with ascites when they are treated with loop diuretics or cortical diluting segment diuretics alone (100,108). Two factors contribute to the occurrence of hypokalemia in this clinical setting. First, the increased delivery of sodium to the distal tubule and thus to the site of action of aldosterone, and second, the activation of the renin–angiotensin–aldosterone system which is characteristic of most cirrhotic patients with ascites. With few exceptions (11), the combined use of an aldosterone antagonist and a loop diuretic will prevent the onset of hypokalemia in these patients. When aldosterone antagonists or other potassium-sparing diuretics are used alone, especially in cirrhotic patients with renal function impairment, hyperkalemia may occur. In these patients a combined diuretic therapy, aldosterone antagonist plus a loop diuretic, is advisable. Aldosterone-antagonist-induced hyperkalemia is classically thought to be related to a decrease in urinary potassium excretion. In patients with cirrhosis and ascites spironolactone was, however, shown to produce an increase in potassium excretion (64,109). The shift of potassium from the intracellular to the extracellular space due to the inhibition of aldosterone activity on internal potassium balance may represent the most effective mechanism of hyperkalemia in these patients (110).

Hyponatremia is another frequent complication of diuretic therapy in patients with cirrhosis and ascites. It occurs in 20–40% of hospitalized patients with cirrhosis and ascites treated with diuretics (100,101). In 204 consecutive hyponatremic patients cirrhosis with ascites was the second cause of hyponatremia after cardiac failure (18% vs. 25%, respectively) (111). Hyponatremia in cirrhotic patients is usually mild and asymptomatic. In a series of 137 patients admitted to the hospital for ascites and then followed prospectively for at least 6 months, we found a serum sodium concentration < 120 mEq/l and 115 mEq/l in 6.5% and 3%, respectively (P. Angeli, unpublished data). The symptoms of hyponatremia (anorexia, apathy, nausea, vomiting, seizures) associated with cirrhosis and ascites do not differ from those of hyponatremia in other diseases in which hyponatremia is coupled with an increase of extracellular fluid volume (dilutional hyponatremia). In almost half of patients with hyponatremia a precipitating factor may be detected, bacterial infection being the most common (112,113). The survival of patients who develop hyponatremia is reduced mainly in those who develop it spontaneously (112). Most patients with spontaneous hyponatremia die due to hepatorenal syndrome (112). The treatment of hyponatremia in cirrhotic patients with ascites is discussed extensively in another chapter (see Chapter 26).

The administration of collecting duct diuretics has also been associated with the development of metabolic acidosis in some cirrhotic patients with ascites (114). The reduction of $H^+$ excretion due to the lumen's negative voltage resulting from the inhibition of $Na^+$ reabsorption is the cause of this disorder. However, metabolic acidosis seldom represents a clinical problem. The use of loop diuretic alone may induce hypocloremi alkalosis (100) due to an increase in distal $H^+$ secretion, which may be related to three factors: (i) increased plasma level of aldosterone, (ii) increased delivery of sodium to the distal tubule, and (iii) hypokalemia.

The most frequent complication related to the use of spironolactone in cirrhotic patients with ascites is probably gynecomastia. This disorder is probably due to the alteration in sexual steroid metabolism induced and/or exacerbated by the antiandrogenic activity of spironolactone (115). At high doses, spironolactone reduces testosterone biosynthesis and increases the peripheral conversion of testosterone to estradiol, thus producing estrogen-like side-effects (116). It has been also observed that spironolactone inhibits the binding of testosterone to cytosolic and nuclear receptors in the target organs (117). Clinically, a lower incidence of antiandrogenic effects and particularly of gynecomastia has been observed in cirrhotic patients treated with potassium canrenoate rather than with spironolactone (118,119). Gynecomastia induced by spironolactone may disappear or be reduced when spironolactone is substituted by potassium canrenoate (120). Accordingly, a reduction of androgen-receptor activity was observed in animals as well as in patients treated with spironolactone, but not in those treated with potassium canrenoate (119,121). As a consequence, the higher antiandrogenic activity of spironolactone is probably related to active compounds other than canrenone. Finally, diuretic therapy in cirrhotic patients with ascites is frequently associated with muscle cramps. It has been recently shown that true muscle cramps occur more frequently in cirrhotic patients than in the general population. They often appear before the onset of ascites (99,122) but they become more frequent and severe after

the start of diuretic treatment for ascites. A reduced effective arterial blood volume, which characterizes the cirrhotic patients, may play a role in the pathogenesis of muscle cramps. So, diuretics may worsen cramps by causing a further decrease in arterial blood volume. For patients with severe incapacitating muscle cramps, diuretics should be decreased or even stopped. In clinical practice, the oral administration of quinine or quinidine (123) or zinc sulfate (124) or weekly human albumin infusion (99) may reduce the frequency and the severity of muscle cramps in cirrhotic patients.

## Contraindications to diuretic therapy

Diuretics are contraindicated in patients with renal failure, hyponatremia, and ongoing bacterial infection. With respect to renal failure, there are no data to support the withdrawal of diuretics in patients with primary renal disease (i.e. diabetic nephropathy), although it is agreed that diuretics should be withheld from cirrhotic patients in whom renal failure is related to a reduction in arterial blood volume (i.e. prerenal failure or hepatorenal syndrome).

There are no data on the level of serum sodium at which diuretics should be withdrawn, but most experts agree that diuretics should be stopped, at least temporarily, when serum sodium is lower than 120 mmol/l (19). Diuretics should be stopped temporarily if hepatic encephalopathy worsens by two grades and their use re-assessed. Finally, loop diuretics should be reduced or stopped when serum potassium is < 3.5 mmol/l, while potassium-sparing diuretics should be reduced or stopped when serum potassium is > 5.5 mol/l or > 6 mol/l respectively (19).

## The effects of diuretics on portal pressure

It is well established that portal hypertension induces several changes in the systemic circulation, the most important being peripheral vasodilation, expanded plasma volume, and hyperdynamic circulation. Hyperdynamic circulation, which can be observed also in well-compensated cirrhosis with portal hypertension, is characterized by a decrease in arteriolar resistance and by an increase in systemic and regional blood flow mainly in the splanchnic area. Arterial vasodilation *per se* is not sufficient for the development of the hyperdynamic circulation, since it would result in no changes in systemic and regional blood flow. Thus, the accompanying increase in cardiac output, secondary to cardiac afterload reduction, is an important component of the hyperdynamic circulation of cirrhosis. The increased plasma volume, related to renal sodium retention resultant from activation of sodium-retaining factors secondary to arterial underfilling, may play an important role in the maintenance of hyperdynamic circulation and may worsen portal hypertension (125). In support of this hypothesis, it has been demonstrated that dietary sodium restriction blunts the development of plasma volume expansion, resulting in a reduction of portal pressure and in an improvement of the hyperdynamic circulation in rats with portal hypertension (126,127). The effect of a low-sodium diet and of oral administration of spironolactone on lowering portal pressure was confirmed also in cirrhotic patients without ascites (128,129). Recently, it was shown that chronic spironolactone administration effectively lowers esophageal variceal pressure in cirrhotic patients without ascites (130). In contrast, no hemodynamic effect was observed in these patients after the oral administration of furosemide (131). The results of clinical and experimental studies on the potential synergistic effect of diuretics and β-blockers or nitrates in lowering portal pressure are still controversial. The acute administration of a β-blocker induces a further decrease in portal pressure in cirrhotic patients treated with spironolactone (129), while the acute administration of furosemide does not potentiate the decrease of portal hypertension in patients treated with a β-blocker (132). The chronic administration of nadolol, a β-blocker, and spironolactone does not increase the efficacy of nadolol alone in the prophylaxis of the first variceal bleeding in cirrhotic patients (133). No synergistic effect was observed in reducing portal pressure when propranolol and spironolactone were administered in combination to rats with bile duct ligation (127). Similarly, the combination of spironolactone with nitrates did not have any additive effect on portal pressure in partial portal vein ligated rats (134). These preliminary reports suggest that spironolactone and furosemide may interact differently with the hepatic circulation and that the effect of spironolactone on portal pressure may involve factors other than diuretic efficacy. Three more observations strongly support this conclusion. First, it has been observed that the reduction in portal pressure induced by spironolactone is dose-independent (134). Second, the effect of spironolactone on portal pressure is not related to changes in plasma volume (128). Third, aldosterone antagonists have been shown to induce relaxation of isolated rat aorta rings precontracted by different agonists (135). Several lines of evidence suggest that the reduction of portal pressure could be due to direct effects of aldosterone antagonists on factors affecting intrahepatic portal circulation rather than being mediated by a decrease in plasma volume (136). It has been hypothesized that this class of drugs may antagonize the profibrogenic effect of aldosterone, particularly highlighted within the cardiovascular system (137). This hypothesis was recently supported by the observation that canrenone, an aldosterone antagonist, may reduce the contractility of activated human hepatic stellate cells and may be active as an antifibrogenic drug in the liver (138). Further stud-

ies are required in order to elucidate the complex mechanism of action of aldosterone antagonists on splanchnic hemodynamics in cirrhosis.

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# Chapter 19
# Paracentesis for Cirrhotic Ascites

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## Introduction

Ascites is the most common complication of cirrhosis. It develops late during the course of the disease, when there is hepatic insufficiency and severe portal hypertension. Therefore, the development of ascites is associated with a significant worsening of the prognosis (1). The 1-year survival probability rate of patients admitted to hospital for the treatment of an episode of ascites has been reported to be only 56% (2).

Since the introduction of modern diuretics in the 1960s, the treatment of ascites in patients with cirrhosis has been based on the combination of low-sodium diet and the administration of these drugs (3). This therapeutic schedule, however, is not entirely satisfactory, since it is frequently associated with complications, particularly hepatic encephalopathy, renal impairment, and dilutional hyponatremia (4). In addition, between 5 and 10% of cirrhotics admitted to hospital for the treatment of an episode of tense ascites do not respond to diuretics. This condition, known as refractory ascites or diuretic-resistant ascites, is an important challenge and has stimulated investigations of alternative treatments for ascites in patients with cirrhosis. Until very recently, the prosthesis designed by LeVeen *et al.* in 1974 (5) was the only alternative treatment to diuretics for the management of cirrhotic patients with ascites. However, this procedure may induce serious complications, is associated with a high rate of obstruction of the prosthesis, and does not improve survival (6–8).

Since the reintroduction of therapeutic paracentesis by Ginès *et al.* (9) in 1985, numerous studies have demonstrated that this procedure is a rapid, effective, and safe therapy for tense ascites in patients with cirrhosis. It is, therefore, not surprising that contrary to previous clinical practice, which was dictated by fear of complications, at present therapeutic paracentesis is widely used in these patients.

## Comparison of paracentesis plus intravenous albumin vs. diuretics in the treatment of tense ascites

The first study re-evaluating paracentesis as a treatment of ascites was a randomized controlled trial comparing repeated large-volume paracentesis (4–6 l/day until complete mobilization of ascites occurs) associated with intravenous albumin infusion (40 g after each tap) with standard diuretic therapy (furosemide plus spironolactone at increasing doses) in 117 cirrhotic patients with tense ascites and urinary sodium excretion of < 10 mEq/day (9,10). Patients not responding to the highest scheduled doses of diuretics were treated by peritoneovenous shunting. Patients with severe renal impairment (serum creatinine concentration > 3 mg/dl) or severe liver failure (serum bilirubin concentration > 10 mg/dl, prothrombin time of < 40%) were not included. After treatment patients were discharged with diuretics. Patients developing ascites during follow-up were treated according to their initial schedule. The main findings of the study were: (i) paracentesis was more effective than diuretics in eliminating ascites and significantly shortened the duration of hospital stay; (ii) paracentesis had no deleterious effect on systemic hemodynamics and renal function; (iii) the incidence of hyponatremia, renal impairment, and hepatic encephalopathy was much lower in patients treated by paracentesis (5, 3, and 10%, respectively) than in patients receiving diuretics (30, 27, and 29%); and (iv) during follow-up there were no differences between the two groups in probability of readmission to a hospital and probability of survival.

The conclusions of the study were that paracentesis is more effective than diuretics in the mobilization of tense ascites, is associated with a lower incidence of complications, shortens the duration of hospital stay, and does not affect the long-term course of the disease. Most of these results were later confirmed in four further controlled trials, reported by Salerno *et al.* (11), Hagève *et al.* (12), Acharya *et al.* (13), and Solà *et al.* (14).

The issue of whether paracentesis should be repeated daily with 5-l paracentesis or a single total paracentesis has been resolved. In 38 cirrhotic patients with tense ascites, Tító *et al.* (15) investigated whether ascites could be safely mobilized by total paracentesis (complete removal of ascites in a single tap) plus intravenous albumin infusion (6-8 g/l of ascites removed). The incidence of hyponatremia, renal impairment, and hepatic encephalopathy in this series of patients (3, 0, 10 respectively) and the

clinical course of the disease, as estimated by the probability of survival, and causes of death, were comparable to those reported by the same group of investigators in patients treated with large-volume paracentesis (9).

Based on these studies, paracentesis is currently considered the treatment of choice of tense ascites (16). Paracentesis is a treatment of ascites, but it does not improve circulatory or renal function. Therefore, patients treated by paracentesis require diuretic treatment to prevent the reaccumulation of ascitic fluid. In a randomized controlled trial in 36 patients with tense ascites treated by total paracentesis plus intravenous albumin infusion, the incidence of ascites recurrence within 4 weeks after the procedure was only 18% in patients receiving spironolactone (225 mg/day) immediately after paracentesis compared with 93% in patients receiving a placebo (17).

## Therapeutic paracentesis without volume expansion

There are only two randomized controlled trials aimed at investigating whether intravenous albumin administration is necessary in cirrhotic patients treated with therapeutic paracentesis. Albumin is given to correct the decreased effective arterial blood volume that drives sodium retention in cirrhosis and maintains ascites formation. In the first study, by Ginès *et al.* (18), 52 cirrhotic patients with tense ascites were treated by repeated large-volume paracentesis with intravenous albumin and 53 by paracentesis without albumin administration. There were no differences between the two groups in the number of patients with complete removal of ascites, loss of body weight, number of paracentesis procedures per patient, and duration of hospitalization. Confirming previous studies, paracentesis plus intravenous albumin did not induce significant changes in standard renal function tests, plasma renin activity, and plasma aldosterone concentration. In contrast, paracentesis without albumin was associated with a significant increase in blood urea nitrogen, a constant and marked elevation in plasma renin activity and plasma aldosterone concentration, and a significant reduction in serum sodium concentration. Side-effects occurred in 17% of patients treated with albumin and in 30% treated without albumin. This difference was due to a significantly higher incidence of hyponatremia and/or renal impairment in patients not receiving albumin (20% vs. 2%). The incidence of other complications was similar in the two groups. Although the probability of survival was similar in the two groups, a multivariate analysis identified the development of hyponatremia, renal impairment or both after paracentesis, and the occurrence of other complications during first hospitalization (encephalopathy, gastrointestinal tract bleeding, and severe infection) as the only independent predictors of mortality.

The second study, by García-Compeán *et al.* (19), was performed in 35 cirrhotic patients with tense ascites treated by total paracentesis: 17 patients were treated with albumin and 18 without volume expansion. There were no significant differences in the incidence of side-effects between the groups. Unfortunately, the period of observation in this study was only 24 h following paracentesis, which is too short to assess the development of paracentesis-induced side-effects. Twenty-four hours after paracentesis, plasma renin activity decreased in patients treated with albumin and increased in patients not receiving albumin. These changes between the two groups were significant.

The results of these studies suggest that complete mobilization of ascites by paracentesis without plasma volume expansion is followed by impairment in effective intravascular arterial volume. This contention is supported by findings of several investigations assessing the effects of complete mobilization of ascites by large-volume paracentesis with and without albumin infusion on systemic hemodynamics, vasoactive hormones, and renal function (20–28). Despite the impairment in systemic hemodynamics in almost all patients treated with paracentesis without albumin infusion, only 20% of patients develop renal or electrolyte complications, or both, probably because of intrarenal compensatory mechanisms that antagonize the effects of circulating hypovolemia on kidney function. Because renal impairment and dilutional hyponatremia after paracentesis are not reversible in many cases and are associated with a poor prognosis (18), the prevention of the impairment in effective arterial blood volume by the intravenous infusion of albumin is an important measure in cirrhotic patients with tense ascites treated with repeated large-volume or total paracentesis.

## Use of other types of plasma expanders in cirrhotic patients treated with paracentesis

Although treatment of ascites by paracentesis with albumin is cost-effective in comparison with diuretic therapy, the use of albumin is limited by its high price. In addition, albumin is currently derived from human plasma, and therefore there is limited availability of this product. Finally, in underdeveloped countries human serum albumin cannot be routinely prescribed in public hospitals. Therefore, it is not surprising that several groups have investigated whether albumin could be substituted by the less expensive synthetic plasma expanders in these patients. Five randomized controlled trials and one pilot study have so far been published concerning this aspect.

The first study was performed by Planas *et al.* (29) and compared albumin vs. dextran 70 (6 g per 100 ml of dextrose solution) in 88 cirrhotic patients with tense ascites

treated by total paracentesis. Salerno *et al.* (30) randomized 54 cirrhotic patients with refractory ascites treated by total paracentesis to receive albumin (6 g per liter of ascites removed) or polygeline (Hemaccel: 3.5% saline solution of polygeline, 150 ml per liter of ascitic fluid removed). Fassio *et al.* (31) performed a randomized controlled trial comparing dextran 70 (containing 6 g of dextran 70 per 100 ml isotonic saline solution) with human albumin in 41 cirrhotic patients and tense ascites treated daily with 5-l paracentesis until resolution of ascites. Solà *et al.* (14) treated 49 cirrhotic patients with tense ascites with total paracentesis plus intravenous dextran 40 infusion (10 g per 100 ml of dextrose solution; 8 g per liter of ascitic fluid removed) in the setting of a randomized study that compared paracentesis vs. diuretic treatment. Finally, Cabrera *et al.* (32), in one pilot study performed in only 14 patients, have suggested that isotonic saline can also be a safe and cost-effective alternative plasma expander in cirrhotics with tense ascites treated with paracentesis.

The above-mentioned studies did not clarify sufficiently whether albumin could be substituted by the less expensive synthetic plasma expanders. Some studies reported a high incidence of paracentesis-induced circulatory dysfunction, whereas in other studies no significant changes in the activity of the renin–angiotensin–aldosterone system were observed. Moreover, the incidence of renal impairment or dilutional hyponatremia and the probability of hospital readmission for ascites and survival following paracentesis appear to be similar in patients with albumin or synthetic plasma expanders.

A recent study by Ginès *et al.*, which represents the most extensive investigation to date assessing the role of synthetic plasma expanders and albumin in cirrhotic patients with tense ascites treated by paracentesis, has clarified most aspects concerning this issue (33). In this multicenter, randomized, controlled trial 289 cirrhotic patients were randomized to treatment by total paracentesis plus albumin (97 patients), dextran 70 (93 patients) or polygeline (99 patients). The patients were followed during a mean period of approximately 1 year and when tense ascites developed during follow-up, patients were treated with total paracentesis and the same plasma expander that was assigned at inclusion. The aim of the study was to investigate the clinical importance of paracentesis-induced circulatory dysfunction and compare the efficacy of the different plasma expanders in preventing this complication. Paracentesis-induced circulatory dysfunction (defined as an increase in plasma renin activity of more than 50% of the pretreatment value to a level > 4 ng/ml/h on the sixth day after paracentesis) occurred significantly more frequently in patients treated with dextran 70 (34%) or polygeline (38%) than in those receiving albumin (18%).

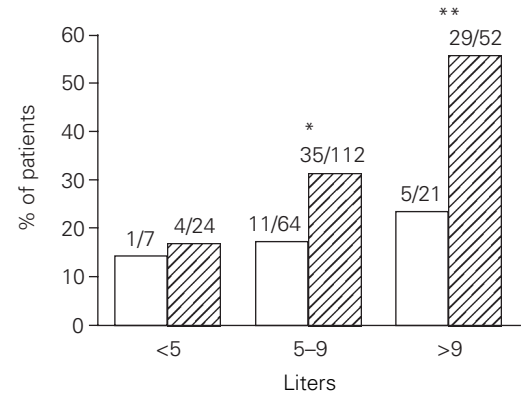



**Figure 19.1** Incidence of paracentesis-induced circulatory dysfunction according to the plasma expander used (albumin: empty bars; dextran 70 or polygeline: dashed bars) and the volume of ascitic fluid drained (*$P$ = 0.04; **$P$ = 0.02 with respect to the incidence in patients receiving albumin). The figures above the bars represent patients developing paracentesis-induced circulatory dysfunction and patients at risk, respectively. (Reproduced by permission from Ginès A, Fernández-Esparrach G, Monescillo A *et al.* Randomized trial comparing albumin, dextran 70, and polygeline in cirrhotic patients with ascites treated by paracentesis. Gastroenterology 1996; 111:1002–10.)

Among 18 parameters analyzed, only the volume of ascites removed and the type of plasma expander had predictive value for the development of paracentesis-induced circulatory dysfunction (Fig. 19.1). The incidence of paracentesis-induced circulatory dysfunction in patients receiving either albumin or synthetic plasma expanders was similar when the volume of ascitic fluid removed was ≤ 5 l. However, it was clearly higher in patients treated with synthetic plasma expanders when the volume of ascites removed was > 5 l. Differences were particularly striking in patients in whom more than 9 l of ascites were drained. In contrast to the results obtained by Salerno *et al.* (30), in the study of Ginès *et al.* paracentesis-induced circulatory dysfunction was not spontaneously reversible but persisted during the entire follow-up period.

Paracentesis-induced circulatory dysfunction was associated with higher diuretic requirements during follow-up, shorter time to first readmission for ascites or for any reason, and shorter survival (Fig. 19.2). Taken together, these data indicate that postparacentesis circulatory dysfunction is not spontaneously reversible and is associated with an increased probability of ascites recurrence and reduced survival time. Albumin, which is more effective than dextran 70 and polygeline in the prevention of this abnormality, should be considered the plasma expander of choice in patients in whom more than 5 l of ascitic fluid are removed.

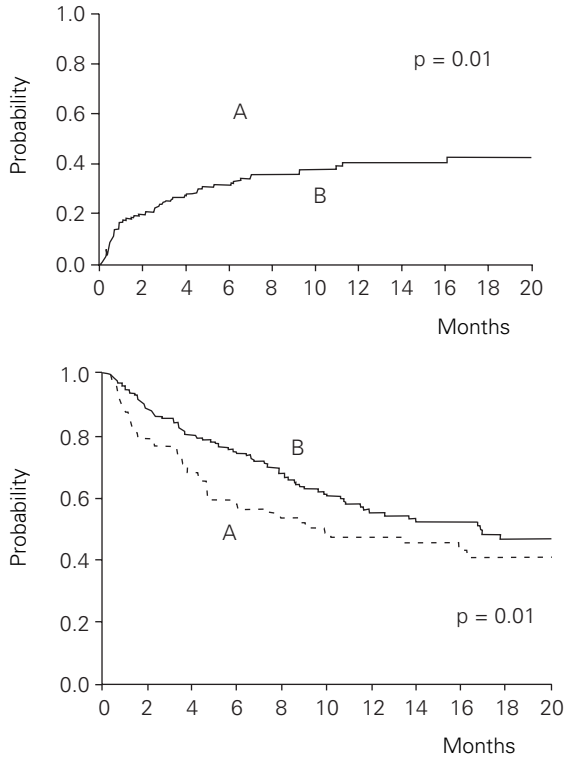



**Figure 19.2** Probability of readmission to the hospital during follow-up for ascites (top) and probability of survival (bottom) in patients who did (dashed line) and did not (solid line) develop paracentesis-induced circulatory dysfunction. (Reproduced by permission from Ginès A, Fernández-Esparrach G, Monescillo A *et al*. Randomized trial comparing albumin, dextran 70, and polygeline in cirrhotic patients with ascites treated by paracentesis. Gastroenterology 1996; 111:1002–10.)

## Changes in circulatory function following therapeutic paracentesis

Numerous studies have been published assessing the effects of paracentesis on systemic hemodynamics, vasoactive hormones, and renal function in cirrhotic patients with tense ascites not receiving plasma volume expansion (19–22,24,26,28,34–38), and they have clearly demonstrated that paracentesis induces marked changes in cardiocirculatory function.

The mobilization of tense ascites by paracentesis is rapidly followed by a significant reduction in intra-abdominal, inferior vena cava, pulmonary artery, pulmonary capillary wedged, and right atrial pressures; an increase in cardiac output and stroke volume; and a reduction in systemic vascular resistance (19–21,24,35,37). These changes are detectable after the extraction of the first liter of ascitic fluid, increase as the ascitic fluid is removed up to a certain stage of drainage (5 l), and persist during a period of 12–24 h (19–21,24,35,37).

These initial and beneficial cardiocirculatory changes can probably be explained by mechanical factors (24). Tense ascites acting on the diaphragm increases intrathoracic pressure to such an extent that the transmural filling pressure of the heart is reduced. This leads to a decreased filling of the heart, a decrease in venous return and cardiac output, and an increase in cardiopulmonary pressures. This sequence of events is comparable to that seen during pericarditis. Paracentesis, therefore, hypothetically improves cardiac function in a way similar to that of pericardiocentesis in patients with pericardial effusion. The fall in intraperitoneal pressure acting on the diaphragm reduces intrathoracic pressure and increases transmural filling pressure, venous return, heart volumes, and cardiac performance. The reduction of systemic vascular resistance would be a homeostatic response of the systemic circulation to accommodate the increased cardiac output and may be mediated by endothelial vasodilators sensitive to shear stress. These circulatory changes are associated with a marked suppression of the plasma levels of renin and aldosterone (19,20,24,34), norepinephrine (38), and antidiuretic hormone and a significant increase in the plasma concentration of atrial natriuretic peptide (34). Taken together, all these data indicate that therapeutic paracentesis in cirrhotic patients with tense ascites is followed by an immediate improvement in cardiocirculatory function that suppresses the endogenous vasoconstrictor systems.

However, when paracentesis is performed without plasma volume expansion, when cardiocirculatory function is assessed 12–24 h following total paracentesis, different findings are observed in many hemodynamic and neurohormonal measurements. Cardiac output decreases below baseline values (19–23,25,35), and central venous, right atrial, pulmonary artery, and pulmonary capillary pressures continue to be below baseline levels or decrease further (20,21,23–25). Systemic vascular resistance also remains below pretreatment values or even decreases further despite a significant increase in the plasma levels of renin, aldosterone, and norepinephrine over baseline values, indicating an accentuation of arteriolar vasodilation and compensatory activation of the endogenous vasoconstrictor systems. The plasma concentration of atrial natriuretic peptide decreases with respect to baseline values (19–21,23,24,38). Finally, renal perfusion and glomerular filtration rate fall (20,35). The initial improvement in cardiocirculatory function, therefore, is transient, being followed by a deterioration of circulatory function, activation of the renin–angiotensin and sympathetic nervous systems and antidiuretic hormone, suppression of atrial natriuretic peptide, and impairment in renal function. An important observation in some investigations is that this impairment in circulatory function can be prevented or reversed by the expansion of plasma volume with albumin (19,23) or dextran 70 (22).

## Mechanism of paracentesis-induced circulatory dysfunction

The traditional concept that therapeutic paracentesis causes a deterioration of systemic hemodynamics as a result of a rapid reformation of ascites from the plasma volume and, therefore, of a decrease in circulating plasma volume has not been confirmed in recent investigations. The complete mobilization of ascites by paracentesis is rapidly followed by reformation of ascites. However, this is due to a shift of peripheral edema to the intraperitoneal cavity. In fact, plasma volume and the transvascular escape rate of albumin, which estimates the passage of intravascular volume to the extracellular space, do not change after therapeutic paracentesis even in patients who develop circulatory dysfunction (28,36).

In contrast, paracentesis-induced circulatory dysfunction is associated with a profound reduction in systemic vascular resistance (37). Although systemic vascular resistance usually falls after therapeutic paracentesis, the decrease is greater in patients with circulatory dysfunction. It is important to note that this feature occurs in the setting of a marked activation of the renin–angiotensin and sympathetic nervous systems, which are potent vasoconstrictors. The decrease in vascular resistance measured, therefore, would have been greater if these homeostatic neurohormonal mechanisms were not activated. These data indicate that paracentesis-induced circulatory dysfunction is predominantly caused by an accentuation of the arterial vasodilation already present in untreated cirrhotic patients with ascites. The mechanism by which paracentesis induces arterial vasodilation, the site where arteriolar vasodilation occurs, and the method by which the expansion of plasma volume with albumin almost totally prevents the development of paracentesis-induced circulatory dysfunction are unknown.

## Paracentesis as a treatment for refractory ascites

Refractory ascites is an infrequent condition in cirrhosis. Only 5–10% of cirrhotic patients admitted to hospital for tense ascites do not respond to diuretics or present complications that preclude the administration of adequate doses of these drugs. The prognosis in this category of patients is therefore usually poor. While the survival rate of patients with ascites is approximately 50% at 2 years, the survival rate in cases of refractory ascites is reduced to 50% at 6 months.

Although specific studies on the pathophysiology of refractory ascites are non-existent, the clinical impression is that the pathophysiological factors leading to this condition are an exaggeration of the factors primarily responsible for retention of sodium and water in cirrhosis. Until the last decade peritoneovenous shunting was the only therapeutic alternative in cirrhotic patients with refractory ascites. However, peritoneovenous shunting does not improve survival in these patients, has serious complications, and is associated with a high rate of obstruction of the shunt. More recently, large-volume paracentesis, the transjugular intrahepatic portosystemic shunt, and liver transplantation have been used in the treatment of refractory ascites. Most cirrhotic patients with refractory ascites do not respond to diuretic therapy or develop complications that preclude the use of an effective diuretic dosage. However, some may show an acceptable natriuretic response. Therefore, diuretics may be continued in such patients if there is a significant natriuresis (> 30 mEq/day), but for those who do not respond to diuretics it is probably safer to stop their use altogether.

Currently, large-volume paracentesis with concomitant administration of intravenous albumin constitutes the standard therapy for refractory ascites. Since therapeutic paracentesis is a local therapy that does not modify the mechanisms that lead to ascites formation, ascites will always recur in patients with refractory ascites unless there is an improvement in liver disease (i.e. alcoholic hepatitis).

Transjugular intrahepatic portosystemic shunt (TIPS) works as a side-to-side portacaval shunt and, from a theoretical point of view, it should correct the two pathophysiological arms of ascites formation. By reducing portal pressure, it should improve the splanchnic arterial vasodilation, as well as the arterial vascular underfilling, thereby suppressing the endogenous vasoconstrictor systems, improving renal perfusion and glomerular filtration rate, and increasing the response to diuretics. On the other hand, by decompressing both the splanchnic and hepatic microcirculation, it should decrease the formation of lymph both in the liver and in the other splanchnic organs. Therefore, TIPS is theoretically a more definitive treatment for refractory ascites than therapeutic paracentesis plus albumin and, by preventing other complications of portal hypertension such as variceal hemorrhage or spontaneous bacterial peritonitis, it could have a beneficial effect on survival. Despite its potential advantages for the treatment of ascites, TIPS has important limitations. Like surgical portosystemic shunts, TIPS can lead to liver failure and hepatic encephalopathy as a result of diversion of blood away from the liver into the systemic circulation. Another important problem of TIPS is its high rate of dysfunction/occlusion, which occurs in 50–70% of patients during the first year. This is an important limitation of TIPS requiring frequent retreatments.

### Paracentesis vs. peritoneovenous shunting

Two multicenter randomized studies comparing repeated large-volume paracentesis associated with

intravenous albumin and LeVeen shunt have been published (39,40). In these two investigations a total of 171 patients with refractory or recidivant ascites were included. Seventy-one patients had renal failure. Eighty-four patients were treated with paracentesis and 87 with LeVeen shunt (39 of these with a titanium tip to assess whether it is effective to prevent obstruction of the venous end of the prosthesis). Although LeVeen shunt was clearly superior in the long-term control of ascites, it had no major impact on the course of the disease. Patients from both therapeutic groups did not differ in the time of first readmission to hospital, total time in hospital during follow-up, and survival. Furthermore, frequent reoperations were required in the surgical group to keep the shunt patent. For all these reasons, there is little role for the use of peritoneovenous shunting in the treatment of refractory ascites. Peritoneovenous shunting may be useful only in patients who are not candidates for liver transplantation, TIPS placement, or repeated large-volume paracentesis.

## Paracentesis vs. transjugular intrahepatic portosystemic shunt

Since an increase in sinusoidal pressure is a prerequisite for ascites formation in cirrhosis, surgical portacaval shunts, either side-to-side or end-to-side, aimed at reducing the portal pressure and stemming the ascites formation, were initially used and rapidly abandoned for the management of these patients. Although surgical portal decompression is very effective for the treatment of ascites, it is associated with an extremely high early postoperative morbidity, significant mortality, and a high incidence of hepatic encephalopathy. However, portosystemic shunting in the treatment of refractory ascites has been reintroduced in recent years in the form of the TIPS.

Pathophysiological studies in cirrhotic patients with ascites have shown that immediately after TIPS the systemic vascular resistance is further reduced and the blood volume and cardiac output are significantly increased, while the right atrial, the pulmonary artery, and the wedged pulmonary artery pressures increase. The increases in cardiopulmonary pressures and cardiac index are probably due to an increased venous return secondary to the portacaval fistula, and the decrease in systemic vascular resistance is probably a physiological response to accommodate the increase in cardiac index (41,42). These circulatory changes have been interpreted as indicative that TIPS impairs systemic hemodynamics and the hyperdynamic circulation in cirrhosis. Nevertheless, studies assessing the effects of TIPS on the degree of activity of endogenous vasoconstrictor systems do not support this assumption. In fact, they indicate that effective arterial blood volume is markedly improved following TIPS in patients with cirrhosis and ascites. TIPS insertion is associated with a significant suppression of the plasma levels of renin, aldosterone, norepinephrine, and antidiuretic hormone (41,43-47). The renal blood flow and the glomerular filtration rate increase, whereas the proximal tubular reabsorption of sodium is reduced, resulting in a higher urinary sodium excretion, which persists even long-term. A significant increase in serum sodium concentration is also observed. So, TIPS ameliorates renal perfusion and solute-free water excretion. However, these changes require 1–3 months to occur.

Although numerous studies suggest that TIPS is an effective therapy of refractory ascites in cirrhosis (48), only four randomized controlled trials on the efficacy of TIPS in refractory ascites have been performed to date (49–52).

The first study, by Lebrec *et al.* (49), included only 25 patients with refractory ascites (13 randomized to TIPS and 12 to paracentesis). This trial demonstrated that intrahepatic shunts are more effective than paracentesis for the treatment of refractory ascites. Compared with repeated paracentesis, TIPS procedure was effective in improving ascites in all Child class B patients but not in the class C patients. By contrast, severe hepatic encephalopathy was observed in 20% of the patients in the TIPS group, but not in any of the paracentesis group. Patients randomized to TIPS had a significantly higher mortality than patients in the paracentesis group (at 2 years the overall survival rate was 29 ± 13% in the TIPS group and 56 ± 17% in the paracentesis group; $P < 0.05$). Further analysis revealed that this higher mortality was caused by a higher mortality in Child class C patients.

In the second study, by Rössle *et al.* (50), 60 cirrhotic patients with refractory or recidivant ascites were randomized to TIPS (29 cases) or to large-volume paracentesis (31 cases). TIPS was, again, more effective than paracentesis for the control of ascites (at 3 months, 61% of the patients in the shunt group and 18% of those in the paracentesis group had no ascites; $P = 0.006$). Although the study included a larger number of patients than that by Lebrec *et al.*, it was not large enough to show a significant difference in the primary end point, which was survival without liver transplantation. The 1- and 2-year probabilities of survival without liver transplantation were 69% and 58% in the TIPS group, respectively, compared with 52% and 32% in the paracentesis group, respectively ($P$ = NS). Although multivariate analysis revealed that TIPS was independently associated with survival ($P = 0.02$), these results should be taken with caution given the small number of outcome events (38 deaths) and the relative large number of variables analyzed (over 15). Moreover, in this study, paracentesis of ≥ 4 l was followed by administration of albumin only "when clinically indicated". As mentioned above, large-volume paracentesis without concomitant plas-

ma volume expansion is associated with postparacentesis-induced circulatory dysfunction in a majority of cases and its presence implies a poorer survival rate. It is therefore conceivable that the tendency for an improved survival in the TIPS group would be eliminated if all patients randomized to paracentesis had received albumin concomitantly. In addition, and somewhat surprisingly in view of the results of previous studies, the incidence of hepatic encephalopathy in the shunt group was not greater than that in the paracentesis group. Finally, mean urinary sodium excretion was 45 and 61 mmol/day, respectively, in the TIPS group and in the paracentesis group, indicating that sodium retention and the mechanisms driving sodium retention were not very important.

In the third trial, by Ginès *et al.* (51), a total of 70 cirrhotic patients with refractory ascites were included in the study (35 in each group). Patients in the TIPS group showed a better control of ascites compared with patients treated with paracentesis, as indicated by a lower number of patients developing ascites during follow-up (17 vs. 29; $P = 0.003$), and a lower number of episodes of ascites ($3.6 \pm 1$ vs. $11.7 \pm 3$; $P = 0.03$). Moreover, TIPS reduced the incidence of hepatorenal syndrome (three vs. 11 patients; $P = 0.03$). However, 13 patients in the TIPS group required angioplasty and/or the insertion of a new stent on 21 occasions due to dysfunction of TIPS. The incidence of encephalopathy during follow-up was higher in patients treated with TIPS compared with those treated with paracentesis. The differences were significant in relation to the mean number of episodes of encephalopathy per patient ($2.2 \pm 0.4$ vs. $1.1 \pm 0.2$; $P = 0.01$) and the incidence of severe (grade III–IV) encephalopathy (21 vs. 12 patients; $P = 0.03$). The probability of survival without liver transplantation was similar in both groups (26% at 2 years in the TIPS group, and 30% in the paracentesis group) (Fig. 19.3). A multivariate analysis of survival, including variables obtained at inclusion as well as treatment modality, showed that baseline BUN levels and Child–Pugh score were the only independent predictors of survival. Finally, the calculated costs were higher in the TIPS group than in the paracentesis group.

In the North American multicenter, randomized, controlled trial (52), 109 cirrhotic patients with refractory ascites were randomized to either medical therapy (sodium restriction, diuretics, and total paracentesis with intravenous infusion of albumin) (57 patients) or medical therapy plus TIPS (52 patients). Again, TIPS plus medical therapy was significantly superior to medical therapy in preventing recurrence of ascites (Fig. 19.4), whereas the total number of deaths in the two groups was identical (21 patients in each group). There was no difference in the frequency of adverse events, apart from a trend towards more moderate and severe hepatic encephalopathy in the TIPS group (20 vs. 12 patients; $P = 0.058$).

The results of these studies demonstrate that although TIPS facilitates the control of ascites in patients with refractory ascites, its use does not improve the overall results obtained with repeated paracentesis and intravenous albumin, as it usually increases the severity of hepatic encephalopathy and does not modify the natural history of the disease. Moreover, the probability of TIPS malfunction is similar to that of peritoneovenous shunt obstruction and its costs are higher than repeated paracentesis plus albumin. Considering these results, the International Ascites Club proposes paracentesis as the first-line treatment of refractory ascites. TIPS could be indicated in those patients requiring very frequent paracentesis (more than three times per month), without

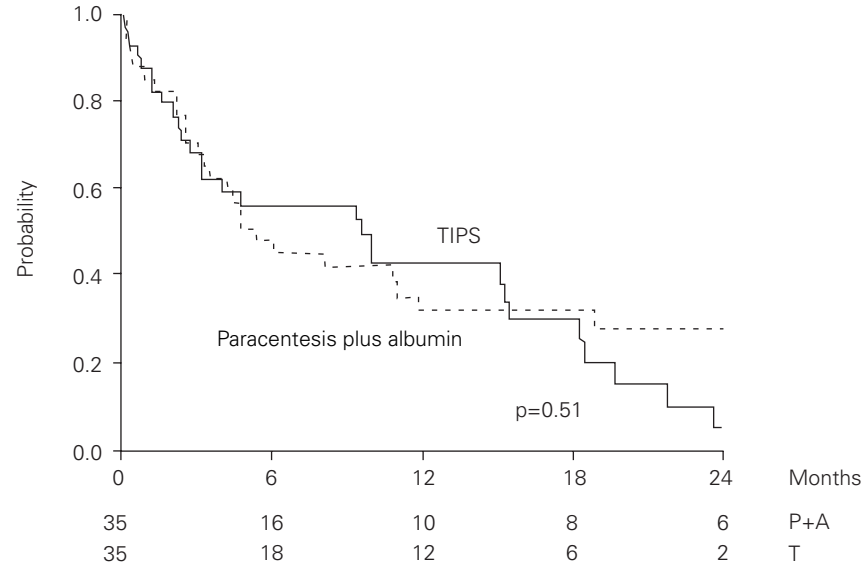
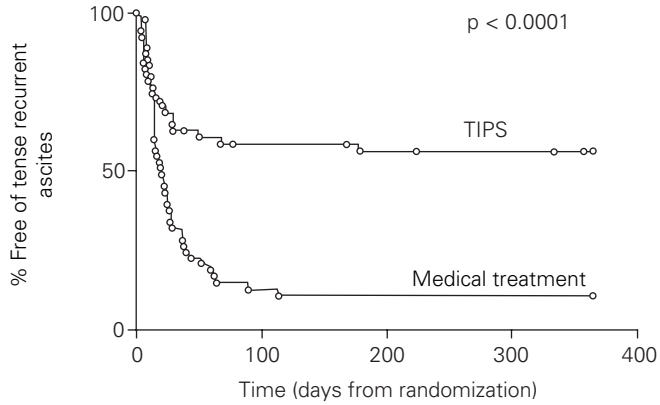



**Figure 19.3** Probability of survival after entry into the study in the two groups. Numbers below the graph are patients at risk at each time point (P+A, paracentesis plus albumin; T, TIPS). (Reproduced by permission from Ginès P, Uriz J, Calahorra B *et al*. Transjugular intrahepatic portosystemic shunting versus paracentesis plus albumin for refractory ascites in cirrhosis. Gastroenterology 2002; 123:1839–47.)




| | Time (days) | | | | |
|---|---|---|---|---|---|
| | 0 | 30 | 60 | 180 | 360 |
| TP | 57 | 23 | 10 | 6 | 5 |
| TP + TIPS | 52 | 36 | 29 | 26 | 20 |

**Figure 19.4** Probability of being free of tense recurrent ascites in patients managed with medical therapy and TIPS vs. medical therapy. The number of subjects at risk in each arm is shown below the figure. (Reproduced by permission from Sanyal AJ, Genning C, Reddy KR *et al*. The North American Study for the treatment of refractory ascites. Gastroenterology 2003; 124:634–41.)

previous episodes of hepatic encephalopathy or cardiac dysfunction, age > 70 years, and Child–Pugh score > 12.

## Liver transplantation

In patients with cirrhosis, refractory ascites is associated with a poor prognosis, which is determined mainly by the impaired liver function and the risk of bleeding from esophageal varices. The ultimate treatment for this condition is therefore liver transplantation. Patients who are otherwise good candidates for transplantation should certainly be referred for evaluation for transplantation as soon as ascites refractory to diuretic treatment develops, or even earlier. Since most of these patients have some degree of renal impairment, they probably have a slightly reduced long-term survival after transplantation. However, a systematic evaluation of the treatment success after liver transplantation in patients with cirrhosis and refractory ascites is not available.

## Technique of paracentesis

Although paracentesis is a very simple procedure, several precautions should be taken to avoid local complications. It is advisable to perform the paracentesis under strict sterile conditions. The abdomen should be cleaned, disinfected, and draped in a sterile fashion, and the physician should wear sterile gown, gloves, mask, and cap during the entire procedure. We use a modified Kus needle, which is a sharp-pointed blind metal needle, with a 7-cm 17-G metal blunt-edged cannula with side holes. Similar needles are now commercially available. Under local anesthesia, the Kus needle is inserted in the left abdominal quadrant. Once the needle enters the peritoneal cavity, the inner part is removed and the cannula is connected to a suction pump with a large volume capacity. The physician remains at the bedside throughout the treatment.

With this technique, the duration of the procedure ranges between 20 and 90 min, depending on the amount of ascitic fluid removed. In cases submitted to total paracentesis, the procedure is finished when the flow from the cannula becomes intermittent despite gentle mobilization of the cannula within the abdominal cavity and turning the patient to his or her left side. After the paracentesis, the patient should recline for 2 h on the side opposite to the paracentesis site to prevent leakage of ascitic fluid. Samples of ascitic fluid should be routinely taken for cell count, biochemical examination, and culture. The intravenous administration of albumin (8 g per liter of ascitic fluid removed) is initiated at the end of the procedure and given for 4–6 h. The patient can be discharged within the first day of the procedure.

Peripheral edema rapidly reabsorbs following the mobilization of ascites and usually disappears within the first 2 days after treatment. Most of this fluid goes to the peritoneal cavity in the form of ascites. It is therefore not uncommon for patients with massive peripheral edema to require a second paracentesis to remove the fluid shifted from the interstitial space to the intraperitoneal compartment.

## Contraindications and complications of paracentesis

Despite the fact that almost all published studies on paracentesis have excluded patients with spontaneous bacterial peritonitis, severe hepatic encephalopathy, thrombocytopenia, or severe jaundice, there is no evidence that these complications should be considered as contraindications for paracentesis in clinical practice. There are no data to support the correction of mild coagulopathy

with blood products prior to therapeutic paracentesis, but caution is needed when severe thrombocytopenia is present.

Acute complications following paracentesis are sporadic, although bleeding occasionally occurs and may be fatal. The most common complication is paracentesis-induced circulatory dysfunction and renal impairment. To date, there are no studies identifying factors that predict the development of these complications.

## Addendum

After writing this chapter, a fifth trial comparing paracentesis vs TIPS in patients with cirrhosis and refractory ascites has been published (53).

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# Chapter 20
# Transjugular Intrahepatic Portosystemic Shunt (TIPS) for the Management of Refractory Ascites in Cirrhosis

Guadalupe Garcia-Tsao

The ideal treatment of cirrhotic ascites should: (i) act upon the pathophysiological mechanisms that determine ascites formation, namely sinusoidal hypertension and sodium retention; (ii) be effective not only in eliminating ascites but in preventing its recurrence; (iii) be accompanied by a low morbidity; (iv) improve survival by preventing the development of complications of ascites such as spontaneous bacterial peritonitis (SBP) and the hepatorenal syndrome (HRS); (v) improve quality of life; and (vi) be cost-effective.

In the majority of cases (80–90%), ascites resolves with the use of diuretics, which act by producing a negative sodium balance (see Chapter 18). It is the 10–20% of patients in whom ascites is refractory to diuretics in which other therapies should be contemplated.

Refractory ascites is defined as ascites that cannot be mobilized or the early recurrence of which cannot be satisfactorily prevented by medical (i.e. diuretic) therapy. Refractory ascites assumes either diuretic-resistant ascites (ascites that cannot be mobilized even with maximal diuretic therapy and proper sodium restriction) or diuretic-intractable ascites (ascites that cannot be mobilized because maximal doses of diuretics cannot be reached given the development of diuretic-induced complications) (1).

The transjugular intrahepatic portosystemic shunt (TIPS) has been considered a therapeutic option in refractory ascites. By reversing the mechanisms responsible for the formation of ascites, TIPS should be effective not only in resolving ascites, but more importantly, in preventing its recurrence and its complications, thereby improving patient survival.

## Mechanism of action of TIPS in the treatment of ascites

As mentioned in Chapters 13 and 16, two major mechanisms participate in ascites formation in cirrhosis: hepatic sinusoidal hypertension and sodium retention. In turn, sodium retention results from splanchnic and peripheral vasodilation, leading to a decreased effective arterial blood volume and activation of sodium-retaining neurohumoral factors (renin, angiotensin, aldosterone).

Sinusoidal portal hypertension plays a fundamental role in the development of ascites. It has been shown that ascites is present only in patients in whom the hepatic venous pressure gradient (HVPG; which reflects sinusoidal pressure) is above 12 mmHg (2,3). In fact, two recent studies, one prospective (4) and one retrospective (5), showed that the development of ascites was significantly lower in patients in whom the HVPG decreased either < 12 mmHg or more than 20% from baseline values. In another study of TIPS for portal hypertension, it was shown that the portosystemic pressure gradient increased to 12 mmHg in all patients who developed ascites post-TIPS placement (6). The measurement of portosystemic gradient in this setting can also be considered an indirect measure of sinusoidal pressure.

Surgical portosystemic shunt therapy has been shown to be effective in the treatment of cirrhotic ascites. A retrospective study of trials in which one of the arms included a surgical shunt (either end-to-side portocaval shunt or distal splenorenal shunt) showed that the development of ascites was significantly lower in patients treated with surgical shunts compared with nonshunted patients (7). Although an end-to-side portocaval shunt would decrease the development of ascites by decompressing splanchnic capillaries and decreasing blood flow into the sinusoids, it can also lead to greater ascites formation, particularly in patients with advanced cirrhosis in whom the outflow block is such that the portal vein becomes the outflow tract. In a study by Voorhees *et al.* (8) that compared different types of portocaval shunts, it was noted that although the end-to-side portocaval shunt relieved ascites in over two-thirds of the patients, there was a proportion of patients (12%) in whom ascites appeared after

the shunt. This did not occur in patients with the side-to-side portocaval shunt or with the mesocaval shunts, with which there was 100% resolution of ascites and no patients developed ascites after shunting. This is concordant with experimental studies in dogs with hepatic vein ligation in which side-to-side portocaval shunting results in lesser ascites formation and lesser ascites reaccumulation compared with an end-to-side portocaval shunt (9). An end-to-side portosystemic shunt will decompress collaterals but may lead to greater formation of ascites, as it will close off the distal portion of the portal vein that, in refractory ascites, may act as an outflow tract and relieve intrasinusoidal pressure. On the other hand, the side-to-side portocaval shunt (and the mesocaval shunt), by connecting the side of the portal vein (or the mesenteric vein) to the low-pressure inferior vena cava, effectively decompresses not only the collaterals but also the sinusoids. However, given a high operative morbidity and mortality in this patient population and a high rate of severe hepatic encephalopathy (10), the surgical side-to-side portocaval shunt is practically never used for the treatment of refractory ascites.

TIPS is a nonsurgical procedure by which an intrahepatic artificial communication between the portal and the hepatic vein is created (11). It is performed by advancing a catheter introduced through the jugular vein into an hepatic vein and into a main branch of the portal vein. An expandable stent is then introduced connecting hepatic and portal systems and blood from the splanchnic and sinusoidal beds (high pressure) will be shunted to the hepatic vein (low pressure). It is performed by an interventional radiologist with experience in angiography and invasive hepatic procedures. TIPS decompresses the portal venous system, and thereby it decompresses portosystemic collaterals such as esophageal varices, and it has largely replaced the use of surgically placed shunts in the treatment of variceal hemorrhage. In fact, various randomized clinical trials that compare endoscopic therapy, another nonshunting therapy for variceal bleeding, vs. TIPS demonstrate a significant beneficial effect of TIPS in preventing recurrent variceal hemorrhage (12–14). However, these trials have identified hepatic encephalopathy and shunt dysfunction as long-term problems of TIPS without any survival benefit. The overall rate of post-TIPS encephalopathy in these trials is around 35% (a pooled risk increase of 16% compared with endoscopic therapy) and the rate of TIPS dysfunction is 55% in a mean follow-up period of 16 months (12).

In addition to decompressing the portal venous system, TIPS has theoretical advantages in the treatment of refractory ascites because (i) it decompresses the sinusoids and therefore should be as effective as a surgical side-to-side portocaval shunt in treating ascites without the morbidity and mortality associated with major surgery; and (ii) by connecting the portal vein with a systemic vein, the blood volume that is sequestered in the splanchnic circulation is transferred to the systemic circulation, effectively refilling the decreased effective arterial blood volume that leads to sodium retention.

## Effects of TIPS on hemodynamics and sodium-retaining mechanisms in cirrhotic patients with refractory ascites

Tables 20.1 and 20.2 summarize the results of several studies assessing the short-term (0–14 days) (15–19) and long-term (1–14 months) (15,16,18,20,21) effects of TIPS on systemic hemodynamics, liver and renal function, and neurohumoral systems in cirrhotic patients with refractory ascites.

As shown in Table 20.1, immediately after TIPS there is an increase in cardiac output and a decrease in systemic vascular resistance (already decreased at baseline in these patients), without changes in mean arterial pressure. These changes persist for at least 1 month but are no longer present 3 months or more after TIPS insertion. The increase in cardiac index is most probably the result of an increase in venous return secondary to shunting of blood sequestered in the splanchnic circulation into the systemic circulation through the newly created portosystemic fistula. The decrease in systemic vascular resistance (SVR) is probably the result of the increase in flow secondary to the increase in cardiac index (as shear stress increases, there is an increase in the synthesis of vasodilators such as nitric oxide). Table 20.1 also shows

**Table 20.1** Effects on hemodynamics after TIPS in patients with refractory ascites.

| | 30 min to 1 day | 7 days | 12–14 days | 1 month | 3–6 months | 7–14 months |
|---|---|---|---|---|---|---|
| CO | ↑ (15,16) | | ↑ (18) | ↑ (15,16,20) | Unchanged (18,20,21) | Unchanged (20,21) |
| SVR | ↓ (15) | | ↓ (18) | ↓ (15,16,20) | Unchanged (18,20,21) | Unchanged (20,21) |
| MAP | Unchanged (15,16) | Unchanged (16) | Unchanged (18) | Unchanged (15,16,20) | Unchanged (18,20,21) | Unchanged (20,21) |
| HVPG (mmHg) | 24.4→7.5 (16) | 22→11.4 (17) | 20→13 (18)<br>23.6→10.4 (19) | 30→19.2 (20) | 30→19 (20) | 30→16.8 (20) |

CO, Cardiac output; SVR, systemic vascular resistance; MAP, mean arterial pressure; HVPG, hepatic venous pressure gradient. (References are shown in parentheses.)

**Table 20.2** Effects on liver/renal/hormonal parameters after TIPS in patients with refractory ascites.

| | 1 day | 7 days | 12–14 days | 1 month | 3–6 months | 7–14 months |
|---|---|---|---|---|---|---|
| CPS | ↑ (15) | ↑ (16,17) | ↑ (16) | ↑ (15,16) or unchanged (20) | Unchanged (20) or ↓ (21) | ↓ (20,21) |
| $U_{Na}$ | Unchanged (15) | Unchanged (16) or ↑ (17) | ↑ (16,19) or unchanged* (18) | ↑ (15,16,20) | ↑ (18,20,21) | ↑ (20,21) |
| PRA | Unchanged (15) | Unchanged (16) or ↓ (17) | ↓ (16,19) or unchanged† (18) | ↓ (15,16,20) | ↓ (18,20,21) | ↓ (20,21) |
| Aldo | Unchanged (15) | ↓ (16,17) | Unchanged (16,18,19) | ↓ (15,16,20) | ↓ (20.21) or unchanged (18) | ↓ (20,21) |
| Serum Na | Unchanged (15) | Unchanged (16) | Unchanged (16,18) | Unchanged (15,16,20) | Unchanged (18,20) | Unchanged (20) |
| GFR | Unchanged (15) | Unchanged (16) | Unchanged (16,18,19) | Unchanged (15,16) | Unchanged (18) | No data |
| Creatinine clearance | No data | No data | No data | ↑ (20) | Unchanged (20) | Unchanged (20) |

CPS, Child–Pugh score; $U_{Na}$, urinary sodium; PRA, plasma renin activity; Aldo, aldosterone; Serum Na, serum sodium; GFR, glomerular filtration rate. *Increased but not statistically significantly. †Decreased but not statistically significantly. (References are shown in parentheses.)

that the mean HVPG is significantly reduced compared with baseline at all studied time-points after TIPS placement, although this effect appears to decrease progressively over time, with mean HVPGs at the > 1- month time-points being above the 12-mmHg threshold.

As shown in Table 20.2, up to the first month post-TIPS there is a deterioration in liver function, with most studies showing a significant increase in Child–Pugh score (CPS); however, by 3 months post-TIPS, CPS has been shown to be similar to baseline and most studies show an improvement in CPS 7–14 months after TIPS placement, probably as a result of resolution/improvement of ascites. Despite this early deterioration in CPS and a seeming deterioration in hemodynamics (Table 20.1), urine sodium significantly increases as soon as 7 days and definitely 1 month after TIPS, persisting for at least the next 7–14 months. The increase in urinary sodium correlates closely with a decrease in plasma renin activity (PRA). Plasma aldosterone levels also decrease significantly, but do not mirror the changes in urinary sodium excretion as closely as PRA. Notably, neither urinary sodium excretion nor levels of neurohumoral substances reach normal values at any time post-TIPS. Furthermore, one of the studies (17) showed that this effect on urinary sodium excretion occurs only in cirrhotic patients with ascites (particularly those with refractory ascites) but does not occur in those without ascites. Of note, no significant differences in glomerular filtration rate, creatinine clearance, serum creatinine or serum sodium concentration are observed after TIPS placement.

## Uncontrolled trials of TIPS in refractory ascites

An increase in sodium excretion, a decrease in volume of ascites, and lower diuretic requirements had been noted in patients with hemorrhagic portal hypertension who had undergone placement of TIPS (22,23). Based on these results, Ferral *et al.* first reported in 1993 the efficacy of TIPS in the treatment of refractory ascites (24). In this study that included only 14 patients, complete resolution of ascites was achieved in seven (50%) of the patients, in six patients ascites failed to resolve, and the remaining patient died 72 h after TIPS procedure as a result of peritoneal bleeding, a complication of the procedure. Additionally, as had been recognized in studies of TIPS for variceal hemorrhage, this study recognized the development of new encephalopathy in two patients and shunt dysfunction in four patients. In a mean follow-up of 7.6 months, eight patients died, a 57% mortality.

Since then, four retrospective cohort studies and eight prospective cohort studies of TIPS in patients with refractory ascites, in which patients have been followed for more than 6 months, have been published (25). The results of the eight prospective uncontrolled studies (19,20,26–31) are summarized in Table 20.3, in which a favorable response is defined as elimination (complete response) or reduction (partial response) of ascites with reduction in the frequency of large-volume paracenteses; hepatic encephalopathy (HE) refers to new or worsening encephalopathy after TIPS requiring medical treatment or hospitalization; and TIPS dysfunction refers to narrowing or occlusion of TIPS leading to recurrent ascites and/or need for TIPS revision. Success in TIPS placement in these studies was essentially 100%. In one of the studies, technical failures occurred in 4/50 (8%) patients, three of whom had markedly shrunken liver positioned high in the abdomen.

In these uncontrolled studies, TIPS appeared to eliminate and/or make ascites easier to manage in the majority of patients (70%); however, diuretics were still required

**Table 20.3** Uncontrolled prospective cohort studies of TIPS for refractory ascites.

| Author/ reference number | *n* | Follow-up (months) | Favorable response | Responders requiring diuretics | New or worse HE | Shunt dysfunction | Mortality |
|---|---|---|---|---|---|---|---|
| Quiroga (20) | 17 | 15.5 | 15 (88%) | NR | 3 (18%) | 9 (53%) | 5 (29%) |
| Ochs (26) | 50 | 14.2 | 46 (92%) | 46 (100%) | 8 (16%) | 21 (42%) | 31 (62%) |
| Somberg (19) | 24 | 9.4–11 | 19 (79%) | 2/5 (40%) | 7 (29%) | 9 (38%) | NR |
| Crenshaw (27) | 54 | 9.1 | 40 (74%) | 40 (100%) | 12 (22%) | 11 (20%) | 27 (50%) |
| Martinet (28) | 30 | 8.8 | 26 (87%) | 26 (100%) | 14 (47%) | 8 (31%) | 17 (57%) |
| Nazarian (29) | 50 | 11.6 | 23 (46%) | NR | 20 (40%) | 17 (34%) | 30 (60%) |
| Deschenes (30) | 53 | 17.5 | 25 (47%) | NR | 14 (26%) | 17 (32%) | 23 (43%) (6 months) |
| Peron (31) | 48 | 11.2 | 35 (73%) | NR | 10 (21%) | 18 (38%) | ~ 70% 1-year cumul. |
| Total | 326 | 11.2 | 229 (70%) | 114/117 (97%) | 88 (27%) | 110 (34%) | 133/254 (52%) |

NR, not reported.

(at lower doses) in essentially all the patients. Shunt dysfunction (with consequent recurrence of ascites) occurred in a third of the patients and new or worsened hepatic encephalopathy occurred in over 25% of the patients. In a median follow-up of around 11 months, the observed mortality in these studies was quite variable but averaged around 50%.

As summarized recently (32), the procedure-related mortality of TIPS is very low (1–2%) and causes include hemoperitoneum, hemobilia, hemolysis, and sepsis. During TIPS placement, the liver capsule is frequently punctured (reported frequency around 33%), particularly with small, shrunken livers. However, intraperitoneal bleed only occurs in 1–2% of the cases. Fistulae may develop between the portal vein and an intrahepatic artery or the biliary tree (leading to hemobilia), but this is rare. Hemolysis may develop in 10–15% of patients and usually resolves within 3–4 weeks of TIPS placement. Sepsis has been reported to occur in 2–10% of patients. In uncontrolled studies of TIPS placement in patients with refractory ascites, the procedure-related complication rate is around 9% (29 of 326 patients), distributed as follows: intraperitoneal hemorrhage (3%), acute renal failure (3%) most described as being secondary to contrast media, sepsis (1.5%), and hemolysis (1.2%). A unique complication is the development of strangulated umbilical hernia following resolution after TIPS (33).

## Controlled trials comparing TIPS with serial large-volume paracenteses

Large-volume paracentesis (LVP) is the most commonly used method to treat refractory ascites. It is a local therapy that does not modify any of the mechanisms that lead to ascites formation. As mentioned in Chapter 19, LVP is usually associated with intravenous albumin infusion to increase effective arterial volume, but this effect is transient. Therefore, ascites will always recur in patients with refractory ascites, unless an improvement in liver disease occurs (e.g. resolution of alcoholic hepatitis), and the need for repeated procedures, besides increasing the cost of LVP, also increases morbidity, including an impaired quality of life associated with ascites recurrence and repeated hospital visits. Additionally, LVP plus albumin has not been shown to improve survival (34,35). TIPS, by relieving sinusoidal hypertension and reversing neurohumoral factors involved in sodium retention, is theoretically a more definitive treatment for ascites than LVP plus albumin and, by preventing other complications of portal hypertension and ascites, such as variceal hemorrhage, HRS, and SBP, it could have a beneficial effect on survival. On the other hand, as shown in uncontrolled studies, TIPS has been shown to lead to the development of liver failure and/or hepatic encephalopathy as a result of diversion of blood away from the liver and into the systemic circulation.

Four prospective randomized trials of TIPS vs. LVP have been published in full to date (18,36–38) and are summarized in Tables 20.4 and 20.5.

The first study, by Lebrec *et al.* (18), included only 25 patients (13 randomized to TIPS, 12 to LVP) and showed that treatment was efficacious (no LVP needed for at least 1 month) in 83% of the patients in whom TIPS was placed, compared with none of the patients in the LVP group. Since patients were already requiring more than one LVP per month at the time of inclusion in the study, this result was predictable. However, patients randomized to TIPS had a significantly higher mortality than patients in the LVP group. Further analysis revealed that this higher mortality was due to a higher mortality in Child C patients.

The study by Rossle *et al.* (36) included 60 patients with refractory or recidivant ascites (29 randomized to TIPS, 31 to LVP) and also demonstrated that elimination of ascites and/or elimination of the need for LVP was significantly higher in the group treated with TIPS (84%) compared with the group treated with LVP (43%). Of note, LVP was not routinely followed by the administration of albumin in this study, and although the investigators found a tendency for a lower mortality in the TIPS group, differences failed to achieve statistical significance.

**Table 20.4** Baseline characteristics of patients included in controlled studies of TIPS vs. large-volume paracentesis (LVP) for refractory ascites.

| Author | Tx | *n* | Age | Alcohol | CPS (% Child C) | Bilirubin (mg/dl) | Creatinine (mg/dl) | Serum Na (mEq/l) (% < 130) | $U_{Na}$ (mEq/l) (% < 10) | HVPG (mmHg) |
|---|---|---|---|---|---|---|---|---|---|---|
| Lebrec (18) | LVP | 12 | 52 | 83% | 9.2 (33%) | 1.6 | 1.0 | 130 | < 5 | 22→20 |
| | TIPS | 13 | 50 | 77% | 9.3 (31%) | 2.0 | 1.0 | 130 | < 5 | 20→13 |
| Rossle (36) | LVP | 29 | 61 | 74% | 8.7 (23%) | 1.8 | 1.4 | 131 (13%) | 61 (10%) | – |
| | TIPS | 31 | 58 | 83% | 9.1 (38%) | 1.8 | 1.3 | 130 (17%) | 45 (40%) | 24→10 |
| Ginès (37) | LVP | 35 | 56 | 60% | 9.2 (43%) | 2.4 | 1.4 | – (48%) | 9 | – |
| | TIPS | 35 | 59 | 51% | 9.3 (37%) | 2.0 | 1.4 | – (54%) | 7 | 19.1→8.7 |
| Sanyal (38) | LVP | 57 | 52 | 33% | 9.3 (NR) | 1.9 | 0.98 | NR | NR | – |
| | TIPS | 52 | 56 | 32% | 9.2 (NR) | 1.9 | 1.07 | NR | NR | 19.8→8.3 |

NR, Not reported; CPS, Child–Pugh score; $U_{Na}$, urine sodium; LVP, large-volume paracentesis; TIPS, transjugular intrahepatic portosystemic shunt. Values given are mean values or percentages. (References are shown in parentheses.)

The study by Ginès *et al.* (37) included 70 patients with cirrhosis and refractory ascites (35 randomized to TIPS, and 35 to repeated LVP plus intravenous albumin). As expected, recurrence of ascites was greater in the LVP group. Contrary to other trials, the primary endpoint in this study was survival without liver transplantation. In a mean follow-up of 9.4 (TIPS group) and 10.8 (LVP+ albumin group) months, 20 (57%) patients treated with TIPS and 18 (51%) treated with LVP died (a nonsignificant difference), while seven patients in each group underwent liver transplantation. The probability of survival without liver transplantation was 41% at 1 year and 26% at 2 years in the TIPS group, compared with 35% and 30% in the paracentesis group, respectively ($P = 0.51$). The frequency of severe hepatic encephalopathy was greater in the TIPS group, as were the calculated costs.

In the most recent study, by Sanyal *et al.* (38), 109 subjects with refractory ascites were randomized to either medical therapy (sodium restriction, diuretics, and total paracentesis) ($n = 57$) or to medical therapy plus TIPS ($n = 52$). As shown by all other studies, TIPS was significantly superior to medical therapy alone in preventing recurrence of ascites. There were no significant differences in the two arms with respect to overall and transplant-free survival. There was a higher incidence of moderate to severe encephalopathy in the TIPS group, but no differences in quality of life between study groups.

Meta-analysis of these four studies (D'Amico, personal communication) reveals a significant benefit of TIPS (compared with LVP) in terms of recurrent ascites, with no differences in overall mortality.

Details of these four studies are summarized in Tables 20.4 and 20.5. Table 20.4 shows the general characteristics of patients included in these studies. As can be observed, the cirrhotic patient populations are quite homogeneous among studies, with a median age of 56 years, a CPS around 9.2, bilirubin levels around 1.9, and a similar decrease in HVPG after TIPS insertion. Important differences appear in urinary sodium excretion, highest in the study by Rossle *et al.* (36), in which only 22% of the

**Table 20.5** Controlled studies of TIPS vs. large-volume paracentesis (LVP) for refractory ascites.

| Author | Tx | *n* | Mean follow-up (months) | Favourable response | New or worse HE | TIPS Dysfunction | Mortality | 1-year cumulative survival | 2-year cumulative survival |
|---|---|---|---|---|---|---|---|---|---|
| Lebrec (18) | LVP | 12 | NR | 0 (at 4 months) | 0 | – | 4 (33%) | ~ 72%* | 60% |
| | TIPS | 13 | NR | 5 (38%) (at 4 months) | 3 (23%) | 3 (23%) | 9 (69%) | 29%* | 29% |
| Rossle (36) | LVP | 29 | 44 | 7 (24%) (at 3 months) | 3 (10%) | – | 23 (79%) | 52% | 32% |
| | TIPS | 31 | 45 | 20 (64%) (at 3 months) | 6 (19%) | 13 (42%) | 15 (48%) | 69% | 58% |
| Ginès (37) | LVP | 35 | 11 | 6 (17%) | 12 (34%)† | – | 18 (51%) | 35% | 30% |
| | TIPS | 35 | 9 | 18 (51%) | 21 (60%)† | 13 (37%) | 20 (57%) | 41% | 26% |
| Sanyal (38) | LVP | 57 | NR | 9 (16%) | 12 (21%) | – | 19 (33%) | ~ 70%* | ~ 60%* |
| | TIPS | 52 | NR | 30 (58%) | 20 (38%) | 53% at 6 months | 18 (35%) | ~ 70%* | ~ 60%* |

*Derived from survival curve. Values given are mean values or percentages. †Severe encephalopathy; NR, not reported. (References are shown in parentheses.)

patients had a urinary sodium excretion of < 10 mmol/day, while this was near 100% in all other studies. This difference may explain the higher survival rates in this study, which was only comparable to survival in the TIPS group of the study by Sanyal *et al.* (38), in which unfortunately sodium levels (urinary or serum) are not reported. Hyponatremia was more frequent in patients included in the study by Ginès *et al.* (37), in which patients also had higher serum creatinine levels. This study showed lower survival rates compared with the other two larger studies, which is not surprising as hyponatremia and renal dysfunction have both been described as being predictors of a poor survival in cirrhosis (39,40), and more recently serum creatinine (> 1.7 mg/dl) and sodium (serum sodium < 130 mmol/l) were identified as the only independent predictors of survival in patients undergoing TIPS for variceal hemorrhage (41). Interestingly, while most earlier studies included a majority of patients with alcoholic etiology of cirrhosis, the study by Sanyal *et al.* (38) included mainly patients with non-alcoholic cirrhosis, predominantly hepatitis C. Patients in this study had a reasonably good survival, which is rather unexpected considering that in this group of patients liver disease is assumedly progressive, while in patients with alcoholic cirrhosis, alcohol abstinence would be associated with an improved survival.

In these prospective controlled studies, TIPS was technically unsuccessful in 5% of the cases (7/131). Although all the studies report a decrease in diuretic requirement, it appears that all patients continued to require diuretics. The procedure-related complications were only specified in the study by Gines *et al.* (37) and consisted of heart failure in four (11%) and severe hemolysis in three (9%) patients.

## Other beneficial effects of TIPS in patients with refractory ascites

In the study by Ginès *et al.* (37), a preventive effect of TIPS on the development of HRS was observed. While *de novo* HRS (in patients without HRS at inclusion) or progression from type 2 HRS to type 1 HRS occurred in 11 (31%) patients in the LVP plus albumin group, this only occurred in three (9%) patients randomized to the TIPS group. This beneficial effect is probably related to the effect that TIPS placement has on the hormonal systems involved in the pathogenesis of renal dysfunction in cirrhosis with suppression of the activity of the renin–angiotensin–aldosterone system (Table 20.2).

It has been noted that some of the patients in whom ascites resolves after successful insertion of TIPS also have a remarkable improvement in nutrition. A study performed in 10 patients with refractory ascites who were malnourished at baseline (as indicated by baseline reduced nitrogen index, percentage body fat, and resting energy expenditure) showed a significant increase in dry weight, total body nitrogen (a measure of lean body mass not affected by the presence or absence of ascites), and resting energy expenditure 3 and 12 months after TIPS placement compared with baseline, indicating an improvement in body composition (42). This effect was ascribed to an increase in energy intake or enhanced nutrient absorption, neither of which was confirmed in the study.

TIPS placement has also been associated with an improvement in quality of life (QOL) in patients in whom it is placed for management of refractory ascites. In an uncontrolled study performed in 21 cirrhotic patients (43), QOL (assessed by the QOL index and by the patient's rating of their QOL on a visual analog scale) significantly improved; however, this improvement was observed only in patients with a complete response (elimination of ascites). Oddly, in patients with a partial response (presence of ascites not requiring paracentesis) there was no significant improvement in QOL, indicating that QOL in patients with ascites may be influenced not only by the invasiveness of repeated LVP but also by the presence of ascites, which may cause significant abdominal discomfort. However, these results could not be confirmed in the only randomized controlled study in which QOL was evaluated (38). In this study, the SF-36 questionnaire was performed at baseline and at months 6 and 12 after TIPS placement. Although both components, the physical scale and the mental scale, improved significantly in both study groups compared with baseline, there were no significant differences between patients treated with LVP vs. patients randomized to TIPS.

## Predictors of post-TIPS mortality

Several prognostic models have been developed to predict survival in patients undergoing TIPS placement. The first was the MELD (Model of End-Stage Liver Disease) model that was originally developed for patients undergoing TIPS (44) and was then modified slightly to predict survival in cirrhotic patients in general (45). MELD is a continuous function of serum bilirubin levels, international normalized ratio for prothrombin time, and serum creatinine to predict 3-month post-TIPS survival and was validated in an independent patient population. In another retrospective study from Emory University (46), variables found to be independent predictors of survival were a bilirubin level > 3.0 mg/dl, an alanine aminotransferase level > 100 IU/l, pre-TIPS encephalopathy, and urgency of TIPS. This model was also validated in a separate group of patients. In the MELD study, only 25% of patients had TIPS placed for management of refractory ascites, while in the Emory study 38% had TIPS placed for refractory ascites and 19% had it placed for the treatment of refractory hepatic hydrothorax. Neither of them

showed that the indication for TIPS had an influence on survival.

Several recent studies have compared these models with the CPS and have shown divergent results. One study showed that the MELD score is better than CPS score in predicting 3-month survival but not in predicting long-term survival (47), while another study found no differences in short-term survival and only a marginal advantage of MELD in long-term survival (48). Another study showed that both scores were equally predictive of 1-month, 3-month, and 1-year survival (49), with a cut-off for the Child score of 11. Interestingly, in this study, which included 475 patients, those undergoing TIPS for refractory ascites (that constituted 32% of the patient population) had a significantly poorer survival than patients with variceal bleeding. This study also found that renal function was the strongest independent predictor of survival.

## TIPS for refractory hepatic hydrothorax

Hepatic hydrothorax is produced by the passage of ascitic fluid from the peritoneal cavity into the pleural space through small holes in the diaphragm. In this smaller space, small amounts of fluid (2–3 l) lead to shortness of breath and requirement of therapeutic thoracentesis, which in some patients may be quite frequent despite the use of diuretics. It is considered that these patients have refractory hepatic hydrothorax and small, uncontrolled studies have shown that TIPS is effective in this setting (50–54). These studies are summarized in Table 20.6. The results are comparable to those of uncontrolled studies of TIPS for refractory ascites (Table 20.3). A favorable response (either resolution of the pleural effusion or a decrease in the need for thoracentesis) occurs in 67% of patients, new or worsened hepatic encephalopathy occurs in 30% of patients, and shunt dysfunction in half the patients. The mortality is around 46% in a follow-up period of 7 months, which would suggest that the mortality is higher in this group of patients, particularly in those who are nonresponders to TIPS, in whom a 100% mortality has been described (51,53,54). Therefore, although TIPS seems to be an effective form of therapy for this condition, its use should be limited to patients with hepatic hydrothorax who require repeated thoracentesis for control of symptoms despite inpatient treatment with diuretics.

## Conclusions

Since the publication of the first uncontrolled study of TIPS for the treatment of refractory ascites 10 years ago, advances have been made in our understanding of the effect of TIPS in cirrhotic patients with refractory ascites, including the publication of four prospective randomized trials comparing TIPS vs. LVP. TIPS placement is associated with a reduction in sinusoidal pressure and a significant improvement in urinary sodium excretion that correlates with suppression in plasma renin activity (indicative of an improvement in effective arterial blood volume). It is therefore clearly effective in preventing the recurrence of ascites, recurrence that is practically universal in patients treated with LVP. However, this beneficial effect of TIPS is offset by an increase in the incidence of severe hepatic encephalopathy, a high incidence of shunt dysfunction, and a higher cost. Importantly, TIPS is not associated with a survival benefit or with a quality of life benefit when compared with LVP. Therefore, the consensus recommendation put forward recently is that first-line treatment of refractory ascites is repeated LVP plus albumin and that TIPS should be considered when the frequency of paracentesis is greater than two to three times per month (55). A good predictor of post-TIPS survival is the CPS and a score > 11 should be considered a contraindication for TIPS placement. Patients with alcoholic cirrhosis who are drinking alcohol may improve with abstinence and therefore TIPS should be delayed in these patients. It should be noted that all these studies used bare (uncovered) TIPS stents. New expanded polytetrafluoroethylene (ePTFE)-covered stents have recently become available for TIPS placement and a recent retrospective study of 89 patients in whom ePTFE-covered stents were used (50% for refractory ascites) showed a survival benefit for the covered stent when compared with patients in whom convention-

**Table 20.6** Uncontrolled prospective cohort studies of TIPS for refractory hepatic hydrothorax.

| Author | *n* | Follow-up (months) | Favorable response | New or worse HE/liver failure | Shunt dysfunction | Mortality |
|---|---|---|---|---|---|---|
| Strauss (50) | 5 | 10.4 | 2 (40%) | NR | 3 (60%) | 2 (40%) |
| Gordon (51) | 24 | 7.2 | 19 (79%) | 13 (54%) | 13 (54%) | 13 (68%) |
| Jeffries (52) | 12 | 5.8 | 7 (58%) | 4 (33%) | 7 (58%) | 3 (25%) |
| Siegerstetter (53) | 40 | 16.0 | 28 (70%) | 3 (8%) | 16 (40%) | 17 (42%) |
| Spencer (54) | 21 | 7.2 | 12 (57%) | 9 (43%) | 13 (61%) | 12 (57%) |
| Total | 102 | 7.2 | 68 (67%) | 29/97 (30%) | 52 (51%) | 47 (46%) |

HE, Hepatic encephalopathy; NR, not reported. (References are shown in parentheses.)

al TIPS were placed (56). This benefit needs to be prospectively evaluated in patients with refractory ascites.

## Addendum

After writing this chapter, a fifth trial comparing paracentesis vs TIPS in patients with cirrhosis and refractory ascites has been published (57).

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# Chapter 21
# Prognosis of Patients with Cirrhosis and Ascites

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Cirrhosis is a chronic and progressive disease characterized by the development of complications related to portal hypertension and circulatory dysfunction, including ascites, dilutional hyponatremia, gastrointestinal bleeding, hepatic encephalopathy, bacterial infections, and renal failure. Ascites is the most common complication of cirrhosis and constitutes the first manifestation of the disease in the majority of patients (1,3). The appearance of ascites is a marker of poor prognosis (1–7). However, these patients can be classified in different groups according the severity of ascites and associated renal function abnormalities and their response to treatment. For instance, patients with mild sodium retention and normal solute-free water excretion and glomerular filtration rate (GFR) and good response to diuretics have a good prognosis that is very different compared with that of patients with refractory ascites. The identification of factors predictive of survival in patients with cirrhosis and ascites has been of pivotal importance because it identifies patients that benefit from liver transplantation at an early stage and provides accurate information to patients and their relatives regarding the clinical course and outcome (8). Recently some prognostic models that specifically address prognosis and survival of patients with cirrhosis and ascites have been proposed (7,9). Nevertheless the recent introduction of the Model for End-Stage Liver Disease (MELD) score for organ allocation in the USA that includes variables of liver function and renal function has taken precedence among other prognostic scores in patients with cirrhosis (10). The purpose of this chapter is to review the current knowledge of the natural history and survival of cirrhosis with ascites.

## Compensated cirrhosis

In the past, the diagnosis of cirrhosis was usually made when patients presented major clinical manifestations of the disease, such as ascites, jaundice, variceal bleeding or hepatic encephalopathy. Presently, a significant number of patients are diagnosed before the appearance of these complications, a condition commonly referred to as compensated cirrhosis. The survival and natural history of patients with compensated cirrhosis is well established in several large series of patients (1,2,5,11–17) (Table 21.1). This condition is associated with a long life expectancy. Studies published during the last decade have reported a greater long-term survival than those published during the 1980s. These differences may be related to several factors, including diagnosis of cirrhosis at earlier stages, screening for hepatocellular carcinoma (which could allow for an early therapy), improved therapeutic methods in the management of major complications of the disease, and effects of antiviral therapy on the evolution of patients with hepatitis B or hepatitis C-related cirrhosis. Nevertheless, despite a relatively long survival, many patients with compensated cirrhosis go on to develop major complications that lead to increased morbidity and mortality. The probability of developing ascites in patients with compensated cirrhosis has been calculated to range between 10 and 50% 2–5 years after diagnosis (2,5,17). Risk factors for the development of ascites in patients with compensated cirrhosis have not specifically been assessed. However, portal hypertension is a prerequisite for the development of ascites. This is demonstrated by the fact that patients submitted to surgical portosystemic shunts and transjugular intrahepatic portosystemic shunts (TIPS) for treatment or prevention of variceal bleeding do not develop ascites during the course of the disease (18,19). Therefore, it is likely that a certain level of portal pressure is required for the development of ascites. A definite cut-off level has not been established, but ascites rarely develops in patients with a portal pressure, as assessed by hepatic venous pressure gradient, lower than 12 mmHg (20,21). Nevertheless, other factors undoubtedly play an important role because not all patients with portal pressure above this level develop ascites.

**Table 21.1** Survival of compensated cirrhosis.

| Author | Study period | Patients (*n*) | Survival probability (%) 1 year | 5 years | 10 years | Comments |
|---|---|---|---|---|---|---|
| D'Amico *et al.* (5) | 1959–76 | 90 | 80 | 53 | 30 | All etiologies of cirrhosis |
| Saunders *et al.* (1) | 1974–80 | 435 | 94 | 67 | NR | All etiologies of cirrhosis |
| Ginès *et al.* (2) | 1968–80 | 293 | 96 | 68 | 47 | All etiologies of cirrhosis |
| Tanaka *et al.* (11) | 1958–84 | 582 | 89 | 62 | 42 | All etiologies of cirrhosis |
| Realdi *et al.* (14) | 1973–90 | 366 | 98 | 84 | 68 | HBV-cirrhosis |
| Fattovich *et al.* (15.) | 1982–92 | 384 | 100 | 91 | 79 | HCV-cirrhosis |
| Zoli *et al.* (12) | 1983–84 | 100 | 90 | 45 | | All etiologies of cirrhosis |
| DeJongh *et al.* (13) | 1970–90 | 98 | 95 | 71 | NR | HBV-cirrhosis |
| Fattovich *et al.* (16) | NR | 297 | 97 | 85 | | HBV/HCV-cirrhosis |
| Benvegnu *et al.* (17) | 1986–96 | 312 | > 95 | 90 | 75 | HBV/HCV-cirrhosis |

Some percentages have been estimated from the survival curves included in the different articles. NR, not reported. Numbers given in parentheses correspond to the reference number.

## Cirrhosis with ascites

### Survival of patients with ascites

The development of ascites in patients with cirrhosis is usually indicative of a poor prognosis. In most series, the probability of survival of cirrhotic patients with ascites ranges between 45 and 82% at 1 year of follow-up and decreases below 50% after 5 years (1–4,6–8) (Table 21.2). Long-term survivors (more than 10 years) are very uncommon. It should be noted that most studies analyzing survival of cirrhosis with ascites were performed in patients admitted to hospital for management of ascites but without concomitant complications and included a large proportion of patients with a past history of ascites. Therefore, data may not be applicable to patients with mild or moderate sodium retention in which ascites is easily managed with diuretics usually without hospitalization, or to hospitalized patients with ascites and associated severe complications (i.e. gastrointestinal bleeding, spontaneous bacterial peritonitis, or hepatocellular carcinoma). Furthermore, these figures cannot be extrapolated to estimate survival of patients with first-onset ascites, because these patients have a better survival than those with previous episodes of ascites (Fig. 21.1).

A significant proportion (10–15%) of patients with cirrhosis and ascites develop severe sodium retention that cannot be appropriately treated either because patients do not respond to high doses of diuretics or because they develop side-effects that preclude their use. This condition is known as refractory ascites (22). In most of these patients sodium retention is associated with severely impaired solute-free water excretion and reduced renal plasma flow and GFR. Patients with refractory ascites constitute a subset of patients with ascites with particularly poor prognosis, the 1-year survival probability being of only 50% (6,9,23–26) (Table 21.3).

### Prognostic factors of patients with ascites

A number of factors with prognostic value have been identified in patients with cirrhosis and ascites. Some factors are related to liver dysfunction whereas others are secondary to circulatory and renal dysfunction commonly present in these patients.

**Table 21.2** Survival of cirrhosis with ascites.

| Author | Study period | Patients (*n*) | Survival probability (%) 1 year | 2 years | 5 years | 10 years |
|---|---|---|---|---|---|---|
| Powell and Klatskin (4) | 1951–63 | 182 | 65 | 60 | 52 | NR |
| Saunders *et al.* (1) | 1959–76 | 512 | 45 | 34 | 22 | 10 |
| Llach *et al.* (8) | 1980–85 | 139 | 56 | 49 | 25 | NR |
| Salerno *et al.* (6) | 1983–89 | 134 | 78 | 63 | 38 | NR |
| Fernández-Esparrach *et al.* (7) | 1980–90 | 216 | 59 | 46 | 27 | 16 |
| Planas *et al.* (3) | 1998–2001 | 200 | 82 | 70 | 41 | – |

Some percentages have been estimated from the survival curves included in the different articles. NR, not reported. *Numbers given in parentheses correspond to the reference number.

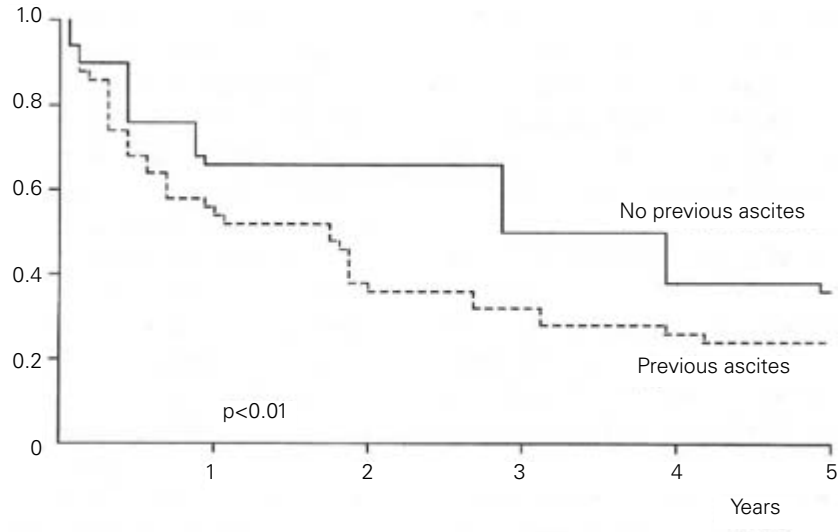


**Figure 21.1** Comparison of long-term survival of cirrhotic patients with first-onset ascites and patients with previous episodes of ascites.

### *Liver function*

In clinical practice, the degree of liver dysfunction is usually assessed by measuring the plasma levels of proteins synthesized by the liver, particularly albumin and coagulation factors, or the plasma levels of substances metabolized by the liver, especially bilirubin and bile acids (27). A reduction in the former and an increase in the latter indicate impaired liver function. A number of studies have shown that parameters of liver function correlate with prognosis and may be useful in clinical practice to estimate survival in the general population of patients with cirrhosis (28–32). It is, therefore, not surprising that some liver function tests have a strong prognostic value in the specific population of patients with cirrhosis and ascites. For example, increased serum bilirubin or reduced serum albumin are associated with a short survival in these patients (6–8,33). By contrast, prothrombin activity has no predictive value in patients with ascites (6–8). This lack of predictive value may be due to the fact that the prolongation of prothrombin time in patients with cirrhosis occurs very late in the evolution of the disease (34). In other disease states such as primary biliary cirrhosis or primary sclerosing cholangitis serum bilirubin levels in conjunction with serum albumin are good markers of prognosis (35,36).

**Table 21.3** Survival of patients with refractory ascites.

| Author | Study period | Patients (*n*) | Survival probability (%) | | |
|---|---|---|---|---|---|
| | | | 6 months | 1 year | 2 years |
| Salerno *et al.* (6) | 1983–89 | 24 | 75 | 58 | 38 |
| Guardiola *et al.* (9) | 1980–92 | 100 | 75 | 42 | 38 |
| Ginès *et al.* (23) | 1987–89 | 121 | 70 | 58 | 40 |
| Ginès *et al.* (24) | 1991–93 | 89 | 70 | 50 | 40 |
| Ginès *et al.* (25) | 1996–2000 | 70 | 50 | 38 | 20 |
| Sanyal *et al.* (26) | 1997–2000 | 107 | 90 | 75 | 60 |

Some percentages have been estimated from the survival curves included in the different articles. Numbers given in parentheses correspond to the reference number.

### *Renal function*

It is now well established that the severity of renal and circulatory dysfunction is of major prognostic significance in patients with cirrhosis and ascites. Hecker and Sherlock published the first report of the prognostic value of renal and circulatory dysfunction in patients with liver disease and ascites in 1956 (37,38). These authors described nine patients with acute or subacute viral hepatitis or cirrhosis who were admitted to hospital with impaired renal function and/or hyponatremia. Most patients developed progressive renal failure and severe hyponatremia during hospitalization and all of them died. The renal and electrolyte disturbances were associated with marked arterial hypotension. Since then, a number of studies have assessed the prognostic value of renal and hemodynamic abnormalities in patients with cirrhosis and ascites.

In 1981, Arroyo *et al.* (39) demonstrated the importance of sodium excretion as a prognostic factor in cirrhosis with ascites. Marked sodium retention was found to be associated with reduced survival. The importance of this simple parameter as guide to prognosis in cirrhosis with ascites has been confirmed in subsequent studies (7,8,40). However it is essential to consider that for the evaluation of prognosis, sodium excretion should be measured in conditions of fixed and controlled sodium intake (usually a low-sodium diet of 70–90 mEq/day during 5–7 days) and off diuretics. Cirrhotic patients who, under these conditions, have a sodium excretion close to or greater than sodium intake have a good prognosis. On the other hand, patients with a markedly reduced sodium excretion (< 10 mEq/l) compared with their intake have a poor outcome (7,8,39,40) (see also Chapter 16).

An impaired ability to excrete solute-free water has also prognostic significance in cirrhotic patients with ascites. In 1965, Shear *et al.* (41) prospectively investigated the relationship between free water excretion and short-term prognosis in a series of 32 cirrhotic patients with ascites. Water excretion was measured after an intravenous water load (dextrose) of 20 ml/kg body weight. All patients with markedly impaired water excretion died within a short period of time. By contrast, patients with preserved renal ability to excrete free water had a markedly better survival. Cosby *et al.* (42) later showed that water excretion correlates with long-term prognosis in cirrhosis with ascites. In this study water excretion was evaluated by measuring diuresis during a 5-h period after the administration of a water load of 20 ml/kg of 5% dextrose i.v. in a group of 21 cirrhotic patients with ascites. Patients with normal (> 80% excretion over 5 h) or moderately impaired water excretion (20–80%) had a relatively good long-term prognosis, whereas patients with markedly impaired water excretion (< 20%) had very poor prognosis. Fernandez-Esparrach *et al.* (7) recently confirmed the predictive value of water excretion in the evaluation of long-term survival in a large series of cirrhotic patients admitted to a single institution for the treatment of ascites. The renal capacity to excrete solute-free water was assessed by measuring diuresis after a water load of 20 ml/kg body weight of 5% dextrose i.v. Survival estimates for patients with normal diuresis after water load (> 8 ml/min) at 1, 5, and 10 years of follow-up were 85, 41, and 32%. Corresponding values for patients with moderately reduced (3–8 ml/min) or severely reduced (< 3 ml/min) diuresis after water load were only 55, 26, and 13%, and 37, 13, and 3%, respectively (Fig. 21.2).

The development of dilutional hyponatremia in patients with cirrhosis and ascites is also a marker of poor prognosis, as it indicates the existence of a markedly impaired renal capacity to excrete solute-free water

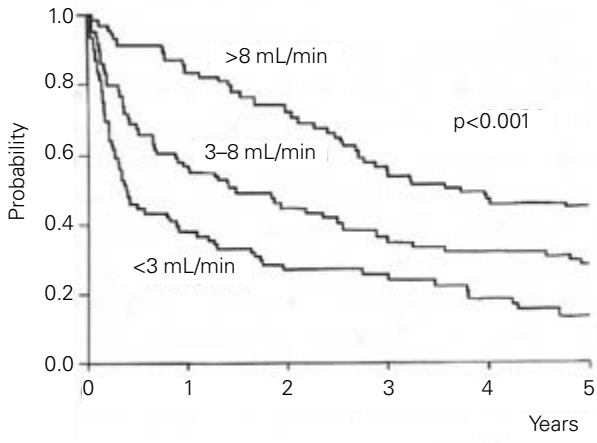


**Figure 21.2** Survival of patients with cirrhosis and ascites as function of pretreatment water excretion, as assessed by measuring diuresis after water load.

(7,8,43,44). As discussed in Chapter 26, this is a common complication among hospitalized patients with cirrhosis and ascites and is associated with a poor survival. The appearance of dilutional hyponatremia after a precipitating event such as hemorrhage or infection is associated with a better prognosis when compared with the spontaneous appearance of this complication (Fig. 21.3) (44). This is possibly related to a higher incidence of renal dysfunction and a more advanced stage of decompensated cirrhosis associated with spontaneous dilutional hyponatremia.

Finally, the intensity of renal vasoconstriction also correlates with prognosis in patients with ascites (45). In clinical practice, renal vasoconstriction is generally estimated by assessing GFR through serum creatinine and/or BUN levels. Slight increases in serum creatinine or BUN (from 1.2 to 1.5 mg/dl and 20 to 30 mg/dl, respectively) are indicative of important reductions in GFR and are associated with reduced survival (Fig. 21.4) (46). Estimation of GFR should be performed in the absence of diuretic therapy as diuretics may significantly increase serum creatinine and BUN levels. Renal vasoconstriction can also be assessed in clinical practice by measuring the resistive index in renal arteries by Doppler ultrasonography. An increased renal resistive index is associated with reduced survival and high risk of developing hepatorenal syndrome (47,48). Therefore, ultrasonographical evaluation of the renal circulation may provide useful prognostic information in cirrhosis with ascites.

### *Circulatory function*

Circulatory dysfunction in patients with cirrhosis and ascites also correlates with survival. Patients with low arterial pressure have a poor prognosis compared with patients with normal arterial pressure (7,8). The activity of vasoconstrictor systems, which are activated as a homeostatic response to maintain systemic hemodynamics, also has prognostic value in cirrhosis with ascites. Patients with increased plasma renin activity or plasma norepinephrine levels have a shorter survival compared with patients with normal values of these parameters (7,8,33,39,40).

### *Pulmonary function*

Since the 1960s, pulmonary consequences of cirrhosis and portal hypertension such as hepatopulmonary syndrome (HPS) have been extensively investigated. HPS is defined by the presence of chronic liver disease, arterial deoxygenation, and widespread intrapulmonary vasodilation (49). The majority of patients have moderate to large ascites; however, HPS may appear in the absence of ascites. In a recent study, Schenk *et al.* evaluated the effect of HPS on survival in patients with cirrhosis (50). The

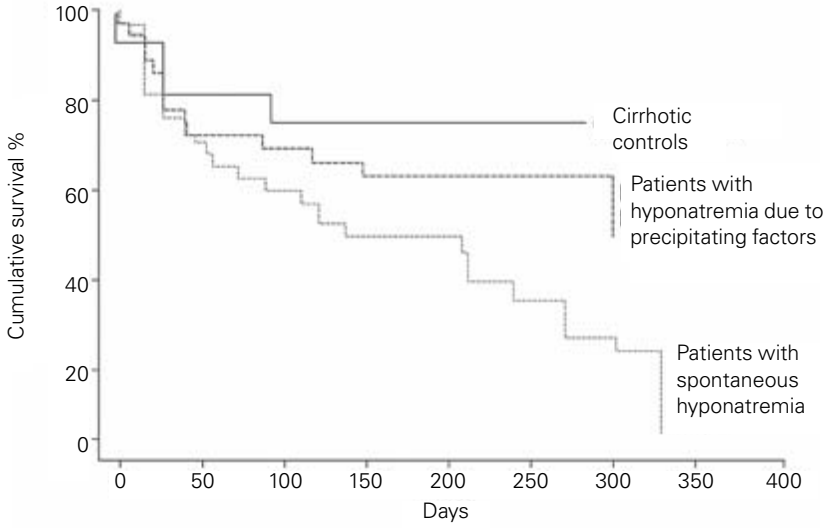


**Figure 21.3** Survival of patients with cirrhosis with and without hyponatremia. The mortality in patients with precipitating factors is less than that in patients with spontaneous dilutional hyponatremia. (Reproduced with permission from Porcel *et al.* (44).)

study showed that the presence of HPS had a major influence on survival in patients with cirrhosis. HPS was an independent prognostic factor, together with age, Child–Pugh class, and renal function. The worst survival was showed in patients with Child–Pugh class C and HPS (five times lower median survival). Thus, the authors suggested that HPS should be carefully sought after and that its presence should influence patient management, scoring systems and accelerate the evaluation process for liver transplantation.

## Assessment of prognosis in cirrhosis with ascites

The prediction of the outcomes of patients with cirrhosis has changed enormously over the past 5 years, particularly with the introduction of the MELD (model for end-stage liver disease) score (10). The main objective of prognostic models such as the Child–Pugh and MELD is to provide precise information in order to make an accurate prediction of survival in a specific patient. For this purpose several prerequisites are needed: (i) assessment of predictive factors of survival in a large group of patients taking into account all possible clinical variables; (ii) development of a prognostic model (an index that combines variables with independent predictive value that allows the estimation of the probability of survival for individual patients) that should ideally use easily available parameters with strong predictive value; and (iii) the accuracy of the prognostic model should be validated and tested in subsequent and prospective series of patients and, preferably, in different settings (51).

As mentioned previously, a number of variables with prognostic value, particularly those that take into account renal and circulatory function, have been identified in

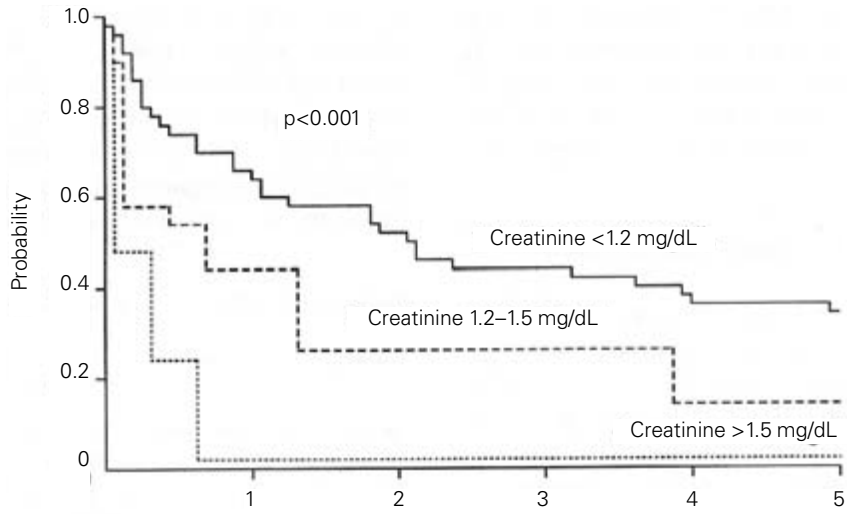


**Figure 21.4** Survival of patients with cirrhosis and ascites as function of serum creatinine measured in conditions of low-sodium diet and without diuretic therapy.

these patients. Nonetheless, only one prognostic model that includes these variables (renal capacity to excrete a water load, mean arterial pressure, Child–Pugh class, and serum creatinine) has been proposed. However, this test has not gained acceptance and may not be easily applicable in all centers (7) (Fig. 21.5). Another prognostic model for patients with refractory ascites included variables of the Child–Pugh score, ascitic fluid, previous episodes of spontaneous bacterial peritonitis, and history of alcohol consumption, but not those of renal or circulatory function (9). Again, this model has not gained acceptance as a model for prognosis in patients with cirrhosis and ascites. Reluctance in accepting these models may be due to the fact that they were published at the time that the MELD score was proposed as a prognostic index for patients with end-stage liver disease and was adopted as a model for organ allocation in the USA in 2002.

For several decades, the Child–Pugh classification has been used in clinical practice to estimate survival of patients with ascites. This classification was originally designed to estimate the risk of death in cirrhotic patients submitted to surgery for the treatment of portal hypertension (52,53). This system includes variables such as ascites, encephalopathy, serum bilirubin, serum albumin, and prothrombin time (Table 21.4). Subsequent to its application to estimate surgical risk, the use of Child–Pugh classification was validated and extended to evaluate long-term prognosis of cirrhosis (28,54,55). The simplicity of the Child–Pugh classification has determined its wide utilization as prognostic model to evaluate survival in cirrhosis. However, the Child–Pugh classification has important drawbacks that limit its use as a prognostic indicator for patients with ascites. First, it does not include variables of renal or circulatory function, which are known to be very important prognostic factors in these patients. Second, prothrombin time which is one of the variables included in the classification has not shown to be of prognostic value in patients with ascites (6,8). Moreover, it can vary from laboratory to laboratory depending on the control measures used, which frequently are not comparable. Third, because of its qualitative na-

**Table 21.4** Child–Pugh classification.

| | Points | | |
|---|---|---|---|
| Variable | 1 | 2 | 3 |
| Encephalopathy | Absent | I–II | III–IV |
| Bilirubin (mg/dl) | < 2 | 2–3 | > 3 |
| Ascites | Absent | Mild | Tense |
| Prothrombin time (%) | > 50 | 30–50 | < 30 |
| Serum albumin (g/l) | > 35 | 28–35 | < 28 |

Group A: 5–6 points; group B: 7–9 points; group C: ≥ 10 points.

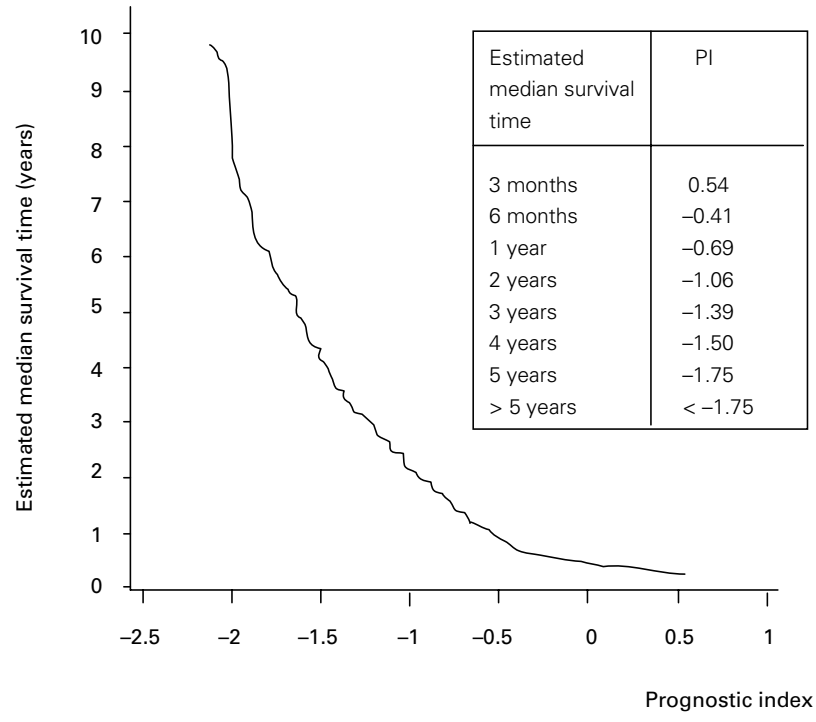


**Figure 21.5** Estimated median survival time as a function of the prognostic index developed for patients with cirrhosis and ascites. Reproduced with permission from Fernandez-Esparrach *et al.* (7). Inset: a sample of estimated median survival times and their corresponding values of prognostic index Two hypothetical examples of calculation of PI are as follows: Case 1. A patient with Child–Pugh class B and a diuresis after water load of 16 ml/min, mean arterial pressure of 90 mmHg, and serum creatinine of 0.6 mg/dl at presentation has a PI of − 2.53, which corresponds to an estimated median survival time of more than 5 years. Case 2. A patient with Child–Pugh class C and a diuresis after water load of 3 ml/min, mean arterial pressure of 80 mmHg, and serum creatinine of 2 mg/dl at presentation has a PI of − 0.47, which corresponds to an estimated median survival time between 6 months and 1 year.

Prognostic index (PI) = −0.071 × diuresis after water load (in ml/min) − 0.0178 × mean arterial pressure (in mmHg) ± 0.4738 × Child–Pugh class (B = 0; C = 1) ± 0.3433 × serum creatinine (in mg/dl).

ture, the score does not distinguish patients with serum bilirubin values > 3 mg/dl, prothrombin ratio < 30%, or serum albumin > 28 g/l. Lastly, the Child–Pugh classification includes hepatic encephalopathy and ascites, two measures that are subject to a wide clinical interpretation and are not objective (56). The main problem with the Child–Pugh classification is for patients that belong to the Child–Pugh class B. It is well known that Child–Pugh class A patients usually show good medium-term survival without transplantation unless other complications occur, while Child–Pugh class C patients are considered the conventional candidates for liver transplantation. However, Child–Pugh class B patients are a heterogeneous group in which patients could remain stable for a long period or on the other hand can suddenly deteriorate into class C. Although these pitfalls were known for years, no other prognostic model of wide applicability and objective measures had been identified until recently.

The MELD scoring system was created with the aims of better predicting survival in patients undergoing a transjugular intrahepatic shunt (TIPS) placement (57). In this model, International Normalized Ratio (INR), total serum bilirubin level, serum creatinine level, and etiology of cirrhosis were used to predict survival following placement of a TIPS for any cause. The formula developed ($3.8 \log_e$ [Bilirubin {mg/dl}] + $11.2 \log_e$ [INR] + $9.6 \log_e$ [Creatinine {mg/dl}] + 6.4) (etiology: 0 if cholestatic or alcoholic and 1 otherwise) was found to accurately predict survival (particularly at 3 months) after TIPS was placed (57). This prognostic index was modified by removing the etiology and then implemented in the USA as the MELD model to establish priority of patients awaiting liver transplantation (10). The advantages of this system are that variables are objective and predictive. For example, bilirubin is a robust variable also included in the Child–Pugh classification; serum creatinine as an estimation of GFR is a well-known variable associated with prognosis in cirrhotic patients (8); and INR is the international normalized ratio for prothrombin time. This model was derived from a heterogeneous group of patients at four medical centers in the USA and validated in an independent dataset from the Netherlands (10). Because survival following portosystemic shunts was predominantly determined by the severity of the underlying disease, the authors of the model, by removing etiology of liver disease, proposed that the same model could be used as a prognostic model in patients with end-stage liver disease as candidates to liver transplantation. The MELD score is therefore a slight modification of the risk score used in the original TIPS model and only includes bilirubin, INR, and serum creatinine. The initial experience with the model revealed good 3-month prediction of survival (Fig. 21.6). An online worksheet is available to calculate the MELD score (http://www.mayoclinic.org/gi-rst/mayomodel5.html).

Recently, MELD was introduced as a tool to predict mortality risk and assess disease severity in patients with cirrhosis. The model's validity was tested with a retrospective dataset obtained from a heterogeneous group of patients with liver disease consisting of hospitalized adult patients, outpatients with primary biliary cirrhosis, and a retrospectively generated cohort of cirrhotic patients from the USA and Italy from a time when liver transplantation was not available (58). In order to validate the MELD score as a prognostic index in determining survival in liver transplant candidates, the authors chose the 3-month survival as the primary outcome. In this study the authors found that the MELD score was useful in grading the risk of death at 1 week, 3 months, and 1 year in patients considered for liver transplantation (58) (Table 21.5). Interestingly, the etiology of liver disease and presence of portal hypertension and its complications did not influence the accuracy of the MELD model. However, most of the patients included in this study had compensated cirrhosis, and 75% of the patients had a MELD score < 20, a cut-off in which patients show better survival. Therefore it is only a small group of patients that made the high-risk group, explaining the poor survival. Another result of this study was that

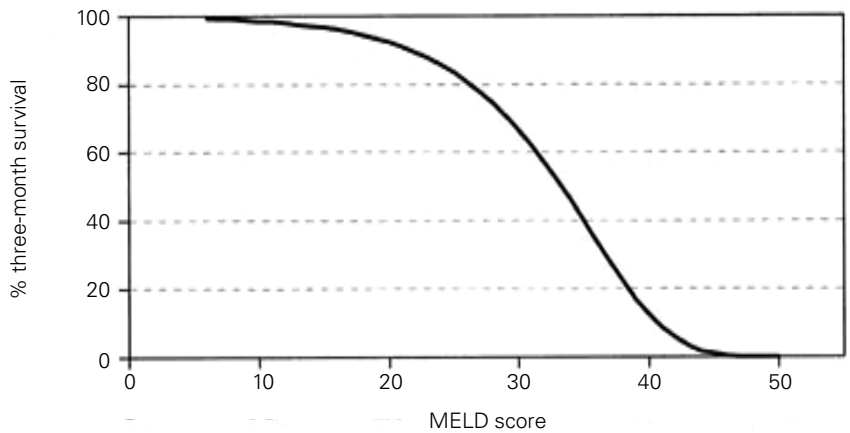


**Figure 21.6** Estimated 3-month survival as a function of Model for End Stage Liver Disease (MELD) score. (Reproduced with permission from Wiesner *et al.* (54).)

**Table 21.5** Concordance of Model for End Stage Liver Disease (MELD) scores in predicting 3-month and 1-year mortality in cirrhotics. PBC, primary biliary cirrhosis. (From Wiesner *et al.* (58) with permission.)

| | No. of patients | No of deaths within 3 months | 3-month mortality concordance | 1-year mortality concordance |
|---|---|---|---|---|
| Hospitalized cirrhotics | 282 | 59 | 0.87 | 0.85 |
| Outpatient cirrhotics | 491 | 34 | 0.80 | 0.78 |
| PBC outpatients | 326 | 5 | 0.87 | 0.87 |
| Historical cirrhotics | 1179 | 220 | 0.78 | 0.73 |

the addition of clinical complications often seen in these patients, such as ascites, encephalopathy, variceal bleeding, or spontaneous bacterial peritonitis did not improve significantly the ability of MELD score in determining prognosis.

In another study, the MELD score was validated in a prospective population of patients with chronic liver disease in a waiting list (59). The authors found that the waiting list mortality increased directly in proportion to the listing MELD score. In contrast with the previous study, most patients presented decompensated cirrhosis. Moreover, according to different cut-offs of MELD, there were a significant number of patients in each group. This fact could explain, at least in part, differences in 3-month survival between the studies (Table 21.6). Another interesting result is that comparing the area under the receiver–operating characteristic curve (ROC) using 3-month mortality as the end point, the curve for the MELD score was significantly higher than those for the Child–Pugh score (Fig. 21.7). These numbers suggest that the MELD score is able to predict 3-month mortality among patients with chronic liver disease on the liver waiting list and can be applied for allocation of donor livers. Finally, the MELD score was also retrospectively evaluated in a group of 129 cirrhotic patients in Italy with a follow-up period of 1 year (60). In this study, MELD score was an excellent predictor of both 6-month and 12-month survival and its performance was as good as the Child–Pugh score.

**Table 21.6** Differences in 3-month survival between two studies validating the Model for End Stage Liver Disease (MELD) score for a population of cirrhotic patients awaiting liver transplantation.

| | 3-month mortality (%) | |
|---|---|---|
| | Kamath *et al.* (10) | Weisner *et al.* (59) |
| MELD | | |
| < 9 | 3.7 (1380) | 1.9 (124) |
| 10–19 | 20 (711) | 6 (1800) |
| 20–29 | 45.5 (137) | 19.6 (1098) |
| 30–39 | 74.5 (41) | 52.6 (295) |
| > 40 | 100 (9) | 71.3 (120) |
| Child–Pugh | | |
| 7–9 | ND | 4.3 (318) |
| 10–12 | ND | 11.2 (2357) |
| 13–15 | ND | 49.5 (588) |

Numbers given in parentheses correspond to the number of patients included in each group. ND, not determined.

In February 2002, the UNOS (United Network for Organ Sharing) changed the rules for the allocation of donor organs by replacing the Child–Pugh classification with the MELD score. The advantages of MELD over the Child–Pugh are that MELD uses objective parameters, and introduces the renal function within the score, a variable which is well known to impact in the prognosis of cirrhotic patients (8). Moreover, there seems to be an important component of prognosis when variation of the MELD score is considered over time. Proponents of this method state that mortality risk on the liver transplant waiting list is predicted more accurately by serial MELD score determinations than by single MELD because measurements at different time points suggests progression of the disease (61). As a result, UNOS has mandated frequent updates of MELD scores every 7 days in patients with MELD score ≥ 25, every 30 days for those between 19 and 24, every 90 days between 11 and 18 and every year for patients with ≤ 10.

An unanswered question is the validity of MELD as a predictor of survival in patients with ascites and cirrhosis. As discussed above, other than abnormalities in liver function, patients with cirrhosis and ascites may develop derangements in renal function and systemic hemodynamics of variable intensity, including sodium and water retention, renal failure, arterial hypotension, and activation of vasoconstrictor systems. Interestingly, it has been shown that in these patients parameters assessing renal function and systemic hemodynamics abnormalities have better prognostic accuracy than liver function tests (6–8). However, these parameters are uncommonly used in the evaluation of prognosis of patients with ascites because of the lack of appropriate prognostic models. Although serum creatinine is included in the MELD score, patients with ascites and severe sodium retention and dilutional hyponatremia, which are known to be associated with a poor outcome (6,8), may have a normal serum creatinine. Unfortunately, some of these patients have low MELD scores, which precludes them from advancing in a transplant list. Unpublished data from our Unit suggest that a different prognostic model, including variables of renal function, serum sodium, and Child–Pugh score, has better predictability of survival than the MELD score in patients with ascites. Recent data from other groups indicate that

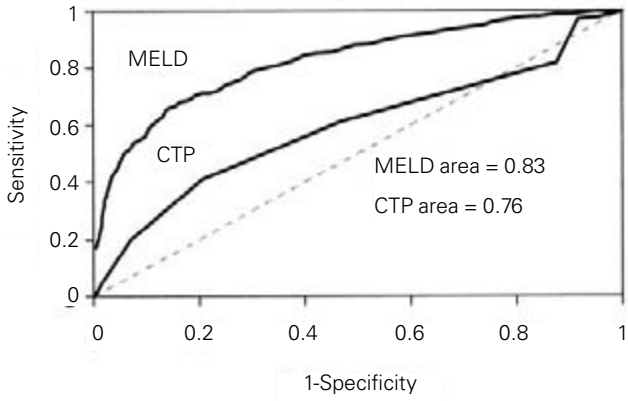


**Fig. 21.7** The area under the receiving–operating curve (ROC) for the Model for End Stage Liver Disease (MELD) score and Child (CTP) score. The end point is 3-month mortality. The dotted line represents the ROC based on chance alone with a c-statistic of 0.5. (Reproduced with permission from Wiesner *et al.* (54).)

a more accurate and improved prognostic model for patients with cirrhosis and ascites may be the addition of serum sodium to the MELD score, which improves its prognostic accuracy.

## Spontaneous bacterial peritonitis

Spontaneous bacterial peritonitis (SBP) is a common complication of cirrhotic patients with ascites. The pathogenesis, clinical findings, and management of SBP are described extensively in Chapters 34 and 35. Although the short-term prognosis of patients with SBP has markedly improved in recent years, the mortality rate until recently was still high, ranging from 20 to 40%. A decrease in these numbers is related to the implementation of i.v. albumin administration as a plasma expander at diagnosis of infection and 48 h later in order to prevent the development of renal failure (62). The incidence of functional renal failure in patients with SBP receiving albumin together with antibiotic therapy is 10%, compared with an incidence of 33% in patients not receiving albumin. Most importantly, hospital mortality was lower in patients receiving albumin (10%) vs. those not receiving plasma expansion (29%). Although resolution of the infection is obtained in most patients, morbidity and mortality may occur due to the presence of advanced liver failure and/or severe concomitant complications, including encephalopathy, gastrointestinal bleeding, or renal failure. The prevention and therapy of this infection-induced renal failure represent a major advance in the management of decompensated cirrhotic patients. Other factors found to be associated with an increased mortality rate in patients with SBP are pretreatment renal failure, old age, positive ascitic fluid culture, and increased bilirubin levels.

## Summary

The development of ascites is an important milestone in the natural history of cirrhosis. Cirrhotic patients that present with ascites have a probability of survival between 45 and 80% at 1 year and it decreases below 50% at 5 years. It is therefore extremely important that these patients be promptly recognized and referred for evaluation of liver transplantation. Of the several prognostic factors of survival identified in patients with cirrhosis and ascites, those parameters measuring renal and circulatory function have the strongest predictability of survival. Although these prognostic factors are very useful, an easily applicable prognostic model based on these parameters has been difficult to establish. The most commonly used prognostic model in clinical practice is the Child–Pugh score; however, this model does not include such variables and does not on its own adequately perform survival prediction in patients with cirrhosis and ascites. In recent years other prognostic models developed in aims of better predicting survival have been introduced into clinical practice. The most utilized is the MELD score, a model that is now used in the USA for establishing priority for organ allocation in liver transplantation. In contrast to the Child–Pugh score, this model incorporates renal function (i.e. serum creatinine) as a variable and therefore may be a better predictor of survival in patients with cirrhosis and ascites. Unfortunately, many patients with cirrhosis and ascites have normal serum creatinine despite having low GFR, low serum sodium, and low arterial blood pressure, parameters with important prognostic information not included in either the Child–Pugh or MELD scores. Further studies are needed to improve the accuracy of prognostic models in patients with cirrhosis and ascites.

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# Chapter 22
# Liver Transplantation for Patients with Cirrhosis and Ascites

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## Preoperative aspects

### General considerations

At present liver transplantation is considered as the optimal treatment for most patients with end-stage liver disease. In this setting, cirrhosis is currently the most important indication for liver transplantation in adult patients (1,2). However, one important problem is the low number of organ donors compared with the increasing number of potential candidates to receive a liver transplant. Therefore, the selection of such candidates is mandatory. Although different approaches may be used for this selection, the most adequate, from the medical point of view, is based on two main desirable goals (3-6):

**1** Transplantation should be useful for candidates in terms of survival expectancy. This objective is achieved by accepting for liver transplantation those patients whose probability of survival after transplantation is estimated to be clearly higher than the survival probability if they received conservative therapy that did not include liver transplantation.

**2** Survival after transplantation should be long enough to obtain reasonable benefit from the scarce liver grafts. This second objective is achieved by rejecting for transplantation those patients with circumstances that represent relevant risk factors for this procedure and which are expected to reduce unacceptably their probability of survival after transplantation. This involves a careful work-up for assessing the existence of possible contraindications for liver transplantation.

In this part of the chapter only specific issues concerning the selection of cirrhotic patients with ascites for liver transplantation will be addressed.

### Adequate timing for transplantation

Adequate timing for liver transplantation in a particular patient is usually established by the calculation of his or her estimated survival probability, both with and without transplantation.

#### *Survival expectancy after liver transplantation*

For a given patient the probability of surviving after transplantation can be extrapolated from the results obtained in patients who have undergone liver transplantation for similar conditions. However, in the case of cirrhotics with ascites there are several considerations to take into account. First, the results achieved after transplantation in cirrhotic patients with ascites have been specifically shown in very few reports (3,7). Furthermore, the results can vary from one center to another according to different factors, such as the experience of the transplant team (8) or the criteria for accepting and rejecting patients for transplantation (9). Therefore, for decision making in selecting liver transplant candidates it is recommended to use the survival obtained in the center where the candidate is evaluated as the reference. As an illustrative example, Fig. 22.1A shows the cumulative survival obtained in cirrhotic patients with ascites who received liver transplantation in our center during the period 1988–2002, which was 84% at 1 year after transplantation and 73% at 3 years.

#### *Survival expectancy with conventional management*

The probability of survival of cirrhotic patients with ascites treated with conservative management without liver transplantation can be estimated from the results obtained in studies investigating the natural history and prognostic factors in these patients. Although the prognosis of cirrhotic patients with ascites as a whole is relatively poor (Fig. 22.1B), these patients represent a nonhomogeneous population. Different parameters have been identified as predictors of survival in these patients (10–16). Several of these parameters correspond to standard clinical and laboratory data and consequently can

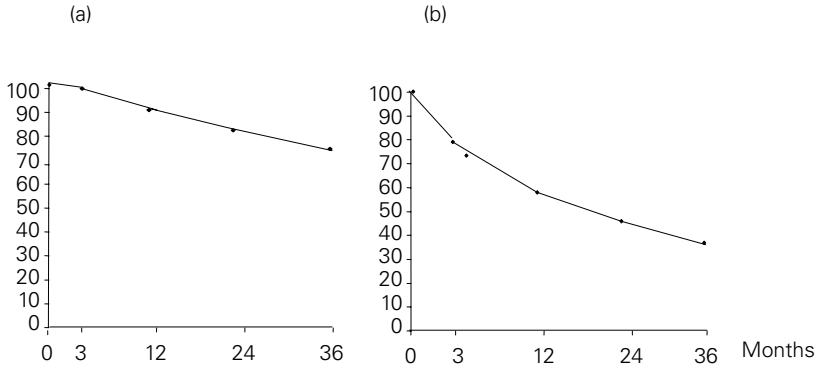


**Figure 22.1** (a) Long-term survival probability after liver transplantation in 486 cirrhotic patients with ascites. Patients underwent transplantation at the Hospital Clinic, Barcelona, during the period 1988–2002. (b) Survival probability in 216 cirrhotic patients with ascites who were conventionally managed, that is, without liver transplantation. Patients were admitted to the Hospital Clinic, Barcelona, during the period 1980–1990.

easily be used for assessing prognosis of these patients in the clinical setting. The most relevant factors associated with poor survival expectancy in cirrhotic patients with ascites are listed in Table 22.1. The presence of any of these factors has been found to be associated with a low survival probability, below 65% at 1 year and 50% at 3 years. Since these figures are clearly lower than the survival currently expected after transplantation, these factors can reasonably be taken as criteria for indicating liver transplantation in cirrhotic patients with ascites.

In the authors' experience, around 80–90% of cirrhotic patients with ascites present one or more of the factors shown in Table 22.1. Therefore, most patients with ascites should be considered as potential candidates for liver transplantation. In the remaining patients, however, survival expectancy with conservative management is similar to that with liver transplantation. Therefore, liver transplantation is not indicated in these patients because no benefit would be obtained if they underwent this procedure.

In the last few years increasing importance has been paid to the Model for End-Stage Liver Disease (MELD) score as a method to establish the priority for graft allocation in patients awaiting liver transplantation (2,17,18). However, no value for the MELD score has been defined yet to indicate liver transplantation in cirrhotic patients with ascites and, therefore, decide to list these patients for transplantation or not. Nevertheless, it should be noted that two of the three variables used to calculate the MELD score, serum bilirubin and serum creatinine (17), have consistently shown to have prognostic significance in cirrhotic patients with ascites (Table 22.1). Therefore, it is possible that future studies could also establish the usefulness of the MELD score in the indication of liver transplantation in cirrhotic patients with ascites.

**Table 22.1** Variables associated with a poor prognosis in cirrhotic patients with ascites.

| |
|---|
| Clinical variables: |
| Ascites refractory to diuretic therapy |
| Resolved spontaneous bacterial peritonitis |
| Poor nutritional status |
| Absence of hepatomegaly |
| Arterial hypotension (mean arterial pressure < 85 mmHg) |
| Hepatic encephalopathy |
| Laboratory variables: |
| Low serum albumin level (< 28 g/l) |
| High serum bilirubin (> 2.5 mg/dl) |
| Low urinary sodium excretion (< 2 mEq/day)* |
| Reduced urinary water excretion (< 6 ml/min)† |
| Low serum sodium concentration (< 133 mEq/l) |
| Renal failure (serum creatinine > 1.3 mg/dl and/or BUN > 30 mg/dl) |
| Combined variables: |
| High Child–Pugh score (> 10) |

*In the absence of diuretics. †Urine output after a water load of 20 ml/kg of body weight.

## Contraindications and risk factors for liver transplantation

Circumstances considered as contraindications for liver transplantation in cirrhotic patients with ascites are essentially the same as those in every liver transplant candidate and are summarized in Table 22.2. However, some of these circumstances, as well as other factors that are particularly frequent in ascitic cirrhotic patients, have a great influence on the post-transplantation results and require specific comments concerning their management while patients are on the waiting list for transplantation.

### *Renal failure*

With few exceptions (19,20), renal impairment, a relatively frequent finding in cirrhotic patients with ascites, has been found to be associated with a marked decrease in survival expectancy after transplantation (7,21–27). In most of these studies, particularly those using multivariate analyses, renal dysfunction has been identified as a variable with a strong significant value for poor prognosis after transplantation (21–23,26,27). As an example, in

**Table 22.2** Contraindications for liver transplantation in cirrhotic patients with ascites.

| |
|---|
| General contraindications: |
| Absolute |
| Massive adhesions precluding hepatectomy |
| Complete portal vein thrombosis* |
| Short-term lethal extrahepatic disease† |
| Severe infectious complications‡ |
| Relative |
| Poor nutritional status |
| Renal failure |
| Premortem stage of liver disease |
| Advanced age |
| Moderately severe extrahepatic disease |
| Adverse psycho-social circumstances |
| Other: history of abdominal surgery or extrahepatic malignancies |
| Specific contraindications in some populations:§ |
| Alcoholic patients |
| Important alcohol-related extrahepatic disease or neuropsychological abnormalities |
| Risk factors for alcohol recidivism: abstinence < 6 months, unfavorable social or familial circumstances |
| Patients with hepatitis B virus infection |
| Positive hepatitis B virus DNA |
| Patients with coexistent hepatocellular carcinoma |
| Uninodular: tumor diameter > 5 cm |
| Two or three nodules: tumor diameter > 3 cm |
| More than three nodules |
| Tumoral invasion of large hepatic vessels |
| Extrahepatic metastases |

*Alternative surgical techniques have been described for allowing transplantation. †There is the possibility of simultaneous transplantations when the extrahepatic disease involves an organ susceptible to transplantation (i.e. kidney, heart, or lung). ‡Contraindication disappears when infection resolves. §Mainly in relation to the risk of recurrence of liver disease after transplantation.

one study which included only patients with nonbiliary cirrhosis (most presenting with ascites at the time of transplantation), the hospital survival rate in patients with normal pretransplantation blood urea nitrogen (BUN) values was 90%, whereas a survival rate of only 65% was obtained in patients with increased BUN values (7). Furthermore, in the same investigation (7), the 1- and 3-year probability of survival after transplantation was 87 and 73%, respectively, in patients with normal preoperative BUN levels, whereas it was only 62 and 49% respectively, in patients with increased BUN levels (Fig. 22.2).

Due to the negative impact of preoperative impaired kidney function on post-transplantation outcome, renal dysfunction is generally considered as a relative contraindication for liver transplantation. Nevertheless, since survival after transplantation in patients with preoperative renal function impairment, although decreased, is substantially better than the survival expectancy without transplantation (< 50% at 1 year and < 25% at 3 years) (11,28,29), most liver transplant groups do not reject patients with preoperative renal failure for transplantation.

The reasons why renal function impairment negatively affects post-transplantation survival are unclear. Nevertheless, several mechanisms may be proposed. First, preoperative renal impairment may contribute to the development of serious postoperative renal failure, a very severe event in liver transplant recipients that, in turn, worsens the prognosis of these patients (7,21,23,26,30–32). On the other hand, since increased mortality has been reported in cirrhotic patients with ascites and renal dysfunction when these patients develop an important adverse event, for example, a severe infection (33–36), it is not surprising that a very aggressive surgical procedure, such as liver transplantation, may also be more harmful to ascitic cirrhotic patients with renal function impairment than to those with normal renal function. Finally, some authors have reported an increased risk of postoperative sepsis in liver transplant recipients with preoperative renal dysfunction (24,37). This feature can also account for the decreased survival rate in these patients, since infections are the most frequent cause of early postoperative death in patients who undergo liver transplantation.

Since renal impairment is associated with a poor outcome in patients who undergo liver transplantation, it seems reasonable for transplantation to be indicated before renal impairment develops. In this setting, it is interesting to remark that low serum sodium concentration, high plasma renin activity, absence of hepatomegaly, and increased resistive index in renal Doppler ultrasonography (reflecting early kidney vasoconstric-

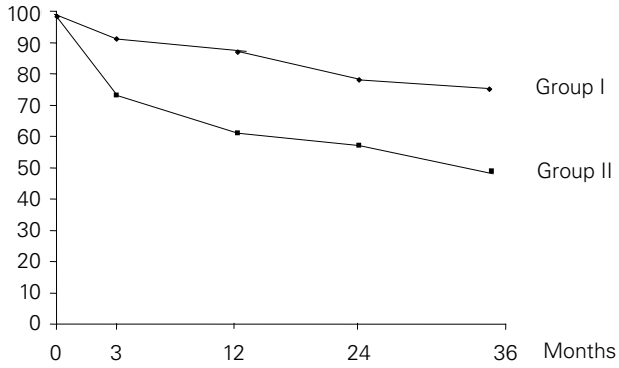


**Figure 22.2** Survival probability after liver transplantation in 162 cirrhotic patients with ascites classified into two groups according to whether their preoperative blood urea nitrogen concentration was normal (≤ 25 mg/dl; Group I, $n = 128$) or increased (> 25 mg/dl; Group II, $n = 34$); $P = 0.0014$. (Reproduced by permission from Gonzalez E, Rimola A, Navasa M *et al*. Liver transplantation in patients with nonbiliary cirrhosis: prognostic value of preoperative factors. J Hepatol 1998; 28:320–8.)

tion) have been identified as powerful predictors of development of renal failure in cirrhotic patients with ascites (28,38). Thus, in cirrhotic patients with ascites and one or more of these factors, early liver transplantation could be indicated. Furthermore, other factors capable of impairing renal function in cirrhotic patients, such as the administration of nephrotoxic drugs (e.g. aminoglycosides) or nonsteroidal anti-inflammatory drugs, aggressive diuretic therapy, or large-volume paracentesis without plasma volume expansion, should be avoided (35,39–45).

Finally, if hepatorenal syndrome, the most common form of renal impairment in cirrhosis with ascites, develops before liver transplantation, treatment with vasoconstrictor agents associated with plasma volume expansion should be attempted to reverse or improve renal dysfunction. The survival of patients who respond to this therapy increases substantially (46,47), and this could permit these patients to undergo transplantation. Furthermore, it has been recently reported that liver transplant recipients with hepatorenal syndrome treated successfully with vasoconstrictors have a post-transplantation outcome similar to that of liver transplant recipients without a history of hepatorenal syndrome (48).

### *Hyponatremia*

Cirrhotic patients with ascites frequently present low concentrations of serum sodium. Preoperative hyponatremia has been reported by several authors to be associated with an increased risk of neurological complications after liver transplantation (49–51). The mechanism suggested for the increased risk of neurological disorders in hyponatremic patients is the rapid correction of the serum sodium concentration during the surgical transplantation procedure with the administration of a large volume of isotonic fluids. The rise in serum sodium concentration in patients developing post-transplantation neurological disorders has been reported to be approximately twice as much as that observed in patients without these complications (49–51). Accordingly, one of the most characteristic neurological complications is central pontine myelinolysis, which has been described in up to 25% of liver transplant recipients dying in the early postoperative period (49,51,52). Nevertheless, other post-transplantation neurological problems, particularly confusion-disorientation disorders, are also observed with increased frequency in hyponatremic patients (50,51).

To minimize the adverse effect of hyponatremia in liver transplant recipients, some authors have proposed the administration of reduced amounts of fluids containing sodium during surgery in patients with low preoperative plasma sodium concentration (51,53). However, the efficacy of this measure has not been established yet. On the other hand, it seems advisable that circumstances known to be associated with the development of hyponatremia, such as overdiuresis, large-volume paracentesis without adequate plasma expansion or the administration of a high volume of fluids without sodium (42,44,54), should be avoided in cirrhotic patients with ascites awaiting liver transplantation. A promising future treatment for hyponatremic patients may be the use of vasopressin antagonists with aquaretic properties (55). Preoperative correction of hyponatremia with the administration of large amounts of sodium is not recommended unless it can be done in the setting of living donor liver transplantation since the timing of transplantation can be precisely selected after the correction of hyponatremia.

### *Refractory ascites*

In the authors' experience, approximately 10–15% of the cirrhotic patients on the waiting list for liver transplantation have refractory ascites. The different therapeutic approaches proposed for this condition are discussed, with special consideration of their impact in patients waiting for liver transplantation.

A first possibility is the use of large-volume paracentesis, which is currently considered as the standard therapy for refractory ascites (45,56). Although the efficacy of paracentesis is only relative because ascites reappears in most patients and requires further and frequent paracentesis, this therapy is safe if adequate plasma volume expansion is used (14,44). In spite of its high cost, the best plasma volume expander is human albumin (57). Repeated large-volume paracentesis without plasma volume expansion is frequently associated with the development of functional renal failure and marked electrolyte disturbances (44), which, as mentioned earlier, can negatively affect the outcome of liver transplantation.

Another alternative for refractory ascites is the insertion of a transjugular intrahepatic portosystemic shunt (TIPS), which has been proposed as a bridge to liver transplantation for these patients (46,56). Several authors have reported that TIPS is highly effective in mobilizing ascites, reducing ascites recurrence, improving renal function parameters, and favorably influencing the pathophysiological factors of ascites formation (58–64). However, some negative aspects regarding the use of TIPS have been raised. One is the risk of impairment of liver function, with the possible development of hepatic encephalopathy (58,63,65), an important problem in patients on the waiting list for transplantation, who usually show a diminished liver function reserve. Another is that, since shunt stenosis or occlusion occurs in a significant proportion of patients, surveillance of TIPS patency must be undertaken regularly and interventions such as balloon dilation or additional stent placement are eventually necessary (66). In one comparative study between TIPS and repeated paracentesis, patients treated

with TIPS survived longer than those treated with paracentesis (62). However, other investigations also comparing TIPS vs. paracentesis have reported that survival was similar in the two therapeutic groups or even worse in patients treated with TIPS (61,63,64). Interestingly, the placement of TIPS does not seem to lead to any major inconveniences for liver transplantation, although it has been reported that patients in whom the position of TIPS is inadequate (e.g. stent extension into the inferior caval or superior mesenteric veins) may have more severe hemorrhage during transplantation surgery and a higher rate of early re-operation compared with patients with a correctly placed TIPS (67).

A third alternative is the use of a peritoneovenous shunt. During the first months after its insertion, peritoneovenous shunting is effective in a large proportion of patients with refractory ascites (14,68). Therefore, it might reasonably be used as a bridge to liver transplantation. Furthermore, this technique can prevent the development of functional renal failure and hyponatremia, or can even reverse these complications if previously existing (14,68,69). However, peritoneovenous shunting is scarcely used at present because this therapy is associated with additional risks, including shunt occlusion at the venous ending with the possibility of superior caval vein thrombosis, the development of infections related to the procedure, and peritoneal fibrosis (68,70). These problems are of relevance not only because they can significantly blunt the beneficial effects of the shunting, but also because they may have possible adverse effects on the practice of liver transplantation. To the knowledge of the authors the impact of peritoneovenous shunts in patients with refractory ascites awaiting liver transplantation has never been specifically investigated.

Surgical options, particularly side-to-side portacaval shunts, have also been used in the therapy of refractory ascites (71). However, since this alternative can be associated with a significant mortality rate and an increased risk of liver function impairment, surgical portosystemic shunting does not appear to be the most adequate bridge therapy to liver transplantation in patients with refractory ascites who are on the transplant waiting list.

Finally, intravenous reinfusion of concentrated ascitic fluid, which has been reported to be as effective and safe as paracentesis in a small series of patients (72), has never been adequately investigated in these patients.

### *Spontaneous bacterial peritonitis*

Spontaneous bacterial peritonitis (SBP) is a frequent complication in cirrhotic patients with ascites (73–77). Since the development of SBP is associated with a short survival, it is a well-established criterion for indicating liver transplantation (78,79). SBP can adversely affect the course of patients waiting for liver transplantation in several ways. First, SBP is considered as a contraindication for liver transplantation while the infection is not under control, although this procedure can be performed without any apparent additional risk in patients with SBP that is not yet resolved but is clearly improving (80). Second, regardless of early diagnosis of the infection and appropriate antibiotic therapy and general management, SBP is currently associated with a 10–30% in-hospital mortality rate (36,79,81). Death can occur during the infectious episode or soon after the resolution of SBP and it is usually caused by a combination of the consequences of the septic process and complications related to the underlying liver disease. Third, a significant proportion of SBP patients develop renal and/or liver function impairment (34), which is probably triggered by the intense local and systemic inflammatory response that usually occurs during the SBP episode and can persist several days or weeks after resolution of the infection (82–84). These features, particularly the impairment of renal function, not only contribute to the high mortality rate of these patients after SBP resolution (34,36,82), but may also negatively influence an eventual liver transplantation performed under these conditions. The administration of nephrotoxic drugs, such as aminoglycosides, increases the risk of renal dysfunction and should be avoided in patients with SBP (35,39,40,85,86).

The possible adverse affects of SBP in patients on the waiting list for liver transplantation make the prevention of this infectious complication very important. Since the causative organisms of most SBP are enteric bacteria, the efficacy of selective intestinal decontamination in preventing this infectious complication has been investigated in several randomized, controlled studies involving different groups of cirrhotic patients with ascites and the highest risk of SBP, such as patients with gastrointestinal hemorrhage, patients recovering from previous episodes of SBP or patients with low total ascitic fluid protein concentration, resulting in a significant reduction in SBP incidence and a significantly prolonged survival in the groups receiving this prophylaxis (86–98). In these studies, selective intestinal decontamination was generally obtained by the oral administration of norfloxacin, a partly absorbed quinolone with a wide coverage against enterobacteria, although some authors have used other oral antimicrobial agents.

Past history of SBP does not represent any special risk factor for post-transplantation infectious complications (80). Furthermore, in several controlled trials, selective intestinal decontamination while patients are on the waiting list for liver transplantation has not been associated with any disadvantage for the post-transplantation period (99–101). Nevertheless, some authors have reported that selective decontamination of the digestive tract affects the type of infection, especially when selective bowel decontamination is extended to the

postoperative period, with a reduction in infections caused by Gram-negative bacteria but with an increase in infections caused by Gram-positive bacteria and fungi (100,102). These data, together with the finding that long-term norfloxacin prophylaxis favors the development of infections caused by quinolone-resistant Gram-negative bacteria (77,103–106), could be important to select empirical antibiotic therapy appropriately if signs of infection develop after transplantation in patients treated with selective bowel decontamination preoperatively.

## Intraoperative and early postoperative aspects

### Outcome of ascites and its pathogenic mechanisms after liver transplantation

Portal hypertension is a major complication of cirrhosis and is responsible for many of the clinical manifestations observed in patients with advanced liver failure (107,108). In the cirrhotic liver, portal hypertension is caused by increased resistance to portal blood flow. However, in advanced states, portal venous inflow also increases because of splanchnic vasodilation, which maintains and aggravates the portal hypertensive state (107,108). Overactivity of vasodilatory factors, including endothelial-derived factors and humoral agents, is a possible contributor to splanchnic vasodilation (107–111) (see Chapters 8 and 12 for detailed review). The systemic circulation is also hyperkinetic, with increased cardiac output and reduced peripheral resistance. Peripheral vasodilation is thought to play a central role in the activation of neurohumoral vasoactive systems, sodium retention, and ascites formation (107,112) (reviewed in Chapters 11 and 16). Different studies have evaluated whether these abnormalities are completely reversed after orthotopic liver transplantation.

#### *Ascites*

Ascites is completely removed during the operation and, although some amount of ascitic fluid can be obtained through abdominal drainage during the first postoperative days in most patients, this problem is usually irrelevant. In some patients, however, ascites may be an important complication and massive ascitic fluid loss may cause coagulation abnormalities, infections of the abdominal cavity, and renal failure (111,113–116). Tissue injury and hemorrhage resulting from surgical manipulation and causing exudation of proteinaceous fluid from inflamed serosal surfaces, and lymph fluid resulting from the transection of lymphatic vessels during extensive abdominal dissection, may contribute to ascitic fluid formation after liver transplantation. However, the most important mechanism responsible for post-transplantation ascites is probably a difficulty in the venous drainage of the graft (111,117,118). In the case of insufficient anastomoses when using the piggyback technique (see later) or stricture of the inferior vena cava, reconstruction of the cavocaval anastomoses or dilation may be necessary.

#### *Splanchnic hemodynamics*

Portal pressure is normalized when the cirrhotic liver is replaced by a graft (115,118,119). However, the splanchnic circulation still shows important abnormalities in the early post-transplantation period consisting of increased azygous and hepatic blood flow (118–123). Azygous blood flow, an index of gastroesophageal collateral blood flow in patients with portal hypertension, remains elevated after liver transplantation, suggesting that portocollateral vessels remain patent for several weeks after the surgical procedure. This phenomenon has also been observed after portacaval shunting and indicates that closure of portosystemic collaterals occurs slowly after the normalization of portal pressure. In the case of liver transplantation, maintenance of increased cardiac output may contribute to delay in the closure of these collaterals (120,124). Another important factor may be the presence of functional stenosis due to poor fixation of the graft, kinking of the vascular anastomoses, or inadequate graft size. This functional stenosis causes increased pressure in the hepatic veins which may result in some degree of liver congestion and increased portal pressure, which, in turn, may contribute to maintaining patent collaterals as alternative pathways for portal blood flow, especially when the splanchnic circulation is hyperkinetic (118). In one study, measurements of azygous blood flow 2 months after liver transplantation showed a significant fall paralleling the normalization of portal pressure (118). In another study there was a significant reduction in azygous blood flow 6 months after transplantation (119).

Acute graft rejection and recurrent hepatitis C virus (HCV) infection may also determine an increase in portal pressure and may also contribute to the maintenance of collateral circulation (111,119). Hepatic blood flow is increased after liver transplantation, but the mechanism is unclear (118–123). It has been suggested that sepsis and the existence of portosystemic shunts may influence post-transplantation hepatic blood flow (119). Although there are some discrepancies, it is currently accepted that the splanchnic circulation (mainly assessed by azygous blood flow and hepatic blood flow) tends to normalize over time (111,120).

#### *Systemic hemodynamics*

As for splanchnic hemodynamics, it is accepted that the hyperkinetic circulation of cirrhosis, with high cardiac index and low systemic vascular resistance, tends to normalize with time (111,118–121). After liver transplantation

there is a clear increase in peripheral resistance. Actually, arterial hypertension is a very common phenomenon that can be observed in up to 70% of liver transplant recipients (125). The increase in peripheral resistance may be due to a combined effect of the liver graft reversing the vasodilation associated with portal hypertension and to the vasoconstrictor effect of the immunosuppressive agents, such as cyclosporin and tacrolimus (118,126). It is important to remark that increased circulating levels of glucagon, a powerful splanchnic vasodilator that can contribute to the hemodynamic abnormalities in cirrhosis, are significantly reduced shortly after liver transplantation, indicating a reduction in glucagon secretion, improved metabolism by the graft, or both. In addition, plasma renin activity and aldosterone levels, which are markedly increased in most cirrhotic patients with ascites before transplantation as a consequence of the activation of the renin–angiotensin system in response to reduced effective arterial blood volume (112), normalize within a few weeks after liver transplantation, indicating that the effective arterial blood volume returns to normal (118). It has been pointed out that sepsis, anemia, acute graft rejection, and persistent portosystemic shunts induce modifications in hemodynamics in liver transplant patients and that these factors may account for the differences observed in the time required for the normalization of the hyperdynamic syndrome after transplantation (119). Furthermore, post-transplantation patients can also be under very different situations that may alter hemodynamic evaluations, particularly the use of cyclosporin or tacrolimus, which promotes an important increase in peripheral resistance and arterial hypertension, with the possible administration of anti-hypertensive drugs.

### *Renal dysfunction*

Hepatorenal syndrome, a syndrome characterized by the spontaneous development of oliguria and marked reduction of renal perfusion and glomerular filtration rate in the absence of significant structural abnormalities of the kidney, is the most extreme manifestation of the continuous process of portal hypertension-induced arterial vascular underfilling (127). Since liver transplantation normalizes effective arterial blood volume and the plasma levels of renin, norepinephrine, and antidiuretic hormone (118), liver transplantation should also normalize renal function in cirrhotic patients with ascites. Since the pioneer study of Iwatsuki *et al*. (128), several investigations have reported a rapid normalization of renal function in patients with hepatorenal syndrome following liver transplantation (19–21,26,129). However, it should be noted that many circumstances occurring during and after the transplant procedure can adversely influence renal function, as well as hemodynamics and endogenous neurohumoral vasoactive systems. Therefore, the outcome after transplantation depends on the interaction of different preoperative, intraoperative, and postoperative factors. The most relevant of the perioperative and postoperative (particularly, early postoperative) factors are discussed later in the chapter.

## The surgical procedure

In its classical technique (130), orthotopic liver transplantation involves the clamping of the recipient inferior caval and portal veins. Occlusion of the inferior vena cava causes blockade of the renal venous outflow and a profound decrease of the venous return to the heart with a subsequent reduction in cardiac output and arterial pressure, which, in turn, is associated with reduced renal perfusion (131–138). Vigorous administration of fluids and vasopressor drugs attenuates these hemodynamic derangements in most patients (138,139), although it does not completely reverse them. Therefore, a significant proportion of liver transplant recipients develop kidney function impairment during the surgical procedure, manifested as both decreased glomerular filtration rate and important tubular damage, which can persist during the early postoperative period (138). Interestingly, patients with lower arterial pressure prior to transplantation are those at highest risk of early postoperative acute renal failure (138), thus suggesting that cirrhotic patients with ascites are particularly prone to this complication since these patients usually exhibit marked baseline hemodynamic alterations.

Three technical modifications have been introduced to reduce the intensity of the hemodynamic derangement and the risk of renal impairment: the use of venovenous bypass, the preservation of the inferior vena cava, and the temporary portocaval shunt, which are discussed below. Since children, particularly those weighing less than 20 kg, tolerate caval and portal vein clamping without important hemodynamic alterations (140,141), these modifications have specially been designed for adult liver transplant recipients.

### *Liver transplantation with the use of venovenous bypass*

Venovenous bypass (VVBP), a support device introduced in 1984 (142) and, since then, routinely used by many liver transplant teams, allows the return of the blood from the inferior vena cava and portal vein territories to the superior vena cava. Therefore, VVBP attenuates the hemodynamic derangement caused by the inferior caval and portal vein clamping during the anhepatic phase of orthotopic liver transplantation (the period during which these veins remain clamped) (132,140,143,144). Furthermore, it has been suggested that VVBP protects the kidneys from damage secondary to the interruption of renal

venous outflow (132,142,145,146) and reduces operative bleeding and transfusion requirements (132,147).

However, in spite of the potential benefits of VVBP, there is no consensus about its usefulness, and several authors have questioned the systematic use of VVBP since a number of patients tolerate the procedure without VVBP support (134,139,148,149). In the only prospective, controlled study performed up to the present to assess the efficacy of VVBP compared with the classical technique in orthotopic liver transplantation (138), the following results were obtained:

**1** During the anhepatic phase, hemodynamic derangement was much less apparent in patients undergoing transplantation with VVBP than in patients undergoing the classical technique (control group). Moreover, around 10% of the control patients needed VVBP to correct adequately the otherwise uncorrectable hemodynamic instability during the surgical procedure.

**2** In spite of these findings, no significant differences were observed in renal function parameters (urine volume, glomerular filtration rate, and markers of tubular damage) measured at different intraoperative and postoperative periods, except during the anhepatic phase, when the control group showed a more marked renal function impairment.

**3** No relationship was found between the development of severe postoperative renal failure and the use of VVBP.

**4** Blood loss and transfusion requirements were similar in the two groups.

Complications related to VVBP (air embolism, pulmonary thromboembolism, hypothermia, and vascular lesions) and the relatively high cost of the device are significant disadvantages of VVBP (138,139,150–152).

### *Liver transplantation with the preservation of recipient inferior vena cava (piggyback technique)*

Essentially, this technique involves the tangential clamping of the inferior vena cava of the recipient at the entrance of the hepatic veins, thus avoiding cross-clamping of the caval vein. Anastomosis of the donor inferior caval vein is then performed end-to-side with the recipient inferior vena cava at the hepatic vein outlet (153–156). The piggyback technique has evident advantages over the classical operation and even over VVBP. Since the venous return to the heart can be physiologically maintained within the normal limits throughout the anhepatic phase, the hemodynamic alterations are dramatically minimized in most patients without the need for fluid overload and aggressive vasopressor drug administration or external support with VVBP (157–166). Furthermore, since the piggyback technique also preserves kidney venous outflow (159,167), the suggestion that this technique protects kidney function during liver transplantation can be raised. This suggestion is supported by the results of a prospective, randomized, comparative trial between the piggyback technique and transplantation supported with VVBP (168), in which a 0% vs. 31% incidence of early postoperative renal impairment was observed, respectively. In addition, lower blood loss and shorter surgery time have been reported with the use of the piggyback technique (162,164,166,168,169).

However, although the piggyback technique seems to be the ideal procedure for orthotopic liver transplantation in adult patients, several considerations should be raised. First, some technical difficulties can be found in the dissection of the liver, particularly in patients with marked collateral circulation or caudate lobe hypertrophy (168). Second, special attention must be paid in the tangential clamping of the inferior vena cava since a generous lateral clamping significantly impairs the blood flow through this vein, thus blunting the hemodynamic advantages of the procedure (170,171). Finally, some problems in hepatic venous outflow have been described, with the development of postsinusoidal portal hypertension and ascites (115,169), although these problems can be avoided by technical modifications, such as the use of the three hepatic veins for donor graft anastomosis or cavocaval anastomosis (172–174).

### *Temporary portocaval shunt*

Besides the preservation of the inferior vena cava, some groups have proposed the systematic use of a temporary portocaval shunt (175). This technique should be performed at the beginning of the operation to facilitate liver dissection during the recipient hepatectomy through a decrease in the pressure in both the portal vein and collateral vessels, thereby reducing operative hemorrhage. Furthermore, it has also been claimed that the temporary portocaval shunt may contribute to the maintenance of kidney function during the anhepatic phase. The only prospective and randomized trial performed up to now showed that the use of a temporary portocaval shunt during orthotopic liver transplantation improved hemodynamic status, reduced intraoperative transfusion requirements, and preserved renal function during the anhepatic phase (176). Moreover, serum creatinine levels during the early postoperative period were lower in the group with temporary portocaval shunt. However, in spite of these beneficial effects, the systematic use of the temporary portocaval shunt is probably not justified.

## Other perioperative factors

Apart from the aforementioned technique-related factors, several other factors can lead to hemodynamic instability during liver transplantation, such as baseline hyperdynamic circulatory status, particularly evident

in cirrhotics with ascites (108), or β-blockade secondary to the variceal bleeding prophylaxis (177), with the consequent decreased capacity to compensate the hemodynamic changes occurring during the operation. Another factor is the blood loss due to both the intensity of the surgical procedure and blood coagulation alterations. Although blood loss is currently mild or moderate (178), perioperative hemorrhage is still important in some patients and can represent a contributory factor to hemodynamic instability. Furthermore, a proportion of patients present a marked and sudden fall in arterial blood pressure immediately after graft reperfusion (179). This alteration usually lasts only a few minutes, but may be more prolonged in some patients, requiring aggressive administration of vasoactive drugs. The pathogenesis of this event, the so-called postreperfusion syndrome, has not been adequately established, although a marked decrease in peripheral vascular resistance is a key feature (131,139,140). Finally, graft failure due to primary nonfunction or arterial thrombosis, which are infrequent but dramatic complications in the perioperative period of transplantation, can also contribute to hemodynamic instability (180–183). All these circumstances, leading to arterial hypotension and to the administration of potent vasoactive agents (most with marked renal vasoconstrictor activity), may also play a role in the perioperative kidney function derangement observed in liver transplant patients.

### Early postoperative factors

Many circumstances occurring after liver transplantation are well known as factors capable of affecting the hemodynamics and renal function in these patients. As mentioned beforehand, the most important is the administration of cyclosporin or tacrolimus, which are powerful vasoconstrictor drugs, specially on the kidney vessels (184). The mechanism of this vasoconstriction is probably multifactorial, with the involvement of different endogenous vasoactive systems, such as endothelin, nitric oxide, sympathetic nervous system, and renal prostaglandins (185). The administration of immunosuppressive agents is a common cause of reduced renal blood flow and glomerular filtration rate in liver transplant recipients, which, in its most severe extent, may be associated with tubular necrosis of ischemic nature (186). However, acute renal failure developing soon after transplantation is usually due to the combination of different pre-, peri- and postoperative factors rather than only the nephrotoxic effect of these immunosuppressive drugs (21,187). Arterial hypertension, a common consequence of the vasoconstrictive effect of cyclosporin, is less frequently observed with the administration of tacrolimus (125,185,188). In spite of numerous data suggesting the possibility of reducing the adverse hemodynamic and renal effects of these drugs, no effective prophylaxis has been introduced in daily clinical practice.

Severe infectious complications, another frequent event in liver transplant recipients, can also contribute to the development of postoperative renal function impairment, as a consequence of an inadequate systemic inflammatory response, shock, or the administration of antimicrobial agents with potential nephrotoxic effects (i.e. aminoglycosides or amphotericin B).

## Summary and conclusions

Liver transplantation in cirrhotic patients with ascites should achieve the resolution of ascites and reverse the mechanisms leading to ascites formation. In the ideal patient, ascitic fluid is eliminated and portal hypertension resolved at the time of the operation, and the remaining hemodynamic alterations, the endogenous vasoactive system derangement, and the possible renal dysfunction preoperatively present in most cirrhotic patients are gradually reversed within a few days or weeks. However, many intraoperative and postoperative factors can interfere with this healing process. The most important of these troublesome factors are those derived from the surgical procedure itself, the administration of drugs with powerful vasoconstrictor activity and nephrotoxic effects, and other factors such as hemorrhage and infection. Therefore, since the final result depends on the balance between all these overlapping factors, efforts should be made in cirrhotic patients with ascites to establish the timing for transplantation before renal failure develops; to avoid events capable of preoperatively worsening the hemodynamics, renal function, and/or general status of the patients; to select the surgical technique that, according to the capabilities and experience of the transplant team, best preserves the hemodynamics and renal function in these patients; and to prevent as much as possible the postoperative circumstances that are potentially adverse for the kidneys.

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# Chapter 23
# A Practical Approach to Treatment of Patients with Cirrhosis and Ascites

Andrés Cárdenas and Pere Ginès

Ascites is the most common complication of cirrhosis, resulting in impaired quality of life, increased risk for infections, dilutional hyponatremia, renal failure, and mortality. This complication marks a defining point in the natural history of cirrhosis because nearly half of the patients will die in approximately 3 years without liver transplantation. The practical management of ascites involves a proper evaluation with laboratory, ascitic fluid, radiological, endoscopic, and in some cases histological tests. One of the most important steps in the initial assessment of patients with ascites is referral of appropriate candidates for liver transplantation, as it offers a definitive cure of cirrhosis and its complications. Taking proper care of patients with ascites can be challenging because they are prone to several complications, such as spontaneous bacterial peritonitis (SBP), dilutional hyponatremia, hepatic hydrothorax, hepatorenal syndrome and some other types of renal failure, hepatocellular carcinoma, bleeding from esophageal or gastric varices, and hepatic encephalopathy. While the initial management of uncomplicated ascites with low-sodium diet and diuretic treatment is straightforward in the majority of patients, there is a group of patients that fail to respond to diuretics and become a real therapeutic challenge. The development of specific associated complications such as dilutional hyponatremia, SBP and hepatic hydrothorax may further challenge proper management of patients with ascites. This chapter will focus on the practical aspects of the evaluation and treatment of patients with ascites and cirrhosis.

## History

Patients with cirrhosis presenting with ascites must always be questioned about precipitating events such excessive salt intake, alcohol consumption, infections, medications such as nonsteroidal anti-inflammatory drugs or steroids, and noncompliance of diuretic therapy if they previously had ascites. Worsening liver disease, portal vein thrombosis, parenchymal renal failure (i.e. glomerular diseases), and development of hepatocellular carcinoma (most often associated with tumor invasion of the portal vein) may also precipitate the development of ascites (1). In severe alcoholic hepatitis, ascites may appear rapidly but in the majority of cases there is resolution following therapy and abstinence. More commonly, ascites develops insidiously over the course of weeks to months. The main symptoms are an increase in abdominal girth often accompanied by lower extremity edema. In patients with a large amount of ascites, respiratory function and physical activity may be impaired. Dyspnea may occur as a consequence of increasing abdominal distension and/or accompanying pleural effusions. Patients with SBP can present with fever, chills, abdominal pain, encephalopathy, and rebound abdominal tenderness (2). However, often they are asymptomatic in the initial stages and the diagnosis relies on the examination of peritoneal fluid.

Other common manifestations of patients with ascites include abdominal discomfort, anorexia, malaise, weakness, malnutrition and jaundice. Increased intra-abdominal pressure favors the formation of abdominal hernias in patients with cirrhosis and longstanding ascites (3). Umbilical hernias increase in size if ascites is not treated and sometimes cause significant complications such as strangulation and rupture due to previous ulcer formation on the surface. Rupture may be complicated with infection of the ascitic fluid and delayed wound healing. Inguinal hernias can also be problematic in patients with ascites. The best treatment is prevention of ascites because surgical treatment is usually not recommended in those with Child C cirrhosis (3). In patients with incisional hernias and ascites, the use of prosthetic meshes should be avoided due to the risk of bacterial infection.

The prevalence of muscle cramps (more than three episodes per week) in cirrhotic patients is approximately 50–60% and is related to the duration of cirrhosis and severity of impairment in circulatory function and presence of ascites (4,5). Muscle cramps commonly occur in the lower extremities and can significantly impair the patients' quality of life (6). Therapies with quinidine (7)

and plasma expansion with albumin (5) were reported to be beneficial in decreasing muscle cramps in patients with cirrhosis.

Painful gynecomastia may occur in patients with cirrhosis and ascites (8). The exact mechanisms are unknown; however, it is postulated that estrogen excess in cirrhotic patients and the estrogenic effects of the spironolactone are possible causes (8). Gynecomastia commonly occurs in association with alcoholic liver disease. In patients with ascites it is more commonly seen when patients are taking spironolactone. In this setting, gynecomastia can be very painful and cause significant discomfort. Tamoxifen (20 mg po b.i.d.) was reported to be successful in this situation but further studies are needed in order to confirm its beneficial effects for mastalgia in patients with cirrhosis (8).

## Examination

Most patients are malnourished and have signs of muscle wasting and atrophy, palmar erythema, spider nevi, jaundice, loss of body hair, splenomegaly, inguinal and umbilical hernias and lower extremity edema. The physical examination is not completely reliable for detecting fluid in abdominal cavity. Patients must have approximately 1500 ml of fluid to be detected reliably by examination (9). The abdomen is distended with bulging flanks; dullness to percussion in these areas indicates ascites. When free fluid is present in the abdominal cavity, it moves to the flanks and the intestines float upward when the patient is supine. The air–fluid level is higher than that normally found on the lateral aspect of the abdomen, and if the patient is turned on his/her side the dullness will shift and percussion over the uppermost part becomes tympanic, because that area is occupied by intestines as fluid shifts to the other side. This maneuver is called shifting dullness and is very sensitive for detecting ascites (10). Mild or moderate pleural effusions are also common in patients with ascites. Large pleural effusions, usually greater than 500 ml, in cirrhotic patients without cardiopulmonary disease are known as hepatic hydrothorax.

The current classification of ascites is described in Table 23.1. In Grade 1 ascites fluid is detected only by ultrasound, in Grade 2 ascites is moderate with symmetrical distension of the abdomen, and in Grade 3 ascites is large or tense with marked abdominal distension (11).

## Diagnostic tests

Standard electrolyte, renal, hematology, and coagulation tests (Table 23.2) should be performed. Liver tests required include aminotransferases, bilirubin, albumin, total protein, alkaline phosphatase, prothrombin time, and α-fetoprotein. An abdominal ultrasound is useful to rule out hepatocellular carcinoma, evaluate the patency of portal flow, presence of collateral veins and in some cases confirm presence of ascites. An upper gastrointestinal endoscopy, to assess the presence and characteristics of esophageal or gastric varices or portal hypertensive gastropathy, should be performed. In patients with previously unknown liver disease, the diagnosis of cirrhosis can be confirmed either histologically or by a combination of exploratory, ultrasonographical, and endoscopic findings. A percutaneous liver biopsy should be performed only after resolution of ascites, because its presence increases the risk of complications. However, if a liver biopsy is required it may be performed through a transjugular approach. Urine collection over a 24-h period with the patient on a low-salt diet and off diuretics for at least 4 days is useful for determining sodium excretion, a parameter that will help evaluate prognosis and determine how patients will respond to therapy. A strong prognostic marker of cirrhotic patients with ascites is the ability to handle water by means of a water load test. In this test a patient receives 20 ml/kg of intravenous 5% dextrose and following this the renal excretion of water is measured. An impaired ability to excrete the water load (urine volume of < 8 ml/min) is associated with a poor prognosis (12).

**Table 23.1** Classification of ascites in cirrhotic patients according to the International Ascites Club (11).

| |
|---|
| Grade 1 ascites: fluid is detected only by ultrasound |
| Grade 2 ascites: moderate amount of fluid in the peritoneal cavity with symmetrical distension of the abdomen |
| Grade 3 ascites: large or tense with marked abdominal distension |

**Table 23.2** Evaluation of patients with cirrhosis and ascites.

| | |
|---|---|
| 1 | General evaluation |
| | A Complete history and physical examination |
| | B Arterial blood pressure, heart rate and pulse oxymetry |
| | C Standard hematology, coagulation, liver tests and α-fetoprotein |
| | D Abdominal ultrasonography and Doppler flow (including the kidneys) |
| | E Upper gastrointestinal endoscopy |
| | F Liver biopsy (selected cases) |
| 2 | Evaluation of renal function* |
| | A 24-h urine sodium |
| | B Serum electrolytes, serum blood urea nitrogen and serum creatinine |
| | C Urine sediment and protein excretion |
| | D Renal water excretion (see text) |
| 3 | Diagnostic paracentesis |
| | A Total protein and albumin measurement |
| | B Cell count |
| | C Culture in blood culture bottles |
| | D Optional tests (see text) |

*Renal and circulatory function should be assessed under low-sodium diet and without diuretic therapy.

Diagnostic paracentesis (approximately 30 ml of fluid) is required in all patients presenting with ascites *de novo*, requiring hospitalization, and in those with any evidence of clinical deterioration such as fever, abdominal pain, gastrointestinal bleeding, hepatic encephalopathy, or hypotension (13). The technique, risks, and complications of performing an adequate diagnostic paracentesis are explained in Chapter 19. The ascitic fluid in cirrhotics is transparent and yellow/amber in color. Basic parameters to be determined in ascitic fluid are cell count, culture in blood culture bottles, albumin, and total protein. Glucose, lactate dehydrogenase, amylase, bilirubin, triglyceride, tuberculosis smear, and cytological analysis of the fluid are optional and provide important information in the differential diagnosis of ascites in selected cases. The cell count is the most helpful test in determining bacterial infection. The ascitic fluid white blood cell count is < 500/mm$^3$ in most cases, with predominance of mononuclear cells (> 75%) and very low number of polymorphonuclear (PMN) cells. An elevated white blood cell count with predominance of PMN cells is indicative of a peritoneal infection. The diagnosis of SBP is made when the fluid sample has a PMN count > 250/mm$^3$ (13,14). Recently the use of reagent strips or "urine dipsticks" was validated for the rapid diagnosis of SBP (15,16). This test has a sensitivity of 96% and a specificity of 89% in diagnosing SBP (16). One potential drawback of this method is that there is no cell count number or differential, therefore it is prudent also to obtain a concomitant cell count and differential if the reagent strips are going to be used. Cultures are positive in approximately 50% of patients if blood culture bottles are used (14,15). Bloody ascites (> 50 000 erythrocytes/mm$^3$) may lead to a higher PMN count in the absence of infection, and in this case a correction factor of one PMN per 250 erythrocytes is recommended (14). A low proportion of patients may have a positive ascitic fluid culture without increased PMN cell count. This condition is known as bacterascites (see Chapter 34). Conversely, as indicated above, many patients with high PMN count suggestive of peritoneal infection may have a negative ascitic fluid culture. This condition is known as culture-negative SBP and should be managed as culture-positive SBP (see Chapters 34 and 35) (14,15).

The difference between serum albumin concentration and ascites albumin concentration (serum–ascites albumin gradient) in patients with cirrhosis and ascites is usually > 1.1 g/dl; values < 1.1 g/dl suggest a cause of ascites other than cirrhosis (15). Most patients with cirrhosis have a total ascitic fluid protein concentration < 1.0 g/dl. However, values > 1.0g/dl are not common. Patients with protein concentration in ascitic fluid < 1.0 g/l have a greater risk of developing SBP. The erythrocyte count in ascitic fluid is usually low in patients with cirrhosis (< 1000 cells/mm$^3$). Bloody ascites may be observed either spontaneously or as a consequence of a traumatic tap or hepatocellular carcinoma.

## Management

The first and most important aspect of the management of all patients with cirrhosis and ascites is an evaluation for liver transplantation (13). Early referral is recommended due to the short survival some patients have once they develop ascites. Although there are no established prognostic models for patients with cirrhosis and ascites, predictive factors related to renal and circulatory function may be useful in identifying candidates for liver transplantation. These factors include dilutional hyponatremia, low arterial blood pressure, serum creatinine > 1.2 mg/dl or 106 µmol/l, and intense sodium retention (urine sodium < 10 mEq/l) (17). In clinical practice, the easiest way of identifying patients that need priority of liver transplantation is to recognize those with severe renal functional abnormalities such as refractory ascites or hepatorenal syndrome and those with SBP. However, patients with these conditions have a short survival and may die while waiting for liver transplantation.

Cirrhotic patients with ascites are at risk of other complications of cirrhosis such as gastroesophageal variceal hemorrhage, hepatic encephalopathy, bacterial infections, and hepatocellular carcinoma, thus preventive measures are recommended to reduce the incidence and severity of these complications. Patients with moderate or large gastroesophageal varices (even without a history of bleeding) should be given oral nonselective β-blockers such as propranolol or nadolol as prophylaxis to prevent variceal bleeding. In those patients without a history of bleeding, controversy exists as to whether variceal band ligation is as effective as medical therapy with β-blockers (18). Patients with a history of hepatic encephalopathy may benefit from oral synthetic disaccharides such as lactulose to prevent recurrences. Screening for hepatocellular carcinoma with serum α-fetoprotein and liver ultrasound every 6 months increases the likelihood of finding small lesions that can be managed promptly. The early identification of bacterial infections in cirrhotic patients with or without ascites with the early institution of antibiotics prevents significant hepatic decompensation and prolonged hospital stays (19). Finally, every patient with advanced liver disease should be vaccinated for hepatitis A and B.

### Nutrition

An evaluation by a nutritionist is recommended for education regarding appropriate caloric and salt intake. A low-sodium diet of 70–90 mmol/day is one of the mainstays of management (13). Sodium restriction below this level is generally unpalatable and compliance is poor.

This restriction causes a negative sodium balance and loss of ascites and edema in those patients with a urinary sodium excretion > 100 mEq/day as measured by a 24-h urine collection. Although only 10–15% of patients reduce ascites with sodium restriction alone, it is essential when diuretics are added (13,15). Cirrhotics with alcoholic liver disease may improve ascites with abstinence.

Improvement of the nutritional status is of key importance given that cirrhotics with advanced disease have decreased intake of nutrients, decreased absorption of nutrients, increased energy expenditure, and altered fuel metabolism with an accelerated starvation metabolism. Ascites *per se* seems to contribute to decreased oral intake and malnutrition (20). Cirrhotic patients with refractory ascites who undergo a transjugular intrahepatic portosystemic shunt (TIPS) for therapy of ascites significantly improve their nutritional status as measured by resting energy expenditure, total body nitrogen, body fat, and food intake (21).

## Uncomplicated ascites

### *Grade 1–2 ascites*

Patients with Grade 1 ascites (only detected by ultrasound) do not appear to require any specific treatment; however, no specific therapeutic or follow-up studies have been performed in this group of patients. Patients with Grade 2 ascites (moderate amount of fluid with symmetrical distension of the abdomen) usually have < 5 l of ascitic fluid and are best treated with a low-sodium diet and diuretics (11,13). There is no need for hospitalization unless patients have associated complications. These patients respond well to low doses of diuretics. The best initial regimen for minimizing ascites is either spironolactone (50–100 mg/day) or amiloride (5–10 mg/day). Low doses of furosemide (20–40 mg/day) may be useful in the beginning (particularly in patients with peripheral edema or anasarca) because they increase natriuresis; however, higher doses may put the patients at risk for volume depletion and prerenal failure because these patients usually do not have severe sodium retention. Another therapeutic strategy is to use spironolactone alone up to 400 mg/day in progressively increasing doses and to add furosemide only when the highest dose of spironolactone has been achieved if there is no response (22). If there is no response, compliance with diet and medications should be confirmed, and then diuretics may be increased in a stepwise fashion every 7 days by doubling doses (ratio of 40 mg : 100 mg); furosemide up to 160 mg/day and spironolactone up to 400 mg/day.

Regardless of the strategy used, the goal is to achieve an average weight loss of 0.5 kg/day in patients without edema and 1 kg/day in those with peripheral edema (11). Urine sodium should be determined in those who do not lose weight; patients with urine sodium > 90 mEq/day (i.e. urine sodium greater than theoretical sodium intake) are not compliant with sodium restriction. After minimizing ascites, sodium restriction can be maintained while the dose of diuretics may be reduced as needed or sodium intake can be mildly increased keeping the same dose of diuretics. Unfortunately, there are no studies assessing which is the best course of action after disappearance of ascites. The recommendations for the treatment of Grade 2 ascites are outlined in Fig. 23.1.

### *Grade 3 ascites*

In patients with Grade 3 ascites (large amount of peritoneal fluid with marked abdominal distension) the treatment of choice is large-volume paracentesis (11,13). The technique, risks, and complications for performing a large-volume paracentesis are explained in Chapter 19. Complete removal of ascites in one tap has been shown to be quick, effective, and associated with a lower number of complications than conventional diuretic therapy (23). Following the paracentesis, plasma expansion with albumin (8 g/l of ascites removed) is recommended in all patients in order to prevent the post-paracentesis circulatory dysfunction (see Chapter 19). Albumin has proven superior to dextran 70, polygeline, and saline for large-volume paracentesis > 5 l but randomized studies have not shown a significant difference in survival between patients treated with albumin and those treated with other plasma expanders, probably due to a low sample size (13,24,25). The use of albumin is controversial because it has not shown improvement in survival and has an elevated cost; however, it has a superior protective effect on the circulatory system compared with other expanders and for this reason it is the preferred plasma expander in patients treated with large-volume paracentesis (11,13). When < 5 l are removed an alternate synthetic plasma expander may be used (11,13,24,25).

Patients with ascites and a known history of cirrhosis and without any associated complications can be managed as outpatients. However, patients in whom tense ascites is the first manifestation of cirrhosis or those with associated hepatic encephalopathy, gastrointestinal bleeding, or bacterial infections require hospitalization. Most of these patients have marked sodium retention and need be started or continued on relatively high doses of diuretics after paracentesis, together with a low-sodium diet. The recommendations for the treatment of Grade 3 ascites are outlined in Fig. 23.2.

## Refractory ascites

Approximately 10% of patients with ascites are refractory to treatment with diuretics (11). In refractory ascites, a significant increase in sodium excretion cannot be achieved, either because patients do not respond to high doses of diuretics (spironolactone 400 mg/day and

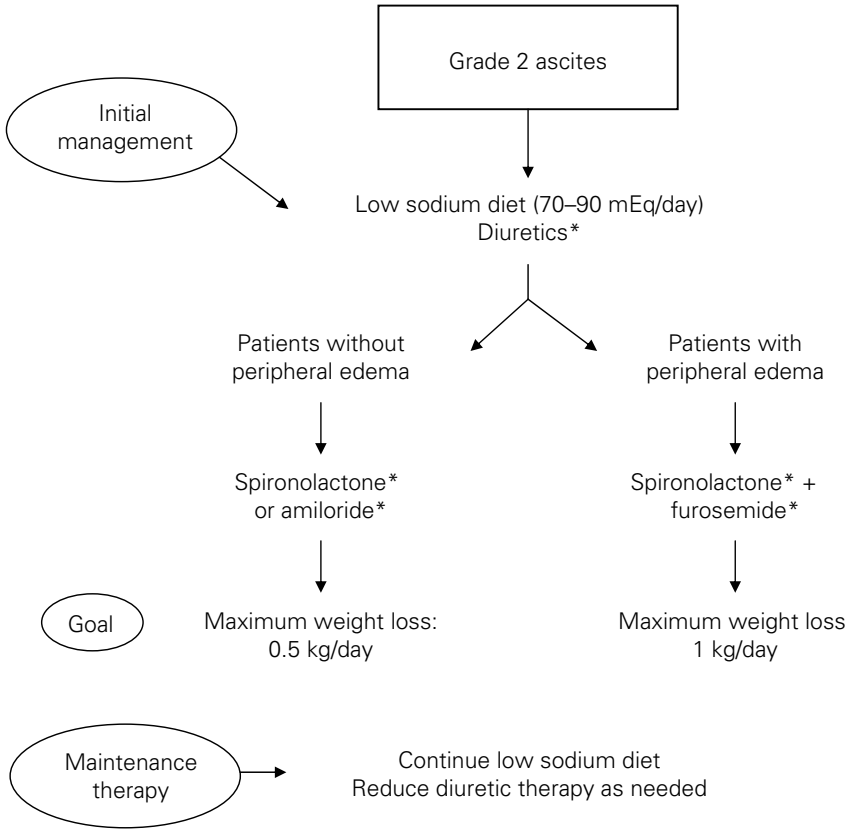


**Figure 23.1** Treatment strategy for patients with cirrhosis and Grade 2 ascites. *Recommended diuretics include spironolactone (50–200 mg/day) or amiloride (5–10 mg/day). Low doses of furosemide (20–40 mg/day) may be added to increase natriuresis in patients with peripheral edema. If there is no response, check compliance with treatment and low-sodium diet. Increase the dose of diuretics in a stepwise fashion to achieve weight loss of disappearance of ascites.

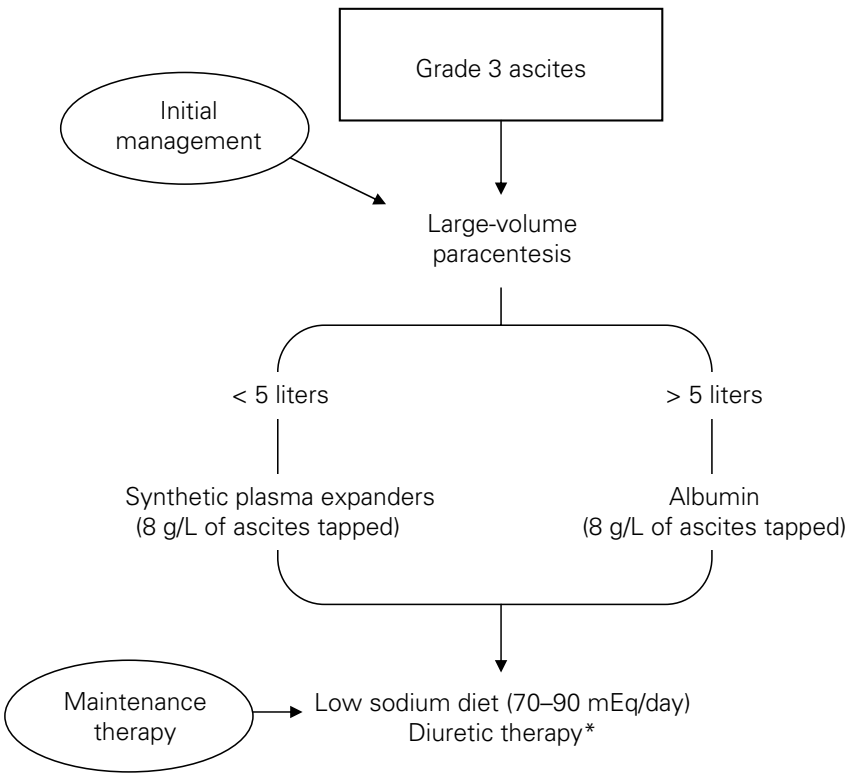


**Figure 23.2** Treatment strategy for patients with cirrhosis and Grade 3 ascites. *If the patient was not on diuretics before the development of large ascites, start with spironolactone (200 mg/day as single dose) and furosemide 40 mg/day and adjust the dose to maintain the patient with mild or no ascites or edema. Check body weight daily and spot urine sodium weekly. Monitor the patient closely during the first 3-4 weeks of therapy. If the patient was on diuretics start with a dose higher than the dose taken before paracentesis. If ascites and edema increase, check compliance with treatment and low-sodium diet. Increase the dose of diuretics in a stepwise fashion every 5–7 days up to 400 mg/day of spironolactone and 160 mg/day of furosemide.

furosemide 160 mg/day) or because they develop side-effects such as hyperkalemia, hyponatremia, hepatic encephalopathy, or renal failure that preclude their use. Treatment options include repeated large-volume paracentesis plus plasma expansion or TIPS. Peritoneovenous shunts, although very effective, were abandoned due to significant complications when compared with paracentesis (26). Therapeutic paracentesis is usually performed as first line of therapy for refractory ascites. Patients, on average, require a tap every 2–4 weeks and the majority may be treated as outpatients, making this option easy to perform, and cost saving. TIPS, a nonsurgical method of portal decompression, acts as a side-to-side portocaval shunt that reduces portal pressure and decreases ascites and diuretic requirements in these patients. The main disadvantage with TIPS is frequent obstruction of the prosthesis (70% in 1 year) (11,27), which precipitates rapid reaccumulation of ascites in some patients; however, newer polytetrafluoroethylene-covered prostheses improve TIPS patency and decrease the number of clinical relapses and reinterventions without increasing the risk of encephalopathy (28). Other major side-effects associated with TIPS include a 30% chance of hepatic encephalopathy, congestive heart failure, hemolytic anemia, and impairment in liver function (27).

TIPS is associated with a lower rate of ascites recurrence when compared with large-volume paracentesis. However, there are no major differences in survival between patients treated with large-volume paracentesis vs. TIPS in randomized trials (29–31), and therefore large-volume paracentesis seems to be the treatment of choice because of its wider applicability and lower cost and less side-effects when compared with TIPS. TIPS placement should be evaluated on a case-by-case basis and may be a good option for patients with loculated fluid or those requiring more than three to four taps per month who have preserved liver function without hepatic encephalopathy. The treatment recommendations for refractory ascites are outlined in Fig. 23.3.

## Dilutional hyponatremia

Patients with cirrhosis and ascites are at risk of developing hyponatremia. A common scenario is that of patients receiving large doses of diuretics and showing excessive diuretic response; in this case, hyponatremia is considered to be hypovolemic in nature. Nonetheless, approximately 30% of patients with cirrhosis and ascites develop a low serum sodium spontaneously. In these cases hyponatremia is secondary to the elevated levels of antidiuretic hormone (see Chapter 25). Dilutional hyponatremia in cirrhotic patients is defined as serum sodium < 130 mEq/l (32). In most patients hyponatremia is asymptomatic, but in some it may be associated with symptoms such as anorexia, poor concentration, lethargy, nausea, vomiting, and occasionally seizures. The clinical implications of dilutional hyponatremia are not well known; however, it appears that in some patients

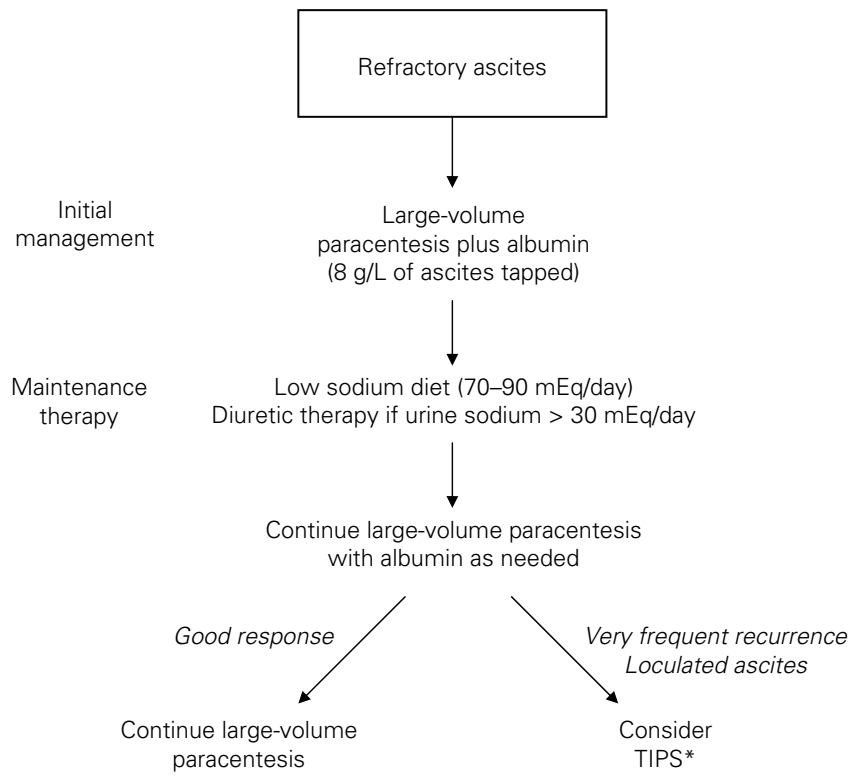


**Figure 23.3** Treatment strategy for patients with refractory ascites. *Consider use of transjugular intrahepatic portosystemic shunt (TIPS) in patients with preserved hepatic function, no hepatic encephalopathy, compartmentalized fluid, and those having repeated paracentesis more than three to four times per month.

there may be an association with hepatic encephalopathy (33,34).

At present there is no pharmacological therapy for dilutional hyponatremia; water restriction to 1 l/day may stop the progressive decrease in serum sodium concentration, but does not seem to improve serum sodium in cirrhotics with ascites and dilutional hyponatremia (35). The administration of hypertonic saline solutions is not recommended because it causes further expansion of extracellular fluid volume, leading to significant accumulation of ascites and edema. Newer treatments with vasopressin antagonists of the antidiuretic hormone seem to be the most promising approach to therapy. Phase II studies with these antagonists of the V2 receptor of antidiuretic hormone indicate that these drugs increase the solute-free water excretion and improve serum sodium concentration in hyponatremic patients with cirrhosis and ascites (see Chapter 26) (35,36). Nonetheless, larger studies and more detailed information regarding the efficacy and safety of these drugs are needed before considering them an established therapy for dilutional hyponatremia in cirrhosis.

### Hepatic hydrothorax

Hepatic hydrothorax, defined as large pleural effusion, is an infrequent complication of cirrhosis with an estimated prevalence among cirrhotic patients of around 5–10% (37). The pathophysiology is not completely understood but it seems to occur due to small diaphragmatic defects, which favor the passage of fluid from the peritoneal to the pleural cavity due to the negative pressure in the pleural space favoring the transfer of fluid across these defects (38). The effusion is usually right-sided, although it can be present on the left side. Patients with hepatic hydrothorax are treated similarly to those with ascites. The first step is starting the patient on a low-sodium diet and diuretics as described above. Contrary to what occurs in the peritoneal cavity where relatively small amounts of fluid (i.e. 2 l) do not cause discomfort, this amount of fluid collection in the pleural cavity causes a significant number of symptoms, such as shortness of breath, cough or chest pain. If these patients with effusions > 1.5–2 l have good natriuresis (urine sodium > 30 mEq/day), a therapeutic thoracentesis followed by diuretics is a fine option. However, in those patients with impaired response to diuretics this measure may need to be repeated very often (about every 1–2 weeks), putting the patient at risk of complications derived from a thoracentesis and causing a significant impairment in the patient's quality of life.

In cases of refractory hydrothorax, which is defined as persistent hydrothorax despite fluid and sodium restriction and use of maximal tolerable doses of diuretics, options include use of TIPS, pleurodesis, and repair of diaphragmatics defects (38,39). TIPS for refractory hydrothorax is beneficial in selected cases as a bridge to transplantation in those with preserved hepatic function (39–41). However, serious adverse outcomes occur, particularly in those who are over the age of 60, have hepatic encephalopathy, and/or those with Child C cirrhosis (41). Pleurodesis is in general unsuccessful because rapid reaccumulation of fluid does not allow the visceral and parietal pleural surfaces to adhere; in addition, it is associated with high morbidity and mortality (42). Chest tube placement should always be avoided, as it is a set-up for infections and massive fluid losses (43). Repair of diaphragmatic defects using videothoracoscopy may be considered in patients with large defects, but experience is limited (44). Finally, suitable patients with hepatic hydrothorax should be considered candidates for liver transplantation.

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# Chapter 24
# Etiology, Diagnosis, and Management of Non-cirrhotic Ascites

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## Etiology and diagnosis

The term ascites is of Greek derivation (*askos*) and refers to a "bag" or "sack." Many diseases are known to lead to the formation of ascites, which is the accumulation of free fluid within the peritoneal cavity (Table 24.1) (1). The most frequent cause of ascites in Europe and in North America is hepatic cirrhosis, followed by neoplasia and congestive heart failure as well as tuberculous peritonitis (1). In this chapter we will only deal with noncirrhotic ascites.

The causes of ascites may be grouped into two different pathological pathways (2). The first group is not associated with peritoneal disease and consists of diseases which (i) cause a general flow block such as sinusoidal portal hypertension (cirrhosis, acute alcoholic hepatitis, fulminant or subacute viral or toxic hepatitis, congestive heart failure, constrictive pericarditis, inferior vena cava obstruction, Budd–Chiari syndrome, and hepatic veno-occlusive disease, i.e. after bone marrow transplantation) (3); (ii) lead to hypoalbuminemia (nephrotic syndrome, protein-losing enteropathy, and malnutrition) (4,5); and (iii) include a variety of disorders which may lead to ascites through different mechanisms such as myxedema, ovarian diseases (carcinoma, benign tumors, ovarian hyperstimulation syndrome) (6), chronic pancreatitis, biliary tract leakage (secondary to liver trauma, biliary tract surgery, or transhepatic cholangiography) (7), diseases affecting the lymphatic system of the splanchnic area, and chronic renal failure.

In the second group ascites is formed as a consequence of primary peritoneal disease or as a result of peritoneal involvement in systemic processes such as tuberculosis, fungal (*Candida albicans, Coccidioides immitis*) infection (8), parasitic and granulomatous peritonitis (sarcoidosis, Crohn's disease, peritoneal granulomatous reaction to talc, cotton and wood fibers, starch and barium) (9), primary or metastatic peritoneal tumors, vasculitis (systemic lupus erythematosus, Wegener's granulomatosis, Henoch–Schönlein purpura) (10,11), eosinophilic gastroenteritis, and Whipple's disease.

Due to the low incidence of the various noncirrhotic ascites syndromes, only case reports and case series have been published over the last years and no randomized controlled studies have been performed. Thus, to describe the best management for the treatment of noncirrhotic ascites the different case reports and series can only be evaluated and compared for the possible best therapeutic approach (12).

## Ascites due to extrahepatic portal and outflow block

In the group leading to ascites caused by mechanical obstruction the most prevalent disorder is the Budd–Chiari syndrome. However, other diseases cause this type of ascites as well (e.g. congestive heart failure, constrictive pericarditis, obstruction of the inferior vena cava) (13). Ascites secondary to postsinusoidal portal hypertension generally shows a high total protein concentration in ascitic fluid and low gradient of serum albumin to ascitic fluid albumin ($< 1.1$ g/dl) similar to those reported in malignant ascites and tuberculous peritonitis (14).

The differentiation between chronic Budd–Chiari syndrome and cirrhosis is often difficult on clinical grounds. The ascites fluid protein concentration may be low in some of these patients with hepatic vein obstruction as a result of the capillarization of the hepatic sinusoids. These patients may also show signs of liver disease, abnormal liver function tests, splenomegaly, and esophageal varices (15). Computed tomography and duplex-ultrasound will demonstrate Budd–Chiari syndrome if major hepatic veins are not visualized and the caudate lobe which drains autonomously into the caval vein is enlarged.

Ascites forms in high-output as well as low-output heart failure. As in cirrhosis, there appears to be decreased effective arterial blood volume and subsequent activation of the vasopressin, renin–aldosterone, and sympathetic nervous systems (4,16). This state leads to renal vasoconstriction and sodium and water retention (2). Fluid then weeps from the congested hepatic sinu-

**Table 24.1** Causes of ascites.

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|---|
| Ascites not associated with peritoneal disease |
| Intrahepatic sinusoidal portal hypertension |
| Cirrhosis |
| Acute alcoholic hepatitis |
| Fulminant hepatitis (toxic or viral) |
| Subacute hepatitis (toxic or viral) |
| Hepatic veno-occlusive disease |
| Massive liver metastasis |
| Schistosomiasis |
| Extrahepatic sinusoidal portal hypertension |
| Congestive heart failure |
| Constrictive pericarditis |
| Inferior vena cava obstruction |
| Hepatic vein obstruction (Budd–Chiari syndrome) |
| Hypoalbuminemia |
| Nephrotic syndrome |
| Protein-losing enteropathy |
| Malnutrition |
| Miscellaneous disorders |
| Myxedema |
| Ovarian diseases: |
| Carcinoma |
| Benign tumors |
| Ovarian hyperstimulation syndrome |
| Pancreatic ascites |
| Bile ascites |
| Chylous ascites |
| Nephrogenic ascites |
| Ascites due to primary peritoneal disease |
| Malignant ascites |
| Primary peritoneal mesothelioma |
| Secondary peritoneal carcinomatosis |
| Granulomatous peritonitis |
| Tuberculous peritonitis |
| Fungal and parasitic peritonitis (*Candida albicans*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Cyptococcus neoformans*, *Schistosoma mansoni*, *Strongyloides stercoralis*, *Entamoeba histolytica*) |
| Sarcoidosis |
| Starch granulomatous peritonitis, barium peritonitis |
| Vasculitis |
| Systemic lupus erythematosus |
| Wegener's granulomatosis |
| Henoch–Schönlein purpura |
| Miscellaneous peritoneal disease |
| Eosinophilic gastroenteritis |
| Whipple's disease |
| Endometriosis |

soids as lymph, as in cirrhotic ascites. If there is no evidence of liver disease the diagnosis can be established by the clinical condition of the patient and the ascitic fluid concentrations of leukocytes, lactic dehydrogenase, cholesterol, and adenosine deaminase, which are low in ascites secondary to hepatic outflow block (17).

Patients with ascites due to constrictive pericarditis often lack symptoms of congestive heart failure. It is therefore important to seek physical findings of this illness (jugular venous distension with early diastolic collapse and inspiratory distension, a diastolic knock that may be confused with a third heart sound, and pulsus paradoxus) in any patient with high protein ascites. Clear lung fields on radiographic examination and low voltage of the electrocardiogram suggest constrictive pericarditis. The diagnosis is confirmed by cardiac ultrasonography or catheterization, computed tomography, or magnetic resonance imaging.

In patients with ascites due to a congenital web in the inferior vena cava, a cava-to-cava collateral circulation can be observed on the abdomen or on the back (13). The final diagnosis is based on magnetic resonance angiography or duplex-ultrasound. Treatment depends on the underlying disease. In the Budd–Chiari syndrome the pathological mechanisms are still not clear. Thus therapy is symptomatic. A surgical shunt between vena porta and inferior vena cava or the insertion of a transjugular intrahepatic portosystemic shunt (TIPS) are the most common treatments. Liver transplantation may sometimes be necessary.

In patients with end-stage liver disease and tense ascites, paracentesis is the best therapy to optimize the situation. Depending on the protein concentration of the ascites fluid, albumin substitution may be required (18).

Schistosomiasis, a disease produced by trematodes that affect the intrahepatic portal bed and cause portal hypertension, may be associated with ascites (19).

All forms of intrahepatic sinusoidal portal hypertension induced by different kinds of hepatic diseases such as fulminant hepatitis, subacute hepatitis, acute alcoholic hepatitis, or even massive liver metastasis, lead to more or less severe ascites formation in the peritoneal cavity. The relationship between sinusoidal pressure and the hepatic production of lymph is such that for every millimeter of mercury increase of sinusoidal pressure there is a 60% increase in hepatic lymph production. The macroscopic consequence of this phenomenon is a marked enlargement in liver size. This means that little interstitial fluid can accumulate without increasing interstitial pressure. This high interstitial pressure explains the two consequences of sinusoidal portal hypertension: an increase in lymph flow and the direct passage of hepatic lymph with high protein levels from the liver into the peritoneal cavity (20).

## Pancreatic ascites

Pancreatic ascites occurs in approximately 3% of patients with chronic pancreatitis as a result of the leakage of pancreatic fluid from a pancreatic duct rupture, or from a pancreatic pseudocyst into the peritoneal cavity, or by a "chemical burn" of the peritoneum. Other less frequent etiologies include acute hemorrhagic pancreatitis, abdominal trauma, and pancreatic cancer. Since most

patients with chronic pancreatitis are affected by alcoholism and rarely may develop massive ascites with little or no abdominal tenderness, the differential diagnosis of pancreatic ascites from cirrhotic ascites may be difficult on clinical grounds. Laboratory analyses are, therefore, essential to establish a correct diagnosis (21,22).

In virtually all cases of pancreatic ascites, serum and especially ascites fluid amylase and lipase values are dramatically increased. The concentration of pancreatic enzymes in ascites fluid is between 5 and 20 times greater than the serum levels obtained simultaneously. The ascites fluid protein concentration is generally > 3 g/dl and the fluid is usually serous, but can be serosanguineous, turbid, or chylous. The concentration of methemalbumin in ascites is markedly increased in patients with hemorrhagic pancreatitis and this finding has prognostic significance for a poor outcome. The concentration of leukocytes in ascites fluid ranges between 70 and 2200 per µl, 80% being lymphocytes (23).

Ultrasound or computed tomography are important diagnostic procedures for pancreatic ascites since they may detect the presence of a pseudocyst and other pancreatic abnormalities. Pseudocysts in patients with pancreatic ascites are usually small due to the continuous leakage of the cystic fluid into the peritoneal space. Treatment aims at eliminating the cause of ascites. In most cases surgery is needed, although transcutaneous drainage of the cyst with and without somatostatin may be used in selected cases (24,25).

Drug therapy is not advisable for pancreatic ascites because of the high proportion of failures. Interventional therapy with surgery or a transpapillary stent have a positive effect on the clinical outcome. The apparent lack of effect of somatostatin analogs could be attributed to the small number of cases and the heterogeneity of the doses used (12).

## Bile ascites

The leakage of bile into the peritoneal cavity may lead to two different clinical pictures. Some patients develop signs and symptoms of peritonitis, including severe epigastric, right upper quadrant, or diffuse abdominal pain; rigidity of the abdomen; rebound tenderness; hypotension and tachycardia; oliguria, and marked leukocytosis. Other cases have no symptoms other than those related to large quantities of bile ascites in the abdominal cavity. In both circumstances paracentesis yields a green ascitic fluid with a bilirubin concentration considerably higher than that in plasma (21).

Between these two extremes there is a wide spectrum of symptoms. Some patients may initially have a very brief period of apparent peritoneal signs followed by rapid spontaneous resolution of symptoms and accumulation of bile ascites, whereas others initially develop ascites with the signs of peritoneal irritation appearing at a later stage. The reason why intraperitoneal bile leakage induces these two types of clinical picture is unknown. Bile ascites usually occurs after biliary tract surgery (mainly cholecystectomy), percutaneous diagnostic procedures (liver biopsy and percutaneous transhepatic cholangiography), and trauma with injuries to the gallbladder, common bile duct, hepatic duct, or liver (26,27). The most common cause of bile peritonitis is the spontaneous perforation of the gallbladder or bile ducts due to erosion by stones or cholecystitis. It has been suggested that in this latter condition the bacterial contamination of the bile or a higher concentration of bile salts (which are capable of inducing chemical irritation of the peritoneum) are important factors in the development of bile peritonitis. The rate of leakage may also be important relative to the degree of symptomatology.

Bile ascites secondary to the spontaneous rupture of the common bile duct can also be observed in neonates as a consequence of congenital malformations of the biliary tract (stenosis, choledocal cyst) or stones. Bile ascites should be considered as a possible diagnosis in any cirrhotic patient accumulating intra-abdominal fluid after a liver biopsy. The diagnosis is made by measuring bilirubin levels in the ascites, which should be significantly higher than those in serum. Treatment consists of surgical or interventional repair of the leak or malformation.

## Chylous ascites

Chylous ascites is an infrequent feature in patients with cirrhosis. In most cases chylous ascites appears spontaneously, but can be induced as well by surgical procedures, in particular on aortic aneurysms (28–30). Chylous ascites is macroscopically turbid and white (milky ascites) and separates into layers when standing. These characteristics are due to a high concentration of chylomicrons very rich in triglycerides. The diagnosis of chylous ascites is based on ascitic fluid triglyceride concentration, which is usually < 110 mg/dl and always higher than the corresponding value in serum. The ascitic fluid concentration of cholesterol and phospholipids is similar to that of nonchylous ascites (21). The proportion of lipids in chylous ascites is very similar to that of intestinal and human thoracic duct lymph after the ingestion of a fat meal. Because patients with cirrhosis have elevated pressure within the splanchnic lymph vessels, it has been suggested that spontaneous chylous ascites in these patients may be a consequence of the rupture of these lymph vessels leading to the leakage of whole intestinal lymph into the peritoneal cavity.

Chylous ascites is a well-recognized complication in the postoperative period in patients who undergo renal transplantation, aortic aneurysm resection, lymphangioectomy, and pancreaticoduodenectomy (7,28–30). He-

patic cirrhosis is a relatively infrequent cause of chylous ascites. Obstruction of the lymphatic system due to malignancies, especially lymphomas, is by far the most common etiology in adults. Other diseases associated with chylous ascites in adults include portal vein thrombosis, nephrotic syndrome, tuberculosis, pancreatitis, Whipple's disease, abdominal trauma, constrictive pericarditis, peritonitis, acquired immunodeficiency syndrome (AIDS), and pulmonary fibrosis with thoracic duct obstruction (8). Chylous ascites can occur in patients with a bile duct tumor, as well as patients receiving automated peritoneal dialysis (31).

Congenital malformations of the lymphatic system, including stenosis or atresia of the lymphatics and lymphatic mesenteric cysts, are the main causes of chylous ascites in children. There are a variety of different diseases associated with chylous ascites or causing the development of chylous ascites. As often with very rare diseases, there are only case reports available on the occurrence of chylous ascites with systemic lupus erythematosus (32), carcinoid tumours (33), HIV/AIDS (34), or Kaposi's sarcoma (35).

Chylous ascites should be differentiated from pseudochylous ascites, in which, although the macroscopic appearance of ascitic fluid may be identical, the triglyceride concentration is < 110 mg/dl.

The treatment of chylous ascites depends on the cause, but normally includes repeated abdominal paracenteses, low-fat (medium chain triglyceride) diets and parenteral hyperalimentation (36). Total parenteral nutrition is a primary therapeutic modality in congenital chylous ascites (37). One case report described the successful treatment with somatostatin analog in chylous ascites after radical nephrectomy (38).

## Malignant ascites

The mechanism of fluid retention in patients with malignancy-related ascites depends on the location of the tumor. Peritoneal carcinomatosis appears to cause ascites by exudation of proteinaceous fluid from tumor cells lining the peritoneum (39), which may be induced by cytokines or other poorly defined soluble factors (Fig. 24.1). Extracellular fluid enters the peritoneal cavity to re-establish oncotic balance (40). Although the pathogenesis of ascites formation in patients with massive liver metastases has not received much scientific investigation (41), fluid presumably accumulates owing to portal hypertension caused by stenosis or occlusion of portal veins by tumor nodules or tumor emboli (2). In patients with hepatocellular carcinoma, ascites forms as a result of the underlying cirrhosis-related portal hypertension and/or tumor-induced portal vein thrombosis (42,43).

The macroscopic appearance of malignant ascites is generally similar to that of cirrhotic ascites; less than 10% of malignant ascites are macroscopically hemorrhagic. Thus the differential diagnosis between these two conditions must be based on exploratory findings and laboratory tests. If the total protein concentration of the ascitic fluid is > 3.0 g/dl, the ascites is defined as exudate. Exudates are often malignant. Cytological examination of the ascitic fluid for malignant cells, which is the initial laboratory test to differentiate malignant ascites from other causes, has a sensitivity of only 65%. Even tests for tumor necrosis factor (TNF), lactic acid dehydrogenase (the activity of lactic acid dehydrogenase in malignant ascites may be higher than in cirrhotic ascites due to the leakage of the enzyme from malignant cells lining the peritoneum), interleukin-2 receptors, $\beta_2$-microglobulin, and neopterin are not useful for the differentiation because the sensitivity is as low as for cytological testing (44). Only the concentration of fibronectin, which is significantly higher in malignant ascites than in most other forms of ascites (Fig. 24.2), has a sensitivity and specificity above 95% (45). A little less specific but as sensitive is the cholesterol concentration in the ascitic fluid, which is increased in malignant ascites (46).

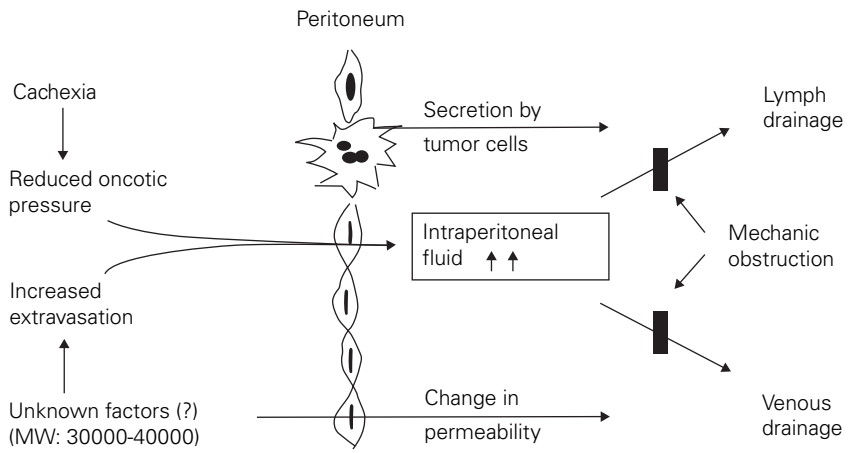


**Figure 24.1** Changes of fluid-transport in malignant ascites.

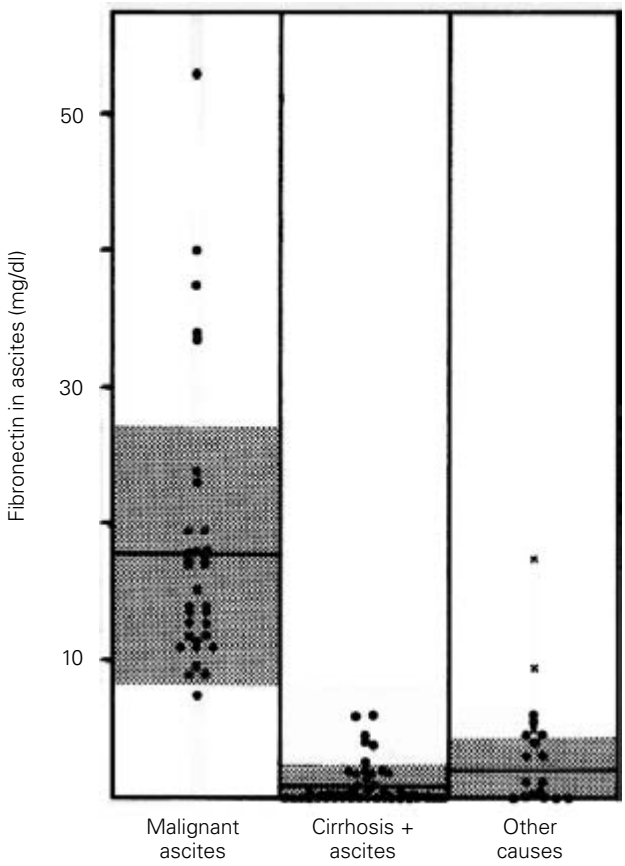


**Figure 24.2** Fibronectin concentration in ascites of patients with malignant ascites, cirrhosis and ascites, and other causes of ascites.

False-negative results of these tests are the rule when ascites is due to portal hypertension secondary to massive liver metastases with little or no peritoneal involvement. In this type of ascites the total ascitic protein concentration is usually < 2.5 g/dl. The differential diagnosis between cirrhotic ascites and the ascites secondary to massive liver metastases, however, can easily be achieved by ultrasound or computed tomography (47) (Fig. 24.3). There is no specific therapy for malignant ascites. As an effective supportive treatment, paracentesis can be used in malignant ascites without substitution of albumin (48). Rarely, a peritoneovenous shunt can provide relief for otherwise intractable malignant ascites. Intraperitoneal injection of cytotoxic drugs or TNF-α has been used with some success (49).

## Tuberculous peritonitis

The differential diagnosis between cirrhotic ascites and ascites due to tuberculous peritonitis is particularly important since alcoholic cirrhosis may predispose to peritoneal tuberculosis. Clinically, tuberculous peritonitis is characterized by fever, abdominal pain, anorexia, abdominal tenderness, and ascites. However, none of these symptoms is invariably present. The proportion of patients with pleural or pulmonary tuberculosis and with reactive tuberculin skin test ranges between 21% and 78% and between 30% and 89%, respectively, in different series (50). In women without active pulmonary tuberculosis, peritoneal tuberculosis may present the local extension of a tuberculous salpingitis (51). However, in many cases no active focus of tuberculosis, apart from the peritoneal disease, can be detected. Results of examination of the peritoneal fluid are often suggestive of tuberculous infection. There is an increased concentration of proteins (> 3 g/dl) and lymphocytes. It has been shown that the ascitic fluid may be a transudate, particularly in cirrhotics with ascites and tuberculous peritonitis (52). But there is also evidence that tuberculosis, chlamydia infection, and coccidioidomycosis probably cause ascites as a result of exudation of proteinaceous fluid as in peritoneal carcinomatosis (2).

Ziehl–Neelsen-stained smears usually fail to show acid-fast bacilli. The rate of positive cultures of ascites fluid for *Mycobacterium tuberculosis* varies markedly from series to series (from 8 to 69%), probably reflecting differences in the technical approach. It has been suggested that the rate of positive cultures may be increased up to 80% by concentrating 1 l of fluid by centrifugation. Nevertheless, diagnosis of tuberculous peritonitis cannot be based on cultures of ascitic fluid since the usual techniques of culturing for acid-fast bacilli may require several weeks to obtain a definite result. The activity of lactic dehydrogenase in ascitic fluid is increased in tuberculous peritonitis compared with cirrhosis (53). As in malignant ascites, the activity of this enzyme in tuberculous ascites is higher than in serum.

Recently, the activity of adenosine deaminase in the peritoneal fluid has been proved to have good accuracy but poor sensitivity and imperfect specificity in a patient population in which the prevalence of tuberculosis is low and underlying cirrhosis is common. Adenosine deaminase is an enzyme involved in the catabolism of purine bases (catalyzing the deamination of adenosine with formation of inosine). It participates in the proliferation and differentiation of lymphocytes and is increased in tuberculous effusions, probably as a consequence of the stimulation of cell-mediated immunity and T lymphocytes (17,54,55).

Open peritoneal biopsy during laparotomy or minimal invasive laparotomy, blind and guided needle biopsy of the peritoneum, and laparoscopy with direct biopsy of the affected areas have been methods used to confirm the diagnosis of tuberculous peritonitis. Characteristically, the peritoneum shows scattered or confluent miliary nodules of uniform size, with adhesions between bowel loops, liver capsule, and abdominal walls. Histological appearance is characterized by the presence of

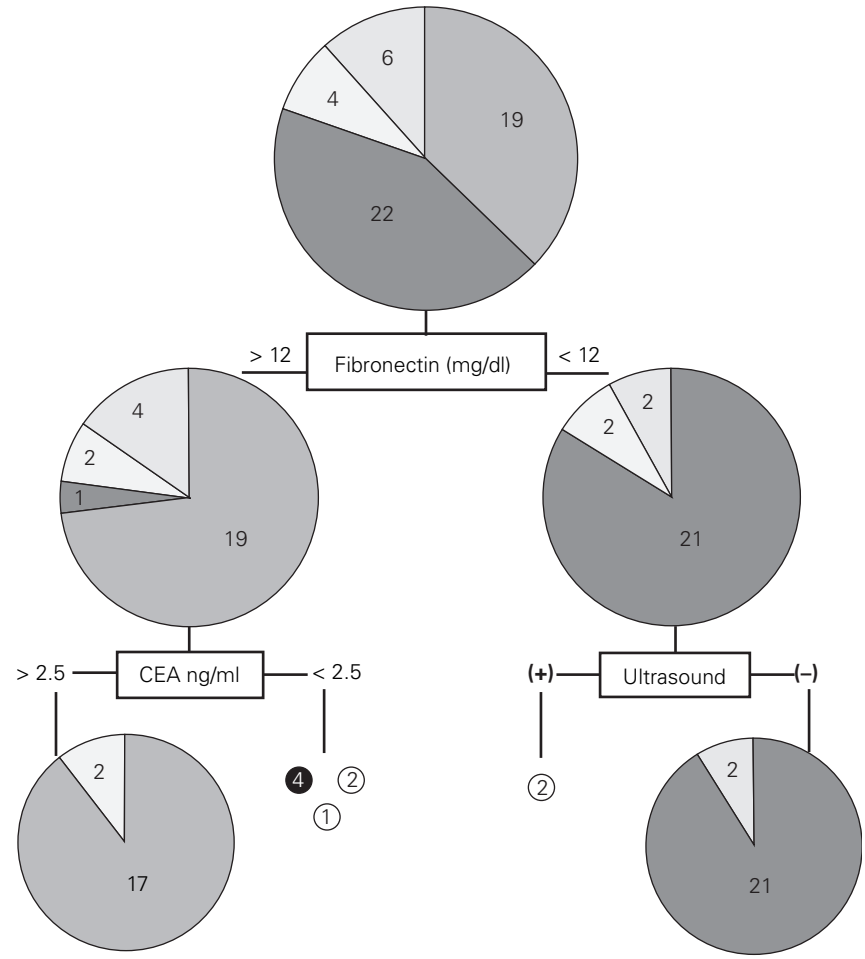


**Figure 24.3** Distribution between the different causes of ascites by fibronectin, CEA (carcinoembryonic antigen), and ultrasound.

caseating granulomas. In some instances mycobacteria may be seen by staining with auramine-rhodamine and performing microscopy under ultraviolet light. *Mycobacterium tuberculosis* can be cultured from the biopsy specimen of the peritoneum. Although there is no definitive study to prove this, a test using PCR for mycobacterium tuberculosis is probably the best way to detect this cause of ascites (56).

The macroscopic and microscopic appearance of tuberculous peritonitis is similar to that of other conditions causing granulomatous peritonitis, such as sarcoidosis, Crohn's disease, and iatrogenic granulomatous peritonitis. The latter condition occurs after 0.15% of abdominal operations and is usually caused by a cell-mediated immune response to starch, talc, cotton fibers, and wood fibers originating from disposable surgical gowns and drapes (9). Iatrogenic granulomatous peritonitis appears 2–9 weeks postoperatively and is characterized by abdominal pain, tenderness, and fever, and frequently by the accumulation of ascites. The observation of starch granules in the ascitic fluid obtained by paracentesis can be diagnostic. Treatment for tuberculous peritonitis is the same as for pulmonary tuberculosis, and should be adapted to the current resistance status.

## Other rare types of ascites

Other causes of ascites easily differentiated from cirrhotic ascites include nephrogenic ascites, myxedema, Meig's syndrome, and yellow nail syndrome (57–59).

Nephrogenic ascites may become a severe problem in approximately 5% of patients maintained on chronic hemodialysis. It is postulated that hemodialysis and in nephrotic syndrome loss of protein in the urine lead to decreased effective arterial blood volume with activation of vasopressin, renin–aldosterone, and sympathetic nervous systems with resulting renal sodium and water retention (4).

The ascitic fluid protein concentration is usually > 3 g/dl and the white blood cell count ranges between 30 and 1500/µl. The amylase and lactic dehydrogenase activities in ascitic fluid are lower than the serum levels obtained simultaneously. Peritoneal biopsy shows minor inflammation or fibrosis. The diagnosis of nephrogenic ascites is one of exclusion. Myxedema is a cause of gross ascites (about 3–4% of cases with myxedema develop significant ascites). Because systemic changes in myxedema may be mild, it may not be identified as the cause of ascites, thus leading to unnecessary diagnostic procedures. The ascitic fluid in myxedema may be serous or gelatinous, and characteristically shows a high protein concentration. Pathogenesis of myxedematous ascites is unknown. Meig's syndrome consists of the association of ascites and hydrothorax with various types of benign ovarian tumors (fibroma, cystadenoma, struma ovarii). Another type of Meig's syndrome is ovarian hyperstimulation syndrome occurring in patients treated with clomiphene and human menopausal gonadotrophins (6). This syndrome is characterized by massive ascites, pleural effusions, signs of tachycardia, hemoconcentration, and oliguria. The formation of ascites and pleural effusions is probably due to increased capillary permeability in the ovarian vessels. However, recent work has shown that the pathogenesis of severe ovarian hyperstimulation syndrome (OHSS) is more complex. Peripheral vasodilation may alter microvascular hemodynamics and permeability, leading to a circulatory dysfunction with marked homeostatic activation of endogenous vasoactive systems having vasoconstrictor and sodium- and water-retaining activity (60,61). In this hyperstimulation syndrome the ovaries become enlarged and may exceed 10 cm in diameter. The patients should be treated by strict bed rest, low-sodium diet, plasma volume expansion with albumin 50 g/day and diuretics, i.e. 20 mg furosemide every 8 h given intravenously (6).

Treatment of all these forms of ascites is aimed at the underlying cause (62–66).

In patients with AIDS, ascites is a relatively common finding. There is a 14% prevalence of ascites in patients with AIDS referred for abdominal complaints and elevated liver enzymes (67). A very rare ascites form is ascites due to endometriosis (68).

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# Part 5
# Hyponatremia and Water Retention in Cirrhosis

# Chapter 25
# Pathogenesis of Hyponatremia: the Role of Arginine Vasopressin

San-e Ishikawa and Robert W. Schrier

Patients with liver cirrhosis have been shown to have impaired water and sodium excretion. In this setting, the non-osmotic release of arginine vasopressin (AVP) is an integral component of the comprehensive response to disorders of arterial vasodilation. Hyponatremia is the clinical hallmark of the non-osmotic release of AVP; however, the vasoconstrictor property of AVP cannot be excluded. Specifically, the non-osmotic release of AVP in cirrhosis occurs secondary to arterial vasodilation in the splanchnic circulation which is mediated through alteration in baroreceptor tone. This AVP release also occurs in concert with the activation of the renin–angiotensin–aldosterone system (RAAS) and the sympathetic nervous system.

## Secretion of arginine vasopressin

AVP is the peptide hormone of the posterior pituitary gland which is synthesized in both magnocellular and parvocellular neurosecretory neurons of the hypothalamus (1). Cell bodies of these neurons reside primarily in the supraoptic nuclei (SON) and the paraventricular nuclei (PVN) (2). The major projection of magnocellular neurosecretory neurons is to the posterior pituitary gland. In the posterior pituitary gland a large amount of AVP, the associated neurophysin (NPII) and glycopeptide are stored in neurosecretory granules. The magnocellular neurons in the SON and PVN respond to both osmotic and non-osmotic stimulation and are involved in the control of body water content and blood pressure. The axon terminals of parvocellular neurons, which originate in the PVN, reach the zona externa of the median eminence of the hypothalamus (3). AVP is secreted from these axons into the pituitary portal circulation, which stimulates adrenocorticotropic hormone (ACTH) via AVP $V_{1b}$ receptor activation (4). Physiological studies suggest that there is independent control of AVP secretion between magnocellular and parvocellular neurons.

Osmotic and non-osmotic stimulations are the two major factors that control AVP release. Osmoreceptors reside in the anteroventral third ventricle (AV3V) region of the hypothalamus, particularly in the organum vasculosum of the lamina terminalis (OVLT) (5,6), and are very sensitive to changes in plasma osmolality (Posm). This region is located outside the blood–brain barrier. There are neural inputs from the osmoreceptors to the PVN and SON, probably mediated via the cholinergic pathway (7). It is evident that other pathways from the brainstem to the magnocellular neurosecretory neurons of the SON and PVN are present (8). These pathways are based on catecholaminergic neurons (9). Afferent fibers from arterial baroreceptors terminate in the nucleus of the tractus solitarius of the dorsomedial medulla oblongata (10). Chemical inhibition and lesions of this nucleus increase plasma AVP levels, suggesting an effect attributable to the interruption of tonic baroreceptor inhibition of AVP release (11). A series of studies with interruption of the glossopharyngeal and vagal pathways from arterial baroreceptors also demonstrated potent non-osmotic AVP stimulation (12,13). The $A_1$ adrenergic cell group of the ventrolateral medulla is suggested to be involved in the afferent pathway from the nucleus of the tractus solitarius to neurosecretory AVP cells of the SON and PVN.

### Biosynthesis of arginine vasopressin

AVP is generated from a precursor form of prepro AVP, which is encoded by the AVP gene on chromosome 20 (14). The AVP gene has three exons. Exon A encodes the signal peptide, AVP, and the N-terminal region of NPII. Exon B encodes the highly conserved central part of NP II. Exon C encodes the C-terminal region of NP II, and the glycoprotein domain (15). The hormone precursor, prepro AVP, contains signal peptide, AVP, NP II, and glycoprotein domains. Pro AVP is generated by removing the signal peptide from prepro AVP and by adding a carbohydrate chain to the glycoprotein domain. Additional post-translational processing of prepro AVP, which yields AVP, NPII, and glycoprotein, occurs within neurosecre-

tory granules during their transport to axon terminals in the posterior pituitary. AVP, NP II, and the glycoprotein are stored in neurosecretory granules in axon terminals of the posterior pituitary, and are released into the bloodstream in response to osmotic or non-osmotic stimulation. The functional properties of NP II and glycoprotein are not completely understood.

The 5′-flanking region of the AVP gene is thought to contain several putative regulatory elements. Mohr and Richter (16) showed the sequences of glucocorticoid responsive element (GRE) and cAMP response element (CRE) in the promoter region along with activator protein-2 (AP-2) binding sites. Glucocorticoid hormones suppress AVP mRNA expression in the parvocellular neurons of the PVN (17). Also, cAMP induces the expression of AVP mRNA, and may be involved in osmotically stimulated AVP gene expression (18). A progressive increase in mRNA has been found in the magnocellular neurosecretory neurons of PVN and SON in rats receiving hypertonic saline or in dehydrated rats (19). Mature mRNA is produced from the precursor RNA by splicing to remove the intron regions and adding the 7-methylguanine cap structure in the 5′-flanking region and a polyadenylate tail [poly (A)] in the 3′-flanking region. Only 2 h of water deprivation is necessary to induce an increased length of poly(A) tail of the AVP mRNA, an effect which occurs prior to any detectable change in Posm. On the other hand, the AVP mRNA accumulation occurred after dehydration for 2 days. Thus, at least two modes of mRNA regulation may be involved in AVP gene expression.

## Osmotic and non-osmotic control of arginine vasopressin release

There is a close correlation between Posm and plasma AVP levels in healthy subjects and in subjects with various states of hydration (20). Linear regression analysis has yielded the osmotic threshold for AVP secretion and the sensitivity of osmoreceptors (Fig. 25.1). The osmotic threshold for AVP secretion is the point of the interception on the horizontal axis, i.e. approximately 280 mmol/kg. Several factors potentially affect the osmotic threshold. Posm decreases by 8–10 mmol/kg during pregnancy, and this decrease is followed by a decrement in the osmotic threshold for AVP release (21). The osmotic threshold is also influenced by non-osmotic stimuli (22). Decreases in circulatory arterial blood volume and blood pressure enhance the secretion of AVP by the osmotic stimulus, which shifts the osmotic threshold to the left in the absence of any change in the sensitivity (22). The sensitivity of osmoreceptors is rather extraordinary. A 1-mmol/kg change in Posm will alter AVP release; this sensitivity, however, is influenced by the nature of solutes. Increases in Posm produced by sodium, sucrose and mannitol exert comparable osmotic effects, but this is not the case with urea or glucose (23,24). Also, other factors, such as the rate of change in Posm, age and the drinking behavior, may affect osmotic sensitivity.

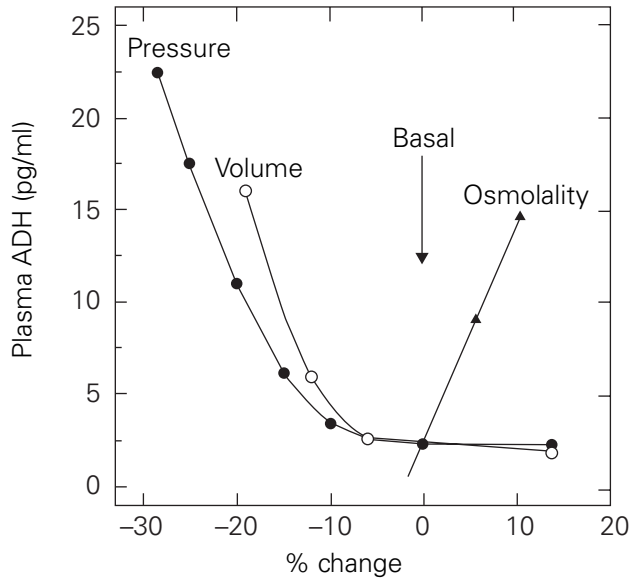


**Figure 25.1** Comparative sensitivities of osmoregulatory and baroregulatory arginine vasopressin (ADH, antidiuretic hormone) efferent mechanisms. (Reproduced by permission from Robertson GL. Regulation of vasopressin secretion. In: Seldin GW, Giebisch G, eds. *The Kidney: Physiology and Pathophysiology*. New York: Raven Press, 1992; 1595–613.)

Decreases in arterial blood pressure and circulating blood volume are potent non-osmotic stimuli for AVP secretion, mediating the high-pressure and low-pressure (left atrial) baroreceptors. It has been generally accepted that a decrement in blood pressure or blood volume of the order of 8–10% is necessary to stimulate AVP secretion. Baylis (25) demonstrated that the relationship between plasma AVP concentrations and the percent fall in mean arterial blood pressure is exponential. Several factors, including low cardiac output, left atrial distension, atrial tachycardia, nicotine, and hypoxia are also evident as the non-osmotic stimulants for AVP release (26).

It is of value to note the separate osmotic and non-osmotic control of AVP release. Electrophysiological studies verified that the osmotic and non-osmotic pathways independently enter the same magnocellular neurosecretory neurons of the PVN and SON (27). The term "reset" osmostat suggests an intrinsic alteration in osmoreceptor cells so that a lower or higher level of Posm is sensed as normal (22). However, many clinical disturbances of Posm can be interpreted by competitive inputs of baroreceptor and osmoreceptor pathways into the same population of neurosecretory cells, independent of any intrinsic

alteration in osmoreceptor sensitivity. In hyponatremic patients with syndrome of inappropriate ADH secretion (SIADH), cardiac failure and liver cirrhosis with ascites, the non-osmotic release of AVP may override the effect of hypo-osmolality.

## Hydro-osmotic action of arginine vasopressin

AVP receptors on the basolateral membranes of renal collecting duct cells are functionally coupled to the Gs protein, leading to the activation of adenylate cyclase (28,29). These receptors are classified as $V_2$ receptors. Lolait *et al.* (30) and Birnbaumer *et al.* (31) independently cloned AVP $V_2$ receptors in rat and human kidney, respectively. The human $V_2$ receptor cDNA encodes 371 amino acids and has seven transmembrane domains in its structure, which is characteristic of G-protein-coupled receptors. Receptor occupancy with AVP leads to a conformational change of guanosine diphosphate to guanosine triphosphate in the α-subunit of Gs. This allows the activation of adenylate cyclase to promptly produce cyclic AMP. Cyclic AMP is the cellular second messenger that activates cAMP-dependent protein kinase A and is catabolized by cAMP-dependent phosphodiesterase. Phosphorylation of protein kinase A then mediates the cellular signaling of AVP to the aquaporin-2 (AQP-2) water channels of collecting duct. This leads to increased AQP-2 mRNA expression and translocation of AQP-2 water channels from the membranes of cytoplasmic vesicles to the apical plasma membranes (Fig. 25.2).

Sasaki and his group (32,33) cloned the cDNA of rat and human AQP-2, by using a polymerase chain reaction (PCR) cloning strategy. This protein is included in the members of the major intrinsic protein (MIP) family (34). Rat and human AQP-2 are 271-amino acid proteins, with 91% amino acid identity with one another and with 43% amino acid homology with AQP-1 (35). These water channels have six putative transmembrane domains, an internal tandem repeat, and a conserved NPA box. Chromosomal mapping of the AQP-2 gene assigned its location to chromosome 12q13.

Northern blot analysis showed that AQP-2 mRNA is extensively expressed in the kidney. Reverse transcriptase-PCR (RT-PCR) along the nephron segments revealed that the expression of AQP-2 mRNA is limited to the collecting duct from the cortical collecting duct (CCD) to inner medullary collecting duct (IMCD). Immunohistochemical studies using the antibody against a synthetic peptide corresponding to C-terminal of AQP-2 showed that AQP-2 is localized only in principal cells of the collecting duct. The AQP-2 staining was strong at apical and subapical regions (36). Immunoelectron microscopy demonstrated that AQP-2 resides in cytoplasmic vesicles in the subapical region in the normally hydrated condition (36). Either the administration of exogenous AVP or dehydration produced translocation of AQP-2 from the cytoplasmic vesicles to the apical plasma membrane (36–38). An abrupt reduction in plasma AVP concentrations or the AVP $V_2$ receptor antagonist causes a prompt endocytosis of AQP-2 into the cytoplasmic vesicles (37,39). This observation supported AQP-2 as the AVP-regulated water channel and confirmed the shuttle hypothesis for water channel trafficking induced by AVP (40).

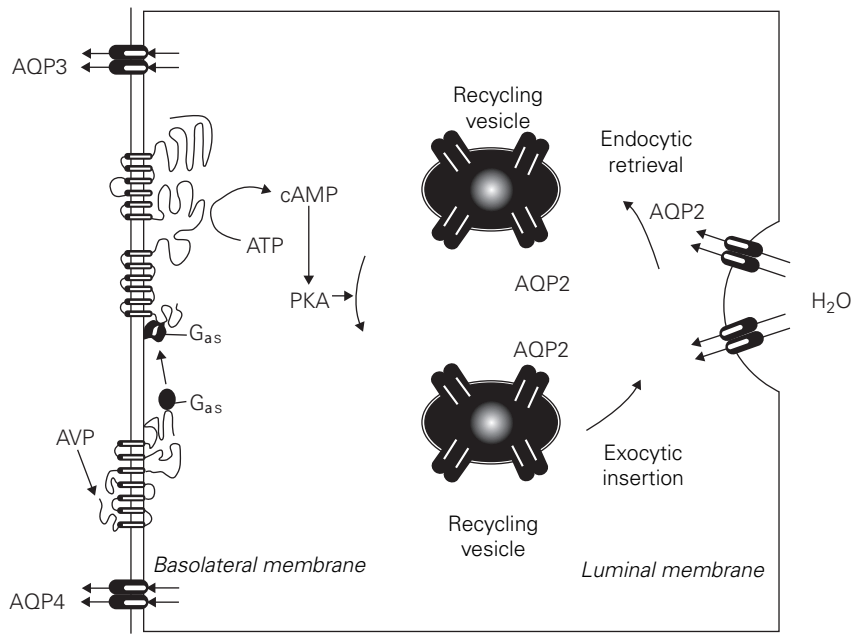


**Figure 25.2** Schematic representation of the intracellular action of arginine vasopressin (AVP) in collecting duct cells.

## Hypersecretion of arginine vasopressin in cirrhosis

Water and sodium retention have been shown to occur in decompensated cirrhotic patients with ascites and edema (41–53). The role of AVP in mediating the abnormal water excretion has been demonstrated in clinical and experimental models of liver cirrhosis (42–45,47,50,52). Removal of the endogenous source of AVP by acute hypophysectomy increased renal water excretion in the dog with acute portal vein constriction (49). However, a residual defect in water excretion persisted, implicating intrarenal factors. Radioimmunoassay techniques directly showed the elevation of plasma AVP concentrations in cirrhotic rats and humans, and AVP secretion was not suppressed by an acute water load (42–47,50,51,54). In addition, hypothalamic AVP mRNA expression is significantly elevated in the cirrhotic rat (55).

Bichet *et al.* (42,43,45) studied the mechanisms of impaired water excretion in patients with liver cirrhosis. An impaired response to an acute water load is present in decompensated cirrhotic patients with ascites, but not in compensated patients without ascites. The decompensated cirrhotic patients exhibited hyponatremia, ascites, and peripheral edema. Basal concentrations of plasma AVP were 2.5 ± 0.5 pg/ml in the decompensated cirrhotic patients and 1.0 ± 0.5 pg/ml in the compensated patients. After the acute water load (20 ml/kg), the minimal concentrations of plasma AVP were significantly higher in the decompensated cirrhotic patients (1.3 ± 0.3 vs. 0.3 ± 0.2 pg/ml) in spite of hypo-osmolality. Activations of the sympathetic nervous system and the RAAS were also presented and correlated closely with the sodium and water retention in the cirrhotic patients.

Several mechanisms for the exaggerated secretion of AVP and other neurohumoral hormones in patients with cirrhosis have been proposed. The elevation of plasma AVP, the activation of RAAS and sympathetic nervous system in cirrhosis are in conflict with the overflow hypothesis (42,43,45,48,56,57), in which it is suggested that primary renal sodium and water retention occurs and leads to total blood volume expansion. If this were the case, these hormonal systems should be suppressed and not stimulated. Alternatively, peripheral arterial vasodilation has been proposed to account for the neurohumoral stimulation and the initiation of water and sodium retention in patients with cirrhosis (58,59). Arteriovenous fistulas and a decrease in peripheral arterial vascular resistance, primarily in the splanchnic circulation, have been shown to occur in clinical and experimental cirrhosis. The peripheral vasodilation is accompanied by sodium and water retention, which increases absolute plasma volume, but this volume expansion is not sufficient to refill totally the enlarged arterial vascular compartment. Thus, the elevated plasma hormones, including plasma AVP, renin, aldosterone, and norepinephrine, are not suppressed. The relative arterial underfilling, which occurs as a result of the systemic arterial vasodilation, thus plays a key role in the baroreceptor-mediated secretion of these vasoactive substances in cirrhotic patients (Fig. 25.3) (60). There are results which indicate that nitric oxide is the mediator of the splanchnic vasodilation in cirrhosis and that inhibition of nitric oxide synthesis reverses the hyperdynamic circulation, suppresses plasma AVP, renin and aldosterone, and reverses the sodium and water retention (61).

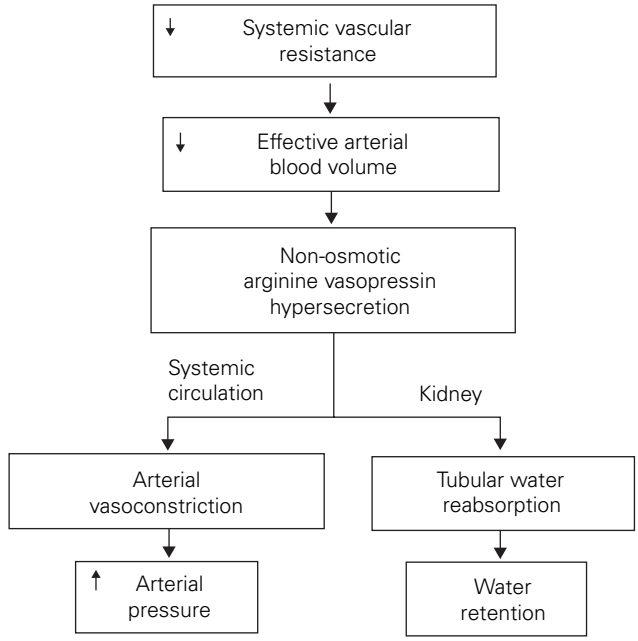


**Figure 25.3** Proposed mechanism of hypersecretion and renal and systemic effects of arginine vasopressin in cirrhosis with ascites.

## Water retention and dilutional hyponatremia

Water retention has been clearly shown to occur in liver cirrhosis in rats, particularly those with ascites (Fig. 25.4) (49–55). An acute water loading test (30 ml/kg body weight) was associated with impaired urinary dilution and a decrease in the percent of water load excreted in the experimental rats with liver cirrhosis. The administration of either the peptide or nonpeptide AVP antagonist can reverse this impairment in water excretion (54).

In cirrhotic rats with nonsuppressible plasma AVP concentrations the expression of the AQP-2 mRNA was augmented by a magnitude 1.6 times greater than in control rats (62,63). The animals were made by administering $CCl_4$ subcutaneously at 1-week intervals for 3 months, and the rats had ascites at the time of the experiment. The upregulation of the AQP-2 mRNA was associated with an increase in the AQP-2 protein in the cirrhotic rats. The

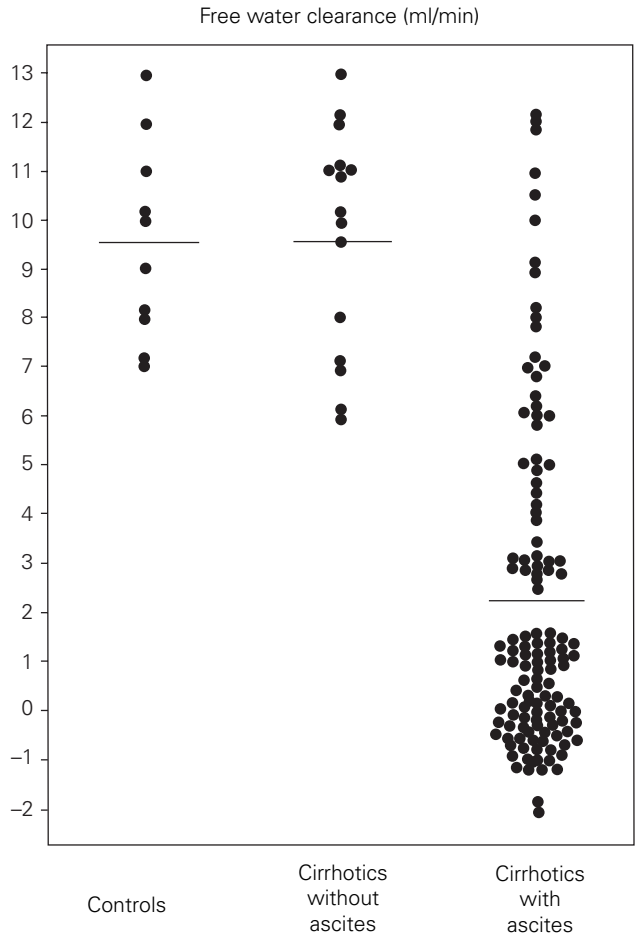


**Figure 25.4** Solute-free water clearance after a water load of 20 ml/kg body weight in healthy subjects, cirrhotic patients without ascites, and cirrhotic patients with ascites. $P < 0.001$ when comparing patients with ascites vs. without ascites and healthy subjects.

expression of AQP-2 mRNA was still increased in the cirrhotic rats 1 h after an acute water load, and plasma AVP levels were also not suppressed. In contrast, the administration of the nonpeptide AVP antagonist OPC-31260 totally reduced AQP-2 expression in the cirrhotic rats. Thus, AQP-2 water channels play an important role in the water retention of hepatic cirrhosis, and these water channels are stimulated by nonsuppressible levels of plasma AVP. The study in cirrhotic rats of $CCl_4$ inhalation showed somewhat different results; that is, cellular redistribution of AQP-2 in collecting duct comparable to trafficking to the plasma membrane was found without an increase in cellular AQP-2 abundance (64). The critical difference between these $CCl_4$ models of cirrhosis may be the degree of liver and damage its decompensation.

Head-out water immersion induces an increase in central blood volume (65), and this maneuver has been performed in patients with liver cirrhosis to determine whether the impaired water excretion was reversible (45). Head-out water immersion did suppress plasma AVP, renin activity, aldosterone, and norepinephrine in the decompensated cirrhotic patients and this suppression was followed by an increase in renal sodium and water excretion. Hemodynamic monitoring showed that head-out water immersion produced increases in cardiac index, right atrial pressure, and pulmonary capillary wedge pressure, and decreased systemic vascular resistance (66). However, head-out water immersion did not normalize renal water and sodium excretion in these patients with liver cirrhosis and ascites. There are at least two potential explanations for the remaining abnormality in renal water and sodium excretion. First, any hepatorenal reflex that might be initiated by increased intrahepatic pressure would not be expected to be corrected by head-out water immersion (67,68). Second, mean arterial pressure did not rise, in spite of the improvement of cardiac output, during head-out water immersion, because of a further decrease in peripheral vascular resistance in these cirrhotic patients (45).

Shapiro *et al.* (48) further evaluated the effect of peripheral vasodilation on renal water excretion in these cirrhotic patients. To avoid the decrease in systemic vascular resistance, the patients were infused with norepinephrine during head-out water immersion. This combined maneuver normalized water and sodium excretion in decompensated cirrhotic patients. No differences were observed in inulin clearance, pulmonary capillary wedge pressure, right atrial pressure, and cardiac output, but mean arterial pressure rose in association with the norepinephrine-mediated effect on vascular resistance. These results therefore supported the peripheral vasodilation hypothesis of sodium and water retention in cirrhosis.

The treatment of ascites and hepatorenal syndrome has been major research topic, but many aspects of the problem remained undetermined. Recent results of clinical trials of AVP $V_2$ receptor antagonists and AVP analogs are promising. Clinical use of AVP receptor antagonists would be expected to improve water retention in cirrhotic patients. Several groups have used nonpeptide AVP $V_2$ receptor antagonist practically (69–71). VPA-985, in concert with water restriction of 1000 ml/day, significantly decreased urinary osmolality and increased solute-free water clearance, and resulted in elevation of serum sodium concentration. In addition, new treatments have been proved to reverse hepatorenal syndrome in cirrhosis, i.e. the simultaneous administration of plasma volume expansion and vasoconstriction. The administration of AVP analogs ornipressin or terlipressin, together with plasma volume expansion with albumin, dramatically improves circulatory function and serum creatinine levels in patients with severe hepatorenal syndrome (72,73).

## Arginine vasopressin as a vasoconstrictor hormone in cirrhosis

Peripheral arterial vasodilation therefore appears to be

the primary event mediating water and sodium retention in decompensated cirrhosis (58,59). Elevations of plasma AVP, angiotensin, and norepinephrine therefore apparently maintain arterial blood pressure in the face of this relative arterial underfilling. The $V_1$ antagonist has been shown to reduce mean arterial pressure in cirrhotic animals after blockade of the renin–angiotensin system (Fig. 25.5), but this effect has not been consistent (53). Angiotensin II has been shown to have a role in maintaining arterial blood pressure in cirrhotic patients. Either saralasin, an angiotensin II antagonist, or inhibitors of angiotensin converting enzyme cause a reversible fall in blood pressure in cirrhotic patients (74). Similarly, the sympathetic nervous system plays a role in maintenance of blood pressure, since cirrhotic patients are very sensitive to α-adrenergic blockade (75).

## Other factors involving water retention in cirrhosis

### Renin–angiotensin–aldosterone system

There is evidence that the RAAS is important for sodium retention in cirrhosis. Removal of the source of aldosterone by adrenalectomy or administration of spironolactone, an inhibitor of aldosterone, is associated with a natriuresis in cirrhosis (76–79). Also, spironolactone augments the natriuretic response to head-out water immersion in cirrhotic patients (65). Saline loading can decrease plasma renin activity and aldosterone concentration to normal values in cirrhotic patients, but the same volume expansion reduces these hormonal levels to below normal ranges in healthy subjects (80). Nonsuppressible concentrations of plasma aldosterone are closely related to the disorder of sodium retention in cirrhosis which is accompanied by iso-osmotic water retention.

### Catecholamines

Plasma norepinephrine concentrations were found to be higher in decompensated cirrhotic patients after an acute water load than in cirrhotic patients who normally excrete the same water load (43). In this study there was a negative correlation between plasma norepinephrine and the percent of water load excreted as well as between plasma norepinephrine and urinary sodium excretion (43). As mentioned earlier, the maneuver of head-out water immersion in combination with norepinephrine administration markedly improved water and sodium excretion in the decompensated cirrhosis, compared with norepinephrine or head-out water immersion alone (48). The increased activity of the sympathetic nervous system could result in enhanced renal water and sodium reabsorption in the proximal tubule in cirrhosis.

### Atrial natriuretic peptide

Plasma levels of atrial natriuretic peptide (ANP) are elevated in cirrhotic patients (81,82). This finding was associated with an increase in ANP mRNA expression in the ventricle of cirrhotic rats (83). However, the natriuretic effect of ANP was blunted in patients with decompensated cirrhosis (84–88). Specifically, infusion of pharmacological doses of ANP did not increase renal sodium excretion, but its natriuretic effect improved when arterial blood pressure was maintained in the cirrhotic patients (86). Other studies showed the inability to mount an adequate sodium excretory response to head-out water immersion in patients with liver cirrhosis (85,88). During head-out water immersion plasma ANP levels increased further, and plasma renin activity and plasma aldosterone were suppressed. In these studies the urinary excretion of cyclic GMP increased in parallel with an increase in plasma ANP, yet no natriuresis was observed (85). Possible mechanisms for this renal resistance to ANP are as follows: (i) downregulation of renal ANP receptors, (ii) enhanced renal neutral endopeptidase activity limiting ANP delivery to the hormone's

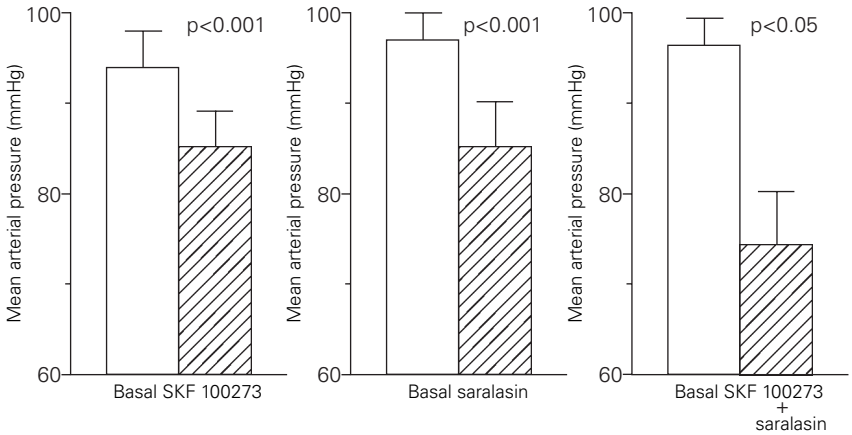


**Figure 25.5** Changes in mean arterial pressure induced by the administration of a V1 AVP receptor antagonist (SKF 100273), the administration of an angiotensin II antagonist (saralasin), and both in conscious rats with cirrhosis and ascites and impaired water excretion. (Reproduced by permission from Claria *et al*. Effect of V1-vasopressin receptor blockade on arterial pressure in conscious rats with cirrhosis and ascites. Gastroenterology 1991; 100:494–501.)

collecting duct site of action, (iii) hyperaldosteronism which increases sodium reabsorption in the distal nephron, and (iv) diminished delivery of sodium to the distal renal tubule site of ANP action. The study by Abraham *et al.* (89) demonstrated that infusion of mannitol, which increased distal delivery of tubular sodium as assessed by lithium clearance, improved the natriuretic effect of exogenous ANP in decompensated cirrhosis. This study supported a normal biological responsiveness of renal ANP receptors in cirrhotic patients, but indicates that the natriuresis is limited by diminished distal sodium delivery.

### Prostaglandins

The renal production of prostaglandins is increased in cirrhotic patients with ascites (90–92). These hormones are known to antagonize the renal vascular effect of endogenous vasoconstrictors and the tubular action of AVP (93,94). Nonsteroidal anti-inflammatory drugs therefore should be administered with caution in these patients because they may induce acute renal failure and water retention (95). In this regard, the administration of indomethacin has been found to be associated with a poor prognosis of liver cirrhosis (96).

In summary, arterial underfilling secondary to splanchnic vasodilation in cirrhotic patients causes non-osmotic release of AVP and increased AQP-2 synthesis and trafficking to the apical membrane of the collecting duct. These events primarily account for the water retention and hyponatremia and can be reversed by AVP $V_2$ receptor antagonists. Diminished fluid delivery to the distal nephron also limits normal water excretion and causes resistance to the action of ANP in cirrhotic patients.

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# Chapter 26
# Management of Hyponatremia in Cirrhosis

Andrés Cárdenas and Pere Ginès

Sodium is critical in the regulation of intra- and extravascular volume and osmolality (reviewed in Chapter 1). Its concentration in blood is maintained by a complex interaction between baroreceptors, osmoreceptors, and central neurohormonal systems, which include the thirst drive and the antidiuretic hormone or arginine vasopressin (AVP). An alteration of sodium concentration may lead to a range of symptoms, including mild cognitive and motor dysfunction, seizure, coma, and death. Abnormalities in AVP arise from increased or decreased production or a deranged action. Diseases such as cirrhosis cause hyponatremia by nature of an increased production of this hormone. In cirrhosis, splanchnic vasodilation leads to arterial underfilling which unloads high-pressure baroreceptors that stimulate a non-osmotic activation of AVP leading to water retention and dilutional hyponatremia, a condition defined as serum sodium concentration < 130 mEq/l (reviewed in Chapter 25). The severity of water and sodium retention in cirrhosis varies considerably from patient to patient; therefore it is important to recognize these derangements early in order to predict response to therapy and survival. While there is treatment for sodium retention with low-sodium diet and diuretics, there is no available therapy yet for dilutional hyponatremia other than fluid restriction. Dilutional hyponatremia is a significant complication in the natural history of cirrhosis as it is associated with a poor prognosis (see Chapter 21). The recent discovery of aquaretic drugs (i.e. drugs that selectively increase solute-free water excretion) for the therapy of dilutional hyponatremia in experimental and human cirrhosis is promising and will probably be of great significance in managing these patients. This chapter will focus on the epidemiology, clinical consequences, diagnosis, current management, and new treatment modalities for dilutional hyponatremia in cirrhosis.

## Definition and epidemiology

Dilutional hyponatremia in cirrhosis is defined as a reduction in serum sodium concentration in the setting of an expanded extracellular fluid volume as evidenced by the presence of ascites and/or edema. This is a common complication with 30–35% prevalence among hospitalized patients with cirrhosis and ascites (1,2). The presence of dilutional hyponatremia in a cirrhotic patient is associated with a poor survival (Fig. 26.1) (1–4). The development of dilutional hyponatremia after a precipitating event such as hemorrhage or infection is associated with a better prognosis when compared with the spontaneous appearance of this complication (Fig. 26.1) (2). This is possibly related to a higher incidence of renal dysfunction and a more advanced stage of decompensated cirrhosis associated with spontaneous dilutional hyponatremia. Dilutional hyponatremia in cirrhosis occurs concomitantly with marked sodium retention; however, water is retained in excess of sodium, which leads to the dilution of body fluids and decrease in serum sodium concentration. The level of serum sodium concentration that defines dilutional hyponatremia has been arbitrarily set at a value below 130 mEq/l (1–5). This dilutional disorder must be distinguished from hypovolemic hyponatremia, which may develop in patients who are maintained on inappropriately high doses of diuretics and sodium restriction after resolution of ascites and edema.

Although patients with hyponatremia arising from hypervolemic states such as cirrhosis, cardiac disease, syndrome of inappropriate antidiuretic hormone secretion (SIADH) usually develop major clinical consequences from non-neurological causes, the brain can be severely affected with significant morbidity and mortality. Hyponatremia can lead to devastating complications such as pulmonary edema, cerebral infarction, cortical blindness, respiratory arrest, and coma (6,7). Therefore the understanding of the underlying mechanisms in cerebral adaptation to hyponatremia is of clinical relevance.

## Cerebral adaptation to hyponatremia

As plasma osmolality falls due to hyponatremia the osmotic equilibrium between cells is preserved by either the removal of intracellular solutes or the dilution of intracellular solutes by the influx of water from the extracellular space (8). When plasma sodium falls, water moves into cells in order to attain osmotic equilibrium. In the brain,

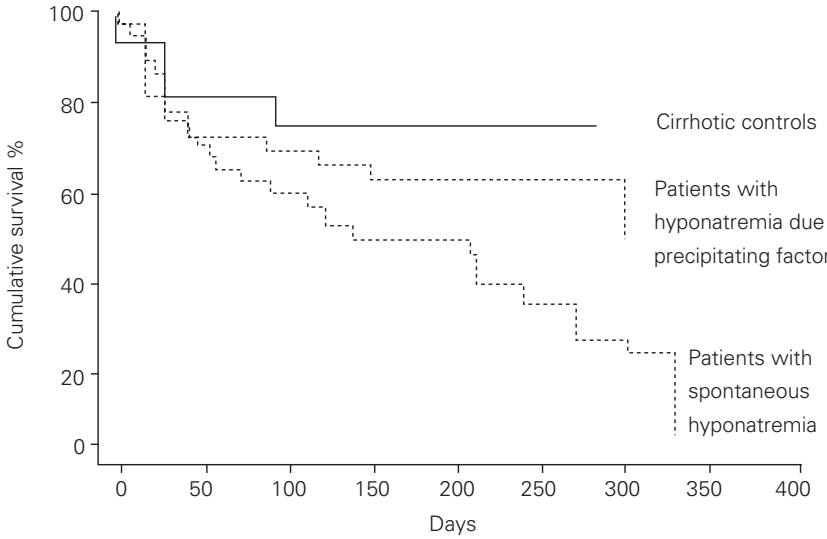


**Figure 26.1** Survival of patients with cirrhosis with and without hyponatremia. The mortality in patients with precipitating factors is less than that in patients with spontaneous dilutional hyponatremia. (Adapted with permission from Porcel A *et al.* Dilutional hyponatremia in patients with cirrhosis and ascites. Arch Intern Med 2002; 162:323–8.)

cell swelling leads to extrusion of intracellular solutes in order to decrease brain osmolality and therefore to try to match that of plasma. Once the solute removal is successfully achieved, osmotic equilibrium will be maintained between brain and plasma, and no symptoms arise from hyponatremia. The mechanisms are complex and involve movement of water in the brain by selective aquaporin water channels, in particular aquaporin 4 (9). If solute extrusion is inadequate, water will continue to influx into cells and increased intracranial pressure, cerebral edema, and eventual tentorial herniation will ensue. Normally, in brain cells, initial swelling begins a process of efflux of intracellular solutes to decrease brain osmolality toward that of plasma. If solutes are extruded an osmolar equilibrium will be reached between brain and plasma (i.e. patient will be asymptomatic despite having a low plasma sodium and osmolality). The mechanisms responsible for protecting the brain from edema have been previously studied (10). In animal models there is a rapid loss (within 24 h) of both electrolyte and organic osmolytes after the onset of hyponatremia; however, water efflux becomes more marked after 1 day (10–13) (Fig. 26.2). The process that follows after recovery of hyponatremia is that of regain of electrolytes and osmolytes; sodium and chloride correct very quickly but the organic osmolytes take longer to recover (14) (Fig. 26.2). Organic osmolytes are intracellular compounds involved in the long-term adaptation of osmotic changes. These osmolytes, which include glutamine, glutamate, taurine, and myoinositol, account for about one-third of the solute loss in chronic hyponatremia (13,15). In cases where dilutional hyponatremia occurs slowly there is a process of adaptation where solutes leave the brain tissue (13). The reason why this occurs has not been completely elucidated but may be related to a protective mechanism that involves an initial increase in the interstitial hydraulic pressure, which produces a gradient for the extracellular fluid to move out of the brain into the cerebrospinal fluid (12). In cases where correction of hyponatremia occurs rapidly (either after aggressive treatment or water restriction alone) lack of adequate adaptation may lead to osmotic demyelination syndrome (15,16). In this severe complication called central pontine myelinolysis (CPM), shrinkage of cerebral tissues leads to demyelination of pontine and extrapontine neurons. This is associated with severe neurological deficits such as quadriplegia, pseudobulbar palsy, seizures, and coma (16).

In advanced cirrhosis with and without hepatic encephalopathy there is an alteration in brain osmolytes as demonstrated by magnetic resonance spectroscopy in human and experimental studies (17–20). There is an increase in brain glutamine and reduction in other organic osmolytes, particularly myoinositol (18,19). Hyperammonemia, which commonly occurs in advanced cirrhosis, can give rise to brain glutamine levels (a consequence of the metabolism of ammonia which can only be metabolized in astrocytes) and contribute to cell swelling and, possibly, cell dysfunction (Fig. 26.3) (17,19). The high levels of glutamine lead to a compensatory loss of myoinositol and other organic osmolytes (17,20). Similarly in chronic hyponatremia of cirrhosis, there is a marked decrease in myoinositol and other osmolytes (Fig. 26.3) (21).

The relationship between hyponatremia and hyperammonemia, although less studied, has shed light on the complex interactions between these two states which commonly occur in cirrhosis (Fig. 26.3). Córdoba *et al.* studied the effects of hyponatremia on osmolytes and its relationship to hyperammonemia in an animal model of acute liver failure (22). In this study hyponatremia was induced by desmopressin (DDAVP); afterwards a portocaval anastomosis and infusion of ammonia was performed. This resulted in a significant increase in brain water in hyponatremic animals compared with that in nonhyponatremic controls. Hyponatremic animals showed a marked decrease in brain organic osmolytes. The release of

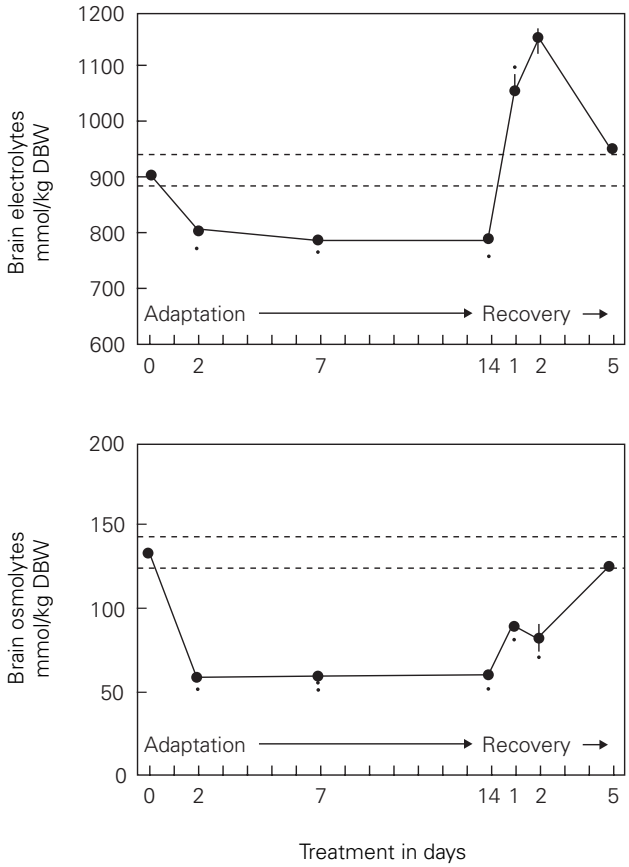


**Figure 26.2** Changes in brain water, brain sodium and potassium and osmolytes after induction of hyponatremia with desmopressin (DDAVP). Brain $Na^+$, $K^+$ and $Cl^-$ and multiple organic osmolytes (glutamate, creatinine, taurine, myoinositol, glutamine and glycerophosphoryl-choline) decreased markedly by day 2 of hyponatremia. Brain electrolyte and most organic osmolyte contents then remained at these reduced levels throughout the duration of the hyponatremia (14 days). After rapid correction of the hyponatremia on day 14, electrolytes re-accumulated rapidly after first 2 days of correction, before returning to normal levels by day 5 after correction. In contrast, brain organic osmolytes recovered much more slowly. Organic osmolytes constitute a significant proportion of the brain solute losses that take place during hyponatremia, indicating that reductions in both organic osmolyte and electrolyte contents are necessary to accomplish brain volume regulation. (Adapted from Verbalis JG, Gullans SR. Hyponatremia causes large sustained reductions in brain content of multiple organic osmolytes in rats. Brain Res 1991; 567:274, and Verbalis JG, Gullans SR. Rapid correction of hyponatremia produces differential effects on brain osmolyte and electrolyte reaccumulation in rats. Brain Res 1993 ; 606:19.)

osmotically active solutes leading to an intracellular depletion of these may limit the total amount of osmolytes available to be released or compensate for brain swelling, as indicated in this study by exacerbation of brain edema after the infusion of ammonia. In the only other study reported so far in patients with cirrhosis and hyponatremia, levels of organic osmolytes, as measured by magnetic resonance spectroscopy, correlated directly with serum sodium and osmolality; however, brain glutamine levels were higher in patients with cirrhosis when compared with those of healthy subjects and correlated with ammonia levels but not with serum sodium. The high levels of glutamine, which in noncirrhotic patients with hyponatremia are low, may be the consequence of a prolonged exposure of the brain to high ammonia levels (21). These findings indicate that organic osmolytes are likely to play an important role in cerebral fluid homeostasis in cirrhosis. Hyponatremia in cirrhosis is accompanied by significant changes in osmolytes that possibly prevent significant neurological damage and occur as high levels of glutamine trigger a compensatory response against cell swelling.

## Clinical consequences

The most frequent symptoms in hyponatremia in patients without liver disease are neurological in nature and related to the rapidity with which the serum sodium concentration falls. In the majority of cases symptoms attributable to hyponatremia occur in association with a rapid reduction in the plasma sodium concentration and reflect neurological abnormalities secondary to cerebral edema, where a fall in plasma osmolality creates an osmolal gradient favoring movement of water arising from plasma and cerebrospinal fluid into brain cells.

Usually patients with cirrhosis develop dilutional hyponatremia over several days to weeks, although occasionally some may present with an acute onset of hyponatremia (23). In most patients the degree of hyponatremia is mild, with levels ranging from 125 to 130 mEq/l. Nonetheless, some patients may have lower values reaching 110–125 mEq/l. Although a detailed assessment of symptoms associated with dilutional hyponatremia in cirrhosis has not been made, patients may have symptoms such as nausea, vomiting, apathy, anorexia, lethargy, muscle cramps, disorientation, headaches, and confusion similar to those found in dilutional hyponatremia of different etiology. Neurological symptoms related to hyponatremia may be difficult to distinguish from hepatic encephalopathy, which also may occur concomitantly with dilutional hyponatremia. Complications of severe hyponatremia such as seizures, coma, respiratory arrest, brainstem herniation, severe brain damage, and death rarely occur in the setting of cirrhosis (23). A better and reliable characterization of symptoms related to dilutional hyponatremia in cirrhosis could be performed if this condition were effectively reversed with newer pharmacological agents. A major clinical consequence of hyponatremia is what may occur following its correction. After adaptation to hyponatremia the excessive brain volume has returned toward normal and rapid correction of severe hyponatremia (< 115 mEq/l) may lead to the development CPM. As mentioned previ-

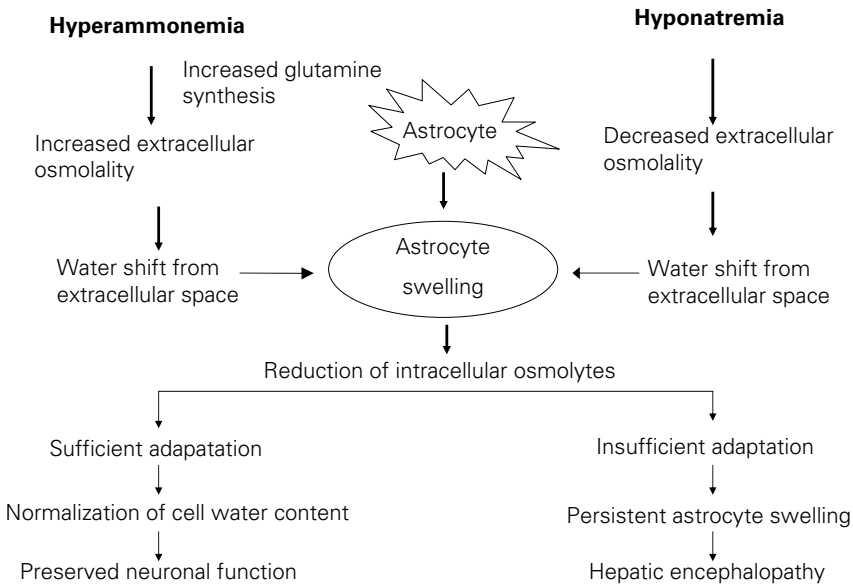


**Figure 26.3** Proposed interaction between hyperammonemia and hyponatremia on brain astrocytes and possible pathogenic relationship with hepatic encephalopathy. High levels of brain ammonia in advanced cirrhosis are detoxified in astrocytes. Here glutamine is produced and leads to increased intracellular osmolality and water shift from extracellular space with subsequent astrocyte swelling, which is further regulated by a reduction in osmolytes, leading to preserved neuronal function. In hyponatremia from cirrhosis a reduction mainly in myoinositol also leads to regulation and preserved neuronal function. In hepatic encephalopathy a hyponatremic state leads to more exacerbation of brain edema due to a decrease in organic osmolytes induced by hyponatremia. The release of osmotically active solutes leading to an intracellular depletion of these may limit the total amount of osmolytes available to be released or compensate for brain swelling (22).

ously, in CPM shrinkage of cerebral tissues leads to demyelination of pontine and extrapontine neurons. This is a severe complication due to the rapid correction of sodium in hyponatremia and has devastating consequences that can rapidly lead to seizures and coma (16). Therefore, if therapy is indicated for hyponatremia, three basic questions to be addressed should include: (i) what is the risk of osmotic demyelination? (ii) what is the appropriate rate of correction and best way of raising serum sodium concentration? and (iii) what is the sodium deficit? If these questions are answered appropriately then the risk of developing CPM can be significantly reduced.

In patients undergoing liver transplantation donor serum sodium is an important predictor of graft survival (24). Many donors have hypernatremia and hyperosmolarity and there is evidence that significant differences between donor serum sodium and recipient serum sodium predispose to early graft dysfunction, which in part could be related to the development of CPM given the rapid correction to which the recipients would be submitted (24). Additionally, rapid correction in serum sodium in the recipient may lead to neurological complications such as CPM or seizures after liver transplantation (25). The development of CPM and extrapontine myelinolysis after liver transplantation has been well described and in all cases post-mortem retrospective review revealed that there were marked perioperative rises in serum sodium levels exceeding 20 mEq/l, with the maximal rate of change occurring on the day of surgery (26). Fortunately, advances in liver transplantation have permitted a better identification of patients at risk for developing CPM; nonetheless, a low serum sodium in the recipient remains a common scenario and therefore efforts to avoid significant serum sodium fluctuation during the perioperative period remain a key element in preventing this devastating complication (27,28).

Another major clinical problem with dilutional hyponatremia is related to the challenge this complication poses on treating ascites effectively, because diuretics may worsen hyponatremia and precipitate hepatic encephalopathy. In addition, compliance with water restriction is difficult to achieve and impairs quality of life in an already sick patient.

## Treatment

### Nonpharmacological therapy

The most accepted therapy for dilutional hyponatremia in cirrhosis and other water-retaining states is fluid restriction of 1000 ml/day in order to prevent a further increase in total body water (23,29). Unfortunately, fluid restriction in patients with cirrhosis and dilutional hyponatremia is not very effective in raising serum sodium levels. In a recent randomized, placebo-controlled study by Gerbes *et al.* (30), fluid restriction did not increase serum sodium, but it did prevent it from decreasing further. Administration of hypertonic saline solution is not recommended because additional expansion of the extracellular fluid can worsen edema and ascites and its effect in increasing serum sodium is modest (31). In addition to water

restriction, patients must also follow a low-sodium diet (70–90 mmol/day) because they have marked sodium retention. Precipitating factors for developing dilutional hyponatremia are the use of nonsteroidal anti-inflammatory agents and large-volume paracentesis without volume expansion (32,33).

## Pharmacological therapy

Pharmacological therapies for dilutional hyponatremia in cirrhosis have focused on inhibiting the actions of AVP. One of the first drugs used for dilutional hyponatremia was demeclocycline, a tetracycline that inhibits the tubular effect of AVP, thereby increasing solute-free water clearance and serum sodium concentration (34). This drug was previously used in patients with SIADH and those with cirrhosis and ascites. One of the drawbacks with this agent was the development of renal failure and it was therefore abandoned in patients with cirrhosis (35,36). In a report from Decaux *et al.*, urea was used to treat cirrhosis patients with ascites and hyponatremia. Urea at a dose of 30–90 g/day improved the renal response to diuretics with an increase in serum sodium concentration and urine volume (37). Although not reported, it is plausible that urea administration could possibly trigger or perpetuate hepatic encephalopathy in patients with advanced liver disease. Despite the initial results of Decaux *et al.*, no further studies have been published regarding the use of urea in this disorder.

The aim of therapy in dilutional hyponatremia is to increase solute-free water excretion. Different approaches to antagonizing the action of AVP include: (i) blockade of the V2 receptor for AVP with specific antagonists, (ii) inhibition of central release of AVP with κ-opioid agonists, and (iii) amelioration of the effect of AVP in the collecting duct. The latter option is not feasible because demeclocycline causes renal failure. Therefore, only V2 receptor antagonists and the κ-opioid agonists have been studied in animals and humans with water-retaining states such as cirrhosis, congestive heart failure (CHF), and SIADH. Nevertheless, none of these drugs is available for use in clinical practice.

### *κ-Opioid agonists*

The effects of κ-opioid agonists on water excretion have been known for several years. These agents increase urine volume in man and experimental animals with a decrease in urine osmolality and without changes in electrolyte excretion (38,39). The mechanism of diuresis of these agents is complex and has not been completely elucidated. In contrast to the μ-opioid agonists, which have an antidiuretic effect, κ-opioid agonists inhibit AVP release from the neurohypophysis. Interestingly, these agonists can increase urine flow in isolated perfused kidneys, which also supports the existence of a local intrarenal mechanism of action (40). In addition, it has been successfully documented that κ-opioid agonists exert an aquaretic effect since the blockade of the receptor with opioid antagonists (i.e. naloxone) causes antidiuretic effects (41).

The effects of these agents on water excretion have been studied in experimental and human cirrhosis. In 1995, Bosch-Marcé *et al.* studied the effects of the κ-opioid agonist niravoline in rats with cirrhosis, ascites, and impaired water excretion. This compound produced a significant increase in urine volume and solute-free water excretion without changes in urinary electrolytes or arterial blood pressure (42). In 1996, Moreau *et al*. also studied the effects of niravoline on renal function in rats with cirrhosis (43). After a single dose (3 mg/kg i.v.) urine flow and serum sodium increased significantly with niravoline when compared with a vehicle; in addition, it significantly decreased urinary osmolality and increased plasma osmolality and solute-free water clearance. However, this agent did not significantly change urinary sodium excretion. The long-term efficacy (10 days) of two aquaretic agents was also assessed by Bosch-Marcé *et al*. in a comparative study of OPC 31260 (a V2 receptor antagonist) and niravoline in rats with experimental cirrhosis (44). The effect of OPC 31260 resulted in a transient increase in urine flow and reduction of urine osmolality that lasted for 2 days, whereas the administration of the κ-opioid agonist displayed aquaretic activity throughout the entire period of treatment. Although these findings suggest that niravoline has a more prolonged action than OPC 31260, this has not been the case with other V2 receptor antagonists (see below).

In the only human study of cirrhosis with a κ-opioid, Gadano *et al*. reported the use of different single doses (0.5–2 mg i.v.) of niravoline in 18 patients. This compound induced a marked aquaretic effect with a significant increase in urine volume and serum sodium and a significant decrease in urine osmolality (45). These effects were observed between 1 and 2 h after administration of a single dose with return of these parameters to basal values within 24 h. In two patients receiving the highest dose, personality disorders and mild confusion occurred within the first hour with reversal of these effects after 8 h of dosage. Although these drugs have a potent aquaretic effect, their potential side-effects on the central nervous system are worrisome and should be evaluated carefully in studies including large numbers of patients. No recent studies have been performed with this group of drugs, probably due to the discouraging side-effects patients developed.

### *V2 receptor antagonists*

Manning *et al*. discovered the first peptide V2 receptor antagonists in the 1970s (46). However, these agents could not be used in the clinical setting due to their low oral bioavailability and their partial antidiuretic agonist activity when given to humans. Further modifications to the

parent molecule permitted the development of nonpeptidic compounds that could hold on to the ability to bind to V2 receptors without activating a signal transduction cascade. In 1992 Yamamura *et al*. reported on an orally effective nonpeptidic V2 receptor antagonist, OPC 31260, 100 times more selective for V2 than for V1 receptors (47). Recently, several other nonpeptide V2 receptor antagonists, including SR 121463, OPC 41061, conivaptan, and VPA 985 with greater affinity for V2 receptors than OPC 31260, have been the focus of basic and clinical studies (48).

Yamamura *et al*. investigated the effects of OPC 31260 in water loaded rats; this agent inhibited the antidiuretic action of exogenous AVP, increased urine flow, and decreased urine osmolality in a dose-dependent manner. Moreover, when given to Brattleboro rats (animals with a congenital AVP deficiency) it was not associated with antidiuretic effects, indicating that it did not have any effects as a partial V2 receptor agonist (47). In another study, Fujisawa *et al.* induced SIADH in rats by the administration of the AVP analog DDAVP; the administration of OPC 31260 increased serum sodium and osmolality levels and caused a marked increase in solute-free water excretion (49). Further studies by Ohnishi *et al.* demonstrated that in healthy men OPC 31260 also induced an increase in urine volume, a decrease in urine osmolality, and a remarkable rise in solute-free water excretion in a dose-dependent fashion without altering electrolyte excretion, blood pressure, or heart rate significantly (50,51). Saito *et al.* also assessed the aquaretic effect of OPC 31260 in 11 patients with SIADH; urine volume and serum sodium concentration increased whereas urine osmolality decreased for 4 h after OPC 31260 was given intravenously (52).

In 1996 Serradeil-Le Gal *et al.* examined the effects of SR 121463. This compound caused a marked aquaresis without changes in electrolyte excretion in experimental animals (53). Epstein *et al.* also reported the effects of VPA 985 in 60 healthy subjects; after a single dose (up to 400 mg), mean urine flow, serum sodium and solute-free water clearance increased, whereas urine osmolality decreased in a dose-related fashion (54). In 2003, Gheorghiade *et al.* evaluated the aquaretic effects of OPC 41061 (tolvaptan) at different doses in patients with CHF in a large, double-blind, randomized trial (55). In this study the agent was well tolerated and caused a significant decrease in body weight, edema and major elevation in serum sodium when higher doses of 45 and 60 mg were used. New V2 receptor antagonists continue to be developed and recently the aquaretic effects of YM087, conivaptan, and YM471 have been reported in animals and humans (56–58).

### *V2 receptor antagonists in experimental cirrhosis*

More than a decade ago, Clària *et al.* investigated the role of a peptidic V2 antagonist [d(CH2)5Tyr(Et)VAVP] in the pathogenesis of water retention in cirrhotic rats (59). The agent normalized water excretion in nine out of 10 rats without significant changes seen in a control group. Unfortunately, these peptidic V2 antagonists turned out to display very poor oral bioavailability and could not be used in humans. In the mid-1990s nonpeptidic V2 antagonists were discovered, as discussed above, and Tsuboi *et al.* examined the role of OPC 31260 in rats with carbon tetrachloride-induced cirrhosis and controls (60). Water excretion after a water load increased fourfold and minimal urinary osmolality decreased by 30% after the administration of this agent. Additionally, serum sodium and urinary sodium excretion increased significantly when compared with the control group. However, in another study by Bosch-Marcé *et al.* the maximum effect this drug was limited to the first 2 days of treatment, probably due to development of tachyphylaxis (44). Jiménez *et al.* in 2000 reported the long-term (10 days) aquaretic efficacy of SR121463 in cirrhotic rats with ascites and impaired water excretion after a water load. The administration of the drug led to a significant increase in urine volume and reduced urine osmolality when compared with a vehicle (Fig. 26.4) (61). This also resulted in a greater renal ability to excrete a water load and normalization in serum sodium and osmolality. In contrast to OPC 31260, the effects of SR121463 were sustained, and there was no tachyphylaxis. Finally, Fernández-Varo *et al.* in 2003 reported the effects of conivaptan, a combined receptor V1a/V2 antagonist, on renal water handling and systemic hemodynamics in cirrhotic rats with ascites (62). In this study the chronic

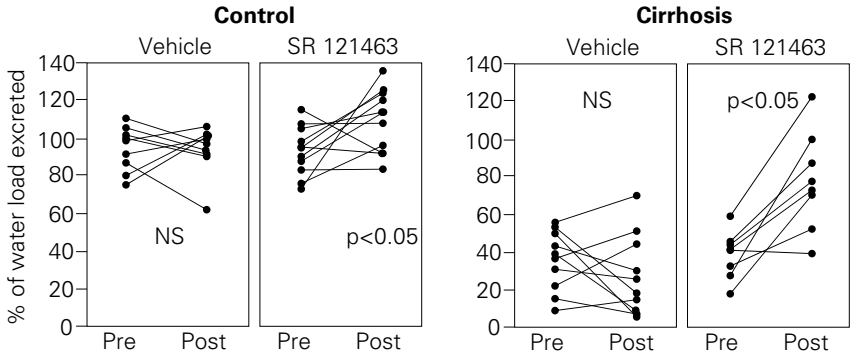


**Figure 26.4** Individual values of the percentage of the water load excreted observed in control rats and in cirrhotic rats with ascites and impaired water excretion before and after completing oral administration of vehicle or the vasopressin V2 receptor antagonist SR121463 (0.5 mg/kg body weight). (Reprinted with permission from Jiménez W *et al*. J Pharmacol Exp Ther 2000; 295:83–90.)

administration (10 days) of this agent to rats with severe water retention and ascites produced an acute increase in urine volume and a reduction in urine osmolality. In this study the aquaretic effect lasted for only 5 days. Interestingly, there was a remarkable improvement in sodium excretion that was probably due to the effect of conivaptan on the renin–angiotensin system. Most importantly, these effects occurred without affecting creatinine clearance or arterial pressure, an effect that could be expected due to the vasoactive action that the V1a receptor exerts over blood vessels mediating vasoconstriction.

*V2 receptor antagonists in human cirrhosis*

Inoue *et al.* reported in 1998 the first study in humans examining the therapeutic effect of OPC 31260 in patients with cirrhosis and ascites (63). The oral administration 30 mg/day to eight patients with cirrhosis and ascites without hyponatremia was associated with an increase in the urine volume and solute-free water clearance at 2 and 4 h, respectively, and a decrease in urine osmolality at 2 h after administration. Since patients did not have hyponatremia at baseline, the effect on serum sodium was not assessed. Furthermore, neither the normal serum sodium rise, nor urinary sodium excretion changed. In 2002, Guyader *et al.* reported the pharmacodynamics, safety, and pharmacokinetics of ascending single doses of VPA 985 (25–300 mg) in 27 patients with cirrhosis and ascites in a Phase I randomized, double-blind, placebo-controlled trial (64). VPA 985 produced a marked dose-related rise in urinary output and a dose-related decrease in urine osmolality when given at 300 mg/day (Fig. 26.5). Urine volume

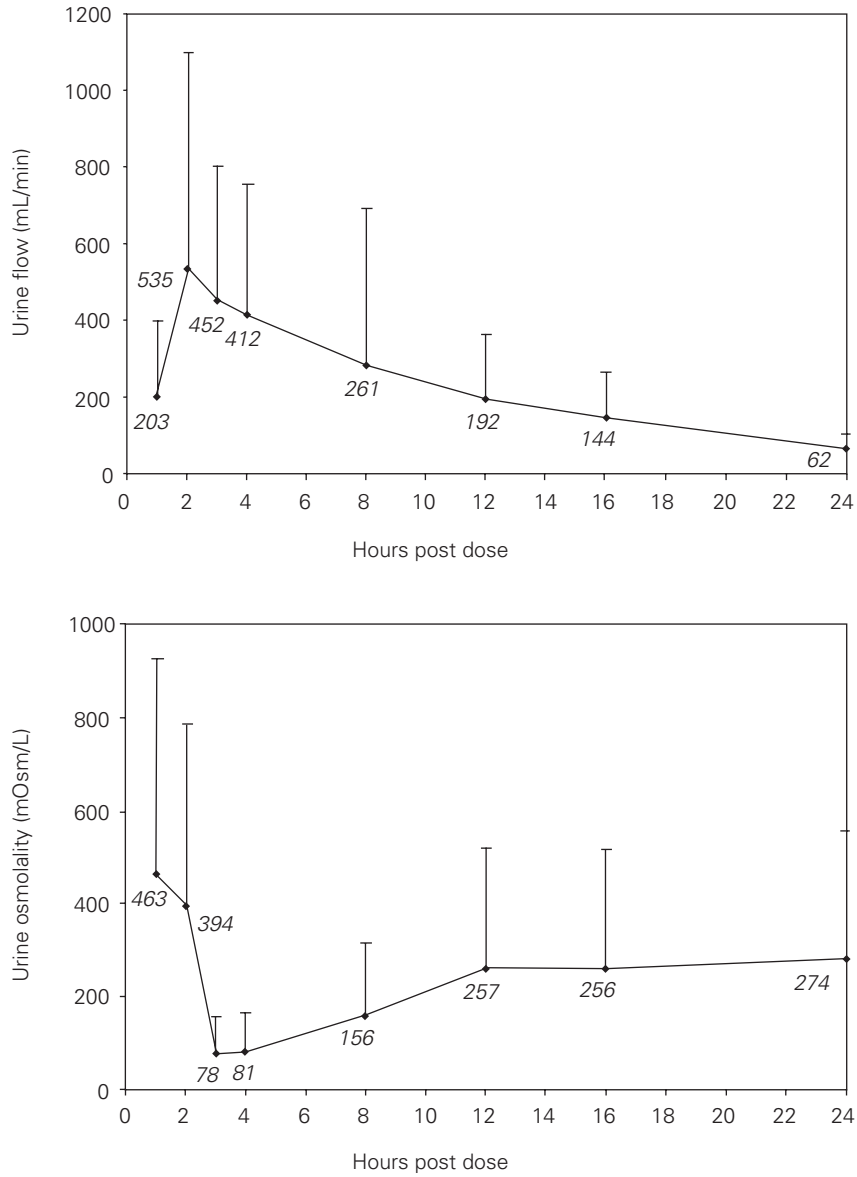


**Figure 26.5** Effects of the vasopressin V2 receptor antagonist VPA 985 at 300 mg on urine flow and osmolality in patients with cirrhosis and ascites. (Reprinted with permission from Guyader D *et al*. Pharmacodynamic effects of a nonpeptide antidiuretic hormone V2 antagonist in cirrhotic patients with ascites. Hepatology 2002; 36:1197–205.)

increased significantly with the 300-mg dose. Solute-free water clearance increased to levels > 3 ml/min for doses 100 mg and higher. In addition, significant increases in urine sodium excretion and serum osmolality, sodium, and vasopressin levels were observed. In this relevant pharmocodynamic study the authors demonstrated the potential of VPA 985 as a therapeutic agent for water retention in cirrhosis. However, since the main end-point was to evaluate the safety and efficacy of this compound, they did not examine its role in managing patients with dilutional hyponatremia; in fact, all patients had serum sodium levels > 130 mEq/l. Both agents (VPA 985 and OPC 31260) in the above studies were clinically well tolerated and did not produce any significant side-effects or changes in systemic hemodynamics.

Two multicenter, randomized, placebo-controlled trials published in 2003 evaluated the use of VPA 985 in cirrhotic patients with dilutional hyponatremia. In the first trial, Wong *et al.* investigated the effects of VPA 985 on serum sodium during a 7-day period in 44 hospitalized patients with dilutional hyponatremia (33 with cirrhosis, five with CHF, and five with SIADH) (65). Patients with cirrhosis and CHF were kept on diuretics and escalating doses of VPA 985 ranging from 50 to 500 mg/day were given. VPA 985 had a significant aquaretic response compared with placebo in those on diuretic therapy as well as patients not on diuretics (SIADH group). There was a dose-related increase in the net fluid volume (urine output minus fluid intake) and solute-free water clearance leading to significant increases in serum sodium and serum osmolality (Fig. 26.6). Regrettably, there was a high dropout rate (12 patients or 27%; six due to dehydration and the other half due to other reasons). The highest doses of the drug (250–500 mg/day) were poorly tolerated and associated with dehydration, as assessed by systemic postural hypotension, increased thirst, and marked sodium eleva-

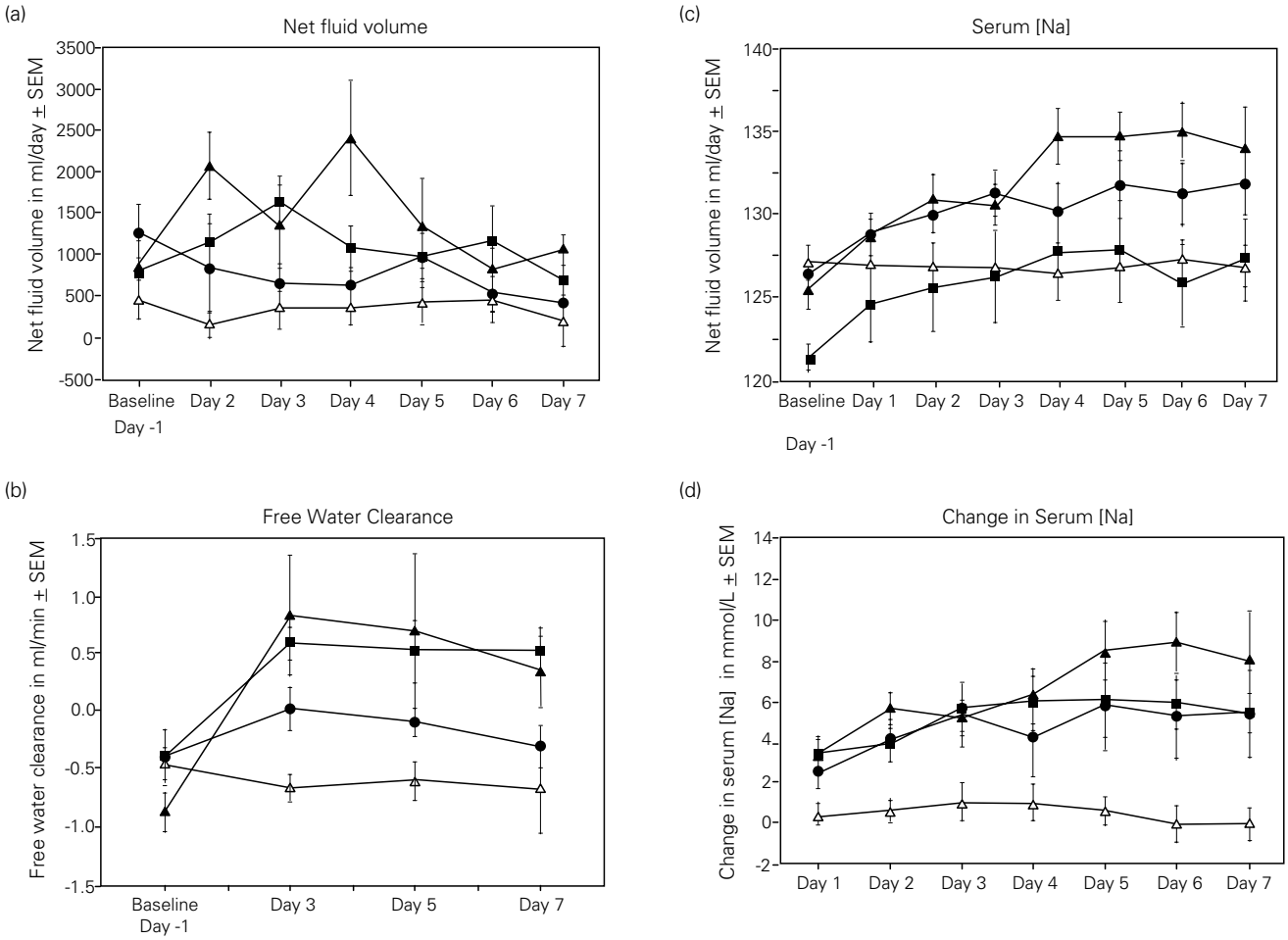


**Figure 26.6** Aquaretic effects of the vasopressin V2 receptor antagonist VPA-985 in patients with dilutional hyponatremia. Twenty-four hour net fluid volume (urine output minus fluid intake) (A) and renal solute-free water clearance (B) in four different study groups. (△) Placebo; (●) 25 mg twice daily; (■) 125 mg twice daily; (▲) 250 mg twice daily. Serum sodium (C) and change in serum sodium (D) in the four study groups. Change in serum sodium value is the difference between the day in question and previous days' serum sodium values. (△) Placebo; (●) 25 mg twice daily; (■) 125 mg twice daily; (▲) 250 mg twice daily. (Reprinted with permission from Wong *et al*. Hepatology 2003; 37;182–91.)

tion. As a result, half of the patients on the 500-mg/day dose had the medication held on several occasions. VPA 985 was effective and safe when given at a dose of 125–250 mg/day. The second study by Gerbes *et al.* included 60 patients with cirrhosis and dilutional hyponatremia on fluid restriction who were randomly assigned to receive 100 or 200 mg/day of VPA 985 or placebo for 7 days (30). There was a significant dose-dependent increase in serum sodium concentration as well as a significant reduction in urine osmolality and body weight in both groups receiving VPA 985, whereas no changes in these parameters were found in the placebo arm. Complete response defined by reaching a serum sodium > 136 mEq/l was observed in 27% in the 100-mg dose and 50% in the 200-mg dose (Fig. 26.7). Thirst sensation was the main side-effect in the 200-mg group, but not in the other groups. The 50% response in the 200-mg group would therefore be difficult to improve by increasing this dose due to limiting side-effects of thirst and volume depletion. Two problems with this study were the small number of patients ($n = 20$) in each group, and that the effects of VPA 985 were evaluated only until serum sodium normalized without information on long-term response.

The findings of these studies suggest that the effects of VPA 985 seem to be independent of sodium reabsorption proximal to the renal collecting tubules. This compound was administered in combination with diuretics without any significant problems. In fact, other aquaretics such as tolvaptan and conivaptan have a significant natriuretic effect in disease states like CHF. This makes VPA 985 an attractive drug for potential combination therapy with diuretics in diseases where there is concomitant sodium retention and dilutional hyponatremia, such as cirrhosis and ascites. Nonetheless, extreme caution would be needed when administering VPA 985 and diuretics such as furosemide. These two drugs may produce a marked aquaretic effect and lead to volume depletion and subsequent dehydration and/or renal failure.

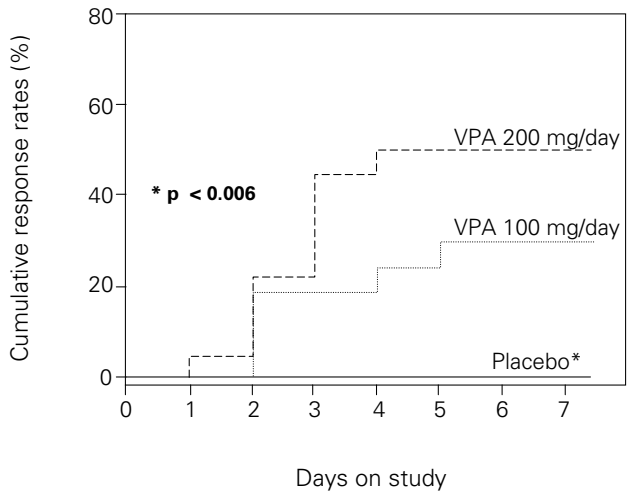


**Figure 26.7** Time to response after administration of the V2 receptor antagonist VPA 985 at two different doses to patients with cirrhosis and ascites and dilutional hyponatremia. Response was defined as normalization of serum sodium to ≥ 136 mmol/l. Mean time to response was 4.8 days in the 200-mg group and 5.7 days in the 100-mg group. There was no effect in the placebo group. (Reprinted with permission from Gerbes *et al*. Gastroenterology 2003; 124:933–40.)

Until now, studies with V2 receptor antagonists in patients with cirrhosis and dilutional hyponatremia have demonstrated that these drugs have a dose-related response when correcting hyponatremia over 1 week. The effects of the V2 receptor antagonists in cirrhotic patients with dilutional hyponatremia are summarized in Table 26.1. Results of further Phase 2 and Phase 3 trials are needed in order to define the indications and contraindications regarding the use of V2 receptor antagonists in clinical practice.

## Summary

The presence of dilutional hyponatremia is a poor prognostic indicator of survival in patients with cirrhosis and ascites. All efforts should be made to prevent worsening of hyponatremia by prescribing fluid restriction. A better understanding of the mechanisms involved in water regulation has enabled researchers to define critical steps in the metabolism of water, mainly the regulation of AVP synthesis and release, the characterization of the V2 receptor of collecting duct cells of the kidney where AVP exerts its action, and the discovery of a selective water channel, AQP2, which inserts water molecules from the luminal membrane of the collecting duct cells into circulation. The demonstration of these events responsible for the non-osmotic AVP hypersecretion and subsequent water retention in cirrhosis and ascites has been the background for the development of the aquaretic drugs discussed above. These drugs have been shown to enhance water excretion selectively in experimental animals and healthy subjects. Furthermore, in animals and patients with cirrhosis similar results have been observed.

Several conclusions may be drawn regarding the use of aquaretics in cirrhosis: (i) effective and safe treatments are sorely needed in an aim to improve prognosis in patients with cirrhosis and dilutional hyponatremia; (ii) the type of aquaretics for cirrhotic patients differs significantly. Current data on V2 receptor antagonists indicate that they are promising and probably safe at low doses, but there are few data on κ-opioid agonists and the side-effects arising from the only human study published so far are troublesome; (iii) V2 receptor antagonists used in dilutional hyponatremia seem to be safe and effective in normal subjects, but may cause important side-effects such as volume depletion in cirrhotic patients. Although this problem seems to be dose related, the best aquaretic effects are achieved with high doses and therefore these

**Table 26.1** Published clinical studies using AVP V2 receptor antagonists in patients with cirrhosis and dilutional hyponatremia.

| Author (ref.) | Year | Compound | Dose | Phase | Patients (*n*) | Findings |
|---|---|---|---|---|---|---|
| Inoue (63) | 1998 | OPC-31260 | 30 mg po single dose | I | 8 | Increased U vol and lowered U osm. $CH_2O$ increased. UNa did not change. Patients did not have hyponatremia |
| Guyader (64) | 2002 | VPA-985† | 25–300 mg po single dose | I | 27 | Increased U vol, $CH_2O$, UNa, S osm and serum Na. Patients did not have hyponatremia. No serious side-effects |
| Wong (65) | 2003 | VPA-985† | 50–500 mg/day po for 7 days | II | 44* | Increased urine output, $CH_2O$, S osm and serum Na. Dehydration and thirst were seen in patients at doses of 500 mg. Drop-out rate was 27% |
| Gerbes (30) | 2003 | VPA-985† | 100–200 mg/day po for 7 days | II | 60 | Increased serum Na, decreased U osm and body weight.Thirst appeared as a side-effect in patients at the 200-mg dose |

U osm, urinary osmolality; $CH_2O$, solute-free water clearance; serum Na, serum sodium; U vol, urine volume; UNa, urine sodium; S osm, serum osmolality; po, per ora; i.v., intravenous. *Included five patients with cardiac disease and five with syndrome of inappropriate antidiuretic hormone secretion. †Randomized, double-blind, placebo-controlled trial.

drugs should be used with caution; (iv) the rapid correction of serum sodium poses a theoretical risk of producing pontine and extrapontine myelinolysis. Therefore, correction of hyponatremia should be done slowly. Finally, the clinical utility of these agents in cirrhosis has been assessed only in short-term studies. In the case of VPA 985, the aquaretic effects reached a plateau after 5 days of treatment and therefore data on the efficacy after 7 days and potential long-term side-effects remain unknown.

The use of these agents in clinical practice has not been defined completely. However, the fact that solute-free water excretion is enhanced in the presence of diuretics makes it very attractive for patients with cirrhosis and ascites with dilutional hyponatremia. In this case, management of chronic hyponatremia would be easier and simpler. Unfortunately, these drugs have not been extensively studied in humans with cirrhosis, ascites, and dilutional hyponatremia. Several of the V2 receptor antagonists discussed above are currently being evaluated in Phase 3 studies; therefore the future of these drugs is promising. Future research with these compounds should not only focus on the serum sodium and water excretion, but must focus also on the changes in cerebral osmolytes (with the aid of magnetic resonance) that occur after correction of hyponatremia for a better understanding of the underlying neuropathophysiological processes that occur with this condition. Although good preliminary data exist on the efficacy of these drugs, further large-scale efficacy and safety trials are needed in order to consider aquaretics a real addition to the therapeutic armamentarium for management of complications of cirrhosis.

## Acknowledgements

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# Part 6
# Renal Failure and Hepatorenal Syndrome in Liver Disease

# Chapter 27
# Pathogenesis of Renal Vasoconstriction in Cirrhosis

Mónica Guevara, Rolando Ortega, Pere Ginès, and Juan Rodés

A normal perfusion of the kidneys is crucial to the maintenance of kidney functions, including not only glomerular filtration rate (GFR) but also excretory functions. Under normal conditions, renal blood flow is maintained constant through a delicate equilibrium between vasoconstrictors and vasodilator factors acting on the renal circulation in such a way that any increase in the activity of vasoconstrictor factors is rapidly followed by compensatory activation of renal vasodilators, which helps maintain renal perfusion within normal limits (1,2). It has been known for many years that such equilibrium in the renal circulation is of crucial importance in the setting of cirrhosis. In some instances, the equilibrium in the renal circulation can no longer be maintained, so that there is predominance of vasoconstrictor factors and a reduction in renal perfusion occurs. Investigations performed by several groups between the 1950s and 1970s using specific methods to assess renal plasma flow and GFR provided conclusive evidence of a reduced renal perfusion and GFR in patients with advanced cirrhosis and demonstrated that hepatorenal syndrome (HRS) was due to a vasoconstriction of the renal circulation (3–6). Later studies showed that patients with less advanced cirrhosis also have some degree of renal vasoconstriction that tends to progress over time (7–9). While patients with pre-ascitic cirrhosis have normal renal perfusion with no evidence of renal vasoconstriction, patients with ascites show reduction of renal blood flow and GFR of variable degrees (Fig. 27.1) that usually follow other abnormalities of renal function such as sodium and solute-free water retention that are further impaired by the reduction in GFR. The aim of the current chapter is to discuss the mechanisms involved in the regulation of renal blood flow and GFR in patients with cirrhosis and the pathogenesis of renal vasoconstriction in patients with cirrhosis.

## Mechanisms involved in the regulation of renal hemodynamics in cirrhosis

The exact mechanisms leading to renal vasoconstriction in cirrhosis are not completely known. Several factors, including disturbances in systemic hemodynamics, increased activity of vasoconstrictor systems, and reduced activity of vasodilator factors acting on the renal circulation play a major role (10). The hemodynamic pattern of patients with HRS is characterized by hypervolemia, high cardiac output, low arterial pressure and total systemic vascular resistance, and marked activation of vasoconstrictor systems (3,11–15). This pattern of highly increased renal vascular resistance in the setting of low total systemic vascular resistance and compensatory overactivity of vasoconstrictor systems is very characteristic of HRS, although not exclusive, as it may be observed in renal failure associated with sepsis (16). By contrast, this pattern is markedly different from that of hypovolemia, low cardiac output, and high total systemic vascular resistance characteristic of most clinical conditions associated with renal hypoperfusion of prerenal origin (17). The abnormalities in systemic circulation of cirrhosis are discussed in detail

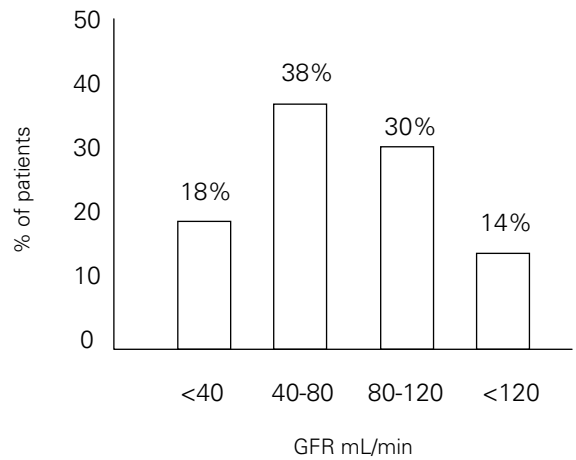


**Figure 27.1** Glomerular filtration rate, measured by inulin clearance, in a series of 216 cirrhotic patients hospitalized for the treatment of an episode of ascites. Values in healthy subjects are 110–140 ml/min. (Reproduced by permission from Ginès P, Fernández-Esparrach G, Arroyo V, Rodés J. Pathogenesis of ascites in cirrhosis. Semin Liver Dis 1997; 17:175–89.)

in Chapters 11, 12 and 15. This chapter will review the systemic and local vasoactive factors that are known to act on the renal circulation in cirrhosis. Factors that may potentially participate are also discussed. For simplicity, the vasoactive factors have been divided into vasoconstrictors and vasodilators. The former are the effectors of renal vasoconstriction, while the latter have a role as counterbalancing systems to maintain renal perfusion.

## Renal vasoconstrictors

### *Renin–angiotensin–aldosterone system*

The renin–angiotensin–aldosterone system (RAAS) was the first major vasoconstrictor system to be investigated as a possible factor responsible for renal vasoconstriction in cirrhosis. It is now well known that the activation of RAAS is particularly intense in patients with HRS compared with patients with cirrhosis and ascites without HRS (see Chapter 4). Moreover, there is an inverse relationship between plasma renin activity (PRA), which estimates the activity of the RAAS, and renal plasma flow and GFR (18–20). Further indirect evidence of the role of the RAAS in the pathogenesis of renal vasoconstriction in HRS derives from clinical studies showing that treatment of patients with HRS with vasoconstrictor drugs and albumin or transjugular intrahepatic portosystemic shunts (TIPS) is followed by a marked improvement of renal plasma flow and GFR that occurs together with a suppression (complete normalization in some cases) of the increased activity of the RAAS (12,15,21–26). An additional piece of evidence supporting the role of the RAAS in the pathogenesis of HRS derives from the assessment of the temporal relationship between the activation of the RAAS and the development of renal failure in patients with cirrhosis and spontaneous bacterial peritonitis (27). In this condition, the development of HRS is associated with a striking activation of the RAAS (Fig. 27.2). The administration of albumin prevents the activation of RAAS during the infection and reduces the rate of development of HRS. Interestingly, a recent study has shown that the beneficial effect of albumin in this setting is due to an increase both in total systemic vascular resistance and in cardiac output (28).

Despite all this indirect evidence, the definite confirmation of a role for the RAAS in the pathogenesis of renal vasoconstriction in cirrhosis has not been provided and may be impossible. This would require the demonstration that the pharmacological interruption of RAAS activity is associated with a reversal improvement of the renal vasoconstriction. Unfortunately, this approach cannot be used in clinical practice, as the blockade of the activity of the RAAS is associated with marked arterial hypotension in patients with cirrhosis and ascites due to the important effect of the RAAS in the maintenance of arterial pressure in this condition (29,30).

### *Sympathetic nervous system*

As occurs with the RAAS, in advanced cirrhosis there is an increased activity of the sympathetic nervous system (SNS) (see Chapter 5). The plasma concentration of norepinephrine (NE) in the systemic circulation, an index of the activity of the SNS, is increased in most patients with ascites and normal or only slightly elevated in patients without ascites (19,31,32). Because the SNS is a powerful vasoconstrictor of the renal circulation (33), it is reasonable to presume that the increased renal sympathetic nervous activity in cirrhosis may be important in the pathogenesis

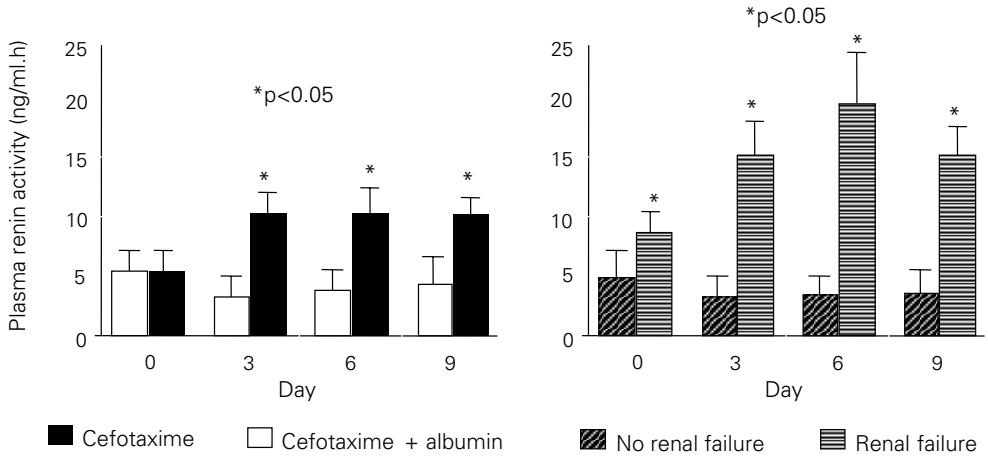


**Figure 27.2** Left, Plasma renin activity on days 0, 3, 6, and 9 in patients with cirrhosis and spontaneous bacterial peritonitis treated with cefotaxime plus albumin (1.5 g/kg body weight at the time of diagnosis and 1 g/kg body weight on day 3) and in patients treated with cefotaxime alone. Right, Plasma renin activity on days 0, 3, 6, and 9 in patients with cirrhosis and spontaneous bacterial peritonitis divided into those who developed renal failure during the infection and those who did not. (From Sort P, Navasa M, Arroyo V *et al.* Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. N Engl J Med 1999; 341:403–9.)

of renal vasoconstriction and HRS. In fact, patients with HRS have significantly higher plasma levels of NE than do patients with ascites without renal failure, and peripheral venous, arterial, and renal venous NE correlate inversely with renal blood flow (18,34,35). Further evidence in favor of the participation of the SNS in the pathogenesis of the HRS is that the improvement in renal blood flow and GFR after pharmacological treatment with vasoconstrictor drugs or TIPS placement in patients with HRS is paralleled by a marked suppression of the activity of the SNS (12,15,22,25) (Fig. 27.3). Unfortunately, as occurs with the RAAS, the inhibition of SNS activity in patients with cirrhosis, using the central $\alpha_2$-adrenergic clonidine, is associated with a marked fall in arterial pressure, which makes it impossible to test directly whether the SNS plays a major role in renal vasoconstriction in cirrhosis (36). The recent observation that the administration of norepinephrine intravenously together with albumin improves renal function in patients with HRS casts some doubt on the role of endogenous NE as renal vasoconstrictor in HRS (24). Nevertheless, it appears that exogenous NE produces renal vasoconstriction only when given directly into the renal artery or at doses that produce arterial hypertension (37,38).

Neuropeptide Y, a neurotransmitter with a potent renal vasoconstrictor effect that is released in the setting of a marked activation of the SNS, may also have a role as renal vasoconstrictor in cirrhosis, as peripheral plasma levels of neuropeptide Y are increased in patients with HRS but not in patients with ascites without renal failure (39).

### *Endothelins*

The endothelin family comprises three homologous peptides (ET-1, ET-2, and ET-3) with a very potent vasoactive action synthesized by a number of cell types, including endothelial cells from the systemic arterial and venous circulation, sinusoidal endothelial cells and hepatic stellate cells from the liver, and mesangial cells from the kidney (see Chapter 9 for review). The effects of ETs are mediated through two types of receptors, $ET_A$ and $ET_B$, that exhibit distinct selectivity for ET isopeptides. The $ET_A$ receptor binds ET-1 and ET-2 with a higher affinity than ET-3, while $ET_B$ displays similar affinities for all three isopeptides. $ET_A$ is responsible for the vasoconstrictor effect of ETs, whereas the stimulation of $ET_B$ causes mainly vasodilation through the activation of nitric oxide (NO) and prostaglandins. ET synthesized by endothelial cells is thought to participate in the regulation of vascular tone by acting as a paracrine substance on the underlying vascular smooth muscle cells (40).

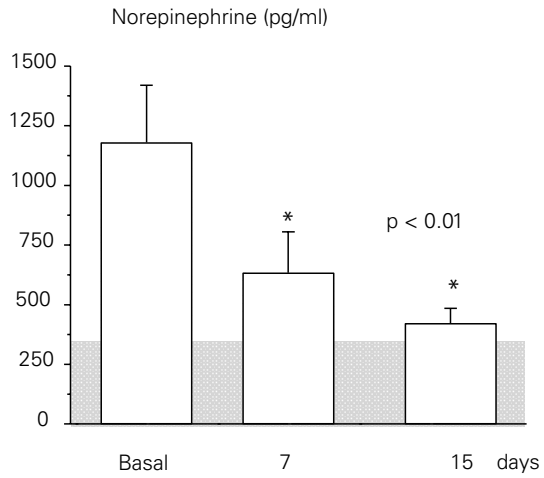


**Figure 27.3** Effect of the administration of ornipressin plus albumin on plasma levels of norepinephrine in a peripheral vein in cirrhotic patients with hepatorenal syndrome. Shaded area represents values in healthy subjects.

Patients with cirrhosis have increased circulating levels of ET (reviewed in Chapter 9). Because of this and its marked vasoconstrictor effect, it has been proposed that ET contributes to the pathogenesis of renal vasoconstriction in cirrhosis. An inverse relationship between plasma levels of ET and renal plasma flow and GFR has been demonstrated in patients with ascites in some (41,42) but not all studies (43–46). However, the correlation between plasma levels of ET and renal function is weak in most studies. Moreover, there is no consistent relationship between plasma levels of ET and the presence or absence of HRS (43,47–49). Nevertheless, it should be emphasized that because ET is a substance with a paracrine mode of action, its plasma levels do not necessarily reflect levels of ET within the kidney, which so far have not been measured in human cirrhosis. Interestingly, improvement of renal function was reported in a small group of patients with HRS after administration of BQ-123, a selective antagonist of $ET_A$ receptors (50). Unfortunately, this interesting finding has not been followed by studies in larger groups of patients. In summary, a role for ET in the pathogenesis of HRS is possible but has not been demonstrated thus far. This confirmation awaits studies using newly developed ET receptor antagonists in patients with HRS.

### *Adenosine*

Adenosine is an adenosine triphosphate (ATP) breakdown product that in most vessels causes vasodilation and contributes to the metabolic control of organ perfusion (51). In contrast to its vasodilatory effect in other organs, adenosine produces vasoconstriction in the renal vasculature (51). For this reason, adenosine has been investigated as possible mediator of renal vasoconstriction in cirrhosis. Adenosine-1 receptors are present on the afferent arteriole in the kidney and cells of the proximal tubule, and stimulation of these receptors inhibit adenyl cyclase, resulting in renal vasoconstriction and sodium and water retention. Adenosine-2 receptors are

found in the vasculature of the systemic circulation and stimulation leads to vasodilation (51). The possible role of adenosine in the pathogenesis of renal functional abnormalities in human cirrhosis has been assessed using several approaches. The acute administration of methylxanthines, which act as nonspecific adenosine antagonists, to patients with cirrhosis and ascites is associated with an increase in renal blood flow, GFR, and sodium and water excretion (52), while the acute administration of an adenosine-1 receptor antagonist to patients with cirrhosis and ascites induces a marked increase in sodium excretion and urine flow, without changes in renal hemodynamics (53). Conversely, the acute administration of dipyridamole, a drug that acts, at least in part, by increasing the levels of adenosine in the extracellular fluid due to inhibition of the cellular uptake of this substance, is associated with renal vasoconstriction and increased sodium and water retention, particularly in patients with ascites and increased activity of the RAAS (54) (Fig. 27.4). Taken together, these results indicate that adenosine may have a role in renal vasoconstriction and associated renal abnormalities of cirrhosis.

### *Vasoconstrictor eicosanoids*

In addition to the vasodilating prostaglandins, the kidney synthesizes a number of eicosanoids which have antinatriuretic and/or vasoconstrictor activities (see Chapter 7). The renal production of some of these eicosanoids with vasoconstrictor activity has been investigated in patients with cirrhosis. It was first suggested that HRS could be the consequence of an imbalance between the renal synthesis of vasodilator and vasoconstrictor eicosanoids based on the observation of reduced urinary excretion of prostaglandin $E_2$ ($PGE_2$) and 6-keto-PGF1α [a metabolite of prostaglandin $I_2$ ($PGI_2$)] and increased urinary excretion of thromboxanes (i.e. $TXB_2$) in patients with HRS (55). These findings, however, were not confirmed by subsequent investigations in which the urinary excretion of $TXB_2$ in patients with HRS was found to be decreased (56–58). Moreover, the inhibition of thromboxane synthesis does not improve renal function in these patients (59).

Eicosanoids other than thromboxanes with vasoconstrictor effect may potentially participate in the reduction of renal perfusion in cirrhosis. The possible role of leukotrienes (LTs) in HRS has been somehow overlooked. Indeed, studies performed in the late 1980s and early 1990s showed that the urinary excretion of LTE4, the major metabolite of LTC4, and LTD4, and a useful measure of whole body production of cysteinyl-leukotrienes, is elevated in HRS (60,61). More recently, the concentration of the vasoconstrictor metabolite 20-hydroxyeicosatetraenoic acid has been shown to be markedly increased in the urine of patients with cirrhosis and ascites and inversely correlated with renal blood flow (62). Finally, the possible role of a family of newly discovered prostaglandin $F_2$-like compounds, termed $F_2$-isoprostanes, that are produced *in vivo* as products of free radical catalyzed lipid peroxidation independent of the cyclooxygenase enzyme, has been explored. Patients with HRS have markedly increased plasma levels of $F_2$-isoprostanes in the peripheral blood compared with those of patients with decompensated liver disease without renal failure and patients with chronic renal failure without liver disease (63,64). The potential role of all these vasoconstrictor eicosanoids in the pathogenesis of renal vasoconstriction in cirrhosis deserves further investigation.

## Renal vasodilators

### *Prostaglandins*

Prostaglandins have a protective effect on renal function in pathophysiological conditions, such as cirrhosis with ascites, associated with increased activity of renal vasocon-

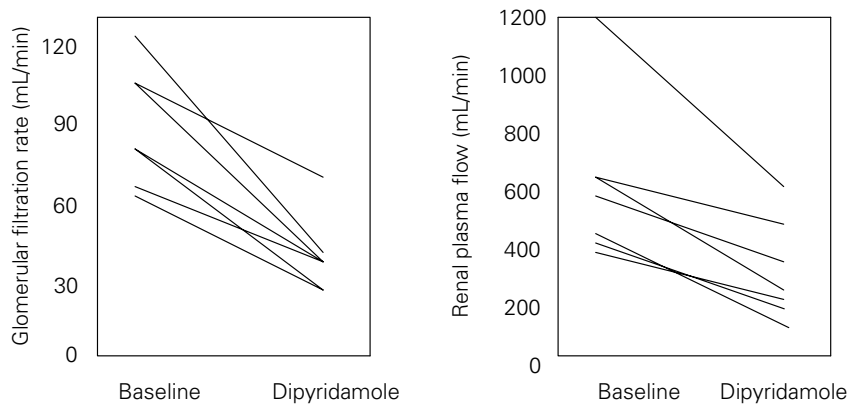


**Figure 27.4** Effect of dipyridamole, 0.4 mg/kg i.v., on glomerular filtration rate and renal plasma flow in cirrhotic patients with ascites. (Reproduced by permission from Llach J, Ginès P, Arroyo V *et al*. Effect of dipyridamole on kidney function in cirrhosis. Hepatology 1993; 17:59–64.)

strictor systems (65). A number of studies in patients with cirrhosis have provided data supporting the existence of an increased renal synthesis of PGs (reviewed in Chapter 8). Urinary excretion of $PGE_2$ and 6-keto-prostaglandin $F1_{\alpha}$, which estimate the renal synthesis of $PGE_2$ and $PGI_2$, respectively, are increased in patients with cirrhosis and ascites without renal failure compared with healthy subjects and patients without ascites (18,56,58,66–70). Studies in experimental cirrhosis have extended these observations, showing an increased activity and expression of cytosolic phospholipase $A_2$ and cyclooxygenase (COX), the first two enzymes of the metabolic cascade of eicosanoid synthesis (71–73). Of the two isoforms of COX, only COX-2, the inducible isoform, is overexpressed in the kidneys of rats with carbon tetrachloride-induced cirrhosis, while the constitutive COX-1 is expressed normally. Nevertheless, COX-1 appears to be more important for the maintenance of renal function because its selective inhibition in rats with cirrhosis is associated with impairment in renal function, while the selective inhibition of COX-2 does not cause significant effects on renal function (73,74). No data are available on the expression and activity of the different COX isoforms in human cirrhosis.

Support for the importance of renal PGs in the maintenance of renal blood flow and GFR in cirrhosis with ascites derives from studies assessing the effects of nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit renal PG synthesis. The administration of classic NSAIDs, which cause a nonselective inhibition of COX activity, even in single doses, to cirrhotic patients with ascites causes a profound decrease in renal blood flow and GFR, particularly in patients with overactivity of endogenous vasoconstrictor systems (in clinical practice these are patients with marked sodium retention requiring continuous administration of moderate or high doses of diuretics) (70,75–78) (Fig. 27.5). Therefore, NSAIDs should not be given to patients with cirrhosis and moderate or severe ascites. Classic NSAIDs have little or no effect on renal blood flow and GFR in patients without ascites or with ascites but without overactivity of vasoconstrictor systems. However, in these patients the administration of NSAIDs may impair sodium excretion and lead to the development of ascites or increase ascites volume (P. Ginès, unpublished data). Besides, inhibition of PG synthesis may also impair solute-free water excretion and lead to the development of dilutional hyponatremia (79). Therefore, the use of classic NSAIDs should be avoided in all patients with cirrhosis.

The available information on the renal effects of drugs that inhibit selectively COX-2 activity in patients with cirrhosis and ascites is very limited. Two recent studies show that the administration of these drugs to patients with cirrhosis and ascites is not associated with deleterious effects on renal function (80,81). However, in these studies drugs were given for a short period of time. Therefore, the effects of prolonged selective COX-2 inhibition on renal function in cirrhosis with ascites remain to be established.

Following the demonstration of an increased renal production of PGs and their participation in the maintenance of renal function in cirrhosis and ascites, it was proposed that the low GFR characteristic of HRS could be consequence of a spontaneous reduction in renal PG synthesis. Several studies reported that patients with HRS have lower urinary excretion of $PGE_2$ and 6-keto-PGF1α than do patients with ascites without renal failure (18,56,58,67–69,82,83), while other studies reported a normal urinary excretion of vasodilator PGs in patients with HRS (84). Nevertheless, even a "normal" synthesis of PGs may be low relative to the increased activity of vasoconstrictor systems present in cirrhosis. Because patients with HRS have the greatest activation of renal vasoconstrictor sys-

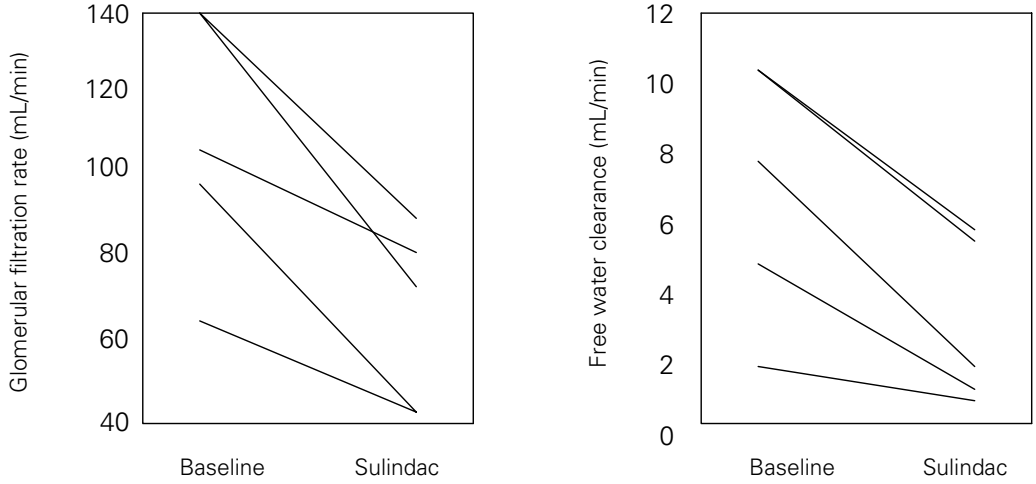


**Figure 27.5** Effect of sulindac, 400 mg for 3 days, on glomerular filtration rate and free water clearance in cirrhotic patients with ascites. (From Quintero E, Ginès P, Arroyo V *et al*. Sulindac reduces the urinary excretion of prostaglandins and impairs renal function in cirrhosis with ascites. Nephron 1986; 42:298–303.)

tems, an imbalance between vasoconstrictor systems and the renal production of vasodilator PGs could account, at least in part, for the marked reduction of renal blood flow and GFR that occur in this condition (82).

### *Natriuretic factors*

There is evidence for an increased production of peptides from the natriuretic peptide family (atrial natriuretic peptide, brain natriuretic peptide, C natriuretic peptide) in the setting of cirrhosis, particularly in patients with ascites (85–92). Since the major biological effect of these peptides is to increase the renal excretion of sodium, a role of these peptides in the pathogenesis of sodium retention in cirrhosis has been proposed (reviewed in Chapter 6). Because these peptides have also vasodilator properties, they could participate in the pathogenesis of arterial vasodilation and maintenance of renal perfusion. Data from studies in experimental cirrhosis suggest that they play an important role in the maintenance of renal perfusion and modulation of RAAS activity. In fact, the selective blockade of the natriuretic peptide A and B receptors causes renal vasoconstriction and increased PRA and aldosterone levels in rats with cirrhosis and ascites (93). No data are available on the effects of inhibition or antagonism of natriuretic peptides in human cirrhosis. Therefore, the possible role of these peptides to counteract the activity of renal vasoconstrictor systems remains speculative.

### *Nitric oxide*

NO has been extensively studied as potential mediator of arterial vasodilation in cirrhosis (94) (reviewed in Chapter 8). There is experimental evidence indicating that the production of NO is increased in the kidney of rats with cirrhosis, because kidneys from cirrhotic rats have an enhanced endothelium-dependent vasodilator response compared with control animals (95) and infusion of L-arginine, the precursor of NO, causes a greater increase in renal perfusion in cirrhotic rats compared with controls (96). Increased expression of NO synthase (NOS) in kidney from cirrhotic rats has been demonstrated in two studies, but there is disagreement on the isoform of NOS responsible for the increased synthesis of NO (96,97).

The importance of this increased renal production of NO in experimental cirrhosis is not known. Inhibition of NO in this setting does not cause renal hypoperfusion, but it is associated with an increased synthesis of PGs. By contrast, simultaneous inhibition of NO and PGs results in marked renal vasoconstriction (98,99). Therefore, it is possible that NO interacts with PGs to maintain renal hemodynamics in experimental cirrhosis. No information is available on renal NO production and its possible role in the modulation of renal function in human cirrhosis.

### *Renal kallikrein–kinin system*

Some studies have reported that urinary kallikrein excretion is increased in patients with ascites without renal failure and reduced in patients with HRS and correlates directly with GFR, suggesting that the renal kallikrein–kinin system, an intrarenal system with vasodilator and natriuretic activities, may contribute to the maintenance of renal hemodynamics in cirrhosis (100,101). Other investigations, however, have found reduced urinary kallikrein excretion in patients with ascites (102,103). More specific methods to evaluate the activity of the kallikrein–kinin system are needed before the role of this system in the homeostasis of renal function in cirrhosis can be defined.

## Pathogenesis of hepatorenal syndrome

The pathophysiological hallmark of HRS is a severe vasoconstriction of the renal circulation (5,6,104–108). Any theory aimed at explaining the pathogenesis of HRS should provide an explanation for the existence of a marked renal vasoconstriction in the setting of a very pronounced arterial underfilling in the systemic circulation characteristic of cirrhosis. In addition, it should provide an explanation for the continuum from compensated cirrhosis with normal or near-normal renal function to decompensated cirrhosis with ascites and sodium retention, severely decompensated cirrhosis with ascites and spontaneous dilutional hyponatremia and, finally, renal failure due to renal vasoconstriction (i.e. HRS).

The theory that best fits with the observed alterations in the renal and circulatory function in HRS is the arterial vasodilation theory, which proposes that HRS is the result of the effect of vasoconstrictor systems (see above) acting on the renal circulation. These constrictor systems are activated as homeostatic mechanisms to counteract the extreme underfilling of the arterial circulation and thus to maintain arterial pressure (104,109–111) (see also Chapter 16) (Fig. 27.6). As a result of this increased activity of the vasoconstrictor systems, renal perfusion and GFR are markedly reduced but tubular function is preserved. This is different from what occurs in acute tubular necrosis (ATN), in which renal failure is associated with a markedly impaired tubular function. The activated vasoconstrictor systems are also responsible for the sodium retention and impaired solute-free water excretion that occur in the setting of HRS (109,110,112).

Most available data suggest that arterial underfilling is due to a marked vasodilation of the splanchnic circulation related to an increased splanchnic production

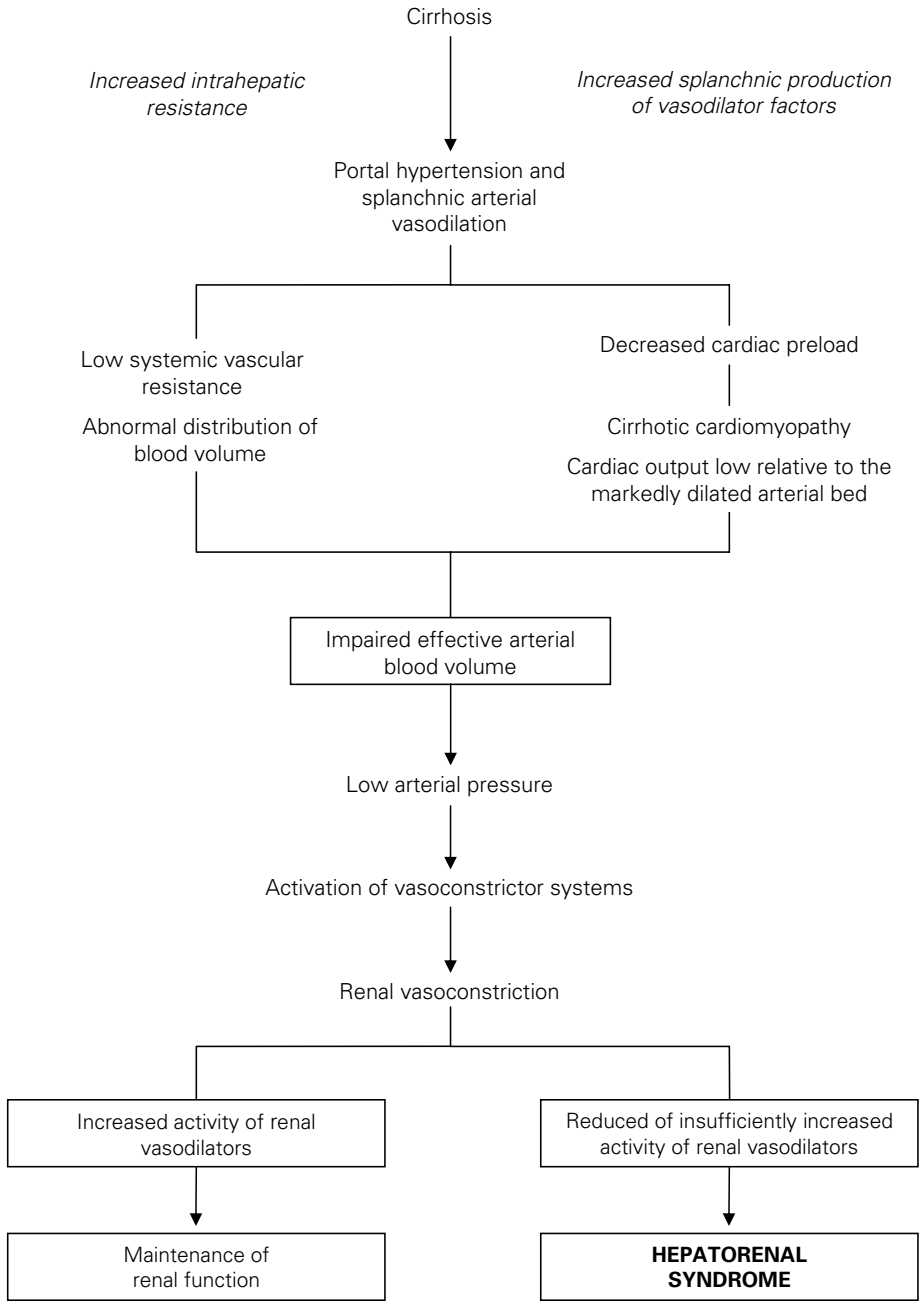


**Figure 27.6** Hemodynamic changes and pathogenesis of hepatorenal syndrome in cirrhosis.

of vasodilator substances, particularly NO (reviewed in Chapter 8) (94,113). Sodium retention and ascites and edema formation would develop as a consequence of homeostatic activation of vasoconstrictor and antinatriuretic mechanisms acting on the systemic circulation and kidneys to maintain effective arterial blood volume constant. In initial phases of decompensated cirrhosis, renal perfusion would be maintained within normal levels despite the activation of potent vasoconstrictor factors because of an increased synthesis of renal vasodilators (mainly prostaglandins). In this setting, the equilibrium between vasoconstrictor and vasodilator factors is very delicate and the kidney is very sensitive to further impairment in the systemic arterial circulation and/or decreases in the renal production of vasodilator factors. Therefore, any impairment in arterial underfilling, either spontaneous, due to progression of splanchnic vasodilation, or triggered by specific clinical circumstances (i.e. bacterial infections, especially spontaneous bacterial peritonitis, large-volume paracentesis without plasma expansion or gastrointestinal bleeding) would result in a further activation of vasoconstrictor systems.

If this is followed by a compensatory increase in the production of renal vasodilator factors, GFR would be maintained within normal or only moderately reduced levels and HRS would not develop. By contrast, if there is no increase in renal vasodilator factors or the activation of vasoconstrictor factors overrides the effect of the increased production of renal vasodilators, then HRS would develop (Fig. 27.6).

The marked activation of vasoconstrictor systems not only causes vasoconstriction in the kidneys but also results in vasoconstriction of some extrarenal vascular beds, including lower and upper limbs and brain (11,114–116). The splanchnic area would escape from the effect of vasoconstrictors probably because of a markedly enhanced local production of vasodilators. The recent observation by several groups that the administration of vasoconstrictor drugs with a preferential effect on the splanchnic circulation, such as ornipresin or terlipressin, together with albumin administration improves renal function in patients with HRS is consistent with series of events proposed by the arterial vasodilation hypothesis (12,15,21,22,117,118).

Although arterial underfilling in HRS is mainly due to splanchnic arterial vasodilation causing an abnormal distribution of arterial blood volume, recent evidence suggests that an impaired cardiac function due to cirrhotic cardiomyopathy (reviewed in Chapters 15 and 28) may also play a role. Although cardiac output is high in most patients with HRS, it may be low relative the to markedly dilated splanchnic vascular bed (Fig. 27.6). It has been proposed that a reduction in cardiac output from the high values characteristic of cirrhosis with ascites to normal or moderately reduced values may trigger the development of HRS, particularly type 1 (14,119–121). The possible role of impaired cardiac function in the pathogenesis of HRS should be investigated further.

## Conclusion

Vasoconstriction of the renal circulation is a common finding in patients with cirrhosis. It may already occur in early stages of the disease, but it becomes more marked over time. In most patients, marked vasoconstriction of the renal circulation occurs after patients have developed an impaired excretion of sodium and solute-free water (which are manifested clinically by development of ascites and dilutional hyponatremia, respectively). It is now well established that vasoconstriction of the renal circulation occurs as a consequence of a marked impairment of the systemic arterial circulation, characterized by arterial vasodilation, that predominates in the splanchnic area, and compensatory activation of the endogenous vasoconstrictor systems. These activated vasoconstrictor systems cause an effect on the kidney circulation, which is roughly proportionate to their degree of activation. Vasodilators of renal and extrarenal origin are subsequently activated to compensate for the increased activity of vasoconstrictor factors in the kidney. This situation results in a very delicate equilibrium in the renal circulation, which is then very sensitive to changes in the activity of vasoactive factors. Either an increase in the activity of vasoconstrictor factors (generally as a result of a further impairment in the systemic arterial circulation) or a decrease in the activity of vasodilator factors may lead to the development of HRS, which is the extreme expression of renal vasoconstriction in the kidney.

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# Chapter 28
# Hepatorenal Syndrome in Cirrhosis: Clinical Features, Diagnosis, and Management

Vicente Arroyo, Carlos Terra, Aldo Torre, and Pere Ginès

Hepatorenal syndrome (HRS) is an outstanding complication in cirrhosis, with an annual incidence in patients with ascites of approximately 8% (1). It develops at the latest phase of the disease and, although initially considered without impact in prognosis (patients would die with and not by the renal failure), there is now evidence that it is an important determinant of survival. During the last decade great advances have been made on HRS. First, it has become clear that it is a much more complex syndrome than initially thought. In addition to a functional renal failure due to severe renal vasoconstriction, HRS is characterized by a splanchnic arterial vasodilation and decreased heart function, vasoconstriction in other vascular territories such as the brain, muscle, skin, and the liver, an increased resistance to the portal venous flow and of portal hypertension, and an impairment in hepatic and cerebral function. Second, there are now reliable diagnostic criteria for HRS and, therefore, investigations using a common terminology and homogeneous groups of patients have been possible. Finally, effective treatments of HRS have been developed. The aim of the current chapter is to review the pathogenesis, diagnosis, and treatment of HRS. The reader is referred to other review articles on this topic (2–10).

## Historical hallmarks

The term HRS was created in the 1930s to define the occurrence of renal failure after biliary surgery or hepatic trauma (11–13). Subsequently it was extended to other types of acute renal failure in liver diseases. During the 1960s and 1970s American nephrologists popularized the term for the renal failure of cirrhosis, and it was finally accepted to define the functional renal failure that spontaneously occurs in cirrhosis in a consensus conference of the International Ascites Club in Chicago in 1995, in which investigators from the five continents proposed a new definition and diagnostic criteria for HRS (2).

HRS in cirrhosis was first recognized by Hecker and Sherlock in 1956 (14) in an article describing nine patients who developed azotemia, progressive oliguria, and hyponatremia in the setting of a severe hepatic insufficiency. All patients died and post-mortem examination of the kidneys showed normal histology. This study is remarkable because it proposed 50 years ago current concepts on the pathogenesis and treatment of HRS. Since patients had arterial hypotension and highly oxygenated peripheral venous blood, and cardiac output was very high in the only patient in whom this parameter was measured, they proposed that HRS is due to a reduction in renal perfusion secondary to peripheral arterial vasodilation. They treated three patients with volume expansion and norepinephrine. In the only patient in which this treatment was applied during several days there was a decrease in blood urea and an increase in urine volume and serum sodium concentration. Finally, they suggested that not only renal function but also hepatic function could be affected by the circulatory dysfunction.

Studies during the 1960s showed that glomerular filtration rate (GFR) in decompensated cirrhosis decreases with the progression of the disease in parallel with a fall in renal perfusion, HRS being the extreme expression of this process (15–20). Because the plasma volume is increased in cirrhosis (21,22), the concept of effective hypovolemia was proposed to explain the pathogenesis of the syndrome (23). Although plasma volume is increased, a substantial part of this volume is used to refill the dilated splanchnic venous bed (splanchnic pooling) and the effective circulating blood volume is actually decreased. Despite this hypothesis not being in agreement with previous studies showing high cardiac output and low peripheral vascular resistance in cirrhosis with ascites (24), thus suggesting an arterial vasodilation, it was the generally accepted mechanism of renal dysfunction in cirrhosis over many years. On this concept, treatments of HRS designed during the 1960s and 1970s were mainly directed to expanding the blood volume. The Rhodioascit apparatus and the LeVeen shunt were the main exponents of this tendency (25,26).

The current concept of the pathogenesis of HRS was delineated during the 1970s and 1980s. It was shown that

the kidneys from patients with HRS regained normal renal function when transplanted to patients with chronic renal failure (27) and that HRS disappeared after liver transplantation (28). HRS was, therefore, a reversible renal impairment. The vasoactive systems were studied in depth (29–38) and the concept that HRS was due to an imbalance between an increased activity of the renin–angiotensin and sympathetic nervous systems and antidiuretic hormone, which are renal vasoconstrictors, and a reduced renal synthesis of vasodilators, such as prostaglandins or bradikinin, was first suggested (39). Great advances were also made on circulatory function in cirrhosis. Investigations using specific antagonists of angiotensin II (AII) and vasopressin and inhibitors of the sympathetic nervous activity showed that arterial pressure in cirrhosis with ascites is critically dependent on the stimulation of the renin–angiotensin and sympathetic nervous systems and antidiuretic hormone (40–43). The activation of these systems represents a concerted response to counteract the arterial vasodilation present in these patients and, therefore, to maintain arterial pressure. The potential role of endogenous vasodilators (glucagon, prostaglandins) in the circulatory dysfunction and in the resistance to the effect of vasoconstrictors was also explored (44–48). Finally, investigations in patients and in experimental animals with cirrhosis and ascites demonstrated that circulatory dysfunction was related to an arterial vasodilation and not to a reduced circulating blood volume (42,45,49–51). Based on these studies, a new hypothesis (the Peripheral Arterial Vasodilation Theory) of renal dysfunction in cirrhosis was proposed (52).

The Peripheral Arterial Vasodilation Theory received great support during the 1990s. Nitric oxide (NO) and other vasodilatory substances (carbon monoxide and endogenous canabinoids) were found to be of major importance in the pathogenesis of circulatory dysfunction in cirrhosis (53–58). It was also demonstrated that the site of arterial vasodilation in cirrhosis is the splanchnic circulation, there being vasoconstriction in other organs such as the muscle, skin, kidneys, and brain (59–64). Since splanchnic arterial vasodilation is due to portal hypertension and NO synthesis is increased in this vascular compartment (54) in cirrhosis, a link between the diseased liver, circulatory dysfunction, and HRS was clearly established.

Although therapeutic paracentesis was reintroduced in the 1980s and represented a major advance in the management of refractory ascites in patients with type 2 HRS (65,66), no effective treatment for HRS was designed until the 1990s. Several attempts to reverse HRS pharmacologically by short-term administration of dopamine, prostaglandins, α-adrenergic antagonists, AII antagonists, metaraminol, and octapressin failed to improve renal perfusion and GFR (20,67–76). The delineation of the mechanism of HRS in cirrhosis was essential for the design of effective treatments. If circulatory dysfunction in HRS is due to a splanchnic arterial vasodilation secondary to portal hypertension and to a decreased cardiac output, the administration of vasoconstrictors with preferential effect on the splanchnic circulation and the expansion of central blood volume (which increases cardiac preload and cardiac output) should be effective (64,77–79). Fortunately, this hypothesis was proved to be true. At present there are several studies indicating that HRS can be reversed by the simultaneous administration of i.v. albumin and vasoconstrictors, such as ornipressin or terlipressin. The same effect has been obtained after relieving portal hypertension with the use of transjugular intrahepatic portosystemic shunts (80).

## Diagnosis and clinical types of hepatorenal syndrome

The first step in the diagnosis of HRS is the demonstration of a reduced GFR, and this is not easy in advanced cirrhosis. The muscle mass, and therefore, the release of creatinine, is considerably reduced in these patients and they may present normal serum creatinine concentration in the setting of a very low GFR (Fig. 28.1). Similarly, urea is synthesized by the liver, and may be reduced as a consequence of hepatic insufficiency. Therefore, false-negative diagnosis of HRS is relatively common (81–83). Nevertheless, there is consensus to establish the diagnosis of HRS when serum creatinine has risen above 1.5 mg/dl or creatinine clearance has decreased to < 40 ml/min (2).

The second step is the differentiation of HRS from other types of renal failure. For many years this was based on the

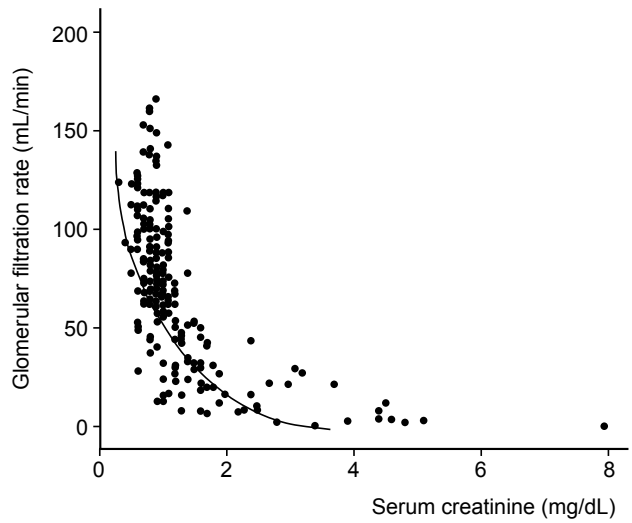


**Figure 28.1** Relationship between glomerular filtration rate as assessed by inulin clearance and serum creatinine. (Reprinted from Zakin, Boyer, eds. *Hepatology: A Textbook of Liver Disease*, 3rd edn, Ginès P *et al*., Disorders of renal function in cirrhosis: pathophysiology and clinical aspects, 650–68, ©1996, with permission from Elsevier.)

**Table 28.1** International Ascites Club's diagnostic criteria of hepatorenal syndrome.

Major criteria
- Chronic or acute liver diseases with advanced hepatic failure and portal hypertension.
- Low glomerular filtration rate as indicated by serum creatinine of > 1.5 mg/dL or 24-hour clearance creatinine < 40 mL/min.
- Absence of shock, ongoing bacterial infection and current or previous treatment with nephrotoxic drugs. Absence of gastrointestinal fluid losses (repeated vomiting or intense diarrhea) or renal fluid losses (weight loss > 500 g/day for several days in patients with ascites without peripheral edema or 1000 g/day in patients with peripheral edema).
- No sustained improvement in renal function (decrease in serum creatinine to 1.5 mg/dL or less or increase in creatinine clearance to 40 mL/min or more) following diuretic withdrawal and expansion of plasma volume with 1.5 L of isotonic saline.
- Proteinuria < 500 mg/dL and no ultrasonographic evidence of obstructive uropathy or parenchymal renal disease.

Additional criteria
- Urine volume < 500 mL/day.
- Urine sodium < 10 mEq/L.
- Urine osmolality greater than plasma osmolality.
- Urine red blood cells < 50 per high power field.
- Serum sodium concentration < 130 mEq/L.

traditional parameters used by nephrologists to differentiate functional renal failure from acute tubular necrosis or rapidly progressive glomerulonephritis (oliguria, low urine sodium concentration, urine-to-plasma osmolality ratio greater than unity, normal fresh urine sediment, and no proteinuria). However, subsequently this was found to be clearly unsatisfactory. Acute tubular necrosis in patients with cirrhosis and ascites is usually characterized by oliguria, low urine sodium concentration, and urine osmolality greater than plasma osmolality (84). In contrast, relatively high urinary sodium concentration has been reported in some patients with HRS (85).

Because of this lack of specific tests, diagnosis of HRS should be based on the exclusion of other disorders that can cause renal failure in cirrhosis (2) (Table 28.1). Acute renal failure of prerenal origin due to renal fluid losses (excessive diuretic treatment) or to extrarenal losses should be ruled out. If renal failure is secondary to volume depletion, renal function improves rapidly after volume expansion, whereas no improvement occurs in HRS. Even if there is no history of fluid losses, renal function should be assessed after diuretic withdrawal and volume replacement (1.5 l of isotonic saline) to rule out any subtle reduction in plasma volume as the cause of renal failure. The presence of shock before the onset of renal failure points towards a diagnosis of acute tubular necrosis. Hypovolemic shock due to variceal bleeding or to other causes of gastrointestinal hemorrhage is easily recognized. However, septic shock may be more difficult to diagnose because of the lack of symptoms of bacterial infections in some patients with cirrhosis and the fact that arterial hypotension is common in patients with HRS without bacterial infections. Conversely, cirrhotic patients with infections may develop transient renal failure, which resolves after resolution of the infection. Therefore, HRS in cirrhotic patients with bacterial infections should be diagnosed in patients without septic shock and only if renal failure persists following infection resolution. Cirrhotic patients are predisposed to develop renal failure in the setting of treatments with aminoglycosides (86), nonsteroidal anti-inflammatory drugs (87), and vasodilators (renin–angiotensin inhibitors, prazosin, nitrates) (88). Therefore, treatment with these drugs in the days preceding the diagnosis of renal failure should be ruled out. Finally, patients with cirrhosis can develop renal failure due to intrinsic renal diseases, particularly glomerulonephritis. These cases can be recognized by the presence of proteinuria, hematuria, or both.

HRS is classified into two types according to the intensity and form of presentation of renal failure (2). Type 1 HRS is characterized by a severe and rapidly progressive renal failure, which has been defined as doubling of serum creatinine reaching a level > 2.5 mg/dl in less than 2 weeks (2). Although type 1 HRS may arise spontaneously, it frequently occurs in close relationship with a precipitating factor such as severe bacterial infection, gastrointestinal hemorrhage, major surgical procedure, or acute hepatitis superimposed on cirrhosis. The association of HRS and spontaneous bacterial peritonitis has been carefully investigated (89–91). Type 1 HRS develops in approximately 25% of patients with spontaneous bacterial peritonitis despite a rapid resolution of the infection with non-nephrotoxic antibiotics. Patients with intense inflammatory response and high cytokine levels in plasma and ascitic fluid are especially prone to develop type 1 HRS after the infection. Besides renal failure, patients with type 1 HRS after spontaneous bacterial peritonitis show signs and symptoms of severe liver insufficiency (jaundice, coagulopathy, and hepatic encephalopathy) and circulatory dysfunction (arterial hypotension, very high plasma levels of renin and norepinephrine) that worsen with the impairment in renal function (89–91). Type 1 HRS is the complication of cirrhosis with the poorest prognosis, with a median survival time after the onset of HRS of only 2 weeks (1) (Fig. 28.2).

Type 2 HRS is characterized by a moderate and steady decrease in renal function (serum creatinine < 2.5 mg/dl). Patients with type 2 HRS show signs of liver failure and

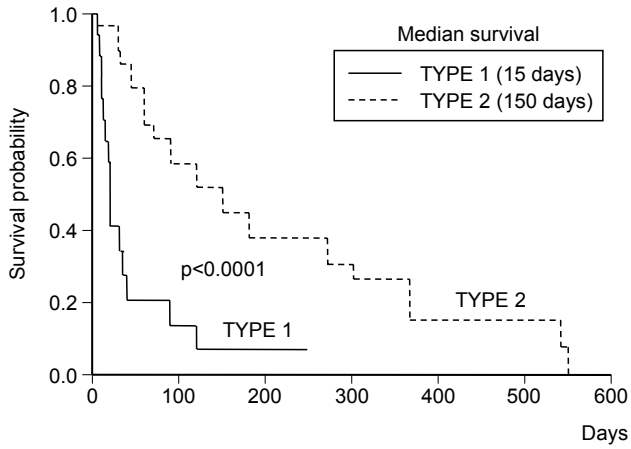


**Figure 28.2** Survival of patients with cirrhosis after the diagnosis of type 1 or type 2 hepatorenal syndrome (Ginès P *et al.* Hepatorenal syndrome. Lancet 2003; 362:1819–27.)

arterial hypotension but to a lesser degree than patients with type 1 HRS. The dominant clinical feature is a severe ascites with poor or no response to diuretics (a condition known as refractory ascites). Patients with type 2 HRS are specially predisposed to develop type 1 HRS following infections or other precipitating events (89–91). The median survival of patients with type 2 HRS (6 months) is worse than that of patients with non-azotemic cirrhosis with ascites (92).

## Pathogenesis of hepatorenal syndrome

The simplistic concept that HRS is a functional renal failure developing in cirrhotic patients with ascites should no longer be maintained. It is a much more complex syndrome. In addition to these abnormalities, which are the most evident clinically, these patients present a severe circulatory dysfunction characterized by vasodilation in the splanchnic circulation, vasoconstriction in all extra-splanchnic territories so far explored, such as the brain, liver, muscle, skin and the kidneys, an impaired cardiac function which may lead to a low cardiac output, and the disappearance of the hyperdynamic circulation, and an increased intrahepatic resistance to the portal venous flow. These changes in extra-splanchnic organs may account for other clinical manifestations of the syndrome such as rapid deterioration of hepatic function, encephalopathy, and aggravation of portal hypertension.

### Renal dysfunction

Chronologically, impairment of renal sodium metabolism is the first renal function abnormality in cirrhosis. It can already been detected when the disease is still compensated (no ascites). For example, although patients with compensated cirrhosis are capable of excreting a regular sodium intake, they may be unable to escape from the effect of mineralocorticoids and to eliminate an acute sodium load normally (93). These features are observed only in patients with significant portal hypertension and hyperdynamic circulation (high cardiac output and low systemic vascular resistance). As the disease progresses, the impairment in sodium metabolism increases and a critical point is achieved at which the patient is unable to excrete their regular sodium intake. Sodium is then retained with water and accumulates as ascites. GFR and the plasma concentrations of aldosterone and norepinephrine are normal at this stage of the disease (94). Sodium retention is therefore unrelated to the renin–aldosterone system and sympathetic nervous system, the two most important sodium-retaining systems so far identified. The plasma levels of natriuretic peptides are markedly increased (95), indicating that sodium retention is not due to a reduced production of endogenous natriuretic substances. The most accepted mechanism of sodium retention at this early stage of decompensated cirrhosis is a decrease in the effective arterial blood volume secondary to splanchnic arterial vasodilation. This circulatory dysfunction, although greater than in compensated cirrhosis, is not intense enough to stimulate the sympathetic nervous activity and the renin–angiotensin–aldosterone system. However, it would activate a still unknown extremely sensitive sodium-retaining mechanism (renal or extrarenal) (96).

With the exception of alcoholic cirrhosis, in which renal function may improve after alcohol withdrawal, the degree of sodium retention increases with the progression of disease. When it is intense, the plasma renin activity and the plasma concentration of aldosterone and norepinephrine are invariably elevated (30,97–99). At this stage of the disease the cardiac output and peripheral vascular resistance do not differ from the previous phase (97). Circulatory dysfunction, however, is greater since an increased activity of the sympathetic nervous and renin–angiotensin systems is needed to maintain the arterial pressure. Renal perfusion and GFR are also normal or only moderately decreased and they are critically dependent on an increased renal production of prostaglandins and NO (30,39,100–102). These are vasodilators that antagonize the vasoconstrictor effect of AII and norepinephrine on the renal circulation. A syndrome indistinguishable from HRS can be produced in patients with cirrhosis, ascites, and increased plasma renin activity following prostaglandin inhibition with nonsteroidal anti-inflammatory drugs (30,100). The renal ability to excrete free water is reduced due to a non-osmotic hypersecretion of antidiuretic hormone (36). However, only few patients show significant hyponatremia (serum sodium concentration < 130 mEq/min). Water retention and dilutional hyponatremia develop when renal water metabolism is severely impaired (free water clearance after water load

< 1 ml/min; normal 6–12 ml/min), and this rarely occurs in cirrhosis in the absence of renal failure (103).

HRS develops at the latest phase of the disease when patients already have severe impairment of circulatory function, arterial hypotension, marked stimulation of the sympathetic nervous and renin–angiotensin systems, intense sodium retention, and dilutional hyponatremia. In fact, arterial pressure, plasma renin activity, plasma norepinephrine concentration, and dilutional hyponatremia in non-azotemic patients with cirrhosis and ascites are important predictors of development of HRS (14–20). Impairment in GFR in HRS is due to renal vasoconstriction (20). Renal histology shows no lesions, or lesions that do not justify the decrease in renal function. Since AII and norepinephrine are powerful renal vasoconstrictors (29,73,74,104–106), renal failure in HRS is thought to be related to the extreme stimulation of the endogenous vasoconstrictor systems. The plasma concentration of endothelin, a vasoconstrictor peptide of endothelial origin, is increased in cirrhosis with ascites (107). However, it is probably not involved in the pathogenesis of HRS since plasma endothelin is similar in patients with and without HRS (108,109). Furthermore, plasma endothelin does not decrease following pharmacological resolution of HRS (64). The urinary excretion of prostaglandin $E_2$ and 6-keto prostaglandin F1α (a prostacyclin metabolite) are decreased in patients with HRS, which is compatible with a reduced renal production of these substances (30,33). Renal failure in HRS could, therefore, be the consequence of an imbalance between the activity of the systemic vasoconstrictor systems and the renal production of vasodilators. Finally, renal hypoperfusion in HRS could also be amplified by the stimulation of intrarenal vasoconstrictors. For example, renal ischemia increases the generation of AII by the juxtaglomerular apparatus, the production of adenosine which, in addition to being a renal vasoconstrictor, potentiates the vascular effect of AII, and the synthesis of endothelin. The observation that dipyridamole, an inhibitor of adenosine metabolism, impairs renal perfusion in patients with cirrhosis and ascites but not in normal subjects, indicates an increased sensitivity to intrarenal vasoconstrictors in decompensated cirrhosis (110). Other intrarenal vasoconstrictors potentially implicated in HRS are leukotrienes and F2-isoprostanes (111).

HRS is usually associated with an extremely low urinary sodium excretion. Sodium retention in patients with HRS is due to a decreased filtered sodium and an increased sodium reabsorption in the proximal tubule. The amount of sodium reaching the loop of Henle and distal nephron, the sites of action of furosemide and spironolactone, respectively, is very low. The delivery of furosemide and spironolactone to the renal tubules is reduced due to the renal hypoperfusion. It is, therefore, not surprising that patients with HRS respond poorly to diuretics (112).

## The cardiocirculatory dysfunction in cirrhosis and hepatorenal syndrome

Portal hypertension in patients with cirrhosis is associated with a circulatory dysfunction characterized by increased cardiac output and heart rate and reduced peripheral vascular resistance (74,97). Despite the increase in cardiac output, the central blood volume, that is the volume of blood contained in the heart, pulmonary circulation and aorta prior to the renal arteries, is reduced due to the extremely short transit time of the circulation from the right atria to the aorta (113). These features define what is called hyperdynamic circulation: an increased blood volume flowing very rapidly within the central vascular compartment as a consequence of a high cardiac output. This hyperdynamic circulation is a compensatory mechanism to maintain the effective arterial blood volume and arterial pressure when there is arterial vasodilation. When the hyperdynamic circulation is insufficient to compensate the changes in circulatory function induced by the arterial vasodilation, arterial hypotension occurs, leading to activation of pressure receptors in the aorta and carotid sinus and reflex stimulation of the sympathetic nervous system, renin–angiotensin system and vasopressin release, and compensatory increase in arterial pressure (52). In fact, the administration of inhibitors of the renin–angiotensin and sympathetic nervous systems or V1 vasopressin antagonists to patients or experimental animals with cirrhosis and high plasma levels of renin is associated with a marked decrease in arterial pressure, an effect not seen in healthy subjects, patients with compensated cirrhosis, or patients with ascites and normal plasma levels of renin and norepinephrine (40,41,114–117). Systemic hemodynamics in cirrhosis with ascites should, therefore, always be considered together with activity of these neurohormonal systems. For example, the small differences in peripheral vascular resistance between patients with compensated cirrhosis and patients with cirrhosis and ascites do not mean small differences in the degree of arterial vasodilation, since it is obscured in the patients with ascites by the vascular effect of the endogenous vasoconstrictor systems.

Splanchnic arterial vasodilation is a constant feature in portal hypertension and plays a major role in many abnormalities associated with this condition (118–123). It is the main determinant of the hyperdynamic circulation in cirrhosis (119–121). Also, it increases the inflow of blood into the portal venous system, and by this mechanism portal pressure remains elevated despite the development of porto-caval collateral circulation (124). Finally, it is the main factor in the increased hydrostatic pressure and permeability in the splanchnic capillaries and in the formation of ascites (125,126). There is a splanchnic resist-

ance to the vasoconstrictor effect of AII, catecholamines, and vasopressin in cirrhosis (127,128). This explains why the splanchnic vasodilation and the hyperdynamic circulation is maintained during the progression of the disease when there is stimulation of the renin–angiotensin and sympathetic nervous systems and antidiuretic hormone. In contrast, these systems may induce arterial vasoconstriction in other organs, such as the liver (129), kidneys, brain, muscle and skin in patients with ascites (73–75,130) (Figs 28.3 and 28.4). NO is an important effector of the splanchnic vasodilation in cirrhosis. The synthesis of this local vasodilator is increased in the splanchnic circulation in cirrhosis (131,132). On the other hand, NO inhibition normalizes the hyperdynamic circulation and the response to vasoconstrictors in experimental cirrhosis (133–140). It has been proposed that vascular endothelial and smooth muscle cells are the sites of NO hyperproduction in cirrhosis, and that this is related with cytokines released locally as a consequence of bacterial translocation from the intestinal lumen (141,142). NO and other vasodilators such as substance P, vasoactive intestinal peptide, and calcitonin gene-related peptide are neurotransmitters of the splanchnic non-adrenergic, noncholinergic nervous system (143). Therefore, it may also be possible that changes in the splanchnic organs secondary to portal hypertension stimulate the non-adrenergic, noncholinergic nervous system and the release of vasodilator neurotransmitters by the nervous terminals. In fact, the circulating plasma levels of vasoactive intestinal peptide, substance P, and calcitonin gene-related peptide are increased in decompensated cirrhosis (144–148). Other vasodilatory substances that may also participate in the splanchnic arterial vasodilation of cirrhosis are carbon monoxide and endogenous cannabinoids (57,58,149,150).

Investigations of circulatory function in cirrhotic patients with renal failure suggest that a decrease in cardiac output is of major importance in the impairment of circulatory function that characterizes HRS. Tristani and Cohn in 1967 (151) showed that cardiac output was normal or reduced in a significant number of patients with HRS. The peripheral vascular resistance in these patients (although below normal values) was higher than that reported by other authors in patients without HRS. In 1979, the same group of investigators assessing cardiovascular function in patients with refractory and with diuretic-responsive ascites reported that the cardiac output was lower and the peripheral vascular resistance higher in the former group of patients (152). Finally, Ruiz del Arbol *et al.* (129) have recently studied patients with spontaneous bacterial peritonitis at infection diagnosis and following infection resolution. The development of HRS was associated with a significant decrease of cardiac output (Fig. 28.5), a feature not observed in patients who maintained serum creatinine within normal levels. At the end of treatment, mean arterial pressure and cardiac output were 10% and 30% lower, peripheral vascular resistance 32% higher and the plasma levels of renin and norepinephrine between 5 and 10 times higher in patients developing HRS compared with those without HRS. These studies indicate that HRS develops in the absence of an aggravation of the arterial vasodilation already present before the syndrome and suggest that the most likely mechanism of HRS is a combination of arterial vasodilation and decreased cardiac output.

A specific cardiomyopathy characterized by impaired ventricular contractility has been described in cirrhosis (153). The chronotropic function of the heart is also impaired in cirrhosis due to autonomic dysfunction (153). Therefore, the reduction in cardiac output in HRS could

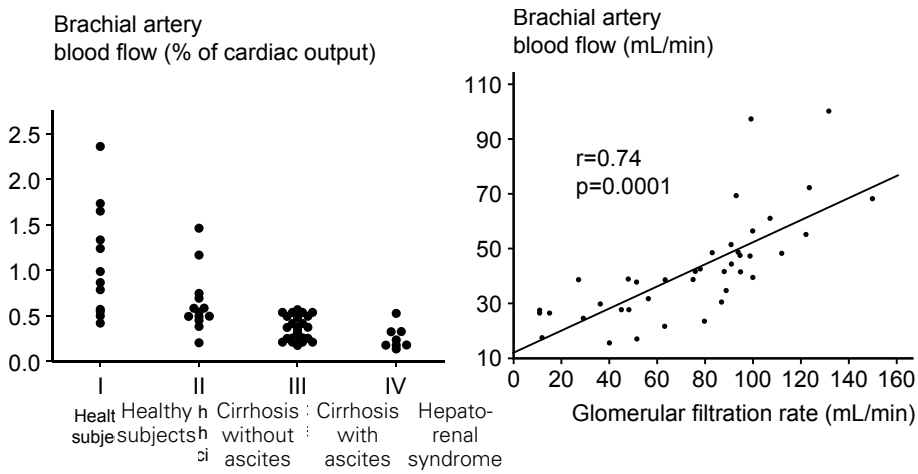


**Figure 28.3** Brachial artery blood flow in three groups of cirrhotic patients and healthy subjects (left graph) and relationship between brachial artery blood flow and glomerular filtration rate (right graph). (Maroto A *et al*. Brachial and femoral artery blood flow in cirrhosis: relationship to kidney dysfunction. Hepatology 1993; 17:788–93.)

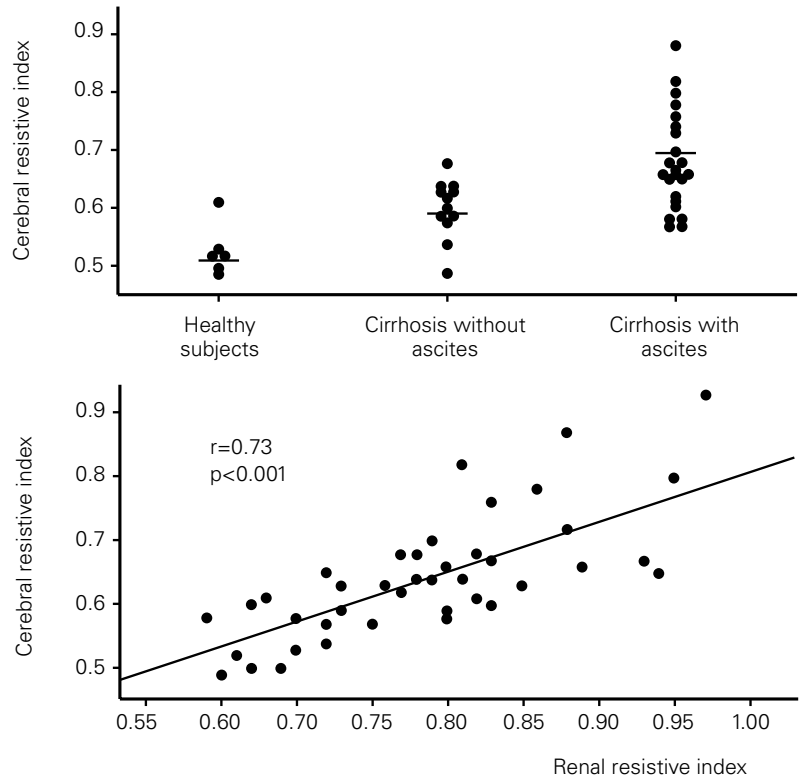


**Figure 28.4** Upper graph: Resistive index in the middle cerebral artery in patients with compensated cirrhosis, patients with ascites, and healthy subjects. Lower graph: Relationship between the renal resistive index and the resistive index in the middle cerebral artery in cirrhotic patients. (Guevara M *et al*. Increased cerebrovascular resistance in cirrhotic patients with ascites. Hepatology 1998; 28:39–44.)

be related to these problems. Another possible mechanism is a decreased venous return secondary to an increased venous compliance. Several features support this latter hypothesis. Cardiopulmonary pressures are normal or reduced in HRS (151). Plasma volume expansion is associated with a marked increase in cardiac output in HRS (151). The i.v. administration of albumin in association with vasoconstrictors but not the treatment with vasoconstrictors alone improve circulatory and renal function in HRS (76,154).

## Influence of systemic circulatory dysfunction on hepatic hemodynamics

The mechanisms by which portal hypertension induces splanchnic arterial vasodilation and the hyperdynamic circulation has been investigated in depth. However, the reverse, i.e. that systemic circulatory dysfunction may impair the intrahepatic hemodynamics in cirrhosis, has received little attention. A significant part of the increased intrahepatic vascular resistance in cirrhosis is unrelated to architectural changes (155,156), and there are data that in decompensated cirrhosis the renin–angiotensin and sympathetic nervous systems may be involved in this functional component of portal hypertension. AII and catecholamines reduce hepatic blood flow and increase intrahepatic vascular resistance and portal pressure (157). The hepatic arterioles and venules and the sinusoidal pericytes (stellate cells) are the sites of action of these substances (155,156). The degree of portal hypertension in cirrhosis correlates directly with the plasma levels of renin and norepinephrine (97,158). The blockade of AII with saralasin or the inhibition of the sympathetic nervous activity with clonidine in cirrhotic patients with ascites decreases the wedged hepatic venous pressure in the absence of changes in hepatic blood flow, indicating a reduction in intrahepatic vascular resistance (41,158,159). Finally, dur-

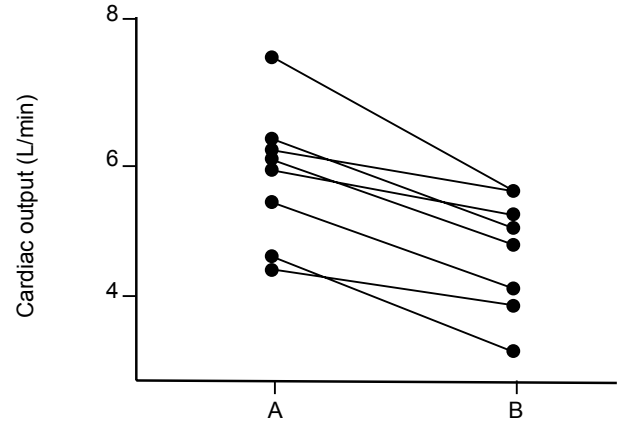


**Figure 28.5** Cardiac output in patients developing hepatorenal syndrome after spontaneous bacterial peritonitis at infection diagnosis (A) and after resolution of the infection (B). (Adapted from Ruiz del Arbol L *et al*. Systemic, renal, and hepatic hemodynamic derangement in cirrhotic patients with spontaneous bacterial peritonitis. Hepatology 2003; 38:1210–18.)

ing paracentesis, stimulation of the renin–angiotensin and sympathetic nervous systems is associated with an increase in hepatic venous pressure gradient (160).

Impairment in circulatory function associated with HRS during spontaneous bacterial peritonitis has been shown to be associated with an increase in intrahepatic resistance to the portal venous flow and portal pressure, a feature not observed in patients who maintain serum creatinine within normal levels (Fig. 28.6). With infection resolution, the hepatic venous pressure gradient was 43% higher in patients who developed HRS after spontaneous bacterial peritonitis compared with those who did not develop HRS. The increase in hepatic venous pressure gradient in patients developing HRS correlated closely with the increase in plasma renin activity and norepinephrine concentration. Circulatory dysfunction during spontaneous bacterial peritonitis in patients developing HRS is also associated with a decrease in hepatic blood flow (Ruiz del Arbol, unpublished observations). Circulatory dysfunction in HRS, therefore, affects not only the renal circulation and the circulation in other organs such as the brain, muscle and skin, but also the intrahepatic circulation (Fig. 28.7). This may explain the rapid deterioration of hepatic function and the frequent development of hepatic encephalopathy in type 1 HRS.

## Treatment of hepatorenal syndrome

During decades many vasoactive drugs (dopamine, fenoldopan, prostaglandins, misoprostol, saralasin, phentolamine, dazoxiben, norepinephrine, metaraminol, octapressin) have been assessed in patients with HRS either to improve systemic hemodynamics or to vasodilate the intrarenal circulation (161). In no case, however, did renal function improve, leading to a general impression that HRS was an intractable terminal event of cirrhosis. It is important to remark that in these studies drugs were given during hours or a few days and we now know that this is insufficient to reverse HRS. The intractability of HRS was reinforced after the demonstration that the LeVeen shunt (26), a prosthesis designed in 1974 that communicates between the intraperitoneal cavity and the systemic circulation, allowing the continuous passage of ascites to the circulation, also failed to improve renal function in patients with HRS despite a significant suppression of the renin–angiotensin and sympathetic nervous systems (162). The concept that the poor prognosis associated with HRS was due to liver failure rather than to renal failure and that any improvement in renal function would have little impact on survival was an additional feature supporting the belief that the only possible therapy for patients with HRS was liver transplantation.

A better understanding of the pathogenesis of HRS, the recent observation that reversal of HRS requires a sustained improvement in circulatory function over a relatively long period of time (1–2 weeks) and, finally, the demonstration that renal failure is an important determinant of the poor prognosis of patients with HRS has increased the interest in the treatment of this complication.

### Liver transplantation

Liver transplantation is the treatment of choice for patients with HRS (163–167). Immediately after transplantation a further impairment in GFR may be observed and many patients require hemodialysis (35% of patients with HRS compared with 5% of patients

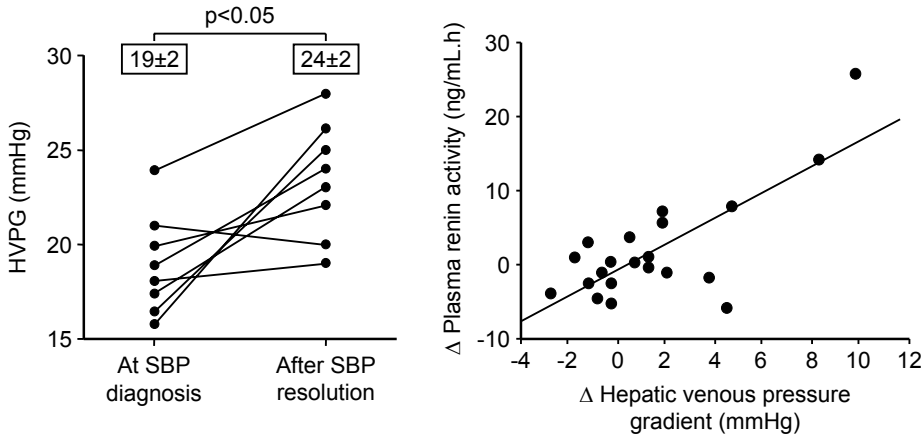


**Figure 28.6** Changes in hepatic venous pressure gradient (HVPG) (left graph) and its relation with plasma renin (right graph) in patients with circulatory dysfunction after spontaneous bacterial peritonitis (SBP). (Adapted from Ruiz del Arbol L *et al.* Systemic, renal, and hepatic hemodynamic derangement in cirrhotic patients with spontaneous bacterial peritonitis. Hepatology 2003; 38:1210–18.)

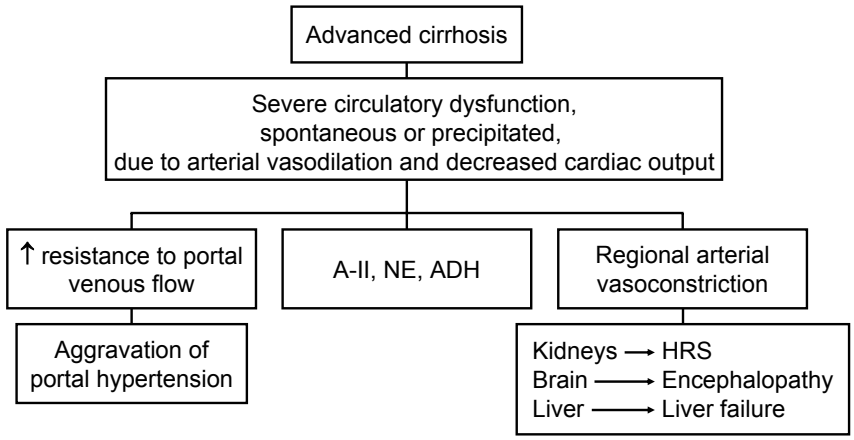


**Figure 28.7** Pathogenesis of hepatorenal syndrome. HRS, Hepatorenal syndrome; A-II, angiotensin II; NE, norepinephrine; ADH, antidiuretic hormone.

without HRS). Because cyclosporin or tacrolimus may contribute to this impairment in renal function, it has been suggested to delay the administration of these drugs until a recovery of renal function is noted, usually 48–72 h after transplantation. After this initial impairment in renal function, GFR starts to improve and reaches an average of 30–40 ml/min by 1–2 months postoperatively. This moderate renal failure persists during follow-up, is more marked than that observed after transplantation of patients without HRS, and is probably due to a greater nephrotoxicity of cyclosporin or tacrolimus in patients with HRS prior to transplantation. The hemodynamic and neurohormonal abnormalities associated with HRS disappear within the first month after transplantation and the patients regain a normal ability to excrete sodium and solute-free water (168).

Patients with HRS who undergo transplantation have more complications, spend more days in the intensive care unit, and have a higher in-hospital mortality rate than transplanted patients without HRS (163–167). The long-term survival of patients with HRS who undergo liver transplantation, however, is good, with a 3-year probability of survival of 60% (163–167). This survival rate is only slightly reduced compared with that of patients transplanted without HRS (which ranges between 70 and 80%). A recent study showed a clear inverse relationship between preoperative serum creatinine or creatinine clearance and survival after liver transplantation (169) (Fig. 28.8), indicating that not only the existence of HRS but also the severity of HRS is important in determining post-transplantation outcome.

Cirrhotic patients with type 2 HRS have a sufficiently prolonged survival to enable them to receive a liver graft. However, this is not the case in patients with type 1 HRS, in whom the expected survival is less than 2 weeks. This poor prognosis makes the applicability of liver transplantation very unlikely in this subset of patients unless survival could be increased by other measures.

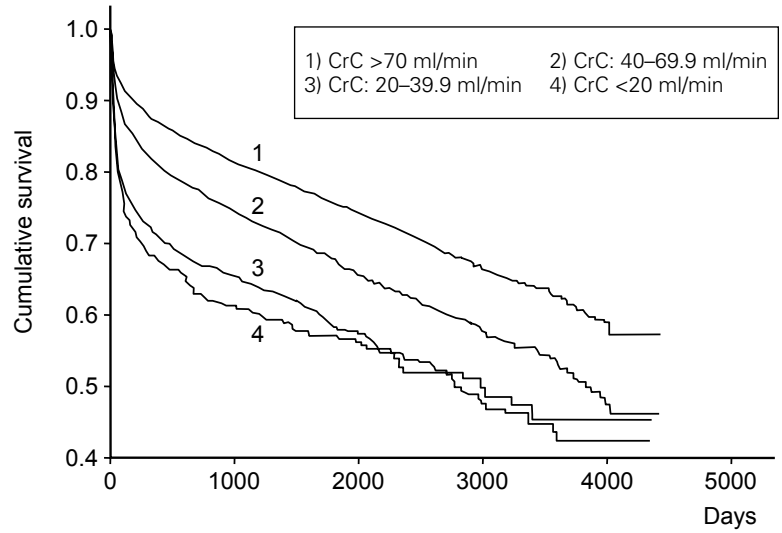


**Figure 28.8** Relationship between survival after orthotopic liver transplantation and pretransplant renal function. Cr C, creatinine clearance. (Nair S *et al.* Pretransplant renal function predicts survival in patients undergoing orthotopic liver transplantation. Hepatology 2002; 35:1179–85.)

## Volume expansion and vasoconstrictors

The first study showing that HRS can be reversed pharmacologically was performed by Guevara *et al.* (76). These authors assessed the hemodynamic, neurohormonal, and renal function in 16 patients with HRS, most of them with type 1 HRS. Eight patients were treated for 3 days (albumin was given at a dose of 1 g/kg on the first day and 20–60 g/day for the next 2 days; ornipressin was given as an i.v. stepped dose infusion of 2–6 IU/h). A normalization of the plasma levels of renin and norepinephrine was obtained, indicating a marked improvement in circulatory function (Fig. 28.9). However, only a slight increase in GFR (from 15 ± 4 ml/min to 24 ± 4 ml/min; normal values > 100 ml/min) was observed. The remaining eight patients were treated for 15 days. Ornipressin was given at a dose of 2 IU/h. Albumin was given at a dose of 1 g/kg during the first day. The amount of albumin during the following days was adjusted according to values of plasma renin activity. In four patients treatment was stopped between 4 and 9 days after initiation of therapy due to ischemic complications in three cases and bacteremia in one. In these four patients a marked decrease in serum creatinine during therapy and a progressive impairment of renal function after treatment withdrawal were observed. In the remaining four patients who completed treatment, there was a significant elevation in mean arterial pressure, a normalization of plasma renin activity, a marked decrease in plasma norepinephrine concentration, an increase in GFR, and a normalization in serum creatinine concentration (Fig. 28.10). These four patients died 12, 60, 62, and 133 days after treatment; HRS did not recur in any of them during follow-up.

In a subsequent study, the same group treated nine patients with HRS (six with type 1 and three with type 2 HRS) with terlipressin (0.5–2 mg/4 h i.v.) and i.v. albumin during 5–15 days (170). Reversal of HRS (normalization of serum creatinine) was observed in seven patients. No case developed ischemic complications. HRS did not recur in any patient. Five cases were transplant candidates and three were transplanted 5, 12, and 99 days after treatment. The two other patients died 30 and 121 days after the inclusion. The remaining four patients died 13–102 days after treatment. In both studies dilutional hyponatremia was corrected with the normalization of serum creatinine.

These observations have been confirmed by other groups (Table 28.2). Gülberg *et al.* treated seven patients with type 1 HRS with ornipressin (6 IU/h), dopamine (2–3 μg/kg/min), and i.v. albumin (171). HRS was reversed in four patients after 5–27 days of treatment. In one patient treatment had to be stopped due to intestinal ischemia. The remaining two patients did not respond. In two of the four patients responding to treatment, HRS recurred 2 and 8 months later and they were retreated. HRS was reversed in one patient. In the other, treatment had to be stopped because of ventricular tachyarrhythmia. In total, two patients reached liver transplantation and one was alive 1 year after inclusion after two successful treatments. Mulkay *et al.* treated 12 patients with type 1 HRS with terlipressin (2 mg every 8–12 h) and albumin infusion (0.5–1 g/kg day during 5 days) for 1–9 weeks (172). HRS was reversed in seven patients. In the remaining five cases serum creatinine also decreased but did not reach normal levels. Withdrawal of terlipressin without recurrence of HRS was observed in six patients. No patient developed complications related to treatment. Three patients were transplanted 34, 36, and 111 days after inclusion. The remaining patients died with a median survival time of 42 days. Finally, Moreau *et al.* (173) in a retrospective study collected 99 patients with type 1 HRS

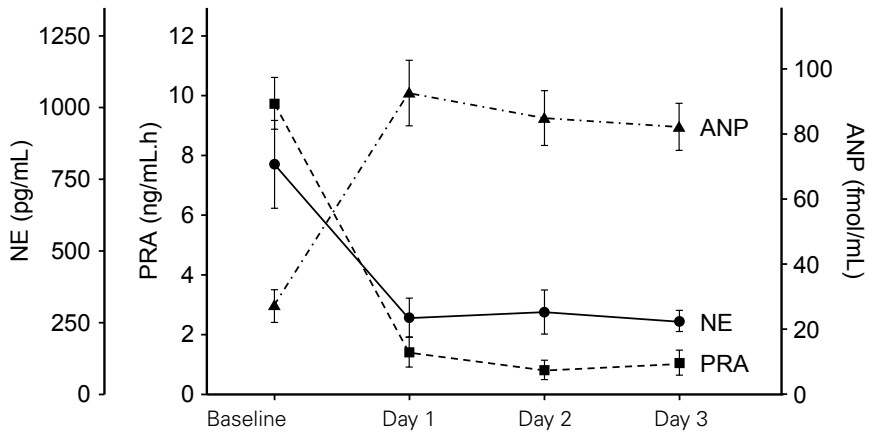


**Figure 28.9** Plasma renin activity (PRA) (dashed line), plasma norepinephrine (NE) concentration (solid line), and atrial natriuretic peptide (ANP) level (dotted line) in eight patients with hepatorenal syndrome treated for 3 days with ornipressin and plasma volume expansion. (Guevara M *et al.* Reversibility of hepatorenal syndrome by prolonged administration of ornipressin and plasma volume expansion. Hepatology 1998; 27:35–41.)

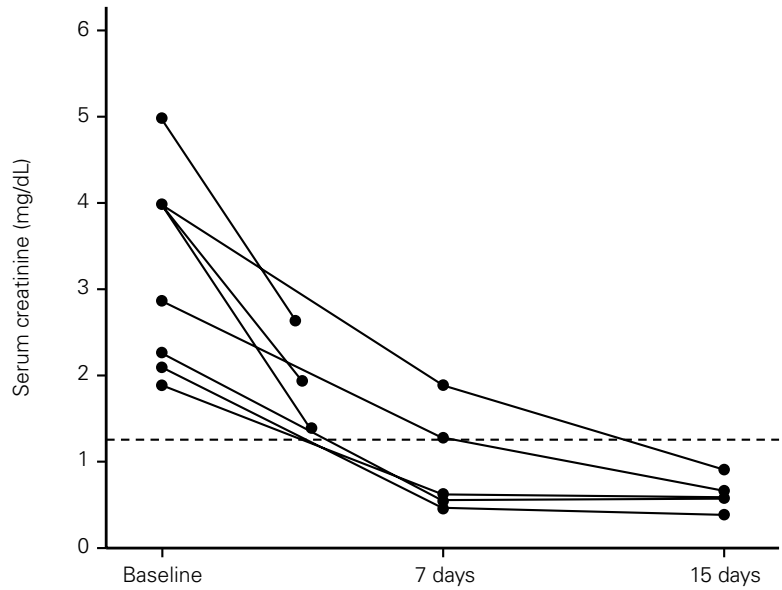


**Figure 28.10** Individual values of serum creatinine in eight patients with hepatorenal syndrome treated with ornipressin and albumin for 15 days. (Guevara M *et al.* Reversibility of hepatorenal syndrome by prolonged administration of ornipressin and plasma volume expansion. Hepatology 1998; 27:35–41.)

from 24 centers treated with terlipressin. Most of them also received albumin. Improvement in renal function was obtained in 58% of cases. The probability of survival was markedly improved in patients who responded to therapy.

Catecholamines are also effective for the treatment of HRS. Angeli *et al.* used oral midodrine, an α-adrenergic agonist, i.v. albumin and subcutaneous octreotide (to suppress glucagon) in five patients with type 1 HRS (174). Midodrine dosage was adjusted to increase mean arterial pressure 15 mmHg or more. Patients received treatment for at least 20 days in hospital and subsequently continued treatment at home. In all cases there was an improvement in renal perfusion, GFR, blood urea nitrogen, serum creatinine, and serum sodium concentration and a suppression of plasma renin, aldosterone, and antidiuretic hormone to normal or near normal levels. Two patients were transplanted 20 and 64 days after inclusion while on therapy. One patient who was not a candidate for liver transplantation was alive without treatment 472 days after discharge from hospital. The remaining two patients died 29 and 75 days after inclusion. These results were compared with those obtained in eight patients with type 1 HRS treated with i.v. albumin plus dopamine (2–4 μg/kg/min). In all these eight patients a progressive worsening in renal function was observed. One patient was transplanted but died 15 days after transplantation because of a fungal infection. The remaining seven patients died within 2 weeks after the initiation of treatment. Duvoux *et al.* treated 12 patients with type 1 HRS with intravenous albumin (to maintain central venous pressure > 7 mmHg) and norepinephrine (0.5–3 mg/h) for a minimum of 5 days (175). A significant improvement in serum creatinine in association with a marked

**Table 28.2** Rate of response, recurrence, transplant, and survival in different series of patients with cirrhosis and hepatorenal syndrome treated with vasoconstrictors and iv albumin.

| | Response (%)* | Recurrence (%)** | Liver transplantation (%) | One-month survival (%) |
|---|---|---|---|---|
| Angeli *et al.* 1999† | 5/5 (100) | NR | 2/5 (40) | 4/5 (80) |
| Uriz *et al.* 2000 | 7/9 (77) | 0/7 (0) | 3/9 (33) | 6/9 (67) |
| Mulkay *et al.* 2001 | 11/12 (92) | 6/11 (55) | 3/12 (25) | 10/12 (80) |
| Moreau *et al.* 2002 | 53/91 (58) | NR | 13/99 (13) | 40/99 (40) |
| Colle *et al.* 2002 | 11/18 (61) | 7/11 (64) | 2/18 (11) | 7/18 (40) |
| Halimi *et al.* 2002 | 13/18 (72) | NR | 2/18 (11) | NR |
| Alessandria *et al.* 2002 | 8/11 (73) | 8/8 (100) | NR | NR |
| Ortega *et al.* 2002 | 14/21 (66) | 2/14 (14) | 3/21 (14) | 11/21 (52) |
| Duvoux *et al.* 2002‡ | 10/12 (84) | 0/10 (0) | 3/8 (37) | 11/19 (58) |
| Solanki *et al.* 2003 | 5/12 (42) | NR | NR | NR |

*The definition of response varies among studies. ** Recurrence of hepatorenal syndrome after treatment withdrawal in responder patients. The definition of recurrence also varies among studies. † Vasoconstrictor used: midodrine. ‡ Vasoconstrictor used: norepinephrine. Terlipressin was used in the other studies. NR, not reported.

suppression of plasma renin activity was observed in 10 patients. Transient myocardial ischemia was observed in one patient. Three patients were transplanted and three were still alive after 8 months of follow-up.

Finally, Ortega *et al.* have recently assessed whether albumin is necessary in the treatment of HRS with vasoconstrictors (154). Twenty-one patients with HRS were studied. The first 13 were treated with terlipressin (0.5–2 g/4 h) and albumin (1 g/kg the first day; 20–40 g/day thereafter). The last eight patients received terlipressin alone. Treatment was given until normalization of serum creatinine or for a maximum of 15 days. In patients treated with terlipressin plus albumin there was a significant increase in mean arterial pressure, a marked suppression of plasma renin activity and a decrease in serum creatinine. In contrast, no significant changes in these parameters were observed in patients treated with terlipressin alone. A complete response (normalization of serum creatinine) was achieved in 10 patients treated with terlipressin plus albumin and in only two treated without albumin. Recurrence of HRS occurred in only two patients. One-month survival without transplantation was 87% in patients receiving terlipressin plus albumin and 13% in patients receiving terlipressin alone.

These studies show that: (i) type 1 HRS is reversible following treatment with i.v. albumin and vasoconstrictors; (ii) the two components of the treatment are important since HRS does not reverse when vasoconstrictors or plasma volume expansion are given alone; (iii) the constant infusion of vasoconstrictors (ornipressin or norepinephrine) is associated with ischemic complications, a feature not observed when they are given intermittently (terlipressin); (iv) there is a delay of several days between the improvement in circulatory function and the increase in GFR; (v) reversal of HRS improves survival and a significant number of patients may reach liver transplantation.

There are few data on the effectiveness of vasoconstrictors in type 2 HRS. Alessandria *et al.* (176) treated 11 patients with type 2 HRS with terlipressin and albumin. Normalization of serum creatinine was observed in eight cases. However, in all of them HRS recurred after discontinuation of therapy. It appears that treatment with volume expansion and vasoconstrictors is effective only in type 1 HRS.

## Transjugular intrahepatic portosystemic shunt (TIPS)

Since portal hypertension is the initial event of circulatory dysfunction in cirrhosis, the decrease of portal pressure by porto-caval anastomosis is a rational approach for the treatment of HRS. There are several case reports showing reversal of HRS following surgical porto-caval shunts (177,178). However, the applicability of major surgical procedures in patients with HRS is small. The development of TIPS has reintroduced the idea of treating HRS by reducing portal pressure.

Four studies assessing TIPS in the management of type 1 HRS have been reported (179–182) and recently reviewed by Brensing *et al.* (80). In total, 30 patients were treated. In two series no liver transplantation was performed, whereas in the other two series three out of nine patients were transplanted 7, 13, and 35 days after TIPS. TIPS insertion was technically successful in all patients. Only one patient died as a consequence of the procedure. GFR improved markedly within 1–4 weeks after TIPS and stabilized thereafter. In one study specifically investigating the neurohormonal systems, improvement in GFR and serum creatinine was related to a marked suppression of the plasma levels of renin and antidiuretic hormone (178). The suppression of plasma norepinephrine is lower than that of renin, a feature also observed in refractory ascites treated by TIPS. Follow-up data concerning hepatic function was obtained from 21 patients. *De novo* hepatic encephalopathy or deterioration of pre-existing hepatic encephalopathy occurred in nine patients, but in five it could be controlled with lactulose. Survival rates based on the 27 patients without early liver transplantation at 1 month, 3 months, and 6 months were 81, 59, and 44%, respectively. These studies strongly suggest that TIPS is useful in the management of type 1 HRS and improves survival.

## Sequential treatment with vasoconstrictors and TIPS

One of the intriguing issues in the treatment of type 1 HRS with vasoconstrictors is the observation that, despite marked suppression of the renin–angiotensin and sympathetic nervous systems and normalization of serum creatinine, renal function does not reach normal levels in the majority of patients, and there is persistence of low GFR, which ranges between 30 and 50 ml/min in most cases (normal 120 ml/min). The reason for this feature is not known, but could be the existence of a component of renal failure unresponsive to changes in circulatory function, or the fact that the effective arterial blood volume, although improved, is not normalized with pharmacology therapy. A recent study by Wong *et al.* (183) is consistent with this latter hypothesis. Treatment with TIPS in patients responding to pharmacological treatment (midodrine, octreotide, and albumin) was associated with normalization in GFR in most cases (Fig. 28.11). Whether the effect of TIPS on the normalization of GFR was due to the correction of the arterial vasodilation, to an increase in cardiac preload and ventricular function, or both, remains to be investigated.

## Other treatments

Hemodialysis and arteriovenous or veno-venous hemo-

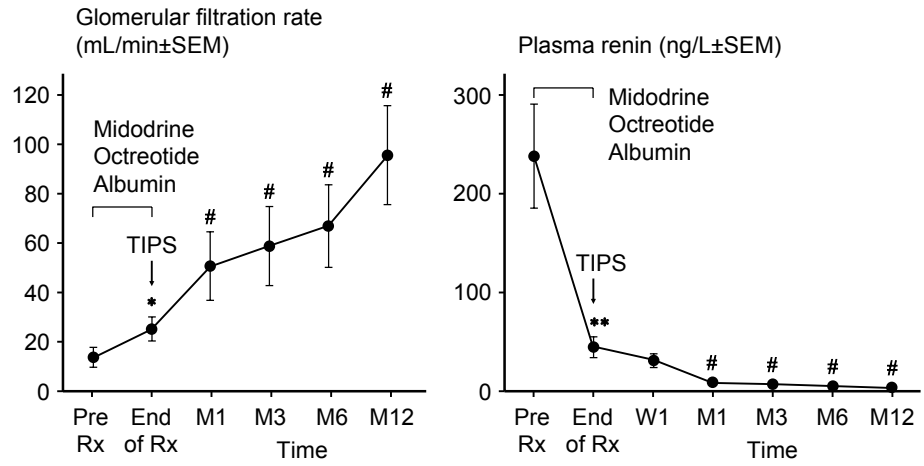


**Figure 28.11** Glomerular filtration rate and plasma renin concentration in five patients who received transjugular intrahepatic portosystemic shunt (TIPS) after medical treatment consisting of midodrine, octreotide, and albumin (Adapted from Wong F *et al.* The use of midodrine, octreotide and transjugular intrahepatic portosystemic stent shunt in the treatment of cirrhotic patients with ascites and renal dysfunction including hepatorenal syndrome. Hepatology 2004; 40:55–64.)

filtration are frequently used in patients with HRS, but their efficacy has not been adequately assessed (184). Recently, extracorporeal albumin dialysis, a system that uses an albumin-containing dialysate that is recirculated and perfused through charcoal and anion-exchanger columns, has been shown to improve systemic hemodynamics and reduce the plasma levels of renin in patients with type 1 HRS (185,186). In a small series of patients an improved survival has been reported (185). Further studies are needed to confirm these findings.

## Prevention of hepatorenal syndrome

Two randomized controlled studies in large series of patients have shown that HRS can be prevented in specific clinical settings. In the first study (187), the administration of albumin (1.5 g/kg i.v. at infection diagnosis and 1 g/kg i.v. 48 h later) together with cefotaxime in patients with cirrhosis and spontaneous bacterial peritonitis markedly reduced the incidence of impairment in circulatory function and the occurrence of type 1 HRS compared with a control group of patients receiving cefotaxime alone (10% incidence of HRS in patients receiving albumin vs. 33% in the control group). Moreover, the hospital mortality rate (10% vs. 29%) and the 3-month mortality rate (22% vs. 41%) were lower in patients receiving albumin (Fig. 28.12). In a second study (188), the administration of the tumor necrosis factor inhibitor pentoxifylline (400 mg t.i.d.) to patients with severe acute alcoholic hepatitis reduced the occurrence of HRS (8% in the pentoxifylline group vs. 35% in the placebo group) and hospital mortality (24% vs. 46%, respectively). Since bacterial infections and acute alcoholic hepatitis are two important precipitating factors of type 1 HRS, these prophylactic measures may decrease the incidence of this complication.

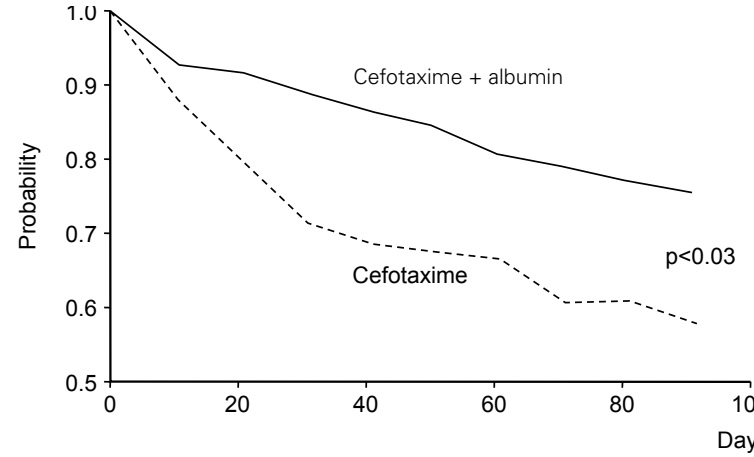


**Figure 28.12** Probability of survival in patients with spontaneous bacterial peritonitis treated with cefotaxime alone or cefotaxime plus albumin. (Sort P *et al.* Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis in cirrhosis. N Engl J Med 1999; 341:403–9.)

## Summary

HRS is a pivotal clinical event in cirrhosis. Although the most characteristic feature of the syndrome is renal failure due to renal vasoconstriction, it is a more generalized process affecting the liver, heart, brain, and the splanchnic organs. There are two types of HRS. Type 1 HRS is characterized by a rapidly progressive impairment in circulatory and renal function. It usually develops in close relationship with a precipitating event, particularly severe bacterial infections, superimposed acute hepatitis or surgical procedures, and is associated with a poor prognosis (median survival < 2 weeks). Type 2 HRS is characterized by a moderate and steady impairment of circulatory and renal function. Patients with type 2 HRS have a median survival of 6 months and their main clinical problem is refractory ascites. The pathogenesis of HRS is deterioration in effective arterial blood volume due to splanchnic arterial vasodilation and reduction in venous return and cardiac output. It is therefore not surprising that the syndrome can be reversed by the simultaneous administration of i.v. albumin and vasoconstrictors. Intrarenal mechanisms are also important and require prolonged improvement in circulatory function to be deactivated. Vasoconstriction in the brain, muscles and skin, increases in intrahepatic vascular resistance and portal pressure, and impairment of hepatic blood flow and hepatic function are other components of the syndrome. Long-term administration of i.v. albumin and vasoconstrictors or the correction of portal hypertension with a transjugular intrahepatic porto-caval shunt are effective treatments of HRS, improve survival, and may serve as a bridge to liver transplantation, which is the treatment of choice in these patients.

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# Chapter 29
# Glomerular Disease in Cirrhosis

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The association between renal disease and cirrhosis has been well established. It has become apparent that several distinct glomerulopathies can exist in the setting of cirrhosis. This chapter will examine the relationship between alcoholic as well as viral induced liver disease and glomerulonephritis including IgA nephropathy, cryoglobulinemic glomerulonephritis, and membranous glomerulonephritis.

## Prevalence

Renal dysfunction associated with cirrhosis was first reported by Flint in 1863 (1) and subsequently glomerular abnormalities have been described in over 50% of patients with cirrhosis (2). Fisher and Hellstrom identified both membranous and proliferative glomerulonephritis associated with cirrhosis (3), while Bloodworth *et al.* coined the term "cirrhotic glomerulosclerosis" based on finding glomerulosclerosis in 78% of autopsied, cirrhotic patients in a series from Boston City Hospital and Ohio State University Hospital (4). More sophisticated immunomorphological staining techniques have subsequently revealed the presence of γ-globulins along glomerular basement membranes (5) and in the mesangium of patients with cirrhosis and hepatitis (6). Callard *et al.* demonstrated the presence of IgA, IgG, IgM, and C3 within electron-dense deposits in the mesangium and capillary walls of glomeruli in 10 patients with liver cirrhosis (7). Six of these patients had "marked" glomerulosclerosis, two had cellular proliferation, and three had "thickening of the capillary loops". Interestingly, none of the patients had proteinuria or renal insufficiency. Other studies, however, have demonstrated renal dysfunction especially when associated with a membranoproliferative glomerulonephritis (8). Subsequent autopsy and biopsy studies confirmed the presence of glomerular pathology, often associated with mesangial IgA deposition in over 50% of cases (9,10). However, two unselected series of orthotopic liver transplant recipients demonstrated a lower prevalence of IgA deposition despite an almost universal presence of glomerular abnormalities on biopsy (11,12).

## Glomerular pathology in cirrhosis

Since the description of "cirrhotic glomerulosclerosis" by Bloodworth *et al.* in 1959 (4), a variety of glomerular nephropathies have been described in cirrhotic patients and it is now recognized that there is no single glomerular disease of cirrhosis (11). Glomerular nephropathies associated with cirrhosis are presented in Table 29.1.

### Renal function

Given the variety of glomerular lesions that can occur in cirrhotic patients, it is not surprising that urinary findings and the level of renal dysfunction are also variable. Nakamoto *et al.* noted nephritic urine in 9.2% of cirrhotic patients and nephrotic urine in 1.6%. The impact of hepatitis on renal disease was not assessed in this series (9). In a series of 34 patients with glomerulonephritis, and chronic alcoholic liver disease, Nochy *et al.* found 11 patients with nephrotic syndrome due to membranoproliferative glomerulonephritis (MPGN), membranous nephropathy (MGN), glomerulosclerosis, and pure endocapillary proliferative glomerulonephritis. Renal insufficiency was more common in patients with IgA-associated MPGN vs. patients with glomerulosclerosis. Microscopic hematuria was present in 85% of patients, while gross hematuria was found in 26% (8). Other studies, however, have reported the finding of glomerular abnormalities without any significant proteinuria or decline in renal function (7,11).

Acute renal failure (ARF) in patients with alcoholic cirrhosis has been reported, with biopsy findings indicating the presence of mild mesangial glomerulonephritis, IgA deposition by immunofluorescence, and marked tubular necrosis (17). It should be recognized, however, that renal insufficiency or ARF is unusual in patients with glomerular abnormalities and cirrhosis (7,8,11).

### Treatment

Treatment of glomerular disease in cirrhotic patients is typically determined by the specific lesion. However, general measures such as restoration of normovolemia,

**Table 29.1** Glomerular pathology described in cirrhosis. GN, glomerulonephritis.

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| Minor glomerular abnormalities (11,12) |
| IgA nephropathy/mesangial IgA deposition (7,11,12) |
| Membranoproliferative GN associated with IgA deposition (8) |
| Membranoproliferative GN +/– cryoglobulinemia (13) |
| Membranous GN (8,14–16) |
| Focal segmental mesangiolysis/aneurysms associated with mesangial IgA deposition (14) |
| Glomerulosclerosis (4,8,11,12,15) |
| Proliferative or necrotizing GN |
| Rapidly progressive glomerulonephritis (9) |

discontinuation of nephrotoxins such as nonsteroidal anti-inflammatory medications, and control of hypertension often lead to improvement in renal function regardless of the underlying glomerular pathology. Proteinuria may increase the progression of renal failure (18). Therefore, treatment of proteinuria (19) and hypertension may reduce the rate of progression of renal disease (20). In this regard, angiotensin-converting enzyme (ACE) inhibition is particularly useful in patients without cirrhosis. Captopril protected against a decline in renal function in insulin-dependent diabetic nephropathy and was shown to be more effective than blood-pressure control alone (21). ACE inhibition also slows the progression of renal disease in nondiabetic renal disease (22). In nonazotemic, cirrhotic patients with ascites, ACE inhibition induced a significant fall in blood pressure and improvement in creatinine clearance (23). However, other studies suggest that ACE inhibition in both ascitic and non-ascitic cirrhotic patients reduces glomerular filtration rate (24). ACE inhibition may be beneficial in patients without azotemia or in the earliest stages of cirrhosis, but cautious observation of renal function, or complete avoidance should be considered in patients with advanced or decompensated cirrhosis (25). Angiotensin II (AII) blockade has been shown to protect against the progression of nephropathy due to Type 2 diabetes independent of the reduction in blood pressure (26). These agents have also been shown to be safe and highly effective in the treatment of portal hypertension in well-compensated cirrhotics (27). AII inhibitors may therefore not have a deleterious effect on systemic or renal hemodynamics in this group of patients. However, these agents may have deleterious effects on mean arterial pressure and renal hemodynamics in patients with established cirrhosis (25). Therapies for individual glomerular lesions will be discussed in more detail in the following sections.

## The association between cirrhosis and glomerular IgA deposition

Several autopsy series reports in the 1970s and 1980s identified the presence of IgA deposits in patients with cirrhosis (14,28). Berger *et al.* studied 100 cirrhotic patients at autopsy, 90 of whom had alcoholic cirrhosis. Mesangial, granular IgA deposits were found in 58 patients, while three cases demonstrated endomembranous IgA deposits. IgA was the primary immunoglobulin identified in these 61 cases. The clinical outcome of these findings was not clear (28). Sinniah *et al*. (14) found mesangial IgA in 36% of 75 consecutive autopsies with liver cirrhosis. A history of alcoholism was present in 13 of the cases. Other than two cases of nephrotic syndrome, there was no history of renal disease in any of the subjects. A more recent report of 250 consecutive autopsy cases without known renal disease demonstrated diffuse, granular, mesangial deposits of IgA in only 4.8% (29). Biopsy studies have yielded similar results. Callard *et al.* (7) obtained liver and kidney biopsies from 10 patients with cirrhosis during portocaval shunt procedures and found glomerular lesions were detected in nine of the patients, all associated with IgA deposition. However, none of these patients had hypertension, proteinuria, or renal insufficiency. These reports suggest that renal IgA deposition can occur in patients with cirrhosis but appears in the vast majority of cases to be clinically latent.

Several reports suggest a link specifically between alcoholic cirrhosis and IgA deposition. Woodroffe found a much higher frequency of mesangial IgA deposition in autopsies of alcoholic cirrhosis compared with controls (30). Smith and Hoy suggested a possible association between ethanol consumption, mesangial glomerulonephritis, and IgA deposition (31). There are also experimental models of alcohol-induced liver injury in which mesangial IgA deposition occurs (32,33). However, other studies have not demonstrated a similar association. Bene *et al.*, in a series of 98 patients with alcoholic liver cirrhosis, did not find an incidence of mesangial IgA nephropathy that was different from the general population (34). Sinniah was also unable to demonstrate a direct correlation between mesangial IgA deposition and alcoholism (14).

Despite the finding that IgA deposition in cirrhosis is usually clinically latent, some biopsy series suggest a different outcome. This is not surprising, since biopsies are typically performed for renal dysfunction. Because of selection bias, the true incidence and clinical relevance of IgA nephropathy cannot be determined by these studies. Berger *et al.* reported biopsy findings from 11 patients with cirrhosis and "renal involvement". Ten of the cases were due to alcoholic cirrhosis. Membranoproliferative

glomerulonephritis with IgA as the main immunoglobulin was present in five of the patients, all of whom had microscopic hematuria and nephrotic range proteinuria. Other changes seen with mesangial IgA deposition were focal glomerulosclerosis and mesangial proliferation (28). Nochy *et al.* reported IgA mesangial deposits in 26/34 patients with cirrhosis and "overt glomerulonephritis", although only 19 patients had renal insufficiency. Furthermore, 11/34 patients had cryoglobulins and 12/34 had a low C3 suggesting other possible etiologies for the cases of renal failure (8). Montoliu *et al.* could find only two cases of IgA deposition associated with mesangiocapillary glomerulonephritis in a series of 12 HBsAg-negative cirrhotic patients with proteinuria, hematuria and/or renal failure. Eight of these patients had alcoholic cirrhosis (35). There are case reports of acute renal failure associated with IgA nephropathy (17,36). These studies illustrate the tremendous variability in the effect of IgA deposition on renal function. They also illustrate that, contrary to earlier reports, a variety of glomerular disorders in addition to IgA nephropathy may cause renal dysfunction in cirrhotic patients.

## Pathogenesis

The pathogenesis of IgA-mediated renal injury in cirrhosis has not been fully elucidated. Several studies suggest that reduced clearance of polymeric, aberrantly glycosylated IgA1 may lead to increases in systemic levels of the antibody, with mesangial cell deposition, complement activation, and resultant renal mesangial cell injury. The majority of, if not all, mesangial IgA in IgA nephropathy is polymeric IgA subtype 1 (pIgA1) (37). IgA1 is unique among circulating immunoglobulins in having O-glycosylation as well as N-glycosylation sites that are restricted to the hinge region. Removal of carbohydrates from the IgA1 molecule results in noncovalent self-aggregation and a significant increase in adhesion to the extracellular matrix (ECM) proteins (38). Clearance of polymeric IgA is reduced in patients with IgA nephropathy (IgAN) (39). The main catabolic pathway for IgA1 is the hepatic asialoglycoprotein receptor (ASGPR) (37). Roccatello *et al.* suggested that an abnormal IgA glycosylation pattern in patients with IgAN or with cirrhosis allowed IgA-containing immune complexes to escape clearance by the asialoglycoprotein receptor system (40). An animal model of liver injury due to bile duct ligation demonstrated deposition of circulating IgA immune complexes in the glomerular mesangium, presumably due to decreased hepatic clearance from serum (41). Bridging fibrosis and nodule formation induced in rats by a lipotrope-deficient diet, intragastric infusions of commercial whiskey, or both was associated with mesangial IgA deposition, hematuria, and proteinuria (32,33).

How the aberrantly glycosylated IgA results in glomerular injury is less clear. Human mesangial cells bind IgA and subsequently proliferate and produce cytokines (42). Aberrantly glycosylated IgA prepared *in vitro* modulates human mesangial cell integrin expression which affects cell growth, differentiation, and survival (43). Abnormally glycosylated IgA impairs vascular endothelial growth factor synthesis (44), which may lead to impaired vascular repair and sclerosis in IgAN. Aberrant IgA glycosylation may promote complement activation via the alternative pathway (45). Polymeric IgA but not monomeric IgA can induce glomerular injury via alternative complement pathway activation (45). In human subjects, elevated plasma levels of C3 were associated with more severe renal injury (46). IgA glycoforms with altered glycosylation isolated from patients significantly depressed the proliferation and increased the apoptotic rate of cultured mesangial cells, in comparison with fractions isolated from control sera. Also, IgA from healthy control subjects treated *in vitro* with glycosidases significantly depressed the proliferation and enhanced the apoptosis rates of cultured mesangial cells (47).

## Treatment

There are no studies of the efficacy of therapy for IgAN specifically in the setting of cirrhosis. For IgAN in general, treatment is typically determined by prognosis. The prognosis for IgAN is generally favorable, but some patients will experience progressive renal failure. One study suggested that even in patients with initially normal GFR, 32% have a subnormal GFR within 5 years and 25% develop end-stage renal failure within 20 years (48). Risk factors for progression include the presence of hypertension, proteinuria > 1 g/day, and renal insufficiency (49–51). Even minimal (< 400 mg/day) proteinuria can lead to progression (52). A number of studies have suggested a benefit of ACE inhibition in IgAN (53–55). These agents should therefore be considered early in the course of the disease except in patients with established or decompensated cirrhosis (see section above). Corticosteroids have been used with variable success in small uncontrolled trials (56,57). However, in recent 5- and 10-year follow-up studies of a randomized controlled trial of patients with IgAN, proteinuria of 1–3.5 g/day, and plasma creatinine levels of ≤ 1.5 mg/dl, steroids significantly reduced proteinuria and prevented renal function deterioration (58,59). Combination therapy employing cytotoxic agents is usually reserved for crescentic glomerulonephritis. The combination of intravenous cytoxan, pulse methylprednisolone and oral prednisone in 12 patients with crescentic, proliferative IgAN reduced proliferative lesions, proteinuria and led to a lower incidence of end-stage renal disease vs. historical controls (60). Needless to say, the potentially severe side-effects of

such regimens must be considered carefully before using them in patients with cirrhosis. Several trials have examined the effect of omega-3-polyunsaturated fatty acid fish oil on progression of IgAN with conflicting results, and a meta-analysis of five trials suggested that fish oil did not have a discernible benefit on progression (61–65).

## Hepatitis C virus and renal disease

### Introduction

The hepatitis C virus (HCV) is a lipid enveloped, positive-sense, single-stranded RNA virus in the family *flaviviridae*. Houghton *et al.* first cloned the virus in 1989 and subsequently it has been identified as the major cause of non-A, non-B hepatitis (66,67). Infection with HCV is followed by an incubation period of 6–12 weeks and generally characterized by a mild, anicteric hepatitis. The majority of cases develop persistent viremia with up to 70% of patients progressing to chronic hepatitis (68). Those patients developing chronic hepatitis have a risk between 2% and 40% of developing cirrhosis within 20 years of infection (69).

HCV has been associated with a number of extrahepatic manifestations that are probably secondary to the persistent viremia with concomitant antibody production that promotes immune complex diseases. In a case–control study, Pawlotsky reported the presence of rheumatoid factor in 70% of patients, cryoglobulins in 36% of patients, antismooth muscle antibody in 21%, and a positive antinuclear antibody in 13% (70). Clinically, HCV has been associated with autoimmune thyroiditis (71), porphyria cutanea tarda (72), lichen planus (70), Sjögren's syndrome (73), as well as renal disease. In the kidney, HCV most often manifests as cryoglobulinemic glomerulonephritis in association with Type II cryoglobulinemia (13,74).

### Cryoglobulinemia

Cryoglobulins are immunoglobulins that precipitate in the cold and become soluble upon warming. Three types of cryoglobulins have been described (Table 29.2) (75). In Type I cryoglobulinemia, the cryoprecipitate contains one monoclonal antibody and is generally associated with a plasma cell dyscrasia such as multiple myeloma or Waldenstrom's macroglobulinemia. In Type II, which is termed a mixed cryoglobulinemia (MC), there is a polyclonal IgG, as well as a monoclonal anti-IgG, that is generally an IgM antibody with rheumatoid factor activity. This form can be secondary to lymphoma or chronic lymphocytic leukemia, but is termed "essential" when there is no identifiable cause. Type III is also a mixed cryoglobulin, but both the IgG and rheumatoid factor IgM are polyclonal. Both types of MC typically present as a systemic vasculitis with the triad of palpable purpura, arthralgia, and weakness (76,77). Renal disease occurs in over 50% of patients, but is most common with Type II MC. The presence of renal disease carries the worst prognosis (77,78).

### Hepatitis C virus and cryoglobulinemia

The prevalence of MC in patients with HCV infection ranges from 36 to 50%, with Type III MC being the most common (70,79,80). It is now thought that the majority of essential MC is secondary to infection with HCV. Patients with MC are overwhelmingly positive (> 90%) for the presence of anti-HCV antibodies (74,81). Furthermore, HCV RNA as well as anti-HCV antibody has been found in a higher concentration in the cryoprecipitate than in the serum (13,81). In a study by Misiani (74), of 51 patients with essential mixed cryoglobulinemia associated with glomerulonephritis, 98% were positive for HCV when tested using an ELISA directed against the c22/c200 antigens. These antigens are structural (c22) and nonstructural proteins (c200) that are found in the HCV. Furthermore, they found that the cryoprecipitates contained anti-HCV in 41% of the patients, but this increased to 94% when rheumatoid factor activity was abolished by dithiothreitol. The authors hypothesized that the rheumatoid factor somehow masked the anti-HCV in the cryoprecipitate. Given the very high prevalence of HCV infection in patients with cryoglobulinemia, it suggests a strong association and is likely to be pathogenic.

### Cryoglobulinemia and glomerulonephritis

As noted previously, renal involvement occurs in over 50% of patients with MC and it generally presents with a moderate degree of renal insufficiency as well as microscopic hematuria and proteinuria that is often nephrotic in

**Table 29.2** Classification of cryoglobulins. HCV, hepatitis C virus. HBV, hepatitis B virus.

| Type | Composition | Etiology |
|---|---|---|
| I | Monoclonal IgG, IgA, or IgM | Multiple myeloma, Waldenström's macroglobulinemia, lymphoproliferative disorders |
| II | Polyclonal IgG and monoclonal IgM with rheumatoid factor activity | HCV, hematological malignancies |
| III | Polyclonal IgG and polyclonal IgM | Autoimmune disease, HCV, HBV, infective endocarditis |

nature (13). Occasionally, a more fulminant presentation with an acute nephritic syndrome and severe renal failure will occur. Renal biopsy most frequently reveals a Type I MPGN with subendothelial deposits(13), but has some unique features that differentiate it from the idiopathic form. The glomerulus is markedly hypercellular secondary to both endocapillary proliferation and massive monocyte infiltration. This leads to accentuation of the normal lobular pattern and double contours of the basement membranes that are more pronounced than in the idiopathic form. Furthermore, the subendothelial deposits sometimes fill the capillary lumen, especially in those patients with a fulminant presentation. These large, eosinophilic, periodic acid–Schiff-positive deposits are termed intraluminal thrombi. By immunofluorescence, the composition of these deposits is identical to that of circulating Type II cryoglobulins containing IgG and IgM as well as C3. Electron microscopy often reveals ultrastructural features of the deposits such as granular, finely fibrillar, or immunotactoid features. In addition, approximately 30% of patients biopsied for renal dysfunction will have evidence of a small/medium vessel vasculitis that can be associated with systemic signs of vasculitis (82).

These typical histological features are termed cryoglobulinemic MPGN and occur in approximately 80% of patients with HCV-associated Type II MC and renal involvement. In the remaining 20% with cryoglobulinemic MPGN, the renal biopsy reveals a mild mesangial proliferative GN with moderate or no infiltration of leukocytes. In these patients clinical suspicion must be high to make the diagnosis of cryoglobulinemic GN (83).

## Pathogenesis

Johnson *et al.* first reported the association of HCV and MPGN in 1993 in their series of eight patients, all of whom were HCV+ with evidence of renal disease (13). Renal biopsies in all eight patients revealed MPGN. Approximately 66% were positive for MC, but all were positive for IgM rheumatoid factor that was able to bind anti-HCV antibodies *in vitro*. In this study it was not possible to detect HCV antigens in the glomeruli, but recently HCV-related proteins have been isolated from the glomeruli of patients with cryoglobulinemic MPGN. In a study by Sansonno (84), HCV-related antigens were identified in the immune deposits in the glomerular capillary wall.

Therefore, a hypothesis of the pathogenesis of cryoglobulinemic GN is that HCV infects peripheral blood mononuclear cells and stimulates them to produce polyclonal cryoglobulins (85). In some patients there is a shift allowing the abnormal proliferation of a clonal set of B lymphocytes, that then induce the production of monoclonal rheumatoid factor IgMκ (86,87). It has been shown that this IgMκ has inherent affinity for an unidentified component of the glomerulus (88). Therefore, it is thought that deposition of this antibody, probably in association with HCV antigens, is responsible for the activation of complement and the subsequent influx of leukocytes that are mostly monocytes. This subsequently results in intense inflammation and renal pathology.

## Noncryoglobulinemic glomerulonephritis

There is some controversy over the association of noncryoglobulinemic HCV and MPGN. Johnson *et al.* reported on 34 patients with MPGN and HCV in whom 40% had no detectable cryoglobulins (89). The majority of these patients subsequently became cryoglobulin-positive, but five remained persistently negative. The clinical course in the cryoglobulin-negative group was similar to the cryoglobulin-positive patients. In opposition to these findings is a retrospective autopsy study of HCV+ patients with unreported cryoglobulin status that reported the prevalence of MPGN in HCV+ patients to be much lower than previously thought. In that study, only three out of 114 (2.6%) biopsies were consistent with MPGN and the authors conclude that there is unlikely to be an association between HCV and noncryoglobulinemic MPGN (90).

While it is apparent that MPGN is commonly associated with HCV infection and cryoglobulinemia, there are reports of other glomerulopathies as well. Membranous glomerulonephritis has also been reported in association with HCV, and HCV-related proteins have also been found in renal biopsies of patients with HCV and membranous GN (16,91,92). These patients present with nephrotic syndrome, renal insufficiency, and no detectable cryoglobulins. Furthermore, focal segmental glomerulosclerosis (93), thrombotic microangiopathy (94), fibrillary glomerulonephritis (95), IgAN (96), and crescentic glomerulonephritis have all been reported in association with HCV (97).

## Clinical features and course

As noted previously, patients typically present with moderate renal insufficiency and urine sediment that reveals microscopic hematuria and proteinuria. Hypertension is also very common. On laboratory investigation patients are generally hypocomplementemic, most notably with a very low C4 and normal or mildly reduced C3 level. Most will also exhibit a positive rheumatoid factor in addition to cryoglobulins (83).

The outcome in patients with idiopathic MPGN has generally been favorable. The course is usually prolonged, with a slow rate of disease progression. Up to 60% of untreated patients will progress to end-stage renal disease within 10–15 years, while 25–40% will maintain apparently normal renal function. As with other glomerular diseases, bad prognostic signs at presentation include the

nephrotic syndrome, renal insufficiency, hypertension, and, on renal biopsy, crescents. Even within this group, however, some patients will undergo spontaneous remission. The long-term prognosis tends to be relatively good in patients who present with asymptomatic hematuria and proteinuria and who have focal, rather than diffuse, glomerular involvement on renal biopsy (98).

### Therapy

Prior to the recognition that the majority of MC was secondary to HCV, the therapy consisted of plasmapheresis to remove circulating cryoglobulins as well as steroids and cytoxan to decrease production (82). With the recognition that HCV is the etiological agent, focus has shifted to antiviral therapies. In liver-limited HCV disease, the combination of pegylated interferon-alpha (IFN-α) and ribavirin has led to sustained virological responses in 40–80% of patients depending on the causative HCV genotype. Patients with genotype 1 are less likely to respond than those patients with genotype 2 (99,100). Unfortunately, the use of ribavirin is limited in patients with renal dysfunction due to its renal clearance and its ability to cause hemolytic anemia.

In patients with HCV-related renal disease, early studies with standard IFN-α therapy for 6 months showed efficacy in achieving clearance of viremia accompanied by improvement of renal function, levels of cryoglobulin, as well as proteinuria. However, upon discontinuation of the drug there was a relapse, with recurrence of renal dysfunction and proteinuria in most patients (13,101).

Recently, reports of using combination therapy in patients with cryoglobulinemic MPGN have emerged (102–104). With careful monitoring of the ribavirin dose based on hemoglobin levels, and in some cases using long-term IFN-α therapy, it has been possible to induce a sustained remission as well as improve renal histology. In a study of three patients (103) with cryoglobulinemic MPGN and moderate renal insufficiency, the use of IFN-α and ribavirin for 1 year was associated with improved renal function and renal histology, as well as a sustained virological response for up to 2 years. Importantly, in studies of liver-limited HCV, the single best predictor of response was the genotype of HCV. In the majority of cases, the evidence of response to combination therapy in cryoglobulinemic MPGN has been evaluated in genotype 2, which is much less treatment-resistant than genotype 1.

## Hepatitis B virus and renal disease

### Introduction

The hepatitis B virus (HBV) is a DNA virus in the *hepadna* virus family. The complete viral particle is known as the Dane particle and consists of an outer envelope, an inner core, as well as viral DNA. There are three antigens, the surface antigen (HBsAg), the core antigen (HBcAg), and the e antigen (HBeAg), to which there is an immune response resulting in the formation of antibodies (105).

HBV is one of the most common infectious diseases in the world. The incidence is lowest in the western hemisphere, with a much higher prevalence in Asia (106). Infection generally results in transient viremia with minor illness, followed by viral clearance in several weeks. In a minority of patients the infection is not cleared, resulting in chronic hepatitis that can progress to cirrhosis. The formation and tissue deposition of antigen and antibody in immune complexes is responsible for the extrahepatic manifestations such as glomerulonephritis (GN) and vasculitis associated with infection by HBV.

### Hepatitis B virus and glomerulonephritis

The association between membranous GN (MGN) and HBV was first noted in 1971 by Combes *et al.* in a patient with transfusion-related hepatitis. The patient had the Australia antigen, now identified as HbsAg, in his serum, and subsequently developed nephrotic syndrome with a biopsy consistent with MGN. Immunofluorescence (IF) revealed the Australia antigen and it was hypothesized that the deposition of the antigen in the glomerulus was responsible for the renal pathology (107). This report was followed by other confirmatory reports, but these early investigators were not able to elute HBV antigens from the glomeruli and therefore relied upon IF for diagnosis. Unfortunately, this method has been shown to be nonspecific secondary to endogenous IgM with antiglobulin activity that resulted in false-positive staining for HBsAg (108). When a more specific method of detecting HBV antigens was developed, it was determined that it was not HBsAg responsible for immune complex deposition, but rather HBeAg. In this method, a monoclonal antibody to the e antigen (HBeAb) was developed and then digested to the $F(ab')_2$ fragment. This avoided the nonspecific binding present with the complete immunoglobulin.

In a study of 16 patients who were seropositive for HBsAg and a renal biopsy consistent with MGN, it was discovered that the immune deposits in the glomeruli of 10 out of 16 patients with MGN were positive for HBeAg, whereas no patients were positive for HBsAg or HBcAg. Using controls of patients with idiopathic MGN or lupus-associated MGN there was no false-positive staining for HBeAg. In the patients negative for antigen staining it was noted that they had seroconverted from HBeAg positivity to HBeAb positivity. The authors postulate that these patients may have been recovering and lost the HBeAg deposits. This led to the idea that it was the HBeAg responsible for development of MGN (109).

A later study by Lai *et al.* (110) reported on 100 patients with a biopsy-proven glomerulonephritis who were also

seropositive for HBsAg. Using monoclonal Fab antibodies it was found that 39 patients had evidence of HBV antigens deposited in the kidney, thereby meeting the stricter criteria for HBV-associated glomerulonephritis. When using these stricter criteria, there were three morphological entities identified: MGN, MPGN, and IgAN. These entities were often seen as mixed lesions with HBeAg present in subepithelial deposits and HBsAg present in the mesangium. When correlating morphological findings with that of antigen specificity it was found that, in those patients in whom HBsAg was found, the diagnosis of MPGN or IgAN was more likely; alternatively, in patients with HBeAg positivity, MN was most common. In patients with both antigens there was generally a mixed lesion. Therefore, it appears that the specific antigen deposited plays a role in the pathology and therefore the histological diagnosis.

## Therapy

In a trial designed to investigate the clinical features and outcome of HBV-associated MGN, Lin *et al.* (111) studied 34 children with HBV-associated MGN defined morphologically, as well as requiring the renal presence of HBV antigens via monoclonal Fab antibodies. They found that 88% of children had detectable HBeAg in the glomeruli and all were seropositive for HBsAg. Nearly all patients initially had nephrotic range proteinuria and many were also hypocomplementemic. In those patients treated with steroids, they noted a poor response rate with frequent relapses or persistent proteinuria. There were four cases that presented with decreased renal function and had persistent or relapsed proteinuria that underwent repeat biopsy. They found that these patients had progressed histologically.

The efficacy of corticosteroids has been further investigated by Lai *et al.* (112), who found that not only did corticosteroids fail to improve patients clinically, but they stimulated viral replication as well. In this study, 6 months of therapy with steroids was associated with early regression of the nephrotic syndrome in three of eight patients, whereas five of eight patients had persistent but reduced proteinuria. In historical controls, two of seven patients experienced spontaneous remission of proteinuria. Furthermore, steroid therapy was associated with increased serum levels of HBeAg as well as serum HBV DNA titers.

Given these disappointing results with steroids, focus has shifted to the efficacy of antiviral therapy. The most experience is with the use of IFN-α. Lin *et al.* (113) has reported on the use of IFN in children with HBV-associated MGN in a prospective, controlled trial. These patients had nephrotic range proteinuria with normal renal function, were seropositive for HBsAg and HbeAg, and had all failed a trial with corticosteroids. They were randomized to receive IFN 5 million units three times weekly for 1 year or supportive therapy only. After 3 months, 80% of the treated group had resolved their proteinuria and at 2 years there were no relapses. In comparison, the control group at 3 months had no remissions. While there was a 35% remission rate in the control group at 2 years, 15% continued with nephrotic range proteinuria and 50% continued with relapsing periods of proteinuria. Furthermore, the patients in the control group remained seropositive for HBsAg as well as HBeAg, while in the treatment group there was significant seroconversion. Unfortunately, with the limited follow-up it is unclear whether the control group would have also resolved their proteinuria as was the trend, and whether becoming seronegative for antigens is a significant event.

## Hepatitis B virus and polyarteritis nodosa

Classical polyarteritis nodosa (PAN) is a systemic, necrotizing arteritis involving small to medium-sized arteries that presents as a systemic illness with multiorgan involvement including the skin, nervous system, gastrointestinal tract, coronary arteries, and kidneys. In the kidneys it is the renal arteries that are affected by focal vessel wall fibrinoid necrosis and infiltration of inflammatory cells. Typically, the glomeruli are not involved. Therefore, PAN will not present as a glomerulonephritis and is associated with bland urine sediment. If a patient presents with an arteritis and glomerulonephritis, one should consider the diagnosis of a small-vessel vasculitis such as microscopic polyarteritis, which is more closely associated with the antineutrophil cytoplasmic antibody (ANCA)-positive vasculitides such as Wegener's granulomatosis (114).

The association of HBV infection and PAN was first demonstrated in 1970 by Gocke (115), who detected the Australia antigen (HBsAg) in the vessels involved by vasculitis. In an autopsy series by Michalak (116) it was found that the most involved vessels had immune complexes containing HBsAg, whereas those that were healed had no evidence of the immune complexes. This was taken to support the idea that the HBsAg and its antibody are deposited into vessel walls and thus responsible for the pathological vasculitis, and that resolution of the vasculitis occurs when the immune complexes are removed.

Therapy for HBV-associated PAN has been evaluated by Guillevin *et al.* (117). They studied 33 patients with PAN, all of whom were seropositive for HBsAg and HBeAg along with histological evidence of active vasculitis. The patients were treated with prednisone to control the active vasculitis and then vidarabine to decrease viral replication and induce seroconversion. Furthermore, patients were treated with plasma exchange to remove immune complexes. At 6 months, the majority (78%) of

patients were under control and after 5 years over 70% of patients had made a complete recovery. Seroconversion to either HBsAb or HBeAb occurred in > 50% of patients.

## Liver disease and renal tubular acidosis

Renal tubular acidosis (RTA) refers to a group of disorders characterized by an inability of the renal tubules to reabsorb filtered bicarbonate, excrete hydrogen ions, or generate ammonium. These disorders are characterized by a normal anion gap (hyperchloremic) metabolic acidosis. Shear *et al.* observed that hepatic encephalopathy occurred more frequently in patients with cirrhosis and RTA than in those without this tubular abnormality. The cause of the cirrhosis was not reported. Some patients were found to have impaired urinary acidification with glucosuria, suggesting a proximal defect, while others had features typical of distal RTA (118).

### Prevalence

A defect in urinary acidification has been found in 21–45% of cirrhotic patients (119–121). Golding found renal tubular acidosis in 32% of 117 patients with chronic liver disease due to active chronic hepatitis, primary biliary cirrhosis, cryptogenic cirrhosis, and alcoholic cirrhosis (121). The acidification defect was either an incomplete or an "overt" distal RTA (gradient type), with the incomplete form being by far the most common. The RTA was seen most frequently with primary biliary cirrhosis and active chronic hepatitis, although other reports have suggested alcoholic cirrhosis is the most common cause (119).

### Etiology

There are several disorders which cause cirrhosis and RTA (see Table 29.3). Type I distal RTA has been associated with cryptogenic cirrhosis (119,122), primary biliary cirrhosis (123,124), and alcoholic cirrhosis (118,119). Proximal RTA with aminoaciduria has also been described with primary biliary cirrhosis and cirrhosis associated with the Fanconi syndrome (122). Incomplete distal RTA, so called because it is clinically silent and is only exposed upon measuring the urinary pH after an acid load, has been described in primary biliary cirrhosis (121, 125) and alcoholic cirrhosis (119). Wilson's disease can cause both proximal (126) and distal RTA (127).

### Pathogenesis

Copper deposition in the tubules may cause RTA in both Wilson's disease (128,129) and primary biliary cirrhosis (125). Decreased distal delivery of sodium has also been implicated as a cause of RTA in cirrhosis (119). Others have attributed the development of RTA in cirrhosis to immunological causes (121). Of note, cholestyramine has been implicated as a pathogenic factor in a 70-year-old female with primary biliary cirrhosis, hyperchloremic metabolic acidosis, and no evidence of renal tubular dysfunction. Medication effects should therefore be considered in the evaluation of normal anion gap metabolic acidosis in cirrhosis (130).

**Table 29.3** Types of cirrhosis associated with renal tubular acidosis (RTA).

| |
|---|
| Proximal RTA |
| Primary biliary cirrhosis (132) |
| Cirrhosis associated with the Fanconi syndrome (122) |
| "Chronic active hepatitis and cirrhosis" (120) |
| Wilson's disease (126) |
| Incomplete and complete distal RTA |
| Cryptogenic cirrhosis (119,122) |
| Primary biliary cirrhosis (123–125) |
| Alcoholic cirrhosis (118,119) |
| Wilson's disease (127) |

### Treatment

The aims of treatment are to correct the acidemia and to prevent progression of nephrocalcinosis and the development of chronic renal failure. Therapy requires appropriate amounts of alkali in the form of either bicarbonate or citrate. The amount of alkali should be equivalent to the amount of bicarbonate lost in the urine plus the amount of acid generated by protein catabolism. Proximal RTA is characterized by massive urinary loss of bicarbonate (up to 10–20 mmol/kg per 24 h) and is best treated with a mixture of sodium and potassium citrate. In distal RTA, the goal is to balance hydrogen ion production so less base is required for this disorder, typically 1–2 mmol/kg per 24 h (131).

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# Chapter 30
# Drug-induced Renal Failure in Cirrhosis

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## Mechanisms of drug-induced renal failure

The kidney is a highly vascularized organ whose function is largely dependent on blood supply. The regulation of renal hemodynamics depends on changes in arteriolar resistances that govern both the renal blood flow (RBF) and the glomerular filtration rate (GFR) by means of autoregulation and tubuloglomerular feedback mechanisms (1). Autoregulation is an intrinsic mechanism present also in denervated kidneys, namely in afferent arterioles. When systemic arterial pressure increases, afferent arterioles constrict, maintaining a constant hydraulic pressure within the glomerular capillaries and avoiding abrupt changes of GFR. On the other hand, a decrease of arterial pressure is followed by the relaxation of afferent arterioles that preserves GFR and RBF. The maximal relaxation of afferent arterioles is reached with a mean arterial pressure of approximately 70 mmHg, and further reductions result in a striking fall of GFR and RBF. The mechanisms governing the autoregulation of renal vessels are not completely known, but the stretch receptors of the arteriolar wall, regulating $Ca^{2+}$ influx across the cell wall, are considered to play a major role (1,2).

Tubuloglomerular feedback is regulated in the macula densa of the early distal tubule, in juxtaposition with the glomerulus and afferent and efferent arterioles of the same nephron (2). The macula densa is sensitive to changes in chloride ion concentration and transport. When GFR rises, the increased delivery of chloride to the macula densa is followed by vasoconstriction of afferent arterioles and reduction of the filtration surface area, probably through mesangial cell contraction (3). This mechanism restores GFR to the normal range. Intrarenal angiotensin II (AII) may be the hormonal mediator of the tubuloglomerular feedback system, but changes of osmolarity or chloride concentration in the interstitium probably also play a role in regulating the arteriolar resistance (3).

The glomerulus contains receptors for many substances that can affect the filtration rate (2,4,5). AII has vasoconstrictor properties that predominantly act on the efferent arterioles, whereas norepinephrine increases the tone of both afferent arterioles (by a direct mechanism) and efferent arterioles (mediated by the renin–angiotensin system). The final result is a renal vasoconstriction and reduction of total RBF with GFR remaining unchanged or only slightly decreased. Under certain circumstances, this phenomenon may contribute to shifting large amounts of blood to coronary or brain vascular beds.

The glomerulus is also capable of the *de novo* synthesis of a number of vasoactive substances that may markedly affect renal function in the absence of significant alterations in systemic hemodynamics (6). Among them, some intrarenal vasodilators, such as prostaglandins and bradykinin, preserve the kidney from excessive ischemia due to hyperactivity of vasoconstrictors (7). Many drugs can affect the synthesis and the release of the mediators of this autoregulatory pathway, namely AII and prostaglandins. Overt clinical renal failure can occur when such drugs are given to patients with hypovolemia including patients with advanced cirrhosis. The nonsteroidal anti-inflammatory drugs (NSAIDs) are of paramount importance among these drugs.

On account of its anatomical and functional characteristics, the kidney presents a series of pathways for injuries caused by drugs. On this basis nephrotoxic drugs can be classified as: (i) affecting renal hemodynamics; (ii) directly damaging tubular cells; (iii) interfering with the immune system or causing immune complex-mediated glomerular or tubular disease.

In the first type of drug-mediated renal damage, GFR and RBF both fall because the drug interferes with AII or prostaglandins. In fact, both substances act to preserve renal perfusion and function under different situations such as sodium depletion (overuse of diuretics, low-sodium diet), arterial underfilling (heart failure, chronic liver disease, dehydration), chronic parenchymal diseases of the kidneys and renal artery stenosis (8). Under all such conditions, the pharmacological inhibition of the renin–angiotensin system and/or prostaglandin synthesis causes acute renal failure.

The second group of drugs exerts a direct nephrotoxic activity independent of renal hemodynamics (9,10). The most common mechanism of damage involves intracel-

lular accumulation of the drug by pinocytosis, such as occurs with aminoglycosides. In most cases the affected cells are those of the proximal tubule, where a high proportion of reabsorption occurs. These drugs can interact with the components of the cellular wall; the damage is documented by the urinary loss of enzymes of the renal tubular brush border, but damage to mitochondria, lysosomes, and nuclei can also occur. The toxicity is dose-dependent and the mechanism involves drug interference with phospholipases and calcium metabolism, degeneration of proteases and phosphatases, and production of free radicals with peroxidation of membrane phospholipids; the generation of autacoids and prostaglandins may be impaired (11). The derangement in prostaglandin metabolism may explain the relationship between tubular damage and reduction in GFR.

Many factors predispose the kidney to these two pathways of drug injury. Nephrotoxic insults that are not injurious to healthy individuals may cause severe damage in dehydrated patients, elderly subjects, and patients with vascular diseases. Anoxic injury can be exacerbated when drug-induced membrane damage or mitochondrial uncoupling cause increased oxygen consumption precisely when oxygen delivery is low. Drugs interfering with prostaglandins or AII may worsen the oxygen deprivation.

A third type of drug-induced kidney injury is immunologically mediated and may lead to glomerulonephritis, small vessel vasculitis, or tubulointerstitial disease (12). The traditional pathogenic hypothesis of activation of humoral immunity is valid only in a small number of drug-induced kidney diseases (e.g. tubular basal membrane antibodies disease), whereas a primary role for the cell-mediated immune system with a predominant activity of the cytotoxic T lymphocytes has been substantiated (12).

## Increased risk of nephrotoxicity in cirrhotic patients

Liver cirrhosis involves a complex series of hemodynamic changes with associated organ derangements that can increase the risk of drug nephrotoxicity. In patients with cirrhosis and portal hypertension the total blood volume is increased, but an abnormal distribution leads to splanchnic vascular bed congestion at the expense of central blood volume (13). This reduces RBF and activates the intrarenal mechanisms that protect GFR.

In some cases of liver disease glomerular cells may be damaged by deposition of antibodies or antigen–antibody complexes. This has been reported for patients with diseases related to hepatitis B and hepatitis C virus infection and for patients with alcoholic liver disease (14). Anemia, diabetes, and frequent bacterial infections are additional uninvestigated risk factors.

Moreover, the diseased liver can lead to abnormal drug metabolism, and the bioavailability of drugs may change in patients with liver disease because of different distribution volumes and altered hepatic clearances. A defect of hepatic clearance causes drug accumulation and may enhance toxicity. It is also worth noting that, due to hypoalbuminemia, the protein binding of drugs is often reduced in cirrhosis, so there is a larger proportion of unbound active drug. The free fraction of some NSAIDs is increased in patients with liver disease (15). This calls for careful dosage and regimen adjustment.

## Nephrotoxicity of drugs employed in cirrhotic patients

### Diuretics

Diuretics are frequently prescribed to cirrhotic patients. In view of the presence of hyperaldosteronism, aldosterone antagonists are employed alone or combined with loop diuretics to treat cirrhotic patients with edema (16). The kidney dysfunction caused by diuretics is due to intravascular fluid depletion. As ascites may be mobilized at a limited rate (17), overdiuresis easily leads to hypovolemia and prerenal azotemia. Conversely, leg edema is more easily reabsorbed than ascites. This explains why diuretic-induced renal failure is less frequent in ascitic patients with ascites and leg edema than in those who have only ascites (18) (Fig. 30.1). Approximately 25–30% of cirrhotic patients with ascites treated with diuretics develop worsening of renal function due to

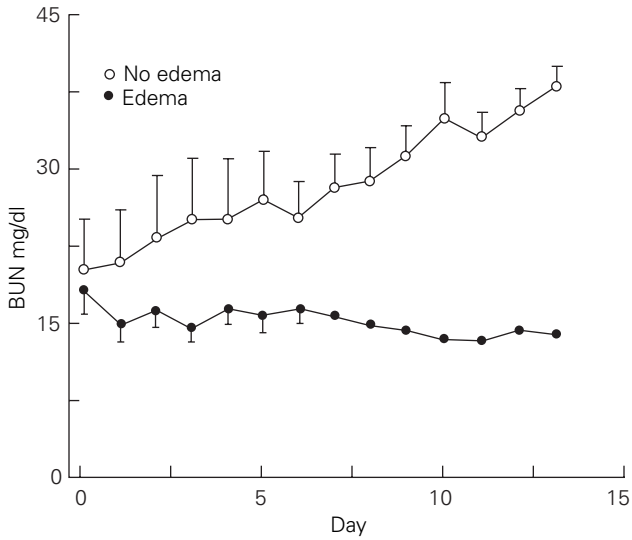


**Figure 30.1** Behavior of blood urea nitrogen (BUN) in cirrhotic ascitic patients with edema (closed circles) and without edema (open circles) during rapid diuresis induced by diuretic therapy. (From Pockros PJ, Reynolds TB. Rapid diuresis in patients with ascites from chronic liver disease: the importance of peripheral edema. Gastroenterology 1986; 90:1827–33.)

intravascular volume depletion (19). This type of renal dysfunction is almost always reversible when diuretics are tapered or withdrawn and/or fluids are given. However, if the impairment of renal function persists because of an uninterrupted administration of diuretics, tubular necrosis may occur (20). Moreover, prerenal azotemia that is sustained by overdiuresis easily enhances the nephrotoxicity of other drugs or of contrast media. Due to the high rate of renal dysfunction in cirrhotic patients treated with diuretics, it is advisable to identify patients at higher risk and closely monitor their kidney function during therapy. However, due to altered muscle and hepatic metabolism, the serum concentrations of urea and creatinine in cirrhotics are often lower than in healthy subjects and thus underestimate incipient renal failure (21–23). Therefore, until more accurate methods of GFR estimation are adequately validated in cirrhotic patients, care should be exercised in interpreting serum creatinine and urea-dependent measures.

## Nonsteroidal anti-inflammatory drugs

NSAIDs are widely used, and many of them are over-the-counter drugs. Their wide use is due to their multiple actions, analgesic, antipyretic, anti-inflammatory, and platelet-inhibitory. The anti-inflammatory activity is mainly due to the inhibition of cyclooxygenase, the key enzyme in prostaglandin synthesis (24). NSAIDs reduce the release of prostaglandin $E_2$ ($PGE_2$) and prostacyclin ($PGI_2$) in different tissues including the kidney. In the diseased kidney, $PGE_2$ and $PGI_2$ participate in the regulation of GFR, sodium excretion, and solute-free water excretion. This activity is protective in case of renal ischemia. Indeed, in many cirrhotic patients with ascites the renal production of prostaglandins is increased to counteract the activity of vasoconstrictors (25,26). Therefore, in such patients NSAIDs frequently induce renal ischemia with fall of GFR, but sometimes may also cause dilutional hyponatremia.

The renal effects of several NSAIDs in cirrhotic patients have been investigated with the purpose of identifying if any agent can spare prostaglandin synthesis and renal function. These studies showed that renal failure frequently occurs in cirrhotic patients receiving indomethacin (27), ibuprofen (28), acetylsalicylic acid (29), and naproxen (30). Even sulindac, a drug reported to spare kidney function in patients with Bartter's syndrome (31), chronic glomerulonephritis (32) and systemic lupus erythematosus, reduced urinary prostaglandin excretion, GFR and solute-free water clearance when given to cirrhotic patients with ascites for three consecutive days (33). The mechanism by which sulindac affects the renal synthesis of prostaglandins in cirrhotics with ascites, but not in patients with other diseases, was attributed to changes in the kinetics of the drug in patients with chronic liver disease (33).

A strategy to prevent the nephrotoxicity of NSAIDs in cirrhotics could be that of combining the drug with a compound with a protective renal activity. As prostaglandins are important factors in defending renal blood perfusion, some suggested to give misoprostol in combination with NSAIDs. Misoprostol was shown to prevent the indomethacin-induced nephrotoxicity in compensated cirrhotic patients (34). However, when misoprostol was given to cirrhotics with ascites, it was unable to prevent the renal dysfunction caused by indomethacin (35), or it had a minimal and insignificant effect against the renal complications induced by ibuprofen (36). In addition, several side-effects, like fever and nausea, made uncertain the chronic administration of misoprostol to patients with cirrhosis.

Imidazole-salicylate was investigated in 10 cirrhotic patients with ascites in a placebo-controlled study (37). Salicylate is a weak inhibitor of prostaglandin synthesis that appeared to be effective in controlling the inflammation of patients with rheumatoid arthritis (38), and imidazole inhibits the enzyme thromboxane-synthase (39), and therefore may cause shunting of endoperoxides toward the synthesis of vasodilator prostaglandins. After two doses of the drug, renal prostanoid production, under basal conditions and after stimulation with furosemide, remained unmodified. Accordingly, the diuretic activity of furosemide and the patients' renal function were spared (Fig. 30.2). Whether or not a more protracted administration would similarly spare renal function is still unknown.

Another way to spare renal function in patients with advanced liver disease who require analgesic or antipyretic therapy is to use acetaminophen combined with codeine (40). This drug might also avoid gastrointestinal complications that occur with NSAIDs. Acetaminophen, however, is highly toxic for the liver and the kidney if overdosed. Kidney toxicity may occur even with normal doses of the drug in patients who are glutathione-depleted (e.g. from chronic alcoholism or prolonged starvation) or who take drugs inducing the P-450 microsomal oxidase enzymes, such as anticonvulsants. Acute renal failure caused by acetaminophen manifests as acute tubular necrosis (41). *N*-acetylcysteine is recommended for hepatic injury due to acetaminophen, but has not been shown to improve renal tubular damage (42). This suggests the existence of additional unidentified factors that contribute to acetaminophen nephrotoxicity.

New approaches to spare the renal function of cirrhotic patients treated with NSAIDs include the use of selective inhibitors of cyclooxygenase-2 (COX-2) and that of nitric oxide-releasing drugs. Anti-COX-2 drugs might be of particular interest for the hepatologist also for their potential antifibrotic effect in the liver (43). These compounds selectively inhibit the inducible isoform of COX-

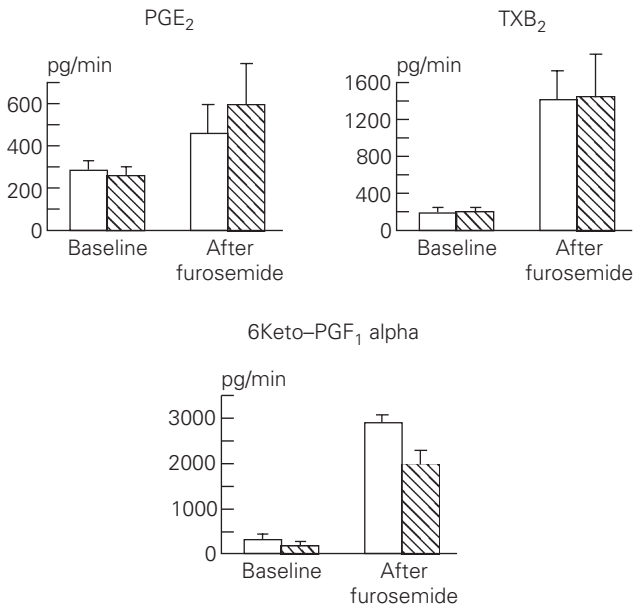


**Figure 30.2** Urinary excretion of prostaglandin $E_2$ ($PGE_2$), 6-keto-prostaglandin $F_1\alpha$ (6Keto-$PGF_1$ alpha), and thromboxane $B_2$ ($TXB_2$) in nine patients with cirrhosis and ascites before and after 80 mg of furosemide. Placebo, open bars; imidazole-salicylate, hatched bars. (From Salerno F, Lorenzano E, Maggi A *et al*. Effects of imidazole-salicylate on renal function and the diuretic action of furosemide in cirrhotic patients with ascites. J Hepatol 1993; 19:279–84.)

2, but spare the constitutive form of the enzyme (COX-1). Since COX-2 is mainly activated during inflammatory processes by cytokines, mitogens and endotoxin, it was suggested that the selective inhibition of this enzyme may achieve its therapeutic goal without affecting the physiological processes governed by the constitutively expressed COX-1 enzyme, such as prostaglandin production by the kidney. In a controlled study carried out in rats with experimentally induced cirrhosis, Bosch-Marcé *et al.* (44) showed that a compound structurally related to celecoxib, the most prescribed COX-2 inhibitor, spared the renal function of the animals whereas the nonselective drug ketorolac produced a profound and reversible impairment of both renal hemodynamics and excretory function. Much late evidence, however, suggests that COX-2 also modulates renal hemodynamics and function (45–47). Fluid retention and renal dysfunction have been recently described in a patient with cirrhosis (48) as well as in patients with heart failure and hypertension receiving COX-2 inhibitors (49). In addition, great concern has been recently raised because the use of some of these compounds was associated with an increased risk of coronary injury. Therefore, great caution should be exercised prescribing COX-2 inhibitors to cirrhotic patients, particularly those with ascites, until the effects of such drugs on renal function are better investigated in the future.

Finally, since endothelial nitric oxide (NO) has been shown to protect the gastric mucosa, NO-releasing derivatives of standard NSAIDs have been synthesized and tested in experimental animals (50). Such drugs might be tolerated by the kidney better than their parent compound that does not release NO, as NO has been shown to exert an important paracrine function in the kidney controlling renal blood flow, renal autoregulation, GFR, renin secretion and salt excretion (51). Their use in patients or experimental animals with liver damage has not yet been investigated.

## Aminoglycoside antibiotics

Despite their potential nephrotoxicity, aminoglycoside antibiotics still retain a prominent role in the therapy of serious Gram-negative infections. This family of drugs (streptomycin, gentamicin, amikacin, kanamycin, tobramycin, and netilmycin) has a narrow toxic–therapeutic ratio, and it has been estimated that renal dysfunction occurs in 10–20% of all courses of therapy (52). Several factors increase susceptibility to aminoglycoside nephrotoxicity, such as older age, prolonged therapy, frequent doses, concurrent use of other nephrotoxic drugs, potassium depletion, and decreased intravascular volume, particularly secondary to overzealous use of loop diuretics. In patients with renal disease, drug accumulation may lead to ototoxicity and additional renal damage with tubular necrosis; with very high blood drug levels, neuromuscular blockade may occur. Nephrotoxicity is due, at least in part, to the fact that these drugs are filtered freely by the glomerulus and are concentrated in the proximal tubular cells. In the rat, high tissue levels of gentamicin persist in the renal cortex for up to 28 days (53). This explains why acute tubular necrosis may occur after drug administration has been discontinued. Acute tubular necrosis is related to the number of cationic amino groups of the molecule. Streptomycin, with three cationic amino groups, is less nephrotoxic than neomycin, with six, whereas gentamicin, tobramycin, netilmycin, amikacin, and kanamycin exert intermediate nephrotoxicity. The cationic amino groups favor both the interaction between cell membrane and the cell surface receptor, recently identified as glycoprotein 300 or megalin, and that between the drug and the consequent accumulation in lysosomes (54). Within lysosomes, aminoglycosides inhibit phospholipases, causing deposition of phospholipids and formation of myeloid lamellar bodies. Finally, the release of lysosome enzymes into the cytoplasm causes tubular cell necrosis (55). The first laboratory feature of this type of acute tubular necrosis is the increased urine excretion of low-molecular-weight proteins such as $\beta_2$-microglobulin because of reduced reabsorption in the proximal tubule level (56). Measuring urinary $\beta_2$-microglobulin concentrations, Cabrera *et al.* (57) found a high incidence of aminoglycoside

nephrotoxicity in cirrhotic patients (31%), particularly in those with pre-existent renal impairment (56% of patients with pretreatment creatinine concentration > 1.2 mg/dl developed renal damage). This suggests that urinary excretion of $\beta_2$-microglobulin should be monitored when aminoglycosides are given to patients with liver disease (Fig. 30.3). However, patients with advanced liver disease, jaundice, and functional renal failure may spontaneously develop asymptomatic tubular damage, thus reducing the specificity of urinary $\beta_2$-microglobulin as a marker of aminoglycoside toxicity (58).

Besides the acute tubular damage, aminoglycosides may also impair renal function by decreasing prostaglandin synthesis. In a retrospective analysis of a double-blind controlled trial of tobramycin vs. cefotaxime, Moore *et al.* (59) found that cirrhotic patients had a five times higher risk of developing renal failure with tobramycin than the general population with infection. To explain this susceptibility, the investigators proposed that aminoglycosides inhibit renal phosphatidylinositol-specific phospholipase C, an enzyme important for the release of arachidonic acid and prostaglandin synthesis (11,60). Many animal studies have provided some indications for preventing aminoglycoside toxicity in humans. Among them, promising data have been reported for free radical scavengers, calcium loading or polyaminoacid administration (61).

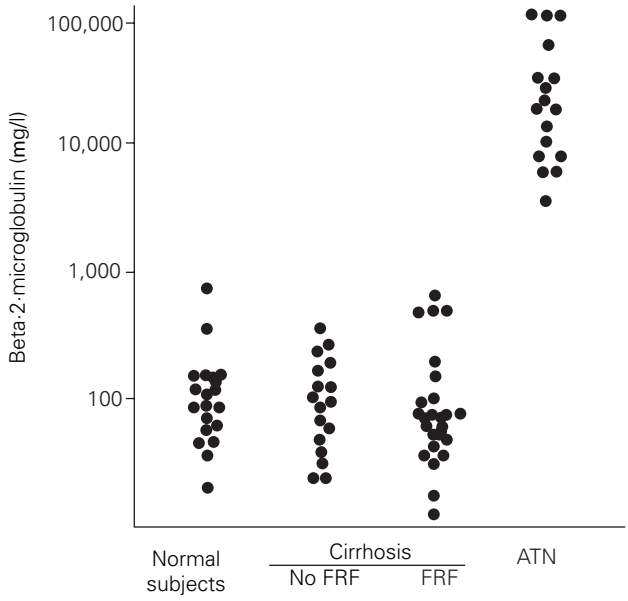


**Figure 30.3** Urinary concentration of $\beta_2$-microglobulin (μg/l) in normal subjects, cirrhotic patients with and without functional renal failure (FRF), and in noncirrhotic patients with acute tubular damage (ATN). (From Cabrera J, Arroyo V, Ballesta AM *et al*. Aminoglycoside nephrotoxicity in cirrhosis. Gastroenterology 1982; 82:97–105.)

In conclusion, considering the frequency of tubular damage, the difficulties of differential diagnosis, and the availability of new antibiotics effective against Gram-negative bacteria, it is advisable to avoid aminoglycosides in cirrhotic patients whenever possible.

## Antihypertensive drugs

### *Angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists*

Angiotensin-converting enzyme (ACE) inhibitors, such as captopril, are potent inhibitors of the generation of AII. It was suggested that these drugs may be useful in treating cirrhotic patients with avid sodium retention and incipient renal failure, because AII causes constriction of cortical renal blood vessels, increases portal pressure, and stimulates aldosterone release. However, two human studies, at least, showed that captopril given to non-azotemic cirrhotic patients at doses from 25 to 150 mg decreased blood pressure and impaired renal function and the natriuretic response to furosemide (62,63). The adverse renal effects of captopril in cirrhotics were attributed to its ability to dilate the efferent arterioles of the glomerulus through inhibition of AII, but other mechanisms were not excluded because captopril acts on other vasoactive systems as well (64,65). A further study, employing a low dose (12.5 mg) of captopril, confirmed that inhibition of AII is deleterious for the kidney function of cirrhotic patients even when basal renin activity is normal and when blood pressure is not decreased by the drug (66). This result confirms that AII exerts a protective role on the renal mechanism of autoregulation even in cirrhotic patients with normal plasma renin activity. Therefore, ACE inhibitors are harmful in cirrhotic patients with ascites and should be given with great caution to patients even with compensated cirrhosis.

According to the demonstration that AII induces contraction and proliferation of hepatic stellate cells (67,68), it was speculated that the administration of specific AII receptor antagonists to patients with chronic liver disease could produce two important clinical beneficial effects: reduction of portal pressure, and inhibition of hepatic fibrosis. Losartan and irbesartan have been investigated in cirrhotic patients with the aim of preventing esophageal varices rupture (69–73). Unlike ACE inhibitors, these drugs selectively inhibit AII activity, without affecting other vasoactive systems such as that of kinins (74). Their ability to reduce portal pressure seems to be excellent also at doses lower than those employed in patients with arterial hypertension (69). However, their efficacy in preventing variceal bleeding is still debated (70), the dosage of the drug being essential to obtain a significant effect (75). With regard to their tolerability, it is worth mentioning that in one study 22% of cirrhotic patients

receiving irbesartan developed severe hypotension and renal function impairment (71). Such patients tended to be Child class C patients with more severe liver disease, lower serum sodium levels, and highly elevated plasma renin activity (PRA). Thus, if the prophylactic utility of AII receptor antagonists in liver cirrhosis is confirmed, their chronic use in cirrhotic patients will require careful monitoring of renal function and sodium handling.

### *Calcium-channel blockers*

Unlike ACE inhibitors, calcium-channel blockers, another group of widely employed antihypertensive drugs, do not seem to be dangerous for the kidney of cirrhotic patients. Wong *et al.* (76) gave 10 mg of nifedipine to 11 cirrhotic patients without ascites. Despite a fall in renal perfusion pressure, renal plasma flow increased and GFR was preserved. The investigators suggested that nifedipine lowered renal vascular resistance by preferentially affecting the tone of the afferent arterioles. However, the drug's effects in patients with decompensated cirrhosis or with hepatorenal syndrome have not been studied. In such patients the beneficial effects on renal circulation might be counteracted by a significant fall in arterial pressure and systemic vascular resistance.

Some calcium-channel blockers, such as verapamil, were considered for treating portal hypertension because of their ability to decrease hepatic resistance in experimental cirrhosis (77), but two consecutive studies in cirrhotic patients failed to confirm any beneficial effect of verapamil on portal circulation and liver function (78,79).

### *Nitrates*

Nitrates may be prescribed to patients with cirrhosis. In particular, patients who do not respond to β-blockers with reduction of portal pressure are given isosorbide-mononitrate in association with β-blockers. This is because nitrates lower hepatic vascular resistance and portal pressure (80–82) and may reduce the risk of bleeding from esophageal varices, as confirmed by some trials (83,84).

Because nitrates are powerful venous and mild arterial vasodilators, they cause hypotension and decreased cardiac output. These effects can be harmful for the renal hemodynamics of cirrhotic patients who suffer from effective arterial hypovolemia. Indeed, Salmerón *et al.* (85) showed that the short-term administration of isosorbide-5-mononitrate to cirrhotic patients caused hypotension, increased PRA and plasma aldosterone concentration, and reduced GFR, urinary sodium excretion, and solute-free water excretion. These effects were more marked in patients with ascites and were confirmed by Salerno *et al.* (86), who also explored the effect of long-term administration of isosorbide-5-mononitrate by measuring renal function and the activity of vasoactive systems after 3 months of therapy with 40 mg twice a day. The negative short-term renal effects of the drug waned surprisingly at 3 months, suggesting that long-term treatment can be tolerated by most cirrhotic patients. The different short- and long-term effects of isosorbide-5-mononitrate can be explained by the following two mechanisms: (i) pharmacological tolerance, described during long-term administration of nitrates (87), may attenuate also the adverse effects; (ii) the untoward effects on renal hemodynamics and GFR may be of short duration and rapidly reversible, hence insignificant for daily renal function. The safety of long-term nitrate use was confirmed by evidence that using a combination of isosorbide-5-mononitrate and propranolol or nadolol to prevent esophageal bleeding did not affect renal function (88,89).

## Somatostatin and octreotide

Both somatostatin and octreotide, its analog with a longer half-life, are employed in cirrhotic patients to stop active bleeding from gastroesophageal varices (90–92). Their ability to reduce splanchnic blood flow and portal pressure is thought to be mediated, at least in part, by inhibition of glucagon release (93,94). However, the hemodynamic effects of somatostatin and octreotide are not limited to the splanchnic area. Indeed, they can affect renal circulation, which is probably also due to inhibition of glucagon release. Several studies in healthy subjects showed that somatostatin and octreotide impair GFR, RBF (95,96), and the ability to excrete solute-free water (97). Cirrhotic patients who are actively bleeding from varices can be treated with an infusion of somatostatin at the rate of 250–500 μg/h for 3–5 days. It is difficult to assess the renal effects of a drug in bleeding patients because systemic hemodynamics may be unstable and many variables can affect renal perfusion and function, including blood loss, blood or plasma transfusion, and other concomitant drug therapies. To avoid such interference, Ginès *et al.* (98) studied the renal effects of a 2-h infusion of somatostatin in 23 non-azotemic cirrhotic patients who were not bleeding. Somatostatin significantly reduced RBF, GFR, solute-free water clearance, and, in patients with ascites, sodium excretion. Because the drug did not affect systemic hemodynamics, PRA, sympathetic nervous activity, vasopressin, and atrial natriuretic peptide, the investigators attributed the renal effects to a direct vasoconstrictor effect of the drug on the renal circulation. The notion that this vasoconstriction was mediated by the inhibition of glucagon release was weakened by the lack of correlation between changes in renal function and plasma glucagon levels. In clinical practice, the adverse effects of somatostatin on renal function do not preclude its use in bleeding patients, taking into account that the drug is given for only a few days, the renal damage appears to

be a reversible functional impairment without permanent consequences, and thus the drug's efficacy in controlling bleeding overrides its side-effects.

Some somatostatin analogs with long-term activity, such as octreotide, have superior pharmacokinetic properties that make them useful for long-term prophylaxis of portal hypertension (99). In contrast to somatostatin, octreotide improved systemic hemodynamics and improved sodium retention in portal hypertensive rats (100). In 11 cirrhotic patients receiving 100 μg of octreotide subcutaneously before every meal, renal perfusion and function were unaffected after 2 weeks of therapy, although the postprandial glucagon release was still blunted (101). This result suggests that long-term octreotide administration is safe for patients with liver disease.

## Other drugs employed infrequently

### *Dipyridamole*

Adenosine is an endogenous nucleoside derived mainly from the intracellular catabolism of adenosine triphosphate, with vasodilator properties in most vascular territories, particularly the coronary circulation (102,103), but with vasoconstrictor activity in the kidney. Adenosine enhances the responsiveness of afferent arterioles to AII and may therefore lower GFR, especially when the release of AII is increased (104). Dipyridamole is a drug that blocks the cellular uptake of adenosine, resulting in increased extracellular levels (105,106). Its only current recommended therapeutic use is for primary prophylaxis of thromboembolism in patients with prosthetic heart valves (107).

Llach *et al.* (108) investigated the renal effects of dipyridamole (0.4 mg/kg i.v.) in 20 patients with cirrhosis. The drug did not affect the renal function in patients with compensated cirrhosis or in those with ascites and normal PRA, but markedly reduced RBF, GFR, urine flow, solute-free water clearance, and sodium excretion in patients with ascites and overactivity of the renin–angiotensin system. This suggests that dipyridamole should not be given to patients with severe liver disease. Indirect confirmation was provided by Milani *et al.* (109), who reported that theophylline, a competitive receptor antagonist of adenosine, increased RBF and GFR in cirrhotic patients with impaired renal perfusion.

### *Demeclocycline*

In humans and laboratory animals, demeclocycline interferes with the action of antidiuretic hormone (ADH) on the collecting duct and may cause nephrogenic diabetes insipidus (110). This tetracycline has therefore been employed for the treatment of symptomatic hyponatremia due to inappropriate secretion of ADH in patients not responsive to simple water restriction (111). Since patients with advanced liver disease may develop progressive dilutional hyponatremia related to ADH hypersecretion, they have been treated with demeclocycline (112–115); however, although effective in raising serum sodium levels, the drug markedly decreased GFR in cirrhotic patients.

The most accurate of these studies (115) showed that the decrease of GFR was related to a decrease in RBF and was reversible after discontinuation of the drug. This, along with a normal result of urinalysis and normal excretion of $\beta_2$-microglobulin, suggested that the renal damage was of functional origin.

Because the urinary excretion of kallikrein decreases during demeclocycline treatment, the investigators proposed that the antibiotic might cause intrarenal vasoconstriction through an inhibitory effect on vasodilators kinins. Why this nephrotoxic effect is frequent in patients with chronic liver disease, but not in other conditions associated with hyponatremia, was attributed to the high plasma levels of the drug found in cirrhotic patients because of impaired biliary excretion. On account of the high incidence of untoward renal effects, demeclocycline is now considered unsafe for patients with liver cirrhosis.

### *Amphotericin B*

Amphotericin B is the most active agent against life-threatening systemic fungal infections. A full course of therapy is associated with a derangement of renal function in most patients (116). Amphotericin B complexes with cholesterol in cell membrane of distal tubule causing a variety of tubular alterations, as resistance to vasopressin and tubular acidosis; moreover, it causes renal vasoconstriction and renal insufficiency, which is generally dose-related. There is increasing evidence that in cirrhotic patients with ascites, not only spontaneous bacterial peritonitis, but also spontaneous mycotic peritonitis may occur (117), requiring antifungal therapy, although the frequency appears to be extremely low. Different formulations of the drug with phospholipids or liposomes as vehicle are reported to attenuate nephrotoxicity in humans (118), but no data are yet available in cirrhosis.

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# Chapter 31
# Clinical Disorders of Renal Function in Acute Liver Failure

John G. O'Grady

Acute liver failure (ALF) is a core term that identifies a heterogeneous group of patients developing encephalopathy less than 12 weeks after the onset of jaundice in the absence of underlying pre-existing liver disease (1). These patients have important differences in clinical features and prognosis depending on the etiology, the time from the onset of jaundice to the development of encephalopathy, and the functional hepatic reserve.

The development of renal failure in such patients is commonplace: early studies in patients with severe encephalopathy (grade III–IV coma) reported an incidence of between 38 and 53%, depending on etiology (2). Despite improvements in the management of patients with ALF, the incidence of renal failure remains essentially unchanged. Renal dysfunction is a useful predictor of outcome in patients with paracetamol-induced liver failure both with and without transplantation and it is one of the criteria contributing to the selection of patients for liver transplantation in this variant of ALF (3).

There are fundamental differences between paracetamol-induced disease and other etiologies of ALF. In paracetamol toxicity, renal failure is a prominent early feature and is probably a reflection of direct renal toxicity rather than a sequel to liver failure. In viral-induced liver failure, renal dysfunction is a late complication that appears to progress from functional renal failure to acute tubular necrosis. The most useful parameters available to assess renal dysfunction are urinary volume, urinary sodium concentration, and serum creatinine concentration. The measurement of serum urea concentration is of limited value as the failing liver struggles to synthesize urea in amounts that reflect substrate available.

Research into the pathogenesis of the renal injury in ALF has been limited by the comparative rarity of the condition, and much of the available data have come from studies carried out principally in patients with cirrhosis and hepatorenal syndrome. Functional renal failure or the hepatorenal syndrome share many features with the renal impairment seen in ALF. The key pathophysiological features associated with the development of renal failure are a decrease in renal blood flow as a consequence of systemic hypotension, and vasoconstriction within the renal circulation. The causes of vasoconstriction are multifactorial, including activation of the sympathetic nervous system and the renin–angiotensin–aldosterone system (RAAS) and the development of endotoxemia with production of eicosanoids and free radicals.

## Definitions of acute liver failure

The terminology used to describe patients with acute liver dysfunction sufficient to cause encephalopathy has changed over the past 25 years. Key changes concern the use of time between onset of symptoms and development of encephalopathy and, more recently, the period between the appearance of jaundice and encephalopathy to identify patients with differing prognoses and risks for the major complications, including cerebral edema, renal failure, and multi-organ failure. To avoid confusion in the discussion of different authors' work, reference will be made to the definition used in individual studies. Those definitions in most common use are outlined below and compared in Fig. 31.1.

**1** *Fulminant hepatic failure (FHF)—Trey and Davidson*. A potentially reversible condition, the consequence of severe liver injury, with the onset of hepatic encephalopathy within 8 weeks of the first symptoms and in the absence of pre-existing liver disease (4).

**2** *Late-onset hepatic failure (LOHF).* The development of hepatic encephalopathy between 8 and 24 weeks from the onset of symptoms in the absence of underlying liver disease (5).

**3** *Fulminant liver failure.* The development of encephalopathy within 2 weeks of the onset of jaundice in the absence of underlying liver disease (6).

**4** *Subfulminant liver failure (SFLF).* The development of encephalopathy between 2 and 12 weeks after the onset of jaundice in the absence of underlying liver disease (6).

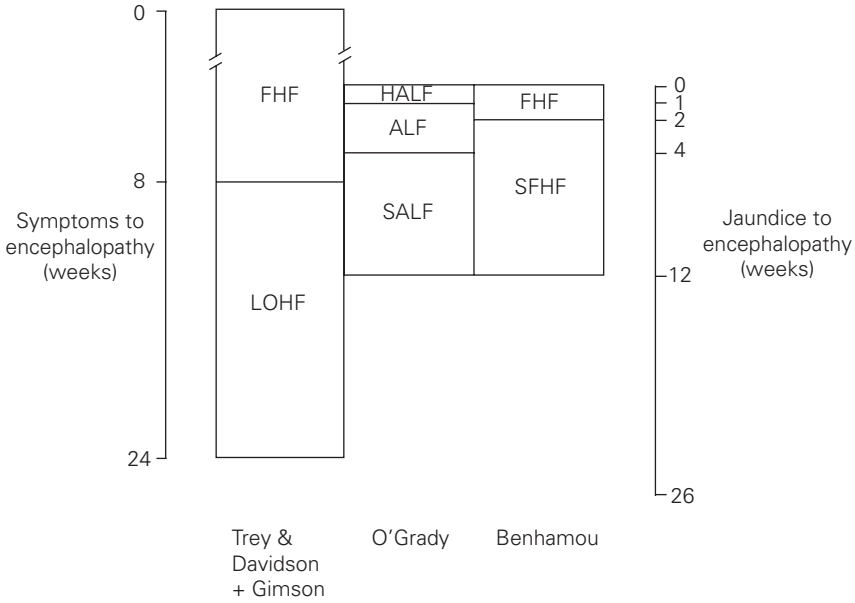


**Figure 31.1** A comparison of the terminology in common use to describe patients with acute liver dysfunction characterized by the appearance of encephalopathy. FHF, Fulminant hepatic failure; LOHF, late-onset hepatic failure; HALF, hyperacute liver failure; ALF, acute liver failure; SALF, subacute liver failure; SFHF, subfulminant hepatic failure.

**5** *Acute liver failure (ALF).* This is a core term applied to patients with acute liver dysfunction who develop encephalopathy. A prefix is applied to the term according to the time period between the onset of jaundice and encephalopathy. Hyperacute liver failure is used to describe those who develop encephalopathy within 8 days, acute liver failure describes those who develop encephalopathy within 9–28 days, and subacute liver failure describes those with a development period of 4–12 weeks (1).

## Definitions of renal failure

Early studies of the hepatorenal syndrome in cirrhosis characterized two types of renal failure, namely functional renal failure and acute tubular necrosis (7).

**1** *Functional renal failure.* Functional renal failure is an otherwise unexplained impairment of renal function that is potentially reversible and is characterized by a low urinary sodium concentration (10 mEq/l), a urine to plasma creatinine ratio > 20, a normal urinary sediment, and normal histological appearance on renal biopsy (8). This term may be applied only when other causes of renal impairment, such as hypovolemia, sepsis, and drug nephrotoxicity, have been excluded.

**2** *Acute tubular necrosis*. Acute tubular necrosis (ATN) is characterized by acute, usually reversible, renal failure in which the urinary sodium concentration is > 20 mEq/l, the urine to plasma creatinine ratio is < 20, and urinalysis reveals mild proteinuria, with granular or tubular cell casts. Abnormalities on renal biopsy include tubular cell necrosis and tubular obstruction by cell debris and casts.

Whether these two forms of acute renal failure represent distinct entities or opposite ends of a clinical spectrum is unclear, as intermediate forms occur commonly in patients with ALF (9).

### Definition of hepatorenal syndrome

In those patients with ALF and functional renal failure it has been suggested that the pathogenesis is similar to those patients with chronic liver disease with hepatorenal syndrome (HRS). A new definition for this syndrome has been proposed, which separates patients into two groups: type 1 HRS characterized by a rapidly progressive reduction of renal function as defined by a doubling of the initial serum creatinine to a level of > 2.5 mg/dl or a 50% reduction of the initial 24-h creatinine clearance to < 20ml/min in less than 2 weeks; and type 2 HRS being that in which the renal failure does not have such a rapidly progressive course (10).

## Incidence

It has long been recognized that there is an association between ALF and renal dysfunction. Three early series described renal dysfunction in between 42 and 80% of patients (11–13). The important relationship between renal impairment and etiology was not immediately recognized. In one large series of 160 patients with fulminant hepatic failure (FHF) and grade IV encephalopathy published in 1977, renal failure occurred in 53% of cases secondary to paracetamol overdose compared with 38% in those with viral hepatitis and other causes. This difference did not reach statistical significance, and the authors concluded that renal failure was not higher in paracetamol cases and that paracetamol was not directly nephrotoxic (2). This conclusion could not be easily reconciled

with the observations that renal failure may occur in the absence of significant liver damage after paracetamol ingestion and that renal dysfunction often precedes the other clinical manifestations of liver failure (14–16). The first clear distinction between renal failure complicating paracetamol hepatotoxicity and other causes of acute liver failure was seen in the controlled trials of charcoal hemoperfusion in patients with grade III or grade IV encephalopathy (17). Of 137 patients studied between 1982 and 1985, 75% of those patients with paracetamol-induced FHF developed renal failure compared with 30% in the nonparacetamol group, as defined by a urine output of < 300 ml and a serum creatinine > 300 μmol/l (3.4 mg/dl). This study also demonstrated the potential for recovery in the paracetamol patients with renal failure. After the cohort with a severe metabolic acidosis, which was a very strong independent determinant of poor outcome, was excluded, the study reported a 50% survival rate in patients developing cerebral edema and renal failure.

A subsequent review of 560 patients admitted to the Liver Unit at Kings' College Hospital with paracetamol-related hepatotoxicity between 1987 and 1993 found the incidence of renal failure requiring dialysis had decreased from 60 to 46% over that time period (18). This change may reflect an alteration in case mix rather than a true reduction in the incidence of renal dysfunction. Over a similar time period, a study of patients with nonparacetamol-related liver failure reported the incidence of renal failure as 36% in FHF and 41% in LOHF (19).

## Sodium retention and decreased solute-free water clearance

Abnormalities in plasma sodium and effective arterial circulating volume occur commonly in patients with severe hepatic necrosis and impending ALF. In patients with grade IV encephalopathy and a glomerular filtration rate (GFR) of > 40 ml/min, marked renal retention of sodium occurs with decreased solute-free water clearance in 50% of cases (20). In addition to the non-osmotic release of vasopressin, this may represent increased sodium reabsorption in the proximal part of the nephron so that insufficient sodium is delivered to the ascending limb of the loop for generation of solute-free water. Aldosterone, which acts in the collecting ducts, would not contribute to this pattern of sodium reabsorption, although plasma levels are increased (21). Angiotensin II (AII) enhances proximal sodium reabsorption, and a close inverse relationship is seen between AII and sodium excretion. Hyponatremia not only reflects a decrease in solute-free water excretion but also the frequent administration of large volumes of hypotonic crystalloid to correct hypovolemia. Another proposed cause of hyponatremia is impairment in sodium pump function within the cell membrane that leads to a high intracellular sodium concentration (22). A circulating toxin has been identified that suppresses membrane sodium-potassium adenosine triphosphatase (Na/K ATPase) activity, which may explain this finding (23).

## Pathophysiology of renal impairment in acute liver failure

The pathophysiological abnormalities that result in sodium retention and decreased solute-free water excretion and in some cases progression to ATN are best considered by compartmentalizing the disease process into three specific phases before considering each abnormality individually. There are many interactions among the proposed mechanisms below; these are summarized in Fig. 31.2.

**1** *Onset of liver disease.* Prior to the development of HRS, it has been suggested that there are early disorders of sodium and solute-free water excretion (20). Studies in paracetamol-related liver injury suggest a role for a decrease in the synthesis of renal prostaglandins (24).

**2** *Functional renal failure*. As liver failure develops, a phase of avid conservation of sodium with a urine to plasma creatinine ratio of > 20 is observed. Hypovolemia arises due to systemic vasodilation, which leads to a decrease in renal blood flow as renal perfusion pressure falls. Renal vasoconstriction is seen as a consequence of overactivation of the RAAS (21,25,26) and stimulation of the sympathetic nervous system (SNS) (27,28). In addition to these changes, endotoxin appears in the systemic circulation, and there is a pathological increase in circulating and intrinsic vasoconstrictors, including cysteinyl leukotrienes and endothelins (29,30).

**3** *Acute tubular necrosis*. As renal blood flow and renal perfusion pressure become critically reduced, the clinical picture of ATN evolves, with an increase in urinary sodium concentration and decrease in urine to plasma creatinine ratio to < 20. The most important feature associated with the development of ATN is the degree of endotoxemia.

### Renal blood flow and the systemic circulation

As ALF develops there is a fall in systemic vascular resistance (SVR), which initially is accompanied by an increase in cardiac output so that the mean arterial pressure (MAP) is preserved. When the cardiac output can no longer increase to compensate for the fall in SVR, the MAP falls (31). The renal perfusion pressure (RPP) is dependent on the MAP and to a lesser extent on the renal venous pressure (RVP) (RPP = MAP – RVP). The RVP is normally < 5 mmHg. Whether there is any change in RVP in ALF is unknown, as no studies to date have addressed this issue. Normally, alterations in renal blood flow arise from changes in smooth muscle tone within the

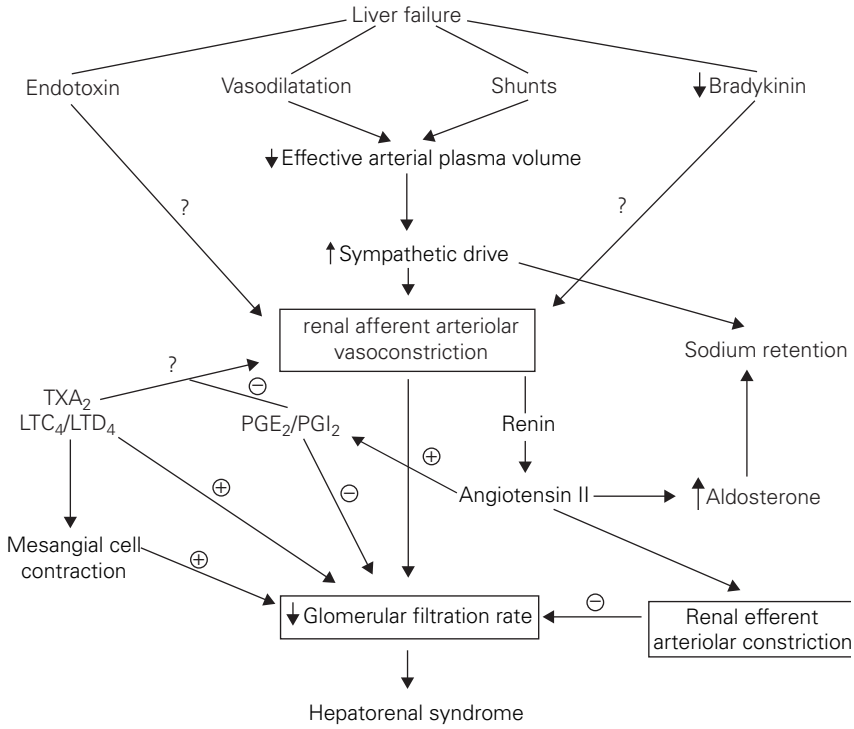


**Figure 31.2** Diagram showing the proposed mechanisms for the development of functional renal failure in acute liver failure. $TXA_2$, thromboxane $A_2$; $LTC_4$, leukotriene $C_4$; $LTD_4$, leukotriene $D_4$; $PGE_2$, prostaglandin $E_2$; $PGI_2$, prostaglandin $I_2$.

afferent arteriole. Autoregulation of renal blood flow occurs until the RPP falls below 60–70 mmHg. Patients with ALF typically have a MAP of between 60 and 80 mmHg early in the illness, and thus any fall in MAP will result in a pressure-dependent decline in renal blood flow (Fig. 31.3). Hemodynamic studies in patients with ALF invariably demonstrate such reductions in renal blood flow (13,20,32).

## Peripheral vasodilation

Nitric oxide (NO) is a potent vasodilator synthesized from L-arginine by a family of complex enzymes known as nitric oxide synthases (NOS), which include at least three isoforms (33). NO synthesis occurs within many tissues, but in ALF the important sources are thought to be endothelial cells, hepatocytes, and macrophages—specifically, Kupffer cells. Activation of the NOS family is mediated by many exogenous factors, including bacterial toxins, tumor necrosis factor (TNF), and interleukin (IL)-1, all of which are increased in ALF (34–37). Indirect evidence shows NO to be present in increased amounts in ALF via the demonstration of elevated circulating levels of nitrite and nitrate, the end products of its metabolism (38), and increased citrulline levels, a by-product of its synthesis

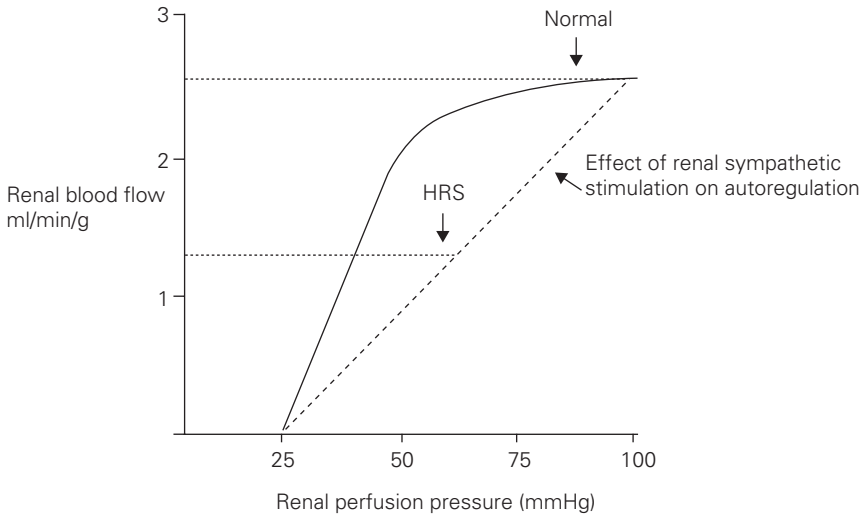


**Figure 31.3** Renal blood flow autoregulation and the effect of activation of the sympathetic nervous system in acute liver failure. HRS, hepatorenal syndrome.

(39). The half-life of NO is less than 1 s, but NO can react with thiol groups and hemoglobin to form nitrosothiols and nitrosohemoglobin, respectively, products that are more stable and retain their vasoactive effect (40,41).

The infusion of L-NMMA, an agent that blocks NO synthesis, has been undertaken in a small number of patients with ALF. Improvements in MAP and SVR were seen, but larger doses were required than previously reported in sepsis. Infusion of L-NMMA resulted not only in a fall in cardiac output but also in oxygen consumption. Studies in isolated perfused kidneys have shown that infusion of L-NMMA decreases renal blood flow and medullary oxygenation (42). In ALF it may be that increased NO synthesis, although causing systemic vasodilation, may still be of benefit to microcirculatory flow, especially within the renal circulation.

## Splanchnic pooling

In patients with cirrhosis, there is pooling of blood within the splanchnic circulation due to vasodilation. Such vasodilation is partly related to portal hypertension, the development of portosystemic shunts, minor arteriovenous fistulae, and portovenous fistulae. A single study in ALF patients reported an increased portal pressure gradient in 24 out of 25 patients The mean hepatic venous pressure gradient (HVPG) was 12.8 mmHg (normal value 5 mmHg). Measurements of HVPG were higher in those patients with ascites (15.1 vs. 9.3 mmHg) and in those with renal failure (14.4 vs. 10.1 mmHg). Transjugular liver biopsies taken at the same time as the pressure measurements revealed a correlation between portal pressure and the degree of liver parenchymal collapse seen on reticulin staining and measured by a morphometric analysis. In all patients there was an associated decrease in SVR, increased cardiac output, and a decreased MAP (43).

## Sympathetic nervous system

The kidney is richly innervated by the SNS. Stimulation of the SNS leads to afferent arteriolar constriction, a reduction in renal blood flow, and increased sodium reabsorption by a direct effect on the proximal tubule epithelium (44). If renal hypoperfusion occurs, activation of the RAAS will lead to an additional increase in proximal and distal tubular sodium reabsorption. The precise role for SNS stimulation in the pathogenesis of HRS is unclear, as studies of patients with cirrhosis have shown that SNS blockade does not reverse the renal vasoconstriction (45), and surgical denervation in an animal model does not invariably result in a significant increase in renal perfusion pressure and natriuresis (46).

Acute liver failure is associated with activation of the SNS with elevated levels of catecholamines in the peripheral blood (47,48). The mechanism by which SNS activation occurs is probably due to a decrease in the effective arterial blood volume seen as the SVR falls. There is additional experimental evidence for a hepatorenal reflex through the stimulation of hepatic baroreceptors by intrasinusoidal portal hypertension (49). As portal hypertension has been identified in some patients with ALF, this may be of relevance.

## Renin–angiotensin–aldosterone system and atrial natriuretic factor

The RAAS is of major importance in the control of systemic blood pressure and extracellular fluid volume. Increased renin production as a result of renal hypoperfusion results in increased production of AII. AII causes predominantly efferent arteriolar vasoconstriction, which, although decreasing renal blood flow, preserves glomerular filtration. AII also stimulates the production of aldosterone, which promotes renal retention of sodium.

Whether the RAAS is of pathological significance in the evolution of HRS or is merely a compensatory mechanism secondary to a decreased effective arterial plasma volume and decreased renal blood flow is unclear. Evidence for the latter comes from studies in patients with chronic liver disease that show that the infusion of saralasin, an AII receptor blocker, or an angiotensin-converting enzyme inhibitor failed to improve renal function (50). Nevertheless, since both types of drugs caused a reduction in arterial pressure and renal perfusion pressure, the role of the RAAS in the pathogenesis of HRS could not be established.

Plasma renin activity (PRA) increases progressively after ingestion of paracetamol and tends to be higher in those with severe liver damage (25). However, the proportionate rise in PRA did not differentiate between those patients who did and did not develop renal failure. Although high levels of plasma renin, AII, and aldosterone have been observed, renin substrate concentration are markedly decreased (in some cases to less than one-fortieth of normal), with increased conversion of AII to inactive peptides. This indicates that the full expression of the stimulated system had not necessarily occurred (21). The former observation was not unexpected given that renin substrate is synthesized within the liver (51,52). An inverse relationship has been seen between systolic blood pressure and PRA, suggesting a homeostatic response to hypotension, in the absence of a correlation between plasma aldosterone concentrations and renal sodium excretion. This suggests that a deficiency of a putative circulating natriuretic factor, or resistance to such a factor, may be contributing to the sodium retention (21).

Human atrial natriuretic factor (hANF) is such a substance and falling circulating levels could contribute to

sodium retention. It is secreted by the cardiac atria in response to changes in pressure as measured by stretch receptors in the atria wall. No deficiency of hANF was seen after paracetamol ingestion, but, to the contrary, levels rose with increasing severity of renal impairment (26). This finding might be due to the fact that hANF is excreted by the kidney.

## Endotoxin

Endotoxin, a constituent of the cell wall of Gram-negative bacteria, is absorbed into the portal circulation from the gastrointestinal tract and cleared by the reticuloendothelial cells of the liver. If small bowel permeability increases or liver failure occurs, endotoxin appears in the systemic circulation. The renal and hemodynamic abnormalities that follow are similar to those seen in Gram-negative sepsis and ALF. In a pilot study of 22 patients with FHF, 14 (63%) were found to have circulating endotoxin by the Limulus assay (53). There was a strong correlation between the presence of endotoxin and the development of renal failure with highly significant falls in GFR and RPF in those patients with detectable endotoxin. In nine cases of functional renal failure, there was progression to ATN in six. A further four patients were initially found to have ATN, and in the final case the type of renal failure was indeterminate. All 14 patients with endotoxemia died, whereas six of eight patients who were persistently endotoxin negative survived. In a follow-up study, these findings were confirmed, with 13 (93%) of 14 patients with FHF testing positive for endotoxin compared with one (14%) of seven controls (2). Renal vasoconstriction that occurs in the presence of endotoxemia may be caused by a primary effect of endotoxin on the kidney, or more likely as a result of macrophages and endothelium activation with release of potent vasoconstrictors, including TNF-α, cysteinyl leukotrienes, endothelins, and thromboxane $A_2$.

## Endothelin

Endothelins are a family of peptides with 21 amino acids that are initially synthesized as propeptides and then cleaved to give big endothelin and, subsequently, endothelins. Endothelin 1 is found principally in vascular endothelium and causes long-acting vasoconstriction. Previous studies have shown the renal circulation to be particularly sensitive to this peptide (54,55). In healthy individuals, endothelins increase when an upright posture is assumed, and one stimulus to their increased production may therefore be hypovolemia. Other stimuli to endothelin synthesis may include bacterial endotoxemia, hypoxia, and oxidant stress (56,57). No study to date has examined the role of endothelins specifically in ALF, although in one study of HRS that included five patients with ALF endothelin levels were found to be significantly elevated (30).

## Lipid peroxidation

Disturbances in microcirculatory flow have been postulated in ALF as a consequence of endothelial cell activation and the formation of white cell and platelet plugs that culminate in tissue ischemia (58). In addition, patients have depleted antioxidant levels (59). The combination of these features will promote local oxidant stress and free radical formation, resulting in cell damage by lipid peroxidation and DNA damage. Oxidant stress increases both phospholipase $A_2$ activity and the availability of lipid hydroperoxides, which may explain the increased production of prostaglandins, thromboxane, and leukotrienes. Reliable evidence in human subjects of free radical-mediated cell damage is now available by measuring $F_2$ isoprostanes, a recently discovered product of lipid peroxidation. $F_2$ isoprostanes are a group of prostaglandin $F_2$-like compounds produced by cyclooxygenase-independent, free radical-mediated catalyzed peroxidation of arachidonic acid. $F_2$ isoprostane levels are markedly increased measured in patients with paracetamol-induced hepatotoxicity and coexisting renal failure but decrease after infusion of superoxide dismutase, suggesting a pathway of stimulation involving superoxide, a toxic free radical. NO, which, as already discussed, is found in supranormal levels in ALF, may react with superoxide to form peroxynitrite, a potent free radical that will stimulate lipid peroxidation.

## Cysteinyl leukotrienes and thromboxane $A_2$

Studies in animals have shown that leukotrienes $LTC_4$ and $LTD_4$ and thromboxane $A_2$ ($TXA_2$) act as vasoconstrictors within the kidney, reducing both RBF and GFR (60–62). In addition, $LTD_4$ causes mesangial cell contraction that reduces glomerular capillary permeability. Urinary $LTE_4$ levels are increased in ALF by a factor of up to 20-fold once levels have been corrected for the severity of renal dysfunction (29). Thromboxane $B_2$ ($TXB_2$), a stable metabolite of $TXA_2$, has been measured serially after paracetamol administration (40 mg/kg/day) and levels were found to be reduced transiently by up to 40–50% before returning to normal levels at 12 h post-ingestion (24). These findings argue against $TXA_2$ causing vasoconstriction soon after paracetamol overdose.

## Renal prostaglandins

In patients with cirrhosis, renal function has been shown to be dependent on renal prostaglandin (PG) synthesis (63). The administration of nonsteroidal anti-inflammatory agents to these patients reduces RBF and GFR significantly (64). In addition, patients with cirrhosis and preserved

renal function have increased levels of $PGE_2$, suggesting a protective role for vasodilatory prostaglandins within the kidney (65). In ALF the urinary excretion of 6-keto-$PGF_{1a}$ was increased in patients with preserved renal function compared with patients with renal failure or healthy controls, suggesting a protective role for 6-keto-$PGF_{1a}$ in the pathogenesis of renal dysfunction in ALF.

## Specific etiologies of acute liver failure

### Paracetamol

The incidence of renal dysfunction is highest in paracetamol-induced ALF with rates of 50–70% in patients with advanced encephalopathy (2,66). However, renal failure can develop in the absence of any significant degree of liver injury (67,68). Paracetamol is concentrated five- to seven-fold in the renal medulla, where it can be metabolized by mixed-function oxidases to *N*-acetyl-*p*-benzoquinone imine (NAPQI), the agent responsible for hepatotoxicity. NAPQI may cause local damage by arylation and oxidation with subsequent interference with $Ca^{21}$ homeostasis. By restoring levels of glutathione, *N*-acetylcysteine (NAC) may protect against renal failure in addition to the established benefit with respect to liver toxicity (69). The histological lesion is that of ATN. Examination of renal biopsies and autopsy specimens shows evidence of coagulative necrosis of proximal tubular cells, tubular dilation, collection of cellular debris and casts within damaged tubules, rupture of tubular membranes, interstitial edema, and infiltration with lymphocytes and plasma cells (15,70,71). There have also been reports of interstitial nephritis and distal tubular damage (68,72,73).

### Other drugs

Many drugs cause simultaneous injury to both hepatocytes and renal tubular cells, resulting in the most severe cases of combined liver and renal failure. The mechanisms for the renal damage seen are varied and include tubular damage and a hypersensitivity reaction leading to acute tubulointerstitial nephritis. The pathological conditions caused by individual drugs are outside the scope of this chapter, and therefore important drugs and their principal pathological features are summarized in Table 31.1.

### Pregnancy

Acute fatty liver of pregnancy is a disease predominantly of the third trimester of pregnancy, characterized by nausea, vomiting, and abdominal pain, and progressing if untreated to hypertension, jaundice, encephalopathy, renal failure, and bleeding. Historical reports suggest an incidence of renal failure as high as 60%, but earlier recognition has led to a decrease in prevalence (74). Renal histological results may be within normal limits or limited to fatty vacuolization and other nonspecific changes. In those patients with the most severe disease, ATN occurs, most likely as a result of hypoperfusion and sepsis. Patients with eclampsia may have changes compatible with disseminated intravascular coagulation (DIC) with extensive fibrin deposition (75).

## Differential diagnosis of renal failure in patients with acute liver failure

In patients who present with hyperacute liver failure there is normally little doubt as to the cause of renal impairment, and in the majority of patients there is no significant past medical history to advocate a second pathological condition. Heavy proteinuria is unusual in ALF, and if found on urinalysis, a full series of renal investigations should be undertaken. Numerous drugs that may be taken as part of an overdose can cause renal damage, and a full history should be taken when possible from the patient. If possible, a corroborative history should be obtained from close relatives, partners, or friends who may be able to look for evidence of other drug ingestion, such as empty prescription bottles. In cases of doubt, a serum sample should be saved for toxicology analysis.

An assessment of potential iatrogenic nephrotoxicity from drugs prescribed for patients with ALF is impor-

**Table 31.1** Drugs and toxins that produce combined hepatic and renal damage.

| |
|---|
| *Acute tubular necrosis* |
| Halogenated hydrocarbons |
| Carbon tetrachloride |
| Chloroform |
| Tetrachloroethylene |
| Amatoxins |
| Tetracycline |
| Rifampicin |
| Sulfonamides |
| Halogenated anesthetics |
| Halothane |
| Methoxyflurane |
| Fluroxene |
| Arsenic |
| Copper sulfate |
| *Interstitial nephritis* |
| Sulfonamides |
| Rifampicin |
| Allopurinol |
| Phenytoin |
| *Other renal injury* |
| Salicylates |
| Methoxyflurane |

tant, for example, aminoglycosides, antifungal agents, and diuretics. In patients with oliguria, it is becoming common practice to administer loop-acting diuretics, such as furosemide, intravenously, in an attempt to convert patients with oliguric renal failure into a polyuric state. The clinical relevance of renal function early in the time course of paracetamol-induced liver injury cannot be overstated, as serum creatinine concentration is one of the criteria used to decide in which patients liver transplantation should be considered.

## Prognosis of renal failure in acute liver failure

Historically, renal impairment has been associated with a poor prognosis in the context of ALF. In early studies, renal failure requiring dialysis was invariably associated with death (12,13). A subsequent multivariate analysis of 588 patients with ALF was undertaken to determine factors of prognostic significance (3). The time period of this study was 1973–1985. Of these 588 patients, 570 (98%) developed severe (grade IV) encephalopathy, and the remainder developed moderate (grade III) encephalopathy. In patients with paracetamol-related FHF, a serum creatinine level of < 100 mol/l (1.3 mg/dl) on admission was associated with a survival of 65%, compared with 40% in those with a level of 100–300 mol/l (1.3–4 mg/dl) and 23% in those in whom the creatinine level was > 300 mol/l (3.4 mg/dl). No such correlation was seen in patients with nonparacetamol-related ALF. In a further validation exercise in patients with paracetamol-induced FHF, serum creatinine concentration was the least discriminating variable as a single predictor of outcome, but a value of > 300 mol/l (3.4 mg/dl) in combination with grade III encephalopathy and a prothrombin time of > 100 s resulted in an outcome sensitivity of 67%. Serum creatinine is also of prognostic significance in patients with *Amanita phalloides* (mushroom) poisoning.

In those patients who recover from their illness without needing liver transplantation, renal function can be expected to return to normal. There has been no longitudinal follow-up of these patients with tests other than serum electrolytes to confirm a complete biochemical recovery, and renal biopsy would be unethical in this group with normal renal function due to the risks of the procedure.

## Renal function as a preoperative risk factor for early post-transplant mortality

Emergency liver transplantation is now an established treatment for patients with ALF, with survival figures reported to be between 65 and 90%. We utilize the King's College Hospital criteria to define the group of patients in whom transplantation is appropriate (3). Once a patient has been identified as in need of liver transplantation, attention turns to the maintenance of the patient in a stable condition until a donor liver becomes available. The management of the patient over this period is of critical importance because the severity of illness at the time of surgery correlates with outcome. In nonparacetamol-induced ALF, serum creatinine is the most powerful determinant of short-term survival after liver transplantation, while the grade of encephalopathy, Acute Physiology and Chronic Health Evaluation (APACHE) III score, and organ system failure scores are also relevant (76). In cases of paracetamol-related ALF, bilirubin and APACHE III scores were higher in nonsurvivors, but renal function measured by serum creatinine concentration was of no prognostic value at the time of transplantation. In a 2-year experience including 181 consecutive patients with ALF, in whom 27 patients proceeded to transplantation, pretransplant renal failure was associated with prolonged mechanical ventilation (13 vs. 6 days; $P < 0.05$) and prolonged intensive care stay (17 vs. 8 days; $P < 0.05$). Pretransplant renal failure did not predict renal dysfunction at 1 year (77).

## Treatment

In all cases treatment should initially focus on the identification of any precipitating causes, such as hypovolemia, the administration of nephrotoxic drugs, or sepsis. Patients with ALF will have a warm periphery due to vasodilation and may mistakenly be thought to have adequate organ perfusion. This vasodilation is also the reason why the routine use of dopamine to increase renal perfusion has largely been abandoned. Urine output should be monitored from the outset and, if < 40 ml/h, appropriate invasive hemodynamic monitoring should be instituted and intravascular pressures should be optimized. Once rehydration has been achieved, a sample of urine is analyzed for urinary sodium, protein, cells, casts, and culture. If the patient remains oliguric despite these measures, some physicians advocate the administration of furosemide intravenously in an attempt to restore urine output, but this approach should be regarded as controversial until supported by convincing data. Intra-abdominal hypertension is now clearly identified as a risk factor for renal impairment and this may be of relevance to patients with ascites in whom renal function may benefit from paracentesis (78).

It is widely assumed that there is merit in instituting renal support therapy early in patients with coexisting liver and renal failure. Hence, the development of anuria is often the trigger to commence renal support. Other indications for dialysis include hyperkalemia refractory to medical therapy, volume overload, hyperlactatemia or acidosis. Intermittent dialysis is hazardous in the patient

with severe ALF and grade III/IV encephalopathy. The rapid osmotic and electrolyte shifts of intermittent dialysis are risk factors for the development of cerebral edema (79). In addition, this mode of renal support is frequently accompanied by transient hypotension on commencement of dialysis, which may compromise the cerebral circulation and provoke cerebral edema. Continuous peritoneal dialysis is ineffective and may lead to bleeding and intra-abdominal sepsis. Continuous venovenous hemodiafiltration (CVVHD) is thus the modality of choice for renal support.

Anticoagulation of the extracorporeal circuit is mandatory and is tailored according to the patient's platelet count and bleeding tendency. For those patients with a platelet count > 60 000/μl heparin is utilized to maintain the activated clotting time between 150 and 200 s. In those patients with a platelet count < 60 000/μl who are not actively bleeding, prostacyclin is used in a dose of 2.5–5 ng/kg/min (Flolan, Epoprostenol). Patients with a low platelet count and associated bleeding may benefit from heparin-bonded circuitry that obviates the need for systemic anticoagulation. Patients on a CVVHD circuit may develop cerebral edema requiring treatment with 20% mannitol. A two- to three-fold increase in volume of ultrafiltrate should be removed over the subsequent hour to protect against a paradoxical worsening in intracranial pressure and to increase the efficacy of CVVHD.

There has been considerable recent interest in the role of biological and nonbiological liver support devices in ALF and other types of liver failure. Neither the ELAD nor BAL systems, based on human hepatoma cells and porcine cells, respectively, have been shown specifically to improve renal function in patients with ALF. The MARS system of albumin dialysis has been shown to improve renal function in patients with chronic liver disease with coexisting hyperbilirubinemia and renal failure. Although these patient characteristics are very relevant to patients with ALF, a similar effect has not been demonstrated in this clinical situation.

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# Chapter 32
# Renal Dysfunction in Obstructive Jaundice

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Patients with obstructive jaundice (OJ) have an increased risk for a wide array of postoperative complications, namely, bleeding, infections, poor wound healing, and renal failure (1,2) (Table 32.1). The three major consequences of biliary obstruction—immunosuppression, malnutrition, and hemodynamic disturbances—are all implicated in the pathogenesis of these complications. Several branches of the immune afferent and efferent pathways have been found to be impaired in animals and in humans with OJ (3,4). Anorexia, fat malabsorption, and metabolic/inflammatory disturbances induced by malignant tumors lead to vitamin deficiencies and malnutrition of the marasmus or the mixed marasmus–kwashiorkor type, the latter associated with marked hypoalbuminemia.

Hemodynamic disturbances and septic complications make patients with OJ susceptible to acute renal failure, the most dreaded postoperative complication in this setting due to its refractoriness to conventional therapies and its high mortality rate. Multivariate analysis of factors predicting postoperative mortality in OJ (5–7) have shown that creatinine and urea plasma concentrations are two of the more relevant markers of risk together with serum albumin and the presence of cholangitis. In this chapter, evidence linking biliary obstruction with renal dysfunction will be reviewed, emphasizing the pathogenesis of this association according to the most recent experimental and clinical data.

**Table 32.1** Frequency of postoperative complications and their relationship to mortality in a consecutive series of 373 patients with obstructive jaundice (2).

| | Frequency | | Mortality | |
|---|---|---|---|---|
| Complication | Patients | % | Patients | % |
| Wound infection | 36 | 9.7 | 3 | 8.3 |
| Upper GI hemorrhage | 27 | 7.2 | 13 | 48.1* |
| Sepsis | 24 | 6.4 | 6 | 25 |
| Intra-abdominal abscess | 18 | 4.8 | 5 | 27.8* |
| Pancreatitis | 14 | 3.8 | 8 | 14.3 |
| Laparotomy wound dehiscence | 12 | 3.2 | 3 | 25 |
| Acute renal failure | 13 | 3.5 | 8 | 61* |

*Significantly different death rates between patients with and without the complication. GI, Gastrointestinal.

## Historical background

Hans von Haberer had trained with von Eiselberg in Vienna and was a leading surgeon and gastric physiologist of his time (8). In 1911, he and Paul Clairmont (9) described a series of five patients with OJ due to lithiasis of the common bile duct, who died of acute renal failure in the postoperative period although, apparently, kidney function was normal before surgery. The association of liver disease with renal dysfunction received several names in the years that followed this first publication on the subject. In 1932 the term "hepatorenal syndrome" was introduced by Helwing and Schutz (10). Caroli *et al.* (11) used the term of "angiocholites urémigènes" to emphasize the association of acute cholangitis with acute renal failure. In the following years, the term "hepatorenal syndrome" was specifically ascribed to renal dysfunction complicating liver cirrhosis with ascites and became separated from renal failure in connection with OJ.

Zollinger in the USA and Dawson in the UK were at the origin of the modern history of research on the syndrome of hypotension, oliguria, and death following surgery for OJ. In a series of clinical observations and experimental studies, Zollinger and Williams observed that morbidity and mortality associated with surgical procedures on the biliary tract were low, except in the presence of jaundice. Dogs were found to suffer from a severe weight loss after bile duct ligation, to be more prone to anuria after bleeding, and to have a reduced plasma volume measured with iodinated albumin (12,13).

Dawson, at the time a senior surgical registrar at King's College in London, suggested that jaundice made the kidneys more susceptible to fail when exposed to ischemia. In an elegant experimental study in monorenal rats with bile duct ligation, he showed that kidneys which had undergone clamping of the pedicle failed more often and more severely in jaundiced than in control animals. The deleterious effect of ischemia could be partially re-

lieved by the osmotic diuretic mannitol (14). Dawson then carried out a pilot nonrandomized study in which seven jaundiced patients received perioperative mannitol with apparent success. Accordingly, he recommended perioperative forced diuresis with mannitol (15) which during the 1970s and 1980s enjoyed much popularity in the UK (16).

## Prevalence of renal dysfunction and acute renal failure

### Baseline assessment

Many studies have reported measurements of the glomerular filtration rate (GFR) in the preoperative period of patients with OJ. A selection of those with more than 20 cases is shown in Table 32.2. As can be observed, roughly one-third of patients coming to surgery because of biliary obstruction have renal impairment (creatinine clearance < 60–70 ml/min) before the operation. In addition, OJ is usually associated with mild hyponatremia in the range of 135–137 mEq/l (17).

The prevalence of preoperative renal dysfunction is not influenced by the benign or malignant etiology of the biliary obstruction, nor does it seem to be related to the duration of jaundice. The main limitation of human studies is that variables such as the degree of bile duct obstruction, the precise onset of jaundice, or the presence of associated diseases or treatments that may affect renal function are difficult or even impossible to assess. This is why the more relevant data on the characteristics and time phases of renal dysfunction in OJ have been obtained from animal studies.

**Table 32.2** Prevalence of preoperative renal dysfunction and acute postoperative renal failure (ARF) in patients undergoing surgery for obstructive jaundice.

| Author | Year | No. of patients | % creatinine clearance < 60 ml/min | % with ARF | Mortality of ARF |
|---|---|---|---|---|---|
| Braasch | 1977 | 279 | – | 8 | – |
| Allison | 1979 | 24 | 25 | 17 | 72 |
| Pitt* | 1981 | 155 | 16 | 6 | 32 |
| Cahill | 1983 | 30 | 53 | – | – |
| Dixon | 1983 | 373 | – | 3 | 61 |
| Armstrong | 1984 | 120 | – | 6 | 100 |
| Thompson | 1986 | 40 | 42 | – | – |
| Pain | 1986 | 24 | 21 | 29 | 14 |
| Thompson | 1987 | 334 | – | 11 | 74 |
| Gubern | 1988 | 31 | 45 | 10 | 100 |
| Pain | 1991 | 102 | 21 | 1 | |
| Parks | 1995 | | 17* | 0 | 0 |
| Gallardo | 1997 | 30 | 33* | – | – |
| Padillo** | 2003 | 63 | 44 | – | – |

*Cut-off for creatinine clearance set at 70 ml/min.**Unpublished observations.

### Postoperative acute renal failure

The prevalence and mortality of postoperative acute renal failure in patients with OJ are shown in Table 32.2. There are some limitations in interpreting these data since renal failure was not defined uniformly. The most common presentation form is the rapid development of oliguria and anuria in the postoperative period, usually in connection with an episode of hypotension due to severe sepsis, bleeding, or inadequate fluid replacement. Patients with acute cholangitis are particularly susceptible to acute renal failure, especially if they develop septic shock (18). High blood urea nitrogen is an independent risk factor for complications and death in this setting (19).

The prognosis of postoperative acute renal failure associated with OJ is dismal. In fact, it is worse than postoperative renal failure due to other causes. Amerio *et al.* (20) compared 67 sequential cases of acute renal failure and jaundice with 168 without jaundice. The mortality rates were 57 and 42% respectively ($P < 0.05$). Patients with a serum bilirubin > 20 mg/dl had a mortality rate of 85%, whereas patients with bilirubin < 10 mg/dl had a mortality of only 33% ($P < 0.01$). Average blood pressure was lower in jaundiced than in nonjaundiced patients. In recent years, however, more careful perioperative fluid therapy, and improved surgical techniques have substantially reduced this complication (21).

## Renal pathophysiology in obstructive jaundice

It is likely that acute renal failure, the most dramatic form of renal dysfunction associated with OJ, is the final manifestation of underlying subtle renal changes that decompensate when renal function is challenged by a stressful perioperative insult such as sepsis, hypotension, hypovolemia, or nephrotoxic drugs. Thus, it is essential to know the baseline renal function changes induced by OJ that make jaundiced patients particularly susceptible to acute renal failure.

### Animal studies

Most data on the pathophysiology of renal function in OJ come from animal studies. As discussed above, studies in man are limited because patients usually seek consultation when jaundice is well established, they present with different degrees of biliary obstruction, and they may suffer either from renal diseases or conditions that may influence the renal physiology (heart failure, chronic liver disease, hypertension, administration of diuretics or nonsteroidal anti-inflammatory drugs).

### *The models*

The dog and the rat with bile duct ligation were the most employed animal models in early studies and data from those investigations have been reviewed by Better and Bomzon (22). These models, however, were comparable to the human setting only during the first week. Later on, the high death rates, the reversibility of jaundice or the development of ascites or progressive hepatic failure made these models too different from the usual clinical picture. The introduction of the rabbit model (23) helped significantly in advancing our knowledge about renal pathophysiology in OJ, particularly when balance studies in metabolic cages were introduced allowing studies in the conscious animal and avoiding the hemodynamic and renal alterations induced by general anesthesia (17,24,25). The rabbit model has consistently shown striking behavioral, compositional, and endocrine similarities with human OJ. This can now be better understood in the light of recent phylogenetic studies which have shown that the order of *Lagomorpha* is significantly more closely related to primates than it is to rodents (26).

### *A biphasic renal response*

A common finding from studies in different animal species is that the renal response to bile duct ligation has two distinct phases in time (22).

#### *Early renal dysfunction*

Shortly after biliary obstruction there is a rapid weight loss with inappropriate diuresis and natriuresis, and a preserved or even increased GFR. Although the nature of weight loss was initially poorly understood, it now seems clear that immediately after biliary obstruction there is volume depletion as a result of marked reduction in water and chow intakes while diuresis and natriuresis are inappropriately preserved. Careful balance studies carried out in metabolic cages have shown that very low water and chow (sodium) intakes are early features of bile duct ligated animals (24,25,27). In studies performed during the first 4 days after bile duct ligation or sham operation, water and chow intakes in OJ rabbits were 60 and 8%, respectively, of those observed in the sham-operated controls. As early as 24 h after bile duct ligation, animals experience profound hypodipsia (27). Obstructive jaundice downshifts the linear correlation existing between water intake and diuresis (Fig. 32.1); thus, for a given water intake, diuresis is higher in OJ animals than in controls (24). As a result of poor *ad libitum* intakes, 4 days after bile duct ligation, jaundiced rabbits had a largely negative cumulative water balance. Despite this, both the solute-free water clearance and the fractional sodium excretion were the same as in control animals (Table 32.3). These findings were confirmed in a subsequent work (27) in which a natriuretic/diuretic drive could be detected already at 24 h after bile duct ligation (Table 32.4). It is

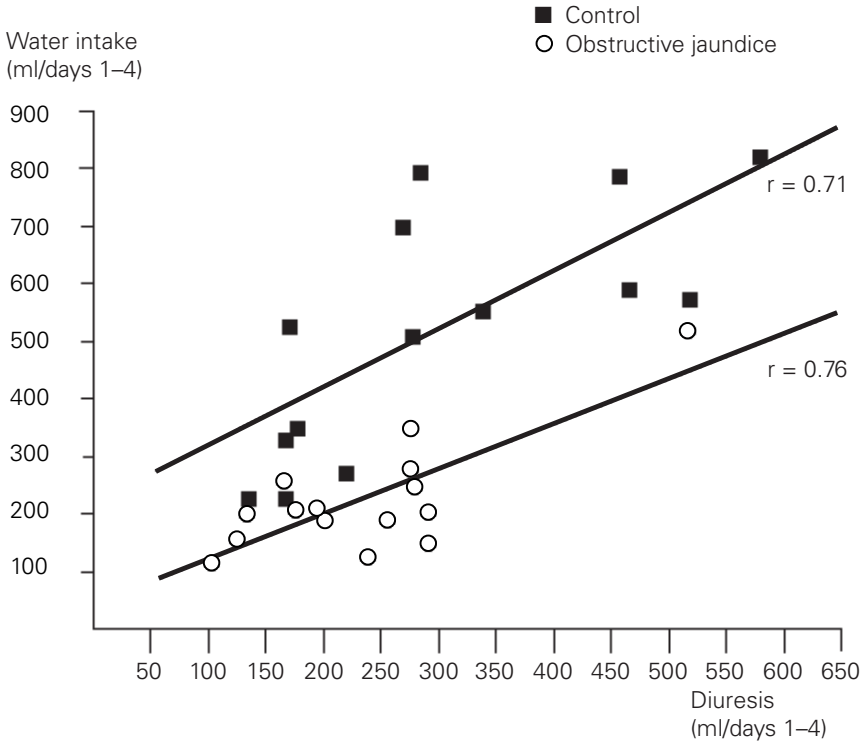


**Figure 32.1** Correlation between cumulative water intake and diuresis in bile duct ligated (open circles) or sham-operated rabbits (black squares). Although in both groups correlation was highly significant ($P < 0.001$), downshifting of the regression line is obvious for rabbits with OJ (from (24)). OJ, obstructive justice.

**Table 32.3** Water balance (days 1–4) and renal function studies in rabbits on the fourth day after bile duct obstruction (OJ) or sham operation (SO) (24).

| | OJ Group ($n$ = 17) | SO Group ($n$ = 14) | $P$-value |
|---|---|---|---|
| Water balance (ml)* | – 2.4 ± 65 | 219 ± 146 | 0.0001 |
| Bilirubin (mg/dl) | 3.1 ± 0.5 | 0.19 ± 0.1 | 0.0001 |
| Creatinine clearance (ml/min) | 6.8 ± 3.6 | 10.5 ± 4 | NS |
| Serum osmolality (mOsm/kg) | 270 ± 18 | 269 ± 8 | NS |
| Urine osmolality (mOsm/kg) | 840 ± 242 | 945 ± 502 | NS |
| Plasma Na (mEq/l) | 139 ± 1 | 141 ± 3 | NS |
| Free water clearance (ml/min) | – 0.10 ± 0.04 | – 0.16 ± 0.09 | NS |
| Na excretion (mEq/24 h) | 1.9 ± 1.5 | 4.7 ± 1.75 | 0.0001 |
| Na intake (mEq/24 h) | 0.73 ± 0.27 | 7.1 ± 1.7 | 0.0001 |
| Na Fe (%) | 0.29 ± 0.31 | 0.38 ± 0.30 | NS |

*Insensibles losses (approx. 80 ml/day) have not been subtracted.

clear from these data that crude sodium excretion at a given point cannot be appropriately interpreted if fluid and sodium intakes and balances and fractional sodium excretion are not concomitantly measured.

To elucidate the role of reduced water and sodium intakes in the pathophysiology of early renal dysfunction, experiments were made comparing jaundiced rabbits with paired-fed paired-watered controls (25). Similar reductions were observed in weight and in creatinine clearance (Table 32.5). Urine osmolality and diuresis, however, were higher in control than in the jaundiced animals, suggesting that, in addition to a poor intake, a defective renal concentration mechanism was also present. Ozawa *et al.* (28) did also remark an early reduction of the osmolar clearance and solute-free water reabsorption after bile duct ligation in the rabbit model.

### *Late renal dysfunction*

Between 3 and 7 days after complete biliary obstruction, depending on the species and maybe on individual variations, a second phase of renal derangements can be demonstrated. The kidneys try to compensate the fluid depletion that has occurred shortly after biliary obstruction by increasing sodium reabsorption and reducing water excretion. A sodium-retaining response is observed with reduction of the solute-free water clearance and the fractional sodium excretion (Table 32.6). The renal plasma flow is reduced and so is the GFR (23,24). However, a low urine osmolality indicates that this response is only partially successful. It is likely that this is due to the very limited sodium intake and to the persistence of a defective urine concentration capability. This unstable situation between an early natriuretic/diuretic drive linked to biliary obstruction, and a late compensatory renal response goes on for some time until hepatic function deteriorates. Data on renal function beyond the second week are mainly restricted to animal models that develop ascites, portal hypertension, or hepatic insufficiency and thus bear little similarity to the usual patients with OJ.

In summary, experimental data indicate that the most striking features of the early derangements of water and sodium metabolism after bile duct ligation are hypophagia, hypodipsia, and inappropriate diuresis. Later on, a partially successful compensatory response can be observed, with a tendency towards water and sodium retention. At this time, renal function is impaired with a low plasma renal flow and decreased GFR.

**Table 32.4** Water and sodium metabolism parameters 24 h after common bile duct ligation (OJ group) or sham operation (SO group) in the rabbit (27).

| | OJ group ($n$ = 17) | SO group ($n$ = 14) | $P$-value |
|---|---|---|---|
| Water intake (ml) | 9 ± 15 | 66 ± 30 | 0.0008 |
| Diuresis (ml) | 63 ± 20 | 58 ± 30 | NS |
| Water balance (ml)* | – 54 ± 30 | 3 ± 37 | 0.0001 |
| Bilirubin (mg/dl) | 2.9 ± 1.3 | 0.1 ± 0.09 | 0.0001 |
| Weight loss (%) | – 5.1 ± 1 | – 3.8 ± 2 | 0.05 |
| Serum osmolality (mOsm/kg) | 290 ± 9 | 299 ± 6 | NS |
| Plasma Na (mEq/l) | 145 ± 4 | 145 ± 6 | NS |
| Free water clearance (ml/min) | – 0.08 ± 0.04 | – 0.08 ± 0.05 | NS |
| Na excretion (mEq/24 h) | 4.8 ± 4.2 | 1.7 ± 1.8 | 0.06 |
| Na intake (mEq/24 h) | 0.78 ± 0.33 | 15.81 ± 4.8 | 0.0001 |
| Na Fe (%) | 0.27 ± 0.31 | 0.61 ± 0.30 | 0.001 |

*Insensibles losses (approx. 80 ml/day) have not been subtracted.

| | SO group ($n$ = 8) | OJ group ($n$ = 8) | Group SO-2 ($n$ = 8) | $P$-value* |
|---|---|---|---|---|
| Cumulative balances | | | | |
| Weight loss (%) | 6 ± 2 | 14 ± 4 | 13 ± 2 | < 0.01 |
| Water intake (ml) | 898 ± 221 | 280 ± 146 | 280 | < 0.01 |
| Chow intake (g) | 363 ± 57 | 57 ± 85 | 57 | < 0.01 |
| Total diuresis | 604 ± 181 | 316 ± 57 | 238±60 | < 0.01 |
| Biochemistry | | | | |
| Bilirubin (mmol/l) | 3.4 ± 1.7 | 152 ± 49 | 3.4 ± 1.7** | 0.01 |
| Plasma Na (mEq/l) | 143 ± 5 | 138 ± 6 | 145 ± 4** | < 0.05 |
| Serum osmolality (mOsm/kg) | 286 ± 11 | 293 ± 19 | 290 ± 26 | NS |
| Urine osmolality (mOsm/kg) | 817 ± 417 | 935 ± 288 | 1373 ± 373** | < 0.05 |
| Total urinary Na excretion (mEq) | 47 ± 9 | 17 ± 7 | 16 ± 6 | < 0.01 |
| Renal function | | | | |
| Δ Creatinine clearance (%) | + 4 ± 36 | – 37 ± 19 | – 44 ± 28 | < 0.01 |
| Osmolar clearance (ml/min) | 0.2 ± 0.05 | 0.1 ± 0.04 | 0.1 ± 0.02 | < 0.01 |
| Free water clearance (ml/min) | – 0.01 ± 0.06 | – 0.01 ± 0.04 | – 0.1 ± 0.02 | NS |
| Fractional Na excretion (%) | 1 ± 0.4 | 0.3 ± 0.1 | 0.5 ± 0.6 | < 0.01 |

**Table 32.5** Renal profiles of sham-operated, bile duct-ligated (OJ) and sham-operated paired-fed and paired-watered paired-fed rabbits (SO-2) on the sixth postoperative day (adapted from (25)).

*Overall $P$-value. **Significant differences between OJ and SO-2.

## Human studies

Gallardo *et al.* (29) investigated 43 patients with OJ not complicated by cholangitis at admission, before any treatment (fluids, radiographic examinations, drainage) was started. Accurate creatinine clearance determinations were possible in 30 patients, of whom 10 showed values below 70 ml/min. These patients showed the same diuresis (1360 vs. 1511 ml/24 h) and a higher fractional sodium excretion (0.84 ± 0.6 vs. 0.38 ± 0.3%; $P$ = 0.02) than patients with creatinine clearances > 70 ml/min. They also showed a marginally lower plasma sodium (136 vs. 138 mEq/l; $P$ = 0.06) with similar daily sodium urinary excretion. Interestingly, patients with a lower creatinine clearance showed marked hypoalbuminemia (29 vs. 34 g/l; $P$ = 0.01). All other renal parameters, and water and sodium-regulating hormone levels were similar in the two groups. When comparing patients with malignant with those with benign OJ, the first had more pronounced hypoalbuminemia (30 vs. 35 g/l; $P$ = 0.01), lower plasma sodium (136 vs. 138 mEq/l; $P$ = 0.05), and lower values for plasma aldosterone and vasopressin, suggesting an extracellular water space relatively expanded in comparison with patients with benign OJ. Creatinine clearances and fractional sodium excretions were similar.

## Pathogenesis of renal dysfunction

There is an ongoing debate on the mechanisms by which OJ induces alterations in the renal handling of water and sodium. Less is known about the mechanisms involved in early hypophagia and hypodipsia. It is likely that a single "pathogenetic factor" cannot be identified and that renal dysfunction in OJ is the result of several mechanisms. The current hypotheses on the pathogenesis of renal dysfunction/failure in OJ can be grouped into four

| | OJ Group ($n$ = 17) | SO Group ($n$ = 14) | $P$-value |
|---|---|---|---|
| Water balance (ml)* | 11 ± 65 | 379 ± 146 | 0.0001 |
| Bilirubin (mg/dl) | 5.3 ± 2.3 | 0.11 ± 0.3 | 0.0001 |
| Creatinine clearance (ml/min) | 3 ± 1 | 11 ± 6 | 0.0001 |
| Renal plasma flow (ml/min)** | 15 ± 4 | 25.6 ± 3.7 | 0.0001 |
| Serum osmolality (mOsm/kg) | 266 ± 23 | 268 ± 17 | NS |
| Urine osmolality (mOsm/kg) | 653 ± 151 | 1103 ± 561 | 0.004 |
| Plasma Na (mEq/l) | 139 ± 3 | 139 ± 5 | NS |
| Free water clearance (ml/min) | – 0.05 ± 0.02 | – 0.16 ± 0.10 | 0.002 |
| Na excretion (mEq/24 h) | 1.1 ± 0.7 | 8 ± 4.4 | 0.0001 |
| Na intake (mEq/24 h) | 0.78 ± 0.33 | 15.81 ± 4.8 | 0.0001 |
| Na Fe (%) | 0.27 ± 0.31 | 0.61 ± 0.30 | 0.001 |

**Table 32.6** Water balance (days 7–10) and renal function studies in rabbits on the tenth day after bile duct obstruction or sham operation (from reference (24)).

*Insensibles losses (approx. 80 ml/day) have not been subtracted. **Measured on day 7.

main categories: (i) direct nephrotoxicity of biliary products; (ii) endotoxemia-induced microcirculatory changes in the renal vascular bed; (iii) hemodynamic (central and peripheral) changes induced by biliary products; and (iv) extracellular fluid depletion and hypovolemia.

## Nephrotoxicity of biliary products

Dawson (15) found that there was a close correlation between the plasma bilirubin concentration and the magnitude of postoperative fall of creatinine clearance and suggested that bilirubin exerted a direct toxic action on the kidney. He also noted that deaths due to acute renal failure were most commonly observed in patients with plasma bilirubin > 20 mg/dl. He did not prove, however, that this correlation existed before surgery. In fact, several studies have failed to demonstrate a correlation between preoperative plasma bilirubin concentrations and creatinine clearance or its postoperative fall, although in some of them mannitol was administered perioperatively (30–32).

A seminal experiment on the potential nephrotoxicity of bile products was carried out by C. Topuzlu, a surgical resident at the University of Vermont (33). This author injected 15 ml of bile intravenously to anesthetized dogs. A prompt natriuretic and diuretic response with an unchanged GFR was noted, associated, in some cases, to a reduction of cardiac output and hypotension. The authors inferred that bilirubin decreased proximal tubule sodium and water reabsorption. In this study, however, the plasma bilirubin concentration was near normal since its average increase was only 0.6 mg/dl. No morphological studies were carried out. As will be discussed later, the current interpretation of this experiment may be different from that afforded initially by the authors.

Aoyagi and Lowenstein (34) could not demonstrate the nephrotoxic effects of conjugated bilirubin or bile salts alone, but showed that these biliary products could impair renal function if superimposed on renal ischemia. Bile salts, however, were administered in doses resulting in plasma concentrations of cholate and taurocholate much higher than those attained in the clinical setting. In studies in the baboon, Bomzon *et al.* (22) did not observe intrarenal hemodynamic changes or alterations in the pressor responses when taurocholate was administered intra-arterially in similar concentrations to those observed in human OJ. However, similar to the study of Topuzlu and Stahl (33), a natriuretic response was noted without changes in the GFR.

In summary, although biliary components may induce functional changes at the proximal tubule level in acute experiments, there is lack of proof that, at the usual concentrations seen in OJ, they can be toxic to renal glomerular or epithelial cells.

## Endotoxemia

Rats with OJ are very sensitive to the acute administration of endotoxin that causes death associated with widespread fibrin deposition in the microcirculation, including the kidney (35–37).

Bailey (30) determined blood endotoxin using the Limulus test in 24 patients with OJ. Sixteen had portal and 13 had peripheral endotoxemia. Patients with OJ and endotoxemia had a lower creatinine clearance than those without (70 vs. 92 ml/min), although the difference was not statistically significant. The magnitude of fall of the creatinine clearance, however, was more marked in the endotoxemic patients. In an experimental study (30), the death rates of jaundiced rats given intravenous lead acetate plus oral endotoxin were determined. Mortality was 13/15 in the control group but only 1/15 rats died if fed sodium taucholate for 2 days before the administration of endotoxin. The beneficial effect of bile salts was supposed to be due to inhibition of endotoxin absorption in the gut. However, endotoxemia was not determined and the cause of death was not ascertained. Renal function was not assessed.

Cahill (38) carried out a nonrandomized trial of anti-endotoxin therapy based on oral bile salts. Twenty-five patients with OJ were studied, and compared with 25 nonjaundiced patients, matched for age and sex, undergoing upper abdominal surgery of similar magnitude. An unselected sequential group of jaundiced patients given sodium deoxycholate for 48 h preoperatively was compared with the first two groups. None of the eight patients given preoperative bile salts had portal or peripheral endotoxemia whereas 12 of 25 patients with OJ not given sodium deoxycholate had portal and systemic endotoxemia and they suffered from a significant deterioration of postoperative renal function. However, some of the results of this study are paradoxical: (i) endotoxemic jaundiced patients had similar preoperative renal function to controls; (ii) the jaundiced patients given oral bile salts fared even better than the nonjaundiced controls, in terms of postoperative decrease in creatinine clearance; and (iii) when bile salts were measured in the bowel contents, the same concentration was found in jaundiced patients given or not sodium deoxycholate. It is worth noting that in these studies, jaundiced patients were given perioperative mannitol infusions, making interpretation of data even more difficult.

Thompson *et al.* (39) carried out a randomized trial of oral administration of ursodeoxycholic acid. Portal endotoxemia was significantly reduced in the treated patients, but there was no difference in systemic venous endotoxemia, renal function, or postoperative morbidity and mortality. Again, perioperative mannitol infusions were administered to all patients. This study was criticized on the basis that ursodeoxycholic acid is one the bile salts with less anti-endotoxin properties. In another randomized trial, in which jaundiced patients were given the bile salts deoxycholate or chenodeoxycholate (40), the prevalence of endotoxemia at operation was significantly lower in patients given deoxycholate

but not in those receiving chenodeoxycholate. Postoperative fall in creatinine clearance was less pronounced in the deoxycholate group, but no statistical analysis was given. No explanation was afforded on the lack of effect of chenodeoxycholic acid, which, *in vitro*, seems to have very similar anti-endotoxin properties to those of deoxycholate.

Administration of lactulose has also been tried to diminish the potential noxious effects of endotoxin on renal function. In one experimental study, increased survival after renal ischemia was noticed but lactulose did not prevent deterioration of renal function (41). In a randomized trial, patients with OJ received preoperative oral lactulose, deoxycholate, or acted as a control group. Only one patient in the control group developed severe postoperative oliguria. Postoperative deterioration of renal function was reduced in both therapeutic groups (42). The authors pointed out that the incidence of renal failure in the control group was lower than in previous studies and that this might be due to the introduction of adequate preoperative hydration.

There is no doubt that biliary obstruction is associated with increased plasma levels not only of endotoxin but also of some other cytokines such as the proinflammatory interleukin-6 and tumor necrosis factor-α (43). This cytokine response appears to be more significant in patients with malignant obstruction and bears some relationship to both increased liver enzymes and hypoalbuminemia. However, its precise link with impaired renal function remains controversial, particularly from a therapeutic point of view.

## Hemodynamic changes

The crucial element of the hemodynamic and the hypovolemic hypotheses is that the kidney is an innocent bystander that fails secondarily to circulatory derangements. The hemodynamic hypothesis has put particular emphasis on the effects of bile components on the heart and on the peripheral arteries, whereas the hypovolemic hypothesis has stressed the presence of an extracellular water and plasma volume deficit. In the end, both theories would explain renal dysfunction as a result of the synergistic effects of low circulatory performance and underfilling of the vascular tree. The absence of morphological renal changes specific for kidney failure in OJ has also given further support to the hemodynamic and hypovolemic hypotheses, which emphasize the functional characteristics of renal alterations rather than the direct damage to the nephron from bile components.

### *Effects of jaundice on the heart*

The Haifa group initially reported blunted *in vitro* contractile response to β-agonists in myocardial tissue taken from dogs with choledochocaval shunts (44). In another study (45), dogs with choledochocaval shunts were investigated *in vivo*. This experimental model does not cause liver damage, to which bile duct-ligated dogs are particularly prone, but induces more severe cholemia than bile duct ligation. After 2 weeks of deep jaundice, at the time of heart function assessment, mean bilirubin and alkaline phosphatase values were 33 mg/dl and 17 000 U, respectively. Maximal rate of increase of left ventricular pressure (dp/dt) decreased after choledochocaval shunt from 4543 ± 593 mmHg/s to 3666 ± 648 mmHg/s. The same group has shown that rats with bile duct ligation for 3 days have impaired indices of basal cardiac contractility but the affinity and number of heart β-receptors and the heart responses to norepinephrine and β-agonists were unaffected by jaundice (46).

In agreement with these experimental hemodynamic findings, our group described increased plasma levels of atrial natriuretic peptide both in experimental and clinical OJ, which may indicate the presence of a subclinical heart insufficiency (see below).

### *Effects of jaundice on the peripheral arteries*

Peripheral resistance to pressor hormones has been well documented in liver cirrhosis (47). In OJ there is also a blunted response of the vascular smooth muscle to pressor stimuli (48). Cioffi *et al.* (49) investigated the renal artery contractile response to norepinephrine in rabbits after bile duct ligation or sham operation. Kidneys and renal arteries from bile duct-ligated animals were removed after 7–14 days of jaundice and studies were done in an *ex vivo* renal perfusion model and on isolated renal arteries. At any concentration of norepinephrine, renal perfusion pressure was lower in kidneys removed from jaundiced animals. Isolated segments of renal arteries had also a significantly blunted response to the amine. These effects could be reversed by indomethacin and it was suggested that they could be mediated by prostaglandins. However, the fact that baseline perfusion pressure was higher in jaundiced rabbits than in controls suggests that the pressor response may also be blunted due to the exhaustion of the local constrictor mechanisms due to prolonged hypersecretion of vasoconstrictor prostaglandins (thromboxane) shown to occur in OJ (50). Utkan *et al.* (51) confirmed a blunted contractile response of femoral arteries to norepinephrine and serotonin in a dog model, which could be reversed by removing the vascular endothelium. This would suggest that excessive release of the endothelium-derived relaxing factor might be involved in the pathogenesis of abnormal vascular reactivity in OJ.

More recently, an increased secretion of the potent vasoconstrictor endothelin-1 by renal vascular endothelial cells has been implicated in the pathogenesis of renal artery vasoconstriction in OJ (52).

## Volume depletion

### *Extracellular fluid and plasma volume reduction*

Abnormalities of fluid volume were suspected of being at the origin of renal failure in OJ already during the mid-1950s, when Zollinger suggested that a reduced blood volume might be present in patients with OJ. A decade later, Topuzlu and Stahl (33) demonstrated a diuretic and natriuretic response in anesthetized dogs given an intravenous infusion of bile and hypothesized that cholemia could cause a defective renal concentration capacity and excessive water and sodium losses and hypovolemia. In the first systematic experimental study of blood volume in OJ, Gillett measured blood volume in bile duct-ligated rats with radioiodinated albumin (53). There were no changes in the blood volume of sham-operated controls; in the bile duct-ligated animals, however, blood volume was found to be reduced by about 15%.

To elucidate further the presence of volume depletion in OJ, body composition studies using a multi-isotope tracer dilution technique were carried out in our laboratory. In the rabbit, a 15% reduction of total body water was noted 6 days after common bile duct ligation, mostly due to depletion of the extracellular water compartment which was decreased by 30% (17). The plasma volume remained unaffected and did not show the physiological postoperative expansion observed in sham-operated animals. Twelve days after common bile duct ligation there was a 35% reduction of the extracellular fluid compartment which then became associated with a 15% decrease of plasma volume (Fig. 32.2). Percentage of body weight loss after bile duct ligation correlated closely with the degree of water loss ($r^2 = 0.85$). The intracellular water volume remained unchanged. These compositional alterations were mimicked by limiting the volume of fluid intake in sham-operated rabbits to that drunk by bile duct-ligated animals (pair-watered).

Similar studies were carried out in humans (54). Total body water was reduced in patients with obstructive jaundice compared with controls matched for age, sex and weight [41.8 vs. 46.2% body weight (b.w.), $P < 0.02$]. Extracellular water depletion was documented owing to a reduction of the interstitial space (16.1 vs. 19.5% b.w., $P < 0.004$) and, to a lesser extent, of the plasma volume (4.2 vs. 4.8% b.w., $P = 0.1$). There was a close correlation between plasma volume and creatinine clearance ($r^2 = 0.56$) and between plasma volume and extracellular volume (Fig. 32.3). In a subsequent study, body composition assessment was carried out by means of bioimpedance analysis (55). Again, it was found that total body water was reduced by 10% in OJ and also the extracellular water compartment expressed as percentage of body weight (21 vs. 23.8% b.w.; $P < 0.05$). There were no compositional differences between those with benign and those with malignant obstruction.

### *Derangements of water and sodium-regulating hormones*

Two types of hormonal alterations, potentially involved in the pathogenesis of renal dysfunction, have been observed in OJ: (i) "primary" endocrine derangements triggered by biliary obstruction, and (ii) "secondary" hormonal changes in response to water and sodium depletion.

In anesthetized dogs, Levy and Finestone (56) showed that bile duct obstruction was followed in the next 4 h by an increase in urine and sodium output. This effect could be reproduced by cross-circulation and thus the presence of a humoral mediator with a diuretic and natriuretic activity was hypothesized. Oms *et al.* (24) first noted a three-fold increase in the plasma atrial natriuretic peptide (ANP) concentrations 4 days after bile duct ligation in the rabbit. This was associated with low sodium intake and low urinary osmolarity. In fact, an inverse correla-

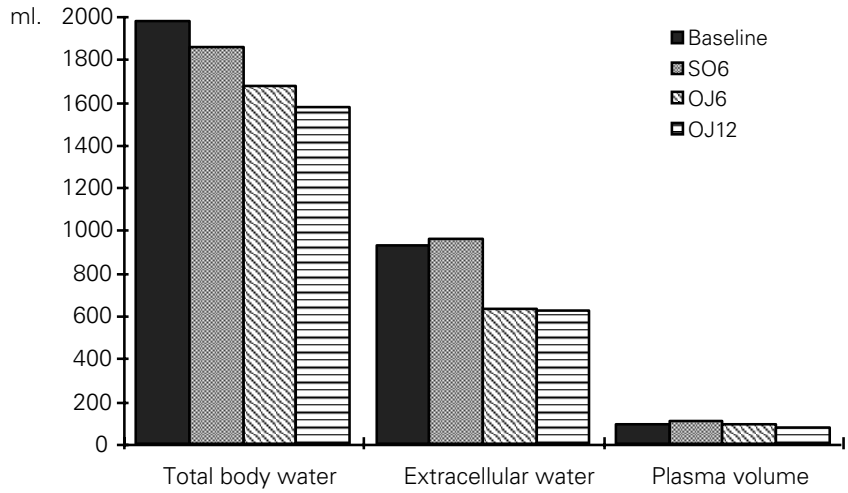


**Figure 32.2** Body water compartments in normal rabbits (baseline), sham-operated rabbits on the 6th postoperative day (SO6) and rabbits with obstructive jaundice (OJ) investigated at days 6 or 12 (OJ6 and OJ12) after common bile duct ligation (from (17)).

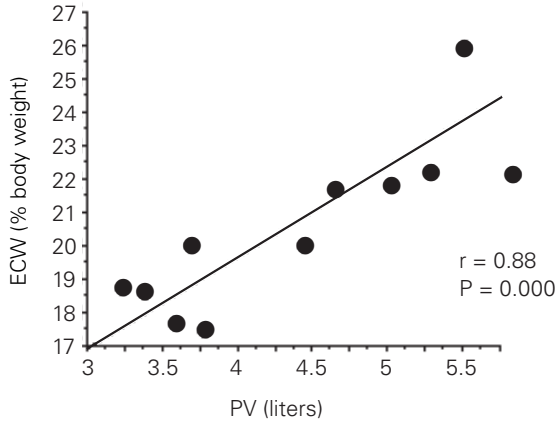


**Figure 32.3** Correlation between plasma volume (PV) and extracellular water (ECW) volume in patients with obstructive jaundice (OJ) (from (54)).

tion was found between plasma ANP concentrations and sodium and water intakes. The authors postulated that ANP could be a major mediator of hypodipsia, loss of sodium appetite, and inappropriate natriuresis occurring after bile duct ligation. Subsequent studies (27) found that 24 h after biliary obstruction, plasma ANP concentrations were already higher in jaundiced animals than in sham-operated controls (41 vs. 10 fmol/l; $P = 0.02$). Intravascular blood sampling showed that the heart was the likely source of ANP. To clarify further the origin of high plasma ANP concentrations in OJ, Pereira *et al.* (57) carried out immunohistochemistry studies of the heart of jaundiced rabbits and could demonstrate that both at 24 and 72 h after biliary obstruction, the number of cells staining positive for ANP was increased in both atria and atrial appendages (Table 32.7). Also, the atrial ANP content was greater in jaundiced animals than in controls (437 vs. 83 pmol/mg of protein; $P < 0.01$).

**Table 32.7** Percentage of atrial natriuretic peptide (ANP)-positive cells per 200-power field according to groups (OJ: bile duct ligation; SO: sham operation) and anatomic heart regions (from (57)).

| | OJ ($n = 11$) | SO ($n = 5$) | *P*-value |
|---|---|---|---|
| At 24 h | | | |
| Right atrial appendage | 62 ± 11 | 31 ± 12 | < 0.003 |
| Right atrial wall | 67 ± 10 | 41 ± 11 | < 0.004 |
| Left atrial appendage | 81 ± 4 | 62 ± 12 | < 0.005 |
| Left atrial wall | 75 ± 5 | 48 ± 17 | < 0.002 |
| At 72 h | | | |
| Right atrial appendage | 56 ± 18 | 31 ± 12 | < 0.01 |
| Right atrial wall | 60 ± 18 | 27 ± 12 | < 0.003 |
| Left atrial appendage | 75 ± 10 | 53 ± 16 | < 0.01 |
| Left atrial wall | 68 ± 15 | 40 ± 13 | < 0.004 |

To investigate the mechanisms leading to ANP increase in OJ, plasma ANP levels were determined in rabbits with external biliary fistula, bile duct obstruction, or bilio-venous shunt (58). The increase in plasma ANP in animals with bilio-venous shunt was nine-fold that observed after bile duct obstruction, whereas no changes were observed after external biliary diversion (Fig. 32.4). Relief of biliary tree obstruction was associated with a return of ANP levels towards baseline values. Thus, elevation of plasma ANP in OJ is not the result of an increased biliary pressure *per se* or absence of bile in the proximal duodenum, but of the passage of bile components into the circulation. It is suggested that the cardiodepressor effect of cholemia noted by Green *et al.* (45) translates into a "subclinical" heart failure, atrial distension, and ANP hypersecretion. ANP has been postulated as one of the most sensitive markers of asymptomatic heart dysfunction (59).

The ANP response after bile duct obstruction seems to be a primary phenomenon not linked to hypervolemia and atrial stretching, the only well-known stimulus for atrial ANP release (60). Thus, high ANP levels in OJ represent a paradoxical phenomenon, since they are associated with an extracellular water depletion. A secondary endocrine response to isotonic volume depletion has also been observed both in animals (late phase) and in man. In acute studies in rabbits after 10 days of biliary obstruction, Hishida *et al.* (23) observed an increase in plasma renin activity and aldosterone associated with a 30% reduction of creatinine clearance and a reduction of renal plasma flow. Valverde *et al.* (27) observed a significant plasma aldosterone increase at both 24 and 72 h after biliary obstruction (Table 32.8). At 72 h, plasma renin activity was also increased. These hormonal changes could be induced by restricting water and chow intake in sham-operated rabbits to the intake levels of jaundiced rabbits.

Gallardo *et al.* (29) investigated 43 patients with OJ. A renal profile was obtained at admission and plasma ANP, renin, aldosterone, and vasopressin were determined. In a subset of 18 patients, studies were repeated 3 days after endoscopic biliary drainage and changes in extracellular volume were measured. OJ patients had higher levels of plasma ANP (118 vs. 40 pg/ml; $P = 0.001$) and aldosterone (156 vs. 43 pg/ml; $P = 0.001$) than matched controls. Renin elevations were observed in 24% of the patients and were associated with impaired creatinine clearance. After biliary drainage plasma ANP and aldosterone decreased in association with an expansion of the extracellular volume (see below). No correlations were found between serum bilirubin or days of jaundice and the plasma concentrations of any of the water and sodium-regulating hormones. This study first showed that plasma ANP is increased in human OJ. It also demonstrated that endocrine markers of hypovolemia are activated in OJ and that after biliary drainage there is an improvement of endocrine and fluid derangements.

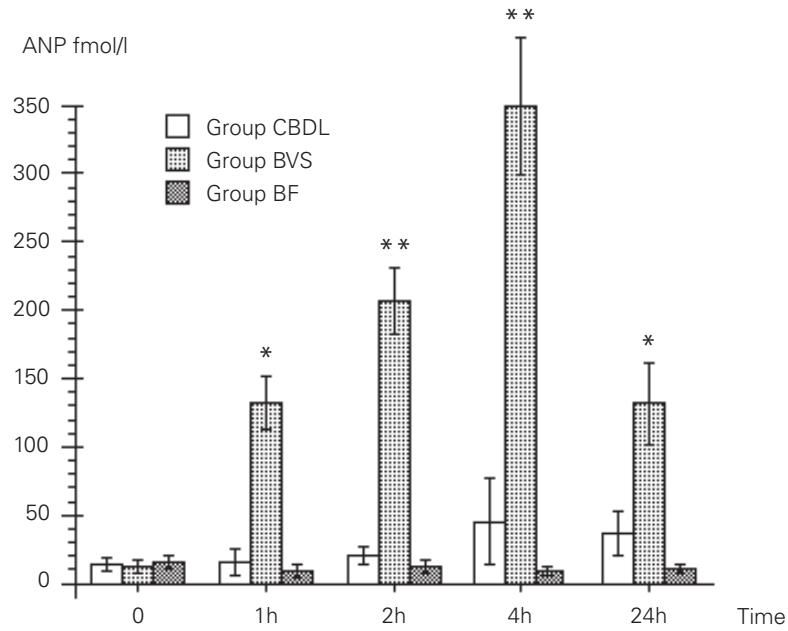


**Figure 32.4** Plasma atrial natriuretic peptide (ANP) concentrations in rabbits with common bile duct ligation (CBDL), biliary-venous shunt (BVS) and external biliary fistula (BF) at different time periods (*$P < 0.01$ and **$P < 0.001$: ANOVA test) (from (58)).

## The human "jaundiced heart"

After increases in plasma ANP had been consistently found in experimental and clinical studies, the next step was to elucidate the most probable origin and mechanism for this endocrine derangement. From experimental studies involving selective catheterization (27) and cardiomyocyte immunohistochemistry (57), it was shown that the heart was the most likely source of ANP hypersecretion. Thus, a clinical prospective study was designed to investigate the presence of hemodynamic abnormalities in patients with OJ, their relationship to plasma ANP levels and their possible reversal by biliary drainage. In a prospective study, 13 OJ patients were investigated by the insertion of a Swan-Ganz catheter (61). Hemodynamic and hormonal determinations were performed before and after internal biliary drainage. Patients with biliary obstruction displayed a subclinical myocardial dysfunction that improved after relieving the biliary obstruction (Table 32.9). Proof of the presence of impaired myocardial contractility in OJ was provided by the following findings:

**1** Differences observed in the handling of a saline load before and after biliary drainage. Once a biliary drainage was established, the volume required to raise the pulmonary capillary pressure was almost double that of the predrainage period.

**2** After internal biliary drainage, a significant reduction of the central venous pressure and the pulmonary capillary pressure was observed despite some fluid retention, as indicated by a 13% decrease of albumin concentrations. This can be attributed to the improvement of contractility parameters.

**3** The decrease in plasma renin and aldosterone concentrations indirectly indicate a better renal perfusion after biliary drainage in the setting of improved myocardial contractility parameters and slight elevation of the systolic blood pressure.

**4** Plasma total bilirubin concentrations correlated with pulmonary vascular resistance ($r = 0.69$; $P = 0.009$), mean arterial pulmonary pressure ($r = 0.67$; $P = 0.01$), and right ventricular systolic work ($r = 0.56$; $P = 0.05$).

Briefly, this study allows the conclusion that increased plasma levels of ANP in OJ reflect a subclinical myocardial dysfunction correlating with the severity of jaundice. After internal biliary drainage there is a measurable improvement of cardiac function.

**Table 32.8** Atrial natriuretic peptide (ANP), aldosterone, vasopressin (ADH), and renin activity plasma concentrations (PRA) at 24 and 72 h after common bile duct ligation (OJ group) or sham operation (SO group) in the rabbit (from (27)).

| | 24 h | | | 72 h | | |
|---|---|---|---|---|---|---|
| | OJ group | SO group | *P*-value | OJ group | SO group | *P*-value |
| ANP (fmol/ml) | 41 ± 7 | 10.7 ± 2 | 0.0001 | 28.4 ± 5 | 11.6 ± 3 | 0.02 |
| Aldosterone (ng/dl) | 14.5 ± 5 | 3.6 ± 3 | 0.001 | 18.6 ± 9 | 2.6 ± 1 | 0.002 |
| ADH (pg/ml) | 21.8 ± 7 | 12.3 ± 6 | 0.008 | 25.8 ± 8 | 9.3 ± 7 | 0.009 |
| PRA (ng/dl) | 7.4 ± 5 | 7.8 ± 7 | NS | 12 ± 5 | 3 ± 1.6 | 0.003 |

**Table 32.9** Atrial hemodynamic parameters before and after biliary drainage ($n$ = 13, two-tailed, paired Student's $t$-test; from (61)).

| | Before drainage | After drainage | *P*-value |
|---|---|---|---|
| Heart rate (b.p.m.) | 71 ± 9 | 73 ± 13 | NS |
| Mean arterial pressure (mmHg) | 93 ± 12 | 97 ± 13 | NS |
| Systemic vascular resistance (dyn. seg. $m^2/cm^5$) | 1304 ± 228 | 1213 ± 169 | NS (0.1) |
| Mean pulmonary artery pressure (mmHg) | 16 ± 4 | 14 ± 4 | NS |
| Pulmonary capillary pressure (mmHg) | 9 ± 4 | 6 ± 3 | 0.01 |
| Central venous pressure (mmHg) | 7 ± 4 | 5 ± 2 | NS (0.1) |
| Pulmonary vascular resistance (dyn. seg. $m^2/cm^5$) | 181 ± 91 | 186 ± 94 | NS |
| Cardiac output (l/min) | 5.9 ± 1.05 | 6.5 ± 1.3 | 0.04 |
| Cardiac index (l/min/$m^2$) | 3.4 ± 0.6 | 3.8 ± 0.7 | 0.03 |
| Systolic volume (ml) | 83 ± 13 | 89 ± 15 | 0.04 |
| Systolic index (SV/$m^2$) | 48 ± 6 | 52 ± 7 | 0.05 |
| Left ventricle work (g/m/$m^2$) | 94 ± 20 | 112 ± 27 | 0.02 |
| Right ventricle work (g/m/$m^2$) | 10 ± 3.5 | 11 ± 3.8 | NS |
| Volume required to reach a PCP of 14 mmHg (ml) | 427 ± 355 | 703 ± 40 | 0.01 |

## Prevention of renal dysfunction

### Forced diuresis

Dawson (14,15) advocated the use of mannitol given immediately before the operation and for the first postoperative days to prevent the decrease of the effective renal blood flow and the increased sensitivity of the renal parenchyma to anoxia. Hypertonic mannitol acts, basically: (i) as a powerful osmotic diuretic inhibiting the reabsorption of water and sodium in the proximal tubule and Henle's loop, and (ii) preventing cell swelling in the ischemic kidney. Mannitol infusion was widely used after Dawson's initial reports (16) although it did not subsequently undergo a critical analysis and despite its use continuing to be associated with a high incidence of postoperative acute renal failure (30,62,63). Gubern *et al.* (31) performed the single randomized prospective clinical trial available on perioperative mannitol infusion. In their study, the postoperative creatinine clearance was significantly impaired in the mannitol group and remained almost unaltered in the group of patients not receiving mannitol. Three patients (9.7%) died of/with acute renal failure, two in the mannitol group, and one in the group of patients not receiving mannitol. The authors concluded that mannitol infusion does not improve the postoperative renal function in OJ and may even be harmful to those patients with poor preoperative renal function.

In a randomized trial, Parks *et al.* (64) compared a preoperative bolus of furosemide vs. furosemide plus dopamine for 48 h after surgery; both groups received preoperative hydration with glucose 5%. There were no differences in the postoperative renal function between the two groups and there was not a single case of acute renal failure. This was attributed to a more careful control of fluid and electrolyte balance rather than to the specific effects of the medications given.

### Anti-endotoxin therapy

Oral bile salts (as discussed above), lactulose and non-absorbable antibiotics (41,42,65–67) have been experimentally tested and used in humans to reduce endotoxin absorption and improve the perioperative renal function. Overall these strategies have not gained popularity and their potential benefits seem rather low. A clear-cut relationship between anti-endotoxin therapy, reduced endotoxin plasma levels, and improved postoperative renal function in the clinical setting has not been conclusively proven.

### Preoperative biliary drainage

In the 1980s, several studies showed that external biliary drainage did not improve the postoperative outcome of patients with OJ due, to a large extent, to complications of the technique (68–70). Probably, the absence of bile in the intestine was also responsible for an inappropriate metabolic and nutritional response to biliary drainage. Internal biliary drainage prior to surgery, however, is very appealing and in some countries has already been implemented as a management routine (71). Trede and Schwall first reported improved outcome after pancreatectomy with prior internal biliary drainage (72). Several important physiological functions seem to improve after internal biliary drainage, such as immunity (73), hepatic function and regeneration (71–74), sensitivity of the kidney to an ischemic insult (41), and intestinal barrier function (75).

Gallardo and coworkers (29) investigated the short-term (72 h) effects of endoscopic internal biliary drainage on the water and sodium metabolism in 18 patients with OJ. A significant drop of water and sodium-retaining hormones was observed accompanied by a 10% extracellular fluid expansion detected both by bioimpedance analysis and by the fall in serum albumin concentrations (Table 32.10). The volume of fluid gained

**Table 32.10** Metabolic changes in 18 patients with obstructive jaundice undergoing endoscopic internal biliary drainage (from (29)). BIA, Bioimpedance analysis.

| | Baseline | 72 h after biliary drainage | *P*-value |
|---|---|---|---|
| Total bilirubin (mg/dl) | 15 ± 5.7 | 8 ± 4 | 0.0001 |
| Alkaline phosphatase (U/l) | 562 ± 446 | 358 ± 255 | 0.008 |
| Creatinine clearance (ml/min) | 85 ± 41 | 72 ± 30 | NS |
| Hematocrit (%) | 39.8 ± 5 | 36 ± 3 | 0.0002 |
| S-albumin (g/l) | 33.2 ± 4.7 | 29.8 ± 3.6 | 0.003 |
| Extracellular volume-BIA (% of body weight) | 20.5 ± 3.4 | 23.1 ± 3 | 0.001 |
| Natriuresis (mEq/24 h) | 52 ± 20 | 63 ± 37 | 0.04 |
| Na-excretion fraction (%) | 0.53 ± 0.3 | 0.82 ± 0.7 | NS (0.07) |
| ANP (pg/ml) | 110 ± 45 | 67 ± 46 | 0.004 |
| Plasma renin (pg/ml) | 31.2 ± 42 | 12.6 ± 10.4 | NS (0.1) |
| Aldosterone (pg/ml) | 182 ± 85 | 85 ± 45 | 0.0002 |
| Vasopressin (pg/ml) | 7.3 ± 3 | 7.6 ± 4 | NS |

did correlate with vasopressin levels after drainage, but not with plasma renin or aldosterone. There was also a good correlation between diuresis 3 days after biliary decompression and the increase of the fractional sodium excretion. Thus, from the pathophysiological point of view, it seems that better fluid homeostasis can be achieved in patients with OJ if bile flow is re-established before surgery.

Although there is ongoing controversy about the indications of preoperative internal drainage, patients with cholangitis due to choledecholithiasis clearly benefit from this procedure (19). In this subset of patients, it has been demonstrated that a high blood urea is a risk factor for mortality, and this further emphasizes the role of aggressive perioperative fluid therapy. It is also possible that malnourished patients and patients with malignant distal obstruction rather than proximal (76) may also benefit from preoperative drainage.

## Avoidance of nephrotoxic drugs

Lucena *et al.* (77) investigated the incidence of aminoglycoside-induced renal impairment in patients with or without OJ. There was a 32% incidence of impaired renal function in patients with OJ who received either gentamicin or tobramicin, an 11% incidence in patients with OJ who did not receive aminoglycosides and a 5.6% incidence in non-OJ patients receiving aminoglycoside antibiotics. The only independent predictive variable of renal failure in patients with OJ was the severity of hyperbilirubinemia. It thus seems appropriate to avoid nephrotoxic drugs in patients with OJ as part of their overall metabolic perioperative care. The availability of new betalactam antibiotics associated with betalactamase inhibitors, such as piperacillin-tazobactam (78), makes it unnecessary to administer aminoglycosides to patients with OJ, with the added advantage of good coverage against *Enterococcus faecalis*, a common pathogen in biliary tree infections.

## Fluid replacement

Experimental and clinical evidence indicates that a substantial proportion of patients with OJ may present with water and sodium depletion at the time of diagnosis. In patients with cholangitis, this may be exacerbated by translocation of plasma volume, increased insensible losses due to fever and sweating, and vasodilation due to severe sepsis. Indirect proof of the relevance of appropriate rehydration comes from several studies in which it has been demonstrated that blood urea and creatinine are independent risk factors for mortality in OJ (5–7,19). Thus, careful perioperative fluid and electrolyte balance seems of utmost importance for the appropriate maintenance of the renal function. Further support for this assertion comes from a trial (64) in which patients with OJ were generously rehydrated with glucose 5% 24 h before surgery. In this study none of the 23 patients operated on developed acute renal insufficiency.

Although no controlled studies have been carried out to determine the preferred way of rehydration and monitoring of patients with OJ, our studies suggest that normal saline would be the ideal fluid-replacing solution because volume depletion in OJ is isotonic or even slightly hypertonic. Careful monitoring of diuresis and central venous pressure are warranted for patients undergoing surgery without prior biliary drainage or resuscitation and for patients undergoing surgery for acute cholangitis.

## Summary

Almost a century has elapsed since an association between renal dysfunction and OJ was first reported and much investigation has been carried out on the intricate relationships between these two events. Recent studies in improved animal models and in the clinical setting implicate hemodynamic and fluid abnormalities as being the major contributors to renal dysfunction and postoperative renal failure in patients with OJ. These seem to be caused

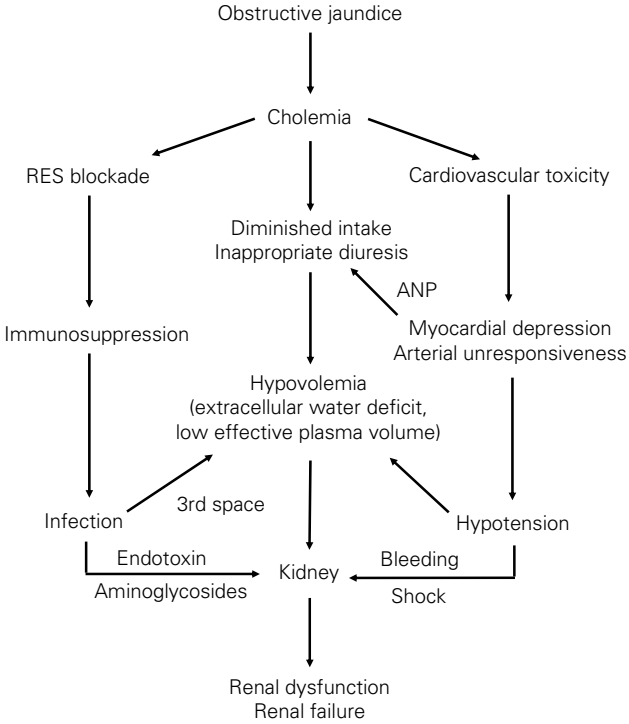


**Figure 32.5** Diagrammatic representation of the current knowledge on the pathophysiology of renal dysfunction and renal failure in obstructive jaundice (OJ). RES, Reticuloendothelial system.

by biliary products refluxing into the circulation and acting directly on the cardiovascular system and indirectly (through ANP and appetite-regulating hormones) on the thirst and hunger centers. The most evident hemodynamic and fluid derangements are blunted contractile response of the heart and peripheral vessels, reduced water and sodium intakes, negative water and sodium balances, loss of extracellular water, ANP hypersecretion, and activation of endocrine markers of volume depletion. Thus, renal failure would be the final result of nephrotoxic stimuli (aminoglycosides, hypotension, severe infection, bleeding) superimposed on a previously impaired renal function due to marginal hypovolemia and a slowly reacting cardiovascular system (Fig. 32.5). Prevention of perioperative renal dysfunction is best achieved by accurate monitoring of fluid replacement and diuresis, and avoidance of all potentially nephrotoxic drugs, particularly of aminoglycoside antibiotics. Preoperative biliary drainage coupled with appropriate rehydration and metabolic support appears to be a promising adjunct to improve the condition of patients with OJ, particularly of those with severe hyperbilirubinemia, sepsis, or advanced malnutrition.

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# Part 7
# Spontaneous Bacterial Peritonitis in Cirrhosis

# Chapter 33
# Experimental Models of Spontaneous Bacterial Peritonitis

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Spontaneous bacterial infections, such as spontaneous bacterial peritonitis (SBP), are a leading cause of death in cirrhotic patients. Many of the pathogenic features of SBP have been inferred from long-standing clinical studies, which started with the first systematized description of the disease in 1971 (1). These clinical studies have led to the hypothesis: (i) bacteria of an enteric origin cause SBP after reaching the systemic blood circulation, and (ii) SBP is the result of the uncontrolled growth of these bacteria in opsonin-deficient ascites. A rat model of cirrhosis and infected ascites was established to test this hypothesis and experimentally analyze the underlying mechanisms for the development of SBP (2). The most relevant achievement of this model has been the identification of gut bacterial translocation (BT) to mesenteric lymph nodes (MLN) as a key pathogenic step contributing to the development of infected ascites (3,4). The availability of an animal model of gut BT has fostered an interest in investigating bacterial entrance and spreading mechanisms in cirrhosis. Recent research has revealed that the barrier to enteric BT goes beyond an exclusively anatomical concept. Intestinal bacterial ecology, controlled permeability of the intestinal mucosa, and competent host immune defenses are three inseparable pillars of the emerging view of the stepwise, functional gut barrier concept. Most episodes of BT in cirrhosis are the result of serial breakdown of two or more of the above pillars of the gut barrier (5,6). Last, but not least, recent evidence indicates that the outcome of SBP depends not only on the infection itself but also on the hemodynamic derangement of patients with advanced cirrhosis, which leads to renal failure (7). Besides being a potential cause of overt infection, BT also promotes the activation of cellular components of the immune system and an abnormal profile of cytokine release. Both these factors have been found to play a role in the pathogenesis of the clinical complications of cirrhosis (8,9).

## Longstanding experience in the clinical setting: the starting hypothesis

The cirrhotic patient is highly susceptible to developing spontaneous bacterial infections, SBP being the most characteristic. Here, we briefly discuss the major features of human SBP, since it is essential to consider the clinical setting in the design of animal models that accurately mimic the patient situation.

The prevalence of SBP in unselected cirrhotic patients with ascites admitted to hospital ranges between 10 and 30% (10). SBP is defined as the infection of a previously sterile ascitic fluid, with no apparent intra-abdominal source of infection. SBP is diagnosed on the basis of a polymorphonuclear cell count > 250 cells/μl, and is confirmed by a positive culture in approximately 70% of cases (10,11). In the absence of symptoms, the presence of bacteria in ascitic fluid along with a polymorphonuclear cell count < 250 cells/μl is not considered SBP, but bacterias-cites. The pathogenic mechanisms for SBP originally proposed were based on clinical clues long before an animal model was established in 1991. The most relevant clinical observations can be summarized as follows: (i) SBP is nearly always a monomicrobial infection usually caused by *Escherichia coli* or other aerobic Gram-negative bacillus (11); (ii) the organism responsible for the infection is also isolated from blood cultures in about 50% of patients with culture-positive SBP (10,11); (iii) intestinal decontamination with quinolones reduces the rate of recurrent SBP by 71% (12); (iv) a reduced opsonic activity in the ascitic fluid (identified by a total protein content ≤ 1 g/dl ascitic fluid) and/or marked impairment in the phagocytic activity of the reticuloendothelial system (identified by advanced liver insufficiency) are associated with an increased risk of developing SBP in patients with cirrhosis and ascites (13). SBP has several other distinct characteristics. The concentration of live extracellular bacteria in the infected ascitic fluid is usually low, < 1 organism/ml, which explains the high rate of culture-negative SBP. This low bacterial load contrasts with the increased concentration of polymorphonuclear cells in ascitic fluid. In the last decade, the mechanism whereby intense inflammatory reactions can be triggered in the relative absence of live bacteria has attracted considerable attention. Current knowledge demonstrates that bacterial wall

components such as endotoxin [lipopolysaccharide (LPS)] stimulate the release by peritoneal macrophages of chemoattractants, proinflammatory cytokines, nitric oxide, and vascular endothelial growth factor (VEGF), among other regulatory and effector molecules (14). These molecules attract polymorphonuclear cells to the peritoneal cavity and have potent vasoactive effects, which can accentuate the arterial vasodilation and increased vascular permeability already present in the cirrhotic patient. This enhanced vasodilation leads to a further reduction in the effective arterial plasma volume and an increased activity of endogenous vasoactive systems, and eventually gives rise to renal failure. In fact, renal impairment is the strongest predictor of patient mortality in episodes of SBP responsive to antimicrobial therapy (15). The development of kidney failure in SBP is related to the extent of the inflammatory response triggered by the infection, estimated by the level of proinflammatory cytokines in ascitic fluid and in serum (7).

Based on this clinical evidence, it was originally postulated that SBP is the consequence of the seeding and uncontrolled growth of blood-borne bacteria of enteric origin in opsonin-deficient ascites. However, recent clinical studies have underlined the relevance of the bacteria–host immune proinflammatory response. The latter is proposed to cause further deterioration in the hemodynamic status of the cirrhotic patient with ascites, a decisive factor in the outcome of SBP.

## Contribution and limitations of the only available animal model of spontaneous bacterial peritonitis

Proctor and Chatamra induced cirrhosis in rats by weekly intragastric administration of $CCl_4$ at a dose adjusted to body weight loss (16). In an attempt to gain insight into the fundamental processes involved in the pathogenesis of SBP, Runyon *et al.* modified the method and designed the first model of cirrhosis and infected ascites in 1991 (2). Their method of cirrhosis induction led to a high rate of early mortality (53%), a high yield of cirrhosis and ascites formation in the surviving animals (93%), and acceptable rates of ascitic fluid infection (55%) (Table 33.1). However, SBP was followed by a rapidly fatal course. In fact, most cases were detected on post-mortem examination and less than one-third were diagnosed in live rats, shortly before death.

The rat model of cirrhosis by weekly $CCl_4$ gavage has been the model used by most researchers to explore experimental SBP (Table 33.1) (2,3,5,17–26). Once metabolized in the liver by P450 cytochromes, $CCl_4$ is hepatotoxic (27). Accordingly, phenobarbital (35 mg/dl), a microsome inducer, is administered in the tap water starting 1 week before and throughout $CCl_4$ administration to hasten cirrhosis induction. In our experience, weekly gavage is greatly facilitated by slight isoflurane anesthesia. In experiments using the oral route, each dose of $CCl_4$ is adjusted according to the weight loss caused by the previous dose (16). Though tedious, dose adjusting has the distinct advantages of achieving more homogeneous liver damage and enabling the administered dose to be individually estimated. Early recognition of ascites formation is an important step in the process of cirrhosis induction by this method, since once ascites appears the dose of the hepatotoxin should be reduced (2,16). Ascites can be recognized by repeated paracentesis or by a sharp increase in body weight and bulging of the flanks. There are fewer reports of cirrhosis with infected ascites induced by inhaling $CCl_4$ compared with those using the intragastric administration (4,8,28), although this is otherwise considered an appropriate method.

Induction of cirrhosis by intragastric $CCl_4$ is associated with high overall mortality (range 41–67%), especially in the early phases of the process due to acute hepatonecrosis. Most (75%) of the surviving animals develop cirrhosis. Ascites appears at around 10–12 weeks, but there is considerable variation (range 6–26 weeks) in spite of repeated dosing of $CCl_4$ and cirrhosis induction. Ascites does not develop in 25% of the animals. The variable incidence of infected ascites among studies probably reflects the liver disease stage at the time of ascitic fluid examination. The rate of infection is high (50–70%) when ascitic fluid is examined shortly before death (2,3,18), and lower when the animal is sacrificed at a fixed time after ascites formation (34%) (5) or after the beginning of toxin administration (11%) (4).

Aerobic Gram-negative bacilli, specifically *E. coli*, are responsible for most episodes of ascitic fluid infection in rats, as occurs in human SBP (Table 33.1). There are, however, some interesting differences: (i) the frequency of enteric bacteria other than *E. coli*, such as Proteus sp, Enterococcus, and to a lesser extent, Klebsiella and Pseudomonas, is slightly higher than in patients (2,5,17–22); (ii) up to 10% of experimental infected ascites are polymicrobial (2,5,17–22); (iii) the intensity of the peritoneal inflammatory response is lower in the rat model, as assessed by a lower ascitic fluid polymorphonuclear cell count, accounting for around 30% of the ascites leukocyte counts in rats and 90% in humans (2,17,19). However, it should be mentioned that a limitation of many experimental studies is that they report the incidence of infected ascites, without considering the presence and extent of the ascitic fluid leukocyte response, i.e. they do not make the distinction between SBP and bacteriascites made in the clinical setting.

The suitability of the gavage $CCl_4$ rat model of cirrhosis for investigating infected ascites has been questioned. It is argued that gastric instillation of $CCl_4$ might be harmful to the mucosa of the gastrointestinal tract, increasing its permeability and promoting the passage of enteric

**Table 33.1** Studies of bacterial translocation and infected ascites in the $CCl_4$ cirrhotic rat model.

| Author, year (Ref.) | No. of animals | Experimental groups | Route of $CCl_4$ administration | Mortality of the model | Mean time to ascites, weeks (range) | Positive microbiological cultures | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | MLN | Ascites | Portal blood | Peripheral blood | Enterobacteriaceae in MLN (*E. coli*) |
| Runyon 1991 (2) | 38 | Cirrhosis with ascites | Intragastric | 53% | NR (6–20) | NR | 55% | NR | NR | 96% (75%) |
| Llovet, 1994 (17) | 22 | Cirrhosis with ascites | Intragastric | 58% | 12 (7–19) | 45% | 50% | 32% | 10% | 80% (70%) |
| | 10 | Normal rats | | | | 0 | 0 | 0 | 0 | |
| Runyon, 1994 (3) | 32 | Cirrhosis with ascites | Intragastric | 41% | 8 (4–20) | 78% | 50% | 20% | 0 | 90% (60%) |
| | 23 | Normal rats | | | | 4% | 0 | 0 | 0 | |
| Garcia-Tsao, 1995 (4) | 9 | Cirrhosis with ascites | Inhalation | NR | NR (12–16) | 56% | 11% | NR | 0 | 100% (100%) |
| | 9 | Cirrhosis without ascites | | | | 0 | | NR | 0 | |
| | 12 | Normal rats | | | | 0 | | NR | 0 | |
| Runyon, 1995 (18) | 20 | Cirrhosis with ascites (96%) + no treatment | Intragastric | NR | NR (3–15) | 50% | 70% | NR | NR | 83% (75%) |
| | 25 | Cirrhosis with ascites (96%) + norfloxacin | | | | 28% | 28% | | | 28% (0) |
| Llovet, 1996 (19) | 12 | Cirrhosis with ascites | Intragastric | 67% | 15 (7–26) | 41% | 0 | 8% | 0 | 16% (16%) |
| | 16 | Cirrhosis with ascites + HS | | | | 69% | 6% | 12% | 6% | 38% (10%) |
| | 16 | Cirrhosis with ascites + norfloxacin | | | | 31% | 6% | 6% | 6% | 0 |
| | 10 | Normal rats | | | | 0 | 0 | 0 | 0 | 0 |
| | 10 | Normal rats + HS | | | | 50% | 0 | 0 | 0 | 10% (0) |
| | 10 | Normal rats + HS + norfloxacin | | | | 0 | 0 | 0 | 0 | 0 |
| Guarner, 1997 (20) | 35 | Cirrhosis with ascites | Intragastric | NR | NR | 83% | 60% | NR | NR | 81% (58%) |
| | 10 | Normal rats | | | | 0 | 0 | | | |
| Casafont, 1997 (21) | 33 | Cirrhosis | Intragastric | 56% | NR | 69%† | 48% | 31% | 31% | 85% (87%) |
| Llovet, 1998 (22) | 27 | Cirrhosis with ascites | Intragastric | 60% | NR | 37% | 44% | 15% | 11% | 54% (54%) |
| | 10 | Normal rats | | | | 10% | | 0 | 0 | |
| Guarner, 1999 (23) | 14 | Cirrhosis + no treatment | Intragastric | | | 78% | 50% | NR | NR | 71% (43%) |
| | 17 | Cirrhosis + TMP/SMX | | | | 47% | 29% | | | 30% (0) |
| Wiest, 1999 (8) | 78 | Cirrhosis with ascites | Inhalation | NR | NR | 51% | NR | NR | NR | NR |
| | 26 | Normal rats | | | | 0 | | | | |
| Perez-Paramo, 2000 (5) | 29 | Cirrhosis with ascites | Intragastric | 57% | 10 (6–19) | 48% | 34% | 17% | 14% | 93% (93%) |
| | 20 | Normal rats | | | | 0 | 0 | 0 | 0 | |

†Includes malnourished rats. In nonmalnourished rats, MLN cultures were negative in cirrhotic rats without ascites and positive in 50% of rats with ascites. §Glutamate and vitamin C. HS, Hemorrhagic shock; LGG, Lactobacillus strain GG; La1, *Lactobacillus johnsonii* La1; MLN, mesenteric lymph nodes; NR, not reported;TMP/SMX, trimethoprim/sulfamethoxazole.

**Table 33.1** (Continued.)

| Author, year (Ref.) | No. of animals | Experimental groups | Route of $CCl_4$ administration | Mortality of the model | Mean time to ascites, weeks (range) | Positive microbiological cultures | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | MLN | Ascites | Portal blood | Peripheral blood | Enterobacteriaceae in MLN (*E. coli*) |
| Perez-Paramo, 2000 (5) | 12 | Cirrhosis with ascites + placebo | | | | 58% | NR | NR | NR | 86% (86%) |
| | 13 | Cirrhosis with ascites + propranolol | | | | 15% | NR | NR | NR | 100% (100%) |
| Pardo, 2000 (24) | 15 | Cirrhosis with ascites + placebo | Intragastric | NR | NR | 40% | 7% | 7% | 7% | 83% (50%) |
| | 15 | Cirrhosis with ascites + cisapride | | | | 0 | 0 | 0 | 0 | 0 |
| | 5 | Normal rats | | | | 0 | 0 | 0 | 0 | 0 |
| Chiva, 2002 (25) | 8 | Cirrhosis (87% ascites) + placebo | Intragastric | NR | NR | 62% | 12% | NR | NR | 100% (100%) |
| | 11 | Cirrhosis (64% ascites) + La1 + antioxidants§ | | | | 0 | 0 | | | |
| | 10 | Cirrhosis (40% ascites) + antiioxidants§ | | | | 0 | 0 | | | |
| Bauer, 2002 (28) | 15 | Cirrhosis with ascites | Inhalation | 19% | 17 ± 8 | 93% | 60% | 33% | NR | 67% (NR) |
| | 19 | Cirrhosis with ascites + LGG | | | | 84% (15% LGG) | 32% | 31% | | 63% |
| | 10 | Cirrhosis with ascites + norfloxacin | | | | 70% | 40% | 10% | | 20% |
| | 10 | Cirrhosis with ascites + LGG + norfloxacin | | | | 100% (40% LGG) | 40% | 20% | | 40% |
| | 10 | Normal rats | | | | 10% | | 0 | | 10% |
| Lorenzo, 2003 (26) | 11 | Cirrhosis with ascites + placebo | Intragastric | 60% | NR | 55% | 0 | 0 | 0 | 50% (50%) |
| | 13 | Cirrhosis with ascites + clolylglycine | | | | 15% | 0 | 0 | 0 | 0 (0) |
| | 13 | Cirrhosis with ascites + clolylsarcosine | | | | 23% | 0 | 0 | 0 | 0 (0) |

†Includes malnourished rats. In nonmalnourished rats, MLN cultures were negative in cirrhotic rats without ascites and positive in 50% of rats with ascites. §Glutamate and vitamin C. HS, Hemorrhagic shock; LGG, Lactobacillus strain GG; La1, *Lactobacillus johnsonii* La1; MLN, mesenteric lymph nodes; NR, not reported; TMP/SMX, trimethoprim/sulfamethoxazole.

bacteria to the bloodstream by a mechanism different from the cirrhosis itself. However, it should be considered that $CCl_4$ does not directly exert cellular toxicity, but only induces toxic effects after its absorption and toxic metabolite formation in the liver (27). In agreement with this, the intragastric administration of one to three doses of $CCl_4$ causes no significant changes in the histology, permeability or motility of the bowel in Sprague-Dawley rats (29).

A major achievement of studies performed in the $CCl_4$ cirrhotic rat model has been to establish a close relationship between BT to MLN and the infection of ascites. BT to MLN occurs in most cirrhotic rats with infected ascites (80–100%) (3–5,17,18,20–22,28) and also in 30–60% of cir-

rhotic rats with non-infected ascites. The latter finding is in agreement with recent observations in the setting of human cirrhosis (30). The high rate of species and genotype concordance among the bacteria (mostly Enterobacteriaceae) isolated from the ascitic fluid and MLN further supports the notion that translocation of enteric microorganism to the MLN is an early step in the development of SBP (22). Portal bacteremia is detected on average in one-quarter of rats with BT. In most of these cases, the same microorganism is isolated from blood and MLN (Table 33.1). The occurrence in all series of a small percentage of rats with SBP but without BT might reflect the non-enteric origin of some cases of infected ascites, the low sensitivity of the current method of detecting viable microorganisms in the MLN, and/or the part played by the host immune defense mechanism in clearing translocated bacteria so that clearance is uneven at the different anatomical sites.

The pioneering work of Runyon *et al.* (2) provided a framework for the optimization of models that more accurately attempt to replicate the clinical setting. The demonstration of BT to MLN in the $CCl_4$ cirrhotic rat model and its close relationship with infected ascites has extended the use of the model to evaluating the spread of bacteria in experimental cirrhosis.

## Gut bacterial translocation: concept and general pathogenic implications in disease

BT is defined as the process by which normal microflora cross the gut mucosa and reach the local MLN and eventually the systemic circulation. BT does not only occur in cirrhosis but may also appear in a broad range of clinical conditions such as intestinal obstruction, hemorrhagic shock, sepsis, endotoxemia, severe trauma, and heat injury (31). The results of several studies indicate the major mechanisms promoting BT are intestinal bacterial overgrowth, altered permeability of the gut mucosa, and defects in host immune defenses (6). This means the gut barrier is more of a functional than an anatomical concept.

Under experimental conditions, BT is assessed by isolating, homogenizing, and culturing the entire chain of MLN under strict sterile conditions. The culture of MLN homogenates by broth inoculation instead of spreading on agar plates is more sensitive and renders a greater number of positive samples (28). Thus, the operational definition of BT has relied upon the culture of viable bacteria in the MLN. However, it should be underscored that the currently accepted definition of BT has certain inherent limitations. First, the release of bacterial products such as endotoxin from nonviable bacteria can promote many of the physiopathological consequences attributed to BT (6), and may even represent a relevant pathogenic mechanism in cirrhosis, as discussed below. Second, it is difficult to discriminate whether the increase in viable extracellular bacteria in the MLN is due to increased BT or to an immunodeficient host response.

Gut bacterial ecobiology has a striking part to play in the intestinal barrier that prevents BT. The villi of the epithelial apical surface are covered by a layer of mucus, coated with a biofilm of anaerobic bacteria (32). These anaerobic bacteria prevent adherence to enterocytes and limit the overgrowth of aerobic Gram-negative enteric bacilli (mainly Enterobacteria), the pathogenic elements in BT. Immunological factors such as secretory IgA further prevent the adherence of aerobic bacteria to the enterocytes. Any expansion of the enteric Gram-negative bacterial population or reduction in anaerobic microflora increases susceptibility to BT. The endogenous factors that maintain the microbiological ecology of the gut are gastric acidity, pancreatobiliary secretion, intestinal immunological factors, and (mainly) intestinal peristalsis. The anatomical barrier of the gut is composed of a layer of simple columnar epithelial cells interspersed with specialized cells such as goblet cells, lymphocytes, dendritic antigen-presenting cells, and M cells. The maintenance of normal epithelial cell structure and function, including the preservation of tight junctions, avoids the transepithelial or paracellular migration of bacteria. Contaminating bacteria are phagocytosed by the antigen-presenting cells; other immune mechanisms then contribute to rapid bacterial clearance. BT occurs at a low rate in normal individuals and indigenous bacteria such as *E. coli* continuously translocate when their levels exceed $10^8$/g cecum (33). A markedly increased BT rate and/or host immunocompromise leads to bacterial replication in the MLN and eventual dissemination through lymphatic or vascular channels.

## Bacterial translocation in cirrhosis: contributions of the animal model

The rat model of $CCl_4$ has been a valuable tool for experimentally addressing BT mechanisms and their pathogenic role in cirrhosis. BT occurs in around 50% of cirrhotic rats (range 37–93%), a proportion much greater than the 0–10% observed in normal rats (Table 33.1). This wide range can be attributed to differences in the interval from the beginning of $CCl_4$ administration or ascites appearance to the time of animal sacrifice and to different methods of MLN culture.

A remarkable feature of cirrhosis is that there is a concurrent alteration of the three pillars (microflora content, mucosal integrity, immunity) that support the gut barrier (5,6), thus explaining the high rate of BT observed (Fig. 33.1). Simultaneous damage to the three gut barrier pillars results in a greater susceptibility to BT than do clinical or experimental situations that affect only one

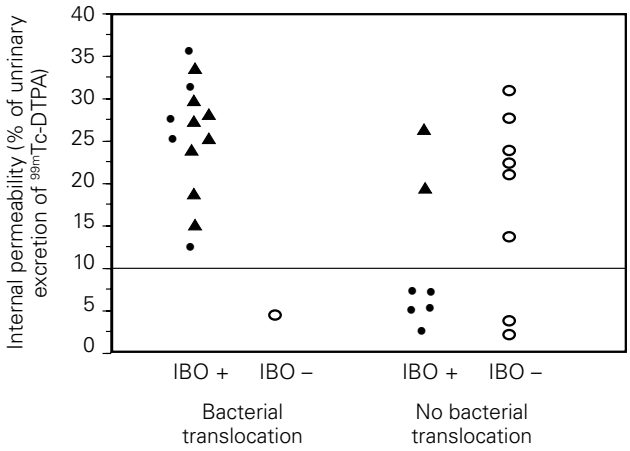


**Figure 33.1** Intestinal permeability of cirrhotic rats with ascites as measured by the fractional urinary excretion of an oral dose of $^{99m}$Tc-DTPA. Filled circles and triangles represent animals with intestinal bacterial overgrowth (IBO+), and empty circles represent animals without intestinal bacterial overgrowth (IBO–). The triangles represent animals with spontaneous bacterial peritonitis. Bacterial translocation was present in most cases of spontaneous bacterial peritonitis. Bacterial translocation to mesenteric lymph nodes required the simultaneous presence of IBO and severe damage to intestinal permeability, as expressed by a urinary excretion of $^{99m}$Tc-DTPA > 10%. Thirteen of the 15 animals with IBO and a urinary excretion of $^{99m}$Tc-DTPA > 10% developed bacterial translocation, and two developed spontaneous bacterial peritonitis without bacterial translocation. (Reproduced with permission from (5).)

alone (34). Moreover, the defective immunological clearance of translocated Gram-negative bacteria that occurs in cirrhosis is accompanied by pronounced endotoxin-driven proinflammatory cytokine release (8,9). Also, tumor necrosis factor (TNF)-α and nitric oxide production promoted by the endotoxin cascade causes further oxidative damage to the bowel wall (35,36).

Patients and animal models of cirrhosis show abnormalities in the coordinated motor function of the small bowel that delay intestinal transit and favor intestinal overgrowth of Enterobacteria (5,24,37–39). Indeed, intestinal motor anomalies are more severe in cirrhotic individuals that develop intestinal bacterial overgrowth (IBO) (3,5). Possible factors proposed as underlying the intestinal dysmotility of cirrhosis are oxidative damage to the intestinal wall, increased activity of the sympathetic nervous system, and enhanced production of nitric oxide (5). Cirrhosis is also associated with structural and functional alterations in the intestinal mucosa, which may increase its permeability to macromolecules and bacteria. Experimental cirrhosis gives rise to oxidative stress in the mucosa of the small intestine, as seen by increased xanthine oxidase activity and altered antioxidant status, increased malondialdehyde levels, increased lipid peroxidation of the brush border membranes, and abnormal intestinal transport (40,41). These lesions resemble those observed in ischemia/reperfusion injury, hemorrhagic shock, and endotoxemia, in which intestinal permeability and susceptibility to BT are increased. However, it should be noted that with respect to cirrhosis, the above concepts form only a working hypothesis, since oxidative damage to the intestinal mucosa, increased permeability, and BT have been separately studied, and firm links among them have not been formally established.

In cirrhosis, immune defense in the MLN is defective, and, together with the marked increase in BT rate, allows enteric bacteria and their products (such as endotoxin) to reach the blood. Several *in vivo* and *in vitro* experimental models of cirrhosis, as well as humans with advanced cirrhosis, have shown deficiencies in the bacteriostatic and opsonic capacity of serum, in phagocytosis by neutrophils, and effector function of immune cells circulating in blood (42–44). The splanchnic hyperemia that follows portal hypertension impairs rolling, adherence, and migration of phagocytic cells in mesenteric venules, providing another contributing mechanism to the impaired immune response in cirrhosis (45). The competence of the immune system to halt BT is further highlighted by the lack of BT to MLN in rats with chronic prehepatic portal hypertension, which, unlike the cirrhotic rat, are immunocompetent (46).

In addition to damage to the epithelial barrier and a deficient immune system, IBO is a prerequisite for BT to develop in the draining lymph node of an intestinal segment. Bacterial overgrowth in cirrhosis has been investigated both in the small bowel and in the cecum. Initial research was prompted by the finding that germ-free mice fed with *E. coli* showed the bacterium in the cecal wall, as detected by immunohistochemistry (47). This led to the demonstration of *E. coli* in the cecal wall of cirrhotic rats, 60% showing BT to MLN (4). Furthermore, morphological analysis of the bowel of cirrhotic rats with ascites revealed submucosal edema and inflammation, most evidently in the cecum (4,17,19). These data support the notion of a transcecal origin for BT in cirrhosis. However, recent evidence is changing the paradigm towards considering the small intestine as an additional origin of BT in cirrhosis. In noncirrhotic animal models of BT, the distal ileum is the primary site of penetration of specific pathogens, although the cecum and large intestine are exposed to a larger number of bacteria for longer periods of time than the small intestine (48). In cirrhotic models, IBO is necessary for BT to occur. More than 90% of rats with BT show small intestine (jejunal or ileal) bacterial overgrowth, which in both cases is mostly caused by the same organism (5,24,26). BT rarely (0–11%) occurs in the absence of IBO (Fig. 33.1). Furthermore, total aerobic bacterial loads in the jejunum and ileum are systematically greater in cirrhotic rats with ascites than in control rats (5,24,26). The cecal bacterial load, however, has been found to be either increased or normal (20,24). Considering the above evidence, we and others have ma-

nipulated the intestinal bacterial load in an attempt to reduce the rate of BT (5,18,19,23,24). Notably, in one of these reports, treatment of ascitic cirrhotic rats with cisapride, a well-known prokinetic agent, decreased the rate of BT and the jejunal—but not the cecal—bacterial load (24).

An exciting feature of this field has been the frequent interplay between experimental and clinical research in terms of the biomedical relevance of BT. According to the current pathogenic hypothesis of SBP, decreasing the enteric load of Gram-negative aerobic bacilli should reduce the rate of SBP. The hypothesis has been confirmed: poorly orally absorbed antibiotics, mainly quinolones, decrease the frequency of SBP in high-risk cirrhotic patients (12). However, in the rat model of cirrhosis, norfloxacin reduces the stool count and BT of Gram-negative organisms, but not the overall rate of BT. This is due to a change in gut flora including the expansion and subsequent BT of Gram-positive cocci (18) (Table 33.1). Moreover, both in humans and animals, the frequent emergence of quinolone-resistant fecal bacteria has led to the search for alternatives to antibiotics. The rat model of cirrhosis has contributed to addressing this problem and several strategies that attempt to overcome it have proved successful (Table 33.1).

Encouraging results have been obtained using agents such as cisapride and propranolol (Table 33.2), which reduce the enteric bacterial load and BT by decreasing the intestinal transit time in animals with cirrhosis (5,24). Sympathetic over-activity, as occurs in cirrhosis with ascites, delays intestinal transit through a $\beta_2$-adrenoceptor-mediated pathway. It is tempting to speculate that blockade of this route by propranolol could accelerate bowel motility. The rate of SBP is reduced in patients receiving nonselective β-blockers as prophylaxis for variceal hemorrhage (49).

Probiotics have yielded less promising results when tested as re-equilibrators of the level of potentially pathogenic Enterobacteria and protective anaerobic bacteria to reduce the BT rate. When administered to cirrhotic rats with ascites, *Lactobacillus rhamnosus* strain GG adequately colonized the bowel, but was unable to reduce the cecal content of aerobic bacteria and the rate of BT (28). The use of probiotics to reduce BT in experimental models of acute liver injury and of prehepatic portal hypertension has led to similarly discouraging results (49,50). It is possible that probiotics may exert their beneficial effect in infectious diseases due to primary disruption of intestinal microecology (51), whereas they are ineffective in processes such as cirrhosis when damage affects several pillars of the gut barrier. Interestingly, *L. rhamnosus* itself translocated to MLN in a quarter of the rats, underlining the severity of gut barrier disruption and immunocompromise in cirrhosis (28).

Feeding cirrhotic rats conjugated bile acids has yielded good results (26). Intraluminal concentrations of conjugated bile acids are reduced in cirrhosis, due to diminished bile acid secretion and deconjugation of bile acids by overgrowing bacteria. In turn, as conjugated bile acids are bacteriostatic, their decreased intraluminal concentration might promote further bacterial overgrowth. A course of conjugated bile acids given to cirrhotic rats with ascites increased bile acid secretion, eliminated IBO, and decreased BT (26).

**Table 33.2** Effect of propranolol on bacterial translocation, intestinal bacterial overgrowth, intestinal transit, and intestinal permeability of cirrhotic rats with ascites (reproduced with permission from (5)).

| | Placebo | Propranolol |
|---|---|---|
| Number of animals | 12 | 13 |
| Portal pressure (mmHg) | 20.9 ± 4 | 17.2 ± 4** |
| Bacterial translocation (%) | 58 | 15** |
| Spontaneous bacterial peritonitis (%) | 33 | 8 |
| Intestinal bacterial overgrowth (%) | 67 | 15** |
| Aerobic bacterial stool count (logCFU/g) | 7.7 ± 0.3 | 7.1 ± 0.3* |
| Intestinal transit (geometric center ratio) | 0.23 ± 0.1 | 0.44 ± 0.1* |
| Intestinal permeability (% urinary excretion of $^{99m}$Tc-DTPA) | 16.4 ± 7 | 19.5 ± 8 |

*$P$ < 0.01 vs. placebo, **$P$ < 0.05 vs. placebo. CFU, Colony-forming units; $^{99m}$Tc-DTPA, $^{99m}$Tc diethylenetriaminepentaacetate acid.

Finally, the use of antioxidants constitutes a preliminary approach to avoid BT. Chiva *et al.* recently reported the ability of the antioxidants vitamin C and glutamate to reduce BT, and the enteric bacterial load and oxidative stress of the bowel wall in cirrhotic rats (25). In accordance with these results, attenuation of intestinal oxidative damage mediated by xanthine dehydrogenase/oxidase reduces BT in bile duct-ligated cirrhotic rats (52). Antioxidants (vitamins C and E, glutamate) have also been proposed to improve intestinal barrier function and ameliorate the gut permeability that occurs after ischemia/reperfusion injury, radiation injury, and parenteral feeding.

The rat model of $CCl_4$ cirrhosis has established the close link among the disturbed gut ecology, disrupted epithelial barrier, and BT to MLN. Although it was originally developed to reproduce human SBP, it has evolved into the preferred tool for exploring the mechanisms underlying BT in cirrhosis and searching for novel therapeutic approaches.

## Lessons learned from the study of bacterial translocation in cirrhotic patients

The rat model has been essential in characterizing some of the mechanisms leading to spontaneous bacterial infection in cirrhosis. Ethical constraints limit the use of the biopsy of MLN in cirrhotic patients to post-mortem studies or during abdominal surgery, including liver transplantation (53). In this setting, overall BT is present in 9%

of cirrhotic patients undergoing liver transplantation or hepatic resection, a value not significantly different from that found in noncirrhotic patients undergoing abdominal surgery (30). BT attains a value of 20% in the group of cirrhotic patients with ascites. In this human study, sampling was limited to a single MLN, so that this could be an underestimate of the prevalence of BT in cirrhosis.

The clinical limitations to culturing bacteria from MLN and blood has driven longstanding efforts to seek surrogate methods of identifying BT in the cirrhotic patient. Until recently, we lacked reliable markers to identify individuals who suffer passage of bacteria or their products (endotoxins) to the circulation. One reason for this may be that the bacteremia that follows BT is episodic and transient in nature, and the half-life of endotoxin (LPS) is short (10–30 min). This limits the use of LPS as a clinical marker for BT in most patients. This drawback is reflected in the rate of LPS detection in cirrhosis, which ranges from 0 to 93% in several studies (cited in (9)) and may explain why most episodes of bacterial seeding remain undetected through common bacteriological methods.

New strategies of measuring LPS-binding protein (LBP) and circulating bacterial DNA fragments have been developed recently to identify patients with cirrhosis who show increased exposure to bacteria or their products. The use of LBP to track BT is based on the biology of the LPS signaling transduction pathway. Once in the circulation, LPS promotes the hepatic synthesis of LBP, a serum protein that enhances the binding of LPS to the CD14 subunit of the LPS receptor. LBP peaks in plasma 2–3 days after transient bacteremia or endotoxemia, and levels remain elevated up to 72 h later (54,55). Circulating levels of LBP are similar in healthy controls (5.62 μg/ml) and in cirrhotics without ascites (5.46 μg/ml) (9) (Fig. 33.2). Interestingly, serum LBP is high (> 9.6 μg/ml) in 42% of cirrhotic patients with ascites. Thus, increased LBP levels are not a feature of cirrhosis itself and are probably triggered by enteric bacteria, since decontamination of the bowel with norfloxacin normalizes the plasma LBP level in this subset of cirrhotic patients with ascites. It is of note that plasma LBP levels remained stable in a group of patients who received placebo over 1 month. The second strategy has demonstrated the simultaneous presence of bacterial DNA with similar fingerprints in the serum and ascitic fluid of one-third of cirrhotic patients with culture-negative, non-neutrocytic ascites, probably representing single clone episodes of subclinical translocation and bacterial seeding in the peritoneal cavity (56).

The development of ascites is a hallmark in the natural history of cirrhosis and is associated with gut BT in addition to markedly increased portal hypertension and exacerbated endothelial disturbances. BT to MLN occurs only in cirrhotic rats with ascites. Notably, frequent passage of bacteria to the systemic circulation in patients with cirrhosis, as assessed by plasma LBP, occurs only in a subset of subjects with ascites. As discussed below, bacteria worsen the prognosis of this subset of patients.

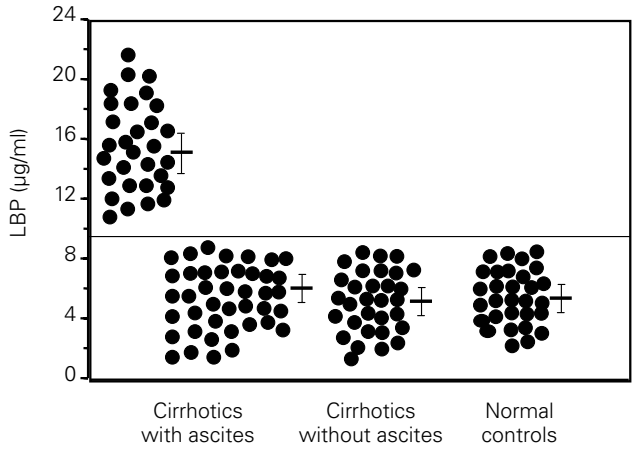


**Figure 33.2** Plasma LPS-binding protein (LBP) levels in cirrhotic patients with and without ascites, and in healthy controls. Plasma LBP was significantly greater ($P < 0.01$) in cirrhotics with ascites than in the other groups. High LBP was defined as > mean + 2 SD in healthy controls (> 9.62 μg/ml, horizontal line). One-third of the patients, all with ascites, showed high LBP. Horizontal bars and squares represent the mean and SEM respectively. (Reproduced with permission from (9).)

## Bacterial translocation aggravates the course of cirrhosis

There is accumulating evidence to support the idea that cirrhosis should be envisaged as a disease in which immune-mediated inflammation may play a prevalent pathogenic role. The threat of BT does not only involve the risk of microbial contamination becoming an overt infection, but is also related to the proinflammatory response of the host immune system to the translocated bacterial products.

In the cirrhotic patient, gut translocated bacteria interact with the innate and acquired branches of the immune system. On the one hand, bacteria and gut products translocated through the intestinal epithelia contain conventional antigens that are processed by the so-called antigen-presenting cells (APC) (such as monocytes, macrophages, and dendritic cells) present in the gut-associated lymphoid tissue. Microbial antigens presented by APC trigger specific T- and B-lymphocyte responses (57). On the other hand, in addition to the antigen-specific receptors clonally distributed in the lymphocytes, the immune system uses polyclonal pattern recognition receptors expressed in the cellular components of the innate immune system, as well as some lymphocyte subsets. For example, APC express receptors for endotoxin and lipoteicoic acid, which are components of the Gram-negative and -positive bacterial wall, respectively. These pattern recognition

receptors are key elements in the discrimination of self and nonself components, and are crucial for the control of the class II immune response triggered upon recognition of the antigen (58). In the clinical and experimental setting, the liver response to etiological factors and the host immune response differ, reflecting polymorphism in the systems processing hepatotoxin, inflammatory cascade molecules, and host immunoregulatory genes.

Upon massive challenge by bacterial products, there is a subset of patients who suffer septic shock. This short, immune response leads to deregulated production of pro- and anti-inflammatory cytokines, which causes lethal tissue damage and immune system paralysis (59). There is emerging evidence for cirrhosis occupying a place at the extreme end of the spectrum of host–bacterial interaction. Recurrent, low load contamination of the host by BT might promote the spiky release of TNF-α, interleukin (IL)-6 and other proinflammatory cytokines (8,9). This cytokine profile has been related to the vascular and renal damage of cirrhosis.

The pioneering work of Groszmann *et al.* revealed that TNF-α is released into the circulation from the MLN of cirrhotic rats with ascites (8). These authors further linked TNF-α production and plasma TNF-α levels in cirrhotic rats with ascites to BT to MLN. Increased TNF-α expression has been recently observed in the MLN of cirrhotic patients at the time of liver transplantation (53). We used plasma LBP to identify cirrhotic patients who suffered frequent BT. This subset represents a third of our patients (Fig. 33.2), but these patients are those with highest circulating levels of TNF-α, IL-6 and other proinflammatory mediators (9). Intracellular cytokine staining and quantitative flow cytometry revealed that activated monocytes are the major circulating cell subpopulation contributing to increased serum TNF-α levels in cirrhosis with ascites and high LBP (60). In these patients, the serum level of TNF-α and the amount of TNF-α spontaneously expressed in monocyte lineage cells were directly correlated. Current evidence indicates that the intense LPS-driven proinflammatory cytokine release observed in cirrhotic animals with BT and in cirrhotic patients with high LBP contributes to the immune and hemodynamic derangement of the cirrhotic patient. Recent research performed in our laboratory indicates that cirrhotic patients with ascites and high LBP show striking alterations in T-helper and T-cytotoxic cell compartments, which might be related to the immunodeficiency of cirrhosis (60). The possible pathogenic role of enteric bacterial products in triggering and/or preserving immune cell abnormalities in cirrhosis is also supported by their amelioration after bowel decontamination with norfloxacin.

The involvement of BT in the hemodynamic disturbance of cirrhosis has also been investigated. Circulatory derangement is more intense in cirrhotic rats with BT to MLN and cirrhotic patients with ascites and high LBP than in their counterparts without BT or with normal LBP, respectively (8,9). The disturbances evaluated were lower arterial pressure and greater vascular hyporeactivity in animals, and lower systemic vascular resistance and concomitant enhanced activation of endogenous vasoactive systems in humans. The close relationship between nitric oxide overproduction and markers of frequent exposure to bacterial products, as well as serum TNF-α observed in these studies, suggests a link between bacteria or bacteria-delivered endotoxin and the release of proinflammatory cytokines, such as TNF-α. TNF-α may stimulate nitric oxide synthase activity and further aggravate peripheral vasodilation in cirrhosis.

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# Chapter 34
# Pathogenesis and Clinical Features of Spontaneous Bacterial Peritonitis

José Such, Carlos Guarner, and Bruce Runyon

## Introduction and historical aspects

Cirrhosis is the most prevalent cause of acquired immunodeficiency in the USA, and in this setting, bacterial infections are a frequent and severe complication. Spontaneous bacterial peritonitis (SBP) is probably the most characteristic infectious complication of cirrhosis. Since an early description by Conn and Fessell in 1964 (1), many different aspects of SBP have been widely investigated during the last decades, such as clinical presentation, diagnosis, pathogenesis, therapeutic and prophylactic measures, and prognosis. Although SBP was initially considered to be a rare event, the routine practice of paracentesis (2) showed that the real prevalence ranged from 7 to 31% in patients with cirrhosis with ascites admitted to hospital (3).

SBP has a poor short- and long-term prognosis, requires urgent antibiotic treatment, and prophylaxis is considered necessary to avoid SBP recurrence. Due to a high mortality rate observed in patients with SBP or survivors of an SBP episode during follow-up, its development is usually considered an indication for liver transplantation.

In this chapter we will outline the basic aspects of SBP, with special emphasis on the pathogenic mechanisms related to the development of this complication. The knowledge of these mechanisms has allowed physicians to achieve an early diagnosis and to improve the treatment and prevention of this severe and life-threatening complication.

## Definition and variants of infected ascites

Diagnosis of ascitic fluid (AF) infection is currently based on the evaluation of AF obtained by a diagnostic paracentesis. Several variants of infected ascites have been described based on the results of AF culture and polymorphonuclear neutrophil (PMN) count (Table 34.1).

### Spontaneous bacterial peritonitis

SBP has been defined as an AF infection, with a positive (usually monomicrobial) culture, with a PMN count $\geq 250/mm^3$, in the absence of any intra-abdominal surgical source of infection. As defined above, this is the most common type of AF infection. Although 15% of patients with culture-positive SBP cases spontaneously become culture-negative, antibiotic administration must be started as soon as possible (4).

### Culture-negative neutrocytic ascites

This SBP variation is diagnosed when AF cultures demonstrate no bacterial growth, with a AF PMN count $\geq 250$ cells/$mm^3$ and without any apparent intra-abdominal source of infection. To diagnose accurately culture-negative neutrocytic ascites (CNNA), other causes of neutrocytic ascites should be excluded, such as peritoneal carcinomatosis, pancreatitis, tuberculous perito-

**Table 34.1** Classification of infected ascites.

| Type of infection | AF PMN/$mm^3$ | AF culture |
|---|---|---|
| Spontaneous bacterial peritonitis | ≥ 250 | Positive (usually monomicrobial) |
| Monomicrobial non-neutrocytic bacterascites | < 250 | Positive |
| Culture-negative neutrocytic ascites | ≥ 250 | Negative |
| Secondary bacterial peritonitis | ≥ 250 | Positive (polymicrobial) |
| Polymicrobial bacterascites | < 250 | Positive |
| Spontaneous empyema | ≥ 250 | Positive (monomicrobial) |

AF, Ascitic fluid; PMN, polymorphonuclear leukocytes.

nitis, and others (5). Although some episodes of CNNA could resolve spontaneously (4), this entity is considered a variant of SBP, with a similar outcome regarding short-term prognosis, probability of recurrence, and long-term survival (6). It is considered that patients with CNNA have a true infection of AF, and must be treated as patients with SBP.

### Monomicrobial non-neutrocytic bacterascites

Monomicrobial non-neutrocytic bacterascites (MNB) is also a common variant of ascitic fluid infection. It is characterized by the isolation of bacteria in AF with a PMN count < 250/mm$^3$. Patients with bacterascites seem to have less severe liver disease (7). In a prospective study of 62% of patients with MNB in whom a second paracentesis was performed before starting antibiotic treatment, ascites became spontaneously sterile (7). Patients with asymptomatic MNB have a similar clinical evolution to patients with sterile ascites (8). In contrast, patients with symptomatic MNB have an infection-related mortality and a hospital-related mortality similar to patients with SBP, as symptomatic MNB patients usually develop a true SBP (7).

### Secondary bacterial peritonitis

Secondary bacterial peritonitis may be suspected when a positive AF culture is obtained (usually polymicrobial) with a PMN count ≥ 250 PMN/mm$^3$. Biochemical characteristics of AF usually show total protein content above 1 g/dl, glucose levels < 50 mg/dl, and lactic dehydrogenase levels > 225 U/ml (or higher than the upper limit of normal in serum) (Runyon's criteria) (9). Determination of ascitic fluid carcinoembryonic antigen and alkaline phosphatase levels (> 5 ng/ml and/or > 240 U/l, respectively) may be helpful to diagnose secondary bacterial peritonitis due to occult intestinal perforation (10). Death is the rule in patients with perforative secondary bacterial peritonitis, if surgical correction is not performed. Runyon's criteria are not so useful in nonperforative secondary bacterial peritonitis. In these patients, a second diagnostic paracentesis performed 48–72 h after the initial diagnosis shows an increase on AF PMN, while it decreases dramatically in patients with SBP treated adequately (11).

### Polymicrobial bacterascites

This is an iatrogenic condition, promoted by a traumatic puncture of the gut in the course of a routine paracentesis (12). Polymicrobial bacterascites does not usually develop an AF PMN response and usually resolves spontaneously without complications. It is recommended, however, to repeat a paracentesis 6–12 h later to rule out the development of a secondary peritonitis (12).

### Spontaneous bacterial empyema

Although extraperitoneal, this complication is an infection of a pre-existing hydrothorax in patients with cirrhosis that has similar characteristics, treatment, and evolution to SBP. This condition is defined by the presence of a positive culture in pleural transudate, or by the presence of more than 500 PMN/ mm$^3$ in pleural fluid, in the absence of pneumonia. It may be associated with SBP, but in 40% of the cases may present alone. A thoracocentesis is therefore necessary to make the diagnosis (13).

## Etiology

Most of the episodes of SBP are monomicrobial and produced by enteric bacteria. Of them, 67% are gram-negative bacteria and *Escherichia coli* is the most frequently isolated organism. Table 34.2 summarizes the microorganisms isolated from AF in 1063 episodes of SBP previously published in the literature (14–16). Most isolates were aerobic gram-negative bacilli or gram-positive cocci. In contrast, anaerobic organisms are the predominant flora in the gut in a proportion to aerobic microorganisms of 1000/1 in health (17). The development of an episode of SBP caused by anaerobic bacteria is so exceptional that secondary peritonitis can be ruled out. This low incidence of SBP caused by anaerobes is probably due to the relatively high oxygen saturation of the intestinal wall, that makes the survival of anaerobic bacteria difficult (18).

Selective intestinal decontamination (SID) consists of inhibition of gram-negative flora with preservation of gram-positive cocci and anaerobes. Since beginning of last decade SID, using oral quinolenes, has been extensively used for preventing bacterial infections and SBP in patients with cirrhosis at high risk of infection (19–22). This preventive treatment has modified the microbiology of bacterial in-

**Table 34.2** Microorganisms isolated from ascitic fluid in 1063 episodes of spontaneous bacterial peritonitis.*

| Microorganism | No. of cases | (%) |
|---|---|---|
| Gram-negative bacilli | 721 | 67.8 |
| *Escherichia coli* | 478 | 44.9 |
| *Klebsiella* spp. | 117 | 11.0 |
| Others | 124 | 11.6 |
| Gram-positive cocci | 289 | 27.2 |
| *Streptococcus pneumoniae* | 80 | 7.5 |
| Other streptococci | 126 | 11.8 |
| *Enterococcus* spp. | 42 | 3.9 |
| *Staphylococcus* spp. | 37 | 3.5 |
| Anaerobes | 40 | 3.7 |

*From references 14–16.

fections in cirrhosis. During prophylaxis, and especially in patients on long-term quinolone treatment, most of the SBP episodes were caused by gram-positive cocci, mainly *Streptococcus pneumoniae* or *Enterococcus* (15). In this setting, it was of interest to assess that the use of norfloxacin implied a high risk for the development of quinolone-resistant gram-negative intestinal strains in stools of patients with cirrhosis (23). Immunosuppression seems to be an important co-factor for the development of episodes of SBP caused by quinolone-resistant gram-negative bacilli, since patients with cirrhosis on immunosuppressive therapy or infected by the human immunodeficiency virus have a higher incidence of infections caused by these organisms (24). Recent data have shown changes in the microbiology of infections (25). More than 50% of SBP episodes are culture negative, probably due to an early diagnosis of the infection. A second observation is that gram-negative bacilli are still the most frequent bacteria isolated in patients with culture-positive SBP, due in part to the emergence of infections caused by quinolone-resistant gram-negative bacilli, especially in patients with community-acquired SBP who were on long-term prophylaxis. In contrast, gram-positive bacteria are more prevalent in nosocomial episodes of SBP, especially in patients submitted to multiple therapeutic invasive procedures and/or admitted to an intensive care unit (26).

In conclusion, gram-negative flora of intestinal origin are the main cause of episodes of SBP, but the routine use of fluorinated quinolones or trimethoprim/sulfamethoxazole in patients with cirrhosis is modifying this pattern, promoting infections caused by gram-positive cocci and by quinolone-resistant gram-negative bacteria.

## Pathogenesis

It is widely accepted that the intestinal lumen is the origin of most of the bacteria causing SBP, since elimination of intestinal bacteria significantly decreases the incidence of bacterial infections (21,27). The decrease in the incidence of episodes of SBP when norfloxacin is given is associated with a significant increase of AF C3 levels in patients with low AF total protein (28), which is probably related to a decreased passage of gram-negative bacteria of gastrointestinal origin to AF.

The mechanisms that allow intestinal bacteria to exit the intestinal lumen, reach the systemic circulation and cause infection of AF are probably multiple, and are still under active investigation. Figure 34.1 depicts the mechanisms that are currently implicated in the pathogenesis of SBP.

### Intestinal bacterial overgrowth

Intestinal bacterial overgrowth (IBO) is a common phenomenon in patients with cirrhosis, and its prevalence varies from 30 to 70% according to the method used to detect its presence (29–32). The prevalence of IBO is high in Child–Pugh C patients (31) and in those with ascites (30).

In an experimental study in rats with cirrhosis, Guarner *et al.* (33) observed that almost all translocating bacteria to mesenteric lymph nodes (MLN) were overgrowing in cecal stools. Similarly, the presence of IBO seems to predispose patients to develop SBP in long-term follow-up (30), and it is more frequent in patients with previous episodes of SBP (32).

Several mechanisms have been considered to explain the presence of IBO in some patients with cirrhosis, and include alcohol abuse (34), hypochloridia related to use of antisecretory drugs (35), a reduced bile acid secretion (36), and an abnormal intestinal transit time (37). Therefore, different investigational therapeutic approaches have been tested in order to reduce IBO. Lorenzo-Zúñiga *et al.* have recently shown that oral administration of conjugated bile acids to rats with cirrhosis and ascites eliminated IBO and increased survival (38), and Pardo *et al.* have observed that administration of prokinetics to patients with cirrhosis decreases both intestinal transit time and jejunal bacterial counts (39).

### Intestinal mucosae morphology and permeability

Patients with cirrhosis and portal hypertension show both intestinal macroscopic and ultrastructural abnormalities (40,41) that might be the reason for the increased intestinal permeability that has been observed in patients with advanced cirrhosis (42).

Endotoxin also increases intestinal permeability (43) and alters host immune defenses (44). Systemic endotoxemia has been observed in patients with cirrhosis (45), and it may be then inferred that alterations in both intestinal structure and function could increase intestinal permeability and facilitate bacterial translocation in some patients. This probably occurs in those patients with more advanced liver disease.

### Bacterial translocation

Bacterial translocation (BT) has been defined as the passage of viable bacteria through the epithelial mucosae into the lamina propria and then to the MLN, and possibly to other tissues (46). Although easily investigated in animal models, it is difficult to assess its real incidence in humans due to obvious ethical reasons.

Molecular identity between bacterial strains isolated in intestinal lumen and in MLN has been reported in rats with cirrhosis (47), which probably supports the concept of BT in this experimental model. Culture-positive MLN have been shown in as much as 30% of Child–Pugh C

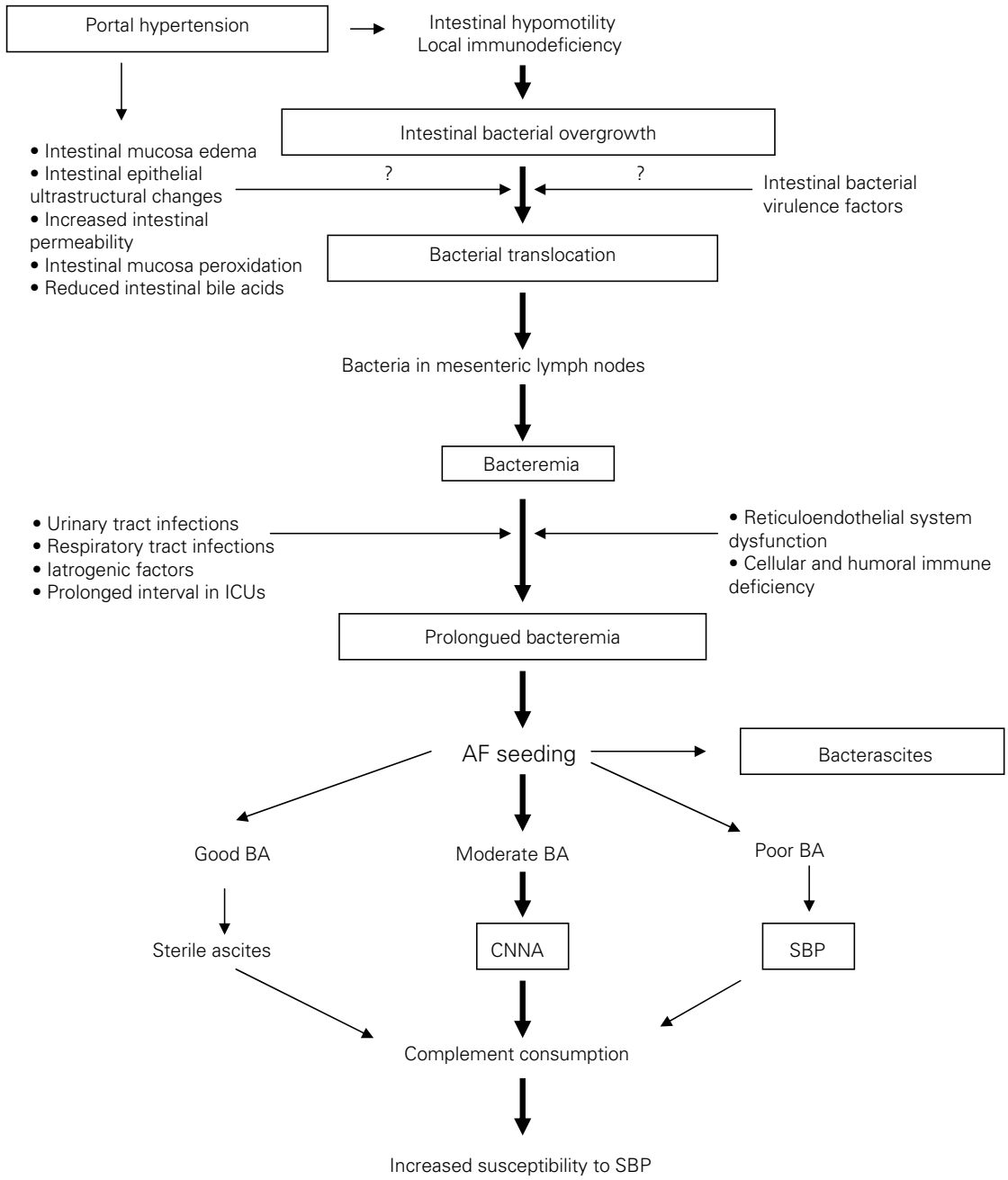


**Figure 34.1** Pathogenesis of spontaneous bacterial peritonitis. AF, ascitic fluid; BA, bacterial activity; CNNA, culture negative neutrocytic ascites; ICU, intensive care unit.

patients undergoing laparotomy due to liver transplantation (48), a figure surprisingly similar to the percentage of patients with cirrhosis and ascites with molecular evidence of BT, as shown by the detection of bacterial DNA in both blood and AF (49).

To translocate, intestinal bacteria must first adhere to specific receptors located in the epithelial cells; the ability to attach is considered a virulence factor (50). *Escherichia coli*, the most prevalent bacterium in SBP, adheres through fimbrial adhesins to epithelial cells promoting initial colonization (51). The presence of capsule, another well-known virulence factor, has been related to a higher incidence of complications in patients with cirrhosis and SBP caused by *E. coli* (52).

In physiological conditions, the presence of IgA in the intestinal lumen impairs the adherence of bacteria to epithelial receptors and, therefore, its penetration through the mucosa (53). However, the intestinal synthesis of IgA

seems to be reduced in patients with cirrhosis (54), and this may favor adherence and subsequent translocation. From a different point of view, it has been suggested that oxidative stress in the mucosa of the small intestine may also favor BT (55), and the administration of antioxidants with or without *Lactobacillus johnsonii* significantly reduces BT compared with placebo-treated rats with cirrhosis (56).

According to different studies (33,57,58), the incidence of BT in rats with cirrhosis varies between 45 and 78%, and the presence of ascites seems to be a prerequisite for BT (57). BT appears to play an important role in the pathogenesis of SBP, and several studies (33,57,58) have demonstrated that almost all rats with cirrhosis and SBP had BT at the time of sacrifice.

Several approaches have been tested to reduce BT. The administration of norfloxacin inhibits intraluminal gram-negative aerobic bacteria, thereby reducing IBO and the incidence of BT and SBP caused by these organisms in rats with cirrhosis with ascites exposed or not to hemorrhagic shock (59,60). This is probably also the reason why the administration of norfloxacin reduces the incidence of SBP in patients with cirrhosis and variceal hemorrhage (21), low-protein AF (20) and patients surviving a previous episode of SBP (19). IBO and BT can also be prevented by administration of oral bile acids (38) or prokinetics (39), but the administration of *Lactobacillus* has given conflicting results (56,61).

Once bacteria arrives in the lamina propria it may be destroyed by the gut-associated lymphoid tissue (62), or translocated to MLN. The reasons why bacteria reach MLN are probably multiple, and may include a decreased functional activity of intestinal macrophages, since their stimulation in bile duct-ligated rats decreases the rate of BT (63).

In normal circumstances, MLN should be capable of killing translocating bacteria. However, MLN in patients with cirrhosis may be repeatedly colonized (48), and an increased synthesis of tumor necrosis factor (TNF)-α has been reported in MLN from patients with cirrhosis, probably as a result of previous episodes of BT and colonization of MLN (64). Besides the fact that some bacteria, such as *E. coli*, are able to survive during at least 48 h in MLN (65), other causes, such as a massive arrival of bacteria to MLN, may favor the subsequent dissemination of bacteria to the bloodstream and other territories, such as AF. The usual detection of antibodies against gram-negative bacteria in these patients (66) may be a reflection of this alteration.

## Systemic immune system

Once bacteria enter the bloodstream, the outcome of blood colonization depends on the capacity of the blood to kill the bacteria. Bacteria must be engulfed by neutrophil leukocytes or macrophages. However, before this occurs, bacteria must be coated or opsonized with IgG and/or the third component of complement (67).

Phagocytic activity, ability for intracellular lysis of bacteria, and chemotaxis of PMN obtained from patients with cirrhosis are abnormal (68–70), and may be improved *in vitro* by the administration of granulocyte macrophage-colony stimulating factor (71). Other immune defects that have been related to the impaired neutrophil function include a reduced production of oxidative metabolites (70) and an impaired synthesis of superoxide anion, platelet-activating factor, and leukotriene B4 (72).

Macrophages are the first-line agents in the host response to foreign agents, because of their capacity to recognize and eliminate the IgG-coated organisms, and their location in the peritoneal cavity, liver, and spleen (73). Gómez *et al.* have found an impaired function of Fcγ receptors in macrophages from alcoholic patients with cirrhosis (74). These alterations, together with the deficient phagocytic activity and functionality of intracellular lysis observed in peripheral monocytes (75), may predispose to the development of bacterial infections in patients with advanced cirrhosis.

## Reticuloendothelial system phagocytic activity

In normal conditions, those bacteria that have reached the systemic circulation are killed by the reticuloendothelial system (RES), mainly located in the liver. However, the functional activity of this essential bactericidal system is impaired in patients with cirrhosis (32). Rimola *et al.* (76) observed that the clearance of $^{99m}$technetium-sulfur colloid by the RES was reduced in patients with cirrhosis compared with healthy subjects, and patients with more severe RES dysfunction developed more episodes of bacteremia during follow-up, and survival was shorter than in patients with preserved or less depressed RES activity. The RES impairment probably favors prolonged episodes of bacteremia, increasing the likelihood of a seeding of the bacteria to AF and the predisposition to SBP.

## Defense mechanisms of ascitic fluid

Regardless of the route by which the bacteria access the AF, the probability of developing an infection is inversely related to the bactericidal capacity of AF. The arrival of bacteria to AF induces the activation of the humoral bactericidal mechanisms of AF, especially the complement system. The nonspecific AF immunity is profoundly altered, with a reduced attractant activity (77), low synthesis of factors of the complement system (16) or fibronectin (78), thus making the first-line immune mechanism less effective.

The development of an SBP episode basically relies on the AF concentration of proteins (79), complement factors (16), and opsonic activity (80). Approximately 70% of patients with cirrhosis show a decreased activity of the

complement system (80), and C3, C4, and total hemolytic activity are reduced in patients with advanced cirrhosis (16,81). The probability of developing the first episode of SBP is also increased in patients with cirrhosis with an AF total protein content < 1 g/dl compared with patients with higher protein levels (24 vs. 4%, respectively, at 3 years of follow-up) (82).

Once gram-negative bacteria enter AF, the activation of the complement system leads to the lysis of bacteria and simultaneous consumption of the implicated complement components. It is likely that repeated arrivals of bacteria to AF will speed the consumption of factors of this nonspecific defensive system, promoting a progressive depression of the functional capacity of this antimicrobial system (83) and, therefore, predisposing to SBP (80).

An effective diuretic treatment increases the concentration of total proteins and the bactericidal capacity of AF (84,85). Diuresis could therefore represent a theoretically effective mechanism for preventing SBP in patients with cirrhosis with ascites. Therapeutic paracentesis is currently a widely used procedure to eliminate AF in patients with cirrhosis, and it causes a loss of all molecular components of AF. However, AF bactericidal capacity does not seem to decrease after repeated paracentesis, apparently at the expense of a decrease in serum complement (86), and does not appear to increase the risk of developing SBP (87).

## Predisposing factors

The currently accepted predisposing factors for the development of bacterial infections and SBP are detailed in Table 34.3. As previously discussed, the severity of liver disease is probably the main predisposing factor for the development of bacterial infections and particularly SBP (87). In fact, a recent study has observed a significantly higher incidence of bacterial infections in Child–Pugh C patients (50%) admitted to hospital compared with that in grade A or B patients (30%) (88). In addition, more than 70% of infected patients admitted to hospital belong to Child–Pugh C class (3).

Twenty percent of patients with cirrhosis with an acute episode of gastrointestinal hemorrhage are infected at the time of admission to hospital (89) and 30% of the previously non-infected patients will develop a bacterial infection during the first 3–4 days of admission (21). This high incidence of infections seems to be directly related to the hemorrhage. An acute episode of hemorrhage increases the incidence of BT from gut to MLN in control rats, but especially in rats with cirrhosis and ascites (61). Moreover, acute hemorrhage depresses reticuloendothelial function (90) and increases intestinal permeability in rats (91). These severe alterations observed during the acute hemorrhage may explain, at least in part, the high incidence of infections of these patients.

Low AF bactericidal capacity of patients with cirrhosis is the main local predisposing factor for the development of SBP and is directly related to AF total protein and C3 levels (16,80,83,92). The incidence of SBP in patients with cirrhosis and low AF total protein (< 1 g/dl) varies between 15 and 27% during a single hospitalization (16,79). In contrast, patients with high AF total protein rarely develop an episode of peritonitis and in this case a secondary bacterial peritonitis must be considered (93). Patients with cirrhosis with low AF total protein (< 1 g/dl) are at risk of developing SBP not only during hospitalization but also during the long-term follow-up (94,95). In addition, a recent study has shown that the probability of developing the first episode of SBP in these patients during follow-up is higher than 50% in those with high serum bilirubin (> 3.2 mg/dl) or low platelet count (< 98 000/mL) (96).

Bacteriuria and urinary tract infections are frequent in patients with cirrhosis, with a high prevalence in females (3), and has been considered as a predisposing factor for the development of SBP (97).

Intravascular catheters are regularly used in hospitalized patients. Between 4 and 20% of bacteremic episodes in cirrhotics are related to a catheter contamination and/or soft tissue infection (98,99). In patients with cirrhosis, prolonged episodes of bacteremia appear to allow AF seeding of the bacteria and eventual development of SBP (9). Therefore, to reduce the incidence of nosocomial infections and the colonization of AF in patients with cirrhosis, urinary, intravascular, and central venous catheters should be used only when necessary and removed as soon as possible.

Other therapeutic maneuvers have been implicated in the development of bacterial infections, including variceal sclerotherapy, gastrointestinal endoscopy, and

**Table 34.3** Predisposing factors for the development of spontaneous bacterial peritonitis in patients with cirrhosis and ascites.

| | |
|---|---|
| **1** | Severity of liver disease: high levels of serum bilirubin (> 3.2 mg/dl), and low platelet count (< 98 000/ml) |
| **2** | Gastrointestinal hemorrhage, and use of vasoactive drugs? |
| **3** | Low ascitic fluid total protein (< 1 g/dl) and/or low $C_3$ (< 13 mg/dl) |
| **4** | Urinary tract infection |
| **5** | Intestinal bacterial overgrowth |
| **6** | Clinical maneuvers: urinary bladder and intravascular catheters and/or admission in Intensive Care Unit |
| **7** | Previous episode of spontaneous bacterial peritonitis |

peritoneovenous shunting (100,101), but a direct relationship to the development of SBP remains unclear. Although initially controversial (102), it is currently considered that the risk of infection is associated with the hemorrhage and not with sclerotherapy (103,104).

In any case, the increasing number of invasive procedures performed in hospitalized patients with cirrhosis may predispose patients to the development of infections, i.e. those caused by gram-positive cocci, and this is even more relevant in patients admitted to an intensive care unit (25,105).

Survivors of an episode of SBP have an increased risk of developing a new episode of SBP during follow-up. Titó *et al.* (106) studied a group of 75 patients with cirrhosis who survived an episode of SBP and found a probability of SBP recurrence of 43% at 6 months, 69% at 1 year, and 74% at 2 years. Patients with severe liver failure and/or low protein content in AF were particularly predisposed to SBP recurrence.

## Clinical findings

### Prevalence

SBP was initially considered a rare complication of cirrhosis with a low incidence rate ranging between 8 and 13% (107–109), probably because of a low suspicion of infection and a reluctance to perform routine paracentesis. In a prospective study of bacterial infections, SBP was observed in 31% of all recorded bacterial infections in patients with cirrhosis, with a high frequency of community acquisition (3).

### Signs and symptoms

Clinical presentation of SBP has been detailed in many studies (9,110). The main signs and symptoms of SBP are abdominal pain and fever. In 8–10% of patients abdominal pain increases with the abdominal palpation and sudden decompression (100,111). Abdominal rigidity may not occur in the presence of large-volume AF. Fever is the most common sign of this complication, being the unique sign of infection in almost 50% of SBP patients (100), or may be associated with the development of septic shock and deterioration of liver function (112).

Other infected patients develop signs or symptoms of hepatic encephalopathy, diarrhea, or shock. However, it is also important to know that 10–33% of patients are asymptomatic during the initial phases of SBP (9,100,108,113). The reason for this paucity of symptoms may be related at least in part to the variability in the diagnosis of SBP and to the occasional presence of hepatic encephalopathy that reduces the response to pain. According to the lack of specificity of clinical signs and symptoms, the clinician must be aware of this complication, and have a low threshold for performing a diagnostic paracentesis to obtain a prompt diagnosis and initiate early treatment.

## Diagnosis

Since clinical manifestations of SBP are nonspecific, the diagnosis of this complication is based on the analysis of AF. Paracentesis is a safe and simple method to diagnose SBP without significant complications if the clinician avoids puncture in the proximity of scars (12). The incidence of other local complications of diagnostic paracentesis is also very low. Routine paracentesis must also be performed in all patients with cirrhosis and ascites admitted to hospital, even in those patients without abdominal symptoms, since 20% of patients with cirrhosis with ascites may present with a community-acquired infection (3).

The diagnosis of SBP is suspected when the AF count of PMN reaches 250/mm$^3$ (9). Antibiotic treatment must be started immediately without waiting for the microbiological results, since 40% of neutrocytic ascites are culture-negative (114). Although the total AF leukocyte count increases with the administration of diuretics without any evidence of bacterial infection, PMN leukocytes do not increase (115) and neutrocytic ascites cannot be attributed to diuresis.

The laboratory should perform the cell count in less than 1 h, but this is unusual. Recent data have demonstrated that the use of urine "dipsticks" to detect neutrophils in ascitic fluid is useful and reduces the time from paracentesis to a presumptive diagnosis of SBP to 90 s (116). A standard cell count and differential should be performed by the laboratory to confirm the results, but empirical antibiotics can be immediately started in patients with a positive urine "dipstick" (117).

Gram's stain of AF identifies the presence of bacteria when the concentration of organisms is > 10 000/ml (118). In SBP patients, AF concentration of bacteria is low (≈ 1/ml) and, therefore, Gram's stain is usually negative (118). However, this technique may be useful to detect multiple bacteria in AF in cases of secondary bacterial peritonitis (93,119).

Before the PMN count of AF became the "gold standard" for the presumptive diagnosis of AF infection, evaluation of pH and lactate in AF and serum were extensively reported in the literature. However, these measurements have lower sensitivity and specificity than PMN count, and are currently not used in clinical practice (105,115).

Culture of AF identifies the responsible organism in 50–80% of AF infections (118,120–122). AF culture was classically performed following the usual criteria for high colony-count polymicrobial infection, with the seeding of a few drops of AF on culture plates. AF culture was negative in approximately half of the cases (120). Cultur-

ing AF in blood culture bottles increases the sensitivity of the method from 51 to 82% (118). The delay of inoculation of AF into blood culture bottles, even for only 4 h, decreases the sensitivity of the culture method; therefore, culture bottles should be inoculated at the bedside of the patient (123). A new culture system (BacT/Alert) based on an automated colorimetric microbial detection method, has recently proven to reduce the time required for the detection of a positive culture in patients with SBP to 13 h (124).

Patients with refractory ascites submitted to repeated large-volume paracentesis as outpatients have a very low incidence of SBP or bacterascites (125). Therefore, it seems reasonable to obtain a cell count and differential on all samples of AF, culturing only samples of AF of symptomatic outpatients (117).

Patients with cirrhosis infected with the human immunodeficiency virus may develop AF fungal and tuberculous infections (126). Therefore, appropriate AF stains and cultures for mycobacteria and fungi should be performed in these patients as a routine.

Bacterial infection promotes the activation of acute-phase reactants such as TNF-α, interleukin-6, $\alpha_1$-antitrypsin, and C-reactive protein. AF and serum levels of these factors have been shown to be elevated in infected patients with cirrhosis (127,128) and also in patients with sterile AF prior to the development of a nosocomial episode of SBP (129).

Tuberculous peritonitis is an unusual cause of ascites, although it may be associated with the presence of cirrhosis, especially in alcoholic, malnourished patients. Tuberculous peritonitis should be suspected in a febrile patient with lymphocytic ascites. Growth of mycobacteria in appropriate culture media may delay the diagnosis up to 6 weeks. Determination of AF adenosine deaminase is useful in the diagnosis of tuberculous pleuritis, and also in TB peritonitis (130,131), but the coexistence of cirrhosis decreases its value dramatically (132).

## Prognosis

In the early 1970s SBP mortality reached 80–90% of the episodes (108,133). Short-term prognosis of SBP has improved during the last decades due to several factors (114,134). Prompt diagnosis of this complication with the routine practice of diagnostic paracentesis (9,134), standardization of diagnostic criteria of AF infection (135,136), and worldwide use of non-nephrotoxic, third-generation cephalosporins are probably the key factors in this improvement (137). However, a recent study has observed that the SBP-associated mortality rate has remained unchanged, despite a significant increase in the mean cost because of increased use of resources by a few patients (138). Interestingly, mortality was associated with age and admission in the intensive care unit.

Although most rapidly diagnosed and treated SBP episodes are satisfactorily resolved, a significant number of patients develop infectious-associated complications, such as hepatic encephalopathy, septic shock, or progressive renal failure, leading in some cases to an irreversible hepatorenal syndrome and death (114,134,139). One-third of patients with SBP develop renal impairment. This can be transient, steady, or rapidly progressive in 25, 33, and 42% of cases, respectively (140). Renal failure and degree of liver insufficiency seem to be the most important predictive factors of hospital mortality (137,140). Half of patients with SBP and renal failure die during hospitalization compared with 6% of patients without renal failure (140). A recent study has observed that administration of albumin (1.5 g/kg at the time of diagnosis of SBP and 1 g/kg on day 3 of treatment) in patients with cirrhosis with SBP reduces the incidence of renal failure and increases short-term survival, especially in patients with blood urea nitrogen (BUN) > 30 mg/dl and/or serum bilirubin > 4 mg/dl (141). Additional trials confirming the results of this important study are required.

Other potential mortality-related factors may be those related to the bacteria. Soriano *et al.* (52) showed that patients with SBP caused by encapsulated strains of *E. coli* developed a higher number of complications compared with that of patients infected by non-encapsulated strains (92 vs. 50%, respectively). However, bacteria-related factors have not been recognized as predictors of mortality in previous studies (114,134).

Long-term prognosis of SBP in patients with cirrhosis continues to be extremely poor. One- and 2-year probability of survival after an episode of SBP is in the range of 30 and 20% respectively (106,142). The severity of the underlying liver disease and the high rate of SBP recurrence in these patients are the main causes of this poor long-term prognosis. Patients with cirrhosis who survive an episode of SBP have a high probability of developing a new episode of SBP, ranging between 32 and 69% at 1 year of follow-up according to the different series (106,142). In addition, patients with cirrhosis with some biochemical characteristics such as AF total protein < 1 g/dl, prothrombin time < 45%, and/or serum bilirubin > 4 mg/dl are at high risk of SBP recurrence (106). Considering the high risk of recurrence and the bad long-term prognosis, patients with cirrhosis who overcome an SBP episode should be evaluated for liver transplantation (143,144).

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# Chapter 35
# Treatment and Prophylaxis of Spontaneous Bacterial Peritonitis

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Spontaneous bacterial peritonitis (SBP) is defined as the infection of a previously sterile ascitic fluid, with no apparent intra-abdominal source of infection (1–7). Diagnosis is established on the basis of clinical signs and symptoms and by a polymorphonuclear cell count in ascitic fluid > 250 cells/mm$^3$. The prevalence of SBP in unselected cirrhotic patients with ascites admitted to hospital ranges between 10 and 30% (1,2). In approximately 50–60% of cases the organism responsible is isolated in ascitic fluid culture or in blood cultures (Table 35.1). The remaining cases are considered culture-negative SBP but are treated in the same way as those with a positive culture (3). The outcome of cirrhotic patients with SBP has dramatically improved during the last 20 years. At present, the SBP resolution rate ranges between 70 and 90% and hospital survival ranges between 50 and 70% (1). An early diagnosis of SBP, the routine use of diagnostic paracentesis in patients admitted to a hospital with ascites, the expansion with albumin and, especially, the use of a more adequate antibiotic therapy, are the most likely reasons for the improvement in SBP prognosis. However, despite the resolution of the infection, the mortality rate of SBP is still high (30%), mainly due to the development of some complications such as renal impairment, gastrointestinal bleeding and progressive liver failure. Cirrhotic patients recovering from an episode of SBP should be considered as potential candidates for liver transplantation since the survival expectancy after this bacterial infection is very poor.

In this chapter we will focus our attention on treatment and prognosis of SBP as pathogenesis and diagnosis are discussed in Chapter 34.

**Table 35.1** Microorganisms responsible for spontaneous bacterial peritonitis.

| | |
|---|---|
| Culture-positive SBP | 67% |
| Gram-negative bacilli | 50% |
| *Escherichia coli* | 37% |
| *Klebsiella* sp. | 6% |
| Others | 7% |
| Gram-positive cocci | 17% |
| *Streptococcus pneumoniae* | 10% |
| Other streptococci | 6% |
| *Staphylococcus aureus* | 1% |
| Culture-negative SBP | 33% |

Source: Navasa M, Follo A, Llovet JM *et al*. Randomized, comparative study of oral ofloxacin versus intravenous cefotaxime in spontaneous bacterial peritonitis. Gastroenterology 1996; 111:1011–17.

## Treatment

Antibiotic therapy must be started once the diagnosis of SBP is established. Empirical antibiotic treatment should cover all potential organisms responsible for SBP without causing adverse effects. At present, third-generation cephalosporins are considered the gold standard in the treatment of SBP in cirrhosis. However, other antibiotics are also effective in the treatment of this infective complication (Table 35.2).

### Treatment of SBP with cefotaxime

The first investigation assessing the efficacy of cefotaxime in patients with SBP was published in 1985 (8). It consisted of a randomized controlled trial comparing cefotaxime vs. the combination of ampicillin plus tobramycin in a large series of cirrhotic patients with SBP or other severe bacterial infections. Cefotaxime was more effective in achieving SBP resolution than ampicillin plus tobramycin, and whereas no patient treated with cefotaxime developed nephrotoxicity and superinfections, these two adverse effects occurred in more than 10% of the patients treated with ampicillin plus tobramycin. Following this study, cefotaxime is considered as one of the first-choice antibiotics in the empirical treatment of SBP in patients with cirrhosis. In addition, further studies have confirmed the susceptibility of cirrhotic patients with bacterial infection to develop nephrotoxicity from aminoglycosides, and therefore their use is considered a

**Table 35.2** Outcome of spontaneous bacterial peritonitis depending on the different antibiotic therapy employed.

| Antibiotic | SBP resolution rate, % | Super-infection, % | Hospital survival, % |
|---|---|---|---|
| Cefotaxime (i.v.) | | | |
| 2 g/4 h | 86 | 0 | 73 |
| 2 g/6 h | 77 | 1 | 69 |
| 2 g/12 h | 79 | 1 | 79 |
| 2 g/8 h/5 days | 93 | 0 | 67 |
| 2 g/8 h/10 days | 91 | 0 | 58 |
| Ceftriaxone (i.v.) | 91 | 0 | 70 |
| Cefonicid (i.v.) | 94 | 0 | 63 |
| Amoxicillin-acid clavulanic (i.v.) | 85 | 7 | 63 |
| Aztreonam (i.v.) | 71 | 19 | 57 |
| Ofloxacin (oral) | 84 | 1 | 81 |

last resort in the treatment of SBP and other infections in cirrhosis (7).

Two randomized controlled trials have assessed the optimal duration of therapy and dosage of cefotaxime in cirrhotic patients with SBP (9,10). In the first one 90 patients with SBP were randomized to receive cefotaxime (2 g i.v. every 8 h) during 10 days or during 5 days. Resolution of the infection (93.1 vs. 91.2%), recurrence of SBP during hospitalization (11.6 vs. 12.8%) and hospital mortality (32.6 vs. 42.5%) were comparable in the two groups. The second one was a randomized, multicenter, controlled trial in 143 patients with SBP treated with cefotaxime comparing two different dosages: 2 g every 6 h vs. 2 g every 12 h. The rates of SBP resolution (77 vs. 79%) and patient survival (69 vs. 79%) were similar in both groups. Therefore, cefotaxime can be employed at a dose of 2 g every 12 h and during a minimum of 5 days in the treatment of SBP, which results in a reduced cost in comparison with other therapeutic schedules.

## Treatment of SBP with other parenteral antibiotics

Several investigations have been carried out to assess the efficacy of other antibiotic regimes in these patients. Ceftriaxone (2 g i.v. every 24 h) is highly effective in the treatment of SBP, with a resolution rate of 100% and a hospital mortality rate of 30% (11,12). Cefonicid (2 g/12 h i.v.) is also effective in the treatment of SBP, with a resolution rate of 94% and a hospital mortality rate of 37% (12). Aztreonam was evaluated in 16 episodes of SBP caused by enterobacteria (13). The overall mortality during hospitalization was 62%. Superinfections due to resistant organisms were detected in three cases (19%). These results, together with the fact that aztreonam is capable of covering only approximately 75% of the potential organisms causing SBP, clearly establish that this antibiotic is not adequate for the empirical treatment of cirrhotic patients with SBP. Finally, two studies have shown that the parenteral administration of amoxicillin associated with clavulanic acid is effective and safe in the treatment of SBP (14,15). The lower cost of this antibiotic in comparison with third-generation cephalosporins is an important advantage.

## Treatment of SBP with oral antibiotics

In most instances, patients with SBP are in relatively good clinical condition and could be treated orally. Two studies have been reported assessing the effectiveness of oral antibiotics in SBP. Both studies used wide spectrum quinolones, which are almost completely absorbed after oral administration and rapidly diffuse to the ascitic fluid (16,17). Oral pefloxacin alone (one case) or in combination with other oral antibiotics (cotrimoxazole, nine cases; amoxicillin, three cases; cefadroxil, one case; and cotrimoxazole plus metronidazole, one case) was administered in 15 SBP episodes. The rate of infection resolution was 87%, two patients developed superinfections, and survival at the end of hospitalization was 60%. The results of a randomized controlled trial in patients with nonseverely complicated SBP (no septic shock, ileus or serum creatinine > 3 mg/dl) comparing oral ofloxacin (400 mg/12 h) vs. intravenous cefotaxime (2 g every 6 h) showed a similar rate of infection resolution and patient survival in the two groups. In addition, the incidence of superinfections and the length of antibiotic treatment were also similar in both groups, suggesting that oral ofloxacin is as effective as intravenous cefotaxime in the treatment of nonseverely complicated SBP in cirrhosis. Of course, quinolones should not be empirically used in patients undergoing selective intestinal decontamination with norfloxacin who develop a SBP episode. In these patients, third-generation cephalosporins are the best therapeutic option.

## Intravenous albumin infusion in SBP

A randomized, multicenter, controlled trial has demonstrated that in patients with SBP treatment with i.v. albumin in addition to an antibiotic reduces the incidence of renal impairment and improves hospital survival (18). The study included 126 patients with SBP, who were randomly assigned to treatment with intravenous cefotaxime (63 patients) or with cefotaxime and i.v. albumin (63 patients). Albumin was given at a dose of 1.5 g/kg of body weight at the time of diagnosis, followed by 1 g/kg of body weight on day 3. Renal impairment developed in 21 patients in the cefotaxime group (33%) and in six in the cefotaxime plus albumin group (10%). The hospital mortality rate was 29% in the cefotaxime group in comparison with 10% in the cefotaxime plus abumin group. The results of this study suggest that cirrhotic patients

with SBP should receive i.v. albumin. However, further studies are needed to determine whether lower doses of albumin have the same effects on renal function and survival, and if albumin can be substituted by artificial plasma expanders. In addition, it would be important to know those patients with SBP who may benefit from albumin infusion or if this treatment should be applied to all SBP patients. In this sense, it should be noted that the incidence of renal impairment among patients with a baseline bilirubin level < 4 mg/dl and a creatinine level < 1 mg/dl was very low in both treatment groups (7 and 0% in the cefotaxime and cefotaxime plus albumin groups, respectively). Therefore, patients with abnormal renal function [blood urea nitrogen (BUN) > 30 mg/dl and/or creatinine > 1.0 mg/dl] and/or high bilirubin levels (> 4 mg/dl) appear to be the subgroup of patients with SBP who derive the most benefit from volume expansion with albumin. Since renal dysfunction is the result of an aggravation of arterial vasodilation and a decrease in effective arterial blood volume, procedures that lead to a decreased effective blood volume should be avoided, such as the use of diuretics and large-volume paracentesis.

## Predictors of SBP resolution and survival

Several studies have been performed in order to identify predictors of infection resolution and patient survival in SBP; those parameters related to kidney function are the most important predictors of survival. In a retrospective analysis of 213 consecutive episodes of SBP empirically treated with cefotaxime in 185 cirrhotic patients, the multivariate analysis identified four out of 51 clinical and laboratory variables considered at the time of diagnosis of infection (band neutrophils in white blood cell count, community-acquired vs. hospital-acquired SBP, BUN and serum aspartate aminotransferase levels) as independent predictors of infection resolution, and six (BUN and serum aspartate aminotransferase levels, community-acquired vs. hospital-acquired SBP, age, Child–Pugh score and ileus) as independent predictors of survival (19). In another study in 252 consecutive episodes of SBP, the development of renal impairment following diagnosis of SBP was the strongest independent predictor of patient mortality in episodes responding to cefotaxime (20). Renal impairment occurred in 83 episodes (33%) and in every instance it fulfilled the criteria of functional renal failure. Renal impairment was progressive in 35 episodes, steady in 27 and transient in 21. The mortality rate was 100% in episodes associated with progressive renal impairment, 31% in episodes associated with steady renal impairment, 5% in episodes with transient renal impairment, and 7% in episodes without renal impairment. Other independent predictors of mortality in this series were age, BUN levels at diagnosis, isolation of the organism responsible in the ascitic fluid culture, and peak serum bilirubin during antibiotic treatment. Plasma and ascitic fluid cytokine levels also have prognostic value in patients with SBP (21–23). Renal impairment in SBP occurs in patients with the highest concentration of cytokines in plasma and in ascitic fluid and is associated with marked activation of the renin–angiotensin system. Therefore, it is considered that renal impairment occurs as a result of a further decrease in effective arterial blood volume, which could be the result of a cytokine-mediated aggravation of vasodilation. This is the rationale for use of plasma albumin administration in SBP. This rationale is also the basis of the recommendation to avoid diuretics and large-volume paracentesis.

## Prophylaxis

Current indications of selective intestinal decontamination in SBP prevention are summarized in Table 35.3. Cirrhotic patients with gastrointestinal hemorrhage are predisposed to develop severe bacterial infections during or immediately after the bleeding episode. Short-term intestinal decontamination is effective in preventing SBP in cirrhotic patients with gastrointestinal hemorrhage (24,25). The usefulness of systemic administration of prophylactic antibiotic agents in cirrhotic patients with gastrointestinal hemorrhage has also been investigated in three controlled studies. In these studies the treated groups received ofloxacin (initially intravenously and then orally) plus amoxicillin-clavulanic acid (before each endoscopy), ciprofloxacin plus amoxicillin-clavulanic acid (first intravenously and then orally once the bleeding was controlled), and oral ciprofloxacin, respectively (26–28). The incidence of bacterial infections was significantly lower in the treated groups (10–20%) than in the corresponding control groups (45–66%). A relative limitation to these studies was the inability to assess the effect of antibiotic prophylaxis specifically on SBP, since the incidence of both SBP and bacteremia were analyzed together. Nevertheless, the marked decrease in the rate of overall infections and the improvement in survival

**Table 35.3** Indications and duration of selective intestinal decontamination for the prevention of spontaneous bacterial peritonitis in cirrhotic patients.

| Indications/duration of prophylaxis |
|---|
| • Cirrhotic patients recovering from a previous episode of SBP (secondary prophylaxis). Indefinitely or until liver transplantation. In patients with absolute control of ascites for a long period, prophylaxis can be discontinued. |
| • Cirrhotic patients with gastrointestinal bleeding. Seven days. |
| • Cirrhotic patients with ascites and low ascitic fluid protein levels (≤ 10 g/l). During hospitalization (no consensus). |

in the groups receiving antibiotic prophylaxis support such prophylaxis, being strongly recommended in cirrhotic patients with gastrointestinal hemorrhage independently of their specific risk of SBP (7). Furthermore, a meta-analysis including all the above-mentioned studies showed a significant benefit in the subgroup of cirrhotic patients with ascites and gastrointestinal hemorrhage: 95% of patients were free of SBP in the treated group vs. 87% in the control group (29).

Patients with cirrhosis and low ascitic fluid total protein concentration may be a second group of patients who may benefit from selective intestinal decontamination. In 63 patients admitted to hospital for the treatment of an episode of ascites with an ascitic fluid total protein concentration < 15 g/l, some of whom had had a previous episode of SBP, the continuous administration of norfloxacin, 400 mg/day throughout the hospitalization period (32 patients), decreased the in-hospital incidence of SBP from 22% in the control group to 0% in the treated group (30). In cirrhotic patients with ascitic fluid protein concentration < 15 g/l and no previous episodes of SBP, the 6-month incidence of SBP was 0% in the group of patients prophylactically treated with norfloxacin, 400 mg/day for 6 months, compared with 9% in patients treated with placebo. Nevertheless, the incidence of SBP caused by Gram-negative organisms (the only one which theoretically can be prevented by norfloxacin prophylaxis) in the two groups was not statistically significant: 0% in the norfloxacin-treated group and 5% in the placebo-treated group (31).

Other antibiotic regimes have been evaluated in the prevention of SBP in high-risk patients. A placebo-controlled study demonstrated that 6-month prophylaxis with ciprofloxacin, 750 mg weekly, was effective in reducing the incidence of SBP in cirrhotic patients with low protein concentration in ascitic fluid: 4% in the treated group vs. 22% in the placebo group (32). In this study, patients with and without a prior history of SBP were included together and no attempt was made to evaluate the development of SBP in these two subgroups of patients separately. Trimethoprim-sulfamethoxazole (one double-strength tablet 5 days a week) is also effective in the prevention of SBP in cirrhotic patients with ascites (33). In a randomized, controlled trial with a median follow-up of only 90 days, the incidence of SBP was 27% in the control group and 3% in the group of patients receiving trimethoprim-sulfamethoxazole prophylaxis. Again, patients with different risk for SBP were analyzed together: patients with low and high ascitic fluid protein and patients who had and had not previous SBP episodes.

Patients recovering from an episode of SBP represent a unique population to assess the effect of long-term intestinal decontamination in the prophylaxis of SBP. In a double-blind, placebo-controlled trial including 80 cirrhotic patients who had recovered from an episode of SBP, the overall probability of SBP recurrence at 1 year of follow-up was 20% in the norfloxacin group and 68% in the placebo group, and the probability of SBP caused by aerobic Gram-negative bacilli at 1 year of follow-up was 3 and 60%, respectively. Only one patient treated with norfloxacin experienced side-effects related to treatment (oral and esophageal candidiasis) (34). Long-term selective intestinal decontamination, therefore, dramatically decreases the rate of SBP recurrence in patients with SBP. Three recent economic analyses have calculated that long-term antibiotic prophylaxis in cirrhotic patients is associated with a reduced cost compared with the "diagnosis and treat" strategy, suggesting that prophylaxis is cost-effective when applied to patients at high risk of developing SBP (35–37).

Taking into account all these prophylactic studies, it can be assumed that antibiotic prophylaxis in cirrhotic patients with ascites is indicated in patients who have had a previous episode of SBP because they are at high risk of SBP recurrence and because prophylaxis is cost-effective. In patients with low protein content in ascitic fluid who have never had SBP the recommendation is difficult to establish due to the heterogeneity of the published studies, which included patients with low and high risk of SBP together. This is the main reason for the lack of consensus since, despite the positive results of all the studies investigating different antibiotics in the prophylaxis of SBP in patients with cirrhosis, they have been unable to identify subsets of patients who clearly benefit from this therapy. On the other hand, it should be noted that three studies have been performed assessing the incidence and predictive factors of the first episode of SBP in cirrhotic patients with ascites, and they may help in deciding whether a patient should initiate antibiotic prophylaxis. In a series of 127 patients admitted to hospital for the treatment of an episode of ascites, the probability of the appearance of the first episode of SBP was 11% at 1 year and 15% at 3 years of follow-up (4). Five variables obtained at admission were significantly associated with a higher risk of SBP appearance during follow-up (poor nutritional status, increased serum bilirubin levels, decreased prothrombin activity, increased serum AST levels, and low ascitic fluid protein concentration), but only one (low ascitic fluid protein concentration) showed an independent predictive value. The 1-year and 3-year probabilities of the first episode of SBP in patients with ascitic fluid protein content < 10 g/l were 20 and 24%, whereas in those with ascitic fluid protein content ≥ 10 g/l they were 0 and 4%, respectively. A clear conclusion from this study is that long-term prophylactic administration of antibiotic is not necessary in patients with a protein content in ascitic fluid > 10 g/l, in whom the risk of developing SBP is negligible. In a similar study performed in 110 consecutive cirrhotic patients hospitalized for the treatment of an episode of ascites

(5), six variables were associated with a higher risk of first SBP appearance during follow-up (serum bilirubin > 2.5 mg/dl, prothrombin activity < 60%, ascitic fluid total protein concentration < 10 g/l, serum sodium concentration < 130 mEq/l, platelet count < 116 000/mm$^3$, and serum albumin concentration < 26 g/l) were identified, but only two (ascitic fluid protein concentration and serum bilirubin) showed an independent predictive value. In a recent study, cirrhotic patients with low ascitic fluid protein levels (≤ 10 g/l) and high serum bilirubin level (> 3.2 mg/dl) and/or low platelet count (< 98 000/mm$^3$) had a 1-year probability of developing a first SBP of 55% compared with 24% of patients with only low ascitic fluid protein levels (6). Three studies, therefore, indicate that cirrhotic patients with ascites who are at risk of developing a first episode of SBP can be identified using routine biochemical parameters and might benefit from selective intestinal decontamination. However, the efficacy of antibiotic prophylaxis in these high-risk patients should be adequately investigated in prospective randomized trials.

A second reason for the lack of consensus in the prophylaxis of SBP, particularly in those patients who have never had a previous episode of SBP, is the problem of the development of quinolone-resistant enterobacteria. A review of the published data indicates that from an initial stage when norfloxacin prophylaxis was considered effective and not associated with the development of quinolone-resistant bacteria, we have moved to a final stage in which quinolone-resistant bacteria may cause severe infections in these patients. Initial studies suggested that the risk of developing SBP or other infections caused by quinolone-resistant strains of Gram-negative bacilli was low, since the majority of SBP recurrences in patients on norfloxacin prophylaxis were caused by Gram-positive cocci, mainly streptococci (34,38,39). Thereafter, a high incidence of quinolone-resistant strains of *Escherichia coli* in stools of cirrhotic patients undergoing long-term quinolone prophylaxis was reported in several studies, although none of these studies reported any infection due to quinolone-resistant *E. coli.* In 1997 a first study was published on long-term norfloxacin prophylaxis in SBP showing a relevant emergence of infections, mainly mild urinary infections, caused by Gram-negative bacilli resistant to quinolones (90% of *E. coli* isolated were resistant to quinolones) (40). More recently it has been shown that 39 out of 106 infections caused by *E. coli* in hospitalized cirrhotic patients were quinolone-resistant, with long-term norfloxacin prophylaxis being significantly associated with the development of infections (mainly urinary tract infections) caused by quinolone-resistant *E. coli*. However, development of SBP due to quinolone-resistant *E. coli* in decontaminated patients was scarcely reported (41).

Data from a study performed in our Liver Unit, which prospectively evaluated all bacterial infections occurring in a 2-year period, show a clear relationship between the development of SBP caused by quinolone-resistant Gram-negative bacilli and long-term treatment with norfloxacin (42). In patients on long-term norfloxacin prophylaxis, 50% of culture-positive SBP was caused by quinolone-resistant Gram-negative bacilli, whereas only 16% of culture-positive SBP in patients not receiving this prophylaxis was caused by these resistant bacteria. Although in this study SBP caused by quinolone-resistant Gram-negative bacilli represented only 26% of the culture-positive SBP, quinolone-resistant SBP seems to have emerged as a real problem in hepatology, and it will probably increase in the near future. This study also showed a high rate of culture-positive SBP caused by trimethoprim-sulfamethoxazole-resistant Gram-negative bacteria in patients on long-term treatment with norfloxacin (44%), suggesting that this antibiotic cannot be used as an alternative to norfloxacin. These results suggest that effectiveness of norfloxacin in the prevention of SBP in cirrhotic patients is decreasing and, therefore, this should be considered as an alarm signal. Actually this situation was expected from what occurred in the general population or in neutropenic patients. An interesting point in the evolution of quinolone resistance in patients with cirrhosis receiving prophylaxis with norfloxacin has been the maintenance of its efficacy despite the evidence that norfloxacin was unable to maintain a selective intestinal decontamination, the main argument proposed for the use of this antibiotic in the prophylaxis of infections caused by Gram-negative bacilli. Different explanations have been proposed for this phenomenon, including a reduction in the intestinal overgrowth, a decrease in the bacterial adhesion resulting in a decreased translocation capacity, and a favorable effect of quinolones upon nonspecific immune defenses. However, it is possible that the continuous use of quinolones has promoted an accumulation of factors involved in quinolone resistance. Actually, *E. coli* quinolone resistance, initially linked to mutation located in a region of *gyrA* known as the quinolone resistance-determining region, has subsequently linked to other factors responsible for quinolone resistance (double mutation in DNA gyrase A, mutations in *gyrB*, mutations in *parC* and changes in the permeation of quinolones). Therefore, it is possible that different factors involved in quinolone resistance acting together are now responsible for the decrease in its efficacy in the prophylaxis of bacterial infections in cirrhosis.

Our study also showed no significant differences in the resolution rate of infections caused by *E. coli* resistant to quinolones in comparison with the resolution rate of those due to sensitive strains (42). The absence of cross-resistance between quinolones and other antibiotics commonly used to treat these bacterial infections, like third-generation cephalosporins, could explain this finding (SBP resolution rate 92 vs. 91%). The fact that none

of the *E. coli* isolated in patients undergoing long-term quinolone prophylaxis was resistant to third-generation cephalosporins reinforces the idea that this antibiotic constitutes the treatment of choice for bacterial infections not only in nondecontaminated cirrhotic patients but also in those undergoing selective intestinal decontamination with quinolones. On the other hand, the high incidence of quinolone and trimethoprim-sulfamethoxazole-resistant strains of *E. coli* isolated in decontaminated cirrhotic patients underlines the necessity of restricting the administration of prophylactic antibiotics only to those patients who are at the greatest risk of SBP. The increasing emergence of infections caused by quinolone and trimethoprim-sulfamethoxazole-resistant strains of Gram-negative bacilli also suggests that the effectiveness of these antibiotics may decrease with time due to their widespread use. In this regard, further studies are needed to evaluate alternative prophylactic measures such as other antibiotic regimes and non-antibiotic procedures in SBP prophylaxis. Finally, it should be kept in mind that SBP carries a poor prognosis. The 1-year and 2-year probability of survival after an episode of SBP is 30–50% and 25–30%, respectively (1). Therefore, patients recovering from an episode of SBP should be considered as potential candidates for liver transplantation.

## Summary

Spontaneous bacterial peritonitis is a frequent, severe complication of cirrhotic patients with ascites. Its diagnosis is established on the basis of a polymorphonuclear cell count in ascitic fluid > 250 cells/mm$^3$. The routine use of diagnostic paracentesis whenever a cirrhotic patient with ascites is admitted to hospital usually allows an early diagnosis of the infection. At present, third-generation cephalosporins are considered the gold standard in the treatment of SBP. Because of the high incidence of quinolone-resistant Gram-negative bacilli isolated in cirrhotic patients on long-term norfloxacin prophylaxis, SBP in these patients should not be treated with quinolones as empirical therapy. Although SBP prognosis has improved in recent years, the mortality rate associated with this bacterial infection is still high. The development of severe complications such as renal impairment and gastrointestinal bleeding is responsible for this poor prognosis. The mechanisms involved in the pathogenesis of these complications are still unknown. Selective intestinal decontamination with quinolones has proven effective in SBP prophylaxis of patients who have recovered from a previous episode of SBP and in patients with gastrointestinal bleeding. The increasing emergence of quinolone-resistant organisms clearly establishes the need to restrict the primary prophylaxis to those subsets of patients at high risk of developing a first episode of SBP. The identification of these patients and the evaluation of alternative prophylactic measures such as other antibiotic regimes and non-antibiotic procedures are under investigation. Because of the poor survival expectancy after this bacterial infection, cirrhotic patients recovering from an episode of spontaneous bacterial peritonitis should be considered as potential candidates for liver transplantation.

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