HEPATIC CIRCULATION: POTENTIAL FOR THERAPEUTIC INTERVENTION

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Abstract—In recent years, knowledge of the physiology and pharmacology of hepatic circulation has grown rapidly. Liver microcirculation has a unique design that allows very efficient exchange processes between plasma and liver cells, even when severe constraints are imposed upon the system, i.e. in stressful situations. Furthermore, it has been recognized recently that sinusoids and their associated cells can no longer be considered only as passive structures ensuring the dispersion of molecules in the liver, but represent a very sophisticated network that protects and regulates parenchymal cells through a variety of mediators. Finally, vascular abnormalities are a prominent feature of a number of liver pathological processes, including cirrhosis and liver cell necrosis whether induced by alcohol, ischemia, endotoxins, virus or chemicals. Although it is not clear whether vascular lesions can be the primary events that lead to hepatocyte injury, the main interest of these findings is that liver microcirculation could represent a potential target for drug action in these conditions.

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1. INTRODUCTION

The mechanisms involved in the regulation of blood flow through the hepatic circulation are incompletely understood. However, in recent years, knowledge of the physiology and pharmacology of the hepatic circulation has grown rapidly. Furthermore, abnormalities of hepatic microcirculation appear to be a major determinant in the physiopathology of many liver diseases.

In this review, we will focus on the structure and the physiological role of the hepatic circulation particularly in relation to substrate exchange and liver metabolism. We will review the effects of pharmacological agents, particularly adrenergic agents and autacoids released by sinusoidal cells, on intrahepatic vascular resistance. We will focus mainly on data obtained recently with the isolated perfused rat liver. Finally, we will examine the nature of the alterations of liver circulation in cirrhosis, alcoholic liver disease and several models of acute liver injury and we will discuss the contribution of these modifications to these disease processes.

2. STRUCTURE OF HEPATIC CIRCULATION

2.1. GENERAL ORGANIZATION

The liver has a dual blood supply. Approximately two-thirds of the blood perfusing the liver is venous and is supplied by the portal vein coming from the splanchnic territory (Fig. 1); this includes the digestive tract below the diaphragm, the spleen and the pancreas. Table 1 shows the relative contribution of the different organs to portal blood flow in the awake rat using the microsphere method. One-third of the blood perfusing the liver is arterial and is provided by the hepatic artery.

In most species, liver blood flow is approximately 1.5 ml/min/g liver, representing 15–20% of cardiac output, although wide variations may ocur. Comparison with other organs (Table 2) shows that the liver receives the largest fraction of cardiac output, although the rate of perfusion is lower than that of other organs such as the brain or kidney.

In terms of its structure and general properties, the hepatic artery is similar to other arteries. It is thus the portal vein that confers its peculiarity to hepatic circulation. A portal system is defined as a set of vessels interposed between two capillary beds. The portal vascular bed is interposed between the capillary network of the splanchnic territory and the hepatic sinusoids. The structure of the portal vein differs markedly from other veins. Valves are lacking and flow can reverse in several pathological conditions, e.g. cirrhosis. Unlike other venous systems, a longitudinal muscle layer showing spontaneous rhythmic activity (Johansson and Ljung, 1967; Johansson and Mellander, 1975) is present in the adventitia. This phenomenon accounts for the stretch-dependent myogenic tone of the portal vein (Bevan, 1985). The myogenic tone can affect the compliance of the portal vein and is therefore an important modulator of portal pressure *in vivo*.

2.2. HEPATIC NERVES

The liver is innervated by sympathetic and parasympathetic nerves which enter the organ closely associated with the hepatic artery and the portal vein (Lautt, 1980; Sawchenko and Friedman, 1979). The extent and localization of adrenergic and cholinergic nerves vary markedly according to the species. In primates, adrenergic nerves extend deeply into the lobule and reach the space of Disse (Friedman, 1982). Nerve endings are in close contact with hepatocytes and sinusoidal cells, particularly perisinusoidal cells. In rats, intrahepatic nerves are confined to the portal space and do not extend into the lobule. Similar findings have been reported in dogs (Friedmann, 1982). However, the largest hepatic veins are innervated at their termination near the inferior vena cava. In rats, the sparse parenchymal innervation is compensated for by numerous gap junctions between hepatocytes (Reilly *et al.*, 1978; Skaaring and Bierring, 1976). This allows propagation of the nervous signal into the lobule to the more distant hepatocytes.

Cholinergic nerves are also in contact with branches of the portal vein and hepatic artery (Gardemann and Jungermann, 1986). The extent and localization of cholinergic nerves in the lobule are less well documented. In rats, cholinergic neurons appear to terminate in the vicinity of periportal hepatocytes (Reilly *et al.*, 1978; Skaaring and Bierring, 1976).

Norepinephrine is released by nerves of the hepatic sympathetic plexus. Using isolated rat livers perfused at constant pressure, Beckh *et al.* (1982), have shown that stimulation of nerves at the liver hilum around the portal vein and the hepatic artery induces a norepinephrine overflow in the perfusate. After stimulation, norepinephrine production is approximately 50 pmol/min/g liver. Taking into account values of perfusion flow (1.5–2 ml/min/g liver), this gives a perfusate norepinephrine concentration in the outflow of 25–33 nM. Concomitantly, sympathetic stimu-
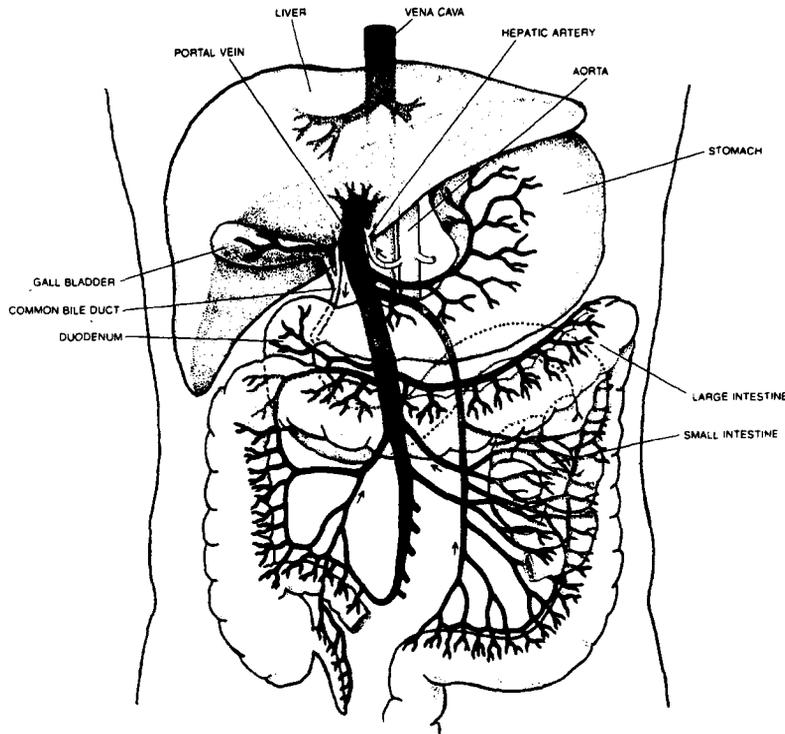
FIG. 1. General organization of liver circulation.

lation induces a production of glucose and lactate and a reduction in portal blood flow. Norepinephrine release is decreased when nervous stimulation is preceded by the administration of norepinephrine (5 nM). The increase observed in the presence of phentolamine (50 μM) is consistent with the presence of presynaptic α_2 adrenergic receptors. Norepinephrine production is decreased by propranolol (10 μM), probably due to presynaptic β_2 adrenergic receptors. Desipramine, an inhibitor of norepinephrine reuptake, augments norepinephrine release, suggesting that neuronal reuptake mechanism (uptake 1) exists in the rat liver. Norepinephrine release is inhibited in the absence of Ca^{2+}, confirming that norepinephrine secretion is Ca^{2+}-dependent (Beckh et al., 1982). The selective stimulation of the arterial plexus induces a significantly higher norepinephrine overflow than the selective stimulation of the portal plexus (Gardemann et al., 1987), indicating that the sympathetic innervation of the hepatic artery is more dense than that of the portal vein.

Norepinephrine release from hepatic sympathetic nerves has also been demonstrated in other species in vivo after direct stimulation or after reflex sympathetic activation (Garceau et al., 1984; Garceau et al., 1985; Yamaguchi and Garceau, 1980).

The liver also plays a major role in norepinephrine metabolism and elimination. In effect hepatic extraction of endogenous norepinephrine reflects both norepinephrine production by sympathetic nerves and norepinephrine elimination by the liver. The estimation of 'true' hepatic extraction and clearance of norepinephrine requires the use of labeled norepinephrine. In the denervated isolated perfused rat liver, norepinephrine hepatic clearance ranges from 0.63 to 0.74 ml/min/g liver in the physiological range of concentration (10^{-9}–10^{-8} M) (Ballet et al., 1985). These values are close to those calculated from the study of Goresky in dogs (Goresky et al., 1989), (0.80–0.89 ml/min/g liver) and to those estimated by Esler in man (0.535 l/min) (Esler et al., 1984). It has been estimated that norepinephrine hepatosplanchnic clearance represents 25% of total plasma norepinephrine clearance (Esler et al., 1984).

TABLE 1. Splanchnic Organ Blood Flow in Awake Rats (301 ± 8g) Determined by the Microsphere Method

	ml/min	% cardiac output
Stomach	0.52 ± 0.04	0.67 ± 0.04
Small bowel	8.01 ± 0.57	10.31 ± 0.71
Colon	2.40 ± 0.20	3.10 ± 0.27
Spleen	1.21 ± 0.13	1.56 ± 0.18
Mesentery-pancreas	2.39 ± 0.15	3.08 ± 0.17
Hepatic portal	14.53 ± 0.80	18.72 ± 1.03
Hepatic arterial	3.21 ± 0.56	4.14 ± 0.71
Hepatic total	17.75 ± 0.93	22.86 ± 1.19

Data from Lee et al., 1985.

TABLE 2. Extrasplanchnic Regional Blood Flow in Awake Rats (301 ± 8g) Determined by the Microsphere Method

	ml/min	% cardiac output
Brain	0.71 ± 0.07	1.56 ± 0.15
Heart	4.38 ± 0.58	4.61 ± 0.58
Lungs	1.31 ± 0.29	2.05 ± 0.31
Kidneys	5.88 ± 0.39	16.91 ± 0.58

Data from Lee et al., 1985.

2.3. MICROCIRCULATION

2.3.1. Organization

Within the liver, the portal vein and the hepatic artery branch are in parallel. After a number of divisions which depend on the size of the liver, terminal branches of these vessels supply blood to the hepatic sinusoids. Portal venules end directly in the sinusoids. However hepatic arterioles join sinusoids through various pathways (Fig. 2) (Bloch, 1970; Grishan and Nopanitay, 1979; Hirooka et al., 1986; Kardon and Kessel, 1980; Koo et al., 1975; Yamamoto et al., 1988). In the rat, they supply a dense peribiliary plexus which drains into the sinusoids. Furthermore, there are numerous anastomoses between hepatic arterioles and portal venules, while no arterioles end directly in sinusoids. In contrast in other species (particularly man), most hepatic arterioles end directly in sinusoids and arterio-portal anastomoses are rare.

The sinusoids are organized into a dense network with extensive anastomoses which connect portal venules to hepatic venules. Anastomoses are more frequent in the periportal area (Fig. 3) (Gumucio and Miller, 1981). Histological studies show that hepatocytes are organized into unicellular trabeculae in contact with two sinusoids. Therefore, in three-dimensional space each hepatocyte is lined by at least three sinusoids. The length of the sinusoids averages 200–500 μm and each is lined by 10–30 hepatocytes. The diameter of the sinusoids averages 6 μm in periportal areas and 7.5 μm in perivenous areas (Gumucio and Miller, 1981; Rappaport, 1981a,b).

Having perfused the sinusoids, the blood collects into hepatic venules, the larger branches of which end in the inferior vena cava. A fraction of plasma entering the space of Disse is filtered by the matrix gel (see Section 2.4.5) and drained into lymphatic ducts in the portal triad (Dumont, 1981).

2.3.2. Flow in Liver Microvasculature

In vivo microscopy of the liver has been used to study the pattern and distribution of flow in the hepatic microcirculation. However, it must be emphasized that most studies have been performed in rodents and are limited to the edge of the liver where hepatic arterioles do not usually terminate (McCuskey, 1986; Rappaport, 1981b).

Flow through arterioles is intermittent due to the cyclic contractions of terminal arterioles and this phenomenon is transmitted to the sinusoids (McCuskey, 1965; McCuskey et al., 1979). The diameter of erythrocytes and white blood cells is larger than that of the periportal sinusoids and strong interactions thus exist between formed blood elements and the sinusoidal lining. Red blood cells are highly deformable and continuously adapt their diameter to that of the sinusoids. By contrast, leucocytes are much more rigid; they can compress the sinusoidal wall and the space of Disse, and can transiently plug the vessels leading to an interruption or a reversal of flow via interconnecting sinusoids (McCuskey et al., 1979). In vivo microscopy, shows a general pattern of flow in the lobule resembling random dispersion rather than steady vectorial motion. The heterogeneity of sinusoidal flow is confirmed by the wide variation of red blood cell velocity in sinusoids as measured by in vivo microscopy (200–400 μm/sec) (Koo et al., 1975) and by the large variations in their transit time estimated from their dilution curve in the plasma (Goresky, 1963).
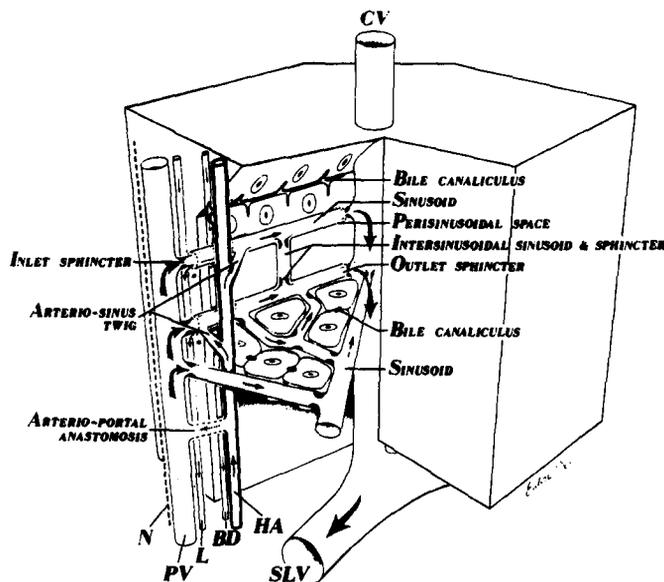

FIG. 2. Diagram of the hepatic microvasculature as determined by the in vivo microscopic techniques. PV, portal venule; HA, hepatic arteriole; L, lymphatic; BD, bile ductule; N, nerve; CV, central venule; SLV, sublobular hepatic venule. From McCuskey, 1988a. Reprinted with permission of the author and copyright holder, Kupffer Cell Foundation, Rjswijk.
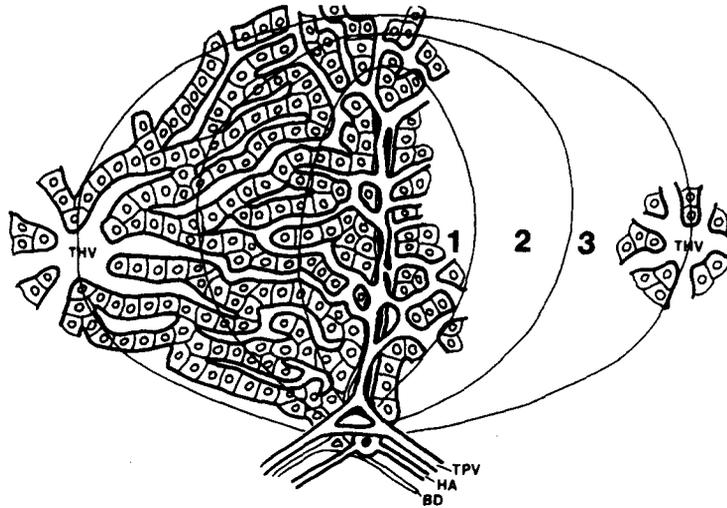
FIG. 3. The hepatic acinus conceived by Rappaport. The acinar axis is formed by the terminal branches of the portal venule (TPV), the hepatic arteriole (HA) and the bile ductule (BD). Blood enters the acinar zone 3, where it exists via the terminal hepatic venules (THV). The sinusoids of zone 1 are highly anastomotic while those of zone 3 are straight and empty into the THV in a radial arrangement. From Gumucio and Miller, 1981. Reprinted with permission of the authors and copyright holder, Elsevier Science Publishers B.V., Amsterdam.

2.3.3. Models of Liver Parenchyma Architecture

Several structural models of liver organization have been constructed. The classic liver lobule described by Kiernan considers that the terminal hepatic venules drain a polygonal area of parenchyma whose corners are occupied by portal spaces (Sasse, 1986). The liver acinus developed by Rappaport (1981a) is a clump of parenchyma oriented around the terminal apparent portal and arteriolar vessels (Fig. 3). Accordingly, the terminal hepatic venules drain the periphery of several adjacent acini.

Whatever the model, the linear arrangement of hepatocytes along the sinusoids creates concentration gradients in the plasma and parenchyma for many of the substances extracted or released by hepatocytes (Gumucio and Miller, 1981). The variations in substrate concentrations may explain some functional differences between periportal and perivenous hepatocytes. It should be noted that the shape of these metabolic zones differs according to the model. For example, in the liver lobule model the perivenous region is circular, while it has an irregular shape in the ascinus model (Fig. 4) (Sasse, 1986). The liver lobule model accounts better for the localization of liver damage while the liver acinus model is better adapted to the shape of metabolic zones. However, as stated by Sasse (1986), it is clear that periportal and perivenous areas together form a continuum and that the liver parenchyma can be described as an irregular interdigitating system of regions related to these terminal vessels.

2.4. STRUCTURE OF SINUSOIDS

Plasma disperses into the liver through specialized fenestrated capillaries called sinusoids. Sinusoids are separated from the hepatocytes by the Disse space which contains the extracellular matrix (Figs 5 and 6). It has been recognized recently that sinusoids and their associated cells are not passive channels but represent a very sophisticated cellular network which controls hepatocellular function.
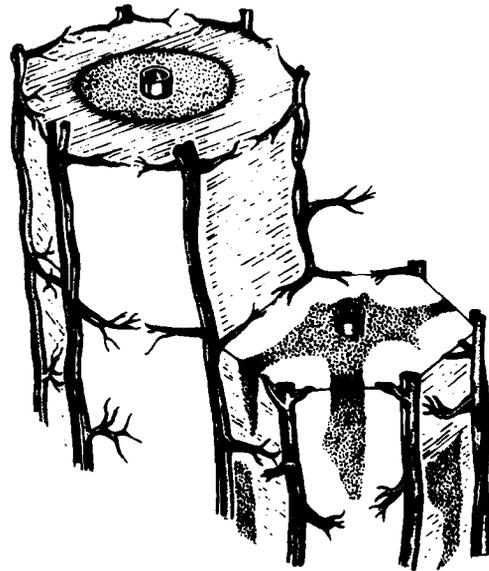
FIG. 4. Schematic drawing of the functional units of the liver. Left: in the liver lobule, a peripheral and a pericentral functional area are discernible, which are oriented concentrically around the central vein. Right: in the liver acinus, the arrangement of metabolic zones depends on their proximity to the terminal afferent vessels. Therefore, the (stippled) perivenous zone is irregularly shaped. From Sasse, 1986. Reprinted with permission of the authors and copyright holder, Plenum Press, New York.
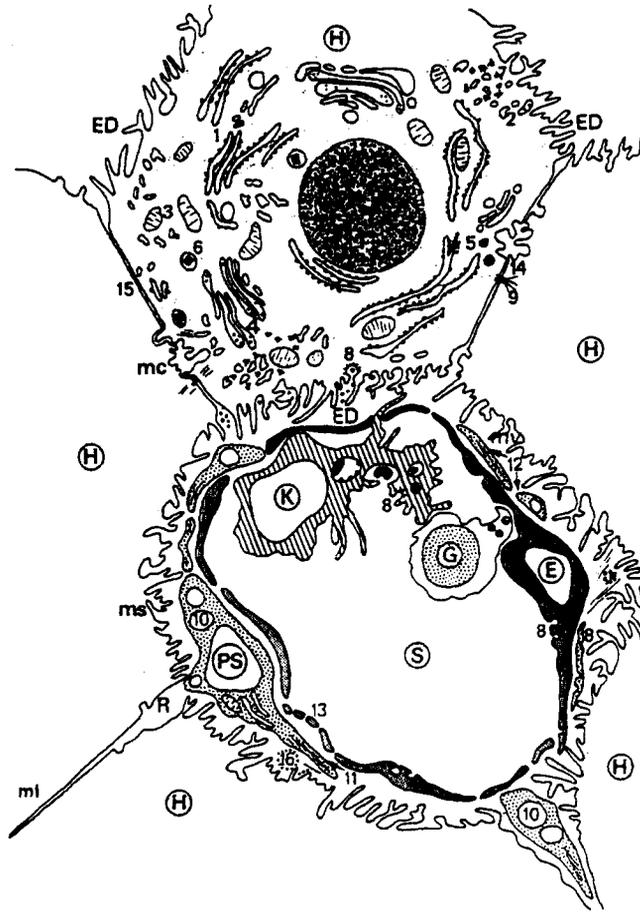
FIG. 5. The wall of liver sinusoids (S) is formed by three cell types: endothelial cells (E), Kupffer cells (K) and perisinusoidal cells (PS). Sinusoids are separated from the hepatocytes (H) by the Disse space (ED); G: pit cells. From Bioulac-Sage and Balabaud, 1985. Reprinted with permission of the authors and copyright holder, Masson, France.

In the adult liver 60–65% of cells are hepatocytes and 35–40% are nonparenchymal cells. Nonparenchymal cells include sinusoidal cells and cells associated with extralobular vascular and biliary structures (portal venules, hepatic arterioles, lymphatics, biliary ducts, connective tissue). These non-parenchymal nonsinusoidal cells can represent 1–3% of liver cells. Hepatocytes are the largest cells, occupying 80% of liver volume, while sinusoidal cells occupy 6%. The remaining 14% represents extracellular spaces: sinusoidal space (10%) and Disse space (4%). The canalicular volume is less than 0.5% of liver volume (Sasse, 1986).

Four sinusoidal cell types have been identified: endothelial cells (44%), Kupffer cells (33%), fat storing cells (10–25%) and pit cells.

2.4.1. *Endothelial Cells* (*EC*)

EC form the wall of liver sinusoids (Figs 5 and 6). They are perforated by large fenestrae often arranged in clusters which have been named 'sieve plates'. The diameter of the fenestrae is approximately 150–175 nm as measured by transmission electron microscopy in the rat. The porosity of sinusoids (fenestrated area/sinusoidal area) is 6–8% in man and in many other species (Wisse et al., 1985). EC are not surrounded by a basal lamina. These characteristics enable the dispersion of plasma into the space of Disse by convection rather than by diffusion (Schafer and Andreoli, 1986; Goresky, 1963). The fenestrae can contract under the influence of hormones and mediators (Gendrault et al., 1988) (Steffan et al., 1987; Wisse et al., 1985); however, there is no evidence that this phenomenon significantly affects exchanges in the liver, at least in normal conditions.

It has been shown that sinusoidal EC can express most of the functions of Kupffer cells (Fahimi, 1982; Summerfield and Jones, 1986) in particular, they have high endocytotic activity (Dan and Wake, 1985; Praaning-Van Dalen et al., 1981, 1987; Steffan et al., 1986) and produce thromboxane and prostaglandins (Eyhorn et al., 1988; Kuiper et al., 1988). The role of sinusoidal EC in coagulation is unknown, but recent data suggest that they do not synthesize factor VIII (Eyhorn et al., 1988) contrary to endothelial cells from other tissues.

Hepatic sinusoids contract after hepatic nerve stimulation or the administration of vasoactive hormones

FIG. 6. Diagram of the sinusoid wall and contiguous hepatocytes (HC). E, endothelial cells; KC, Kupffer cells; SD, space of Disse; SP, sieve plate composed of endothelial fenestrae; PSC, perisinusoidal cells; BC, bile canaliculus. From McCuskey, 1988a. Reprinted with permission of the author and copyright holder, Kupffer Cell Foundation, Rjswijk.

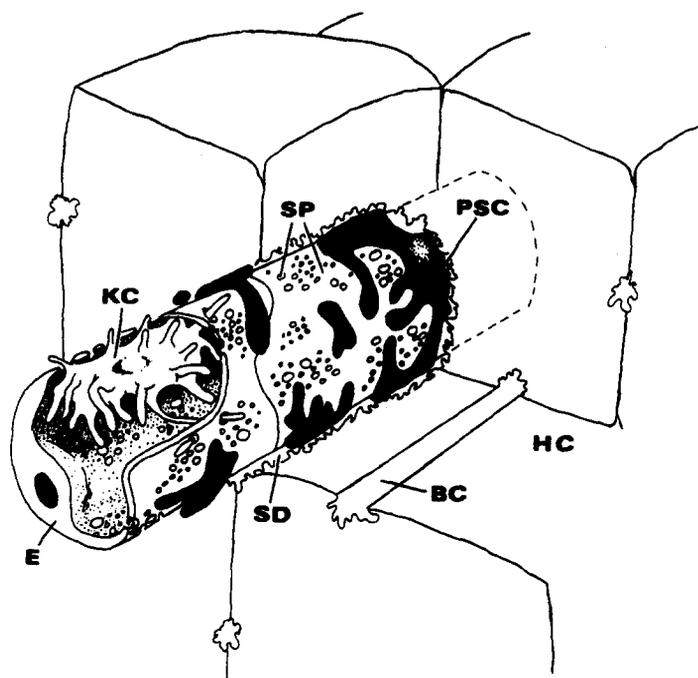or mediators (Reilly et al., 1981, 1982). The mechanism of this phenomenon is unclear, but it could be induced by the contraction of fat-storing cells rather than by EC (Burridge, 1981; Grega, 1986a; Majno et al., 1969; Bioulac-Sage et al., 1990).

2.4.2. Kupffer Cells (KC)

KC are macrophages fixed to EC. They protrude into the sinusoidal lumen and can move across the endothelial barrier (Figs 5 and 6). KC predominate in periportal regions and represent 80–90% of all resident macrophages in the body. KC show tremendous receptor-mediated, nonspecific endocytotic activity (Brouwer et al., 1988; Dan and Wake, 1985; Kolb-Bachofen et al., 1983) and play a major role in the uptake and destruction of foreign particles (mainly microorganisms and bacterial endotoxin). KC express class II major histocompatibility antigens and play a major role in the initiation of immune responses and graft rejection (Fahimi, 1982).

KC are activated by numerous agents including γ-interferon, endotoxins, platelet-activating factor (PAF), phorbol esters, zymosan and Ca^{2+} ionophore A 23187 (Dieter et al., 1986). Their response includes the release of cytokines (interleukin 1, tumor necrosis factor α (TNFα) (Karck et al., 1988), interleukin 6 (hepatocyte stimulating factor), α and β interferon, transforming growth factor β), lipid mediators (PAF, prostaglandins and leukotrienes) (Birmelin and Decker, 1984; Brouwer et al., 1988; Hagmann and Keppler, 1988; Kuiper et al., 1988; Ouwendijk et al., 1988; Sakagami et al., 1988), oxygen-derived free radicals (Dieter et al., 1988) and, finally, lysosomal enzymes (Dieter et al., 1988). The pattern of response depends on the stimulating agent. For example, the production of superoxide is induced by zymosan and phorbol myristate acetate but not by endotoxins or Ca^{2+} ionophore A 23187 (Dieter et al., 1986). The production of TNFα is selectively stimulated by endotoxins (Karck et al., 1988).

The factors released by activated KC may interact secondarily with the microcirculation and/or the hepatocytes inducing vasoconstriction, glucose production, synthesis of acute-phase proteins and cell necrosis. These mechanisms will be discussed in more detail later.

2.4.3. Fat-Storing Cells (FSC)

Fat-storing cells (FSC) (Ito cells, perisinusoidal cells, lipocytes) are located in the space of Disse and are sometimes inserted between adjacent hepatocytes (Figs 5 and 6) (Bioulac-Sage and Balabaud, 1985; Bioulac-Sage et al., 1988). FSC contain numerous intracytoplasmic fat droplets made up of vitamin A stored as retinyl esters (Hendriks et al., 1987). The cell body is prolonged by long processes which extend into the space of Disse surrounding endothelial cells. FSC contain a complex cytoskeleton and it has recently been shown in human liver that these cells are close to nerve endings. This suggests that FSC may contract and regulate flow in the microcirculation (Bioulac-Sage et al., 1988, 1990). FSC can synthesize and secrete collagen and other constituents of the extracellular matrix and play a major role in the development of fibrosis (Arenson et al., 1988;

Gresner and Haerman, 1988). During fibrosis, FSC can transform into myofibroblasts and migrate to pericentral regions, contributing to increased intrahepatic portal resistance (see Section 4.1.4.3) (Rudolph et al., 1979).

2.4.4. Pit Cells (PC)

PC are located in the sinusoidal lumen in contact with EC and KC (Figs 5 and 6). PC contain characteristic intracytoplasmic granules and rod vesicles. The morphology of PC is identical to that of NK cells. Pit cells show spontaneous cytotoxicity and may represent a defense system against tumoral and virus-infected cells (Bioulac-Sage et al., 1988).

2.4.5. Space of Disse

Sinusoids are separated from hepatocytes by the space of Disse. Although optically empty, the space of Disse contains the major components of the extracellular matrix (ECM), i.e. collagens (thin bundles of type I and VI), glycosaminoglycans (80% as heparan sulfates) and glycoproteins (mainly fibronectin) (Martinez-Hernandez, 1984). Under normal conditions, there is no continuous basement membrane surrounding EC and only small amounts of collagen IV and laminin are found (Martinez-Hernandez, 1984). Interestingly, no liver-specific ECM components have been identified as yet.

The ECM plays an important role in (a) cell adhesion, (b) cell differentiation, multiplication and phenotypic expression, (c) elasticity of sinusoids and resistance against compressive forces and (d) water and solute exchange (Granger et al., 1979). Glycosaminoglycans form a hydrated gel with pores which filter molecules according to their size and charge. Estimates of pore size for liver interstitium suggest equivalent pore radii of 180–280 Å (Barrowman et al., 1982). The space of Disse is connected at the periphery of the lobule to the space of Mall and to liver lymphatics (Dumont, 1981).

The development of hepatic fibrosis is associated with a marked increase in the amount of collagens and other components of the EMC deposited in the space of Disse. Significant amounts of collagen type IV and laminin are synthesized and this can result in the formation of a continuous basement membrane surrounding the sinusoids (Martinez-Hernandez, 1985). It has been suggested that the development of perisinusoidal fibrosis could act as a barrier to the normal exchange of nutrients and metabolites across the Disse space (see Section 4.1.5). However, viewed from en face, the basement membrane appears more like a wide-mesh net than an impermeable barrier. It has also been proposed that fibrosis could disrupt the normal cell–matrix relationship and the phenotypic expression of cells.

3. PHYSIOLOGY OF HEPATIC MICROCIRCULATION

The hemodynamic properties of the liver microvasculature can be assessed by studying the relationship between flow and pressure (intrahepatic vascular resistance) and the relationship between vascular volume and pressure (intrahepatic vascular compliance). Another important aspect of liver microcirculation is the regulation of plasma and solute exchanges across the sinusoids. Finally, it has been recognized recently that sinusoids and their associated cells can release a variety of mediators with potent vasoactive and metabolic properties.

In the following section, these different aspects of liver microcirculation will be examined.

3.1. VASCULAR RESISTANCE

3.1.1. Theoretical Concepts

According to the Poisseuille law which applies to an ideal fluid at constant temperature in a nondistensible tube, the relationship between flow (Q) and pressure (P) is

$$Q = \frac{\Delta P \times \pi r^4}{8 \eta l} \qquad (1)$$

where ΔP is the pressure gradient between the extremities of the tube, r radius, l length and η viscosity.

Equation (1) indicates that flow is related to pressure by a constant:

$$\frac{\pi r^4}{8 \eta l}.$$

Therefore, the pressure–flow relationship is theoretically a linear function which intercepts with the origin. By analogy with Ohm flow, it is possible to define the resistance R arbitrarily as:

$$R = \frac{\Delta P}{Q}. \qquad (2)$$

Thus, R is the derivative of the function $P = f(Q)$ and can be easily calculated from the inverse slope of the pressure–flow curve.

If we substitute Eq. (2) into Eq. (1),

$$R = \frac{8 \eta l}{\pi r^4}.$$

This relationship indicates that resistance depends mainly on the radius of the vessel and more accessorily on length and viscosity.

When the pressure–flow curve does not intercept the origin, the intercept with the pressure axis is defined as the zero flow pressure or the critical closure pressure (Mitzner, 1974) (Fig. 7). This value is the pressure that would induce flow in the system. Usually, at low pressure and flow values, the pressure–flow curve becomes curvilinear towards the pressure axis. This indicates that as the pressure increases, resistance decreases. This phenomenon has been interpreted as indicating an increase in the cross-sectional area of the vessels which can result either from a passive elastic distension of all the vessels and/or an increase in the number of open parallel channels with a distribution of closing pressures. According to the latter model, the zero flow pressure corresponds to the closing pressure of the last vessel to collapse when flow stops. Mitzner (1974) has demonstrated mathematically that the intercept
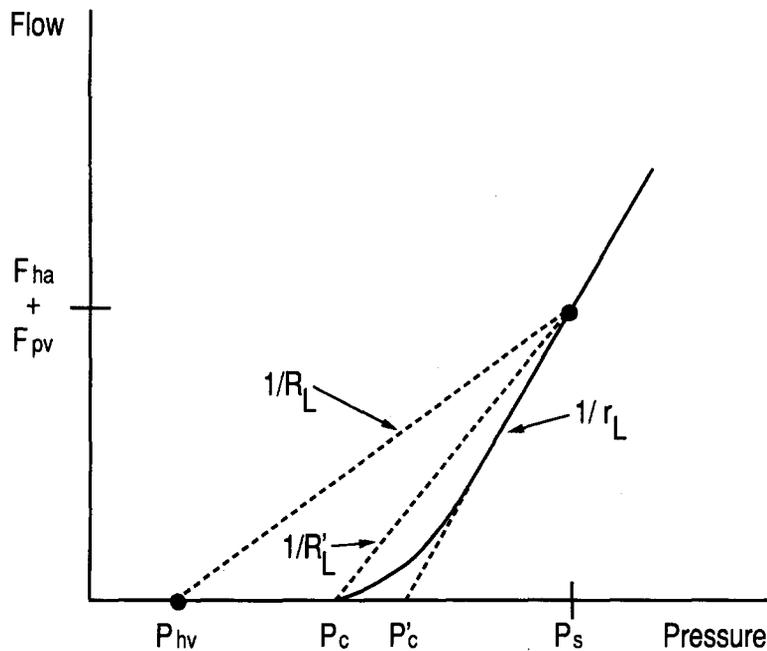
FIG. 7. Idealized pressure-flow characteristic of hepatic resistance showing calculation and interpretation of pertinent resistances and pressures. $F_{ha} + F_{pv}$: hepatic arterial flow + portal venous flow: FL: total liver blood flow at normal sinusoid pressure (P_s). P_{hv}: hepatic venous pressure. P_c: closing pressure. P'_c: weighted arithmetic mean of all closing pressures. R_L: outflow resistance. R'_L: liver resistance taking into account the existence of a closing pressure $(R'_L = P_s - P_c/FL)$. r_L: incremental resistance. Redrawn from Mitzner, 1974.

3.1.2. Pressure–flow curve in the liver

of the extrapolated linear part of the curve with the pressure axis is the weighted arithmetic mean of all closing pressures. Therefore, from the pressure-flow curve, it is theoretically possible to determine whether a vasoactive agent affects resistance and/or critical closure pressure (Greenway et al., 1985).

The pressure–flow relationship in the hepatic artery is approximately linear in most studies, suggesting that autoregulation mechanisms (curvilinearity towards the flow axis, indicating an increase in resistance when flow augments) are minimal (Condon et al., 1962). The intercept of the curve with the pressure axis is positive, indicating a critical closing pressure of 15–30 mm Hg according to Mitzner (1974) and approximately 40 mm Hg to Richardson and Withrington (1978).

Similarly, the pressure–flow relationship in the portal venous system is linear over the range of physiological pressure. There is no autoregulation phenomenon when pressure rises. A curvilinearity towards the pressure axis has been observed at low pressure with a positive intercept on the pressure axis indicating sinusoidal closing pressures of approximately 2–3 mm Hg in vivo (Condon et al., 1962; Richardson and Withrington, 1978).

Brauer et al. (1956) have studied the pressure-flow relationship in the portal venous system in isolated rat livers perfused at constant pressure. The curve was curvilinear towards the pressure axis at perfusion pressures below 10 cm H_2O. At pressures higher than 10–12 cm H_2O, the pressure-flow curve became

linear. From Brauer's data it is possible to estimate a zeroflow pressure of around 2–3 cm H_2O when livers are perfused with plasma and 4 cm H_2O when perfused with whole rat blood ($Ht = 0.45$). According to Brauer, the observed curvilinearity is consistent with a distribution of sinusoids having critical closing pressures between 2–4 and 10–12 cm H_2O which are progressively recruited as pressure increases (Brauer et al., 1956). These data are important in understanding the relationship between flow and the metabolic activity of the liver. The model predicts that the number of perfused sinusoids depends on flow and, accordingly, that variations in flow can significantly affect liver function. There are few studies to confirm these results. Very recently, using isolated rat livers perfused at constant flow with an erythrocyte-free medium, Grossman and Bhatal (1989) found a critical closing pressure of 1.8 mm Hg. Curvilinearity of the pressure flow curve is thus observed at pressures far below the physiological range. Clearly more studies are needed to clarify this point.

3.1.3. Role of Resistance in the Regulation of Flow and Pressure

Flow in the hepatic artery is controlled by resistance in the arterioles. Arteriolar resistance is high, as indicated by the pressure gradient between the artery (110–120 mm Hg) and the sinusoids (1–10 mm Hg). In vivo, portal flow is not regulated by intrahepatic portal resistance but by splanchnic resistance, which controls blood flow through the splanchnic territory. Similarly, portal pressure depends mainly on portal flow and on

the compliance of the portal venous system and appears to be less affected by portal resistance, at least in physiological conditions. As indicated by the low pressure gradient between the portal vein (5–10 mm Hg) and the hepatic veins (0–1 mm Hg), portal resistance is 40–100 times lower than arterial resistance (Richardson and Withrington, 1981a).

A very interesting phenomenon in the liver is the reciprocal relationship between the hepatic artery and the portal vein (Lautt, 1977, 1981). When portal flow decreases, resistance in the hepatic artery decreases and therefore arterial flow increases. Conversely, when portal flow increases, arterial resistance decreases and flow through the hepatic artery decreases. However, according to some investigators, it is not a true reciprocal relationship since variations in hepatic arterial flow do not affect portal flow. This response has been called the "hepatic arterial buffer response" (Lautt, 1983, 1985). The mechanism that might account for this phenomenon is at present unknown. Complete extrinsic denervation does not eliminate the response, but selective adenosine antagonists have an inhibitory effect; this suggests that the mechanism could be mediated by adenosine (Ezzat and Lautt, 1987; Lautt et al., 1985). The 'buffer response' may complicate the study of vasoactive agents in hepatic circulation since the response of the hepatic artery may be secondary both to a direct effect on arterial resistance and an indirect effect on portal blood flow. This mechanism could account for the increase in hepatic arterial flow observed in cirrhosis or after portocaval shunting.

3.1.4. Regulation of Hepatic Resistance

Intrahepatic vascular resistance is regulated by numerous factors: (a) sympathetic and parasympathetic nerves through the release of neurotransmitters such as norepinephrine, ATP or acetylcholine, (b) circulating vasoactive hormones, e.g. angiotensin II, vasopressin and glucagon, (c) vasoactive mediators released by sinusoidal cells.

The effects of nervous stimulation and vasoactive agents on liver circulation in vivo have been extensively studied and reviewed. Most of the data have been obtained in anesthetized dogs or cats (Greenway and Stark, 1971; Lautt, 1977; Richardson and Withrington, 1981b, 1982).

The principal regulation of intrahepatic vascular resistance is by sympathetic nerves. Sympathetic stimulation by electrical stimulation of the hepatic perivascular plexus, reflex nervous activation or systemic administration of norepinephrine increases hepatic arterial resistance, portal resistance and portal pressure (Greenway et al., 1967; Lautt and Greenway, 1987; Richardson and Withrington, 1988a,b, 1981a,b, 1982). Increased portal pressure results both from the increase in portal resistance and from the reduction in the compliance of the portal territory. The effects on portal flow are less clear. The portal flow response depends mainly on the intensity of the vasoconstriction of the splanchnic territory which may vary according to experimental conditions.

The isolated perfused liver is increasingly used in studies of the effect of hepatic nerve stimulation or vasoactive mediators on intrahepatic vascular resistance. This experimental model has many advantages over in vivo studies: (a) it allows the study of the intrahepatic vascular response without interference from hepatic arterial, splanchnic or systemic effects and therefore enables one to determine if vasoactive mediators have a direct effect on hepatic microcirculation, (b) it permits a direct measurement of hepatic blood flow, (c) it allows the construction of pressure–flow curves, (d) it enables the study of high concentrations of agents while avoiding systemic toxic effects; complete dose–response curves can therefore be constructed.

However, it is important to emphasize the major limitations of the model, i.e. (a) the experiments cannot usually exceed 2–3 hr because of the progressive deterioration liver function, (b) the liver is denervated, (c) it is difficult to perfuse through the artery in small animals although different systems to perfuse livers through both the hepatic artery and the portal vein have been recently reported (Ahmad et al., 1984; Gardemann et al., 1987; Reichen, 1987).

In the following section, we will present an overview of the data obtained with the isolated rat liver perfused through the portal vein.

3.1.4.1. Adrenergic agonists

Dose–response curve

Norepinephrine, epinephrine and phenylephrine increase intrahepatic vascular resistance in the isolated perfused rat liver. The response has been studied in different experimental conditions, i.e. constant pressure vs constant flow system, single-pass vs recirculating system, with or without erythrocytes and/or albumin in the perfusion medium. Complete dose–response curves have been obtained in only a few studies. Hems et al. (1976) have studied the effect of epinephrine in a constant pressure system containing rat erythrocytes. From the dose–response curve, it is possible to estimate a minimal effective concentration (MEC) of 3×10^{-8} M and a concentration corresponding to half-maximal effect (ED_{50}) of around 10^{-7} M. Grossman and Bhatal (1984) have studied the response to norepinephrine using a recirculating constant-flow system containing albumin without erythrocytes. From the dose–response curve, the MEC falls between 10^{-9} and 10^{-8} M, the ED_{50} around 10^{-6} M and the E_{max} approximately 370% at 3×10^{-5} M.

We have studied the dose–response effect of norepinephrine and epinephrine on the isolated rat liver perfused at constant pressure with a medium containing rat whole blood diluted 1/3 in Krebs–Ringer bicarbonate buffer (Ballet et al., 1988; Marteau and Ballet, unpublished data) (Table 3). The MEC, ED_{50} and E_{max} derived from the norepinephrine dose–response curve were 10^{-9} M, 3.3×10^{-6} M and 303%, respectively. These values are similar to those obtained by Grossman and Bhatal (1984). With regard to epinephrine, the corresponding values were 10^{-8} M, 1.1×10^{-6} M and 196%. In another study, we found that portal resistance was affected by physiological doses of norepinephrine (Ballet et al., 1987a). Three doses of norepinephrine were perfused and

TABLE 3. *Activity Parameters of Alpha-Adrenergic Agonists on Intrahepatic Portal Resistance in Isolated Perfused Normal Rat Livers*

	Clonidine	UK14304	Phenylephrine	Norepinephrine	Epinephrine
MEC (M)	4.10^{-7}	10^{-7}	10^{-6}	10^{-9}	10^{-8}
E_{max} (%)	63 ± 13	107 ± 20	429 ± 92	303 ± 46	196 ± 22
ED_{50} (M)	1.9 ± 1.4	8.3 ± 2.3	8.7 ± 2.3	3.3 ± 0.7	1.1 ± 0.3
	10^{-6}	10^{-7}	10^{-6}	10^{-6}	10^{-6}
γ	1.16 ± 0.12	1.00 ± 0.10	1.15 ± 0.12	0.70 ± 0.07	0.70 ± 0.04

MEC: minimal effective concentration, E_{max}: maximal effect, ED_{50}: agonist concentration at $E_{max}/2$, γ: Hill coefficient.

Data from Ballet *et al.*, 1988 and Marteau and Ballet, unpublished data.

plasma norepinephrine concentrations in the portal vein, determined by a radioenzymatic method, were 0.47 ng/ml (2.7×10^{-9} M), 2.14 ng/ml (1.2×10^{-8} M) and 10.09 ng/ml (5.8×10^{-8} M). The first two concentrations are at the two extremes of the physiological range (Cryer, 1980; Darlington *et al.*, 1986; Woolf *et al.*, 1988). A dose–dependent increase in resistance was observed to 8.1, 24.2 and 50.6% (Ballet *et al.*, 1987a).

It should be emphasized that in several studies, the MEC of norepinephrine was higher, ranging from 10^{-8} to 10^{-7} M (Hartmann *et al.*, 1982), although the reasons for these discrepancies are unclear. *In vivo*, norepinephrine shows vasoconstrictive activity at 5×10^{-9} M in canine skeletal muscle (Hjemdahl *et al.*, 1979) while a significant response is obtained at 10^{-9} M in smooth muscle *in vitro* (Bevan, 1979). However, it must be borne in mind that a decreased response to catecholamines could result from the photo-oxidation of norepinephrine in the perfusion medium. Furthermore, albumin could modify norepinephrine activity and induce a variation in the response. Finally, the production of active metabolites of norepinephrine could potentiate the response to norepinephrine in a recirculating system. In effect, Noguchi and Plaa (1970a) have shown that normetanephrine and metanephrine have vasoconstrictive properties, although their activity is 1000-fold less than that of norepinephrine or epinephrine. Using a single-pass system with constant flow, we have shown that norepinephrine perfused at 10^{-8} M induces an increase in resistance similar to that obtained in a recirculating system (22% vs 24%). However, an escape phenomenon occurs in the single-pass system after 10–15 min but not in the recirculating system (Ballet and Huet, unpublished data).

Mechanism of action (Tables 4 and 5)

The effects of norepinephrine on resistance are antagonized by phentolamine, in agreement with data in the literature, showing that the response to norepinephrine is mediated through α-adrenergic receptors (Ballet *et al.*, 1987a). Propranolol has no effect on the norepinephrine dose–response curve (Ballet *et al.*, 1987a), while it significantly increases the E_{max} of the dose–response curve to epinephrine. Furthermore, after administration of propranolol, the E_{max} of epinephrine is not significantly different from that of norepinephrine (Marteau and Ballet, unpublished data). This indicates that the lower efficacy of epinephrine as compared to norepinephrine results from its β-agonist activity which

tends to antagonize the α-adrenergic response. In basal conditions, i.e. without α-adrenergic stimulation, isoproterenol (a nonselective β_1–β_2-agonist) and terbutaline (a selective β_2-agonist) reduce intrahepatic resistance by 12.5 and 7.4% respectively at 10^{-5} M. This further confirms that β-adrenergic receptors exist in the hepatic microcirculation. However, isoproterenol does not significantly modify the response to norepinephrine (Marteau and Ballet, unpublished data).

Vasoconstriction induced by α-adrenergic stimulation could result from the stimulation of either α_1 or α_2-adrenergic receptors (Langer and Hicks, 1984; Langer and Armstrong, 1984). We have shown that dose–response curve parameters of an α_1-agonist, phenylephrine, are not significantly different from those of norepinephrine (Table 3). The α_2-agonists clonidine and UK 14304 induce dose-dependent vasoconstriction at 10^{-7} M, i.e. in the range of α_2-adrenergic selectivity. However, the E_{max} of clonidine and UK 14304 (63 and 107% respectively) are much lower than that of phenylephrine (429%) (Table 3). Norepinephrine-induced vasoconstriction is antagonized in a dose-dependent fashion by the α_2-antagonist yohimbine and the α_1-antagonist prazosin. The concentrations of these two antagonists which inhibit the norepinephrine response by 50% (ID_{50}) are not significantly different (2.6 and 2.9×10^{-6} M, respectively); the same is true for the I_{max} (99 and 83%, respectively). However, the yohimbine MEC (10^{-6} M) is higher than that of prazosin (10^{-7} M) (Marteau and Ballet, unpublished data, Marteau *et al.*, 1988b). These data show that α_2-adrenergic receptors are present in the intrahepatic microcirculation and that they contribute to the vasoconstriction produced by norepinephrine. However, their density (E_{max}) is lower than that of α_1-adrenoreceptors and they therefore contribute less to norepinephrine-induced vasoconstriction. This corroborates the findings by Segstro and Greenway (1986) that α_2-adrenergic receptors mediate the contraction of hepatic blood volume induced in cats by sympathetic activation.

The effects of various classes of vasodilators on norepinephrine-induced vasoconstriction have been studied (Marteau *et al.*, 1988a): (a) α-adrenergic antagonists: ifenprodil, isoxsuprine, buflomedil, (b) a nonselective β-adrenergic antagonist: propranolol, (c) a nonselective β-adrenergic agonist: isoproterenol, (d) an α_2-adrenergic agonist: clonidine, (e) calcium channel blockers: verapamil, diltiazem, (f) nitro-vasodilators (nitroglycerine and sodium nitroprusside) and (g) other drugs with miscellaneous modes of action: papaverine, diazoxide, vincamine, cinepazide,

TABLE 4. *Major Vasoconstrictors of Intrahepatic Circulation and their Antagonists as Studied in the Isolated Perfused Normal Rat Liver*

Vasoconstrictor	Vasodilators	Reference
Norepinephrine	Phentolamine	(Ballet *et al.*, 1987a; Beckh *et al.*; Ji *et al.*, 1984)
(Ballet *et al.*, 1987a)	Prazosine	(Marteau and Ballet, unpublished data)
(Ballet *et al.*, 1988)	Yohimbine	—
(Hartmann *et al.*, 1982)	Clonidine	(Marteau *et al.*, 1988a)
(Grossmann and Bhathal, 1984)	Ifenprodil	—
(Noguchi and Plaa, 1970a)	Isoxuprine	—
	Buflomedil	—
	Nitroprusside	(Hartmann *et al.*, 1982)
	Nitroglycerine	—
	Diazoxide	—
	Pentoxifylline	—
	Papaverine	(Hartmann *et al.*, 1982)
Phenylephrine	Nitroprusside	(Haussinger *et al.*, 1987a, b)
(Hill *et al.*, 1987)	Allopurinol	(Hill and Olson, 1987)
(Beckh *et al.*, 1984)	Cytochalasine D	(Fisher *et al.*, 1986)
(Dieter *et al.*, 1987a)	Phenylarsine oxide	(Steinhelper and Olson, 1988a)
(Haussinger *et al.*, 1987b)		(Steinhelper and Olson, 1988b)
Epinephrine	Prazosine	(Mendlovic *et al.*, 1984)
(Mendlovic *et al.*, 1984)	NDGA	—
(Hems *et al.*, 1976)	BPB	—
Sympathetic stimulation	Phentolamine	(Hartmann *et al.*, 1982)
	Prazosine	(Puschel *et al.*, 1987)
(Hartmann *et al.*, 1982)	Ca^{2+} depletion	(Hartmann *et al.*, 1982)
(Haussinger *et al.*, 1987b)	Nifedipine	(Iwai *et al.*, 1988a)
	Indomethacin	(Iwai and Jungermann, 1987)
	BPB	
	Nitroprusside	(Hartmann *et al.*, 1982)
		(Haussinger *et al.*, 1987b)
		(Haussinger *et al.*, 1987a)
	Papaverine	(Hartmann *et al.*, 1982)
ATP	Ca^{2+} depletion	(Buxton *et al.*, 1986c)
(Buxton *et al.*, 1986c)	Nitroprusside + papaverine	(Haussinger *et al.*, 1987a)
(Haussinger *et al.*, 1987a)	Indomethacin	(Iwai and Jungermann, 1987)
		(Haussinger and Stehle, 1988)
	NDGA	(Iwai and Jungermann, 1987)
	BPB	—
	TXA$_2$ antagonist (BM 13177)	(Haussinger and Stehle, 1988)
	Phenylarsine oxide	(Buxton *et al.*, 1986c)
UTP		
(Haussinger *et al.*, 1987a)	Indomethacin	(Haussinger *et al.*, 1988b)
(Noguchi and Plaa, 1970b)	TXA$_2$ antagonist (BM 13177)	(Haussinger and Stehle, *et al.*, 1988)
(Haussinger *et al.*, 1988)	Papaverine	(Haussinger *et al.*, 1987a)
Adenosine	Ca^{2+} depletion	(Haussinger *et al.*, 1988b)
		(Buxton *et al.*, 1987b)
(Buxton *et al.*, 1987b)	Indomethacin	—
	Phenyltheophylline	—
	Phenylarsine oxide	(Steinhelper and Olson, 1988b)
Arachidonic acid	Indomethacin	(Dieter *et al.*, 1987a)
(Dieter *et al.*, 1987a)		
PAF	PAF antagonists	(Buxton *et al.*, 1986b)
	(U 66985, CV 3988)	
(Buxton *et al.*, 1984a)	Indomethacin	(Mendlovic *et al.*, 1984)
(Buxton *et al.*, 1986a)		(Altin *et al.*, 1987)
(Mendlovic *et al.*, 1984)	NDGA	(Mendlovic *et al.*, 1984)
	BPB	(Altin *et al.*, 1987)
	Ca^{2+} depletion	(Buxton *et al.*, 1984a)
	Verapamil	—
	Isoproterenol	(Fisher *et al.*, 1986)
	Allopurinol	(Hill and Olson, 1987)
	Phenylarsine oxide	(Steinhelper and Olson, 1988b)
PGE$_2$		
(Tran-Thi *et al.*, 1988a)		
(Buxton *et al.*, 1987a)	Phenylarsine oxide	(Steinhelper and Olson, 1988b)
(Haussinger *et al.*, 1987c)		
(Altin and Bygrave, 1988)		
(Meren *et al.*, 1986)		
(Iwai *et al.*, 1988c)		

TABLE 4—*Continued*

Vasoconstrictor	Vasodilators	Reference
PGF$_{2\alpha}$		
(Iwai and Jungermann, 1988a)	Nifedipine	(Iwai *et al.*, 1988a)
(Haussinger *et al.*, 1987c)		
(Altin and Bygrave, 1988a)		
(Iwai *et al.*, 1988c)		
TXA$_2$ analogs		
(Fisher *et al.*, 1987)	Ca^{2+} depletion	(Fisher *et al.*, 1987)
(Iwai and Jungermann, 1988)	TXA$_2$ antagonist (BM 13177)	(Haussinger *et al.*, 1988a)
(Haussinger *et al.*, 1988b)	(SQ 29548)	(Fisher *et al.*, 1987)
(Altin *et al.*, 1988a)	Nitroprusside	(Haussinger and Stehle, 1988)
(Haussinger *et al.*, 1988a)	Phenylarsine oxide	(Steinhelper and Olson, 1988b)
LTC$_4$	LTC$_4$/D$_4$ antagonists (CCP 35949 B)	(Iwai and Jungermann, 1988a)
LTD$_4$		
(Iwai and Jungermann, 1988)	(LY 1711883)	(Haussinger *et al.*, 1988a)
(Haussinger *et al.*, 1988a)	Nitroprusside	(Haussinger and Stehle, 1988)
PMA		
(Tran-Thi *et al.*, 1988a)	Ca^{2+} depletion	(Tran-Thi *et al.*, 1988a)
(Patel, 1987)	TXA$_2$ antagonist (BM 13177)	(Tran-Thi *et al.*, 1988b)
(Tran-Thi *et al.*, 1988b)	TXA$_2$ biosynthesis inhibitor (GS13084)	—
Zymosan		
(Dieter *et al.*, 1987a)	Indomethacin	(Dieter *et al.*, 1987a)
	NDGA	—
	BPB	—
Heat-aggregated IgG		
(Buxton *et al.*, 1987a)	Ca^{2+} depletion	(Buxton *et al.*, 1987a)
	Indomethacin	—
	Phenylarsine oxide	(Steinhelper and Olson, 1988a,b)
Complement C3A fraction		
(Puschel *et al.*. 1989)	TXA$_2$ antagonist (BM 13505)	(Puschel *et al.*. 1989)
KCl		
(Hill and Olson, 1987)	Allopurinol	(Hill and Olson, 1987)
(Hill *et al.*, 1987)	Phenylarsine oxide	(Steinhelper and Olson, 1988b)

naftidofuryl and pentoxifylline. The most potent drugs were ifenprodil, phentolamine, isoxsuprine, clonidine, sodium nitroprusside and buflomedil. Diazoxide, papaverine, pentoxifylline and trinitrine were effective to a lesser degree, while verapamil, diltiazem, propranolol, isoproterenol, vincamine, cinepazide and naphtidofuryl were ineffective.

The effect of clonidine could be related to its partial agonist activity on postsynaptic α_2-adrenergic receptors. Pentoxifylline is known to have rheological properties. However, at the low hematocrit level used in the study (15%), this effect was probably small. The lack of effect of naphtidofuryl may be explained by its predominant effect as a serotonin type 2-receptor agonist. An interesting finding was the lack of effect of calcium antagonists, whereas sodium nitroprusside was effective. The inhibitory effect of sodium nitroprusside on the α-adrenergic response has been confirmed in other studies (Hartmann *et al.*, 1982; Haussinger *et al.*, 1987a,b). Norepinephrine-induced contraction of the isolated portal vein of the rat *in vitro* has been shown to be inhibited by calcium channel blockers but not by sodium nitroprusside (Jetley and Weston, 1980). This indicates that the functional properties of intrahepatic portal vessels differ from those of the extrahepatic portal vein.

It should not be inferred from these data that Ca^{2+} channels are lacking in the hepatic microcirculation.

Calcium channel blockers are thought to inhibit voltage–dependent Ca^{2+} channels and these are not activated by norepinephrine (Cauvin and Van Breeman, 1987; Godfrind, 1988; Kamp and Miller, 1987; Van Zwieten and Timmermans, 1988). However, it has been shown recently that, at least in some experimental systems, Ca^{2+} channel blockers can also inhibit receptor–dependent Ca^{2+} channels, particularly after α_2-agonist stimulation (Cauvin *et al.*, 1988). Thus, the inefficiency of Ca^{2+} channel blockers suggests that receptor–dependent Ca^{2+} channels are not involved in the norepinephrine-induced vasoconstriction of the hepatic microcirculation and that other mechanisms may occur, e.g. Ca^{2+} mobilization from the intracellular pools or activation of protein kinase C (Exton, 1985, 1986, 1988; Rasmunsen, 1986a,b). The presence of voltage-dependent Ca^{2+} channels in the hepatic microcirculation is suggested by the vasoconstriction induced by potassium chloride at high concentrations (50 mM) in the isolated perfused rat liver. However, there are some discrepancies in the response of KCl-induced vasoconstriction to Ca^{2+} antagonists. In the study of Taylor *et al.* (1985), verapamil (4 μM) and diltiazem (50 μM) were ineffective, while nifedipine (2 μM) showed significant activity. In the study of Hill and colleagues (Hill *et al.*, 1987b), verapamil (50 μM) and cobalt chloride had significant activity while nifedipine (10 nM and 1 μM) was ineffective. As discussed further KCl-induced

TABLE 5. *Ineffective Antagonists on Intrahepatic Vasoconstriction Induced by Different Agonists as Studied in the Isolated Perfused Normal Rat Liver*

Vasoconstrictor	Inactive agents	Reference
Norepinephrine	Propranolol	(Ballet et al., 1987a)
	Isoproterenol	(Marteau et al., 1988a)
	Verapamil	—
	Diltiazem	—
	Indomethacine	(Iwai and Jungermann, 1988a)
	NDGA	(Iwai and Jungermann, 1988a)
	CGB 35949 B (LTC4/D4 antag)	(Iwai and Jungermann, 1989)
	Atropine	(Noguchi and Plaa, 1970a)
	Hexamethonium	—
	Nicotine	—
	Pyribenzamine	—
	Promethazine	—
	Vincamine	(Marteau et al., 1988a)
	Cinepazide	—
	Naphtidofuryl	—
	Glucagon	(Ballet, unpublished data)
	VIP	(Ballet, unpublished data)
	Somatostatin	(Ballet, unpublished data)
Phenylephrine	Indomethacin	(Dieter et al., 1987a)
	NDGA	—
	BPB	—
	TXA$_2$ antagonist (SQ 29548)	(Fischer et al., 1987)
Epinephrine	Indomethacin	(Mendlovic et al., 1984)
	Atropine	(Noguchi and Plaa, 1970a)
	Hexamethonium	—
	Cocaïne	—
	Ouabaïne	—
	Nicotine	—
	Pyribenzamine	—
Sympathetic activation	Propranolol	(Hartmann et al., 1982)
	Ca^{2+} depletion	(Haussinger et al., 1987b)
	NDGA	(Iwai and Jungermann, 1987)
	CGB 35949 B (LTC4/D4 antag)	(Iwai and Jungermann, 1989)
	Atropine	(Hartmann et al., 1982)
	Glucagon	(Beckh et al., 1982)
	Insulin	—
ATP	Ca^{2+} depletion	(Haussinger et al., 1987a)
	Isoproterenol	(Noguchi and Plaa, 1970b)
	Atropine	(Noguchi and Plaa, 1970b)
	Nicotine	—
	Dibenzyline	—
	Promethazine	—
	Pyribenzamine	—
UTP	Ca^{2+} depletion	(Haussinger et al., 1987a)
	Nitroprusside	—
	Nitroprusside + papaverine	—
	Isoproterenol	(Noguchi and Plaa, 1970b)
	Atropine	—
	Nicotine	—
	Dibenzyline	—
	Promethazine	—
	Pirybenzamine	—
Adenosine	Verapamil	(Buxton, 1988)
Arachidonic acid	NDGA	(Dieter et al., 1987a)
	BPB	—
PAF	TXA$_2$ antagonist (SQ 29548)	(Fischer et al., 1987)
	Nitroprusside	(Fischer et al., 1986)
	Nitroglycerin	—
	5-hydroxytryptophane	—
PGE$_2$	Ca^{2+} depletion	(Altin et al., 1988a)
PGF$_{2x}$	Ca^{2+} depletion	(Altin et al., 1988a)
	TXA$_2$ antagonist (BM 13505)	(Iwai and Jungermann, 1989)
	Nifedipine	—
	Indomethacine	—
TXA$_2$ analogs	Phentolamine	(Fisher et al., 1987)
PMA	Nitroprusside	(Patel, 1987)
	Indomethacin	—
Zymosan	Superoxide dismutase	(Dieter et al., 1987a)
	Catalase	—

vasoconstriction may result from the release of vaso-active mediators by sinusoidal cells, particularly Kupffer cells.

Interestingly, it has been shown in the isolated perfused kidney that norepinephrine-induced vaso-constriction is not inhibited by diltiazem, nitrendipine or nisoldipine while these agents completely antago-nize KCl-induced vasoconstriction (Loutsenhiser and Epstein, 1985, 1988).

Other mechanisms may be involved in α-agonist-induced vasoconstriction. It has been demonstrated recently that intrahepatic vasoconstriction induced by phenylephrine at concentrations between 10^{-6} and 10^{-5} M is antagonized by phenylarsine oxide (10 μM), a trivalent arsenical derivative that com-plexes vicinal thiols and inhibits vesicular trans-port (Steinhelper and Olson, 1988a,b). The effect of phenylarsine oxide was demonstrated at low con-centrations that do not affect energetic metabolism or oxygen consumption. This suggests that the inhibition of the cellular processes which depend on vesicular transport, such as receptor-mediated endo-cytosis, could affect α-agonist-induced vasoactive responses.

Recently, Hill and Olson (1987) have shown that allopurinol (5 μM), a xanthine oxidase inhibitor, is able to antagonize KCl (50 mM) and phenylephrine (10 μM)-induced liver vasoconstriction. In prelimi-nary experiments, we have found that allopurinol is able to inhibit norepinephrine-induced vasoconstric-tion (5 $\times$ 10^{-8} M) (Chazouillères and Ballet, unpub-lished data). However, the effect was obtained at a high allopurinol concentration (1–5 $\times$ 10^{-3} M). These data have been interpreted as suggesting that oxygen-derived free radicals produced by xanthine oxidase could in part mediate vasoconstriction (Hill and Olson, 1987). Xanthine oxidase might be activated during vasoconstriction via an 'ischemia-reperfusion' mechanism, although this remains to be demon-strated. However, at high concentrations, allopurinol also has significant free radical scavenger properties; it could therefore inactivate free radicals generated by other systems than xanthine oxidase, e.g. by autoxi-dation of catecholamines.

Sympathetic hepatic nerves (Tables 4 and 5)

The response to sympathetic stimulation has been extensively studied in the isolated perfused rat liver by the group of Jungermann in Göttingen (Ballé and Jungermann, 1986; Ballé et al., 1987; Beckh et al., 1984; Beuers et al., 1986; Ji et al., 1984; Puschel et al., 1987; Sannemann et al., 1986). Bipolar platinum electrodes were placed around both the portal vein and hepatic artery and nerve stimulation was performed by applying rectangular monophasic impulses. Nerve stimulation increased intrahepatic vascular resistance, i.e. increased pressure at constant flow and decreased flow at constant pressure. The response was maximal 2–3 min after the stimulation and then decreased even when stimulation was main-tained indicating an escape phenomenon.

Gardemann et al. (1987) have studied the effect of a separate stimulation of the arterial and venous portal plexus on arterial and portal resistance. Stimulation of the arterial plexus increases both portal and arte-rial resistance and the response was stronger after portal stimulation. This reflects the higher density of the arterial nervous plexus. Interestingly, the re-sponses obtained after joint portal and arterial stim-ulation did not differ significantly when the liver was perfused through the portal vein alone or through both the portal vein and hepatic artery.

Vasoconstriction induced by nervous stimulation is antagonized by phentolamine (20 μM) and prazosin (0.1 μM), while propranolol (10 μM) and atropine (10 μM) are ineffective. These results show that the vascular response is mediated through α_1-adrenergic receptors and that hepatic cholinergic nerves do not influence significantly the sympathetic vascular re-sponse. This latter is inhibited when the extracellular Ca^{2+} level is lowered to 0.3 mM. However, it should be emphasized that in these conditions nore-pinephrine release can also be supressed. Papaverine (1 mM) and sodium nitroprusside (10 μM) also signifi-cantly inhibit the vascular sympathetic response (Table 5).

3.1.4.2. Circulating vasoactive hormones.
A number of circulating vasoactive hormones have been shown to modulate intrahepatic portal resistance (reviewed in Richardson and Withrington, 1981b). In the iso-lated perfused rat liver, we have shown that angio-tensin II significantly increases portal resistance (Ballet et al., 1988; Hems et al., 1976). However, dose–response curves to angiotensin II are bell-shaped suggesting an escape phenomenon. Vaso-pressin also increases intrahepatic resistance but the E_{max} is 10-fold lower than with angiotensin II (Ballet et al., 1988; Hems et al., 1976).

3.1.4.3. Autacoid mediators (Tables 4 and 5). In this section, we will examine the effects of a series of agents which elicit a vasoconstriction via mediators released by sinusoidal cells.

Platelet-activating factor (PAF)

Platelet-activating factor (PAF) is an alkyl phos-pholipid produced by and acting on a variety of cell types including neutrophils, eosinophils, monocytes, macrophages, platelets and endothelial cells. PAF exhibits a broad spectrum of biological effects. In addition to being a potent activator of platelets and leucocytes, it induces smooth muscle contraction and dramatically increases vascular permeability. PAF has been implicated in several pathological processes, including inflammation and ischemia (Braquet et al., 1987).

In 1984, Mendlovic et al., showed that PAF induces vasoconstriction in the isolated perfused rat liver. This effect was inhibited both by bromo-phenacyl bromide (BPB), a phospholipase A$_2$ in-hibitor and indomethacine, a cyclo-oxygenase inhibitor, and was significantly reduced by nordi-hydroguaiaretic acid (NDGA), a 5-lipooxygenase inhibitor. These data were subsequently confirmed by others (Altin et al., 1987; Buxton et al., 1986a,b). These results suggest that PAF-induced vasoconstric-tion might be mediated by prostaglandins and/or leukotrienes formed from arachidonic acid and re-leased by sinusoidal cells. Indeed, in vitro studies have

shown that PAF stimulates PGE_2 production by rat Kupffer cells. PAF alone does not appear to stimulate cysteinyl leukotriene production by Kupffer cells; however, it significantly potentiates the secretion of cysteinyl leukotrienes elicited by Ca^{2+} ionophore AA 23187 (Sakagami et al., 1988).

Agents stimulating Kupffer cells

Subsequently, it was shown that a variety of agents known to stimulate Kupffer cells in vitro can induce vasoconstriction in the isolated perfused rat liver.

Phorbol myristate acetate (PMA), (Patel, 1987; Tran Thi et al., 1988a,b), zymosan, (Dieter et al., 1987a), heat-aggregated IgG, (Buxton et al., 1987b), and complement C3a fraction (Puschel et al., 1989) increase intrahepatic vascular resistance in the isolated perfused rat liver. The responses elicited by these agents are suppressed by inhibitors of the biosynthesis and antagonists of prostaglandins, thromboxanes and/or leukotrienes. The response to PMA is inhibited by TXA_2 antagonists BM 13177 and CGS 13084 (Tran Thi et al., 1988b). Vasoconstriction induced by heat-aggregated IgG is inhibited by indomethacin (Buxton et al., 1987a). The response to zymosan is significantly reduced by BPB, indomethacin and NDGA (Dieter et al., 1987a). Recently, responses to C3a have been shown to be suppressed by the TXA_2 antagonist BM 13177 (Puschel et al., 1989). The data suggest that intrahepatic vasoconstriction induced by these agents, which are well-known activators of macrophages, could result from the secondary release of eicosanoids. In effect, it has been demonstrated that these agents stimulate PGE_2 and PGD_2 production by Kupffer cells in vitro (see Section 2.4.2.) while PMA has been shown to induce the production of PGD_2, PGE_2 and TXA_2 by the isolated perfused rat liver (Tran Thi et al., 1988a,b). Furthermore PGE_2, $PGF_{2\alpha}$ and TXA_2 analogs are vasoconstrictors in the isolated perfused rat liver while PGE_1, PGD_2 and PGI_2 do not significantly affect intrahepatic vascular resistance in basal conditions (Altin and Bygrave, 1988a,b; Haussinger et al., 1987c; Iwai et al., 1988a; Meren et al., 1986; Fisher et al., 1987). The cysteinyl leukotrienes LTC_4 and LTD_4 also have potent vasoconstrictive properties, while LTE_4 and LTB_4 are inactive (Iwai and Jungermann, 1988; Iwai et al., 1988b). Of all the mediators studied, TXA_2 appears to be the most potent.

There is evidence that the response to eicosanoids involves intracellular Ca^{2+} mobilization. The response to prostaglandins characteristically consists of a brief and transient mobilization of intracellular Ca^{2+} (Haussinger et al., 1987c) which resembles that obtained with α-adrenergic agonists and sympathetic stimulation (Haussinger et al., 1987b), while the response to thromboxanes and leukotrienes consists of a slow and prolonged mobilization of intracellular Ca^{2+} (Haussinger et al., 1987a; Haussinger, 1989).

In conclusion, these findings indicate that eicosanoids produced by sinusoidal cells play an important role in the regulation of hepatic microcirculation. This concept could have major implications in inflammatory conditions characterized by an increased pro-duction of eicosanoids and other lipid mediators. This will be discussed later.

Sympathetic stimulation, norepinephrine and ATP

By analogy, it has been suggested that the vasoconstriction induced by adrenergic agonists could also depend on the secondary release of autacoids. Mendlovic et al. (1984) have shown in the isolated perfused rat liver that vasoconstriction induced by epinephrine (10^{-5} M) in the presence of propranolol (10^{-5} M) is inhibited by NDGA and BPB, while indomethacin is inactive. Subsequently, Dieter and colleagues (Dieter et al., 1987a) reported that NDGA, BPB and indomethacin do not modify the response to phenylephrine ($2\,\mu$M). Iwai and Jungermann (1987) found that BPB has a weak inhibitory effect on the vasoconstriction induced by 1 μM norepinephrine and that BPB and indomethacine are ineffective. The reason for these discrepancies are unclear but could be related to different experimental conditions. However, the overall data suggest that the response to adrenergic agonists is not mediated by eicosanoids.

The situation is different after sympathetic activation. In effect, indomethacin and BPB significantly inhibit vasoconstriction induced by sympathetic stimulation, whereas NDGA is inactive (Iwai and Jungermann, 1987). This suggests that the effect of sympathetic stimulation could be mediated at least in part by prostaglandins and/or thromboxane released secondarily from sinusoidal cells. This hypothesis has recently been supported by the finding that sympathetic stimulation elicits PGE_2 release by the liver (Tran Thi et al., 1988c).

ATP is stored at high concentrations, associated with norepinephrine, in vesicles of the varicosities of terminal sympathetic fibers (Burnstock, 1985). ATP is liberated along with norepinephrine by exocytosis when sympathetic nerves are stimulated. Therefore, it has been suggested that the response to sympathetic stimulation could be mediated or modulated by ATP (Burnstock, 1988). ATP can also be released by platelets under the influence of a variety of mediators, several of which are produced during inflammation.

ATP induces vasoconstriction in the isolated perfused rat liver (Buxton et al., 1986c). There is evidence indicating that the response to ATP is mediated via purinergic P_2 receptors present on smooth muscle and involves an intracellular Ca^{2+} pool identical to that mobilized by norepinephrine, prostaglandins and other Ca^{2+}-mobilizing hormones (Haussinger et al., 1987a; Haussinger, 1989). However, ATP-induced vasoconstriction is inhibited by BPB, indomethacine, the TXA_2 inhibitor BM 13177 and NDGA, suggesting that the effects of ATP on hepatic microcirculation could be mediated, at least in part, by eicosanoids released by sinusoidal cells.

UTP is a more potent hepatic vasoconstrictor than ATP (Haussinger et al., 1987b). Interestingly, it has been shown that the response to UTP is mediated by a pyriminidergic receptor which differs from the P_2 receptor (Haussinger, 1989; Von Kugelgen et al., 1987). Furthermore effects of UTP on Ca^{2+} fluxes are different from those of ATP but similar to

those observed with cysteinyl-leukotrienes or TXA_2 analogs (Haussinger et al., 1987a). In addition, UTP-induced vasoconstriction is inhibited by indomethacin and BM 13177. Lastly, ATP and UTP both elicit PGE_2, PGD_2 and TXA_2 release by the isolated perfused rat liver (Haussinger et al., 1988a; Tran-Thi et al., 1988c). All these findings suggest that the effects of ATP and UTP on hepatic microcirculation are mediated, in part, directly via their specific receptor on smooth muscle and partly by eicosanoids liberated from sinusoidal cells.

3.1.5. Location of Intrahepatic Portal Resistance

The location of portal resistance in basal conditions and under the influence of vasoactive agonists is unknown. It is important to emphasize that the site(s) of resistance may be different in basal conditions and after stimulation. Furthermore, the location may change according to the stimulating agent. Using microscopic methods in rats, Reilly et al. (1981, 1982) have demonstrated that all components of the microvasculature, including sinusoids and central venules, are able to constrict in response to agonist stimulation. However, direct measurement of pressure in the microcirculation in basal conditions indicates that, in the rat, most of the resistance is located in the portal venules, i.e. is presinusoidal (Nakata et al., 1960). The increased sinusoidal volume during retrograde as compared to anterograde perfusion of rat livers also supports the hypothesis that maximum resistance to perfusate flow is located at the presinusoidal level (Bass et al., 1989). In dogs and cats, a recent study suggests that, in basal conditions, the major resistance site is postsinusoidal in the hepatic veins (Lautt et al., 1986; Legare and Lautt, 1986). However after adrenergic stimulation, a presinusoidal component is observed (Lautt and Liegare, 1987; Lautt et al., 1987).

3.2. Hepatic Blood Volume

Another important function of liver circulation is its role as a blood reservoir. This subject has been reviewed extensively and will be discussed only briefly (see Bennett and Rothe, 1981; Donald, 1981; Rothe, 1983, 1984).

The splanchnic vascular bed contains 35–40% of the circulating blood volume and is therefore the major blood reservoir. In dogs and cats, the spleen is an important component while in humans the intestine and the liver play the major role (Greenway, 1983).

Hepatic blood volume can be measured by means of plethysmography (Greenway, 1979). From volume–pressure curves, the compliance of the intrahepatic circulation can be estimated (Rothe, 1983, 1984). Hepatic blood volume has been estimated to range between 0.15–0.3 ml/g liver, i.e. approximately 10% of the circulating blood volume (Greenway, 1983). The hepatic vascular bed is highly compliant, as shown by the steep linear volume–pressure relationship. 30–40% of hepatic blood can be expelled into the systemic circulation after sympathetic nerve stimulation. This represents a major reflex mechanism to compensate for hypovolemia and to maintain

cardiovascular homeostasis. Hepatic blood mobilization by the hepatic vascular bed is not a passive elastic phenomenon due to a decrease in portal pressure and/or portal flow but results from active venoconstriction. This occurs via a change in the unstressed volume (i.e. the volume at zeroflow pressure) but not the compliance of the system (the slope of the pressure–volume curve) (Greenway et al., 1985, 1986a,b; Greenway, 1987).

3.3. Fluid and Solute Exchanges Across the Sinusoids

3.3.1. Control of Exchanges across the Sinusoids

It is generally agreed that vasoactive mediators control transvascular exchange of fluids and solutes (a) by regulating the number of open capillaries, (b) by modifying the permeability–surface product (PS) of individual capillaries and (c) by changing capillary hydrostatic pressure (Crone, 1986; Granger and Barrowman, 1983; Granger et al., 1983).

By contracting portal venules and/or presinusoidal 'sphincters' and/or sinusoids, vasoactive mediators can reduce the number of perfused sinusoids and consequently the number of functioning hepatocytes. It is important to emphasize that vasoactive mediators could modify the number of perfused sinusoids without changing their critical closing pressure, only by changing liver blood flow. Accordingly, a reduction in liver blood flow could lead to a 'derecruitment' of sinusoids. However, as stated previously, it is not clear that this phenomenon can occur in normal conditions in the physiological range of liver blood flow values. Vasoactive mediators could also contract sinusoids without changing the number of perfused channels. However, it is unlikely that a reduction in the surface of sinusoids would significantly affect exchanges across the endothelial barrier inasmuch as sinusoids are perforated by large fenestrations and do not show significant resistance to dispersion (Goresky et al., 1970). However, it is possible that a marked contraction of endothelial fenestrae could restrict the movement of macromolecules. In the most extreme situation, where the fenestrae are completely closed, the dispersion of molecules into the space of Disse would change from flow-limited to diffusion-limited conditions (Schafer and Andreoli, 1986; Goresky et al., 1970). A change in sinusoidal hydrostatic pressure is unlikely to significantly affect exchanges across sinusoids since their permeability is infinitely high and sinusoidal pressure is identical to interstitial pressure. Any change in hydrostatic pressure would be transmitted to the surface of the hepatocytes and would directly affect lymphatic flow.

It should be emphasized that the effects of vasoactive mediators on capillary exchanges cannot be inferred from changes in vascular resistance. For example, in skeletal muscle, norepinephrine increases resistance and concomitantly augments the capillary filtration coefficient and O_2 extraction (Harper et al., 1985). On the other hand, in the intestine, norepinephrine increases resistance, whereas the capillary filtration coefficient and O_2 extraction decrease significantly (Harper et al., 1985; Richardson, 1984).

This indicates clearly that resistance and capillary exchanges are regulated separately.

3.3.2. *Effect of Sympathetic Stimulation and/or Adrenergic Agents on Exchanges across Sinuosids*

A variety of methods have been used to study the effects of sympathetic stimulation and/or adrenergic agents on exchanges across sinusoids.

Methods based on the study of intrahepatic distribution of dyes or contrast medium have shown that sympathetic stimulation or norepinephrine administration cause a 'redistribution' of flow or a 'restricted' circulation, i.e. a strong reduction in flow accompanied by a possible closure of vessels in peripheral vs central regions of the liver (Daniel and Pritchard, 1951; Pritchard and Daniel, 1952). However, it should be noted that these methods show only gross variations in intrahepatic vascular volumes and do not rule out the possibility that contracted vessels are still perfused. A continuing uniform distribution of labeled 15 μm microspheres injected via the hepatic artery or the portal vein in cats and dogs under these conditions suggests that large areas of exclusion are not present (Greenway and Oshiro, 1972). However, it remains possible that smaller areas of vascular collapse, undetectable with the microsphere technique, are present. Transillumination studies of the rat liver have shown that all parts of the microcirculation can contract under the influence of adrenergic agonists (Reilly et al., 1981). In one study, no closure of the sinusoids was seen (Koo et al., 1977). However, as stated previously, such studies are limited to the surface and edge of the liver where hepatic arterioles do not usually terminate.

Measurements of tissue flow and pO_2 with miniature probes positioned on the liver surface show that norepinephrine shifts flow and pO_2 distribution curves to the left. However, no zero-flow or pO_2 values are recorded, again suggesting that no closure of sinusoids occurs (Kessler et al., 1978, 1986).

The capillary filtration coefficient (LpS) can be estimated from the fluid filtering across the surface of the liver and accumulating in a plethysmograph during raised venous pressure (Greenway, 1981). In one study in cats, LpS was not significantly modified by epinephrine. However, Greenway (1981) has shown that sympathetic stimulation decreases LpS when venous pressure is varied over the physiological range. This suggests that sympathetic stimulation impairs filtration across sinusoids but does not elucidate the mechanism of the reduction.

The vectorial transport and metabolism of substrates eliminated by the liver is determined by the number and permeability of perfused sinusoids. Therefore, the study of substrate elimination provides information about the constraints exerted by vasoactive agents on liver microcirculation. More than 30 years ago, Brauer (1958) showed in the isolated rat liver perfused at constant pressure, that the infusion of epinephrine results in progressive decreases in blood flow with no evidence of increased $CrPO_4$ colloid extraction which accompanies similar flow changes brought about by decreasing perfusion pressure. This indicated that epinephrine reduces the elimination efficiency of the liver. These findings were interpreted as being due to a decrease in the number of perfused sinusoids as well as to a reduction in vessel lumen.

Since that time, there have been few attempts to confirm these important findings. Boobis and Powis (1974) have shown that norepinephrine significantly decreases the rate of elimination of hexobarbitone in the isolated rat liver perfused at constant flow. As norepinephrine did not modify hexobarbitone metabolism in liver slices or microsomes, the data were interpreted as being due to an effect on liver microcirculation. Recently, we examined this subject in detail by studying the effect of norepinephrine on portal blood flow, hepatic extraction and the intrinsic clearance of taurocholate, the major bile acid in the rat (Ballet et al., 1987a). Intrinsic clearance is a flow-independent constant reflecting the maximal ability of the liver to remove substrates from the circulation. We found that norepinephrine induced a dose-dependent reduction in portal blood flow and a concomitant dose-dependent decrease in intrinsic taurocholate clearance. Interestingly, the effects of norepinephrine were significant in the physiological range of concentrations $(2 \times 10^{-9}$ and 10^{-8} M). The data showed that norepinephrine impaired the vectorial transport of taurocholate from plasma to bile. These findings suggest that the reduction in taurocholate transport could result from vasoconstriction of the hepatic microvasculature. However, a direct effect on cellular transport was not ruled out. Very recently, Akerboom et al. (1987) have shown that Ca^{2+} ionophore A 23187 also induces vasoconstriction and a significant reduction in hepatic taurocholate transport. The impairment of taurocholate transport was corrected, in part by papaverine, suggesting again that it could have been secondary to vasoconstriction of the hepatic microvasculature.

Recently, we have used the multiple indicator dilution technique to study the effect of vasoactive agents on liver microcirculation (Ballet et al., 1987b). The technique consists of injecting vascular and extravascular reference tracers into the portal vein together with the substrate subjected to extraction. Labeled erythrocytes are confined to the vascular space (vascular reference) and plasma dissolved labeled substances gain access to the extravascular space (space of Disse and hepatocytes) through the fenestrae of endothelial cells: albumin (MW 69,000) and sucrose (MW 342) diffuse in extracellular spaces that are inversely related to their molecular weight and water diffuses in the cellular space. This produces a major delay in, and a decrease in the magnitude of, the labeled albumin, sucrose and water dilution curve recovered at the outflow with respect to that of labeled erythrocytes, which can be analyzed using the flow-limited model of Goresky (1963). Extravascular volumes can be calculated from the displacement of the outflow curves in relation to that of erythrocytes (peak-time method) or more directly from the differences in the mean transit times (transit-time method). The latter method will be accurate, even in a system with reduced permeability, as long as all the tracer is recovered at the outflow. Furthermore, from the dilution curve of tracers sequestrated intracellularly such as taurocholate, it is possible to estimate the

three unidirectional rate constants (influx, efflux and excretion into bile) using the three-compartment elimination model developed by Goresky (Goresky and Bach, 1970).

This technique was used to study the effect of norepinephrine in the isolated rat liver perfused at a constant flow rate (Ballet *et al.*, 1987b). Norepinephrine was administered at a concentration of 10^{-8} M which can be reached during maximal stress (Cryer, 1980). Norepinephrine increased portal pressure and decreased sinusoidal volume, reflecting a vasoconstriction of liver microcirculation. In spite of these modifications, the interstitial space of albumin and sucrose was not modified and the cellular water space was slightly increased. Concomitantly, norepinephrine significantly reduced taurocholate extraction and the influx rate constant k_1. The data therefore indicate that, in spite of its vasoconstrictive properties on hepatic microcirculation, norepinephrine did not close the sinusoids. Indeed, closure would have reduced concurrently the vascular and extravascular volumes.

Furthermore, extravascular volumes calculated according to the transit-time method (a model-independent approach) do not differ significantly from those calculated according to the peak-time method (a model-dependent approach). This suggests that norepinephrine does not create a diffusional resistance across the endothelial cells. Thus, norepinephrine was unable to impair the dispersion of albumin and albumin-bound substrates such as taurocholate in the space of Disse. The reduction in taurocholate extraction was not the consequence of vasoconstriction but a specific effect of norepinephrine on the cellular uptake of taurocholate.

These findings are in agreement with those obtained *in vivo* by Cousineau *et al.* (1983, 1985) and Goresky *et al.* (1986), after the administration of norepinephrine or reflex sympathetic activation in dogs, i.e. a decrease in sinusoidal volume with no change in the water cellular space. Data concerning the interstitial sucrose space were less reproducible, possibly due to changes in liver blood flow. However, the above data are in discrepancy with those of Reichen *et al.* (1987c) who have recently shown in the isolated perfused rat liver that norepinephrine decreases both vascular and extravascular (albumin and sucrose) spaces, although norepinephrine was administered at supraphysiological concentrations.

The multiple indicator dilution technique has been used recently to study the effect of PAF on liver microcirculation. Lapointe and Olson (1989) have shown in the isolated perfused rat liver that PAF at concentrations of 0.2 and 20 nM significantly decreases vascular, sucrose interstitial and urea cellular spaces, suggesting that a substantial fraction of the hepatic parenchyma is excluded from the circulation. However, PAF acts mainly as a local mediator and not as a circulating homrone; thus the observed response to PAF administered intravascularly could be artefactual and irrelevant to the *in vivo* situation.

In summary, there is evidence that sympathetic nervous activation and/or circulating norepinephrine can induce marked contraction of the sinusoids. However, in physiological conditions this constraint does not appear to significantly affect the perfusion of the hepatic parenchyma. Therefore, during sympathetic activation, the redistribution of blood from the liver vascular space into the systemic circulation would not impair the exchange of substrates between plasma and hepatocytes, allowing an optimal metabolic response of the liver to stress.

In pathological conditions, the effects of sympathetic stimulation may be different. It is conceivable that in the case of a concomitant decrease in liver blood flow, sympathetic stimulation could lead to closure of sinusoids and the exclusion of a significant portion of the hepatic parenchyma. In inflammatory conditions, excessive production of vasoactive mediators by leucocytes migrating into the liver parenchyma could modify the effects of sympathetic stimulation on the hepatic microcirculation. Finally, in cirrhosis, the development of fibrosis can create a diffusional barrier in sinusoids, and could markedly change the dispersion regimen of molecules across the endothelium, thus modifying the hepatic response to stress.

3.4. ROLE OF THE MICROVASCULATURE IN THE MODULATION OF HEPATIC METABOLISM

A variety of agents with vasoconstrictive effects on hepatic microcirculation can induce glycogenolysis in the isolated perfused rat liver. The glycogenolysis induced by the administration of norepinephrine and other adrenergic agonists results from a direct effect on the hepatocytes, as demonstrated by the response of cultured or isolated hepatocytes (Hems and Whitton, 1980). An additional mechanism that could contribute to glycogenolysis is tissue hypoxia induced by vasoconstriction. In effect, anoxia is a potent activator of hepatic glycogenolysis (Hems and Witton, 1980; Sharma *et al.*, 1980), and has been shown to be due to stimulation of phosphorylase a (Van de Broeck *et al.*, 1988). However, sodium nitroprusside which inhibits the vasoconstriction induced by adrenergic agonists and sympathetic stimulation does not significantly modify the response, indicating that tissue hypoxia does not contribute to the glycogenolysis induced by adrenergic agonists (Hartmann *et al.*,1982).

Several agents known to stimulate Kupffer cells, such as PAF (Buxton *et al.*, 1984b, 1986a,b; Fisher *et al.*, 1984; Shukla *et al.*, 1983) phorbol myristate acetate (Garcia-Sainz and Hernandez-Sotomayor, 1985; Patel, 1987) zymosan (Dieter *et al.*, 1987b), heat-aggregated IgG (Buxton *et al.*, 1984a, 1987a; Steinhelper and Olson, 1988a), and complement fraction C3a (Puschel *et al.*, 1989) stimulate glucose production in the isolated perfused rat liver. As discussed previously, these agents concomitantly induce vasoconstriction. Glycogenolysis does not result from a direct effect on hepatocytes since these agents are inactive on cultured or isolated hepatocytes. A series of experimental data suggested that activation of glycogenolysis could result, just as vasoconstriction, from eicosanoids release by sinusoidal cells (Altin and Bygrave, 1988b; Garcia-Sainz, 1989): (a) responses are suppressed or significantly reduced by phospholipase A_2, cyclo-oxygenase or 5-lipo-oxygenase inhibitors and TXA_2 antagonists

(Dieter *et al.*, 1987b); (b) agents stimulating glyco-genolysis can induce PGD_2, PGE_2 and TXA_2 release by cultured sinusoidal cells and isolated perfused rat liver; (c) PGE_2 and PGF_{2x} induce glycogenolysis in the isolated perfused rat liver and cultured hepato-cytes, while TXA_2 analogs and leukotrienes induce glycogenolysis in the isolated perfused rat liver but not in cultured hepatocytes (Altin and Bygrave, 1988a,b; Iwai *et al.*, 1988a; Fisher *et al.*, 1987).

There is evidence that endotoxins (Casteleijin *et al.*, 1988a,b,c) could stimulate glucose production by similar mechanisms. Glycogenolysis induced by ATP and UTP could result both from a direct effect on hepatocytes and from prostaglandin production by sinusoidal cells (Haussinger, 1989). Finally, glycogenolysis elicited by sympathetic stimulation could be mediated, at least in part, by prostaglandins (Iwai and Jungermann, 1987).

In summary, these findings show that the hepatic microvasculature can no longer be considered as a passive structure ensuring the dispersion of molecules in the liver but rather as a very sophisticated cellular network releasing a variety of mediators modulating liver metabolism. As discussed later, it is conceivable that an inappropriate production of such mediators, as occurs in inflammatory conditions, could con-tribute to the impairment of liver function (Hagmann *et al.*, 1984; Hagmann and Keppler, 1988; Keppler *et al.*, 1985).

4. HEPATIC MICROCIRCULATION IN DISEASE

Alterations of liver microcirculation and sinusoidal cells occur in many pathological states. Changes in the architecture of the microvasculature will 'pas-sively' affect its hemodynamic properties, as well as the exchange processes. It has also recently been recognized that liver damage induced by a number of agents can be mediated by sinusoidal cells through 'active' mechanism(s). In this section, we will examine the extent and nature of the alteration of liver microvasculature in cirrhosis, alcoholic liver disease and several models of acute liver injury. We will also discuss the contribution of these modifications to the disease process.

4.1. CIRRHOSIS

Cirrhosis is the final common pathway of most types of chronic liver injury and is defined as a widespread fibrosis with the formation of regenerat-ing nodules. It involves a dramatic and irreversible modification of liver architecture.

In cirrhosis, marked modifications of liver micro-circulation have been demonstrated; it is widely accepted that these changes contribute to the in-creased resistance to portal flow and to the develop-ment of portal hypertension. There is also evidence that microcirculatory disturbances could contribute to the reduced function of the cirrhotic liver.

4.1.1. *Morphology of Hepatic Circulation*

We will give a brief overview of the major alter-ations of hepatic microcirculation that have been reported. The morphology of hepatic circulation in cirrhosis has been studied by means of radiological techniques, light microscopy, transmission electron microscopy and, more recently, by corrosion cast-scanning electron microscopy. These methods have been used in animals with experimentally-induced cirrhosis as well as in cirrhotic patients.

In cirrhosis, the branches of the portal vein are usually tortuous, irregular and slightly decreased in caliber but, in general, these modifications are slight (Shibayama and Nakata, 1985, 1989a,b). Portal venules are present in fibrous septa where they can proliferate around the regenerative nodules (particu-larly in macronodular cirrhosis with thin septa) and/or constitute intrahepatic porto-hepatic shunts (mainly in micronodular cirrhosis with thick septa) (Hirooka *et al.*, 1986). A marked proliferation of hepatic arterioles and capillaries is also noted in fibrous septa around the regenerative nodules. The extent of the proliferation appears to be related to the degree of fibrosis. Accordingly, it has been suggested that in micronodular wide septal cirrhosis, regenerative nodules are perfused by terminal arteri-oles and capillaries while in macronodular thin septal cirrhosis nodules are perfused by both arterial capillaries, and portal venules (Hirooka *et al.*, 1986). A marked hyperplasia of the peribiliary vascular plexus has also been observed in several studies, even in the absence of any proliferation of bile neoductules (Terada *et al.*, 1989). Arterio-portal anastomoses have been reported to be significantly increased in cirrhosis, and anastomosis may develop between terminal arterioles or capillaries and terminal portal venules or sinuosids. There is no evidence that intra-hepatic shunts can develop from arterioles (Hirooka *et al.*, 1986).

Branches of hepatic veins are tortuous, irregular in caliber and show stenoses. Abnormalities are more pronounced than in the portal vein. Structural alter-ations are also found in the smallest hepatic venules which appear to be compressed by the surrounding nodules (Shibayama and Nakata, 1985).

Several structural modifications of sinusoids have also been reported. Morphometric studies have shown that the surface area and caliber of sinusoids are usually decreased (Reichen *et al.*, 1987c; Vidins *et al.*, 1985). There is a marked increase in collagen deposited in the space of Disse which, in conse-quence, appears widened (Martinez-Hernandez, 1985; Huet *et al.*, 1982; Varin and Huet, 1985; Reichen *et al.*, 1987a; Gross *et al.*, 1987). However, the degree of perisinusoidal fibrosis can vary greatly inside a given region of the parenchyma (Stenger, 1966): some sinusoids have a normal structure, while others show perisinusoidal fibrosis with no significant structural modification. Finally, sinusoids can take on the appearance of capillaries with a continuous endothelium lacking fenestrae, supported by a con-tinuous basement membrane (Martinez-Hernandez, 1985). Such 'capillarized' sinusoids are usually found at the periphery of the nodules near fibrous septa (Stenger, 1966).

These modifications may also vary according to the etiology of the cirrhosis. Capillarization of the sinusoids appears to be more marked in human alcoholic cirrhosis and in liver fibrosis induced in

rabbits by cholesterol and stilbestrol (Huet et al., 1986). In contrast, in CCl$_4$-induced cirrhosis in rats or in dogs with chronic bile duct ligation, a significant fraction of the hepatic sinusoids may remain structurally unaltered (Huet et al., 1986).

4.1.2. Intrahepatic Portal Resistance

A major consequence of the structural modifications of liver microcirculation in cirrhosis is an increase in intrahepatic portal resistance. However, the exact mechanism and location of portal resistance is not known. Initially, it was thought that the increase in resistance was postsinusoidal, secondary to the compression of hepatic venules by regenerating nodules. However, most of the recent evidence suggests that it is located mainly at the level of the sinusoids (Conn and Groszmann, 1982).

The site of increased vascular resistance in the cirrhotic liver has been studied in rats by Shibayama and Nakata (1985), who measured microvascular pressure by the direct insertion of a glass micropipette (10–20 μm tip diameter) into the microvessels. Cirrhosis was induced by a choline-deficient diet for 6 months. Blood pressure in the portal vein and the terminal portal venules (30–40 μm diameter) were 173 and 100 mm H$_2$O respectively, while those in the terminal hepatic venules and the inferior vena cava were only slightly above normal. These data suggest that the increase in vascular resistance was located in the intrahepatic portal veins, between the portal vein and the terminal portal venule, as well as in the sinusoids, between the terminal portal venule and the terminal hepatic venule, but not in the intrahepatic veins. It was suggested that the cause of increased portal resistance was the stenosis of most peripheral branches of the portal vein, while the cause of increased sinusoidal resistance was thought to be the narrowing of sinusoids by hepatocytes swollen by a marked accumulation of fat droplets. This hypothesis was subsequently confirmed in rats without steatosis. In effect, it was found that in choline-deficient cirrhotic rats fed with an ordinary regimen for a further 2 months, in which steatosis was absent and the caliber of sinusoids was normal, increased sinusoidal resistance and elevated portal pressure were reduced markedly (Shibayama and Nakata, 1989b). These findings have recently been confirmed in the isolated rat liver perfused at constant pressure: in cirrhotic rats with steatosis, portal resistance was 2.7 times the normal while in cirrhotic rats without steatosis, portal resistance was only 1.5 times the normal (Shibayama and Nakata, 1989a).

Microvascular pressures have also been measured in CCl$_4$-induced cirrhotic rats (Shibayama, 1988). Blood pressure in the portal vein, the terminal portal venules, the terminal hepatic venules and the inferior vena cava were 211, 112, 34 and 24 mm H$_2$O respectively. The data again show that the increased vascular resistance is in the intrahepatic portal vein and the sinusoids. The increase in sinusoidal resistance was associated with a narrowing of the sinusoids and was interpreted by the authors as a consequence of the enlargement of hepatocytes. The reduction in vascular volume of the cirrhotic liver has also been observed in rats with CCl$_4$-induced cirrhosis using the

multiple indicator dilution curve technique and was reported to range from −18 to −37% (Varin and Huet, 1985).

Another mechanism contributing to the narrowing of the sinusoids might be the enlargement of the space of Disse by perisinusoidal fibrosis. In effect, working on isolated perfused CCl$_4$-induced cirrhotic rat livers, we have found a significant inverse correlation between intrahepatic portal resistance and liver weight that does not exist in normal livers (Ballet et al., 1988). Since in cirrhosis liver volume is inversely correlated with the degree of fibrosis, intrahepatic portal resistance would appear to correlate with fibrosis. As discussed later, the finding that intrahepatic portal resistance can be modulated in this model by vasoactive agents suggests that part of the increased resistance could also be caused by the contraction of cellular structures.

In alcoholic cirrhosis, most of the evidence also suggests that the increased resistance is of sinusoidal origin. It is well known from hemodynamic studies in alcoholic cirrhotic patients that pressure measured in hepatic veins in the wedged position is identical to that measured directly in the portal vein. This has been generally interpreted as indicating that the main location of resistance is sinusoidal (Conn and Groszmann, 1982). Again, the cause of increased resistance could be the decrease in sinusoidal caliber by perisinusoidal fibrosis. Indeed, a very significant correlation exists between fibrosis in the space of Disse and portal pressure (Orrego et al., 1981).

In alcoholic liver disease induced in baboons by alcohol feeding, early and selective fibrosis around the hepatic venules has been observed (Nakano et al., 1982). However, the contribution of such lesions to the increased resistance is unknown. Israel and Orrego (1987) have proposed that hepatocyte enlargement is the main cause of sinusoidal compression and increased intrahepatic resistance. According to them, the main arguments in favor of this hypothesis are the following: (a) morphometric studies show that hepatocyte size is increased and sinusoidal area is decreased in rats fed chronically with alcohol as well as in patients with alcoholic cirrhosis (Vindins et al., 1985), (b) the swelling of hepatocytes by perfusing isolated rat livers with a hypotonic medium is associated with a significant increase in intrahepatic resistance (Colman et al., 1983) and (c) in patients with alcoholic liver cirrhosis, there is a significant inverse correlation on the one hand between hepatocyte size and sinusoidal area and on the other hand between portal pressure and sinusoidal area, if reduced to values below 10% of normal (Vindins et al., 1985).

The 'cell enlargement' hypothesis has been challenged by Huet et al. (1987), who measured liver vascular and extravascular diffusion spaces using the multiple indicator dilution curve technique in isolated perfused livers from rats chronically intoxicated with alcohol. They found an increase in total liver volume, with an increase in cellular and interstitial volumes but no significant change in sinusoidal volume. Furthermore, intrahepatic portal resistance of the perfused liver and portal pressure in vivo (Mastaï et al., 1989) were not significantly modified. Finally, administration of alcohol, in CCl$_4$-induced cirrhotic rats,

did not further increase portal pressure measured *in vivo* in conscious animals, in spite of a significant increase in liver cell volume assessed by morphometry (Huet *et al.*, unpublished data). This indicates that cell enlargement is not necessarily associated with a reduction in sinusoidal volume and an increase in portal pressure.

In conclusion, most of the evidence suggests that in alcoholic cirrhosis, CCl_4-induced cirrhosis and cirrhosis induced by a choline-deficient diet, increased resistance is mainly due to the reduction in sinusoidal caliber and volume. However, there is some disagreement as to the cause of sinusoidal reduction, i.e. whether it results from hepatocyte enlargement or fibrosis in the space of Disse.

It is important to emphasize that the site of increased resistance may be different in other varieties of cirrhosis. In patients with biliary cirrhosis or posthepatitis macronodular cirrhosis, pressure measured in the portal vein is usually higher than in the hepatic veins in the wedged position, suggesting that in these conditions the main site of resistance is presinusoidal (Conn and Groszmann, 1982).

4.1.3. Physiopathology of Portal Hypertension

In cirrhosis, the main consequence of the increased intrahepatic vascular resistance is increased pressure in the portal system and the development of porto-systemic shunts. Therefore, in this condition, portal resistance consists of two parallel resistance pathways, i.e. (a) intrahepatic resistance where resistance is high and (b) collateral resistance where resistance is low. In spite of the latter low resistance pathway, total portal resistance remains abnormally high (Sikuler *et al.*, 1985; Sikuler and Groszmann, 1986a). Furthermore, a number of studies have found that in cirrhosis splanchnic blood flow, i.e. flow entering the portal system, is increased by approximately 40–50%; this has been shown in a variety of experimental models: partial ligature of the portal vein (Blanchet and Lebrec, 1982; Groszman *et al.*, 1982; Lebrec and Blanchet, 1985; Vorobioff *et al.*, 1984), chemically-induced cirrhosis (Fernandez-Munoz *et al.*, 1985) and biliary cirrhosis (Bosch *et al.*, 1983). The pressure–flow relationship in the portal system is impaired, as when flow is reduced to normal values, portal pressure remains high (Benoit *et al.*, 1985; Kroeger and Groszmann, 1985a; Sikuler and Groszmann, 1986a). The increase in portal inflow is due to vasodilation of arterioles in the splanchnic territory (Benoit *et al.*, 1984, 1986). However, vasodilation is not restricted to the splanchnic territory but also involves other organs such as muscle (Benoit *et al.*, 1985). This leads to a reduction in peripheral vascular resistance and systemic arterial pressure and to an increase in cardiac output, i.e. an hyperkinetic syndrome (Blanchet and Lebrec, 1982; Vorobioff *et al.*, 1983). The mechanism of vasodilation is unknown (Schrier and Caramelo, 1987) but a number of studies suggest that it could be secondary to an increased plasma concentration of a vasodilatory substance (Benoit *et al.*, 1984; Korthuis *et al.*, 1985; Sikuler and Groszmann, 1986b) and/or to a reduction in vascular response to vasoconstrictors (Bomzon and Blendis, 1987; Chiandussi *et al.*, 1962; Kiel *et al.*, 1985; Laragh *et al.*, 1963; Murray and Paller, 1985).

4.1.4. Pharmacology of Portal Hypertension

The major complication of portal hypertension is bleeding from esophageal varices which accompanies the development of porto-systemic shunts. The risk of bleeding in cirrhotic patients with esophageal varices who have never bled is estimated as approximately 30%, reaching 65% in patients with large varices. The mortality rate in this complication is 40% (Burroughs, 1986; Calès and Pascal, 1988). Bleeding is likely to recur in 50–70% of untreated patients, and the mortality rate in such patients is 30% (Calès and Pascal, 1988; Poynard and Chaput, 1989). Since there is evidence that increased portal pressure plays a major part in the development and rupture of esophageal varices (Garcia-Tsao *et al.*, 1985), most of the research has been directed at finding pharmacological agents which lower portal pressure. Theoretically, portal pressure can be reduced either by decreasing splanchnic blood flow or by decreasing portal resistance (Groszmann *et al.*, 1988).

4.1.4.1. Vasoconstrictors.
A reduction in splanchnic blood flow can be obtained either by decreasing cardiac output or by inducing splanchnic vasoconstriction. Vasopressin decreases portal flow by inducing splanchnic vasoconstriction (Groszmann and Atterbury, 1985). Data suggest that somatostatin could act by a similar mechanism (Bosch, 1985; Morgan and Groszmann, 1989). These agents have been used in patients who bleed actively with similar efficacy to that obtained by balloon tamponade. However, the evidence shows conclusively that these drugs do not significantly influence survival.

4.1.4.2. Beta-blockers.
In 1981, Lebrec and co-workers in France showed that propranolol, at doses that decrease the heart rate by 25%, significantly reduced the risk of rebleeding in cirrhotic patients in good overall condition. This study was a landmark in the therapy of portal hypertension, hitherto considered inaccessible to medical therapy. The mechanism of action of propranolol is still unknown but a number of clues have been obtained in experimental studies (Bosch, 1985; Groszmann and Atterbury, 1985). Propranolol has been shown to reduce portal pressure both as an antagonist of β_1-adrenergic cardiac receptors, decreasing cardiac output, and as an antagonist of β_2-adrenergic splanchnic receptors inducing vasoconstriction via α-adrenergic receptors unopposed by β_2-vasodilatory receptors (Kroeger and Groszmann, 1985a,b). Both mechanisms decrease portal flow and, consequently, portal pressure.

So far, seven controlled trials have studied the effect of beta-blockers on the risk of rebleeding in cirrhotic patients (Lebrec and Hadengue, 1989); however, although it was reduced in four studies, in the other three it was not significantly modified. Survival was significantly improved in only one study. The reasons for the discrepancies between these studies have been extensively examined but remain unclear (Lebrec, 1987). For example, alter-

ations in β-adrenergic receptor responsiveness have been demonstrated in these patients and are correlated to the severity of the liver disease (Caujolle et al., 1988; Gerbes et al., 1986, 1987; Ramond et al., 1986). However, the role of this factor in the therapeutic efficacy of beta-blockers is unknown (Caujolle et al., 1988).

Meta-analysis of the trials has shown that beta-blockers decrease the risk of bleeding after two years from 66% in the placebo group to 49% in the treated group. However, heterogeneity among the groups is significant and no definitive conclusion can be drawn (Pignon et al., 1988). By contrast, it remains clear that survival is not significantly modified. These studies show that the efficacy of propranolol in the prevention of rebleeding is nil or, at the most, very modest. However, it remains possible that a subgroup of cirrhotics, so far unidentified, could benefit from the treatment.

β-Adrenergic agents have also been evaluated in the prophylaxis of variceal bleeding in patients who had never bled. Five controlled trials have been published (Conn, 1988; Lebrec and Hadengue, 1989). All show a highly significant reduction in the risk of bleeding. It is generally admitted that, in this group of patients, the risk of bleeding is reduced by approximately 30–40%. Furthermore, a significant effect on one-year survival was found by meta-analysis of the published studies (Pignon et al., 1988).

In conclusion, although the efficacy of propranolol in the prevention of rebleeding is still disputed, it is generally accepted that the drug is beneficial in the prophylaxis of variceal bleeding in high risk patients who have never bled.

4.1.4.3. Vasodilators.

An alternative approach to reducing portal pressure is to decrease intrahepatic vascular resistance. Although it had generally been accepted that the vascular resistance of the cirrhotic liver was fixed and insensitive to vasoactive agents, this concept was challenged by Bhatal and Grossman in 1985. These authors showed in isolated perfused livers from rats with CCl_4-induced cirrhosis that some vasodilators were able to decrease intrahepatic portal resistance. Livers were perfused at a constant pressure (16 mm Hg) with a buffer solution containing albumin. Sodium nitroprusside (3×10^{-3} M), magnesium sulfate (6×10^{-2} M), papaverine (6.4×10^{-4} M) and cytochalasine B (6.3×10^{-5} M) decreased portal resistance by approximately 15%, while prostaglandin E_1 and isoprenaline reduced it by approximately 5% (Bhatal and Grossman, 1985). In the isolated perfused normal rat liver, most of these agents were unable to decrease resistance significantly, only magnesium sulfate and papaverine reducing it by about 5%. According to the authors, a series of arguments suggested that the reduction of resistance by vasodilators could be secondary to the relaxation of myofibroblasts. In effect (a) myofibroblasts have been shown to occur around sinusoids and vessels embedded in fibrous septa (Rudolph et al., 1979), (b) preparations containing myofibroblasts can maintain tonic contractions in vitro and (c) strips of granulation tissue can relax in vitro under the influence of prostaglandin E_1, papaverine and cytochalasine B.

Other data were subsequently provided by Reichen and Le (1986) who studied the effect of the calcium channel blocker verapamil on the isolated perfused liver from rats with CCl_4-induced cirrhosis. Livers were perfused with a system where both flow and pressure can vary when changes in intrahepatic resistance occur. The perfusion medium contained 20% human erythrocytes and albumin. Verapamil at concentrations ranging from 8 to 16 mg/100 ml (1.6 to 3.2×10^{-4} M) decreased the resistance of the livers by approximately 22% while the resistance of normal livers was reduced by 14%. Oxygen consumption of the cirrhotic livers increased significantly by approximately 21%. Intrahepatic shunts, estimated from the recovery of labeled microspheres at the liver outflow, were significantly reduced from $4.9 \pm 3.3\%$ to $3 \pm 3\%$. Interestingly, verapamil did not significantly modify the sinusoidal volume as estimated from the labeled erythrocyte dilution curve. This suggested that the reduction in resistance was not due to an increase in the sinusoidal caliber throughout the length. Subsequently, Reichen et al. (1986) studied the effect of chronic administration of verapamil in rats with CCl_4-induced cirrhosis via the drinking water for 9–11 days. The plasma concentration of verapamil was 81 ± 65 ng/ml (1.6×10^{-7} M). Systemic arterial pressure was not significantly modified, but intrasplenic pressure, which provides an estimate of portal pressure, was reduced by approximately 28%. Again, the sinusoidal volume measured on the isolated perfused rat liver was not significantly modified. These findings suggested that chronic administration of verapamil was able to decrease portal pressure in cirrhotic rats in vivo. However, other mechanisms than a decrease in intrahepatic resistance could have explained these findings, e.g. a decrease in the resistance of portosystemic collaterals or a decrease in portal flow induced by reflex splanchnic vasoconstriction.

These reports led us to investigate the vascular response of the cirrhotic liver to vasoactive agents. The effects of norepinephrine, angiotensin II and vasopressin were studied in isolated perfused livers from CCl_4-induced cirrhotic rats vs normal livers (Ballet et al., 1988). Livers were perfused at constant pressure. In basal conditions, intrahepatic vascular resistance was 2–3-fold higher than that of normal livers. Norepinephrine, angiotensin II and vasopressin induced a dose-dependent increase in vascular resistance in normal as well as in cirrhotic livers. Interestingly, we found that the maximal effect (E_{max}) of normal and cirrhotic livers did not differ significantly. The sensitivity to norepinephrine (ED_{50}) was even increased in cirrhotic livers while that to angiotensin II was decreased and that to vasopressin was not significantly modified. Overall, these data showed that the resistance of the cirrhotic liver could be modulated by the three major circulating vasoactive hormones. This further suggested that the high plasma concentration of these mediators which has been demonstrated in cirrhosis could contribute to increased intrahepatic vascular resistance. Subsequently, we studied the effects of a series of vasodilators using the same experimental model (Marteau et al., 1989). The vasodilators studied were an α_1-adrenergic antagonist (prazosin), an α_2-adrenergic

agonist (clonidine), a $\beta_1-\beta_2$-adrenergic agonist (iso-proterenol), calcium antagonists (verapamil, diltia-zem and nifedipine), nitrovasodilators (sodium nitroprusside and nitroglycerin) and vasodilators with miscellaneous pharmacological properties, i.e. papaverine, pentoxifylline and diazoxide. In basal conditions, isoproterenol, sodium nitroprusside, nitroglycerin, papaverine and pentoxifylline signifi-cantly decreased resistance. However, the maximal response was weak (-3 to 19%) and isoproterenol was the only drug active in the therapeutic range of plasma concentrations ($10^{-7}-10^{-6}$ M). Prazosin, propranolol, verapamil, diltiazem, nifedipine and dia-zoxide were ineffective (Marteau et al., 1989).

Plasma concentrations of norepinephrine are increased in cirrhotic patients (Arroyo et al., 1983; Bernardi et al., 1983; Bichet et al., 1982; Henriksen et al., 1984, 1985; Lenz et al., 1985; Nicholls et al., 1985; Ring-Larsen et al., 1982; Tage-Jensen, 1988; Willet et al., 1985) and, as discussed previously, nore-pinephrine significantly increases intrahepatic resis-tance in cirrhotic livers. We therefore examined the effects of vasodilators after the induction of vaso-constriction by norepinephrine. Prazosin, papaverine and pentoxifylline significantly reduced vasoconstric-tion induced by norepinephrine while it was not significantly modified by clonidine, isoproterenol and propranolol (Marteau et al., 1989). The lack of effect of clonidine in the cirrhotic liver contrasts with its inhibitory effect in the normal liver, suggesting that a significant modification of α-adrenergic activity occurs during the development of cirrhosis.

The lack of response to calcium antagonists was in discrepancy with the results obtained by Reichen and Le (1986), and could be related to differences in experimental conditions. In the Reichen study, per-fusion flow and pressure were able to vary with resistance, while in our system pressure was main-tained constant. Also, our perfusion medium con-tained rat erythrocytes, while in the Reichen study human erythrocytes were used. Since the diameter of rat erythrocytes is much smaller than that of human erythrocytes, verapamil, which increases erythrocyte deformability, would affect resistance more when the liver is perfused with human erythrocytes than with rat erythrocytes.

Rheological factors could also explain the decrease in resistance induced by pentoxifylline in the cirrhotic liver. Pentoxifylline also increases red blood cell deformability; however, at the low hematocrit level (15%) used in our study, the change in viscosity was probably too small and insufficient to account for the decrease in resistance.

In conclusion, these studies show that in spite of fibrosis, the vascular resistance of the cirrhotic liver can be modulated by vasoactive agents. How-ever, the reduction in resistance induced by vaso-dilators is small and is reduced in the presence of norepinephrine. Furthermore, the effect in vivo of vasodilators on portosystemic collaterals or on the splanchnic circulation could antagonize their poten-tial beneficial effect on portal pressure (Blei, 1989). In effect, isoproterenol has been shown to increase portal pressure in normal dogs (Seaman and Green-way, 1984), while clonidine decreases portal pressure in cirrhotic patients (Willet et al., 1986). It has been

suggested that this latter effect could result from a decrease in intrahepatic portal resistance (Willet et al., 1986); however, our experimental studies suggest that it may be due to alternative mechanisms. Nitro-glycerin and nitrates also decrease portal pressure in cirrhotic patients (Blei et al., 1988); however, experimental studies have demonstrated that this is due to a reduction in both splanchnic flow and in portal collateral resistance (Blei and Gottstein, 1986). Finally, hemodynamic studies in cirrhotic patients have shown that verapamil and nifedipine do not significantly modify portal pressure (Navasa et al., 1988).

4.1.5. Vascular Factors in Decreased Liver Function in Cirrhosis

The role of the structural modifications of the microcirculation in the diminished function of the cirrhotic liver is unclear.

Various theories have been proposed to explain the decreased clearance observed in cirrhosis. The 'sick cell' hypothesis states that the function of individual hepatocytes is decreased. The 'capillariza-tion' hypothesis considers that the loss of fenestra-tions of endothelial cells and the constitution of a continuous basal layer beneath the endothelial cells decreases the permeability of sinusoids to diffus-ible substances in the plasma (Huet et al., 1982; Villeneuve and Huet, 1987). The 'intact cell' hypoth-esis holds that intrahepatic shunts account for a reduced number of normally functioning perfused hepatocytes (Wood et al., 1979).

Different approaches have been used to discrimi-nate between the models. It is possible to compare the observed values for different pharmacokinetic parameters under conditions of varying flow, or varying protein binding with the values predicted from the model. For example, the lack of increase in the hepatic extraction of propranolol when flow is reduced has been interpreted as indicating that intravascular shunting is present since, in case of a uniform reduction of enzyme activity, a reduction in flow should increase extraction (Wood et al., 1979). Similarly, the reduction in hepatic blood flow induced by lysine-vasopressin in cirrhotic patients is accom-panied by a decrease in the intrinsic clearance of indocyanin green (Barbare et al., 1984). This reduc-tion is consistent with an increase in intrahepatic shunting when flow decreases. For substances bound to proteins in the plasma, another experimental approach is to study the influence of varying un-bound protein fractions on hepatic elimination parameters. According to the 'intact cell' model, a highly extracted drug will maintain its clearance regimen in spite of decreased extraction: its extraction will only be slightly affected by the variation in the unbound fraction, due to liver regions with normally functioning hepatocytes. By contrast, in the 'sick cell' model, a highly extracted substance would show a modified clearance regimen since all hepatocytes are supposed to have a low extraction: in these con-ditions, extraction will be low and will vary closely with the unbound fraction. In the 'capillarization model' substrates whose diffusion across the sinu-soidal barrier is impaired will behave similarly, i.e.

their extraction will be reduced and highly dependent upon the fraction unbound to proteins. In the isolated perfused liver from rats with CCl_4-induced cirrhosis, we found that the extraction of taurocholate, a substrate bound to albumin, was decreased and strongly influenced by the unbound fraction. These data were consistent either with the 'sick cell' or the 'capillarization' model (Petit and Ballet, unpublished data).

More direct evidence for the 'sick cell' hypothesis has been provided by studying the function of hepatocytes isolated from the cirrhotic liver. It has been shown that the *in vitro* metabolism of a propranolol was impaired and that this is accompanied by a reduction in the cytochrome P-450 content (Villeneuve et al., 1978). Recently, reduced cellular uptake of taurocholate and ouabaine, together with reduced oxygen consumption, have been demonstrated (Reichen et al., 1987b). However, it must be pointed out that *in vitro*, numerous artifacts or experimental constraints may limit the meaning of the data. For example, the very low yield of the dissociation procedure may induce sampling artifacts. In favor of the 'intact cell' hypothesis, Reichen et al. (1987a) have found that reduced aminopyrine N-demethylation assessed by the aminopyrine breath test in rats with CCl_4-induced cirrhosis correlated with liver cell volume. Microsomal aminopyrine N-demethylase and cytochrome P-450 activities expressed per unit of hepatocyte volume were maintained, indicating that the function of individual cells remained unaltered. The galactose elimination capac-

ity has also been found to correlate with liver cell volume in both CCl_4-induced and biliary cirrhosis in the rat (Gross et al., 1987).

The multiple-indicator dilution technique has been used by Huet and Goresky in Montreal to study the influence of microcirculatory alterations on exchange processes in cirrhosis (Huet et al., 1982; Varin and Huet, 1985). As stated previously, the hepatic vascular volume estimated from the diffusion space of labeled red blood cells was significantly reduced. A major finding was that extravascular diffusion space of albumin was decreased in proportion to the degree of fibrosis in the space of Disse. This indicated that fibrosis was able to restrict the extravascular diffusion of albumin (Fig. 8). At least in some cirrhotic patients, the albumin diffusion space can even be superimposed on that of red blood cells, thus providing a direct demonstration of the capillarization of sinusoids (Fig. 8) (Huet et al., 1982). By contrast, in CCl_4-induced cirrhosis in the rat, such a capillarization pattern was uncommon in the study of Varin and Huet (1985), while it was present in most cirrhotic livers in the studies of Reichen and Le (1986) and Reichen et al. (1986, 1988). These discrepancies are probably explained by differences in the severity of liver fibrosis obtained after the administration of CCl_4. In cirrhotic patients, the hepatic extraction of ICG, a dye highly protein-bound is decreased and strongly correlated with the diffusion space of albumin (Huet et al., 1982). This suggests that the restricted diffusion of albumin across the endothelial barrier affects the uptake of the protein-bound dye.
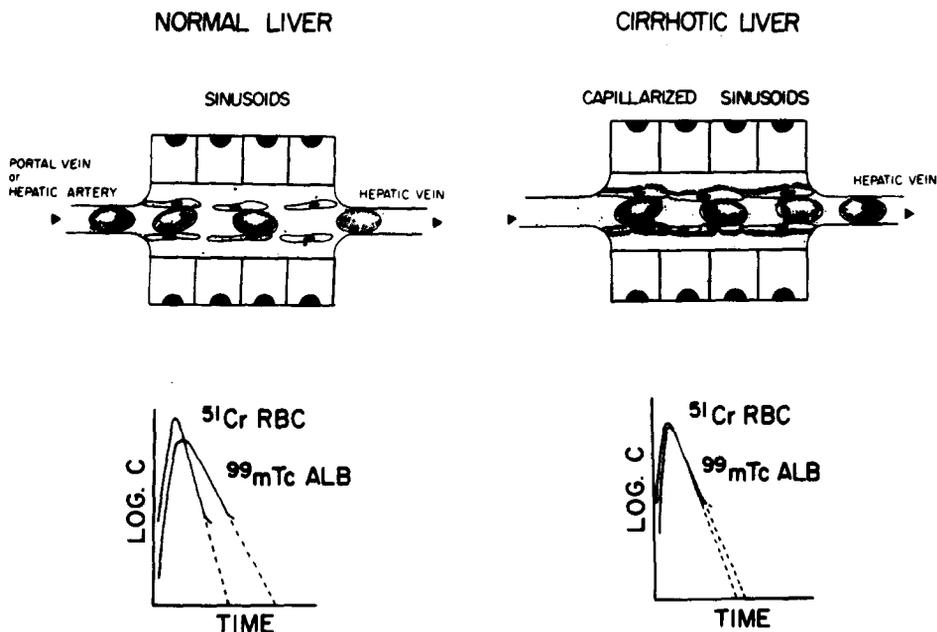

FIG. 8. Schematic representation of the liver microcirculation in a normal liver (left hand panel) and in a cirrhotic liver (right hand panel). In normal liver, the entry of ^{99m}Tc albumin (^{99m}Tc ALB) into the space of Disse produces a major delay in and decrease in magnitude of the albumin curve with respect to that of ^{51}Cr red blood cells (^{51}Cr RBC). In the cirrhotic liver, capillarization limits the diffusion of albumin into the space of Disse and the ^{99m}Tc ALB curve is only slightly delayed in relation to the corresponding ^{51}Cr RBC curve. From Huet et al., 1985. Reprinted with permission of the authors and copyright holder, W. B. Saunders, Philadelphia.

The modifications of the diffusion space of sucrose and water are more difficult to analyze since, at least in some cases, their diffusion in the extravascular space does not appear to be flow-limited, indicating a diffusional barrier at the level of the sinusoid (Huet et al., 1982).

In some cirrhotic patients, as well as in most cirrhotic dogs with chronic bile duct ligation, labeled erythrocyte, water and lidocaine appear as an early peak, related to a bulge on the erythrocyte upslope curve, superimposed with that of 15 μm microsphere (Huet et al., 1986). This early peak has been interpreted as representing predominantly throughput, material coming through vascular shunts poorly permeable to small molecules which diffuse freely across membranes, such as lidocaine and water. In CCl_4-induced cirrhosis, lidocaine outflow patterns have the form of a dilution curve slightly delayed in relation to that of labeled red blood cells (Varin and Huet, 1985). This pattern differed systematically from that observed in noncirrhotic rats, in which unchanged lidocaine emerged substantially later and only in trace amounts. In contrast, only $0.25 \pm 0.34\%$ (range 0–12%) of 15 μm microspheres were recovered in the outflow. These findings were consistent with the development of small ($< 15 \mu$m) intrahepatic shunts. In other studies, intrahepatic shunting measured by the passage of 15 μm microspheres was higher: $3.8 \pm 2.85\%$ and $2.2 \pm 2.6\%$ but highly variable ranging from 0–10% (Reichen and Le, 1986; Reichen et al., 1986, 1988). Again, the discrepancies may be related to differences in the severity of cirrhosis produced in rats using CCl_4.

In hepatic fibrosis induced in rabbits with a cholesterol-supplemnted diet (1%) and stilbestrol (10 mg twice weekly s.c.) for 12 weeks (Mastai et al., 1988a,b) the albumin diffusion space was markedly decreased. By contrast, the water diffusion space and lidocaine outflow curve were unchanged and the recovery of 15 μm microspheres was less than 1%. These data suggest that, in this model, extensive fibrosis of sinusoids restricts the diffusion of albumin but does not affect the diffusion of water and lidocaine, i.e. intrahepatic shunting does not occur. Interestingly, it was found that taurocholate extraction was much more strongly decreased (−45.9%) than propranolol extraction (−3%), suggesting that capillarization selectively impairs the diffusion of hydrophilic protein-bound substances such as taurocholate, by opposition to lipophilic drugs such as propranolol (Mastai et al., 1988b).

In conclusion, data obtained with the multiple-indicator dilution technique suggests different kinds of microcirculation alterations in cirrhosis, i.e. (a) a reduction in the vascular space, (b) a reduction in extravascular diffusion space of albumin with a pattern consistent with sinusoidal capillarization when fibrosis in the space of Disse is extensive and (c) the development of large and small intrahepatic shunts.

The consequences of these alterations for hepatic elimination are unclear. The data suggest that sinusoidal capillarization could affect the uptake of protein-bound hydrophilic substrates such as ICG or taurocholate, without significantly impairing the transport of lipophilic molecules such as propranolol or lidocaine. Intrahepatic shunting may be the major determinant in the decreased extraction of lidocaine. Finally, the decreased extraction of propranolol is probably the consequence of both reduced metabolic cellular activity and intrahepatic shunts.

Therefore, it is clear that in cirrhosis, a multiplicity of factors such as (a) sick cells, (b) intrahepatic shunts, (c) decreased cellular mass and (d) anatomical barriers, all contribute to reduce liver function. However, the relative contribution of each factor may vary according to the duration of the disease and to superimposed events.

Finally, the main interest of these studies is to identify factors that could be the target for therapeutic intervention. In view of the possible pharmacologic modulation of hepatic microcirculation in cirrhosis, it is obvious that microcirculatory factors are of major interest.

The role of microcirculatory disturbances in the impaired function of cirrhotic livers has been examined by Reichen and Le (1986) by studying the effect of verapamil (1.6–3.2×10^{-4} M), a calcium channel blocker, on vascular and extravascular diffusion spaces and liver function. On isolated perfused livers from rats with CCl_4-induced cirrhosis, it was found that verapamil did not affect the hepatic vascular space but significantly increased the albumin extravascular space. This was associated with a 38% reduction in intrahepatic shunting. Concomitantly, a significant improvement in oxygen consumption (+21%) and antipyrine clearance (+20%), but not propranolol clearance, was observed. It was suggested that verapamil could relax endothelial cells and lead to a redistribution of flow to sinusoids with selectively well-maintained exchange characteristics.

These findings were subsequently confirmed in cirrhotic rats in vivo after oral administration of verapamil (1.6×10^{-7} M) for 9–11 days (Reichen et al., 1986). A significant improvement in hepatic microsomal function assessed by the aminopyrine and caffeine breath-test was observed. The data again suggest that the improvement in liver function could result from the effect of verapamil on liver microcirculation.

4.2. ALCOHOLIC LIVER DISEASE

The mechanism by which chronic alcoholism induces cirrhosis is still controversial. There is a general agreement that cirrhosis is preceded by alcoholic hepatitis, a lesion characterized by hepatocyte necrosis in conjunction with an inflammatory cell infiltrate predominantly composed of neutrophils and pericellular fibrosis (Popper, 1974). Necrosis in alcoholic hepatitis occurs characteristically in the hepatic acinus zone 3 (or centrilobular area).

We still do not understand how alcohol causes liver damage, but it is unlikely that the disease results from a single factor. Two interactive mechanisms appear to play a predominant role (Israel and Orrego, 1987): (a) acetaldehyde-induced damage and (b) hypoxic necrosis.

It is generally agreed that liver injury induced by ethanol results from the production of acet-

aldehyde by NAD^+-dependent alcohol dehydrogenase at low blood ethanol levels ($K_m = 0.2$–2 mM) and by cytochrome P-450 isozymes (the 'microsomal ethanol oxidizing system') at high ethanol levels ($K_m = 10$–15 mM) (Lieber, 1988). The latter pathway predominates after long-term use of alcohol which has been shown to be associated with a marked proliferation of hepatic microsomal membranes and increased activity of the microsomal ethanol oxidizing system and in the elimination rate of ethanol. The cytochrome P-450 system requires NADPH and oxygen.

Acetaldehyde is an electrophilic species that can react with nucleophilic sites of other molecules. A number of studies have demonstrated that acetaldehyde can bind *in vitro* to a number of macromolecules such as cysteine, albumin, membrane proteins, hemoglobin, lipids and nucleic acids (Lauterburg and Bilzer, 1988; Lieber, 1988). Furthermore, acetaldehyde adducts with proteins have been demonstrated in alcoholic patients. It is not clear however how these interactions lead to cell necrosis. It has been proposed that acetaldehyde-mediated cell death might have an immunological component. An immune cytotoxic response could be directed towards cellular proteins altered by acetaldehyde. Acetaldehyde could damage either intracellular proteins subsequently transported to the cell surface, or plasma membrane proteins themselves, or both (Lauterburg and Bilzer, 1988).

As stated previously, a characteristic feature of alcoholic liver injury is the predominance of lesions in the centrilobular region of the hepatic acinus. Several factors could account for this pattern (Lieber, 1988): (a) low oxygen pressure, normally prevailing in perivascular zones, may exaggerate the oxidation-reduction shift produced by ethanol which results in various deleterious metabolic effects, (b) a higher level of alcohol-metabolizing enzymes in the perivenular zone could produce higher concentrations of acetaldehyde in these regions.

To account for the centrilobular location of the lesions, it has also been suggested that hepatocellular necrosis could result from hypoxia. According to this hypothesis, centrilobular hypoxia would occur as the consequence of an imbalance between increased oxygen demand and an inadequate oxygen delivery to the liver (Israel *et al.*, 1975; Israel and Orrego, 1987; Thurman *et al.*, 1986).

Indeed, it has been demonstrated that acute and chronic ethanol administration increase the rate of oxygen uptake by the liver. The mechanism of such an increased oxygen demand has not been fully elucidated (Thurman *et al.*, 1986). It has been proposed that the increased availability of NADH for mitochondrial NADH oxidation increases oxidative phosphorylation. Increased oxygen consumption could also result from the increased activity of the microsomal ethanol-oxidizing system which has a direct requirement for oxygen. Alternatively, the increased oxygen uptake could be caused by the release of catecholamines, since (a) epinephrine mimicks the increase observed with ethanol (Thurman *et al.*, 1986), (b) the increased O_2 uptake can be blocked both by α- and β-adrenergic agents (Thurman *et al.*, 1986) and (c) chronic alcoholism is

associated with sympathetic hyperactivity, particularly during ethanol withdrawal. Finally, thyroid hormone function appears to be permissive for such an effect to occur. While T4 or T3 levels are unchanged after acute or chronic ethanol administration, thyroidectomy or the administration of the antithyroid drug propylthiouracil (PTU) suppresses or abolishes the ethanol-induced hypermetabolic state (Israel and Orrego, 1987).

The increased O_2 consumption induced by ethanol administration is associated with a steeper oxygen gradient in the liver lobule. This has been demonstrated by Thurman in perfused livers from ethanol-treated rats by measuring NADH fluorescence and tissue pO_2 in periportal and perivenous regions with micro-light guides and miniature oxygen electrodes (Thurman *et al.*, 1986). The increased oxygen gradient along the sinusoids would lead to centrilobular necrosis in conditions that reduce oxygen availability. In support of this postulate, Israel *et al.* (1975) have shown that brief exposure to hypoxia produces more centrilobular necrosis in ethanol-treated rats than in controls. Pretreatment of rats with propylthiouracil significantly reduces tissue damage. On the other hand, it has been shown that ethanol increases hepatic blood flow by 50–60%. This has been shown to be attributable to vasodilation in the splanchnic territory and has been interpreted as a compensatory mechanism that could play an important role in protecting the liver against hypoxic necrosis (Carmichael *et al.*, 1987; McKaigney *et al.*, 1986). Accordingly, in ethanol-fed rats with minimal liver injury, the enhanced hepatic oxygen consumption is adequately compensated by a concomitant increase in hepatic oxygen delivery (Bredfeldt *et al.*, 1985). However, in a rat model with alcoholic centrilobular necrosis it has recently been shown that the increase in oxygen delivery was much too small to compensate for the marked increase in oxygen consumption (French *et al.*, 1988; Tsukamoto and Xi, 1989). This suggested that centrilobular necrosis could result from enhanced, uncompensated hepatic oxygen consumption.

In conclusion, there is evidence that alcohol-induced liver necrosis could be due to anoxic injury of centrilobular hepatocytes resulting from an imbalance between the increased oxygen consumption and reduced oxygen delivery to the liver.

Theoretically, drugs that decrease oxygen demand and/or increase oxygen delivery to the liver should thus be beneficial.

As stated previously, propylthiouracil decreases oxygen uptake by the liver. Two short-term studies have yielded conflicting results (Orrego *et al.*, 1979; Israel *et al.*, 1978) but a recent long-term study reported lower mortality in patients with alcoholic liver disease (Orrego *et al.*, 1987). It is tempting to speculate that the beneficial effect of propranolol in liver cirrhosis could be related in part to its inhibitory effect on the increase in hepatic oxygen uptake due to ethanol. Drugs that have been shown to be effective in preventing or reducing hypoxic liver injury could also be beneficial in alcoholic liver disease. Finally, drugs that increase liver blood flow and oxygen delivery, i.e. vasodilators, should be tested.

4.3. EXPERIMENTALLY-INDUCED ACUTE LIVER INJURY

4.3.1. Ischemia- and Hypoxia-Induced Liver Injury

The liver is highly sensitive to ischemia. In circulatory shock, a rise in serum transaminase, sometimes accompanied by a significant increase in serum bilirubin, is frequently observed (Lefkowitch and Mendez, 1986). Histological studies show sinusoidal dilation with a variable degree of congestion and hemorrhage together with centrilobular necrosis (Lefkowitch and Mendez, 1986). Experimentally in isolated perfused livers from rats fasted for 24 hr, hypoxia induced by a 75% decrease in portal flow for 60 min or by a stopping flow for 45 min followed by reperfusion, induces damage in pericentral regions of the liver lobule (Bradford et al., 1986). The sequence of changes in cell structure has been extensively studied by Lemasters and Thurman (1981) and Lemasters et al. (1983, 1987). The first lesions to occur are blebs that develop exclusively on the sinusoidal surface of hepatocytes and that project through the fenestrations of the endothelium into the sinusoids. Subsequently, blebs detach from the cells and are released into the microcirculation with a concomitant release of cytosolic enzymes, such as lactate dehydrogenase, into the effluent. Finally, breaks appear in the plasma membrane which leads to membrane hyperpermeability and cellular staining by dyes that are normally excluded, such as trypan blue or propidium iodide. These events indicate an abrupt transition from reversible to irreversible injury (Lemasters et al., 1987).

The sensitivity of the liver to ischemia also accounts for the impossibility of extending the preservation time of liver allografts by cold storage beyond 10 hr with standard preservation solutions (Belzer and Southard, 1988).

4.3.1.1. Mechanism of cell necrosis. The mechanism(s) of hypoxia- or ischemia-induced hepatocyte necrosis are poorly understood (Bonventre, 1988; Leaf et al., 1986). It has generally been assumed that the primary target in injury is the hepatocyte. It has been proposed that cell death could occur as the result of the generation in situ of oxygen-derived free radicals (Bonventre, 1988; McCord, 1983; Parks and Granger, 1988; Granger, 1988). According to this hypothesis, ischemia triggers the conversion of xanthine dehydrogenase to the oxygen radical producing xanthine oxidase (McKelvey et al., 1988). Concomitantly, cellular ATP is catabolized during the hypoxia period to hypoxanthine. On reperfusion or reoxygenation, molecular oxygen reacts with hypoxanthine and xanthine oxidase to produce the highly cytotoxic reactive metabolites, superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^.$). This is mainly based on the observation that allopurinol, a xanthine oxidase inhibitor prevents ischemia- or hypoxia-induced liver injury (McCord, 1983). It has also been proposed that hepatocyte death could result from a massive cellular influx of calcium. According to this hypothesis, the resulting increase in cytosolic calcium could activate several Ca^{2+}-dependent enzymes such as phospholipases, proteases and endonucleases. The activated enzymes could secondarily induce cell killing by mechanisms that have been extensively reviewed (Bonventre, 1988; Orrenius and Bellomo, 1986; Pounds and Rosen, 1988). Accordingly, it has been shown that several Ca^{2+}-channel blockers have protective effects during liver ischemia or hypoxia (Lefer and Stahl, 1987; Lefer and Papanicolaou, 1985; Peck and Lefer, 1981; Thurman et al., 1987, 1988a).

It is well established that, in addition to lesions of parenchymal cells, ischemia and hypoxia induce alterations of the microcirculation. It has been shown in a number of experimental models that ischemia increases vascular resistance and capillary permeability (Korthuis et al., 1988). Different degrees of endothelial cell damage have been demonstrated (Burton et al., 1984): cells swelling, breaks in the cytoplasmic processes extending from the cell body, bulging in the vascular space and, finally, detachment. The increased resistance has been attributed to several factors, i.e. (Braunwald and Kloner, 1985) (a) occlusion of vessels by leucocyte plugging, resulting from their adhesion to the endothelium, (b) swelling of endothelial cells and (c) pericapillary edema. The increased capillary permeability has been attributed to the disruption or widening of intercellular junctions either 'passively', due to cytoskeletal alterations, or 'actively', by cell contraction induced by vasoactive mediators (Svensjo and Grega, 1986). However, it has also been reported that increased capillary permeability can be observed without any structural modifications of interendothelial junctions (Rasio et al., 1987).

Endothelial cell injury has been attributed to the production of oxygen-derived free radicals by xanthine oxidase present in endothelial cells (Burton et al., 1984; Ratych et al., 1987; Rosen and Freeman, 1984; Zweier et al., 1988). Endothelial cells could also be damaged by the joined action of oxygen-derived free radicals and proteases secreted by neutrophils that migrate into the interstitium (Fig. 9) (Ratych et al., 1987; Hernandez et al., 1987; Granger, 1988). Very importantly, it has been shown in different models that the alterations of microvasculature occur early and appear to precede lesions of epithelial cells (Burton et al., 1984; Braunwald and Kloner, 1985). This suggests that parenchymal cell injury could be the consequence of alteration in the microcirculation.

4.3.1.2. Microcirculatory factors in ischemia/hypoxia-induced liver injury

Normothermic ischemia/hypoxia

Experimental evidence also suggests that ischemia and hypoxia induce early and selective damage of the liver microcirculation that could significantly contribute to parenchymal cell lesions. Lesions of endothelial cells have been demonstrated in rat livers stored at 37°C for 1 or 2 hr in a 0.9% NaCl solution containing 2 mM $CaCl_2$ (McKeown et al., 1988). After 1 hr, endothelial cells were swollen and the number and size of fenestrations was increased. After 2 hr, large disruptions in the continuity of the endothelium were seen. Recently, we have shown (Chazouillères and Ballet, unpublished data) using isolated perfused livers from fasted rats that 1 hr of ischemia followed
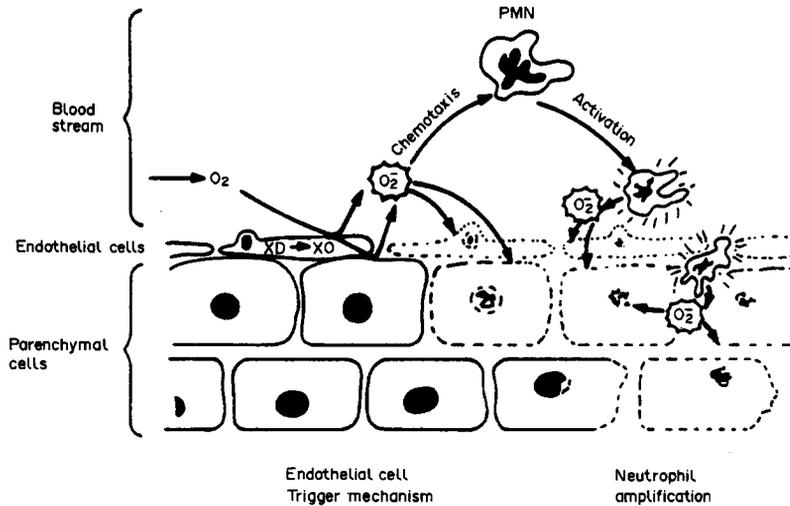
FIG. 9. Proposed endothelial cell trigger mechanism. With the onset of ischemia, xanthine dehydrogenase (XD) is converted to xanthine oxidase (XO) within the endothelial cell. Xanthine oxidase catalyzes superoxide (O_2^-) generation within the endothelial cell itself from molecular oxygen which is reintroduced at reperfusion. This endothelial-cell-generated superoxide can produce direct cellular injury independent of other elements. Neutrophils (PMN) are then attracted to the area secondarily, either by the chemotactic properties of the superoxide and/or by the injured endothelial cell itself. The neutrophils may then undergo activation and serve as an important, but secondary, amplifying system that results in further cellular injury to both endothelial and parenchymal cells. From Ratych et al., 1987. Reprinted with permission of the authors and copyright holder, the C.U. Mosby CO., St Louis.

by 30 min reperfusion induces mediolobular parenchymal cell death, as assessed by trypan blue staining, in agreement with a recent study (Marotto et al., 1988). Significant endothelial cell injury was also observed which, interestingly, was not restricted to midzonal regions but involved the whole lobule. These abnormalities were accompanied by marked alterations in liver microcirculation, as indicated by the reduction in liver blood flow and an increase in intrahepatic vascular resistance, as well as by the heterogenous staining of the liver after trypan blue perfusion (Chazouillères and Ballet, unpublished data). Very recently, Komatsu et al. (1989), and Koo et al. (1989) using an in vivo model of ischemia-reperfusion in the rat with in vivo microscopic techniques have shown that during reperfusion, leucocyte velocity is significantly decreased in the sinusoids, with adhesion of leucocytes to the endothelium and cessation of blood flow in many sinusoids. These abnormalities were prevented, in part, by the administration of superoxide dimutase, thus confirming the role of the superoxide anion in these alterations.

The protective effect of calcium antagonists in various models of hypoxic or ischemic liver injury has been interpreted as being the consequence of a direct effect on hepatocytes (Thurman et al., 1988a). However, there is no evidence that voltage-dependent calcium channels binding dihydropyrimidins exist in hepatoctyes (Mauger and Claret, 1988). By contrast, such channels are present in smooth muscle cells, endothelial cells and macrophages. Therefore, the hepatoprotective properties of these agents could be related to their effect on microcirculation and/or sinusoidal cells. A similar mechanism could also account for the protective effect of prostaglandins (Alvares-Lopez et al., 1987; Araki and Lefer, 1980; Mora et al., 1987; Sikugara et al., 1983).

In conclusion, several lines of evidence suggest that, in addition to necrosis of parenchymal cells, liver ischemia/hypoxia induces marked abnormalities of liver microcirculation together with lesions of endothelial cells. However, both phenomena occur simultaneously and it must be emphasized that the data presented are insufficient to establish a cause–effect relationship.

Hypothermic preservation

Microcirculatory disturbances have also been demonstrated after preservation of the liver by hyperthermic ischemia.

Otto et al. (1986) have observed the effect of graft cooling on the minipig liver ultrastructure. Administration of Ringer's at 2°C for initial flushing of the donor liver induced a complete desquamation of the endothelium together with minor hepatocyte lesions. By contrast, after flushing with Ringer's at 15°C, only minor alterations occurred. After preservation in Eurocollins or in a protein solution for 5–6 hr, the extent of the damage was similar. However, after transplantation and reperfusion of the liver, endothelial damage was more pronounced and parenchymal necrosis was apparent. The authors suggested that liver parenchymal cells were damaged mainly during reflow, as the consequence of microcirculatory disturbances. Myagkaya et al. (1987) have also shown that, after flushing rat livers at 17°C with Eurocollins and preserving small pieces of perfused liver in Eurocollins at 4°C, selective lesions of endothelial cells can occur. After 12 hr, interruptions in the continuity of the endothelial lining were regularly observed and the nuclear chromatin of these cells showed pronounced clumping. After 45 hr, necrosis and desquamation of endothelial cells was seen.

Again, the data suggest that hepatocyte injury could be related to endothelial cell damage. Iu *et al.* (1987) have shown that after 8 hr preservation at 4°C in a 0.9% saline solution containing 2 mM calcium, rat liver grafts are nonviable (100% mortality in recipient animals). When the livers preserved in such conditions were perfused at constant pressure, a significant decrease in flow was observed as compared with control nonpreserved livers. Ultrastructural studies demonstrated necrosis and desquamation of endothelial cells, while hepatocyte lesions were minimal, consisting of blebs with a mild vacuolization (McKeown *et al.*, 1988). It is important to emphasize that in this work the ultrastructural abnormalities were present after ischemia in the absence of reperfusion. This suggests that ischemia alone is able to damage endothelial cells. When the duration of preservation did not extend beyond 4 hr, rat liver grafts were viable (100% survival at 15 days in the recipients). In these conditions, flow was maintained and endothelial cell alterations were minimal (McKeown *et al.*, 1988). These data clearly demonstrate that sinusoidal damage and microcirculatory disturbances are critical determinants in graft failure after transplantation. These findings have been confirmed by other groups. Caldwell-Kenkel *et al.* (1987) have shown that, after 20 hr preservation in Eurocollins at 4°C followed by reperfusion for 15 min, approximately one third of sinusoidal cells are nonvizable as indicated by trypan blue uptake (Belinsky *et al.*, 1984). When the duration of reperfusion was limited to 8 min, only 4% of sinusoidal cells were stained, suggesting that endothelial damage occurred during reperfusion and not during preservation. Thurman *et al.* (1988c) subsequently observed that after a short (< 60 min) preservation in cold Ringer's, a spotty and uneven distribution of trypan blue occurred in the liver 24 hr after transplantation: 15–20% of the hepatocytes were nonviable. In contrast, untransplanted livers were judged normal by the criteria of uniform distribution of dye and dye uptake. The altered microcirculation and cell death were reduced markedly when livers were perfused briefly with nitrogen-saturated buffer for 5 min prior to transplantation. This suggests that oxygen-derived free radicals are involved in parenchymal injury. Protection was even greater if the perfusion medium contained verapamil, a calcium antagonist. As stated previously, the protection offered by verapamil could be related to its effect on microcirculation and/or sinusoidal cells. Interestingly, it was shown that in spite of these lesions, the transplanted animals were able to survive for up to 30 days. This important observation suggests that the extent of cell damage is too modest and/or that other factors are necessary to induce graft failure after transplantation (Thurman *et al.*, 1988c).

In conclusion, endothelial cell injury and microcirculatory abnormalities appear to play a major role in the pathogenesis of liver failure after prolonged cold preservation and/or orthotopic transplantation in the rat.

These obsrvations led us to investigate the effect of two vasodilators, papaverine and a methyl-xanthine derivative, pentoxifylline, on liver hemodynamics and function immediately after 5 and 19 hr preservation (Chazouillères *et al.*, 1989). Rat livers were cooled by *in situ* portal flushing with Ringer-lactate solution and preservation solution (Eurocollins) at 4°C. They were then excized and stored in Eurocollins at 4°C for 5 or 19 hr. Livers were reperfused for 70 min at 37°C, at a constant pressure (10 cm H_2O) through the portal vein in a recirculating perfused organ system. After 5 hr preservation, liver hemodynamics and function were not significantly modified. After 19 hr, bile flow and intrinsic taurocholate clearance were significantly reduced; transaminases and potassium release were markedly increased and histological studies showed mild centrilobular necrosis. Concomitantly, liver blood flow was significantly reduced and intrahepatic vascular resistance was increased. Papaverine and pentoxifylline administered during preservation (10^{-4} M) and at the time of reperfusion (10^{-5} M) significantly improved all parameters. The improvement was more pronounced after pentoxifylline administration, and this group showed no significant difference in any of the studied parameters vs control livers. Thus, papaverine and pentoxifylline significantly protected the liver during long hypothermic preservation. This provides more evidence that abnormalities of liver microcirculation are of major importance in the pathogenesis of liver injury after hypothermic storage (Chazouillères *et al.*, 1989).

However, the precise mechanism by which papaverine and pentoxifylline exert hepatoprotective effects under our experimental conditions can only be speculated on. In view of the increased intrahepatic resistance after hypothermic storage and the vasodilatory properties of both compounds, two mechanisms can be proposed: (a) an inhibitory effect on intrahepatic vasoconstriction of portal venules and/or sinusoidal sphincters preventing the closure of sinusoids, and (b) an action on the sinusoidal endothelium possibly involving relaxation of contracted endothelial cells.

Intrahepatic vasoconstriction may be elicited during preservation by the high potassium concentration in Eurocollins solution (114 mM) and it may persist after reperfusion (Hill and Olson, 1987; Taylor *et al.*, 1985). Vasoconstriction of portal venules or sphincters can lead to closure of sinusoids. This could restrict the vascular and extravascular diffusion of the preservation solution and consequently reduce the number of protected cells. After reperfusion, it might also impair nutrient supply to cells and taurocholate uptake, resulting in decreased bile flow and intrinsic taurocholate clearance.

Microcirculatory disturbances can also result from endothelial cell injury. The swelling of endothelial cells may contribute to increased vascular resistance. Endothelial cell contraction could also be an important mechanism contributing to the increase in portal resistance and sinusoidal injury. Sinusoidal endothelial cells contain actin and myosin and can contract under the influence of vasoactive agonists (Grega, 1986a,b; Grega *et al.*, 1986; Svensjo and Grega, 1986). During reperfusion, active contraction could also be induced by a massive influx of calcium and/or the production of free radicals in endothelial cells. Activated Kupffer cells can secrete vasoactive mediators such as leukotrienes and platelet-activating factor, which might induce endothelial cell contrac-

tion. This would lead to cell bulging and protrusion into the sinusoidal lumen, increasing vascular resistance and facilitating endothelial cell separation and detachment under the influence of the shearing force of the flow. Parenchymal cell injury could occur secondarily as the result of a no-reflow phenomenon worsening cellular ischemia. It is therefore tempting to speculate that papaverine and pentoxifylline act by antagonizing endothelial cell contraction. Indeed, it has been proposed that β-adrenergic agonists, calcium antagonists and xanthine derivatives which inhibit the increase in vascular permeability induced by inflammatory mediators could act by relaxation of mediator-contracted endothelial cells (Grega, 1986b).

Other mechanisms could account for the protection offered by the two agents. Endothelial cells could be damaged by free radicals produced *in situ* or by free radicals and proteolytic enzymes secreted by activated Kupffer cells. Accordingly the inhibition of Kupffer cells could prevent liver injury induced by prolonged preservation. Papaverine and pentoxifylline are potent inhibitors of cyclic nucleotide phosphodiesterase which can increase intracellular cyclic AMP. Agents increasing cyclic AMP are known to inhibit the secretory response of neutrophils and macrophages. We have shown that pentoxifylline inhibits superoxide production by human alveolar macrophages *in vitro* in a dose-dependent fashion (Housset *et al.*, 1988). These findings suggest that the inhibition of Kupffer cells could explain the beneficial effect of pentoxifylline. Very recently, it has been demonstrated that after 24 hr preservation in Eurocollins at 4°C followed by 15–20 min reperfusion, Kupffer cells present morphological modifications consistent with a functional activation (Caldwell-Kenkel *et al.*, 1988). These alterations were not observed in the absence of reperfusion, suggesting that cellular activation is triggered by reperfusion. The same group has also shown in rat livers perfused after 24 hr preservation that hepatic clearance of colloidal carbon is increased two-fold (Thurman *et al.*, 1988b). This again indicates that cold preservation followed by reperfusion activates Kupffer cells and that this mechanism could contribute to endothelial cell damage and microcirculatory disturbances.

4.3.2. Liver Injury Induced by Endotoxins

4.3.2.1. *Endotoxins.* The effects of infection by gram-negative bacteria are mediated by endotoxins. Endotoxins are lipopolysaccharides that reside in the outer membrane of bacteria and consist of a polysaccharide chain associated with lipid A (Van Deventer *et al.*, 1988). The polysaccharide region can be divided into the O-antigenic polysaccharide side chain which is near the nonreducing end, and the 'core' polysaccharide region which binds with lipid A and is near the reducing end. Lipid A has a more constant molecular structure and is thought to mediate most biological effects of endotoxins. In spite of the tremendous concentration of bacteria in the gut, there is little evidence that a physiological portal endotoxemia occurs (Van Deventer *et al.*, 1988). By contrast, in diseases of the gut, particularly the large

bowel, endotoxins can be absorbed through the intestinal wall and reach the liver (Van Deventer *et al.*, 1988). The liver and more specifically, sinusoidal cells are responsible for the uptake and inactivation of endotoxins (Nolan and Cohen, 1988). Kupffer cells appear to be mainly responsible for uptake which is mediated through a specific membrane receptor (Van Bossuyt and Wisse, 1988). In spite of their phagocytic properties, endothelial cells do not appear to play a role in endotoxin uptake in the liver, at least in normal conditions. Van Bossuyt *et al.* (1988) have recently shown that endotoxins taken up by Kupffer cells are subsequently transferred to hepatocytes and excreted in the bile.

Endotoxins activate Kupffer cells. It has been shown *in vitro* that endotoxins induce the release of PGE_2 by Kupffer cells, whereas in basal conditions the major prostaglandin secreted is PGD_2 (Brouwer *et al.*, 1988; Kuiper *et al.*, 1988; Decker *et al.*, 1989). This effect is inhibited by dexamethasone (Dieter *et al.*, 1986). Endotoxins alone do not stimulate superoxide production (Dieter *et al.*, 1986) but they increase superoxide release induced by zymosan (Rieder *et al.*, 1988). They do not affect the secretion of lysosomal enzymes (Dieter *et al.*, 1988). Recently, it has been shown that endotoxins selectively trigger TNF secretion by Kupffer cells. This response is inhibited by dexamethasone and PGE_2 (Karck *et al.*, 1988; Decker *et al.*, 1989).

When clearance of endotoxins by the liver is decreased, significant amounts may reach the systemic circulation and lead to harmful effects such as circulatory shock, intravascular coagulation and metabolic alterations, e.g. increased protein catabolism and lipolysis.

There are some discrepancies as to hepatic alterations caused by endotoxins. It is generally admitted that endotoxins produce cholestasis without evidence of parenchymal cell necrosis. However, liver parenchymal injury has been reported after *in vivo* administration of endotoxins to animals (Fraker *et al.*, 1988). *In vivo* liver damage could result not from a direct effect of endotoxins on the liver, but from shock and/or intravascular coagulation. Indeed, in the isolated perfused rat liver and in hepatocytes *in vitro*, endotoxin administration does not cause cytotoxic effects (Utili *et al.*, 1976). In baboons, mortality and liver necrosis induced by injection of *E. coli* are prevented by administration of C protein, a substance that participates in the endothelial cell anticoagulant function (Taylor *et al.*, 1987). Furthermore, hepatic necrosis induced in rats by endotoxins is prevented by a thrombin inhibitor (Arai *et al.*, 1988). These findings suggest that activation of coagulation plays a role in liver injury. The invasion of liver parenchyma by neutrophils could also contribute to liver damage by endotoxins. Endotoxins have been shown to induce inflammatory reactions in many organs. The major events which follow the administration of endotoxins in a variety of experimental models include (Cybulsky *et al.*, 1988) (Fig. 10) (a) neutrophil adhesion to endothelial cells; this step appears to be mediated by interleukin 1 (IL 1) and tumor necrosis factor α (TNFα) secreted by macrophages, (b) emigration of neutrophils through the endo-
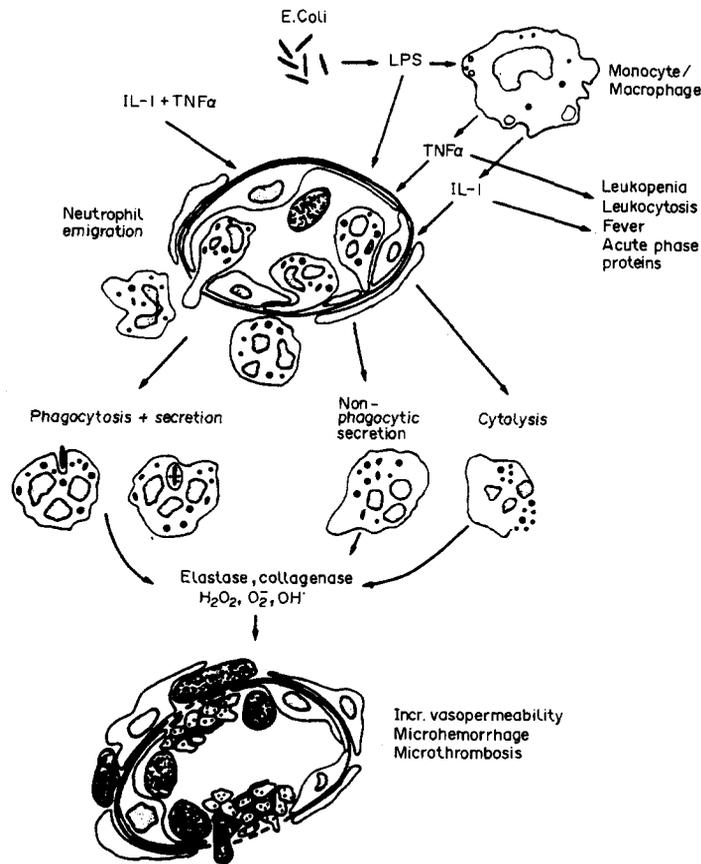
FIG. 10. Diagrammatic representation of events which follows entry of *E. coli* into tissues. The bacteria shed endotoxin (LPS) which TNFα. Through action on the endothelium, IL-1 and TNFα induce neutrophil emigration and accumulation at the site of bacterial multiplication. Endothelium can also synthesize and release IL-1. The emigrated neutrophils release lysosomal enzymes and oxygen radicals. The severely injured microvessels exhibit increases in vasopermeability, hemorrhage and thrombosis. From Cybulsky *et al.*, 1988. Reprinted with permission of the authors and copyright holder, Williams and Wilkins, Baltimore.

thelium and accumulation into the tissue interstitium and (c) release of oxygen radicals and proteases by migrating neutrophils, leading to microvascular hemorrhage and thrombosis.

These mechanisms have been recently explored in the liver. Schlayer *et al.* (1987) have shown that endotoxins induce neutrophil adhesion to sinusoidal endothelial cells *in vitro* (Schlayer *et al.*, 1987) and that this effect is mediated by TNFα (Schlayer *et al.*, 1988). TNFα could act by directly activating neutrophils and/or by an indirect mechanism which depends on TNFα binding to endothelial cells. McCuskey (1988b) has studied the effects of endotoxins on hepatic microcirculation using *in vivo* microscopy and has found that endotoxins trigger neutrophil adhesion to endothelial cells. This leads to partial or complete plugging of sinusoids as well as to a reduction or cessation of blood flow. However, given the highly anastomotic nature of the sinusoidal network this results in a redistribution of flow into adjacent sinusoids. Anastomotic sinusoids eventually become plugged and regions of the liver parenchyma may become ischemic.

The effect of endotoxins on liver hemodynamics are unclear. Endotoxins increase intrahepatic vascu-

lar resistance in isolated rat livers perfused in re-circulating systems (Gaeta *et al.*, 1986; Nolan and O'Connell, 1965; Utili *et al.*, 1976, 1981, 1987) but not in single pass systems (Chrétien and Ballet, unpublished data). In one study, vasoconstriction was observed only when the perfusion solution contained whole blood or a mixture of leucocytes, platelets and plasma (Filkins, 1969). In contrast, no response was obtained when the perfusion medium contained only plasma, Krebs buffer, erythrocytes or leucocytes (Filkins, 1969). In other studies, a weak response was obtained when the liver was perfused with simple buffer solutions (Gaeta *et al.*, 1986; Nolan and O'Connell, 1965). In one, the increase in resistance was inhibited by hydrocortisone (Nolan and O'Connell, 1965). By analogy with their metabolic effect in the intact liver (see Section 4) and taking into account their effects on Kupffer cells *in vitro*, it is conceivable that the effect of endotoxins on liver microcirculation could result from the release of eicosanoids by sinusoidal cells. PGD_2 appears to have no vasoactive effect in the liver. In contrast, TXA_2 and/or LT have potent vasoactive properties and could mediate the hemodynamic response to endotoxins in the liver.

In summary, microcirculatory factors play a major role in the pathogenesis of endotoxin-induced liver injury. Adhesion of neutrophils to the endothelium could stop flow in the sinusoids and lead to parenchymal ischemia. Migration of neturophils through the endothelial lining and their accumulation in liver parenchyma could secondarily induce endothelial injury, thereby increasing the alterations of liver perfusion and activating coagulation mechanisms in the sinusoids.

Although endotoxins *per se* appear to induce moderate liver injury, it has been shown that a massive necrosis of the liver can result in two conditions, i.e. when the susceptibility threshold of hepatocytes is lowered by pretreatment of animals with D-galactosamine at low, noncytotoxic doses, and when the number of hepatic macrophages is increased.

4.3.2.2. Hepatic necrosis induced by galactosamine and endotoxins.

Pretreatment by galactosamine at low, noncytotoxic doses dramatically increases the sensitivity of animals to endotoxins *in vivo* by more than 10^5-fold (Galanos *et al.*, 1979). In this model, the hypersensitivity induced by galactosamine is not the consequence of increased macrophage activity but results from a lowering of the susceptibility threshold of hepatocytes. Other activators of Kupffer cells do not induce necrosis when associated with galactosamine, indicating that cytotoxicity is induced by the secretion of a mediator specifically triggered by endotoxins. Administration of TNFα reproduces liver necrosis in mice pretreated with galactosamine whether they are sensitive (C3H/He N) or resistant (C3H/He J) to endotoxins (Lehmann *et al.*, 1987) implying that in this model, the effect of endotoxins *in vivo* is mediated by TNFα and that resistance to endotoxins results from a deficit in TNFα synthesis by macrophages (Fraker *et al.*, 1988). Tiegs *et al.* (1989) have also known that the administration of TNFα reproduces the cytotoxic effect of endotoxins and that the response to TNFα is dose-dependent. Furthermore, plasma concentrations of TNF are significantly increased and are maximal 2 hr after the administration of endotoxins in this model. The plasma concentrations reach levels that are in the cytotoxic range. Interestingly, TNF alone is not cytotoxic. Again, it has been shown in this model that endotoxins induce a dramatic accumulation of neutrophils in the liver parenchyma (Schlayer *et al.*, 1988). Furthermore, it has been shown that fibrin deposits are often present in sinusoids. These data again suggest that activation of neutrophils and coagulation pathways contribute to liver injury (Schlayer *et al.*, 1988). Endotoxins could stimulate TNF secretion by Kupffer cells, trigger endothelial procoagulant activity and induce adhesion of neutrophils to endothelial cells, leading to plugging of sinusoids and altered microcirculation. After migration into the liver parenchyma, neutrophils could induce hepatocyte and endothelial cell necrosis. This would produce microthrombus formation, microcirculatory disturbances and, finally, tissue ischemia, further aggravating parenchymal necrosis.

Interestingly, it has been shown in this model that hepatic necrosis can be prevented by allopurinol, an inhibitor of xanthine oxidase (Wendel *et al.*, 1987). This implies that a mechanism similar to that seen in ischemia reperfusion-induced damage could occur. The prostacylin analog iloprost antagonizes liver injury when given simultaneously with galactosamine/endotoxin (Wendel *et al.*, 1987). The phospholipase A_2 inhibitor, dexamethasone, the lipooxigenase inhibitors BW 755 C, nafazatrom, RV 5901 and Ebselen, and the LTA$_4$ biosynthesis inhibitor, diethylcarbazine, also prevent galactosamine-endotoxin hepatitis (Tiegs and Wendel, 1988). This suggests that leukotriene synthesis is involved in the mechanism of injury. Cytotoxicity is also decreased when the hepatic glutathione content is decreased by more than 90% following the administration of phorone or diethylmaleate, suggesting that glutathione-derived cysteinyl leukotrienes may be the pathogenic mediators. The gamma glutamyl transpeptidase inhibitor AT 125 which prevents the conversion of LTC$_4$ to LTD$_4$ significantly reduces liver injury. Furthermore, the LTD$_4$ receptor antagonist FPL 55712 has a significant protective effect, while damage is replicated when LTD$_4$ is substituted for the endotoxins. This strongly supports LTD$_4$ as a major pathogenic mediator in this model. LTD$_4$ could induce transitory liver vasoconstriction leading to ischemia followed by liver injury at the time of reperfusion (Tiegs and Wendel, 1988). According to this hypothesis, the protective effect of the PGI$_2$ analog iloprost could be related to the inhibition of vasoconstriction. More recently, Tiegs *et al.* (1989) have shown that allopurinol, iloprost, dexamethasone, BW 755 C and FPL 55712 are ineffective when TNF is substituted for the endotoxins (Tiegs *et al.*, 1989). This indicates that these agents could also act by inhibiting the secretion of TNF and/or by antagonizing its activity. Similarly, the calcium channel blockers verapamil and nifedipine, which significantly prevent liver injury in this model, are ineffective when TNF is substituted for the endotoxins. This indicates that these agents too could act by inhibiting the secretion of TNF and/or by antagonizing its activity. According to the authors, the data suggest that the secretion of TNF could result from the generation of oxygen-derived free radicals at the time of reperfusion following activation of xanthine oxidase during ischemia. According to this hypothesis, ischemia could result from vasoconstriction induced by LTD$_4$. Again, the protective effect of calcium channel blockers could be related to the inhibition of vasoconstriction (Tiegs *et al.*, 1989). However, other mechanisms may be involved. As discussed previously, TNF is not directly cytotoxic but can trigger endothelial procoagulant activity, adhesion of neutrophils to endothelial cells and the secretion of oxygen-derived free radicals by neutrophils. Cysteinyl leukotrienes could increase the permeability of the sinusoids and facilitate the migration of neutrophils into the liver parenchyma (Ford-Hutchinson, 1987; Lewis and Austen, 1984; Samuelsson *et al.*, 1987).

4.3.2.3. Hepatic necrosis induced by Corynebacterium parvum or Propionibacterium acnes followed by endotoxin.

Endotoxins can trigger a massive liver necrosis when the hepatic macrophage content is

increased by prior injection of *Corynebacterium parvum* in rats (Tanner *et al.*, 1981) or *Propionibacterium acnes* in mice (Mizoguchi *et al.*, 1987). Liver necrosis is significantly reduced by the administration of superoxide dismutase (Arthur *et al.*, 1985). Furthermore, endotoxins stimulate superoxide release by hepatic macrophages isolated from rats pretreated with *C. parvum* (Arthur *et al.*, 1988). These data suggest that oxygen-derived free radicals secreted by hepatic macrophages under the influence of endotoxins could induce liver injury. Mizoguchi *et al.* (1987) have shown in hepatitis induced by *P. acnes*-endotoxin in mice, that a PGE_1 analog decreases liver injury and mortality significantly. The mechanism by which PGE_1 exerts hepatoprotective properties is unclear. PGE_1 inhibits the production of cytotoxic mediator(s) by adherent liver cells *in vitro* and also prevents the cytotoxic effect of the supernatant medium conditioned by adherent cells (Mizoguchi *et al.*, 1987). This indicates that PGE_1 could not only inhibit the release of cytotoxic mediators by liver macrophages but could also exert a 'cytoprotective' effect on hepatocytes. Recently, it has been shown that antithrombin III administration induces a significant biological and histological improvement in this model. These findings again suggest that activation of coagulation plays a role in the physiopathology of liver damage (Yamada *et al.*, 1989).

4.3.3. Liver Injury Induced by Viruses

There is also evidence that sinusoidal cells and microvascular factors are involved in two experimental models of hepatitis induced by viruses: Murine Hepatitis Virus 3 (MHV 3) and Frog Virus 3 (FV 3).

4.3.3.1. *Hepatitis induced by MHV 3.* MHV 3 is coronavirus known to exert a direct cytopathic effect on hepatocytes *in vitro*. In susceptible mice, MHV 3 induces focal parenchymal necrosis surrounded by an inflammatory infiltrate. The extent of necrosis varies according to the susceptibility of the strain. Accordingly, the spectrum of liver damage may range from fulminant hepatitis leading to death in 5 days to chronic aggressive hepatitis progressing to death in 6–9 months. Direct observation of the microcirculation by *in vivo* microscopy has shown that marked alterations may occur (McPhee *et al.*, 1985). Importantly, they appear to precede lesions of hepatocytes. An early finding was the deposition of fibrin microthrombi in sinusoids associated with a reduction in sinusoidal flow, the formation of clumped erythrocytes and the dilatation of sinusoids, a pattern qualified as 'granular blood flow'. Subsequently, avascular parenchymal areas occurred, corresponding to necrotic foci. Studies of microcirculation by corrosion cast-SEM techniques have confirmed that sinusoids are obstructed as early as 48 hr after inoculation, leading to the complete occlusion of small spherical zones of liver parenchyma (McPhee *et al.*, 1988). The mean diameter of the lesions was 83 μm which represents the width of about 6 hepatocytes. Sinusoidal obstruction was induced by protrusion of sinusoidal cells and plugging by clumped erythro-cytes and/or platelets and/or cellular debris. Subsequently, the ischemic foci increased in size and reached near-confluence at the time of death 5–7 days postinfection. Importantly, the lesions appeared to predominate in periportal regions. A significant increase in macrophage procoagulant activity correlated with the degree of parenchymal injury in this model (McPhee *et al.*, 1985; Abecassis *et al.*, 1987). Finally, it has been shown that the administration of dimethyl-PGE_2 prevents the stimulation of macrophage procoagulant activity and significantly reduces liver injury (Abecassis *et al.*, 1987). These data suggest the following sequence: MHV 3 infection and/or lesions of endothelial cells could trigger procoagulant activity and activate coagulation. Formation of fibrin microthrombi in sinusoids impairs microcirculation. Concomitantly, the release of oxygen-derived free radicals by Kupffer cells could contribute to sinusoidal and parenchymal damage, in accord with the predominant periportal localization of the lesions. Lesions of sinusoids could lead to their obstruction and to the formation of foci of ischemic necrosis in the liver parenchyma. However, it is also clear that the infection of hepatocytes by MHV 3 can directly damage the cells.

4.3.3.2. *Hepatitis induced by FV 3.* Infection of rats or mice by FV 3 leads to a fulminant hepatitis and death in less than 30 hr. Liver injury predominates in periportal and mid-lobular regions. An early and selective necrosis of endothelial and Kupffer cells has been observed in this model that leads to the destruction of sinusoids (Kirn *et al.*, 1983). The lesions result from the infection of sinusoidal cells by the virus. Parenchymal lesions occur secondarily, with nuclear modifications followed by cytoplasmic lesions leading to hepatocyte necrosis. The sequence of lesions suggests that parenchymal lesions could be secondary to the alterations of sinusoids (Kirn *et al.*, 1983). Data also suggest that endotoxins, normally inactivated by Kupffer cells and endothelial cells, could contribute to this liver injury: (a) endotoxin administration aggravates liver lesions and inoculation with sublethal doses of virus increases the susceptibility of animals to endotoxins; (b) liver injury is significantly reduced by colectomy which suppresses the pool of enteric endotoxins, in germ-free animals, by the administration of polymyxin B which inactivates endotoxins, following the development of endotoxin tolerance by repeated administration of nonlethal doses. However, as discussed previously, endotoxins *per se* are not cytotoxic for normal hepatocytes. The inhibition of RNA synthesis by FV 3 proteins in hepatocytes could thus increase their susceptibility to endotoxins (Kirn *et al.*, 1983).

Recent studies have shown that cysteinyl leukotrienes also contribute to the pathogenesis of FV3-induced hepatitis. In effect, the secretion of cysteinyl leukotrienes into bile is significantly increased and reaches levels similar to those observed after severe traumatic injury (Denzlinger *et al.*, 1985; Hagmann *et al.*, 1987). Furthermore, inhibition of leukotriene biosynthesis by the lipooxigenase inhibitors AA 861 and BW 755 C, significantly decreases liver injury (Hagmann *et al.*, 1987). Finally, it is important to emphasize that endotoxins are potent stimulators

of leukotriene synthesis and secretion in a variety of experimental systems. However, the origin of the leukotrienes secreted into the bile during FV 3-induced hepatitis is unknown. As stated previously, Kupffer cells are the only liver cells known to produce significant amounts of leukotrienes (Keppler et al., 1985, 1988; Hagmann and Keppler, 1988). Accordingly, Kupffer cells could produce leukotrienes early after infection by FV 3, before their destruction by the virus. Another possibility is that leukotrienes are released by polymorphonuclear cells and/or monocytes circulating in the blood or infiltrating the liver parenchyma (Mullane et al., 1988).

The mechanism by which leukotrienes could trigger liver injury is unclear. Leukotrienes could impair microcirculation. In effect, observation of the liver microcirculation by in vivo microscopy has revealed abnormalities in FV3-infected animals similar to those observed after administration of endotoxins. Furthermore, it has been shown that abundant cellular debris resulting from necrosis of sinusoidal cells obstructs the sinusoids and aggravates microcirculatory disturbances (McCuskey, 1988b). Leukotrienes could also exert deleterious effects by increasing the permeability of sinusoids and facilitating the migration of neutrophils and macrophages into the liver parenchyma and/or by activating inflammatory cells (Ford-Hutchinson, 1987; Lewis and Austen, 1984; Samuelsson et al., 1987).

4.3.4. Liver Injury Induced by Chemicals

The contribution of sinusoidal cells and/or microvascular factors to the liver injury induced by toxic agents has also been explored.

4.3.4.1. Galactosamine. Administration of galactosamine to most animal species usually induces an acute hepatitis within 24 hr with histological features similar to those observed in patients with viral hepatitis. Lesions consist of foci of parenchymal necrosis disseminated in the liver lobule surrounded by inflammatory infiltrates mainly composed of polymorphonuclear cells and lymphocytes (Decker and Keppler, 1974).

The mechanism of liver injury has been extensively studied (Decker and Keppler, 1974). Administration of galactosamine leads to the formation of UDP-derivatives of galactosamine together with a depletion in UTP and UDP-hexoses in hepatocytes. This produces an inhibition of macromolecule synthesis dependent on uracil nucleotides, lesions of cellular organelles and, finally, cell necrosis. A major argument supporting this hypothesis is the prevention of liver injury by uridine given in the 3 hr following galactosamine administration. Furthermore, in vitro, galactosamine has a direct cytotoxic effect on isolated or cultured hepatocytes. These data indicate that the hepatotoxicity of galactosamine in vivo may result from its direct cytotoxic effect on hepatocytes.

However, there is also evidence that circulating endotoxins and hepatic macrophages could be involved in the pathogenesis of this liver injury. As discussed previously, liver damage induced by galactosamine is significantly increased by the adminis-

tration of endotoxins (Galanos et al., 1979). Conversely, liver lesions are markedly reduced when prior colectomy is performed (Nolan and Cohen, 1988). Furthermore, the inhibition of Kupffer cells by methylpalmitate (Al Tuwaijri et al., 1981) or administration of latex particles before the administration of galactosamine (Shiratori et al., 1988) significantly decreased liver injury. In contrast, necrosis is significantly increased when Kupffer cells are stimulated by latex particles after the administration of galactosamine (Shiratori et al., 1988). These data suggest that the activation of Kupffer cells by circulating endotoxins plays a major role in the pathogenesis of parenchymal necrosis. Furthermore, the hepatoprotective effect of superoxide dismutase suggests that liver lesions could be mediated by oxygen-derived free radicals released by macrophages. An additional argument was provided by Chojkier and Fierer (1985). It was shown that strains of mice resistant to endotoxins were also partially resistant to galactosamine. Furthermore, the transfer of splenic macrophages isolated from susceptible animals to resistant irradiated animals induced susceptibility to galactosamine. This suggests that parenchymal necrosis induced by galactosamine can result, at least in part, from an activation of hepatic macrophages. Accordingly, it is possible that the protective effect of prostaglandin analogs dimethyl-PGE_2 (Stachura et al., 1980), PGI_2 (Noda et al., 1986) and PGD_2 in this model, could be related to their inhibitory effect on liver macrophages and, more generally, on the inflammatory reaction (Bray, 1987; Grygelwski et al., 1987; Ogawa et al. 1988). Muller-Berghaus et al. (1975) have shown that fibrin microthrombi can form in sinusoids during galactosamine-induced hepatitis in rabbits. The administration of heparin prevents the formation of microthrombi but does not affect liver lesions or animal survival, indicating that activation of coagulation processes occur in sinusoids during galactosamine-induced hepatitis but that this phenomenon does not contribute significantly to liver injury.

4.3.4.2. Carbon tetrachloride (CCl_4). The administration of CCl_4 induces parenchymal necrosis predominating in perivenous regions. Lesions are accompanied by inflammatory infiltrates consisting of polymorphonuclear and mononuclear cells and by fatty changes which can sometimes be extensive (Zimmerman, 1976). Lesions are usually maximal between 24 and 36 hr. CCl_4-induced hepatic injury was probably the first model in which microcirculatory factors were considered to play a role in cell necrosis. An early concept attributed hepatic necrosis to centrilobular ischemia secondary to the decrease in sinusoidal flow. While early transillumination studies provided evidence that microcirculation was impaired after CCl_4 intoxication, subsequent studies showed that microcirculatory disturbances occurred only after centrilobular injury (Brauer, 1963). Furthermore, the demonstration by Brauer (1963) that in the isolated perfused rat liver, administration of $CHCl_3$ led to centrilobular necrosis even when blood flow was reversed indicated clearly that necrosis was independent of any vascular changes and indeed preceded the latter by several hours. It is generally thought

that CCl₄ hepatotoxicity occurs via a direct cytotoxic effect on hepatocytes. A number of studies indicate that CCl_4 toxicity is related to its conversion by cytochrome P-450 to the highly reactive trichloromethyl radical $(CCl_3^•)$ (Anders, 1984).

However, while Brauer (1963) recognized that CCl_4-induced cell necrosis results from a direct cytotoxic effect, he emphasized that impaired microcirculation could lead to tissue ischemia and thus to liver injury. This mechanism could account for the finding that liver lesions produced after CCl_4 administration can be greatly reduced by procedures which interfere with sympathetic activation (Zimmerman, 1976). Indeed, it is conceivable that liver vasoconstriction induced by sympathetic activation could contribute to the microcirculatory disturbances observed after CCl_4 administration. However, alternative mechanisms have been proposed, i.e. (a) a cytotoxic effect of catecholamines resulting from their conversion into free radicals (Singal et al., 1981) and (b) a depletion of hepatic glutathione resulting from the stimulation of glutathione cellular efflux by catecholamines (Sies and Graf, 1985).

Recent studies suggest that endotoxins could also contribute to liver damage. Development of endotoxin tolerance or administration of polymyxin B significantly reduce the extent of liver lesions. Furthermore, Nolan and Cohen (1988) have shown that endotoxin clearance is significantly reduced after administration of CCl_4 which suggests that sinusoidal cells could be injured in this model. In contrast, Fujiwara et al. (1988a,b) found neither sinusoidal lesions nor fibrin microthrombus formation. Furthermore, administration of antithrombin III did not significantly modify liver damage. These data suggest that lesions of the sinusoids and activation of coagulation are not a primary event in the pathogenesis of liver lesions in this model.

Prostaglandin analogs $dmPGE_2$, PGI_2 and $PGF_{2\alpha}$ have been shown to have hepatoprotective properties in CCl_4-induced liver injury (Guarner et al., 1983; Rush et al., 1986; Stachura et al., 1981). It has been suggested that the protection offered by prostaglandins could result from their effect on liver microcirculation. However, it has been shown that $dmPGE_2$ decreases the formation of the reactive metabolite $CCl_3^•$ (Rush et al., 1986). Furthermore, prostaglandins have been shown to prevent the cytotoxicity of CCl_4 on isolated or cultured hepatocytes (Bang et al., 1988). Therefore alternative mechanisms not directly involving the liver microvasculature could account for the effects of prostaglandins.

4.3.4.3. Other models. Recent data suggest that microcirculatory factors could play a role in hepatic injury induced by acetaminophen. Jaeschke and Mitchell (1989) have reported that hepatic injury produced in mice by the arylating metabolite of acetaminophen leads to a rapid 15-fold increase in neutrophil accumulation with a consequent plugging of the hepatic microvasculature. It was suggested that these secondary microcirculatory changes exacerbate the original injury and expand the region of necrosis through an ischemic infarction of the periacinal region. Interestingly, this component of injury was prevented by treatment with antioxidants such as

allopurinol without decreasing the formation of the toxic metabolite of acetaminophene or preventing glutathione depletion.

Fujiwara et al. (1988b) recently showed that lesions of endothelial and fat-storing cells, together with fibrin microthrombi formation in sinusoids, occur in a model of acute liver damage induced by dimethylnitrosamine in rats. Liver injury was accompanied by increased plasma concentrations of soluble fibrin monomer complexes with decreased concentrations of antithrombin III and coagulation factor VIIIc. Administration of antithrombin III significantly decreased liver injury and prevented intravascular coagulation suggesting a cause–effect relationship.

5. CONCLUSION

In conclusion, abnormalities of the microcirculation and sinusoidal cell function occur in several types of acute liver injury. This raises the question as to the role of these modifications in hepatocyte injury. Although several findings suggest that in ischemic or hypoxic liver injury and in hepatitis induced by FV 3, lesions of the microvasculature could be a primary event that leads to hepatocyte injury, most evidence indicates that in other types of liver damage the primary liver lesion is independent of microcirculatory factors. However, as emphasized in 1963 by Brauer this is a vicious circle with "liver injury causing impaired hepatic blood supply, this causing more liver injury, which causes yet more circulatory embarrassment, and so on to tissue death or to resolution by some event breaking the cycle".

Since Brauer, it has been recognized that sinusoids represent a much more complex system than passive channels ensuring the dispersion of molecules. Sinusoidal cells form a very complex network that protects and regulates parenchymal cells through a multiplicity of lipid mediators and cytokines. Sinusoidal cells and, particularly, Kupffer cells also have the potential to cause injury by releasing cytotoxic substances, activating coagulation processes or recruiting inflammatory cells. However, delicate feedback mechanisms exist between the different cell types, maintaining an equilibrium between cytotoxic and inhibitory mechanisms. Disturbances in the regulation of this system, be they excessive stimulation by mediators or critical reduction in inhibitory factors, will cause or amplify tissue injury.

The concept that liver injury is not the consequence of a single factor but results from disturbances in a regulatory network, was recognized by Popper (1987, 1988) who stated that "the response to injury in experimental animals and in humans is usually not a one-step reaction. . . . It is not even a series of stepwise reactions or a cascade but rather a network of interacting events under the influence of regulatory factors and mediators, secondary messengers and transducing signals which determine the response."

If this hypothesis is correct, the complex multicomponent nature of the processes suggests that the most successful therapeutic strategies may consist of combinations of drugs rather than a single pharmaco-

logical agent directed at a putative 'key mechanism', e.g. a mediator antagonist.

Finally, as biological and physiological research reveals the existence of systems of increasing complexity, it becomes essential, in order to understand and to modulate the complex dynamic behavior of such systems, to develop new theories and new models. This is, in the opinion of the author, the major challenge for the investigator at the end of the twentieth century.

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