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Integration of Function in the Hepatic Acinus: Intercellular Communication in Neural and Humoral Control of Liver Metabolism

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The liver is *the* center of metabolism, a center of defense, a control station of the hormonal system and a blood reservoir. As with every organ the liver has to form and to maintain its own cellular and extracellular organ structure (Table 2–1). These many diverse functions are provided by the parenchymal cells, which account for about 90% of the liver cell mass and 60% of the cell number,[1,2] and at least four types of nonparenchymal cells—endothelial cells, resident macrophages (Kupffer cells), perisinusoidal cells (Ito cells, fat-storing cells), and large granular lymphocytes (pit cells)—as well as bile duct cells, which make up the remainder of the cell mass and cell number, respectively (Fig 2–1). These cells perform the various functions either alone or in cooperation. Thus, the liver catalyzes a huge array of synthetic and degradative metabolic pathways, which need to be integrated by complex regulatory systems. The substrate concentrations in blood, the circulating hormone levels and the autonomic hepatic nerves control the functions of all cells. In addition, the parenchymal cells can be regulated by the nonparenchymal cells and vice versa by locally produced and degraded mediators and the biomatrix. Finally, the heterogeneities of the parenchymal and nonparenchymal cells between the periportal or upstream and the perivenous or downstream zones of the liver acinus[3–7] are important factors in the regulation of the various liver functions.[8–15]

This article presents an overview on the role of cell-to-cell communication in the control and integration of liver functions by the hepatic nerves, hormones, and mediators. It focuses mainly on liver metabolism; acute-phase protein synthesis, biomatrix formation, and fibrogenesis are also considered briefly. Thus, it is our goal to promote an understanding of basic liver functions in health and disease.

ARCHITECTURE OF THE LIVER ACINUS

Microscopic View of the Acinus

Cell Types. The smallest functional unit of the liver is the acinus, which extends from a terminal portal venule and a terminal hepatic arteriole to the central vein.[16] The terminal portal vein is accompanied by a bile duct. Branches of the terminal arteriole form a network around the bile duct, ie, the peribiliary plexus.

Table 2–1. *Functions of the Liver*

Service Functions for Nonhepatic Organs	
Center of metabolism	**Center of defense**
Energy supply of the organism	*Xenobiotic metabolism*
Glucose uptake and release	Oxygenation, reduction
Amino acid uptake and release	Conjugation
Urea formation	*Phagocytosis*
Lipid processing	Uptake and biochemical destruction of foreign macromolecules and macromolecular aggregates
Ketone body synthesis	
Biosynthesis and biodegradation	*Elimination of tumor cells*
Plasma protein synthesis and degradation	Acute-phase reaction
Bile formation-excretion of endobiotics and xenobiotics	
Control station of the hormonal system	**Blood reservoir**
Inactivation and elimination of hormones and mediators	Active blood storage
	Passive blood storage
Synthesis and release of (pro)hormones and mediators	
Formation and Maintenance of Organ Structure	
Synthesis and degradation of cellular and extracellular (biomatrix) components	**Protective metabolism**
Biomembrane components	Scavenging of reactive oxygen intermediates
Cytosolic components	Scavenging of electrophilic intermediates
Nuclear components	
Cytoskeletal components	
Biomatrix components	

Portal vein and arteriole deliver their blood into the sinusoids. The region of the acinus close to the terminal portal vein is called the periportal zone, and that close to the central vein is the perivenous zone. Within the acinus the polarized parenchymal cells, the *hepatocytes,* form a trabecular network facing the sinusoids with their basolateral surface and forming the bile canaliculi with their apical membrane (Fig 2–1). The wall of the sinusoids is formed by fenestrated *endothelial cells.* The endothelium lacks a basement membrane. The fenestrae, 100 to 200 nm in diameter,[16] permit free access for macromolecules to the hepatocyte surface but retain cellular components within the sinusoidal lumen. Resident liver *macrophages,* the Kupffer cells, are attached to the sinusoidal wall on the luminal surface especially at branching points. Kupffer cells comprise about 80% of the total number of macrophages of the body.[16] Between the endothelial cells and the hepatocytes is a small space, the space of Disse. In this space protrusions of Kupffer cells, which pass through the fenestrae,[17] are located as well as *perisinusoidal cells,* that are also called Ito cells, lipocytes, or fat-storing cells. Ito cells embrace the endothelial lining with long processes. They contain large, vitamin A–containing fat droplets in the cell body.[18] Because they possess contractile elements their participation in presinusoidal[19] and intrasinusoidal[20] regulation of sinusoidal blood flow has been discussed. During fibrogenesis they dedifferentiate to myofibroblast-like cells.[21, 22] In close neighborhood to the perisinusoidal cells, especially in the periportal zone, varicosities of sympathetic *hepatic nerves* end in the space of Disse.[2, 23, 24] *Large granular lymphocytes,* so-called

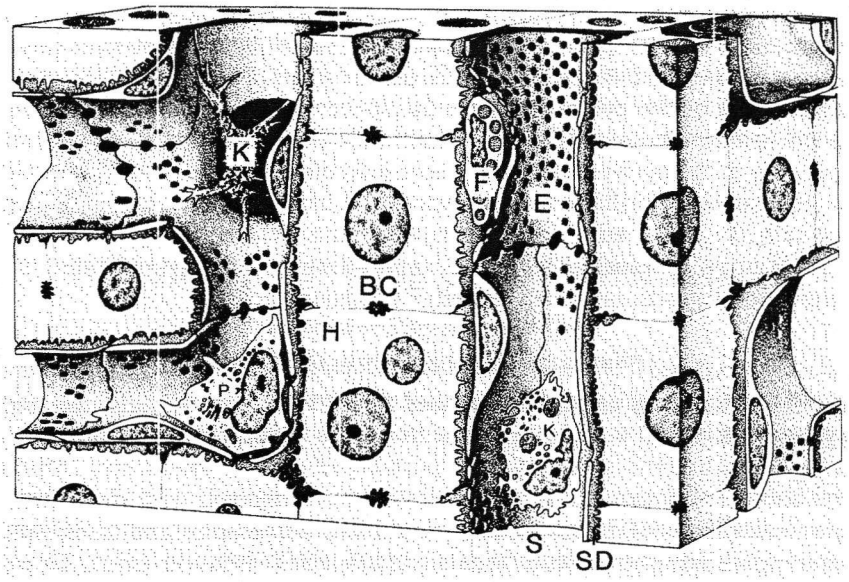

Figure 2–1. Schematic drawing of liver tissue. The parenchyma consists of liver plates formed by adjoining hepatocytes (H). Blood flows through the sinusoids (S) formed by fenestrated endothelial cells (E), which are surrounded by perisinusoidal or fat storing cells (F). Kupffer cells (K) and pit cells (P) bulge into the sinusoidal lumen from the endothelial cells. The space between the endothelial cell lining and the hepatocyte plates is the space of Disse (SD) in which extracellular matrix is located. Adjacent hepatocytes form the bile canaliculi (BC) with their apical membrane. (Reprinted with permission.[122])

pit cells or liver-associated lymphocytes, are loosely attached to the luminal surface of the sinusoids, to Kupffer cells, or endothelial cells.[18] They exhibit natural killer activity.

Cell-to-Cell Contacts. *Hepatocyte-hepatocyte:* Neighboring hepatocytes separate the blood and bile compartment by tight junctions.[25] They are also connected by gap junctions, which seem to play a role in signal propagation from one hepatocyte to the next[24] (discussed below). *Endothelial cell—endothelial cell:* Sinusoidal endothelial cells, in contrast to other capillary endothelial cells, form only loose cell contacts without tight junctions.[26] *Significance of cell proximities:* The circulating concentrations of signal substances such as noradrenaline or eicosanoids are much lower than the concentration of these mediators that elicit biological effects on isolated cells, but because of the close proximity of the different cell types, high local concentrations of these mediators can probably be reached.[27] In this view, the contact of Kupffer cell protrusions with hepatocytes as well as the proximity of Ito cells to hepatocytes and nerve terminals or of perivenous hepatocytes to the endothelium of the central vein seem to be of functional importance (discussed below).

Biomatrix. The highly organized extracellular matrix is composed of proteins (collagen types I, III, IV, and V, elastin), glycoproteins (fibronectin, laminin, undulin, nidogen=entactin, tenascin, and osteonectin), proteoglycans (heparan sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate, and dermatan sulfate) and

glycosaminoglycans (hyaluronan).[21] The exact composition of the biomatrix, ie, its biosynthesis and degradation, are strictly regulated involving an intricate interplay between the different liver cell types (discussed below). The extracellular matrix is most abundant in the portal fields. In the space of Disse it gradually decreases along the acinus. The matrix composition in the periportal zone differs from the composition in the perivenous zone. There is a shift from collagen type IV and V in the periportal to fibrillar collagens types I, III, and VI in the perivenous zone.[28] Periportally the major adhesion protein is laminin, perivenously it is fibronectin. The gradient in extracellular matrix composition has been implicated in the development of functional heterogeneity[28] (discussed below).

Blood and Signal Supply in the Acinus

Site of Confluence of Hepatic Artery and Portal Vein. Two vessels, the hepatic artery and the portal vein, supply the liver with blood. At some point before entering or within the sinusoids, the portal and arterial blood mix. Anatomic studies using serial sections after the vessels were injected with dyes, scanning electron microscopy of microvascular casts, microradiography, and transillumination microphotography indicate that an interspecies variation exists. However, even within a single species conflicting results were obtained (reviewed in reference no. 29). Thus, arterioles were found to drain into the terminal portal vein and the first third of the sinusoid with the same frequency in rabbits, more frequently into the terminal portal vein in rats, and almost exclusively into the sinusoids in hamster, humans, rats, mice, pigs, and rabbits. Arterioles have been shown also to penetrate into the first and middle third of the sinusoid in pigs and monkeys and into the last third in rodents. A recent study in rats addressed the problem in liver perfused in the orthograde and retrograde direction via hepatic artery and portal vein by determining the effect of glucagon and the distribution of acridine orange under these conditions. The study provided evidence for the existence of a presinusoidal site of confluence of arterial and portal blood as well as an intrasinusoidal site of confluence within the first third of the acinus.[29]

Gradients of Signal Input Along the Acinus. Because hepatocytes are metabolically active, the blood compositions in the periportal and perivenous region differ. *Oxygen and substrates:* The oxygen tension falls from about 65 mm Hg periportally to 35 mm Hg in the perivenous region. The concentration of most carbon substrates does not change very much, but ammonia and bile acid concentrations decrease about sixfold and ketone bodies increase twofold.[3] *Hormones and nerves:* During one liver passage adrenaline concentration is diminished by 80%, glucagon and noradrenaline by 50%, and insulin by 50% between meals but only by 15% after meals. Thus, after meals the ratio of the concentrations of catecholamines plus glucagon versus their "antagonist" insulin decreases from the periportal to the perivenous area. Triiodothyronine increases 1.5-fold because of the conversion of tetraiodothyronine, whereas the adenosine concentration increases 10-fold. Hepatic nerves terminate predominantly in the periportal zone.[2] Signal substances are not degraded uniformly along the acinus. For example adenosine triphosphate (ATP)[30] and eicosanoids[31] were degraded predominantly by a small population of perivenous hepatocytes, whereas anaphylatoxin C3a, the small peptide generated during the activation of complement factor

C3, was inactivated predominantly in the periportal zone.[32] *Biomatrix:* The composition of the extracellular matrix changes gradually from the periportal to the perivenous zone[23] (discussed above). Thus, as a result of sinusoidal gradients in substrate concentrations, neural and humoral mediators and biomatrix composition, the signal input at periportal hepatocytes differs considerably from the signal input at perivenous hepatocytes.

FUNCTIONAL ZONATION OF THE LIVER ACINUS

Functions of Parenchymal Cells

Zonal Distribution of Enzymes and Subcellular Structures. Hepatocytes from the periportal and perivenous zones of the liver parenchyma differ in their content of key enzymes and subcellular structures and thus have different metabolic capacities (Table 2–2).[3–5] This was the basis for the model of "metabolic zonation" first proposed for carbohydrate metabolism[33] and later expanded to almost all major hepatic functions (Table 2–3, Fig 2–2).[6,7] The model distinguished, at first arbitrarily, only two zones of about equal size, the periportal and the perivenous compartment, because most enzymes seem to have high activities in the first half and low activities in the second half of the acinus or vice versa. Because meanwhile some enzymes were found to occur only within the first or last quarter, respectively, the periportal and the perivenous compartments have to be subdivided into a proximal and a distal part each (Table 2–3 and Table 2–2 footnotes).

Zonal Heterogeneity of Functions. The functional specialization of the hepatocytes in the different zones has been especially well studied for the metabolism of carbohydrates,[8] amino acids, and ammonia,[9] as well as xenobiotics.[10,11] Strong evidence is also available for bile formation.[12] Different experimental approaches have been used[3,5,34]: zonal flux differences were calculated from enzyme and metabolite distributions measured in vivo. They were determined in periportal- and perivenous-like hepatocytes in cell culture and in hepatocyte populations enriched in periportal and perivenous cells. They were detected during orthograde and retrograde liver perfusion with unlabeled or labeled substrates measuring balances in the perfusate and localizations in the tissue. They were observed also by noninvasive techniques, using surface microlight guides and miniature oxygen electrodes. The various findings support the following schemes: (1) *Carbohydrate metabolism*[3,8]: During the digestive and absorptive phase nutrient glucose is taken up by the perivenous cells to be incorporated into glycogen and degraded to lactate. Lactate returns to the liver via the circulation and is converted in periportal cells via gluconeogenesis to glycogen. During the postabsorptive (fasting) phase glycogen is degraded in the periportal cells to glucose and in the perivenous cells mainly to lactate, which again reaches the periportal cells to be converted via gluconeogenesis to glucose (Table 2–3). (2) *Amino acid and ammonia metabolism*[3,9]: "nitrogen" derived from amino acids including glutamine and free ammonia is removed via ureagenesis in periportal and proximal perivenous hepatocytes. Ammonia escaping ureagenesis in the upstream zones is scavenged via glutamine synthesis in distal perivenous hepatocytes (Table 2–3). (3) *xenobiotic metabolism*[3,10,11,35]: xenobiotics are biotrans-

Table 2–2. *Zonation of Key Enzymes, mRNA and Subcellular Structures in Rat Liver*

		Periportal	Perivenous	Ref. No.
		Oxidative energy metabolism		
Mitochondria volume		++	+	3, 8
Succinate dehydrogenase	Enz	++	+	3, 8
		Carbohydrate metabolism		
Phosphoenolpyruvate carboxykinase	Enz	+++	+	3, 8
	mRNA	++++	+	3, 8
Fructose 1,6-bisphosphatase	Enz	++	+	3, 8
	mRNA	+++	+	‡
Glucose 6-phosphatase	Enz	++	+	3, 8
Glucokinase	Enz	+	++	3, 8
	mRNA	+	+	42, 43
Pyruvate kinase L	Enz	+	++	3, 8
	mRNA	+	+	3, 8
		Amino acid and ammonia metabolism		
Tyrosine aminotransferase	Enz	++	+	3, 8, 9
	mRNA	++	+	8
Glutamate pyruvate transaminase	Enz	++	+	3, 9
Carbamoyl phosphate synthetase*	Enz	++	–	3, 9
	mRNA	++	–	3, 9
Glutamine synthetase*	Enz	–	+++	3, 9
	mRNA	–	+++	3, 9
		Lipid metabolism		
ATP-dependent citrate lyase	Enz	+	++	3
Acetyl-CoA carboxylase	Enz	+	++	3, 13
Fatty acid synthetase	Enz	+	++	3
HMG-CoA reductase†	Enz	+++	–	3
	mRNA	++	–	46
		Xenobiotic metabolism		
Cytochrome P450 (1A1, 1A2, 2B1, 2B2, 2E1, 3A1)	Enz	+	++	3
	mRNA	+	++	38–41
UDP-glucuronate transferase	Enz	+	++	3
		Protective metabolism		
Glutathione peroxidase	Enz	++	+	3
		Bile formation		
Golgi apparatus		++	+	3
Bile canaliculi		++	+	3

NOTE: For a very detailed overview on the zonal distribution of enzymes and subcellular structures see Table 2 in reference no. 3.

*Carbamoyl phosphatase synthease is located in the periportal and proximal perivenous and glutamine synthase reciprocally only in the distal perivenous zone.

†HMGCoA reductase is situated in the proximal periportal zone only.

‡Eilers F, Bartels H, Jungermann K. Unpublished observations, February 1994.

Abbreviation: Enz, enzyme.

Table 2–3. *The Model of Functional Zonation of Parenchymal and Nonparenchymal Liver Cells*

Periportal zone		Perivenous zone	
proximal	distal	proximal	distal
Parenchymal cells			
Oxidative energy metabolism			
Glucose release		***Glucose uptake***	
Gluconeogenesis		Glycolysis	
Glycogen degradation to glucose		Glycogen synthesis from glucose	
(Glycogen synthesis from pyruvate)*		(Glycogen degradation to pyruvate)†	
		Liponeogenesis (fatty acid synthesis)	
Amino acid and ammonia metabolism			
Ureagenesis from amino acid nitrogen		Glutamine formation from NH_3	
Ureagenesis from NH_3 and glutamine			
Amino acid conversion to glucose			
Cholesterol synthesis			
Protective metabolism		***Xenobiotic metabolism***	
Glutathione peroxidation		Monooxygenation	
Glutathione conjugation		Glucuronidation	
Plasma protein formation			
Albumin		α-Fetoprotein	
α_2-Macroglobulin		Angiotensinogen	
Fibrinogen		α_1-Antityrpsin	
Bile formation			
Nonparenchymal cells			
Endothelial cells			
Lectin binding		Porosity (Filtering)	
Kupffer cells (resident macrophages)‡			
Phagocytosis		Cytotoxicity	
Pit cells (large granular lymphocytes)‡			
Tumor cell killing			
Perisinusoidal cells (Ito cells)‡			
Matrix formation			
Lipid storage			

Note: Predominant localization of capacities for metabolic pathways based on the zonal distribution of enzymes and subcellular structures (Table 2–2).

*Periportal glycogen previously synthesized in the absorptive phase via gluconeogenesis is degraded to glucose in the postabsorptive phase.

†Perivenous glycogen synthesized from glucose in the absorptive phase is degraded to pyruvate in the postabsorptive phase.

‡More numerous in the periportal zone.

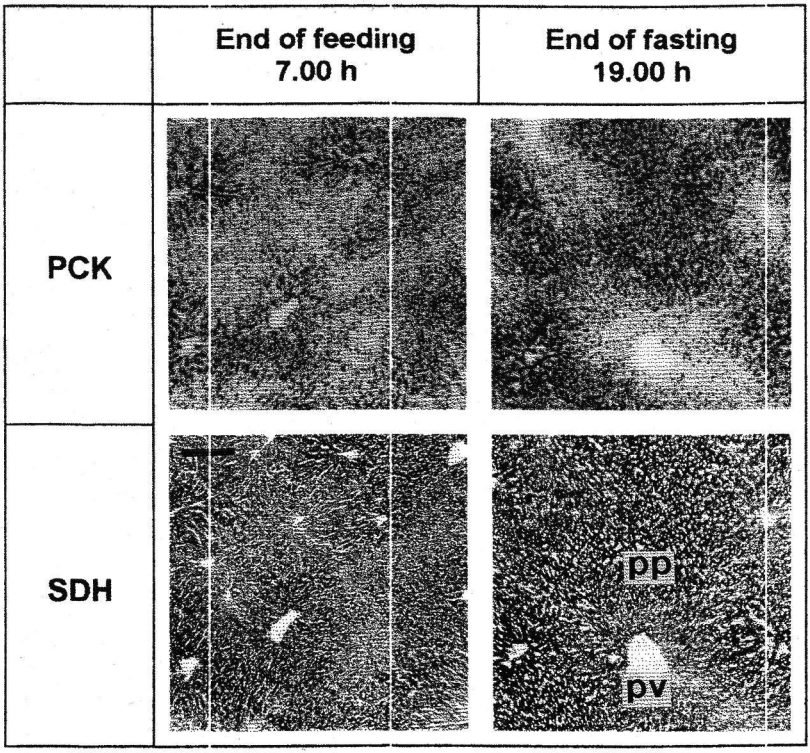

Figure 2–2. Distribution of phosphoenolpyruvate carboxykinase (PCK) mRNA in rat liver during a normal feeding cycle. PCK mRNA was hybridized with a ^{35}S-labeled rat antisense RNA probe; in a parallel section succinate dehydrogenase (SDH) was shown histochemically as periportal marker enzyme. PCK mRNA (high grain density) and SDH (dark precipitates) show higher levels in the periportal (pp) than in the perivenous (pv) zone. Bar = 200 μm. For more details consult reference no. 8.

formed into excretable derivatives via mono-oxygenation and glucuronide formation preferentially by perivenous hepatocytes. Thus, toxic metabolites such as electrophiles also are formed in a P450 side reaction from many xenobiotics mainly in the perivenous zone. However, the potential for detoxification by glutathione conjugation is higher in the periportal zone. The perivenous hepatocytes are most potentially at risk because of their higher P450 content and, at the same time, least protected because of their lower glutathione content.[36] This could be the molecular background for many agents that are well-known causes of perivenous necrosis, eg,, bromobenzene, CCl_4, or ethanol[3] (Table 2–3). (4) *Bile formation* (references no. 3 and 12 and the references therein): bile acids are taken up and secreted into bile mainly in the proximal periportal zone because of both the sinusoidal concentration gradients of bile salts and intrinsic hepatocellular differences in their content of carriers, intracellular binding proteins or enzymes. The Na^+-dependent taurocholate uptake carrier in the sinusoidal as well as the ATP-dependent bile acid export carrier in the canalicular membrane seem to be located mainly in periportal cells, the bile acid–binding protein predominantly in perivenous cells. (5) *Plasma protein formation*[3, 14]: all hepatocytes

produce plasma proteins, but periportal hepatocytes seem to be more active in the synthesis of albumin, α_2-macroglobulin, and fibrinogen, whereas perivenous cells preferentially synthesize angiotensinogen, α_1-antitrypsin, and α-fetoprotein as long as it is produced during the first 4 postnatal weeks (Table 2–3).

Zonation of Gene Expression. All hepatocytes have the same genome. Its different activation along the acinus could be caused by zonal differences in the input and transmission of signaling molecules such as substrates, hormones, or mediators and in cell-to-cell or cell-to-biomatrix interactions.[3, 5, 8, 9, 28] The zonation of enzymes and subcellular structures (Table 2–2) may result from different rates of transcription, messenger (m)RNA degradation, translation or protein degradation. The mRNAs of phosphoenolpyruvate carboxykinase,[8] fructose 1,6-bisphosphatase, tyrosine aminotransferase,[8] carbamoylphosphate synthetase,[9] and hydroxymethylglutaryl-coenzyme A(HMG-CoA) reductase[37] were found by in situ hybridization to be localized mainly in the periportal zone as were the enzyme proteins and activities. Similarly, the mRNAs of glutamine synthetase[9] and many cytochrome P450 isoenzymes[38–41] were situated predominantly in the perivenous zone as are the enzyme proteins and activities. Apparently the zonal expression of these genes is regulated primarily at a pretranslational level. In contrast, the mRNAs of glucokinase and pyruvate kinase L were distributed homogeneously in the acinus during a normal feeding rhythm, whereas enzyme proteins and activities were higher in the perivenous zone. The zonal expression of these genes therefore seems to be controlled mainly at a translational or posttranslational level.[4, 42, 43]

The zonation of the enzymes of carbohydrate metabolism changes "dynamically" with the metabolic state, eg, feeding versus fasting.[8, 42, 43] This may indicate that their zone-specific activation is controlled primarily by "dynamic" changes in humoral and neural signals rather than by "stable" interactions between parenchymal cells and neighboring nonparenchymal cells or biomatrix. In contrast, the zonation of the enzymes of ammonia detoxification does not vary with the metabolic state.[9] This would suggest that their zone-specific activation is not regulated primarily by "dynamic" changes in humoral and nervous signals but by "stable" interactions between parenchymal cells and neighboring nonparenchymal cells or biomatrix. Information on this differential gene activation was gained by studies in primary hepatocyte cultures. The "periportal" phosphoenolpyruvate carboxykinase (PCK) and the "distal perivenous" glutamine synthetase (GS) were used as examples.

The PCK gene was activated by glucagon under the permissive action of glucocorticoids; gene transcription was maximal after 30 minutes, elevation of mRNA after 2 hours, and increase in enzyme activity after 4 to 6 hours. This glucagon-dependent gene activation was higher at all three levels under arterial rather than under venous oxygen tensions[44]; it was antagonized again on all three levels by insulin.[45] A heme protein seemed to function as the oxygen sensor; it could regulate gene activity directly as a transcription factor or indirectly, for example, as a protein kinase, which in turn would modulate transcription factors.[46, 47] In preliminary experiments the oxygen-sensitive region of the PCK gene was located in the 5′-flanking sequence within the first −277 base pairs (J. Bratke, T. Kietzmann, H. Bartels, et al: unpublished observations, March 1994).

In contrast, the oxygen-sensitive region of the erythropoietin gene, the activation of which was higher under venous than under arterial oxygen tensions, was localized in the 3′-flanking sequence.[48]

The GS gene was not activated in hepatocyte mono-cultures but in co-cultures of hepatocytes (inner circle) and epithelial cells of hepatic origin (probably bile duct cells) (outer circle). GS was expressed in the ring of 10 to 20 hepatocytes near the interface to the epithelial cells. The latter apparently produced a diffusible factor inducing GS. Thus, it was proposed that in the intact liver cells of the central vein produce this factor, which could reach the distal perivenous hepatocytes by diffusion within the space of Disse opposite to the blood stream (R. Gebhardt, personal communication, January 1994).

Conclusion: The different hepatocellular complement of carriers, binding proteins, and enzymes is caused by a zonation of gene expression mainly at a pretranslational level. Gradients in oxygen and in glucagon/insulin ratio decreasing from the periportal to the perivenous area seem to be major factors in the dynamic zonation of carbohydrate-metabolizing enzymes; local paracrine cell-to-cell interactions seem to be responsible for the stable zonation of ammonia-metabolizing enzymes.

Functions of Nonparenchymal Cells

Endothelial cells constitute the fenestrated wall of the sinusoids, which allows the free diffusion of solutes but not of particles as large as chylomicrons between the blood and the hepatocyte surface. The endothelial lining therefore functions as a filter, which limits, for example, chylomicron lipid uptake by the liver. The endothelial cells also possess a high endocytotic capacity and are therefore an important constituent of the reticuloendothelial system. Moreover, they actively secrete mediators and extracellular matrix components. The lectin-binding capacity seems to be higher in the periportal zone.[15] The size of the endothelial fenestrae may be modulated by various factors (discussed below), but is somewhat larger in the periportal zone,[8] whereas they are more numerous in the perivenous zone.[15] Therefore, the filtering capacity is larger in the perivenous area[15] (Table 2–3).

Kupffer cells: The resident macrophages of the liver show a very high endocytotic capacity and form a great number of inflammatory mediators, eg, eicosanoids, and cytokines such as interleukin-1, interleukin-6, tumor necrosis factor α (TNFα), or transforming growth factor β (TGFβ).[49] They have an important role in the immune response as antigen presenting cells and a cytotoxic action on parasitic and microbial organisms.[15] They are more numerous in the periportal area. The endocytotic capacity seems to be higher in the periportal cells consistent with higher lysosomal activities, whereas the cytotoxic function is greater in cells in the perivenous zone, reflecting their greater number of galactose receptors that bind tumor cells or effete erythrocytes[15] (Table 2–3).

Ito cells: The perisinusoidal cells, also called fat-storing cells and lipocytes, are the main producers of extracellular matrix components and the main vitamin A reservoir. They are also more numerous in the periportal zone, which may therefore be the major site of biomatrix formation[15] (Table 2–3).

Pit cells: The large granular lymphocytes with a high natural killer activity are

anchored to the endothelial cells by pseudopodia, microvilli, or phylopodia. They are spontaneously cytotoxic to some tumor cells. Because they are more numerous in the periportal than in the perivenous zone, the elimination of certain tumor cells by pit cells may occur primarily in the upstream area[15] (Table 2–3).

TOPOLOGICAL ORGANIZATION OF METABOLIC REGULATION IN THE ACINUS

Differential Effects of Arterial and Portal Signals

Adrenaline and Noradrenaline. The catecholamines are major circulating hormones controlling liver function; noradrenaline can also act locally as a neurotransmitter. In rat liver, single-pass perfused bivascularly through the portal vein with low pressure and two thirds of total flow, and through the hepatic artery with high pressure and one third of total flow, arterial adrenaline and noradrenaline caused an increase in glucose output and a shift from lactate uptake to output that was slower in onset, smaller in peak height, and longer in duration than if adrenaline and noradrenaline were infused via the portal vein only.[50, 51] Arterial catecholamines decreased arterial flow strongly and portal flow slightly. Portal catecholamines decreased only portal flow. During a single pass adrenaline and noradrenaline were extracted more efficiently after infusion into the artery than after infusion into the portal vein (80% and 65% *v* 40% and 30%, respectively). The metabolic and hemodynamic effects were mediated by α_1-receptors after both arterial and portal administration; only after arterial input did β_2-receptors exert an antagonistic effect on hemodynamics and thus increased indirectly the catecholamine effect on metabolism.[50, 51] The differences in the metabolic effects of portal and arterial catecholamines and in their degradation were largely because of the differences in the hemodynamics, and these hemodynamic effects of noradrenaline were almost completely abolished by the calcium antagonist nifedipine. As a consequence, nifedipine rendered the metabolic effects and extraction of noradrenaline similar after both arterial and portal infusion.[52] Thus, the different metabolic actions of arterial and portal catecholamines represent an example of a complex communication from vascular and nonparenchymal to parenchymal cells in liver.

Nucleotides and Nucleosides. Extracellular ATP, uridine triphosphate (UTP) and their degradation products may function as neuro(co)transmitter or as local hormones released from nonspecialized cells. In bivascularly perfused rat liver, portal ATP increased glucose and lactate output to a greater extent than arterial ATP; portal ATP decreased only portal flow, but arterial ATP decreased both arterial and portal flow. Portal ATP acted directly via P_2-receptors; arterial ATP operated indirectly after its degradation to adenosine via P_1-receptors and after the indomethacine-sensitive formation of prostanoids in vascular and nonparenchymal liver cells (Y. Watanabe and K. Jungermann: unpublished observations, 1992). Comparable results have been obtained with ATP and UTP in rat livers perfused classically via the portal vein only.[53–55] Portal adenosine was slightly less effective metabolically than arterial adenosine; both portal and arterial adenosine decreased portal and increased arterial flow. Portal and arterial adenosine acted

in part directly via P_1-receptors and in part indirectly via prostanoids (Y. Watanabe and K. Jungermann: unpublished observations, 1992). Thus, the actions of arterial ATP and of arterial and portal adenosine constitute another example of a complex cell-to-cell communication in the liver.

Acetylcholine is the classical terminal neurotransmitter of the parasympathetic system; it does not act as a circulating signal. In bivascularly perfused rat livers arterial acetylcholine increased glucose and lactate output and decreased flow slightly, whereas portal acetylcholine was without effect. Arterial acetylcholine also caused an overflow into the hepatic vein of adrenaline and noradrenaline, which seemed to mediate only the hemodynamic but not the metabolic alterations, because these persisted in the presence of α- and β-receptor blockers.[56] The nonparenchymal cells involved in the metabolic action of arterial acetylcholine remain to be defined.

Glucagon and insulin, the islet hormones, like catecholamines, are important circulating signals for liver metabolism. In bivascularly perfused rat liver arterial and portal glucagon elicited the same increase in glucose output without any influence on portal or arterial hemodynamics.[29] Insulin increased glucose uptake only if the portal glucose concentration was higher than the arterial glucose concentration, but not if the arterial and portal glucose concentrations were identical or if the arterial glucose concentration was higher than the portal glucose concentration.[57] Similar observations were made in vivo in dogs.[58] Thus it seems that the liver can sense a porto-arterial glucose gradient, which would be a straightforward means to distinguish between exogenous and endogenous glucose. The mechanism by which the liver senses the gradient and modulates insulin action is still unknown; nonparenchymal cells in the inflow vessels could be involved.

Cell-to-Cell Communication From Parenchymal to Parenchymal and From Nonparenchymal to Parenchymal Cells

Eicosanoid-Mediated Action of Zymosan, Endotoxin, Immune Complexes, Anaphylatoxins and Platelet Activating Factor (PAF). Eicosanoids are synthesized only in nonparenchymal liver cells; they seem to control locally the functions of parenchymal and nonparenchymal liver cells.[59]

Impairment of action by eicosanoid synthesis inhibitors and eicosanoid receptor antagonists. In perfused liver glucose and lactate output were enhanced, flow was reduced, or perfusion pressure increased by various effectors to which the liver may be exposed during pathological processes such as zymosan,[60] endotoxins,[61] immune complexes,[62] complement-activated serum,[63, 64] and anaphylatoxins[65] as well as PAF.[66–68] However, neither zymosan nor endotoxins[61] or anaphylatoxins[27] activated glycogen phosphorylase or increased glucose output in isolated hepatocyte cultures or suspensions. For PAF conflicting results were reported.[67, 68] The effects of these stimuli in perfused liver were attenuated by agents that inhibit eicosanoid synthesis or action. These include inhibitors of phospholipase A2 such as bromophenacyl bromide (BPB)[60] and mepacrine,[63] inhibitors of cyclooxygenase (PGH synthase) such as indomethacin[50] and aspirin,[61] and thromboxane receptor antagonists.[64] Inhibitors of 5′-lipoxygenase and thus leukotriene synthesis or leukotriene receptor antagonists were less effective. Their inhibitory effect, for

example, in the case of complement activated rat serum, could be ascribed to unspecific inhibition of cyclooxygenase or interference with thromboxane receptors.[64] Endotoxin,[61] immune compexes,[62] complement-activated rat serum,[64] and PAF[69] caused an overflow of eicosanoids into the hepatic vein in perfused liver. Thus, an eicosanoid-mediated cell-to-cell communication between nonparenchymal and parenchymal liver cells must have been involved in the regulation of liver metabolism by these factors. This proposal is based on the assumption that the inhibition of the metabolic effects of endotoxins, immune complexes, complement-activated serum, and PAF by BPB, indomethacin, or aspirin was caused by the inhibition of eicosanoid synthesis. However, this interpretation has to be regarded with caution. Thus, inhibition of PAF-stimulated glycogenolysis by indomethacin in perfused liver was ascribed to the unspecific interference of the drug with intracellular Ca^{2+} -metabolism. It was concluded that PAF activated glycogenolysis by a direct action on hepatocytes and that the generation of eicosanoids by PAF was an unrelated process. The finding that ibuprofen inhibited PAF-induced eicosanoid formation without affecting the PAF-stimulated glycogenolysis in perfused liver seems to support this interpretation.[69] In these studies the metabolic alterations were interpreted as a consequence of the hemodynamic changes, leaving part of the liver tissue in a hypoxic state. Yet, in the case of the anaphylatoxins, the eicosanoid-mediated increase in glucose and lactate output was independent of the alterations in flow. The flow reduction elicited by complement-activated rat serum was abolished by nifedipine without affecting the metabolic changes.[64]

Eicosanoid production in nonparenchymal liver cells. The model of the eicosanoid-mediated cell-to-cell signal propagation was further corroborated by experiments showing that conditioned media of Kupffer cells treated with endotoxin increased glycogenolysis in hepatocytes.[61] The effect could be abolished by pretreatment of the Kupffer cells with aspirin. Similarly, human C3a did not increase glycogen phosphorylase activity in rat hepatocyte monocultures but it increased glycogen phosphorylase activity in cocultures of rat hepatocytes and Kupffer cells.[27] Furthermore, zymosan,[70] endotoxins,[59] anaphylatoxin C3a,[27] and PAF[71] stimulated prostanoid formation in isolated Kupffer cells.

Eicosanoid action in perfused liver. For an eicosanoid-mediated cell-to-cell communication to be operative, nonparenchymal cell-derived eicosanoids must be able to modulate hepatocyte metabolism. In perfused liver prostaglandins $F_{2\alpha}$, D_2, and E_2,[62, 72–74] the thromboxane A_2 analogs U46619[72, 75] and ONO11113[74] as well as leukotrienes[76] increased glucose and lactate output and reduced flow or increased perfusion pressure. Whereas the metabolic changes observed after infusion of thromboxanes and leukotrienes could be ascribed largely to the drastic hemodynamic alterations, the increase in glucose and lactate output observed after infusion of prostaglandins was independent of flow changes. The prostaglandin $F_{2\alpha}$-elicited flow reduction was abolished by nifedipine, whereas the metabolic effects remained unaffected.[72, 77] In perfused rat liver, prostaglandin $F_{2\alpha}$ was more potent than PGD_2 and PGE_2. At saturating concentrations it stimulated glucose output by sevenfold and reduced flow to 50%. Alterations were half-maximal at concentrations of 3 μmol/L.[72]

Prostaglandin action in isolated hepatocytes. Prostaglandins $F_{2\alpha}$, E_2, and D_2 but

not the thromboxane analog U46619 increased glucose output and glycogen phosphorylase activity in isolated rat hepatocytes in suspension and primary culture.[78–81] Different rank orders of potency and half maximally effective concentration (ED_{50})-values were reported. In hepatocyte suspensions glucose release into a glucose-free medium was half-maximal at 20 nmol/L PGD_2, which was more potent than $PGF_{2\alpha}$ and PGE_2.[81] Under similar conditions an ED_{50} of 1 nmol/L was found for PGE_2.[79] In cell culture or suspension in a medium containing 5 mmol/L glucose, 2 mmol/L lactate, and 0.2 mmol/L pyruvate $PGF_{2\alpha}$, PGE_2, and PGD_2 stimulated glycogen phosphorylase activity half-maximally at concentrations of 1 µmol/L, 0.5 µmol/L, and 10 µmol/L, respectively (G.P. Püschel, A. Schröder, K. Jungermann: unpublished observations, July 1991). $PGF_{2\alpha}$ and PGE_2 but not PGD_2 stimulated glucose release from (^{14}C)-labeled glycogen in hepatocyte cultures.[82]

Mechanism of prostaglandin action in hepatocytes. Prostaglandin $F_{2\alpha}$ and E_2 stimulated the formation of inositol-1,4,5-triphosphate ($InsP_3$) but not of cyclic adenosine monophosphate (cAMP).[78–80] Apparently, prostaglandins activated glycogenolysis via a phospholipase C-linked rather than by an adenylate cyclase-linked signal chain. However, prostaglandins elevated cAMP in cultured hepatocytes in which an inhibition of adenylate cyclase had been relieved by pertussis toxin treatment,[80, 83] or in whole liver plasma membranes where cAMP formation can be attributed to adenylate cyclase of nonparenchymal liver cells.[84] By the use of receptor-specific prostaglandin analogs evidence has been obtained, that $PGF_{2\alpha}$ stimulated glycogenolysis via specific $PGF_{2\alpha}$-receptors and that PGE_2 acted via PGE_2-receptors most probably of the EP_1-subtype. Because the PGD_2 receptor-specific analog ZK118182, which in contrast to PGD_2 does not bind to $PGF_{2\alpha}$ receptors, did not stimulate glycogen phosphorylase, hepatocytes seem not to possess a PGD_2 receptor. The G-proteins involved in the $PGF_{2\alpha}$ and PGE_2 signal chains were pertussis toxin–insensitive (G. P. Püschel, F. Neuschäfer-Rube, C. Kirchner, et al: unpublished observations, November 1994).

Prostaglandin receptors on hepatocytes. A number of prostanoid receptors have been cloned and sequenced in the last 2 years (reviewed in reference no. 85): the thromboxane A_2 receptor from human placenta, three isoforms of the EP_3 subtype of PGE_2 receptors from mouse mastocytoma and bovine adrenal medulla, PGE_2 receptors of the EP_2- and EP_1-subtypes from mouse mastocytoma, and a $PGF_{2\alpha}$ receptor of mouse ovary.[86] However, in liver prostaglandin receptors have so far been characterized only in binding studies. Molecular characterization of these receptors is a subject of current research: an EP_3 receptor from rat hepatocytes has been cloned and sequenced.[123] A PGE_2 binding site in liver plasma membranes with a K_d of about 3 nmol/L and a maximal specific binding (B_{max}) of 175 fmol/mg membrane protein was downregulated in starved animals[87] and upregulated in animals treated with indomethacin,[88] which would indicate that the site was a PGE_2 receptor. PGE_2 and PGD_2 binding sites were reported in hepatocyte cultures and suspensions, respectively, with a rather high density of 10^6 sites per cell.[81, 89] A general drawback of these studies is that in some binding experiments were performed either with whole liver plasma membranes, which contain high amounts of nonparenchymal cell plasma membranes, rather than hepatocyte plasma membranes. In others intact cells were used, which even at low

temperatures accumulate prostaglandins to an extent far exceeding the surface binding yielding erroneously high receptors numbers.[90]

Therefore, in a recent study[90] $PGF_{2\alpha}$ binding was determined on plasma membranes of purified hepatocytes, showing the existence of a high-affinity/low-capacity site with a K_d of 3 nmol/L and about 1,000 copies/cell, and a low-affinity/high-capacity site with a K_d of 500 nmol/L and about 20,000 copies/cell. The binding to the high-capacity but not to the low-capacity site was enhanced by GTPγS, which would imply that this site was coupled to a G-protein, as would be expected for a receptor. A site with similar binding characteristics as the low-affinity/high-capacity $PGF_{2\alpha}$ binding site was downregulated in chronically endotoxin-treated animals,[91] the liver of which is exposed to constantly elevated prostaglandin levels. These results are in favor of the idea that this site might represent a receptor.

Prostaglandin degradation by hepatocytes. Another important feature of an intraorgan cell-to-cell communication chain is the local restriction of the action of the mediator. Because the extrahepatic organs, ie, lung and heart, are very sensitive to prostaglandins, intrahepatically liberated prostaglandins must be eliminated efficiently. Indeed, more than 90% of prostaglandins infused to micromolar concentrations are eliminated in a single pass through the liver.[92] They are rapidly degraded by hepatocytes[78, 81, 93–96] through peroxisomal β-oxidation,[94, 95] ω-oxidation, and oxidation of the hydroxyl group at carbon 15.[96] The degradation can be inhibited by other β-oxidation substrates or inhibitors of the P450 system.[94] The inactivation system seems to be located predominantly in the perivenous zone of the liver acinus, because prostaglandins elicited stronger effects in orthograde perfusions than in retrograde perfusions.[31]

Relevance of cell-to-cell contacts. An argument often brought forward against the relevance of nonparenchymal cell-derived prostaglandins in cell-to-cell communication within the liver was that the prostaglandin concentration in perfusates,[64] portal or hepatic vein blood,[97] or cell culture supernatants of nonparenchymal liver cells[27] were much lower than the concentrations needed to stimulate glycogenolysis in hepatocytes.[78, 80] However, this argument does not take into account the close proximity between nonparenchymal cells and hepatocytes in the space of Disse. Thus, at sites of, for example, Kupffer cell–hepatocyte contacts micromolar prostaglandin concentrations can be expected to occur.[27] The importance of these close proximities or even contact sites is underlined by the observation that supernatants of C3a-stimulated Kupffer cells did not increase glycogen phosphorylase activity in hepatocyte cultures, but that in cocultures of Kupffer cells and hepatocytes, where close contacts between the two cell types can be formed, C3a stimulated glycogen phosphorylase activity.[27]

Conclusion I. These data taken together support the following model for the action of anaphylatoxins and other system-derived mediators of inflammatory processes on liver metabolism (Fig 2–3): anaphylatoxins bind to specific receptors on Kupffer cells and induce the formation of prostaglandins D_2, $F_{2\alpha}$, E_2, and thromboxane A_2. Because thromboxane A_2 analogs did not increase glycogenolysis in hepatocytes,[78] but thromboxane receptor antagonists inhibited the anaphylatoxin-mediated increase in glucose output from perfused livers at least partially,[65] thromboxane A_2 might enhance prostaglandin formation in an autocrine manner

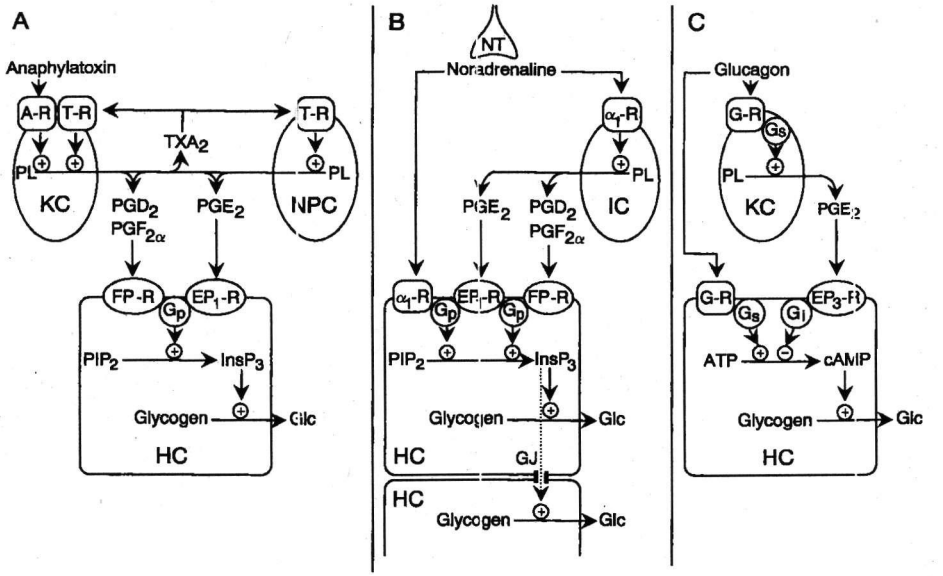

Figure 2–3. Proposed models of the eicosanoid-mediated cell-to-cell communications between nonparenchymal cells and hepatocytes involved in the anaphylatoxin-elicited (A) and nerve stimulation–dependent (B) increase in glycogenolysis as well as in the feedback inhibition of glucagon-stimulated glycogenolysis (C) in liver. (A) Anaphylatoxins increase formation of PGD_2, $PGF_{2\alpha}$, PGE_2, and TXA_2 in Kupffer cells. TXA_2 may enhance prostaglandin formation in Kupffer cells or other nonparenchymal cells. Prostaglandins increase $InsP_3$ formation and glycogenolysis in hepatocytes via G_p-coupled FP and EP_1 receptors. (B) Noradrenaline, released from nerve terminals in the periportal region, increases prostaglandin formation in Ito cells. Nerve terminal-derived noradrenaline and Ito cell–derived prostaglandins increase $InsP_3$ formation and glycogenolysis in hepatocytes via G_p-linked α_1-receptors, FP and EP_1 receptors, respectively. The signal is propagated to more perivenous hepatocytes via gap junctions. (C) Glucagon increases glycogenolysis in hepatocytes and prostaglandin formation in Kupffer cells. Kupffer cell-derived prostaglandins may decrease glucagon-simulated cAMP formation and glycogenolysis in hepatocytes via G_i-linked EP_3 receptors. HC, hepatocyte; GJ, gap junction; NPC, nonparenchymal liver cell; KC, Kupffer cell; IC, Ito cell; NT, nerve terminal; PG, prostaglandin; NA, noradrenaline; PL, phospholipid; TXA_2, thromboxane A_2; A-R, anaphylatoxin receptor; α_1-R, α_1 receptor; EP_1-R, PGE_2 receptor of the EP_1 subtype; EP_3-R, PGE_2 receptor of the EP_3 subtype; FP-R, $PGF_{2\alpha}$ receptor; T-R, TXA_2 receptor; G-R, glucagon receptor; G_p, phospholipase C-coupled trimeric G-protein; $InsP_3$, inositol 1,4,5-trisphosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; G_i, adenylate cyclase-coupled inhibitory trimeric G-protein; G_s, adenylate cyclase-coupled stimulatory trimeric G-protein; Glc, glucose.

in Kupffer cells or in a paracrine manner in other nonparenchymal liver cells. Prostaglandins then may increase glycogen phosphorylase activity in hepatocytes via prostaglandin receptors, probably $PGF_{2\alpha}$ and PGE_2 receptors of the EP_1-subtype that are linked to phospholipase C by a pertussis toxin–insensitive G_p-protein.

Eicosanoid-Mediated Nerve Actions. *Regulation of liver metabolism and hemodynamics by hepatic nerves.* Hepatic metabolism is modulated by the action of sympathetic hepatic nerves (recently reviewed in reference no. 92). In perfused rat liver stimulation of the nerve bundles around hepatic artery and portal vein increased glucose and lactate output, uric acid and allantoin formation, and

decreased oxygen consumption, ketogenesis, urea release, ammonia uptake as well as conjugation of xenobiotics. Nerve stimulation caused a decrease in blood flow or increase in perfusion pressure, depending on the technique used, and a reduction of bile flow and bile acid secretion. The effects on metabolism, except those on urea, glutamine, and ammonia balance as well as bile acid formation, were independent of the hemodynamic alterations, because flow changes could be abolished by nifedipine or sodium nitroprusside[92] without affecting the metabolic alterations. Studies with subtype-specific adrenergic receptor antagonists and agonists provided evidence for the involvement of postsynaptic α_1-receptors but neither presynaptic nor postsynaptic α_2-, β_1-, or β_2-receptors in the action of sympathetic hepatic nerves.[92]

Hepatocyte-to-hepatocyte communication via gap junctions. In rats hepatic nerves terminate predominantly in the periportal zone of the acinus. Therefore it has been proposed that the nerve signal could be propagated to more distal regions of the acinus via gap junctions between hepatocytes,[98] which have been shown to be permeable to Ca^{2+} and $InsP_3$ in hepatocyte couplets.[99] In line with this hypothesis the number of hepatocyte gap junctions and the number of nerve terminals within the acinus were found to be inversely correlated in different species.[25] Two different experimental approaches underline the functional significance of gap junctions in hepatic nerve action: (1) in regenerating rat liver 48 hours after 67% partial hepatectomy, the major gap junction protein connexin 32 was reduced to 25% of its normal level, and nerve stimulation-dependent glucose output to 20%. By contrast, nerve stimulation-induced flow reduction was normal after partial hepatectomy. Nerve stimulation-dependent glucose output returned to normal levels only after gap junctions had been reexpressed on day 11.[100] (2) The function of gap junctions can be inhibited by low doses of phorbol esters or glycyrrhetinic acid. In livers perfused with either of the two agents, the nerve stimulation-dependent increase in glucose output was attenuated, whereas the noradrenaline overflow remained unaffected. This indicated that the attenuation of glucose output was caused by an impairement of gap junctional permeability rather than to an unspecific interference with hepatic nerve function.[98]

Nonparenchymal cell-to-hepatocyte communication via eicosanoids. The nerve stimulation-dependent increase in glucose and lactate output and decrease in flow was attenuated by inhibitors of eicosanoid synthesis like bromophenacylbromide (phospholipase A_2 inhibitor) or indomethacin (cyclooxygenase inhibitor) indicating the involvement of prostanoids in the signal transmission after nerve stimulation.[92] In contrast to endotoxin, complement-activated serum, and other simuli that can activate all nonparenchymal liver cells along the acinus, nerve stimulation did not elicit an overflow of prostaglandins into the hepatic vein.[53, 92] However, this does not argue against the participation of eicosanoids in the action of hepatic nerves, because the overflow represents only the very small fraction of locally released prostaglandins that escape the efficient elimination system of the liver. The total amount of prostaglandins formed in the periportal zone in the proximity of nerve endings may just be too small to cause a significant posthepatic change in prostaglandin concentration.

Noradrenaline- and ATP-stimulated eicosanoid formation in Ito cells. Recently, an additional link in the eicosanoid-mediated signal chain after nerve stimulation has

been established and a possible candidate for the nerve stimulation-dependent prostaglandin formation identified: Ito cells maintained in short-term primary culture for 24 hours produced $PGF_{2\alpha}$ and PGD_2 in response to the sympathetic transmitter noradrenaline and the putative cotransmitter ATP.[101] The noradrenaline effect was α_1-mediated, the ATP effect was mediated via P_2-purinergic receptors. The time course of noradrenaline-stimulated prostaglandin formation in Ito cells fitted the expectations for an involvement of neuronal signaling in that it was rapid in onset with a maximum after 1 minute. In Ito cells that had begun to dedifferentiate to myofibroblast-like cells after 48 and 72 hours of primary culture, noradrenaline or ATP no longer stimulated prostaglandin formation, which would be in line with an impaired metabolic nerve effect in thioacetamide-cirrhotic rat livers.[102]

Conclusion II. Taken together the data on hepatic nerve stimulation support the following model (Fig 2–3): sympathetic hepatic nerves, which end in the proximal periportal zone of the liver acinus, release noradrenaline. Noradrenaline can act directly on nearby hepatocytes and stimulate glycogen phosphorylase activity via α_1-receptors. Additionally, noradrenaline can stimulate prostaglandin formation via α_1-receptors in Ito cells, which are located in close proximity to both hepatic nerves and hepatocytes.[23] Prostaglandins may then stimulate glycogenolysis in hepatocytes via prostaglandin $F_{2\alpha}$ and prostaglandin E_2 receptors of the EP_1 subtype, as described for the circulating mediators such as zymosan, endotoxins, and anaphylatoxins. Because both the direct noradrenaline-mediated and indirect eicosanoid-mediated nerve actions will be restricted to the proximal periportal hepatocytes, the signal can then be further propagated to more distal cells via gap junctions between the hepatocytes, presumably via interhepatocellular movement of $InsP_3$ and Ca^{2+}.

Eicosanoid-Modulated Glucagon Action. *Glucagon-antagonistic effect of prostaglandins in hepatocytes.* Not only can prostaglandins activate glycogenolysis by a phospholipase C-linked increase in $InsP_3$, but they can also inhibit glucagon-stimulated cAMP formation,[83, 103] glycogen degradation,[104, 105] and fatty acid oxidation.[106] This glucagon-antagonistic effect was mediated by a signal chain involving a pertussis toxin–sensitive inhibitory G_i-protein.[80, 83, 107, 108] PGE_2 was more potent than $PGF_{2\alpha}$. PGD_2 was either inactive or less potent than $PGF_{2\alpha}$. The ED_{50} for the PGE_2-dependent inhibition of the glucagon-stimulated adenylate cyclase activity was in the range of 50 to 200 nmol/L. The PGE_2 receptor involved in the glucagon-antagonistic signal chain was of the EP_3 subtype.[80]

Glucagon-stimulated prostaglandin synthesis in Kupffer cells. Glucagon was able to stimulate prostaglandin formation in Kupffer cells. Furthermore, the glucagon-dependent increase in glycogen phosphorylase activity in Kupffer cell/hepatocyte co-cultures was smaller than in hepatocyte monocultures of the same hepatocyte density. Pretreatment of the co-cultures with aspirin abolished this difference (U. Hespeling, G.P. Püschel, J, Jungermann: unpublished observations, January 1994).

Conclusion III. In liver, prostaglandins, primarily PGE_2, might be part of a feedback inhibition loop that limits the glucagon-stimulated activation of hepatic glycogenolysis (Fig 2–3): glucagon, reaching the liver via the portal vein, can increase cAMP formation and hence glycogen phosphorylase activity in hepato-

cytes. At the same time it can increase the formation of PGE_2 (and other prostaglandins) in Kupffer cells and possibly also other nonparenchymal liver cells. PGE_2 in turn can partially inhibit the glucagon-stimulated cAMP-formation in hepatocytes via a PGE_2-receptor of the EP_3-subtype linked to the adenylate cyclase via a pertussis toxin–sensitive G_i-protein and thereby limit the glucagon-mediated glycogenolysis in hepatocytes.

Cooperation Between Nonparenchymal and Parenchymal Cells

Cooperation of endothelial cells and Kupffer cells with hepatocytes. The interaction of nonparenchymal cells and parenchymal cells is not restricted to the modulation of functions by intercellular communication. In addition, metabolism is coordinated by a regulated cooperation between nonparenchymal and parenchymal cells. Thus, sinusoidal endothelial cells and Kupffer cells are integrated in hepatic lipid metabolism. After injection into rat liver acetylated low-density lipoprotein (LDL) was taken up by sinusoidal endothelial cells via specific receptors. Their clearance capacity exceeded the capacity of hepatocytes.[109] The lipoprotein was degraded within the endothelial cells and fatty acids were delivered to the hepatocyte.[110] LDL can also be taken up and then degraded efficiently by Kupffer cells.[111] Sinusoidal endothelial cells also contribute to the regulation of liver lipid metabolism by the modulation of the size of their fenestrae: pantetheine and alcohol at low doses increase and nicotine and serotonin narrow the fenestrae; alcohol at high doses causes a defenestration.[112] By this means, the accessibility to the space of Disse for larger lipoprotein particles such as chylomicron remnants, and the ensuing delivery of cholesterol and fatty acids to the hepatocyte, can be influenced.[112]

Cooperation between Ito cells and hepatocytes. Vitamin A at high concentrations is toxic. Vitamin A is transported to the liver as retinol esters in chylomicron remnants. Retinol esters are taken up by hepatocytes and retinol is transferred to Ito cells either free or bound to retinol-binding protein.[15] Ito cells have a large capacity to store vitamin A in fat droplets and they probably deliver vitamin A according to the metabolic needs of the body.

TOPOLOGICAL ORGANIZATION OF DEFENSE AND ORGAN STRUCTURE REGULATION IN THE ACINUS

Cell-to-Cell Communication in the Acute-Phase Reaction

The acute-phase reaction is a defense response of the organism against bacterial and viral infections, tissue injury, neoplastic growth, and disorders of the immune system.[113–115] It consists of a local reaction at the site of disturbance of homeostasis, comprising invasion and activation of granulocytes and monocytes and platelet aggregation. In addition, it involves blood vessel dilation and leakage, and a systemic reaction that includes, for example, fever, leukocytosis, increased erythrocyte sedimentation rate, complement activation, and dramatic alterations in the concentration of some plasma proteins. These proteins are formed in the liver and are called positive acute-phase proteins if their concentrations increase, and negative acute-phase proteins if they decrease. Locally at the site of injury

bacterial endotoxins (lipopolysaccharides) or viruses cause the formation of *acute-phase cytokines* such as interleukin-6 (IL-6), IL-1 and tumor necrosis factor α (TNFα) in monocytes and endothelial cells, which reach the liver and the pituitary via the circulation. In the liver these circulating cytokines, mainly IL-6, activate or inhibit the synthesis and release of acute-phase proteins in hepatocytes. In addition, the circulating cytokines, primarily IL-1 and TNFα, as well as endotoxins and viruses stimulate the further synthesis of acute-phase cytokines mainly by Kupffer cells, which represent the bulk of the macrophage pool, and by recruited monocytes. Thus, the signal to the hepatocytes is locally amplified.[113] In addition, the circulating IL-6 stimulated the formation of adrenocorticotropic hormone (ACTH) in the pituitary and thus of glucocorticoids in the adrenals. Glucocorticoids feedback inhibit cytokine synthesis in monocytes and macrophages[113] (Fig 2–4). The cytokine receptors involved in the regulation of acute phase protein synthesis such as the IL-6 receptor (R), IL-1R, and TNFαR have been cloned from various nonhepatic sources but not from hepatocytes.

Cell-to-Cell Communication in Biomatrix Formation and Fibrogenesis

The biomatrix of the liver in the space of Disse is made up of proteins (collagens I, II, IV, V, VI, elastin), glycoconjugates (glycoproteins, eg, fibronectin, laminin, entactin = nidogen, tenascin; and proteoglycans, eg, heparan sulfate, chondroitin

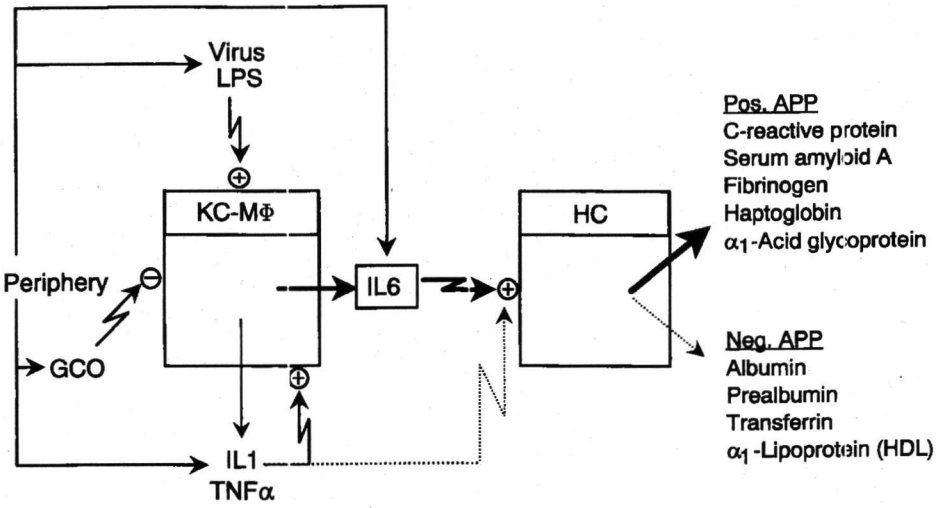

Figure 2–4. Regulation of acute-phase proteins in liver (simplified model). Locally at the site of insult endotoxin or viruses elicit the formation of the cytokines IL-6, IL-1, and TNFα mainly in monocytes. The cytokines as well as endotoxin and viruses reach the liver and there stimulate the further production of IL-6, IL-1, and TNFα in Kupffer cells and recruited monocytes. Thus cytokine synthesis is amplified in the liver. IL-6 is the main signal for the increase in the synthesis of positive and the decrease in the synthesis of negative acute phase proteins.[113–115] HC, hepatocytes; KC, Kupffer cells; MΦ, macrophages; APP, acute phase proteins; GCO, glucocorticoids; IL-1, interleukin-1; IL-6, interleukin-6 (= hepatocyte-stimulating factor, B-cell stimulatory factor-2, interferon-β_2); LPS, lipopolysaccharide (= endotoxin); TNFα, tumor necrosis factor α. +, activation; −, inhibition. Straight arrows (bold or dotted): normal (increased or decreased) production. Lightening arrows (bold or dotted): normal (strong or weak) modulation.

sulfate, dermatan sulfate), and glycosaminoglycans (hyaluronan)[21, 116, 117] (Fig 2–5). Ito cells synthesize all biomatrix components. Current evidence suggests that hepatocytes contribute only very little to collagen formation and proteoglycan, glycoprotein, and glycosaminoglycan synthesis. Kupffer cells and endothelial cells

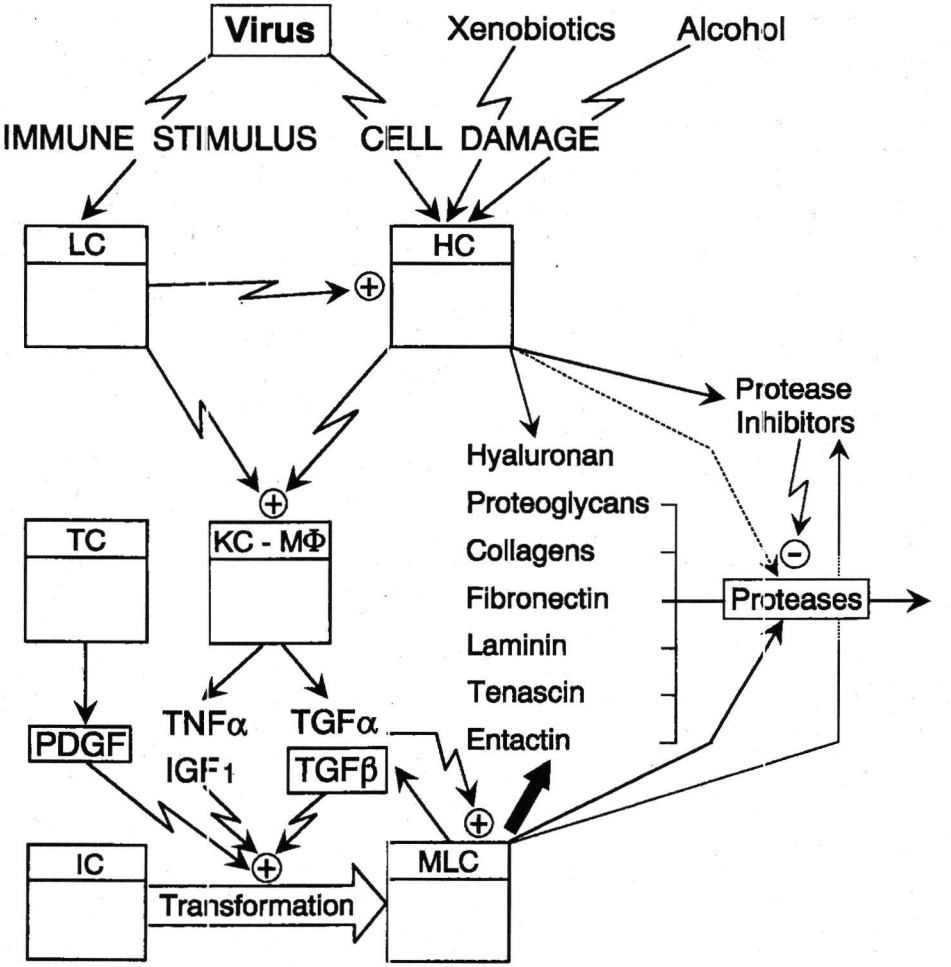

Figure 2–5. Pathogenesis of liver fibrogenesis (simplified hypothesis). The initiating event of fibrogenesis is hepatocyte damage caused by various insults. The necrotic hepatocyte activates Kupffer cells to release *fibrogenic cytokines,* mainly TGFβ. Thrombocytes secrete *proliferative cytokines,* especially PDGF. TGFβ and PDGF together with other cytokines *initiate* via paracrine loops the transformation of Ito cells to myofibroblast-like cells. These produce the various biomatrix components and the proteases that degrade them; they also form cytokines such as TGFβ, which via autocrine loops *perpetuate* fibrogenesis; the process is aggravated by the formation of inhibitors that reduce the breakdown of biomatrix components.[21, 116, 117] HC, hepatocytes; IC, Ito cells; KC, Kupffer cells; LC, lymphocytes; MΦ, macrophages; MLC, myofibroblast-like cells; IGF1, insulin-like growth factor 1; PDGF, platelet-derived growth factor; TC, thrombocytes; TGFα, transforming growth factor α; TGFβ, transforming growth factor β; TNFα, tumor necrosis factor. +, activation; −, inhibition. Straight arrows (bold or dotted): normal (increased or decreased) production. Lightening arrows (bold or dotted): normal (strong or weak) modulation.

seem to produce only quantitatively less important amounts of certain biomatrix constituents.[21, 116, 117] The biomatrix components are subject to a continuous turnover; they are degraded in part after endocytosis by lysosomal enzymes and in part extracellularly by metalloproteinases (eg, collagenase, elastase, gelatinase, stromelysin), which in turn are under the control of tissue inhibitors of metalloproteinases (TIMP). Endothelial cells are mainly responsible for the endocytotic uptake.[21] Ito cells seem to be not only the major producers of biomatrix components but also of tissue metalloproteinases, whereas hepatocytes and Ito cells synthesize tissue inhibitors of metalloproteinases.[118, 119]

During fibrogenesis the amounts of the various types of proteins, glycoconjugates, and glycosaminoglycans increase disproportionately by threefold to sixfold, leading to a greatly changed pattern.[21, 116, 117] The initiating event of fibrogenesis is hepatocyte damage (Fig 2–5). The necrotic hepatocyte together with immune stimuli activates Kupffer cells, the "*effector cells of fibrogenesis,*" to secrete *fibrogenic cytokines* mainly transforming growth factor β (TGFβ) but also TGFα, TNFα, and insulin-like growth factor 1 (IGF1). Moreover, thrombocytes play a role by the local production of the *proliferative cytokines* platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). TGFβ and PDGF perhaps together with other cytokines initiate the transformation of Ito cells to myofibroblast-like cells ("activated" Ito cells), the "*producer cells of fibrogenesis,*" which then increase the production of the various biomatrix components and also synthesize cytokines such as TGFβ and TGFα.[21, 116, 117] Ito cell proliferation and dedifferentiation is under negative control of factors produced by intact hepatocytes and under positive control of factors derived from necrotic hepatocytes.[120] Ito cells, on activation to myofibroblast-like cells, show an increased expression of PDGF and TGFβ receptors. The cytokine receptors involved in fibrogenesis such as the receptors for TGFβ, TGFα, IGF1, PDGF, and EGF have been cloned from various nonhepatic sources but not yet from Ito cells. Thus, besides the paracrine loops acting on Ito cells from Kupffer cells, hepatocytes, myofibroblast-like cells, and thrombocytes, autocrine loops at myofibroblast-like cells are established. The paracrine loops initiate and accelerate and the autocrine loops probably perpetuate fibrogenesis[21, 116, 117] (Fig 2–5). Simultaneously the cytokines may enhance the formation of metalloproteinase inhibitors, which would further aggravate fibrogenesis.[115, 118]

This highly simplified scheme of fibrogenesis (Fig 2–5)[21, 114, 116, 117, 121] illustrates a most important example of intercellular communication and cooperation in liver.

SUMMARY AND PERSPECTIVES

The liver is composed of parenchymal cells, the hepatocytes, and an array of nonparenchymal cells, the endothelial cells, Kupffer cells, Ito cells, and pit cells. The organ is supplied with blood by the hepatic artery and portal vein. In addition to their function as routes for substrate and oxygen supply the two vessels function as separate sites of signal input. Along the functional unit of the liver, the acinus, a substrate, signal, and biomatrix gradient develops that is reflected in a morphological and functional heterogeneity of parenchymal and nonparenchymal cells in the

periportal and perivenous zones. The regulation of liver metabolism, the control of defense reactions such as acute-phase protein synthesis and biomatrix formation depend on an intraorgan cell-to-cell communication between the different nonparenchymal cells, the parenchymal cells, and between nonparenchymal and parenchymal cells. This cell-to-cell communication is mediated mainly by prostaglandins and thromboxanes as well as cytokines such as interleukins, TNF, or TGF, which are formed in the nonparenchymal cells and act in autocrine and paracrine loops. Communication between parenchymal cells is effected by gap junctions.

Future research in cell-free *in vitro* systems and cell culture will have to provide additional molecular concepts and molecular tools. Then the physiological significance of the complex intrahepatic cell-to-cell communications and cellular heterogeneity can be studied in whole organ perfusion systems or *in vivo* to improve the understanding of the regulation of normal liver metabolism and the molecular basis for development of liver disease.

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