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ZONATION OF PARENCHYMAL AND NONPARENCHYMAL METABOLISM IN LIVER

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ABSTRACT

The enormous number of different liver functions are carried out by parenchymal and four main types of nonparenchymal cells, either alone or in cooperation. Although the liver tissue is uniform on the level of histology, it is heterogenous on the level of morphometry and histochemistry. This heterogeneity is related to the blood supply; cells located in the upstream or periportal zone differ from those in the downstream or perivenous zone in their equipment with key enzymes, translocators, receptors, and subcellular structures and therefore have different functional capacities. This is the basis of the model of metabolic zonation, according to which glucose release from glycogen and via gluconeogenesis, amino acid utilization and ammonia detoxification, protective metabolism, bile formation, and the synthesis of certain plasma proteins such as albumin and fibrinogen occur mainly in the periportal area, whereas glucose utilization, xenobiotic metabolism, and the formation of other plasma proteins such as α_1-antitrypsin or α-fetoprotein occur predominantly in the perivenous zone. The mor- phologic and functional heterogeneity is the result of zonal differences in the activation of the cellular genome caused by gradients in oxygen, substrate, hormone, and mediator levels, in innervation, as well as in cell-to-cell and cell-to-biomatrix interactions.

CONTENTS

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INTRODUCTION

In most western societies, a normal meal contains three classes of macronutrients—carbohydrates, fat, and protein (approximately 45, 40, and 15 energy%, respectively)—and three classes of micronutrients—electrolytes, trace elements, and vitamins. In addition, ethanol can become a major macronutrient, and xenobiotics, e.g. drugs or environmental poisons, can constitute important micronutrients. The hydrophilic nutrients such as glucose and amino acids, as well as electrolytes, water-soluble vitamins, and many drugs, enter the circulation by absorption from the intestine into the mesenteric veins that drain into the portal vein and, after passage through the liver, into the inferior vena cava. The lipophilic nutrients such as fatty acids and lipid-soluble vitamins are secreted into the intestinal lymph as components of chylomicrons and then enter the circulation at the left jugular vein, which, in turn, drains into the superior vena cava. Thus, the liver—supplied via the portal vein (about 80% of flow) and the hepatic artery (about 20% of flow)—holds an extraordinary, strategic position for the handling of the hydrophilic nutrients, placed as it is between the intestine, which absorbs nutrients, and all the other organs, which utilize nutrients. For the handling of lipophilic nutrients, the position of the liver is more "ordinary"; it is placed into the circulation in parallel with all other organs.

All organisms need energy: there is a permanent need to maintain structure and basal functions (basal metabolic rate), and a transient need to perform specific work functions (activity energy costs) (30). In principle, energy can be derived from the oxidation of glucose, fatty acids, or amino acids. However, the brain, the erythrocytes, and the working muscles depend on glucose, and therefore, the provision of glucose in all metabolic states is the leitmotif of animal metabolism. Most animals, however, are intermittent eaters, shifting between absorptive and postabsorptive states and, in addition, between rest and physical activity. When taken up in excess of the actual demand after a meal, glucose, fatty acids, and amino acids are used for the syntheses of energy stores such as glycogen (mainly in liver and muscles), triglycerides (in adipose tissue), and protein (mainly in muscles but also in liver), respectively. These processes are predominantly stimulated by insulin and by a higher activity of the parasympathetic system. When the exogenous energy supply has ceased or is too low, utilization of these stores is predominantly stimulated by glu-

Table 1 Functions of the liver

Service functions for nonhepatic organs	
Effector organ	
Center of metabolism	*Center of defense*
Energy supply	Xenobiotic metabolism
Glucose uptake and release	Oxygenation, reduction
Amino acid uptake and release	Conjugation
Urea formation	
Lipid processing	Phagocytosis
Ketone body synthesis	Elimination of tumor cells
Biosynthesis and biodegradation	Acute phase reaction
Plasma protein synthesis and degradation	
Bile formation	
Control station of the hormonal system	*Blood reservoir*
Inactivation of hormones and mediators	Active and passive blood storage
Synthesis and release of (pro)hormones and mediators	
Sensor organ	
Portal sensing	
Energy substrates	Osmolarity
Glucose	NaCl and water
Amino acids	Blood pressure

Formation and maintenance of organ structure	
Synthesis and degradation of cellular and extracellular (biomatrix) components	*Protective metabolism*
Biomembrane components	
Cytosolic components	Scavenging of reactive O_2 intermediates
Nuclear components	Scavenging of electrophilic intermediates
Cytoskeletal components	
Biomatrix components	

cagon during rest and, in addition, during exercise by adrenaline and noradrenaline and by a higher activity of the sympathetic system (30).

Because of its extraordinary location in the circulatory system, the liver functions both as an effector and a sensor organ (Table 1). It is a center of metabolism, maintaining the organism's energy supply in all metabolic states; a center of defense, preparing xenobiotics for elimination and destruction of foreign macromolecules; a control station of the hormonal system, inactivating hormones and releasing (pro)-hormones; and a blood reservoir. The liver senses and reports the state of hydrophilic nutrient supply via vagal and splanchnic afferents to the central nervous system, thus contributing to the

control of food intake. In addition, it catalyzes all processes required for the formation and maintenance of its own cellular and extracellular structures.

The enormous number of metabolic activities are carried out by parenchymal and four main types of nonparenchymal cells, either alone or in cooperation. Although the liver tissue has a uniform histologic appearance, it is heterogenous at the level of morphometry and histochemistry (32, 58) (Figure 1). This heterogeneity is linked to the position of a cell within the functional unit of the tissue, which, in turn, is related to the blood supply: Cells located in the upstream zone differ from those in the downstream zone with respect to subcellular structures, key enzymes, translocators, and receptors and, therefore, have different metabolic capacities (29, 31–34) (cf Table 2). This is the basis for "metabolic zonation," a concept first proposed for carbohydrate metabolism (33, 36) and later expanded to include amino acid and ammonia metabolism, xenobiotic metabolism, protective metabolism, and formation of plasma proteins. The zonal heterogeneity of liver tissue has been reviewed previously, both in general (15, 21, 31, 32, 52) and with focus on specialized areas of metabolism (4, 12, 25, 34, 53, 61, 63). This article presents an overview on the zonation of parenchymal and nonparenchymal metabolism and gene expression in liver, with special consideration for nutritional aspects.

FUNCTIONAL ORGANIZATION OF LIVER TISSUE

The Liver Acinus: a Microcirculatory Functional Unit

THE ACINUS AND ITS ZONES The structural and functional liver unit has been proposed to be the classic lobule, the portal unit and sickle zone concept, and the acinus (58). A generally accepted view has not been reached. The acinus is the smallest functional unit of the liver. It is based on the blood supply, thus representing a microcirculatory unit. It extends from a terminal portal venule and a terminal hepatic arteriole, which deliver their blood into the sinusoids, to the central vein, which delivers the blood to the hepatic vein. The upstream region around the terminal portal vein and arteriole is called the periportal zone; the area around the central vein is known as the perivenous, pericentral, or centrilobular zone (Figure 1). In the original model of metabolic zonation, only these two zones of about equal size were differentiated, because enzyme activities or contents were high in the first half and low in the second half of the acinus, or vice versa. Later, it was learned that some key enzymes are expressed within only the first or last quarter of the acinus. Therefore, the periportal and the perivenous compartments now have to be further subdivided, into a proximal and a distal part each (29).

CELL TYPES The hepatocytes are polarized parenchymal cells that form a trabecular network within the acinus. They face the sinusoids with their baso-

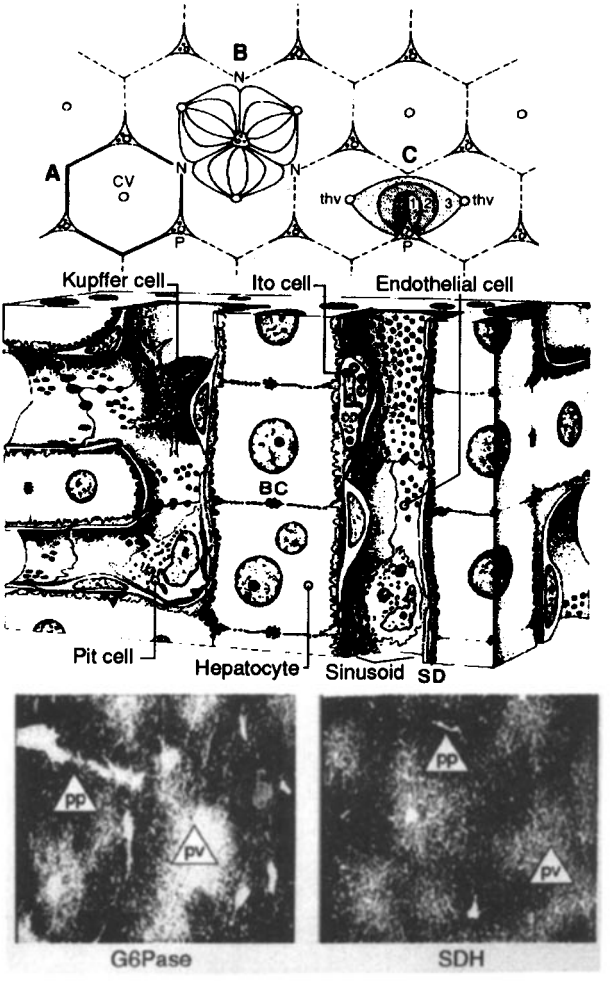

Figure 1 Structural and functional liver unit and liver tissue. (*Top*) *A,* Classic lobule; *B,* portal unit and sickle zone; *C,* liver acinus. *1,* Periportal (afferent) zone in which concentrations of O_2, hormones, and substrates are high; *2,* intermediate zone; *3,* perivenous (pericentral, centrilobular, efferent) zone in which concentrations of O_2 hormones and substrates are low. CV, Central vein, corresponding to terminal hepatic veins (thv); P, portal field; N, nodal point. (*Middle*) The parenchyma consists of trabeculae of adjoining hepatocytes. Blood flows through the sinusoids formed by fenestrated endothelial cells, which are surrounded by perisinusoidal (Ito or fat storing) cells. Kupffer cells and pit cells bulge into the sinusoidal lumen from the endothelial cells. The extracellular matrix is located in the space between the endothelial cells and the hepatocytes, the space of Disse (SD). Adjacent hepatocytes form the bile canaliculi (BC) with their apical membrane. (From Reference 58.) (*Bottom*) Distribution of succinate dehydrogenase (SDH) and glucose-6-phosphatase (G6Pase) visualized by histochemical techniques. Dark precipitates, high activity; pp, periportal; pv, perivenous.

Table 2 Zonation of function in the parenchymal and nonparenchymal cells of the liver: predominant localization of capacities for metabolic pathways based on the distribution of enzymes, translocators, cellular structures, or cell densities

Parenchymal cells					
Periportal zone			Perivenous zone		
Physiologic function	Metabolic function	Enzyme or protein involved	Physiologic function	Metabolic function	Enzyme or protein involved
Oxidative energy metabolism		Succinate dehydrogenase			
Glucose output	1. Glucosefrom pyruvate (gluconeogenesis)		Glucose uptake	1. Glucose to pyruvate (glycolysis)	
	2. Glucose from glycogen	Glucose-6-phosphatase		2. Glycogen from glucose	Glucokinase
	3. Glycogen from pyruvate	Phosphoenolpyruvate carboxykinase		3. Glycogen to pyruvate	Pyruvate kinase type L
Urea formation	Urea from amino acid nitrogen and from NH_3	Carbamoyl phosphate synthetase	Glutamine formation	Glutamine from NH_3	Glutamine synthetase[a]
Protective metabolism	1. Glutathione peroxidation	Glutathione peroxidase	Xenobiotic metabolism	1. Monooxygenation	Cytochrome P450
	2. Glutathione conjugation	(Glutathione level)		2. Glucuronidation	UDP-glucuronosyl transferase
Plasma protein synthesis		Albumin α2-Macroglobulin Fibrinogen	Plasma protein synthesis		a-Fetoprotein Angiotensinogen α1-Antitrypsin
Cholesterol synthesis	Hydroxymethyl glutaryl-CoA reductase[b]				
Bile formation	Taurocholate uptake carrier Bile acid export carrier				

Table 2 (*continued*)

	Nonparenchymal cells				
	Periportal zone		Perivenous zone		
Cell type	Function	Property	Cell type	Function	Property
Endothelial cells		Fenestrae larger & low in number	Endothelial cells	Filtering (porosity)	Fenestrae smaller & numerous
	Lectin binding	Wheat germ agglutinin binding			
Kupffer cells (resident macrophages)	Phagocytosis	Cells more numerous and larger	Kupffer cells (resident macrophages)	Cytotoxicity	Galactosyl receptors
		Lysosomal enzymes			
Ito cells (perisinusoidal cells)	1. Matrix formation	Cells more numerous			
	2. Vitamin A storage	Lipid droplets			
Pit cells (large granular lymphocytes)	Tumor cell killing	Cells more numerous			

[a] Present only in the distal perivenous zone.
[b] Present only in the proximal periportal zone.

lateral surface and form the bile canaliculi with their apical membrane (Figure 1). There are four major types of nonparenchymal cells. (*a*) Fenestrated endothelial cells lacking a basement membrane form the wall of the sinusoids. The fenestrae, 100 to 200 nm in diameter, permit free access for macromolecules to the hepatocyte surface but retain cellular components and large macromolecular aggregates such as chylomicrons within the sinusoidal lumen (4). The small space between the endothelial cells and the hepatocytes is known as the space of Disse. (*b*) Kupffer cells are resident liver macrophages attached to the sinusoidal wall on the luminal surface, especially at branching points. They are variable in shape and possess protrusions reaching the space of Disse (4, 58). (*c*) Perisinusoidal cells, also called Ito cells, lipocytes, or fat-storing cells, encircle the sinusoidal wall with long processes from the space of Disse. (*d*) Large granular lymphocytes, called pit cells, are loosely attached to the luminal surface of the sinusoids, to Kupffer cells, or to endothelial cells. They exhibit natural killer activity (4, 5) (Figure 1).

CELL-TO-CELL CONTACTS *Hepatocyte-hepatocyte* The blood and bile compartment of neighboring hepatocytes are separated by tight junctions (24). Gap junctions seem to play a role in signal propagation from one hepatocyte to the next (see below).

Endothelial cell-endothelial cell The sinusoidal liver endothelia form only loose cell contacts without tight junctions, in contrast to other capillary endothelia (4, 58).

Cell proximities in the space of Disse The contact of Kupffer cell protrusions with hepatocytes, as well as the proximity of perisinusoidal cells to hepatocytes and nerve terminals and the proximity of perivenous hepatocytes to the endothelium of the central vein, allow the buildup of high concentrations of mediators required for intercellular signaling in the liver (51) (Figure 1).

BIOMATRIX The extracellular matrix is highly organized and is composed of proteins (collagen types I, III, IV, and V and 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) (19). The biosynthesis and degradation, and thus the exact composition of the biomatrix, are strictly regulated by an intricate interplay between the different liver cell types (19).

Gradients of Signal Input and Transmission Along the Sinusoid

The functions of parenchymal and nonparenchymal cells are controlled by circulating substrates and products, hormones, nerves, and intrahepatically formed mediators, as well as the biomatrix. With the exception of substrates and products, the signals are transmitted via receptors. Because of liver meta-

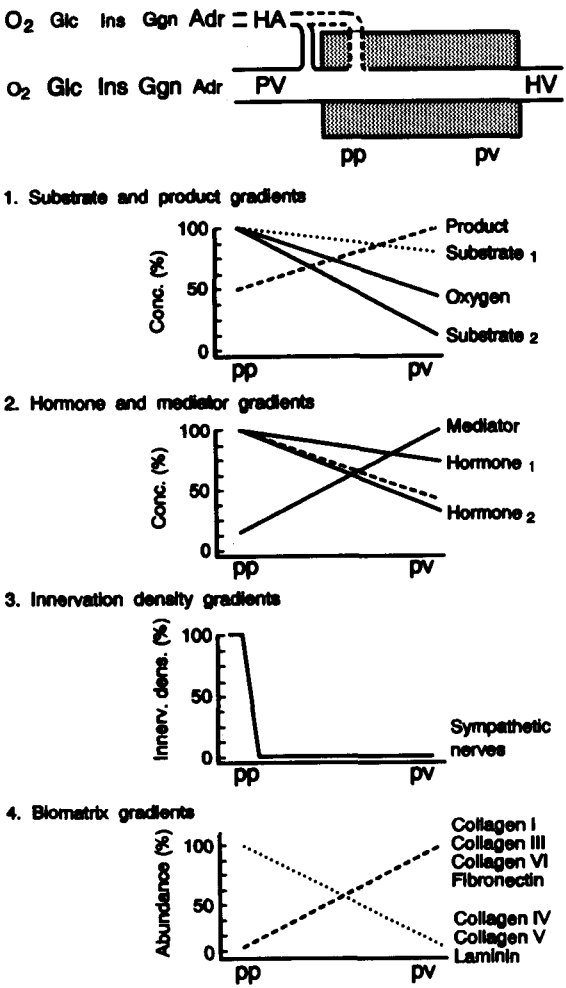

Figure 2 Gradients of signal input. During the passage of blood through the sinusoids, concentration gradients of O_2, hormones, and substrates are established, which in cooperation with the innervation density gradients (very steep in rats and mice) and biomatrix gradients (schematic illustration; the gradients have not yet been quantified) play a major role in the establishment and maintenance of the zonation of parenchymal and nonparenchymal metabolism. Adr, Adrenalin; Glc, glucose; Ggn, glucagon; Ins, insulin; HA, hepatic artery; HV, hepatic vein; PV, portal vein; pp, periportal; pv, perivenous.

bolism, the composition of blood is changed during the passage through the sinusoids. Concentration gradients of, e.g., substrates and hormones are established. Thus, the periportal and perivenous cells receive different patterns of signals.

OXYGEN, SUBSTRATES, AND PRODUCTS The oxygen tension in the periportal zone is about 65 mm Hg and falls to about 35 mm Hg in the perivenous zone. The concentration of ammonia and bile acids falls by about 85%, and that of ketone bodies rises twofold. The concentration gradients of most carbon substrates, such as glucose or amino acids, are rather shallow (32) (Figure 2).

HORMONES AND NERVES During passage of blood through the liver, about 50% each of glucagon, noradrenaline, and cortisol and about 80% of adrenaline is degraded. Passage through the liver causes a 50% drop in insulin between meals but only a 15% decrease after meals. Thus, the ratio of insulin versus its antagonists glucagon plus catecholamines increases from the periportal to the perivenous zone, especially after a meal. Moreover, deiodenation of thyroxin (T_4) decreases T_4 about 40%, whereas triiodothyronine (T_3) increases about 50% (32) (Figure 2). Nerves enter the liver as a plexus of sympathetic and parasympathetic fibers around the hepatic artery and the portal vein. The extent of the intra-acinar innervation varies from species to species. In rat and mouse, only a few sympathetic nerves terminate in the periportal zone and do so at parenchymal and nonparenchymal, mainly Ito, cells. The nerve signals appear to be propagated via gap junctions between hepatocytes (14, 28, 60) (Figure 2).

BIOMATRIX The composition of the biomatrix changes from the periportal zone, where collagen types IV and V and the major adhesion protein laminin dominate, to the perivenous zone, where fibrillar collagen types I, III, and VI and the adhesion protein fibronectin dominate (54) (Figure 2).

RECEPTORS The zonation of the ectocellular receptors of insulin, glucagon, and catecholamines is not yet known. Glucagon-activated adenylate cyclase and cGMP-activated cAMP phosphodiesterase are not zonated (55, 69). The glucocorticoid receptor is distributed homogeneously (1), but distribution of the thyroid receptors is unknown. The small 21-kDa gap junction protein, connexin 26, is localized in the periportal area; the 26-kDa connexin 32 is equally distributed (47, 64).

ZONATION OF PARENCHYMAL CELL FUNCTIONS

Zonation of Metabolism

Different experimental approaches, such as periportal- and perivenous-like hepatocyte cultures, hepatocytes prepared with the digitonin-collagenase

method (enriched in periportal and perivenous cells), ortho- and retrograde liver perfusions with unlabeled or labeled substrates, surface microlight guides, and miniature oxygen electrodes (32), have been used to study the zonal differences in the metabolism of carbohydrates (34), fatty acids (53), amino acids and ammonia (25), and xenobiotics (61), as well as in bile formation (20). All these studies with their different approaches support the metabolic specialization of hepatocytes.

CARBOHYDRATE METABOLISM In humans with sedentary occupations, the average intake of glucose is 100 g per meal and is absorbed within 2.5 h: 16 g/h are taken up by the liver, of which about 10 g/h are used for glycogen synthesis, 6 g/h are degraded to pyruvate, and 5.5 g/h are consumed by the intestine, pancreas, and spleen. The insulin-dependent tissues, skeletal and heart muscle, as well as adipose tissue, eliminate glucose at a rate of 11 g/h, mainly via glycogen synthesis in skeletal muscle. The "insulin-independent" organs, the central nervous system (CNS) and erythrocytes, use glucose at 4.5 and 1.5 g/h, respectively, irrespective of the metabolic situation. The remaining 4 g of glucose causes the transient increase in circulating glucose concentration in the extracellular space (30). In the postabsorptive state at rest, the liver meets the glucose requirements of 7.5 g/h for the CNS, erythrocytes, and some other organs by liberating 4.5 g/h via glycogenolysis and 3 g/h via gluconeogenesis from lactate, amino acids, and glycerol; during exercise, the liver can increase its glucose release to 30 g/h or higher, with 25 g/h produced by glycogenolysis and 5 g/h produced by gluconeogenesis, in order to meet the increased glucose requirements of working muscles in addition to the existing requirements of the CNS and erythrocytes (30).

Studies with various rat liver preparations and different techniques have led to the following concept: During the absorptive phase, glucose is taken up mainly by the perivenous cells. It is used here to synthesize glycogen, and then, with increasing replenishment of the glycogen stores, it is degraded to lactate. Lactate is transported via circulation to the periportal cells, where it is converted by gluconeogenesis to glycogen. In the postabsorptive phase, glycogen first is degraded to glucose in the periportal hepatocytes; later it is degraded to lactate, mainly, in the perivenous cells. Lactate is released into the circulation and when it reaches the periportal cells is used as a substrate for gluconeogenesis (Figure 3, Table 2). This clearly supports the observation that glycogen degradation starts periportally and ends perivenously and that, accordingly, glycogen stores are refilled starting in the perivenous and ending in the periportal zone (57). It also explains the so-called glucose paradox (35), i.e. the finding that in liver, in contrast to muscle, pyruvate via the indirect pathway rather than glucose via the direct pathway serves as the major precursor for the synthesis of glycogen (34). Gluconeogenesis is an endergonic

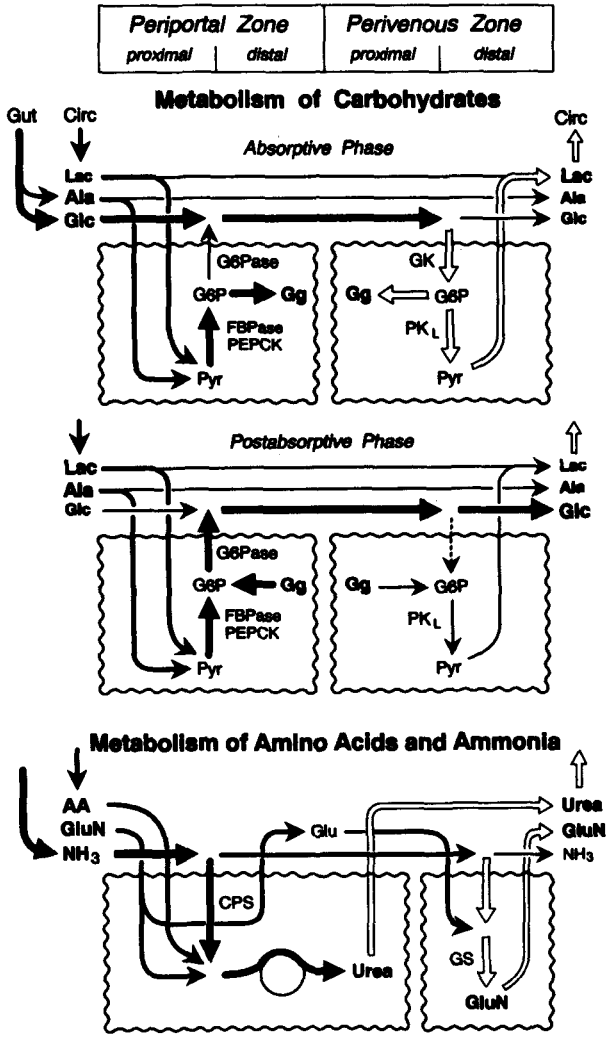

Figure 3 Zonation of carbohydrate, amino acid, and ammonia metabolism. Metabolites: AA, amino acids; Ala, alanine; G6P, glucose-6-phosphate; Gg, glycogen; Glc, glucose; Glu, glutamate; GluN, glutamine; Lac, lactate; Pyr, pyruvate. Enzymes are indicated only in the zones where they are predominantly expressed: CPS, carbamoyl phosphate synthetase; FBPase, fructose-1,6-bis-phosphatase; G6Pase, glucose-6-phosphatase; GK, glucokinase; GS, glutamine synthetase; PEPCK, phosphoenolpyruvate carboxykinase; PK_L, pyruvate kinase type L; Circ, circulation.

process (2 lactate$^-$ + 2 H^+ = glucose; $\Delta G'_o$ + 47.2 kcal/mol of glucose); it has to be linked to oxidative energy metabolism and should therefore be located in the more aerobic periportal zone with a higher capacity for oxidative energy production (Table 2). Glycolysis alone and glycolysis plus liponeogenesis are exergonic processes (glucose = 2 lactate$^-$ + 2 H^+; $\Delta G'_o$ = –47.2 kcal/mol; 4 glucose = palmitate$^-$ + H^+ + 8 CO_2 + 6 H_2O + 2 H_2; $\Delta G'_o$ = –71.1 kcal/mol of glucose); the formation of lactate or the synthesis of fatty acids from glucose in principle do not have to be coupled to oxidative energy metabolism and could therefore be located in the less aerobic perivenous zone, which has a lower capacity for oxidative energy metabolism (32, 33).

FATTY ACID AND ETHANOL METABOLISM During absorption, dietary fat, about 37 g per average meal, is mainly used to refill the triglyceride stores in adipose tissue. The chylomicron triglycerides are hydrolyzed by endothelia-anchored lipoprotein lipases, mainly in skeletal muscle and adipose tissue. The liberated fatty acids are taken up by the adipose tissue and by the liver, where they are used for the resynthesis of triglycerides. In the adipose tissue, the triglycerides are stored; but in the liver they are exported in the form of very low-density lipoproteins (VLDL), or pre-β-lipoproteins. Ultimately, VLDL triglycerides are also stored in the adipose tissue. In addition, fatty acids can be formed de novo from glucose; in humans, this lipacidogenesis (liponeogenesis) appears to occur only with very carbohydrate-rich diets and mainly in the liver. Fatty acids are also formed from ethanol, which, in many western cultures, is consumed at a rate of 20–30 g daily (30). In the early postabsorptive state, fatty acids are liberated from the triglyceride stores in the adipose tissue at a rate of about 5 g/h; 1.5 g/h are taken up by the liver for energy production via β-oxidation. During a prolonged postabsorptive state (starvation), fatty acids are released at a slightly increased rate of 6.6 g/h; the additional 1.6 g/h are utilized by the liver to synthesize ketone bodies; oxidation of ketone bodies by the brain spares glucose. During exercise, fatty acid release can be increased severalfold, depending on the requirements of the working muscles; fatty acid utilization by the liver is not changed (30).

In the absorptive state in rat liver, VLDL synthesis appears to occur preferentially in the perivenous zone (22), whereas in the postabsorptive state, β-oxidation (23) and ketogenesis (62) seem to take place predominantly in the periportal zone. These findings are in line with expectations, but the zonation of fatty acid metabolism seems to be less pronounced than that described for carbohydrate metabolism. Ethanol degradation via acetaldehyde to acetate by alcohol dehydrogenase or the microsomal ethanol oxidizing system (MEOS) and acetaldehyde dehydrogenase appears to occur predominantly in the downstream area (56).

AMINO ACID AND AMMONIA METABOLISM After digestion and absorption, nutrient proteins reach the portal vein as amino acids. They are used mainly to refill the protein stores. There is no specific storage protein that can be converted to amino acids on demand, as glycogen can be degraded to glucose. It is generally accepted that not more than one third of the cellular proteins, mainly those in muscle and liver, can be used as amino acid stores. In humans, 400–500 g of amino acids become available per day as a result of protein turnover; an additional 70–80 g are taken up from the diet. Amino acids from body protein degradation and nutrient protein form a common pool from which 400–500 g/day are used for protein synthesis and 70–80 g/day are used for direct and indirect (via gluconeogenesis) energy production, finally yielding CO_2 and NH_3. NH_3 is detoxified in the liver by the formation of 24–28 g/day of urea; only a minor part, 1 g/day, is excreted into the urine (30).

Based mainly on studies with rat liver perfused in the ortho- and retrograde direction, the following scheme was developed (25, 26) (Figure 3, Table 2). The periportal and proximal perivenous cells convert the NH_3 released by amino acid utilization to urea. Any NH_3 that escapes ureagenesis in the periportal area is scavenged in the distal perivenous hepatocytes by conversion to glutamine. The resulting glutamine is released into the circulation and transported to the periportal cells, where transiently scavenged NH_3 is channeled into ureagenesis. Because ureagenesis in the upstream area is a high-capacity but low-affinity (high K_m) pathway, it is unable to lower the NH_3 level below 20 μM, generally believed to be the threshold for an unimpaired brain function. Effective detoxification of NH_3, thus, requires glutamine synthesis in the downstream zone; the glutamine synthesis must have a low K_m per NH_3. Moreover, ureagenesis (lowered in acidosis) and glutamine synthesis (increased in acidosis) play antagonistic roles in the regulation of the acid-base status of the organism (for a detailed discussion, see Reference 25).

XENOBIOTIC METABOLISM The cytochrome P450 system is responsible for the conversion of many xenobiotics into excretable products via monooxygenation followed by conjugation with either glucuronic or sulphuric acid. Studies with microlight guides in perfused rat liver have shown that monooxygenation occurs preferentially in the perivenous zone, and that glucuronide formation is the major conjugation reaction in perivenous cells, whereas sulfate formation predominates in periportal cells (32, 61).

PROTECTIVE METABOLISM The formation of toxic electrophiles in a P450 side reaction is characteristic for many xenobiotics, such as bromobenzene or carbon tetrachloride. This enhances the production of reactive oxygen intermediates (ROI) such as the superoxide radical anion, hydrogen peroxide, and hydroperoxides, mainly in the perivenous zone, where P450 isoenzymes pre-

dominate. However, the capacity for ROI detoxification by glutathione and reduction of hydroperoxides by glutathione peroxidase is higher in the periportal zone. Thus, the perivenous hepatocytes are much less protected against the toxic effects of electrophiles. This likely explains the long-known perivenous necrosis caused by some hepatotoxins (32, 61).

BILE FORMATION The periportal zone is the major site of bile formation; bile acids reabsorbed from the gut are taken up in this zone and then secreted into bile. The de novo synthesis of bile acids from cholesterol seems to be localized predominantly also in periportal cells (20).

Zonation of Gene Expression

Although all parenchymal cells have the same genome, zonal differences have been observed in the expression patterns of the genes for most key enzymes (Table 3). This zonation of gene activation in the liver acinus could be caused by heterogeneity in the input and transmission of signals such as substrates, including oxygen and products, hormones, mediators, and nerves, and in cell-to-cell or cell-to-biomatrix interactions. The zonal expression patterns may be due to different rates of transcription, mRNA degradation, mRNA translation, or protein degradation. Zonation of mRNA and protein in the same area would indicate that the expression of the gene is regulated mainly at the pretranslational level of mRNA formation or by mRNA degradation. Unequal zonal patterns of mRNA and protein, e.g. a homogeneously distributed mRNA and zonated protein, would imply that the expression is regulated mainly at the translational or even posttranslational level.

The expression of the genes for the rate-controlling enzymes of gluconeogenesis, phosphoenolpyruvate carboxykinase (PEPCK), and fructose-1,6-bisphosphatase (FBPase), as well as for the amino acid metabolizing enzymes serine dehydratase (SerDH) and tyrosine aminotransferase (TAT), which are related to gluconeogenesis, is regulated mainly at the pretranslational level. In situ hybridization studies in rat liver revealed that the mRNAs of PEPCK, FBPase, TAT, and SerDH were found predominantly in the periportal zone, as has been shown for the proteins and enzyme activities (Table 3, Figure 4). The PEPCK, TAT, and SerDH mRNA and enzyme levels were high at the end of the daily fasting period and after starvation, and low at the end of the daily eating period and following refeeding. Only FBPase mRNA and activity were independent of the daily feeding/fasting state. These nutrient-controlled alterations in gene expression during a normal feeding rhythm and during starvation/refeeding are termed dynamic zonations.

In contrast, the genes for the rate-controlling glycolytic enzymes, glucokinase (GK) and pyruvate kinase type L (PK_L), appear to be regulated mainly

Table 3 Zonation of key enzymes and their mRNA in rat liver[a]

Enzymes	Activity/ message	Periportal	Perivenous	Ref.
Carbohydrate metabolism				
Phosphoenolpyruvate carboxykinase	Enz	+++	+	32
	mRNA	++++	+	2
Fructose-1,6-bisphosphatase	Enz	+	+	32
	mRNA	+++	+	10
Glucokinase	Enz	+	++	32
	mRNA	+	+	9, 45
Pyruvate kinase L	Enz	+	++	32
	mRNA	+	+	43
Amino acid and ammonia metabolism				
Tyrosine aminotransferase	Enz	++	+	32
	mRNA	++	+	2
Serine dehydratase	Enz	+++	+	49
	mRNA	+++	+	48
Carbamoyl phosphate synthetase[b]	Enz	++	—	32
	mRNA	++	—	46
Glutamine synthetase[c]	Enz	—	+++	32
	mRNA	—	+++	16, 46
Lipid metabolism				
HMG-CoA reductase[c]	Enz	+++	—	32
	mRNA	++	—	4a
Xenobiotic metabolism				
Cytochrome P450	Enz	+	++	51
(1A1, 1A2, 2B1, 2B2, 2E1, 3A1)	mRNA	+	++	51

[a] For a detailed overview on the zonal distribution of enzymes and subcellular structures, see Table 2 in Reference 32 and Table 2 in Reference 51.

[b] Carbamoyl phosphate synthase is located in the periportal and proximal perivenous and glutamine synthase reciprocally only in the distal perivenous zone.

[c] HMG-CoA (hydroxymethylglutaryl-coenzyme A) reductase is situated in the proximal periportal zone only.

at a translational or posttranslational level during a normal feeding rhythm. The mRNAs of GK and PK_L were distributed homogeneously in rat liver parenchyma, whereas the proteins and enzyme activities showed a clear perivenous predominance (41, 65, 70) (Table 3). GK and PK_L zonation was dynamic, with increasing activities with feeding and decreasing activities with fasting. Yet, during refeeding after a long (60 h) period of starvation, GK mRNA were transiently localized mainly in the perivenous zone (9, 45), indicating that the regulation of zonal gene expression may shift from translational/posttranslational to pretranslational depending on the nutritional conditions.

The genes of the key enzymes of NH_3 detoxification also appear to be regulated mainly at the pretranslational level. The mRNA and the protein of

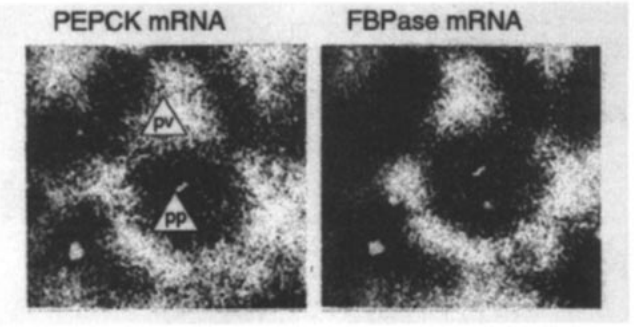

Figure 4 Zonation of phosphoenolpyruvate carboxykinase (PEPCK) and fructose-1,6-bisphosphatase (FBPase) mRNA in rat liver parenchyma. Parallel sections were prepared at 7 a.m. after 12 h of feeding. The mRNAs were localized by in situ hybridization using a ^{35}S-labeled antisense RNA (autoradiographic grains). pp, Periportal; pv, perivenous (for details, see Reference 10).

the key ureagenic enzyme, carbamoylphosphate synthetase (CPS), were localized in the periportal and proximal perivenous zone. The mRNA and the protein for glutamine synthetase (GS) were exclusively in parenchymal cells, so-called scavenger cells, of the distal perivenous area (Table 3). Interestingly, neither the mRNA nor the protein level of either enzyme varies with the nutritional state. This expression pattern is called stable zonation.

DYNAMIC ZONATION Dynamic zonation appears to be the result of varying inputs of humoral and neural signals into the acinus rather than the consequence of constant, stable cell-cell interactions between parenchymal and nonparenchymal cells or biomatrix. The periportal expression pattern of the PEPCK gene represents a typical example of dynamic zonation. When isolated, primary rat hepatocytes are heterogeneous; after 48 h in culture, the pattern of gluconeogenic and glycolytic enzymes was comparable to that of periportal cells in vivo, i.e. high PEPCK and low GK, if the cells were maintained in media containing glucagon. If the hepatocytes were maintained in media containing insulin, the pattern was like perivenous, i.e. high GK and low PEPCK. Thus, the ratio of the two pancreatic hormones may play a role in the zonal expression of glucose metabolizing enzymes (50) (Figure 5).

Cultures incubated under periportal O_2 tensions had higher levels of PEPCK and lower levels of GK compared to cells incubated under venous oxygen tensions (68). Therefore, the periportal-perivenous O_2 gradient could be a major determinant for this dynamic zonation. In primary rat hepatocytes, glucagon activates the PEPCK gene via cAMP; glucocorticoids are permissive. Insulin is an inhibitor (7). Glucagon stimulates transcription maximally after

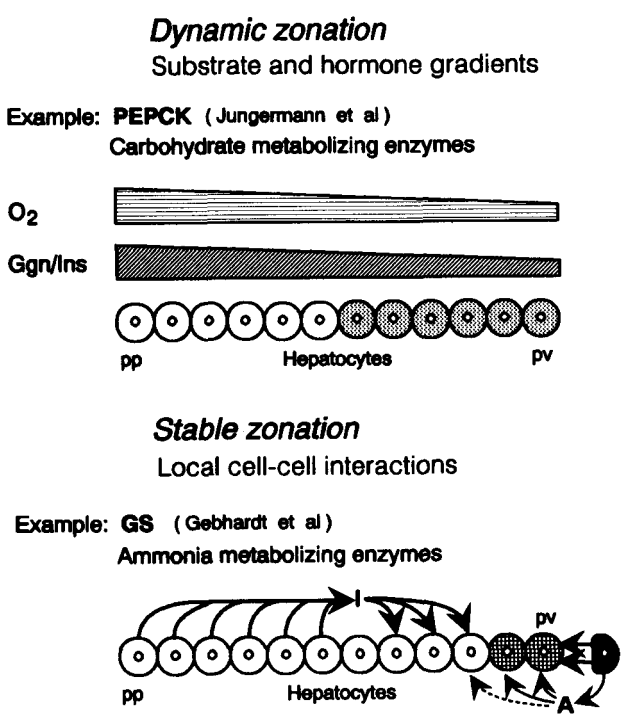

Figure 5 Major determinants for the zonation of gene expression. PEPCK, Phosphoenolpyruvate carboxykinase; GS, glutamine synthetase; Ggn, glucagon; Ins, insulin; A, activator; I, inhibitor.

30 min and enhances mRNA and enzyme activity maximally after 2 and 4–6 h, respectively. This glucagon-dependent induction of the transcription of the PEPCK gene and of PEPCK mRNA abundance and enzyme activity was higher under periportal than under perivenous O_2 tensions (27) (Figure 5).

The hepatocytes sense the glucagon/insulin gradients via the respective hormone receptors; the different oxygen tensions seem to be sensed via a nonrespiratory chain ferro-heme protein (39, 40). This sensor could act directly as a transcription factor in analogy to the steroid receptor or indirectly as a protein kinase or an oxidase producing H_2O_2, which in turn would modulate transcription factors by phosphorylation or oxidation, respectively (Figure 6). Evidence for the involvement of a ferro-heme protein functioning as an oxidase in the oxygen-sensing system comes from the finding that carbon monoxide (CO), like O_2, locks heme in the oxy conformation and, like O_2, counteracts the reduced induction of PEPCK mRNA and PEPCK activity at perivenous oxygen tensions (40). In addition, exogenously added H_2O_2 mimics the peri-

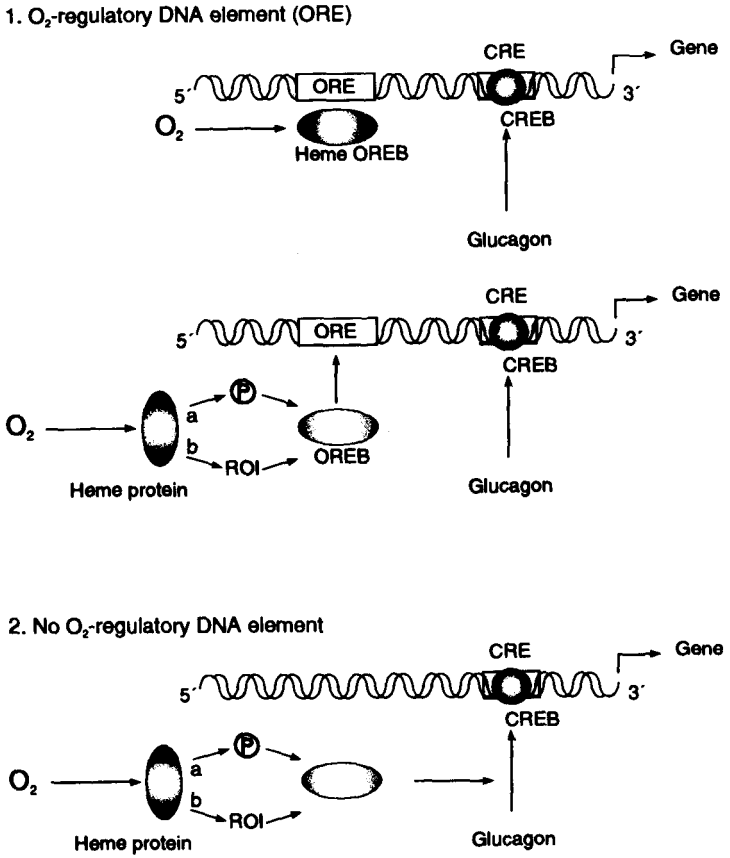

Figure 6 Possible mechanisms of O_2-sensing and O_2-regulated gene transcription in liver. Genes may contain oxygen-responsive elements (ORE), which could be regulated by an ORE binding protein (OREB). OREB could be an O_2-sensing transcription factor (Heme OREB) or it could be modulated by an O_2-sensing heme protein. The genes may also be devoid of an ORE; then components of the hormone signaling chains, e.g. the glucagon signaling chain, could be modulated by O_2. The proposed O_2-sensing heme protein, if not acting as an OREB, might be a protein kinase or an oxidase, which either phosphorylates (*a*) or oxidizes (*b*) an OREB via reactive oxygen intermediates (ROI), such as H_2O_2. The sensor could also induce a phosphorylation (*a*) or oxidation (*b*) cascade to modify components of a hormone signaling chain, e.g. the cAMP regulatory element binding protein (CREB) without the involvement of an ORE.

portal pO_2, counteracting the reduced induction at perivenous pO_2 (T Kietzmann, S Freimann, K Jungermann, unpublished data). The best-known O_2-regulated gene is the erythropoietin (EPO) gene in kidney. It appears to use a similar O_2-sensing mechanism, as its hypoxia-induced expression was repressed by CO (18) and H_2O_2 (11).

For transfection experiments, PEPCK promoter fragments serially deleted from the 5′ end into ends ranging from –2500 basepairs (bp) to +73 bp with respect to the start site of transcription were linked to the chloramphenicol acetyltransferase (CAT) gene. It was shown that modulation of glucagon-dependent induction by O_2 required the 5′-flanking sequence from –277 bp to +73 bp (38). In the EPO gene, the oxygen-sensitive region contained a highly conserved enhancer element and was localized 120 bp downstream of the poly(A) addition site in the 3′-flanking sequence (3, 13, 59). The importance of O_2 gradients for the zonation of gene expression is corroborated by the following findings: Livers of mice transgenic for the human EPO gene expressed EPO, if the animals were made anemic, but only in the perivenous zone (42); transgenic mice carrying a chimeric gene in which the PEPCK promoter (–460 to +73 bp) was linked to the gene for bovine growth hormone expressed bovine growth hormone in the periportal zone (44). In addition, two oxygen-responsive elements (ORE1 and ORE2) have been identified in the 5′-flanking region of the human glutathione peroxidase gene (8), which is expressed mainly periportally (37).

STABLE ZONATION Stable zonation appears to be caused by cell-to-cell or cell-to-biomatrix interactions. The distal perivenous expression pattern of the glutamine synthetase (GS) gene is the main example of a stable zonation. The GS gene could not be induced significantly in rat hepatocyte cultures by relevant substrates or hormones unless the hepatocytes were co-cultured with hepatic epithelial cells. GS was expressed in a small (10–20 cells) population of hepatocytes (inner circle) adjacent to the epithelial cells (outer circle) (17). Apparently, the epithelial cells produce a factor that, upon diffusion to the hepatocytes, triggers GS induction (Figure 5). Therefore, the vascular cells, i.e. mainly the endothelia of the central vein, may produce a factor that would reach the distal perivenous hepatocytes by diffusion against the direction of the blood stream, probably in the space of Disse (16). Consistent with this observation, hepatocytes transplanted into the spleen form sinusoid-like structures; GS is expressed only in those cells close to efferent spleenic blood vessels (6).

Microsurgical techniques in vivo (66), using the source of the afferent hepatic blood was changed so that the livers were perfused only with arterial, portal, or caval blood. The results of these studies support the proposed mechanisms of dynamic and stable zonal gene expression. The zonations of neither PEPCK nor GS mRNA were changed when livers were perfused with blood from different sources. This underscores the fact that the direction of the blood stream, i.e. upstream compartments rich in O_2 and hormones, and downstream compartments low in O_2, and hormones each with different cell-to-cell interactions, is a major determinant in the zonation of gene expression.

ZONATION OF NONPARENCHYMAL CELL FUNCTIONS

Endothelial Cell Functions

The endothelial lining functions as a filter between the blood and the hepatocytes, allowing free diffusion of solutes, controlling the passage of medium-sized particles like chylomicron remnants, and preventing the passage of large particles such as chylomicrons (67). In the perivenous zone, the fenestrae are slightly smaller but clearly more numerous, so that the filtering capacity is larger (4, 5). This may be linked to the predominance of VLDL secretion in that zone. Because of their high endocytotic capacity, the endothelial cells are an important component of the reticuloendothelial system. Moreover, they actively secrete mediators and extracellular matrix components. The functional importance of the higher lectin binding capacity of periportal endothelial cells is so far not known (Table 2).

Kupffer Cell Functions

Kupffer cells, resident macrophages of the liver, are characterized by a very high endocytotic capacity and the production of inflammatory mediators, e.g. cytokines such as interleukin-1, interleukin-6, tumor necrosis factor α, or transforming growth factor β and eicosanoids. They are antigen presenting cells and are involved in the cytotoxic destruction of parasitic and microbial organisms and certain tumor cells. They are more numerous and possess higher endocytotic capacities and lysosomal acitivities in the periportal area, whereas they are endowed with higher cytotoxic activities in the perivenous zone (4, 5) (Table 2).

Ito Cell Functions

The perisinusoidal Ito cells mainly form biomatrix components and store vitamin A. They possess actin filaments and may participate in the control of sinusoidal blood flow (4, 5). They are also more numerous in the periportal zone, which may therefore be the major site of biomatrix formation and vitamin A storage (4, 5) (Table 2). Moreover, they appear to have a role in the propagation of sympathetic nerve actions (14, 28, 60).

Pit Cell Functions

Pit cells are large, granular lymphocytes that are anchored to the endothelial cells by pseudopodia, microvilli, or phylopodia. They are more numerous in the periportal zone. They exhibit natural killer activity on some tumor cells, the elimination of which therefore occurs primarily in the periportal area (4, 5) (Table 2).

CONCLUSION AND PERSPECTIVES

The zonation of gene expression in parenchymal and nonparenchymal liver cells causes a specialization of functions. This heterogeneity of periportal and perivenous cells appears to be a prerequisite for the effective functioning of the whole organ in nutrient metabolism and in a multitude of other roles (Table 1). Although the zonal distribution of some important enzymes, and especially of major hormone receptors, is still unknown, future studies will focus on the regulation of zonal gene expression. In the dynamic zonation, decreasing periportal-perivenous gradients of oxygen tension and of the glucagon/insulin ratio appear to be major factors for the carbohydrate-metabolizing enzymes. Paracrine interactions that involve diffusible mediators could be major determinants in the stable zonation of ammonia-metabolizing enzymes. The molecular characterization of O_2-sensing systems, of O_2-regulated hepatic genes, and of new factors involved in the interaction between hepatocytes and nonparenchymal cells will provide the basis for a deeper understanding of the zonation of parenchymal and nonparenchymal metabolism in the liver.

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