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REVIEW

Mechanisms Determining Phenotypic Heterogeneity of Hepatocytes

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Abstract—This review summarizes results of biochemical and immunohistochemical studies indicating the existence of functional heterogeneity of hepatocytes depending on their localization in the hepatic acinus; this determines characteristic features of metabolism of carbohydrates, lipids, and xenobiotics. The physiological significance of hepatocyte heterogeneity is discussed. According to the proposed model of intercellular communication, the metabolic specialization of hepatocytes is determined by secretory activity of hepatic resident macrophages (Kupffer cells) localized mainly in the periportal zone of the liver acinus. Macrophages participate in secretion of a wide spectrum of intercellular mediators (cytokines, prostaglandins, growth factors) and also in metabolism of numerous blood metabolites and biologically active substances (hormones, lipoproteins, etc.). In the sinusoid and in the space of Disse (also known as perisinusoidal space) they form a concentration gradient of regulatory factors and metabolites inducing the phenotypic differences between hepatocytes.

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It is long known that hepatocytes (liver parenchyma cells) are heterogeneous in structure, enzyme activity, and sensitivity to hepatotoxins. In 1958 Wilson [1] originally proposed the existence of functional heterogeneity of hepatocytes. However, at that time it was impossible to evaluate the functional role of hepatocyte subpopulations in metabolic processes due to lack of adequate methodical basis. Progress in investigation of this phenomenon started in the 1970s is associated with the development of new immunohistochemical and microbiochemical studies. Results obtained using these methods represented a basis for the concept of metabolic zones in hepatic tissue [2, 3]. Biochemical studies employing the isolated subpopulations confirmed the existence of functional specialization of hepatocytes [4, 5]. However, many authors emphasize that results obtained by various methods are contradictory. Some points of the proposed concept require additional experimental validation and specification. The molecular mechanism responsible for differences in gene expression in hepatocyte subpopulations still remains unknown. This review summarizes our own and literature data on the role of hepatocyte subpopulations in metabolism of carbohydrates, lipids, and xenobiotics and also discusses mechanisms determining functional specialization of these cells.

Abbreviations: apo) apolipoprotein; CYP) cytochrome P450; EGF) epidermal growth factor; G6PDH) glucose-6-phosphate dehydrogenase; HDL) high density lipoproteins; HGF) hepatocyte growth factor; IGF) insulin-like growth factor; IGFBP) insulin-like growth factor binding protein; IL) interleukin; LAP) latency associated peptide; LDL) low density lipoproteins; LTBP) latent TGF-binding protein; MCT) 3-methylcholanthrene; PAI-1) plasminogen activator inhibitor-1; PB) phenobarbital; PEPCK) phosphoenolpyruvate carboxykinase; TGF-β1) transforming growth factor-β1; TNF-α) tumor necrosis factor-α; VLDL) very low density lipoproteins.

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STRUCTURAL HETEROGENEITY OF HEPATOCYTES

According to modern concepts, the hepatic acinus is the functional unit of the liver. The simple acinus is the smallest fragment of irregular shaped fragment of parenchyma, which is oriented around the axis and consists of terminal branches of the portal vein (portal venule) and the hepatic artery (hepatic arteriole). The direction of the acinus axis roughly coincides with the so-called peripheral zone of the hexagonal lobule. In accor-

dance with actually existing blood circulation in the liver, Rappaport [6] suggested the existence of three zones of cells in the acinus: the first zone consists of periportal hepatocytes, the second zone is the intermediate or midlobular one, and the third zone consists of perivenous hepatocytes, which correspond to centrolobular cells of the hexagonal lobule (Fig. 1a).

Structural heterogeneity of hepatocytes depends on their zonal distribution in the acinus. The morphometric analysis has shown that hepatocytes of the third zone are characterized by the highest number of mitochondria and lysosomes and the largest surface area of smooth endoplasmic reticulum (EPR) [7, 8]. Cells of the first zone are characterized by predominance of large mitochondria, membranes of rough EPR and membranes of Golgi apparatus [7, 9]. It should be noted that structural differences between periportal and centrolobular hepatocytes are species specific and depend on age and type of diet [8]. For example, in 6- and 10-month-old rats peroxisomes predominated in periportal hepatocytes, and in 16-month-old rats and in older animals peroxisomes predominated in centrolobular hepatocytes [7]. Peroxisome proliferation and activation of its enzymes induced by administration of hypolipidemic preparations was more

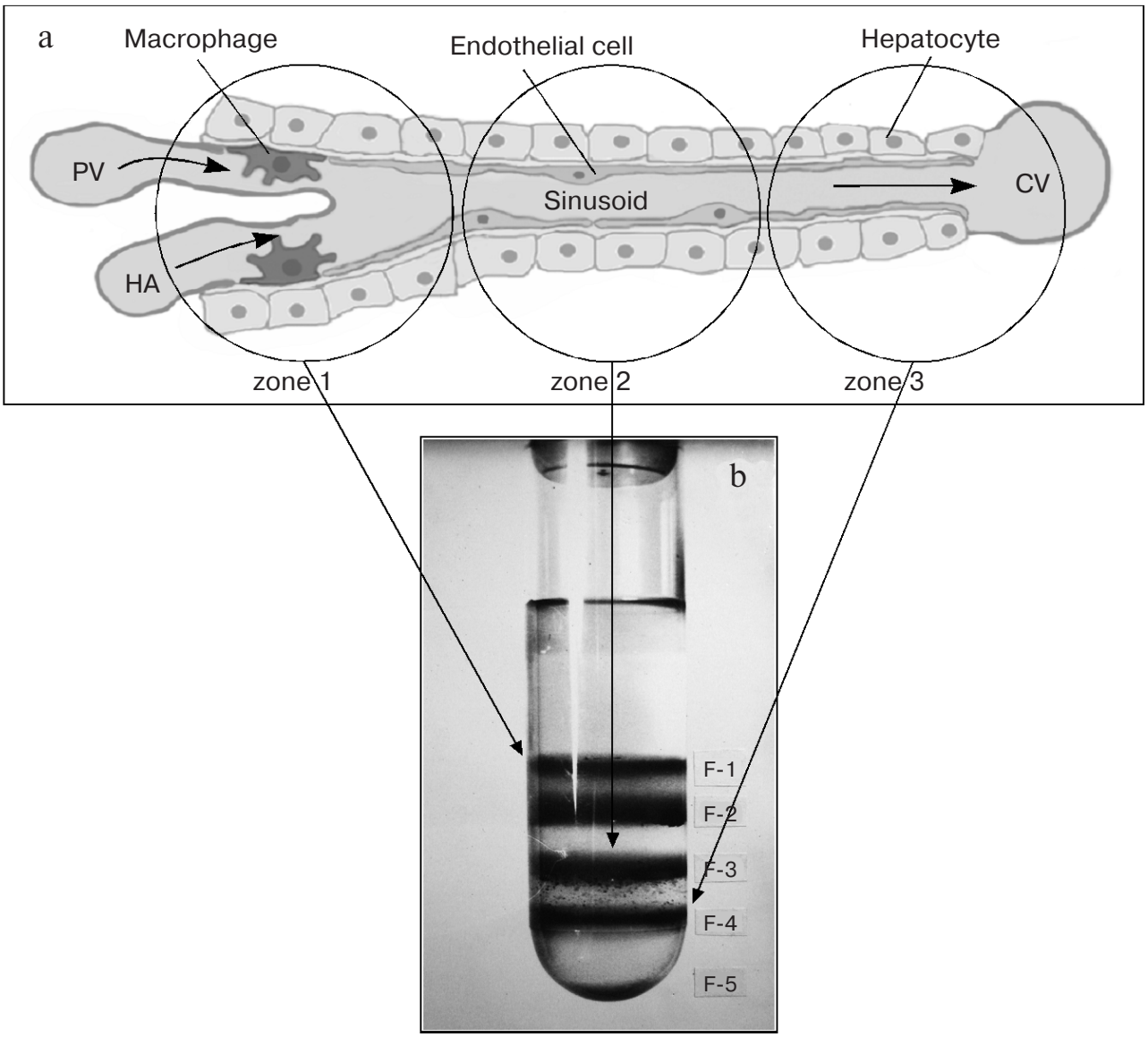

Fig. 1. a) Scheme of hepatic sinusoid structure and its subdivision into zones according to the acinar concept of hepatic structure [6]. PV, portal venule; HA, hepatic arteriole; CV, central vein. Arrows show direction of blood flow in the sinusoid. b) Fractions of hepatocytes (F1-F5) enriched with periportal (zone 1) and centrolobular hepatocytes (zone 3). Hepatocytes were fractionated by means of isopycnic centrifugation of hepatocyte suspension in a Ficoll density gradient and using a J-13 bucket rotor in a J2-21 centrifuge (Beckman, USA). Isolated hepatocytes were obtained after recycling liver perfusion with a collagenase solution [5, 20].

pronounced in the periportal cells [10]. The size of bile tubules was two times larger in the first zone compared with the third one [7]. Activity of alkaline phosphatase involved in secretion of bile acids by hepatocytes was also higher in the first zone [11]. However, administration of bile acid excess to animals was accompanied by the increase of tubule diameter only in the third zone [12]. This suggests that under normal conditions bile is preferentially secreted within the first zone, and under hypersecretion conditions it is secreted in all zones of the acinus [13].

FRACTIONATION OF PERIPORTAL AND CENTROLOBULAR HEPATOCYTES

Results of morphologic studies suggesting significant ultrastructural differences in hepatocytes represented a basis for the development of the methods for preparative isolation of hepatocyte subpopulations enriched with periportal and centrolobular cells. This method is a promising approach for studies of mechanisms responsible for formation of phenotypic heterogeneity of hepatocytes, because it employs many methods of cell and molecular biology.

The first isopycnic fractionation of hepatocytes was carried out by Castagna and Chauveau [14]. Using Ficoll gradient density separation they obtained three fractions of hepatocytes: light cells had higher cholesterol content than heavy cells; they also had high activity of glucose-6-phosphatase and exhibited higher activity in incorporating orotic acid into RNA and acetate into cholesterol. Based on these data, they concluded that the light fraction was enriched with periportal hepatocytes, whereas the heavy fraction contained more centrolobular hepatocytes. However, using similar fractionation conditions and based on morphologic differences between fractions, Wanson et al. [15] came to the opposite conclusion. Analysis of hepatocyte fractions isolated in a metrizamide density gradient did not resolve these contradictions [16]. Such extreme contradictions of results may be attributed to differences in conditions of isolation and fractionation of hepatocytes used in different laboratories. Some studies did not take into consideration the physiological conditions of the experimental animals. For example, some authors fractionated cells obtained from starved animals or animals injected with phenobarbital [15]. We have demonstrated that these conditions influence not only density of floating cells but also redistribution of enzymes between subpopulations [5]. Selection of marker enzymes is important for identification of periportal and centrolobular hepatocytes. Many researchers investigated activity of alcohol dehydrogenase, 5′-nucleotidase, alanine aminotransferase, glutamate dehydrogenase, and lactate dehydrogenase. In our viewpoint selection of these enzymes is not well justified because differences in activities of these enzymes are not well defined in various acinus zones. We have used the key enzyme of gluconeogenesis—phosphoenolpyruvate carboxy kinase (PEPCK)—as the marker of periportal hepatocytes [2] and glucokinase [3], glucose-6-phosphate dehydrogenase (G6PDH) [11], cytochrome P450 [17, 18], and cytochrome b_5 [19] for identification of centrolobular hepatocytes. Biochemical analysis of hepatocyte fractions based on distribution of these enzymes provided convincing evidence that during isopycnic fractionation (Fig. 1b) light fractions are enriched in periportal hepatocytes, whereas heavy fractions are enriched in centrolobular hepatocytes [5, 20]. This is consistent with conclusions made by other authors [4, 14]. From our viewpoint, lipids are one of the main structural components determining hepatocyte difference by floating density; results of morphometric studies indicate that lipid content is higher in periportal (light) cells [7, 11]. Recently, liver perfusion with digitonin solution was used for isolation of periportal and centrolobular hepatocytes. This method is based on selective lysis of cells of the first and the third zones of acinus during anterograde (via *vena porta*) or retrograde (via *vena hepatica*) perfusion of the liver, respectively [21, 22]. However, using this method it is impossible to exclude digitonin effect on integrity of plasma membrane and other cell structures during isolation of particular subpopulation of hepatocytes.

FUNCTIONAL HETEROGENEITY OF HEPATOCYTES

Carbohydrate metabolism. Using microbiochemical analysis of hepatic tissue, it was found that under normal conditions the activity of gluconeogenic enzymes predominated in the first zone of the acinus, whereas glycolytic enzyme activity predominated in the third zone of the acinus. The second zone was characterized by an intermediate level of activity of these enzymes [2]. Use of this method has demonstrated reciprocal mode of distribution of glycolytic enzymes: glucokinase activity was maximal in the third zone, whereas hexokinase activity was maximal in the first zone [23]. Our biochemical analysis of the isolated hepatic cells explained this contradiction. First, studies of isolated subpopulations have demonstrated that the highest activity of hexokinase and glucokinase is detected in centrolobular (“heavy”) hepatocytes [5]. Second, hexokinase activity in sinusoidal hepatic cells is significantly higher than in hepatocytes [24]. Since the number of sinusoidal cells, mainly macrophages, predominates in the first zone [25], one can assume that the mode of hexokinase distribution revealed during microanalysis of this tissue correlates with macrophage distribution in the acinus, rather than distribution of this enzyme between periportal and centrolobular hepatocytes.

Activities of glycolytic and gluconeogenic enzymes were simultaneously detected in all subpopulations [20]. This is not related with contaminations of hepatocyte fractions with periportal and centrolobular cells because the recognized differences between subpopulations were consistent with results of histochemical analysis of the hepatic tissue [2, 3]. These facts indicate that even under conditions of functional specialization substrate (futile) cycles were preserved in hepatocytes.

Functional differentiation of hepatocytes related to carbohydrate metabolism is most pronounced at physiological resting conditions and is changed during metabolic rearrangements. For example, activity of gluconeogenic enzymes increased and activity of glycolytic enzymes decreased during starvation. These changes were especially pronounced in the subpopulation of centrolobular hepatocytes. Differences between these subpopulations decreased or even disappeared depending on duration of starvation [26]. Diabetes mellitus is also characterized by lack of differences in the rate of gluconeogenesis between these subpopulations [27]. Thus the increase in hepatic glucose synthetic capacity is achieved by activation of gluconeogenesis in both periportal and centrolobular hepatocytes. Activity of glucose utilizing processes undergoes more pronounced decrease in the centrolobular hepatocytes as well.

Differences between hepatocytes were not detected in the prenatal period; these were gradually formed during postnatal development. According to histochemical analysis, glycogen is uniformly distributed in the acinus of prenatal hamsters. In neonates glycogen exhaustion simultaneously began in all zones. However, starting 2-3 days after birth there was uniform distribution of glycogen in the acinus [2, 3]. In pubertal rats, the highest glycogen content was found in centrolobular hepatocytes [11]. These cells were also characterized by maximal activity of glycogen synthase and glycogen phosphorylase [28]. High activity of glucose-6-phosphatase observed in neonates and during the fist two days after birth was also coupled to its uniform distribution between hepatocytes. The uniform mode of enzyme distribution typical for adults appeared during the subsequent postnatal week due to the decrease in the enzyme activity in the centrolobular zone [29]. Similar changes were found in the activity of ornithine carbamoyltransferase, succinate dehydrogenase [30], G6PDH, and malate dehydrogenase [29] during the postnatal period.

Lipid metabolism. Taking into consideration functional links between lipogenesis and glycolysis, one may suggest that both processes are localized in centrolobular hepatocytes. Indeed, activity of acetyl-CoA carboxylase involved in fatty acid synthesis from acetyl-CoA is the highest in the centrolobular zone [31]. Fatty acid synthesis is a NADPH-dependent process. Under conditions when glucose is used as the substrate for fatty acid synthesis, the pentose phosphate pathway is the main supplier of reducing equivalents. Activity of dehydrogenases reducing NADPH predominates in the centrolobular zone [11]. Analysis of isolated subpopulations of hepatocytes has shown that distribution of G6PDH between hepatocytes coincided with distribution of glycolytic enzymes [26]. These facts may be considered as indirect evidence for preferential fatty acid synthesis in the centrolobular cells.

Feeding of rats with a carbohydrate diet activated fatty acid synthesis and accumulation of triglycerides in the liver; initially, lipid accumulation was noted in the centrolobular and then in periportal cells [32]. Aspichueta et al. have shown [33] that all hepatocytes are involved in synthesis and secretion of apolipoprotein B (apo B) and very low density lipoproteins (VLDL); however, their secretion after endotoxin administration was preferentially activated in periportal cells.

In rats fed with a balanced diet, significant predominance of lipids was detected in cytoplasm of periportal cells [7, 11]. It is difficult to interpret these results because the amount of lipids (triglycerides) in the cell depends not only on the rate of fatty acid synthesis, but also on the rate of esterification of fatty acids obtained from adipose tissue. The study of distribution of ^{125}I-labeled plasma lipoproteins in hepatocyte subpopulations has shown that HDL (high density lipoproteins) and LDL (low density lipoproteins) are preferentially taken up by centrolobular hepatocytes [34], whereas chylomicron remnants are preferentially taken up by periportal cells [35]. These subpopulations demonstrated no differences in uptake of VLDL [34, 36]. Activity of cytosol and microsomal cholesterol ester hydrolase was higher in centrolobular hepatocytes. However, these subpopulations demonstrated no differences in activities of lysosomal cholesterol ester hydrolase and acyl-CoA:cholesterol acyltransferase. The authors suggest that degradation of intracellular cholesterol esters preferentially occurs in the cells of the third zone, whereas degradation of lipoprotein cholesterol esters equally occurs in all zones [37].

Xenobiotic biotransformation. Significant differences have been found in the content of enzymes involved into oxidative metabolism of endogenous (steroids) and exogenous (xenobiotic) lipophilic compounds. In intact rats, the content of cytochrome P450 and cytochrome b_5 was 2 and 4 times higher in centrolobular hepatocytes than in periportal hepatocytes, respectively [5, 17]. Interestingly, the mode of distribution of these heme proteins slightly differed in these subpopulations. For example, concentration of cytochrome P450 in hepatocyte subpopulations gradually increased with the increase in their flotation, whereas concentrations of cytochrome b_5 equally distributed between "light" (periportal) fractions and sharply increased in the "heaviest" (centrolobular) cells [5].

Histochemical analysis revealed differences in distribution of other components of the monooxygenase sys-

tem. In intact rats the content of cytochrome P450 induced by phenobarbital (PB) was significantly lower in the periportal zone compared with the midlobular and the centrolobular zones, whereas the 3-methylcholanthrene (MCT)-inducible cytochrome P450 equally distributed between the periportal and midlobular zones but predominated in the centrolobular zone [17]. Activity of ethoxycoumarin O-deethylase [38] and the content of CYP1A1 and CYP1A2 mRNA [39] were also higher in centrolobular cells. The amount of NADPH-cytochrome P450 reductase was equal in the centrolobular and midlobular zones, but significantly higher in the periportal zone [40].

During PB administration, induction of cytochrome P450 was preferentially observed in centrolobular cells, whereas induction of reductase was equal in all cells [17]. In contrast to PB, a synthetic steroid, pregnenolone-16α-carbonitrile, caused a 3-fold increase in the reductase content in periportal cells; in the centrolobular and the midlobular cells this effect was weaker, increasing by 73 and by only 45%, respectively [40]. Interestingly, administration of other types of inducers, MCT and β-naphthoflavone, caused higher binding of antibodies to MCT-cytochrome-450 in midlobular and periportal cells. However, the mode of reductase distribution did not change within the acinus [17]. These facts suggest that hepatocyte subpopulations differ not only by content of components of the monooxygenase system, but also by mode of their induction by xenobiotics.

Conjugation of xenobiotics with sulfate or glucuronic acid is an important mechanism of xenobiotic biotransformation. These reactions involve the cytoplasmic enzyme sulfotransferase and the microsomal enzyme uridine diphosphate glucuronyltransferase. Conjugation of acetaminophen with sulfate occurs preferentially in the periportal zone [41], and its conjugation with glucuronic acid preferentially occurs in the centrolobular zone [42]. Interestingly, MCT caused preferential induction of glucuronyltransferase in the centrolobular zone, whereas PB preferentially induced this enzyme in the periportal zone [43]. These differences are obviously associated with existence of several forms of this enzyme, which are preferentially induced in various subpopulations of hepatocytes.

These results suggest the existence of functional specialization among hepatocytes; it includes metabolism of carbohydrates, lipids, and xenobiotics. Functional specialization of hepatocyte subpopulations is rather dynamic: differences between subpopulations are especially pronounced under physiological conditions, whereas activation of expression of the same genes in response to various stimuli (inducers) may occur in all cells. This phenomenon may play an important adaptive role because the liver as an organ involved into numerous homeostatic functions in the body requires activation of metabolism in all subpopulations of cells in response to functional load.

MECHANISMS INDUCING PHENOTYPIC CHANGES BETWEEN HEPATOCYTES

Regulatory role of oxygen. Several hypotheses explaining structural–functional heterogeneity of hepatocytes have been proposed. Novikoff [44] originally associated hepatocyte heterogeneity with parenchymal blood flow direction. He indicated that in the parenchymal cell, distribution of organelles reflects direction of intracellular molecular movement and differences between adjacent cells probably reflect direction of blood flow inside a hepatic lobule. Later Rappaport [6] suggested that the arteriole plays the decisive role in functional heterogeneity of hepatocytes. According to this hypothesis, an oxygen gradient exists in the acinus: the first zone (where the arteriole "falls") is supplied with highly oxygenated blood, whereas the second and the third zones are supplied with poorly oxygenated (and carbon dioxide enriched) blood. Indeed, modeling of such conditions in experiments with hepatocyte culture demonstrated that oxygen content in the medium was crucial for expression of cytochromes CYP2B and CYP3A [45], glucose production, and induction of glucokinase and PEPCK influenced by insulin and glucagon [46]. However, certain evidence exists that under normal conditions oxygen is not a rate-limiting factor for xenobiotic transformation [47]. It was demonstrated that the rate of the reaction catalyzed by glutamine synthetase did not depend on oxygen concentration during anterograde or retrograde perfusion of the liver [48]. Blood flow direction is not the factor determining substrate distribution in the acinus. For example, during liver perfusion with solution containing physiological concentrations of [^{3}H]glutamate, [^{3}H]α-ketoglutarate, and [^{3}H]aspartate, the radioactive label localized in the third zone of the acinus irrespectively of the direction of perfusion [49].

A mechanism of oxygen-dependent changes in intracellular processes remains unknown. There is evidence that oxidative phosphorylation in liver mitochondria depends on oxygen even at its high concentrations [50]. So, one may assume that the effect of oxygen on carbohydrate metabolism is mediated by changes in concentration ratios ATP/ADP·Pi, NAD^+/NADH, and $NADP^+$/NADPH. It should be noted that comparative tissue microanalysis of samples from various zones of the acinus did not reveal any differences in phosphate potential and the state of the redox system [51].

Regulatory role of hormones. The functional state of hepatocytes depends on many factors. Mechanisms of neurohumoral regulation of metabolic processes are actively discussed in the literature. It was shown that insulin, glucagon, catecholamines, and glucocorticoids play major roles in regulation of carbohydrate and lipid metabolism. The distribution of hormone receptors between hepatocytes remains unknown. However, based on data of hormonal regulation of carbohydrate metabo-

lism and also taking into consideration distribution of glycolytic and gluconeogenic enzymes in hepatocyte subpopulations, we may suggest that glucagon and glucocorticoids are dominating hormones for periportal cells, whereas insulin plays a major role in centrolobular cells. Jungermann and Katz indicated [52] that the index of molar ratio insulin/glucagon increases during blood passage from the first to the third zones. From their viewpoint, this explains predominance of gluconeogenesis in the first zone and glycolysis in the third zone. From our viewpoint, the ratio of these hormones is not the determining factor for functional specialization of hepatocytes. For example, in rats with portacaval anastomosis the glucagon/insulin index was 10 times higher than in intact animals [53], but distribution of key enzymes of gluconeogenesis and glycolysis within the acinus remained unchanged [54]. Lack of correlation between blood ratio of glucagon and insulin and distribution of enzymes in the acinus was also found in rats with experimental diabetes [55]. Also, no differences were found during the study of glucagon effect on transmembrane potential of hepatocytes localized in different zones of the acinus [56].

Heterogeneity of sinusoidal hepatic cells. Like any other cells of parenchymal organs, hepatocytes do not have direct contact with the intravascular space; they are separated from this space by sinusoidal cells, which include endothelial cells and resident macrophages (Fig. 1a). Penetration into the space of Disse of various blood plasma components may occur via pores (fenestras) in the endothelial lining or via vesicular transfer (transcytosis) through sinusoidal cells [25]. In the first case, transport of components depends on the number and size of such fenestras, in the second case transport depends on the presence of corresponding receptors in sinusoidal cells. However, in any case functional state will determine exchange of substances between the space of Disse and the blood.

Morphology of sinusoidal cells is heterogeneous; it depends on their localization in the acinus. For example, porosity of the endothelial lining and diameter of fenestras are significantly higher in the third zone of the acinus [25, 57, 58]. The number of macrophages predominates in the first zone; these cells are larger and exhibit the highest secretory activity there [25, 59, 60]. Expression of caveolin-1 (involved in intracellular signaling pathways [61]) and also marker proteins of endothelial cells, CD31 and CD34, is higher in the first zone, whereas expression of CD105 is higher in the third zone [62, 63]. Endothelial cells of the periportal zone are characterized by the highest density of galactose and mannose receptors [64]. The first zone is characterized by the highest content of resident lymphocytes (pit-cells) and fat-storing cells (also known as Ito cells) [57]. These cells are the main source of hepatic extracellular matrix; they accumulate the major proportion of vitamin A in the body. One may suggest that differences of sinusoidal lining by structure and cell composition are important factors forming characteristic features of hepatocyte microenvironment in periportal and centrolobular zones of the acinus.

Recently Hailfinger et al. [65] proposed a model explaining hepatocyte heterogeneity as the result of effects of two opposite signals acting at the cells. One of these signals comes from central venous endothelial cells and activates a β-catenin-dependent pathway, and another one has extrahepatic origin and activates a Ras-dependent pathway. This model is based on similarity of gene expression in two types of hepatic tumors possessing mutations in *Catnb* (encoding β-catenin) and oncogenes *Ha-ras* and also on enzyme distribution between periportal and centrolobular hepatocytes [66]. Results of other authors also suggest involvement of the Wnt/β-catenin in formation of hepatocyte heterogeneity [67]. However, it remains unclear which cells are the source of signals for activation of these pathways in hepatocytes [68].

Gumucio and Gebhardt [69] suggest that contact of centrolobular hepatocytes with the central vein is the signal for expression of glutamine synthetase and ornithine carbamoyltransferase. This suggestion is based on the following facts. The mode of distribution of these enzymes typical for intact animals remains the same during reparative regeneration of the liver after its partial resection. Selective damage of centrolobular cells by means of carbon tetrachloride results in total disappearance of the cells containing these enzymes. Their reappearance during reparation coincides with appearance of contacts between hepatocytes and the central vein.

Role of paracrine factors in regulation of hepatocyte metabolism. Regulatory functions of macrophages are associated with their ability to secrete a wide spectrum of regulators involved in paracrine regulation: interleukins (IL), prostaglandins, lysosomal enzymes, various factors, etc. [70, 71]. Receptors to these mediators have been found in various cell types including hepatocytes [72].

It is long known that peritoneal macrophages incubated in the presence of endotoxin release some factor inhibiting glucocorticoid induction of PEPCK [73]. This has been named the "glucocorticoid antagonizing factor" (GAF). Later it was demonstrated that hepatic macrophages can also secrete products inducing similar effect in isolated hepatocytes [74]. It should be noted that GAF suppressed only glucocorticoid-dependent induction of PEPCK, but did not influence basal level of this enzyme. This may explain the fact that even increased concentration of corticosterone and glucagon (inducers of PEPCK synthesis) observed under conditions of endotoxemia did not overcome PEPCK inhibition [75].

Administration of IL-6 to animals caused induction of α2-macroglobulin in periportal hepatocytes; the authors suggested that this effect might be attributed to selective binding of this cytokine with cells of the periportal zone [76]. In hepatocyte culture, IL-6 [77] and transforming growth factor (TGF) [78] increased content of

PEPCK mRNA and activated gluconeogenesis. Tumor necrosis factor (TNF) [79] and also combined addition of IL-1β and IL-10 [80] caused the opposite effect. Under these conditions, mRNA and catalytic activity of pyruvate kinase remained unchanged; this suggests the key role of PEPCK in regulation of *de novo* glucose synthesis by these cytokines [80]. Recombinant IL-1 did not influence PEPCK activity in hepatocyte culture but significantly decreased glucagon-dependent induction of this enzyme [81, 82]. Injection of IL-1 into animals decreased the number of steroid hormone receptors in the liver and prevented PEPCK induction during starvation [83]. IL-1, IL-6, and nitric oxide significantly decreased insulin-inducible synthesis of glycogen in hepatocytes by inhibiting glycogen synthase and activation of glycogen phosphorylase [84, 85]. IL-6 also influenced glycolysis by preventing glucokinase activation by insulin [86].

There are contradictory data on the effect of prostaglandins on carbohydrate metabolism. Some authors [87-89] found that prostaglandins D_2 and E_2 as well as thromboxane A_2 stimulated glucose production by hepatocytes. However, Okumura and Saito [90] did not find regulatory effect of prostaglandin D_2 on glucose metabolism, but they demonstrated that prostaglandins E_2 stimulated incorporation of glucose into glycogen and inhibited insulin-inducible glycogenolysis in primary hepatocyte culture. Selective blockade of hepatic macrophages by gadolinium chloride almost totally prevented the stimulating effect of adenosine and ATP on glycogenolysis in perfused liver [91].

Involvement of macrophages into regulation of lipid metabolism is supported by numerous facts indicating significant changes in parameters of lipid metabolism during infection and inflammation. Hyperlipidemia and the increase of blood plasma VLDL are the most typical signs for these pathological states [92]. Administration of TNF-α, IL-1, and interferon-α to animals caused increase in fatty acid synthesis in the liver, whereas administration of TNF-β, IL-1, and interferon-γ stimulated cholesterol biosynthesis [93, 94]. Administration of TNF-α was also accompanied by significant increase in hepatic citrate content [95]. Taking into consideration that citrate is an allosteric activator of acetyl-CoA carboxylase, one may assume that TNF-α regulates fatty acid synthesis by changing citrate content in liver cells.

Nonogaki et al. [96] found that administration of IL-6 to rats caused significant increase in blood serum triglycerides. Maximal effect was observed after 2 h. These authors suggest that hypertriglyceridemia may be attributed to increased secretory activity of the liver rather than decreased rate of lipoprotein clearance. Using blockers of α- and β-adrenoreceptors, they demonstrated that this effect did not depend of catecholamine content. Oncostatin secreted by macrophages caused marked stimulation of LDL uptake, the number of LDL receptors, and their mRNA in HepG2 cell culture [97, 98].

Incubation of hepatocytes in a conditioned medium of nonparenchymal liver cells caused significant decrease in apo A-1 mRNA. Interestingly, under these conditions there were minor changes in concentrations of apo B, apo E, albumin mRNA, and activities of hydroxymethylglutaryl-CoA reductase and hepatic lipase [99]. Cultivation of human hepatoma HepG2 cells with TNF-α, IL-1β, and IL-6 caused a decrease in production of apo A-1 and apo B. This was accompanied by a decrease in apo A-1 mRNA in these cells, whereas apo B mRNA remained unchanged [100].

There is evidence that macrophage secretory products influence metabolism of xenobiotics in hepatocytes. Injection of IL-1, IL-2, IL-6, TNF-α, TNF-β, and interferon-γ caused a decrease in cytochrome P450 content and the rate of metabolism of xenobiotics in the liver [101-105]. Studies employing hepatocyte culture have shown that IL-1 not only decreases basal activity of cytochrome P450, but it also significantly suppresses PB-induced increase in ethoxyresorufin and pentoxyresorufin metabolism. IL-1β, TNF-α, interferon-α, and interferon-γ decrease MCT-induced activity of ethoxyresorufin-O-deethylase (EROD) in the hepatocyte culture. Interestingly, under these conditions TGF-β1 totally blocked induction of CYP1A1 and CYP1A2 caused by MCT and benzo(a)pyrene [106]. Suppressive effect of IL-2 on CYP3A required the presence of liver macrophages in the hepatocyte culture [105]. Inhibitory effect on a monooxygenase system was also demonstrated for NO [107]. Thus, secretory products of macrophages caused not only direct regulatory effect but they also modulated effects of hormones and specific inducers on expression of hepatocyte enzymes.

Role of paracrine factors in regulation of hepatocyte proliferation. Normally hepatocytes divide rarely, but hepatectomy or toxic liver damage cause sharp increase in proliferative activity of hepatocytes; this results in restoration of the mass of this organ. Nonparenchymal cells, particularly residential macrophages, play an important role in the growth and proliferation of hepatocytes. Experiments on rats have demonstrated that macrophage stimulation prior to hepatectomy significantly increased the rate of hepatic protein biosynthesis [108] and shifted the peak of proliferative activity of hepatocytes to earlier time intervals [109]. On the contrary, selective blockade of macrophages decreased the rate of hepatic recovery [110, 111]. The regulatory role of macrophages in hepatocyte proliferation is associated with secretion of cytokines and growth factors. It is suggested that cytokines trigger regeneration process and "prepare" hepatocytes to the mitogenic effect of growth factors [112, 113].

Among cytokines, TNF-α and IL-6 attract much attention of researchers. Hepatectomy caused a sharp increase in TNF expression in the liver, which correlated with the increase in DNA synthesis [114, 115]. Neutralization of TNF-α by means of specific antibodies

suppressed regeneration of this organ [116]. In hepatocyte culture, recombinant TNF-α induced proliferation and prevented apoptosis [117]. The effect of TNF-α on the increase in DNA synthesis was comparable with the effect of epidermal growth factor (EGF). However, in contrast to EGF, the action mechanism of TNF-α is related to activation of nuclear factor-κB (NF-κB) [118, 119]. An important role of TNF-α receptors type 1 and type 2 was demonstrated during liver regeneration induced by carbon tetrachloride [120].

Involvement of IL-6 in liver regeneration was demonstrated using IL-6 gene knockout mice [121] and a chimeric protein consisting of IL-6 and its soluble receptor [122]. Using a bone marrow transplantation model, it was shown that replacement of liver macrophages from mice with IL-6(+/+) genotype for the cells obtained from mice with IL-6(–/–) genotype caused a decrease in the regeneration of the organ after partial resection. This process is restored by administration of IL-6 to the IL-6 deficient animals or by cell transplantation from IL-6(+/+) animals [123]. It should be noted that during liver regeneration IL-6 triggered expression of a limited number of genes and itself could not induce DNA synthesis [124].

Hepatocyte growth factor (HGF) is the best-studied mitogenic factor. Its expression increases during liver regeneration and precedes activation of DNA synthesis. Neutralization of HGF by specific antibodies decreased reparative regeneration [125], whereas injection of HGF into animals with partial hepatectomy increased hepatic synthesis of DNA. A similar effect was reproduced during hepatocyte cultivation in the presence of HGF [126]. It was demonstrated that in liver of intact rats HGF mRNA expression occurs in macrophages, endothelial, and fat-storing cells [127, 128]. During liver regeneration involvement of these cells in HGF production depends on the type of damaging factor: after toxic damage with carbon tetrachloride the most pronounced HGF expression was observed in resident macrophages [127], whereas in the case of partial hepatectomy the most pronounced expression was observed in fat-storing cells [129]. Selective blockade of macrophages decreased the level of HGF expression in hepatectomized animals and this also decreased organ recovery. This suggests that during hepatectomy the stimulating effect of macrophages on hepatocyte proliferation is mediated by activation of HGF synthesis in fat-storing cells [129]. Immunohistochemical methods have shown that hepatocyte growth factor activator is synthesized in hepatocytes adjacent to a central vein [130].

The insulin like growth factor (IGF) belongs to a family of proteins involved into regulation of proliferation and differentiation of various cells [131] including hepatocytes [132]. In the liver, it is synthesized in both parenchymal [133] and nonparenchymal cells [134]. IGF binds to cell surface membrane receptors and also to specific IGF-binding protein (IGFBP), involved in transport and modulation of IGF effects. IGFBP-1 is expressed in hepatocytes, IGFBP-2 is expressed in hepatocytes and macrophages; expression of IGFBP-3 is found in macrophages and endothelial cells, whereas IGFBP-4 is expressed in hepatocytes and to a lesser extent in nonparenchymal cells [135, 136]. In a hepatocyte culture, insulin and IGF-1 decrease synthesis of IGFBP-1 and IGFBP-2, but increase synthesis of IGFBP-4 [135, 136]. In contrast to hepatocytes, synthesis of IGFBP-2 and IGFBP-3 in macrophages is not regulated by insulin and IGF-1, and only in the presence of hepatocyte conditioned medium these factors stimulated synthesis of IGFBP-3 [135, 137].

Hepatic resident macrophages can not only stimulate, but also inhibit hepatocyte proliferation. Meyer et al. [138] found the inhibitory effect of macrophage conditioned medium on EGF-induced synthesis of DNA. This effect was determined by the presence of transforming factor-β (TGF-β), because neutralization of TGF-β resulted in the increase in DNA synthesis in hepatocytes. Using competitive blockade of TGF-α and EGF receptors, the authors demonstrated that the activating effect could be attributed to the presence of TGF-α in the macrophage conditioned medium. According to Kimura and Ogihara [139], the inhibitory effect of TGF-β on hepatocyte proliferation induced by TGF-α is realized via the increase in intracellular cAMP level and activation of protein kinase A.

TGF-β is secreted by cells as a latent noncovalent complex including TGF-β, latency associated peptide (LAP), and binding protein (LTBP) [131]. In intact liver TGF-β1 is preferentially expressed by macrophages, TGF-β2 is expressed in macrophages and fat-storing cells, whereas TGF-β3 is expressed only in fat-storing cells. During hepatic fibrosis, the level of TGF-β1 expression in macrophages remains unchanged, but significantly increases in fat-storing and endothelial cells. No expression of any form of TGF-β was found in hepatocytes [140]. Using an immunostaining method, expression of LTBP and LAP was found in macrophages [141] and fat-storing cells [142].

Activation of the latent form of TGF-β occurs in extracellular space and involves proteolytic enzymes. Plasmin plays an important role in TGF-β activation [143]. Plasmin formation from plasminogen depends on the level of plasminogen activator inhibitor-1 (PAI-1). In the liver PAI-1 is secreted by fat-storing [144] and endothelial cells [145], and therefore it is suggested that nonparenchymal cells may modulate TGF-β effect by means of PAI-1 secretion [71].

Regulatory role of resident macrophages. Hepatic macrophages (Kupffer cells) are referred to the system of mononuclear phagocytes and they represent more than half of the total pool of resident macrophages of the body [60]. It is believed that macrophages are important for

tight cell contacts of the liver with bone marrow and macrophages involve liver in complex inter-organ communications [109]. Involvement of resident macrophages in regulation of hepatocyte functions was also demonstrated: *in vivo* experiments by means of specific stimulators [146-148] and macrophage blockers [91, 110, 111], perfused rat liver [149], and combined culture of hepatocytes and hepatic sinusoidal cells [105, 150-152]. Numerous data demonstrate that macrophage secretory products influence metabolism of carbohydrates, lipids, and xenobiotics. It is important to emphasize that macrophage regulatory effects may be associated not only with secretion of paracrine factors, but also with their participation in metabolism of various blood plasma components exhibiting regulatory properties (e.g. hormones and lipoproteins).

Results of our studies [34, 153, 154] and data from other laboratories [155] demonstrate high lipoprotein uptake capacity of hepatic sinusoidal cells especially with respect to HDL. Macrophage stimulation by lipopolysaccharide resulted in reduction of serum HDL_3, LDL, and glucocorticoids [153]. In rats, there was time-dependent redistribution of intravenously administrated ^{125}I-labeled HDL between cells: the decrease in radioactivity in sinusoidal cells was accompanied by its increase in hepatocytes [34]. Employment of isolated rat liver macrophages [156] and also mouse peritoneal macrophages [157] demonstrated that HDL uptake was not accompanied by total degradation of these lipoproteins but was accompanied by their subsequent secretion into the extracellular medium (as well as by secretion of the main HDL protein, apo A-1). These facts suggest existence of metabolic cooperation of macrophages and hepatocytes in lipoprotein metabolism. It is possible that lipoproteins undergo partial degradation in macrophages and lipid and protein components enter hepatocytes independently. This scenario has been proposed for cholesterol taken by hepatic sinusoidal cells in complex with lipoproteins [158].

Various authors have demonstrated that in addition to the main functions of HDL associated with lipid transport, these lipoproteins may also cause regulatory effects on various cell types. For example, HDL exerted mitogenic effect on lymphocytes [159], endothelial [160], smooth muscle [161], and tumor cells [162]. In the smooth muscle cells HDL stimulated cell growth induced by such mitogens as EGF, platelet derived growth factor, and fibroblast growth factor [163]. A sharp increase in expression of placental lactogen induced by apo A-1 was demonstrated using human trophoblast cells [164]. Apo A-1 stimulated synthesis and secretion of prostaglandin E_2 in macrophage [165] and also production of apo E via a mechanism that was independent of cholesterol transport out of the cells [166, 167]. Modulating effect of apo A-1 on intercellular communications included inhibition of induction of TNF-α and IL-1β mRNA in monocytes caused by T-lymphocytes [168].

It is known that in macrophages steroid hormones undergo biotransformation followed by formation of tetrahydrocompounds [169]. Subsequent biotransformation steps yielding water-soluble steroid glucuronide conjugates occur in hepatocytes, and so it is believed that there are cooperative communications between these cell types in metabolism of steroid hormones [170]. Taking into consideration these facts, we have investigated the effect of hydrocortisone, its metabolite tetrahydrocortisol, and the main HDL protein component apo A-1 on protein biosynthesis in culture of hepatocytes, macrophages, and endothelial cells of rat liver. Our experiments demonstrated that neither the hormone nor its reduced form influenced the rate of [^{14}C]leucine incorporation into proteins in all three types of liver cells. Addition of apo A-1 into the incubation medium increased protein biosynthesis only in hepatocytes and macrophages. Addition of apo A-1 together with tetrahydrocortisol into hepatocyte culture increased the rate of protein biosynthesis, which became maximal in these cells. Based on these data it was concluded that in addition to known mechanisms of regulation of protein biosynthesis in hepatocytes by hormones and cytokines, there is another, macrophage-dependent mechanism related to participation of these cells in metabolism of lipoproteins and glucocorticoids [171].

Certain evidence exists in the literature that macrophages modulate effects of various hormones. Brix et al. [172] demonstrated that the induction of lipogenesis and G6PDH activity in hepatocytes by triiodothyronine, thyroxin, and their precursor thyroglobulin required their co-cultivation with macrophages. Addition of all these hormones into cell culture containing only hepatocytes did not produce this effect. Liver sinusoidal cells are characterized by high density of glucagon [173] and insulin receptors [174]. However, hormone uptake by these cells is not obviously accompanied by total degradation of these hormones because specific proteases have been found only in hepatocytes [175].

Based on these facts, one may suggest that hepatocyte interaction with sinusoidal cells in various zones of the acinus is one of the important factors determining functional heterogeneity of hepatocytes [5]. According to our model (Fig. 2), metabolic specialization of hepatocytes depends on secretory activity of macrophages preferentially localized in the first zone of the acinus [25, 59, 60]. Macrophages secrete a wide range of mediators of intercellular communications (cytokines, prostaglandins, growth factors, etc.); these cells are also involved in metabolism of many blood and biologically active compounds including hormones and lipoproteins. High secretory activity of macrophages results in formation of a gradient of regulatory factors and metabolites in the sinusoid and in the space of Disse (concentration of these factors and metabolites decreases from the first to the third zone of the acinus). Based on this model, it is possi-

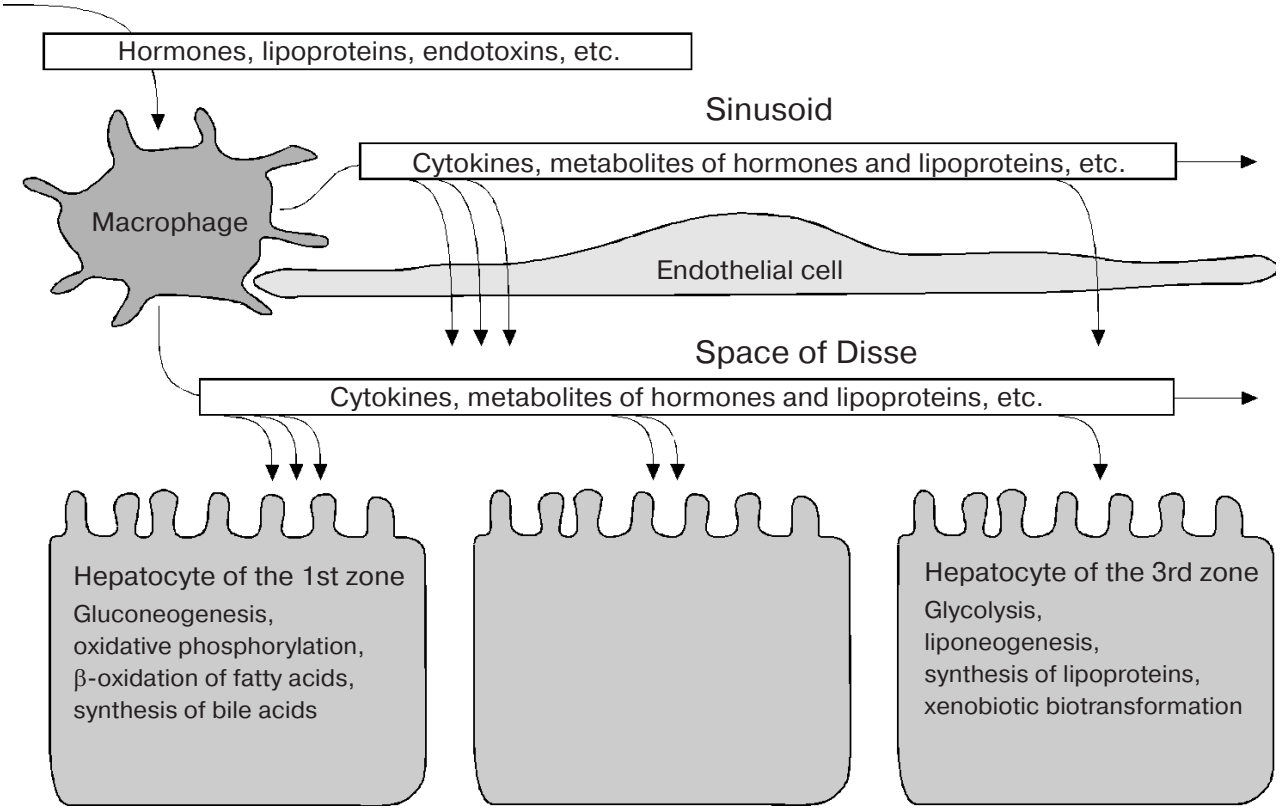

Fig. 2. Scheme illustrating involvement of resident hepatic macrophages in formation of phenotypic heterogeneity of hepatocytes. Metabolic specialization within the acinus depends on secretory activity of macrophages localized preferentially in the first zone of the acinus. Macrophages secrete a wide range of mediators for intercellular communications (cytokines, prostaglandins, etc.); they also participate in metabolism of biologically active compounds of blood plasma (hormones, lipoproteins, etc.). Thus, macrophages form a gradient of regulatory factors and metabolites in the sinusoid and in the space of Disse (concentration of these factors and metabolites decreases from the first to the third zone of the acinus).

ble to explain the fact that metabolism of periportal hepatocytes is directed toward preferential synthesis of glucose from amino acids, which could be supplied by liver macrophages involved in elimination of erythrocytes and blood plasma proteins [176]. The other example is high rate of protein biosynthesis [177] and proliferative activity of hepatocytes of the first zone of the acinus in the intact rats [178, 179] and during reparative regeneration [9, 180]. These processes are controlled by cytokines and growth stimulated factors secreted by macrophages. There is evidence that functional state of hepatocytes also depends on other nonparenchymal liver cells: endothelial, fat-storing, and *pit*-cells also exhibiting high secretory activity. However, the role of these cells in intercellular communications remains poorly investigated.

Thus, there are many facts suggesting phenotypic heterogeneity of hepatocytes depending on their localization along the hepatic sinusoid. Differences between hepatocytes (in ultrastructure, gene expression, metabolic activity, and sensitivity to inducers) probably reflect the existence of a gradient of regulatory factors and metabolites that exists inside the sinusoid. The role of humoral and paracrine factors in regulation of hepatocyte functions is well known. However, the origin of a gradient inside the sinusoid remains unknown. The above-mentioned facts suggest that resident macrophages localized preferentially in the periportal zone of the acinus may play an important role in formation of such gradient. Macrophages secrete a wide range of mediators of intercellular communications; they are also involved in metabolism of many blood compounds and biologically active substances, in the sinusoid and the space of Disse these cells form a concentration gradient of regulatory factors and metabolites inducing phenotypic differences between hepatocytes. Subsequent progress in investigation of this phenomenon will undoubtedly depend on understanding of molecular mechanisms of hepatocyte interaction not only with macrophages, but also with other sinusoidal liver elements, first of all with endothelial cells. Endothelial cells play not only barrier and protective functions for hepatocytes; they may determine their functional activity by secreting regulatory mediators into the extracellular space and also by involvement in transcytosis of various blood components.

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