0270-9139/89/0901-0154$02.00/0
HEPATOLOGY

Vol. 9, No. 1, pp. 154-160, 1989
Printed in U.S.A.

Editorials

Hepatocyte Heterogeneity: The Coming of Age From the Description of a Biological Curiosity to a Partial Understanding of Its Physiological Meaning and Regulation

Morphological and Histochemical Description of Hepatocyte Heterogeneity. Studies on the functional heterogeneity of hepatocytes appeared in the literature as early as 1856 (1). In that publication, Beale described the heterogeneous distribution of "oil" among hepatocytes of the liver lobule as well as the different contribution of hepatocytes to bile secretion. In the 1920's, Noel (2) proposed the division of the hepatic lobule into three functional zones. In the 1950's, the work of Rappaport provided the concept that the simple liver acinus rather than the hepatic lobule represented the structural and functional unit of hepatic parenchyma (3). More recently, other groups have raised the possibility that the acinus, as described by Rappaport, may not fully describe the unit structure of the liver (4, 5). More data are needed to solve this discrepancy between Rappaport's proposal and these studies.

The essential feature of the acinar concept is that, within this unit, blood originating in terminal portal venules and hepatic arterioles perfuses hepatocytes unidirectionally. All hepatocytes of this unit are perfused with blood originating in the same vessels. Within the acinus, exchange of solutes between blood and hepatocytes occurs in a sequential manner, from hepatocytes located at the entry or "periportal" to those located at the exit or "perivenous" region. The splanchnic and arterial blood is progressively modified as it crosses the acinar sinusoids by uptake of substrates into and secretion from hepatocytes. It finally empties into hepatic venules reaching the systemic circulation. This sequential modification of sinusoidal blood implies that hepatocytes located at various points between the entry of blood into the acinus and the exit at the hepatic venules are perfused with blood of varying composition, creating a microenvironmental heterogeneity. In the 1960's, the work of Novikoff (6), Shank, Morrison (7) and others (8), using histochemistry and measurements of enzyme activities in samples microdissected from periportal and perivenous areas, provided evidence for the heterogenous zonal distribution of various enzymes. Maximal differences were seen between hepatocytes located at the entry and exit of the acinus, and thus an arbitrary division of the structural unit into three functional zones was proposed. These were called zones 1, 2 and 3 by Rappaport and are equivalent to the periportal, intermediate and perivenous regions only if the landmark used as reference is a terminal portal venule (3). The general consensus was that the main factor regulating these functional differences was the perfusion of these zones with blood of varying composition. To this extent, hepatocyte heterogeneity was seen as an adaptation to a changing sinusoidal microenvironment, a concept which is still prevalent, although not adequately tested.

Description of the Zonal Heterogeneity for Complex Enzymatic Systems: The Concept of Metabolic Zonation. The work performed in the 1960's described hepatocyte heterogeneity for individual enzymes. In the 1970's, European investigators proposed that there was a zonal organization for groups of enzymes working in specific metabolic pathways. This was one of the first attempts to assign hepatocyte heterogeneity a more definitive role in hepatic physiology. The work of Jungermann, Katz, Sasse, Teutsch, Schmidt and others on the acinar compartmentation of carbohydrate metabolism gave rise to the concept of "metabolic zonation" within the liver. Periportal hepatocytes were seen as glucose-forming cells, whereas perivenous hepatocytes were glucose-consuming cells. In this proposal, the key event, the shift between glucose uptake and glucose release, did not require a change in the direction of the metabolic flux within a single cell. Rather, these apparently antagonistic processes would occur in different hepatocytes (9-13). At this point, interest began to center on the study of the mechanisms regulating this metabolic zonation. Approaches different or complementary to the histochemical techniques previously used were needed. Probst, working in Jungermann's laboratory (14), developed a novel approach by attempting to mimic the microenvironmental conditions of periportal and perivenous hepatocytes existing *in vivo*, using hepatocytes in culture. With glucagon as the major hormone in the media, the relative content of enzymes involved in carbohydrate metabolism in these hepatocytes in culture in some respects "resembled" that of periportal hepatocytes. With insulin as the major enzyme, hepatocytes seem to resemble perivenous hepatocytes.

To address the study of the possible mechanisms involved in the regulation of hepatocyte heterogeneity, it

Address reprint requests to: Jorge J. Gumucio, M.D., Department of Medicine, Veterans Administration Medical Center, 2215 Fuller Rd., Ann Arbor, Michigan 48105.

was desirable to work with hepatocytes isolated from one or another functional zone. Several groups attempted this isolation but with uncertain results. The main difficult was in the assessment of the zonal origin of the isolated hepatocyte subpopulations. By implication, it was difficult to quantitate the degree of "contamination" of these preparations with cells from another functional zone. This is still an unresolved problem, although more recent attempts using selective anterograde (portal to hepatic vein direction) or retrograde perfusion with digitonin, as suggested by Lindros (15) and by Quistorff (16), has been used by several groups. As discussed below, this technique may represent a valid approach to certain studies. However, as with any damaging agent, care should be taken in assessing the morphological and biochemical integrity of the "nondamaged" area specially, in experiments depending on the integrity of plasma membranes. Further assessment of the advantages and limitations of this approach are needed.

In the 1980's, the field exploded with numerous publications emanating from Canada, Europe and this country describing other cellular processes which were also compartmentalized within the liver acinus. Several of these studies moved the concept of hepatocyte heterogeneity into the pathophysiological field by addressing the issue of the perivenous damage observed after hypoxia and drug toxicity (17–20). In an effort to determine the functional implications of zonal damage as seen in drug-induced liver disease, attempts were made to damage predominantly one acinar zone and then to study the influence of zonal damage on bile formation (21). These studies showed that all acinar hepatocytes were capable of taking up bile salts. However, under physiological conditions and due to the limited availability of bile salts, periportal and intermediate hepatocytes contributed predominantly to bile salt uptake and, thus, to the generation of the bile salt-dependent fraction of canalicular bile.

From a physiological standpoint, it became of interest to predict zonal metabolic fluxes. Metabolic fluxes depend not only on the zonal location and activity of rate-limiting enzymes but are modulated also by the availability of substrates, cofactors and other regulators (22). Therefore, delivery of substrates into the various acinar zones was an important issue that was studied by various groups. Goresky (23) provided one of the first studies using autoradiography to visualize the predominant uptake of galactose by periportal hepatocytes. Similarly, LeBouton (24) studied protein metabolism and showed the preferential incorporation of labeled amino acids by periportal hepatocytes. Groothuis et al. (25), also using autoradiography, showed that the uptake of small concentrations of taurocholate occurs in hepatocytes of the periportal region. Increasing the acinar load of taurocholate resulted in the recruitment of intermediate and perivenous hepatocytes to the task of taurocholate transport.

In this issue of *Hepatology*, Burger et al. (26) publish their work on the zonal distribution of the amino acid transport systems A, N and G^- as well as the sodium-independent uptake of some amino acids. In this study, the authors used preparations of hepatocytes obtained after selective damage of the periportal or perivenous zone with digitonin followed by collagenase-induced cell separation (15, 16). The resultant populations had high and low activities for glutamine synthetase and were presumably enriched in perivenous or periportal hepatocytes, respectively. The authors observed a higher rate of sodium-independent transport of histidine by perivenous cell preparations, results which they interpreted as suggestive of a significant role for facilitated diffusion in glutamine export by perivenous hepatocytes. Also, they observed a higher rate of sodium-dependent glutamate uptake (System G-) by the perivenous hepatocytes. These findings are better understood within the context of the previously published work already performed by this and other groups on the acinar organization for ammonia metabolism. Haussinger, in previous studies using the perfused rat liver (27), proposed that glutamate was mainly used for glutamine synthesis by hepatocytes of the perivenous region. A 15- to 20-fold higher transport capacity for glutamate was calculated in hepatocytes synthesizing glutamine than in controls. The interesting study of Burger et al. provides a more direct assessment of the transport capabilities of the perivenous hepatocyte preparation, and the findings on glutamate uptake are essentially in agreement with those of previous studies utilizing more indirect approaches.

The work of Haussinger, Gerok, Gebhardt, Moorman, Lamers and others on the acinar organization for ammonia removal and processing illustrates the importance of hepatocyte heterogeneity on liver function. Thus, Gaasbeek et al. (28) found that carbamylphosphate synthetase, an enzyme involved in urea synthesis, is predominantly located in periportal and midzonal hepatocytes. Gebhardt and Mecke (29) also using immunofluorescence techniques, reported that glutamine synthetase is localized exclusively in one to two hepatocytes surrounding the hepatic venules. After cell dissociation and culture for 3 days, the heterogeneity of expression of glutamine synthetase was maintained, although the intensity of the fluorescence signal had decreased. Induction of this enzyme by dexamethasone and growth hormone in hepatocytes in culture suggested that the same cells expressing glutamine synthetase constitutively, but not other hepatocytes, were inducible by these hormones. Haussinger and Gerok (30) employed the isolated rat liver, perfused either anterograde or retrograde, under conditions in which ammonia supply was rate limiting. Hepatocytes located closer to the terminal portal venule were observed to be involved predominantly in urea synthesis, whereas those near the hepatic venule were mainly involved in glutamine synthesis and secretion, in agreement with the immunofluorescent localization of carbamylphosphate synthetase and glutamine synthetase, respectively. Glutamine released by perivenous hepatocytes enters the systemic circulation and on another passage is taken up by periportal hepatocytes by a sodium-dependent process. This example demonstrates the importance of the intercellular solute cycling in liver function (30). Another interesting aspect of this work which sheds light on the metabolic organization of the liver acinus is the obser-

vation that these two ammonia removal pathways, urea synthesis and glutamine synthesis, differ markedly in their affinity for ammonia. Ammonia removal via urea synthesis seems to be restricted to incoming ammonia concentrations above 50 μM, whereas glutamine synthesis occurs at concentrations of ammonia below 50 μM (30). This represents an example of two enzymatic systems in sequence, one periportal, with a high K_m (carbamyl phosphate synthetase involved in urea synthesis and appropriately exposed to higher concentrations of ammonia), the other with a low K_m, perivenous glutamine synthetase.

Physiological Significance of Hepatocyte Heterogeneity. A pattern of organization begins to emerge from these studies. The unidirectional perfusion of the liver acinus results in the exposure of periportal hepatocytes to high concentrations of solutes. Interaction between solutes and the plasma membrane at the acinar entry is favored by a high surface to volume ratio of sinusoids in this area (31). Some transport systems, such as that for bile salts and ammonia, have a great capacity for uptake and processing. Substances not taken up by these first hepatocytes or refluxing from these hepatocytes back into sinusoidal blood can be taken up by hepatocytes located "downstream." This downstream uptake may be favored by a higher affinity of these systems for some substrates, such as in the case of ammonia. The net result is the sequential modification of incoming blood. However, differences in uptake and processing are both quantitative and qualitative, as exemplified by the apparent selective synthesis and secretion of glutamine by perivenous hepatocytes. Substances can be selectively subtracted or added to sinusoidal blood by perivenous hepatocytes before blood reaches the systemic circulation, thus allowing a final adjustment in solute concentration. Therefore, hepatocyte heterogeneity provides a versatile and complex organization by which the liver can remove, process and deliver substrates of splanchnic origin as well as products of its own synthetic machinery into the systemic circulation and into bile. The various factors in sinusoidal blood which may act as feedback signals for hepatocytes of one zone to alter the pattern of uptake, processing, synthesis or secretion are unknown but may involve, among others, variations in the incoming concentration of substrates, cofactors, hormones and the changing patterns of acinar blood flow, presumably under hepatic nerve regulation (32, 33).

Regulation of Hepatocyte Heterogeneity. The assumption that hepatocyte heterogeneity develops as a consequence of the microenvironmental heterogeneity created by the sequential perfusion of acinar hepatocytes has been generally accepted. Differences in oxygen supply, in hormonal concentrations and in the availability of substrates and cofactors have been mentioned as possible modulators of hepatocyte heterogeneity and metabolic compartmentation. Kessler et al. (34) demonstrated in the late 1970's that the distribution of oxygen tension on the liver surface was heterogeneous; oxygen tension ranged between 0 and 60 torr in the liver *in situ*. It has been estimated that there is a difference of about 50 μM in the concentration of dissolved oxygen *in vivo* between the portal vein and the venous exit of the liver. Therefore, a gradient of oxygen concentration in sinusoidal blood must exist between the periportal and the perivenous region. However, the concentration of oxygen bathing each zone *in vivo* still has to be defined.

Chance et al. (35, 36) developed a system to monitor tissue oxygenation based on the finding that reduced pyridine nucleotides (NADH and NADPH) but not the oxidized forms are fluorescent (450 nm) on excitation with light at 366 nm. Thurman's laboratory has determined zonal oxygen gradients using micro-light guides placed on periportal or perivenous regions. These regions are recognizable on the surface of the hemoglobin-free perfused liver as light and dark areas, respectively (19). In control livers, the oxygen tension measured by this approach was 208 torr in periportal hepatocytes and 67 torr in perivenous hepatocytes (37). By extrapolation of this technique, Belinsky et al. (38) studied mixed-function oxidation in phenobarbital-treated rat livers perfused with 7-ethoxycoumarin. The rates of 7-hydroxycoumarin formation measured on perivenous regions were more than twice those measured on periportal regions. In addition, Thurman et al. (39) tested the importance of the direction of blood flow on metabolic compartmentation within the liver acinus by assessing the preferential contribution of periportal or perivenous hepatocytes to various metabolic processes during anterograde or retrograde liver perfusion. The acinar location of the areas of maximal oxygen consumption, gluconeogenesis, glycolysis and ketogenesis changed immediately following a change in direction of perfusion. Therefore, the periportal or perivenous location of these processes was regulated by the availability of substances which were delivered in highest concentrations to the hepatocytes first perfused. However, the predominant location of mixed-function oxidation in perivenous hepatocytes was not altered by a change in the direction of liver perfusion.

Therefore, at least two types of regulation of hepatocyte heterogeneity can be proposed. Some metabolic processes, as mentioned above, will occur predominantly in an acinar zone due to the availability of a rate-limiting factor carried in sinusoidal blood. In this type of regulation, the direction of blood perfusion will be crucial. The result of a change in the direction of perfusion will be a rapid change in the activity of a metabolic system in one zone. Carbohydrate metabolism is an example of a process subject to this type of regulation. The dynamic nature of the zonal compartmentation for carbohydrate metabolism, as described by several authors, testifies to a dependence on the composition of the sinusoidal blood (10–12). In contrast, the zonal heterogeneity for other processes seem to be differently regulated. For instance, how is the zonal expression of glutamine synthetase or α2-microglobulin, another protein almost exclusively expressed in few hepatocytes surrounding the hepatic venules (40), or cytochrome P-450 induction by phenobarbital regulated? Studies in our laboratory have shown that the most likely molecular mechanism responsible for the selective induction of the phenobarbital-inducible

cytochrome P-450 protein in perivenous hepatocytes, as first shown by Baron et al. (41), is a difference in the rate of transcription of the cytochromes P-450b and e genes, the two major forms of this P-450 family, between perivenous and periportal hepatocytes. Experiments using *in situ* hybridization showed that after 16 hr of phenobarbital administration, both P-450b,e protein and b,e mRNA were induced in approximately 15 hepatocytes located in the midzonal and perivenous regions. About 5 to 8 hepatocytes closer to the terminal portal venules or periportal cells showed minimal induction at both the protein and mRNA level (42). The finding that mRNAs and proteins were induced in the same hepatocytes made translational regulation unlikely and a regulatory process involving the zonal modulation of transcription of P-450b,e genes most likely. Other experiments showed that induction was accomplished by the parent phenobarbital molecule and not by its main metabolite, *p*-hydroxyphenobarbital. Furthermore, the increase in transcription rate of the cytochromes P-450b,e genes measured by runoff studies using nuclei isolated after 1 to 3 hr of phenobarbital administration was abolished by the previous administration of cycloheximide. This result suggested the possible participation of proteins of relatively short half-life in the regulation of the heterogeneous expression of these genes (43). Therefore, zonal regulation of the pattern of gene expression is another mechanism by which the functional heterogeneity of the liver acinus is modulated. But is the increment in transcription rate of cytochromes P-450b,e genes in perivenous hepatocytes or the relative lack of response to phenobarbital of periportal hepatocytes dependent on the direction of blood perfusion? As mentioned, short-term perfusion experiments (39) did not alter the predominant perivenous location of mixed-function oxidation. Efforts in our laboratory (Traber et al., unpublished observations) to induce P-450b,e genes in the perfused liver (and subsequently, to reverse the direction of perfusion) have provided levels of induction too low for an adequate assessment. Using a different approach, we have recently shown (44) that cytochromes P-450b,e genes can be induced by phenobarbital in hepatocytes transplanted into the spleen 6 months prior to the experiments. A 20- to 30-fold induction occurs in some, but not all, transplanted hepatocytes, *i.e.* there seems to be a pattern of heterogeneous induction. It should be noted that intrasplenically transplanted hepatocytes do not show an organization into liver acini and are perfused with blood of a composition that is, most likely, very different from that of sinusoidal blood in the perivenous area. Therefore, these results raise the possibility that the regulation of the zonal rate of transcription of the P-450b,e genes after phenobarbital administration may not be dependent on the direction of blood perfusion or on the composition of sinusoidal blood bathing perivenous hepatocytes. Then, what regulates the possible zonal differences in transcription rate of these genes in response to phenobarbital? Is the capacity for a response to phenobarbital with induction of cytochromes P-450b,e genes imprinted in some hepatocytes, *i.e.* is the information present in the cells, independent of the sinusoidal microenvironment, and dependent on the capability of hepatocytes to maintain transcription of regulatory proteins which act as trans-acting factors? These are possibilities which need to be studied. The elucidation of these questions should provide a better understanding of this second type of regulation of hepatocyte heterogeneity. This type of regulation may not depend on the direction of blood flow but may represent a different state of differentiation or specialization attained by perivenous hepatocytes as these cells presumably move from the original periportal location to the perivenous region (45, 46).

What about glutamine synthetase and α2-microglobulin? The expression of these proteins is constitutive to only a few hepatocytes which are in immediate contact with the hepatic venules. Glutamine synthetase is apparently inducible by dexamethasone and growth hormone only in the same hepatocytes expressing the enzyme constitutively (29). Recent studies by Moorman et al. (47) using *in situ* hybridization have shown that mRNAs for carbamylphosphate synthetase and for glutamine synthetase are present only in the same hepatocytes expressing the corresponding proteins. This suggests that there is a zonal modulation of the expression of these genes which most likely is transcriptional, as also found for P-450 genes (42). α2-Microglobulin is expressed in males but not in females and is inducible by hormones by a process of recruitment of new hepatocytes, starting from the immediate vicinity of the hepatic venules toward the intermediate zone. The pattern of constitutive expression and that of induction suggest that, in spite of the apparent similarities in regulation of the zonal expression of some of these genes, there seem to be differences among them. For instance, glutamine synthetase induction is restricted to the same hepatocytes expressing the enzyme constitutively. In contrast, α2-microglobulin expression can be induced in hepatocytes originally not expressing this protein, but this expression is under the control of sex hormones and induction is still restricted to few hepatocytes, whereas P-450b,e can be induced in most acinar hepatocytes with the exception of a few periportal cells.

As suggested by Gebhardt and Mecke (29), working with glutamine synthetase, and by Roy et al. (40) studying α2-microglobulin expression, it is conceivable that the regulation of the expression of these systems within the acinus may be influenced by contact with mesothelial or endothelial cells of the hepatic venules. Whether the regulation of the expression of genes in the hepatocytes of the periportal limiting plate may be influenced by the endothelial or mesothelial cells of the surrounding vessels needs to be studied. Within this context, the possible influence of other liver cells on hepatocytes of the various acinar zones also remains to be studied.

The Development of Hepatocyte Heterogeneity. A different but relevant question is: when during liver development is the zonal pattern of hepatocyte heterogeneity manifested? This is interesting because the elucidation of this problem can give us some clues as to the mechanisms which may be involved in determining the heterogeneous zonal pattern. The work of Kanai et

al. (48), using morphological approaches to assess the perivenous induction of the smooth endoplasmic reticulum by phenobarbital in newborn mice, proposed that this was a postnatally acquired capability. A similar conclusion was reached concerning the development of the metabolic zonation for carbohydrate metabolism (49). The work of Lamers et al. (50), on the other hand, has shown that the heterogeneous expression of glutamine synthetase is already observed in fetal liver, leading to the proposal that the perivenous pattern of gene expression develops before birth and prior to that of the periportal compartment.

Therefore, it can be proposed that there are at least two or three regulatory patterns responsible for hepatocyte heterogeneity. First, it is conceivable that the adult pattern of liver circulation may influence the development of a zonal pattern of heterogeneity in those metabolic systems which are controlled by the availability of substrates or cofactors (carbohydrate metabolic zonation), as discussed above. Second, an imprinted pattern of zonal gene expression which may not be readily susceptible to the sinusoidal microenvironment may develop very early in life for other systems (glutamine synthetase, P-450b). Finally, it is plausible that other heterogeneous systems, those in which gene expression is clearly influenced, for instance, by the levels of hormones, may represent examples of a third type of regulatory pattern in which the quantity of a protein expressed in an imprinted zone may be regulated by the sinusoidal microenvironment.

The question can now be asked: how many functional zones are there in the hepatic acinus? This is a difficult question which does not have a simple answer. There seem to be at least two elements commanding zonal responses, the sequential delivery of substrates and the zonal patterns of gene expression. Zonation of processes dependent on the availability of rate-limiting factors carried by sinusoidal blood, such as may be the case for carbohydrate metabolism, will follow the pattern of delivery of that regulator(s). A metabolic compartmentation will be established with a gluconeogenic zone at the entrance and a glycolytic zone at the acinar exit. In contrast, the division into functional zones of processes in which the central element responsible for the zonal heterogeneity is differential modulation of gene expression, represents a more complex and less predictable situation. Some genes, such as albumin, are expressed by all acinar hepatocytes. In contrast, other genes are expressed in a rather restricted manner. Glutamine synthetase and α2-microglobulin genes are expressed only in the one to two hepatocytes surrounding the hepatic venules and, as discussed, these may be under the modulatory influence of mesothelial or endothelial cells in these vessels. At the other extreme, although no data are available, it is plausible that the one to two hepatocytes of the periportal limiting plate may also differ in gene expression and may be under the regulatory influence of endothelial or mesothelial cells of the terminal portal venules. What are the hints that this zone may be differently regulated? It is of interest in terms of potential capabilities and differential responses to regulatory signals that these hepatocytes may be precursors of intrahepatic bile ducts (51). Also, the proposal has been made that mesothelial cells of the portal vessels may have a regulatory influence in the generation of bile duct cells from hepatocytes (51). Moreover, several of the first periportal hepatocytes have the capacity for cell replication under physiological cell renewal (45, 52). However, during liver regeneration following partial hepatectomy, the hepatocytes of the portal limiting plate are labeled with ^{3}H-thymidine to a lesser extent than other periportal hepatocytes. The hepatocytes located 40 to 60 μm (two to three cells) distant from the portal venules showed maximal thymidine incorporation (52). Finally, hepatocytes of the portal limiting plate participate in transport processes differently from other periportal hepatocytes (see questions and answers section of Ref. 52). Therefore, gene expression in hepatocytes surrounding terminal portal and hepatic venules may be regulated differently and thus represent different functional zones. What about the rest of hepatocytes?

Between the periportal limiting plate and the perivenous hepatocytes, there are about 20 to 22 hepatocytes in a hemiacinus (distance from one portal to a hepatic venule). It has been proposed that hepatocytes derived from the periportal zone move along the acinus, changing into intermediate and perivenous hepatocytes (45, 46). The question can be raised: are there variations in cell differentiation as hepatocytes move toward the perivenular region? Usually, cells which have the capacity for rapid replication are poorly differentiated. However, these periportal hepatocytes outside of the limiting plate are capable of synthesizing albumin and participate very actively in transport processes, such as Na^+-coupled bile salt uptake, secretion of the bile salt-dependent fraction of bile, urea synthesis, etc. Therefore, these hepatocytes are highly differentiated, a state which seems compatible with the slow replicative process occurring in liver. These first five to eight hepatocytes preferentially express several proteins, such as carbamylphosphate synthetase. However, these hepatocytes do not respond or respond poorly to phenobarbital induction of cytochromes P-450b,e genes. The predominant constitutive-inducive expression of several of the P-450 genes seems to occur in hepatocytes of the distal third of the acinus. Therefore, the expression of different genes in hepatocytes in various locations creates specialized areas contributing to the sequential processing of material within the acinus. However, the analysis of the present data suggests that this specialization represents compartmentation by zonal specialization of hepatocytes which otherwise seem to have attained comparable levels of cell differentiation. To this extent, the liver is different from other organs, such as the small intestine or the epidermis, which have a basal or germinal cell layer that moves and gives rise to a more differentiated cell capable of synthesizing an end product(s), such as skin keratin, which characterizes one of the main functions of the organ. Whether hepatocytes attain higher levels of cell differentiation once in the perivenular location, although not apparent now, will have to be assessed further as new data become available. Regardless, what triggers the appearance of different

patterns of gene expression as hepatocytes apparently move along the acinus is one of the main questions to be answered.

Given this background, the present division of the liver acinus into three functional zones may be inappropiate and restrictive. While it may be possible to characterize various functional zones depending on patterns of expression and types of regulation, it may be more realistic and useful, at this point, to divide the acinus according to the function to be studied. For instance, for studies involving P-450b,e induction by phenobarbital, there are 15 to 20 "responsive" and five to eight "non- or less responsive" hepatocytes. For glutamine synthetase, there seems to be a large zone (possibly 20 of the 22 to 25 hepatocytes of a hemiacinus) in which there is no expression and no induction, and a very narrow zone represented by one to two hepatocytes expressing the enzyme constitutively. A similar example is the narrow pattern of α2-microglobulin expression. For carbohydrate metabolism, lipid metabolism or others, the situation, as already discussed, may be entirely different. As a corollary, one may propose that the experimental approach to the study of zonal heterogeneity has to be tailored to the compartmentation of the process under study. In this regard, screening approaches involving histochemistry may be helpful.

Is the approach followed by Burger et al. (26) in the paper published in this issue of *Hepatology*, suitable to every study? Light microscopy of the liver after digitonin perfusion shows damage to about half of the hepatocytes of the acinus first in contact with this compound. To this extent, this method seems to be a reasonable approach. However, although the damaged and "nondamaged" areas are well demarcated, it is possible that a more subtle morphological or biochemical damage may have been inflicted to hepatocytes distal to the direction of perfusion. This may be important in interpreting experiments performed immediately after isolation especially when integrity of plasma membranes is required. Given that, as discussed above, the extent of a functional zone varies with the system studied, this approach may represent a good initial separation to be followed by a more specific purification of the cells to be studied. Some caution should be exerted in interpreting the purity of hepatocyte subpopulations when using some of the marker enzymes utilized by Burger et al. It may be possible to isolate a population of glutamine synthetase-free hepatocytes but that finding only tells us that the last one to three cells near the hepatic venules are not present in that preparation. If the focus of interest is the hepatocytes located in the periportal limiting plate, this would be a very "contaminated" preparation. Conversely, if expression-regulation of glutamine synthetase are the object of study, a demonstrable high activity of this enzyme only reveals that we have, in that preparation, those few hepatocytes containing the enzyme, whereas the preparation may be quite "contaminated" by other cells. It may represent a good starting point, but further hepatocyte purification will be necessary. These limitations are not unique to the digitonin approach. Every method of isolation of zonal hepatocytes has pitfalls with respect to purity, yield or toxicity. Given these limitations, as well as the importance of cell-to-cell and cell-to-biomatrix interactions in the expression and regulation of functional processes in liver, other investigators have opted for approaches in which the tissue integrity of the liver acinus is preserved. Experiments *in vivo* or those using the liver perfused in an anterograde or retrograde direction preserve these communications. The drawback is in the complexity of the interpretation. Nevertheless, data obtained with a variety of these systems have proved useful. Isolated cells, hepatocyte subpopulations, perfused liver, hepatocytes in culture and surface fluorescence all have advantages and limitations. There is not a single method that can be recommended as a general approach. Rather, given the problems encountered with each system, experimental designs comprising several approaches should be used to be certain that the observation is correct. Given the power of the techniques, experiments utilizing some of these approaches in conjunction with molecular biology techniques are highly desirable.

Hepatocyte heterogeneity is no longer a simple biological curiosity. Rather, it can be seen as a necessary organization for the liver acinus to accomplish the function of regulation of an adequate concentration of substrates, metabolites, proteins and hormones, among others, in the terminal hepatic venules and in bile. To this extent, the study of hepatocyte heterogeneity has opened up new perspectives in the field of hepatic physiology. However, it should be kept in mind that these considerations about heterogeneity and liver function have been decanted mainly from animal experiments. Whether these proposals are also applicable to human liver remains to be established. More studies on the hepatocyte heterogeneity of human liver are needed.

JORGE J. GUMUCIO, M.D.
Veterans Administration Medical Center
University of Michigan School of Medicine
Ann Arbor, Michigan 48105

REFERENCES

1. Beale LS. The minute anatomy of the liver. Med Times Gazette 1856; 13:82–85.
2. Noel R. Sur la cellule hépatique du mammifère. Arch Anat Microsc 1923; 19:1–158.
3. Rappaport AM. The structural and functional unit of the human liver (liver acinus). Anat Rec 1958; 130:637–686.
4. Teutsch HF. Regionality of glucose-6-phosphate hydrolysis in the liver lobule of the rat: metabolic heterogeneity of "portal" and "septal" sinusoids. Hepatology 1988; 8:311–317.
5. Matsumoto T, Komori R, Magara T, et al. A study on the normal structure of the human liver with special reference to its angioarchitecture. Jikekai Med J 1979; 26:1–40.
6. Novikoff AB, Essner E. The liver cell. Some new approaches to its study. Am J Med 1960; 29:102–131.
7. Shank RE, Morrison G, Cheng CH, et al. Cell heterogeneity within the liver lobule (quantitative histochemistry). J Histochem Cytochem 1959; 7:237–239.
8. Gumucio JJ, Miller DL. Liver cell heterogeneity. In: Arias IM, Popper H, Schachter D, et al. The liver: biology and pathobiology. New York: Raven Press, 1982: 647–662.
9. Jungermann K. Metabolic zonation of liver parenchyma: significance for the regulation of glycogen metabolism, gluconeogenesis

and glycolysis. Diabetes Metab Rev 1987; 3:269–293.
10. Katz N, Jungermann K. Autoregulatory shift from fructolysis to lactate gluconeogenesis in rat hepatocytes suspensions: the problem of metabolic zonation of liver parenchyma. Hoppe-Seyler's Z Physiol Chem 1976; 357:359–375.
11. Sasse D. Dynamics of liver glycogen. Histochemistry 1975; 4:237–254.
12. Katz N, Teutsch HF, Jungermann K, et al. Heterogeneous reciprocal localization of fructose-1,6 biphosphatase and glucokinase in microdissected periportal and perivenous rat liver tissue. FEBS Lett 1977; 83:272–276.
13. Schmidt U, Schmid H, Guber W. Liver cell heterogeneity: the distribution of fructose biphosphatase in fed and fasted rats and in man. Hoppe-Seyler's Z Physiol Chem 1978; 359:193–198.
14. Probst I, Schwartz P, Jungermann K. Induction in primary culture of "gluconeogenic" and "glycolytic" hepatocytes resembling periportal and perivenous cells. Eur J Biochem 1982; 126:271–278.
15. Lindros KO, Bengtsson G, Salaspuro M, et al. Separation of functionally different liver cell types. In: Thurman RG, Kaufmman FC, Jungermann K, eds. Regulation of hepatic metabolism: intra- and intercellular compartmentation. New York and London: Plenum Press, 1986: 137–158.
16. Quistorff B, Grunnet N, Cornell N. Digitonin perfusion of rat liver. A new approach in the study of intra-acinar and intracellular compartmentation in the liver. Biochem J 1985; 226:289–297.
17. Israel Y, Kalant H, Orrego H, et al. Experimental alcohol-induced hepatic necrosis: suppression by propylthiouracil. Proc Natl Acad Sci USA 1975; 72:1137–1141.
18. Sies H. Cytochrome oxidase and urate oxidase as intracellular oxygen indicators in studies of oxygen gradients during hypoxia in liver. Adv Exp Med Biol 1978; 94:561–566.
19. Ji S, Lemasters JJ, Christenson V, et al. Periportal and pericentral pyridine nucleotide fluorescence from the surface of the perfused liver: evaluation of the hypothesis that chronic ethanol produces pericentral hypoxia. Proc Natl Acad Sci USA 1982; 79:5415–5419.
20. Pang KS, Terrell JA. Retrograde perfusion to probe the heterogeneous distribution of hepatic drug metabolizing enzymes in rats. J Pharmacol Exp Ther 1981; 216:339–346.
21. Gumuncio JJ, Balabaud C, Miller DL, et al. Bile secretion and liver cell heterogeneity in the rat. J Lab Clin Med 1978; 91:350–362.
22. Gumucio JJ, Miller DL. Functional implications of liver cell heterogeneity. Gastroenterology 1981; 80:393–403.
23. Goresky CA, Baeh GG, Nadeau BE. On the uptake of material by the intact liver. The transport and net removal of galactose. J Clin Invest 1973; 991–1009.
24. LeBouton AV. Heterogeneity of protein metabolism between liver cells as studied by radioautography. Curr Mod Biol 1968; 2:111–114.
25. Groothuis GM, Hardonk MJ, Karleman KPT, et al. Autoradiographic and kinetic demonstration of acinar heterogeneity of taurocholate transport. Am J Physiol 1981; 243:G455–G462.
26. Burger HJ, Gebhardt R, Mayer C, et al. Different capacities for amino acid transport in periportal and perivenous hepatocytes isolated by digitonin/collagenase perfusion. Hepatology 1989; 9:22–28.
27. Haussinger D. Hepatocyte heterogeneity in glutamine and ammonia metabolism and the role of an intercellular glutamine cycle during ureogenesis in perfused rat liver. Eur J Biochem 1983; 133:269–275.
28. Gaasbeek Janzen JW, Lamers WH, Moorman AFM, et al. Immunohistochemical localization of carbamylphosphate synthetase (ammonia) in adult rat liver: evidence for a heterogeneous distribution. J Histochem Cytochem 1985; 33:1205–1211.
29. Gebhardt R, Mecke D. Heterogeneous distribution of glutamine synthetase among rat liver parenchymal cells in situ and in primary culture. EMBO J 1983; 2:567–570.
30. Haussinger D, Gerok W. Metabolism of amino acids and ammonia. In: Thurman RG, Kauffman FC, Jungermann K, eds. Regulation of hepatic metabolism: intra- and intercellular compartmentation. New York and London: Plenum Press, 1986: 253–291.
31. Gumucio JJ. Functional and anatomical heterogeneity in the liver acinus: impact on transport. Am J Physiol 1983; 244:G578–G582.
32. McCuskey RS. Intrahepatic distribution of nerves: a review. In: Popper H, Bianchi L, Gudat F, et al., eds. Communications of liver cells. 1980: 115–120.
33. Friedman MI. Hepatic nerve function. In: Arias IM, Popper H, Schachter D, et al., eds. The liver: biology and pathobiology. New York: Raven Press, 1988: 949–960.
34. Kessler M, Hoper J, Lubbers DW, et al. Local factors affecting regulation of microflow, oxygen uptake and energy metabolism. Adv Physiol Sci 1980; 25:155–162.
35. Chance B, Cohen P, Jobsis F, et al. Intracellular oxidation-reduction states in vivo: the microfluorimetry of pyridine nucleotide gives a continuous measurement of the oxidation state. Science 1962; 137:1–10.
36. Matschinsky FM, Hintz CS, Reichlmeier K, et al. The intralobular distribution of oxidized and reduced pyridine nucleotides in the liver of normal and diabetic rats. In: Srere PA, Estabrook RW, eds. Microenvironments and metabolic compartmentation. New York: Academic Press, 1978: 149–166.
37. Matsumara T, Thurman RG. Measuring rates of oxygen uptake in periportal and pericentral regions of the liver lobule: stop flow experiments with perfused liver. Am J Physiol 1983; 244:G656–G659.
38. Thurman RG, Kauffman FC, Baron J. Biotransformation and zonal toxicity. In: Thurman RG, Kauffman FC, Jungermann K, eds. Regulation of hepatic metabolism: intra- and intercellular compartmentation. New York and London: Plenum Press, 1986: 321–382.
39. Thurman RG, Kauffman FC. Sublobular compartmentation of pharmacological events (SCOPE): metabolic fluxes in periportal and pericentral regions of the liver lobule. Hepatology 1985; 5:144–151.
40. Roy AK, Sakar FH, Nag AC, et al. Role of cytodifferentiation and cell to cell communication in the androgen dependent expression of alpha-2-microglobulin gene in rat liver. Prog Clin Biol Res 1986; 226:401–415.
41. Baron J, Taira Y, Redick JA, et al. Effect of xenobiotics on the distributions of monooxygenase components in liver. In: Coon MJ, Conney AH, Estabrook RW, eds. Microsomes, drug oxidations and chemical carcinogenesis, Vol. II. New York: Academic Press, 1980: 501–504.
42. Wojcik E, Dvorak C, Chianale J, et al. Demonstration by in situ hybridization of the zonal modulation of rat liver cytochrome P450b and P450e gene expression after phenobarbital. J Clin Invest 1988; 82:658–666.
43. Chianale J, Mulholland L, Traber PG, et al. Phenobarbital-induction of cytochrome P-450 b,e genes is dependent on protein synthesis. Hepatology 1988; 8:327–331.
44. Maganto P, Traber PG, Wojcik E, et al. Intrasplenically transplanted hepatocytes: an expression system for genes involved in drug metabolism (Abstract). Hepatology 1988; 8:1249.
45. Grisham JW. A morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver. Autoradiography with thymidine-^{3}H. Cancer Res 1962; 22:842–849.
46. Zajicek G, Oren J, Weinreb M. The streaming Liver. Liver 1985; 5:293–300.
47. Moorman AFM, de Boer PAJ, Geerts W, et al. Complementary distribution of carbamylphosphate synthetase (ammonia) and glutamine synthase in rat liver acinus is regulated at a pretranslational level. J Histochem Cytochem (in press).
48. Kanai K, Kanamura S, Wanatabe J. Peri- and postnatal development of heterogeneity in the amounts of endoplasmic reticulum in mouse hepatocytes. Am J Anat 1986; 175:471–480.
49. Katz N, Teutsch H, Jungermann K. Perinatal development of the metabolic zonation of hamster liver parenchyma. FEBS Lett 1983; 69:23–28.
50. Lamers WH, Gaasbeek Janzen JW, Kortchot A, et al. Development of enzymic zonation in liver parenchyma is related to development of acinar architecture. Differentiation 1987; 35:228–235.
51. Van Eyken P, Sciot R, Desmet V. Intrahepatic bile duct development in the rat: a cytokeratin-immunohistochemical study. Lab Invest 1988; 59:52–59.
52. Rabes HM, Tuczek HV, Wirsching R. Kinetics of hepatocellular proliferation after partial hepatectomy as a function of structural and biochemical heterogeneity of the rat liver. In: Lesch R, Reutter W, eds. Liver regeneration after experimental injury. III. Workshop on experimental liver injury, Freiburg, W. Germany. New York: Stratton Intercontinental Medical Book Corp., 1975: 35–55.