Microanatomy of the Human Liver—Exploring the Hidden Interfaces

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The functional unit of an organ may be defined as the smallest, structurally distinct, "self-sufficient" unit that can independently subserve all known functions of that organ. The nephron is exemplary of such a concept, for it is both a well-defined functional and structural unit. A similar hepatic unit that can reconcile the dual vascular supply and the dual outflow tracts (vascular and biliary) with all known liver functions has not been identified. This reflects the functional complexity of the liver and the sophistication of its microarchitectural interfaces. This review addresses the recent resurgence of investigation into the microanatomy of the liver, emphasizes studies in humans, and highlights the "grey" zones in our existing knowledge.

THE FUNCTIONAL UNIT OF THE LIVER

The classic and portal lobules, defined on structural characteristics as functional units of the liver, have held sway for more than a century. The former, Kiernan's classic lobule,[1] was a hexagonal lobule with portal tracts at the corners of the hexagon and a hepatic vein at the center (Table 1). The portal lobule described by Mall[2] had a portal tract in the center and central venules at the periphery. A parallel concept of biliary lobule was also based on the portal tract.[3] In 1954, Rappaport et al.[4] described the liver acinus, defining the functional unit on the basis of the microcirculatory flow within the liver. The concepts of classic lobule, portal lobule, and acinus have never been completely accepted, but none have been completely discarded. Although each can successfully explain some of the pathological and physiological processes in the liver, none can explain them all.[5-7] It seems certain that the classic lobule as seen in animals and humans has both a structural and metabolic basis, as it highlights a porto-central gradient of metabolic processes, vascular flow, and biliary drainage. However, it does not explain lateral or portal-portal gradients between adjacent portal tracts as seen in animals and in humans.[8-12] The classic lobule is not the smallest functional unit but rather a composite of many smaller units, with the acinus as one conceptual candidate.

PRIMARY AND SECONDARY LOBULE

Two pioneering concepts have emerged over the last 2 decades in our perception of the lobular architecture. In 1979, Matsumoto et al.,[12] by extensive and painstaking 3-dimensional (3-D) reconstruction of serial sections of the human liver, highlighted the role of vascular "septa" within the liver parenchyma in establishing the framework for lobular architecture, which had already been alluded to in the acinar model described by Rappaport et al.[4] Although Rappaport et al. did not describe the vascular septa as Matsumoto et al. did, they offered a similar concept as evidenced by their observation that terminal arterial and portal venous twigs branched out from portal tracts at different levels and in a tridimensional way.[4] The twigs appeared to be perpendicularly oriented to the central veins, and together with accompanying bile ductules formed the axes of the proposed acinar units.

The "vascular septum"[12] is not a fibrous septum, as we generally understand it. Rather, it is the terminal and tertiary branch of the distributing portal vein (PV), devoid of surrounding connective tissue and only slightly larger than the sinusoids that arise from it. This terminal branch of the PV, which we prefer to call a "portal venule," is a septum inasmuch as it defines the boundaries of, and separates adjoining lobules. Thus, divided by portal venules, the hepatic parenchyma is composed of primary and secondary lobules; the latter being made up of 6 to 8 primary lobules in a hexagonal array that corresponds to the classic lobule as seen on 2-D images. Each side of the hexagon is composed of 2 "vascular septa" containing their respective terminal portal venules arising from adjacent portal tracts. These 2 "vascular septa" meet in the midseptal region, midway between adjacent portal tracts.

In 3-D reconstructions,[12] each primary lobule is a cone-shaped unit bordered by vascular septa and composed of 2 zones: portal and septal. The portal zone is a sickle shaped area wedged against the portal tract with its broadest part abutting the portal tract and the tapered edges spreading laterally towards the adjoining portal tracts on either side (Fig. 1A). This zone is supplied by short inlet venules arising directly from the terminal PV within the portal tract, and the proximal portion of the portal venule. These supply venules break up immediately into sinusoidal branches, which spread transversely before turning inward and heading radially towards a central vein. These sinusoids are referred to as "portal" sinusoids in rat livers.[9] In contrast, the septal zone is fed by inlet venules from the distal part of the portal venule. Sinusoids arising in this region, called the "septal" sinusoids in the rat liver,[9] do not have a transverse course, but immediately head radially towards the central vein. In describing these 2 zones of the primary lobule, Matsumoto et al.[12] introduced 2 novel concepts. First, the sinusoidal bed of transverse sinusoids, constituting the sickle zone, provides an "inflow front" for perfusion of the lobule that is dramatically different from a linear or axial supply hitherto described in the acinus model. The second concept regards the distinction of these 2 zones, as a basis for explaining differences in the metabolic properties of hepatocytes across a lateral gradient

Abbreviations: 3-D, 3-dimensional; PV, portal vein; HMS, hepatic microcirculatory subunit; HA, hepatic artery; THV, terminal hepatic vein; BD, bile duct.

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TABLE 1. Glossary of Recommended Terms*

Inlet sinusoid	The entry channel from a portal venule into the sinusoidal network.†
Portal venule	The final branch of the portal venous system that arises from a terminal portal vein or conducting PV, and enters the parenchyma to form a vascular septum. Also called a "septal" PV.
Terminal portal vein	The smallest branch of the portal vein that gives rise to portal venules. Corresponds to the portal vein seen in terminal portal tracts.‡
Conducting portal vein	The system of larger portal veins that supply terminal portal veins.
Hepatic arteriole	Proposed but controversial supply arteriole from terminal hepatic artery into parenchymal sinusoids in the periportal region.
Terminal hepatic artery	The smallest branch of the hepatic artery; corresponds to hepatic artery seen in terminal portal tracts.
Canal of Hering	A biliary channel lined partially by biliary epithelium (cholangiocytes) and partially by hepatocytes situated at the periphery of portal tracts or within the parenchyma in the periportal region.
Bile ductule	The biliary channel linking the canal of Hering to the terminal bile duct within the terminal portal tract.§
Terminal bile duct	The smallest branch of the portal tract-based biliary system; corresponds to the bile duct seen in terminal portal tracts.
Terminal portal tract	A mesenchyme-based structure containing 1 or more parallel sets of (bile duct and hepatic artery), and only 1 portal venous channel; the classic "portal triad." ‖
Terminal hepatic vein	The smallest branch of the hepatic venous system situated in the center of the secondary as well as the classic lobule; also called the central vein.
Classic lobule	The hexagonal lobule as originally described by Kiernan with the terminal hepatic vein (central vein) surrounded by 6 terminal portal tracts.
Primary lobule	The cone-shaped zone of parenchyma formed by the sinusoids given off by 2 adjacent portal venules. The primary lobule thus encompasses 2 portal venules, their inlet sinusoids, hepatic arterioles, bile ductules, and canals of Hering.
Secondary lobule	A unit made up of 6 primary lobules with the terminal hepatic vein in the center and 6 portal tracts at the periphery, and bordered by terminal portal venules. In 2 dimensions, it corresponds to the classic lobule.

*In this proposed terminology, the suffix "-ule" denotes a channel that exits the portal tract to enter the parenchyma. This enables uniformity in designating anatomic location of the venous, arterial, and biliary components of the portal tract system. The concept "terminal" denotes the smallest branches of the portal tract system, which give rise to the multiple sequential final, venular, arteriolar, and ductular branches entering the parenchyma.

†This corresponds to the "inlet venule" of Matsumoto[12] and Ekataksin et al.[15] This structure is without connective tissue stroma or endothelial basement membrane and hence, may appear simply as a larger sinusoidal channel. The "inlet venule" would be the putative location of an inlet sphincter.

‡Terminal portal vein corresponds to the "pre-terminal portal venule" of Ekataksin.[14]

§Bile ductule is not to be used to describe a small terminal bile duct in a terminal portal tract.

‖If multiple portal venous channels are identified in a portal tract, it is probable that one of the channels is a conducting portal vein, and therefore, this is not a terminal portal tract.

as one moves from the portal tract to the midseptal region, in both humans and animals.[8-12]

Matsumoto et al. note that many pathological processes like fatty change, perivenular necrosis, and staining with glucose-6-phosphatase highlight the presence of a sickle zone.[12] However, if we do dare to extrapolate from amphibians and small mammals, an "inflow front" type of flow is not consistent with the variation in blood flow between adjacent sinusoids in the same lobule, as has been observed by direct visualization in living animals under a wet-mount microscope.[14] Similarly, it does not agree with the statements of some investigators who maintain that some sinusoids are perfused wholly by arterial blood and others by venous blood.[13] Therefore, we must allow that dynamic, functional implications cannot be drawn from studies of fixed tissue. However, the presence of an anatomic sinusoidal bed in the portal or sickle zone provides a potential inflow front, the effect of which is seen in pathological states like ischemia or fatty change.[12]

MICROCIRCULATORY SUBUNITS

The second important advance in our understanding of lobular architecture comes from studies in pigs, rats, mice, and hamsters. Using a combination of colored media injec-

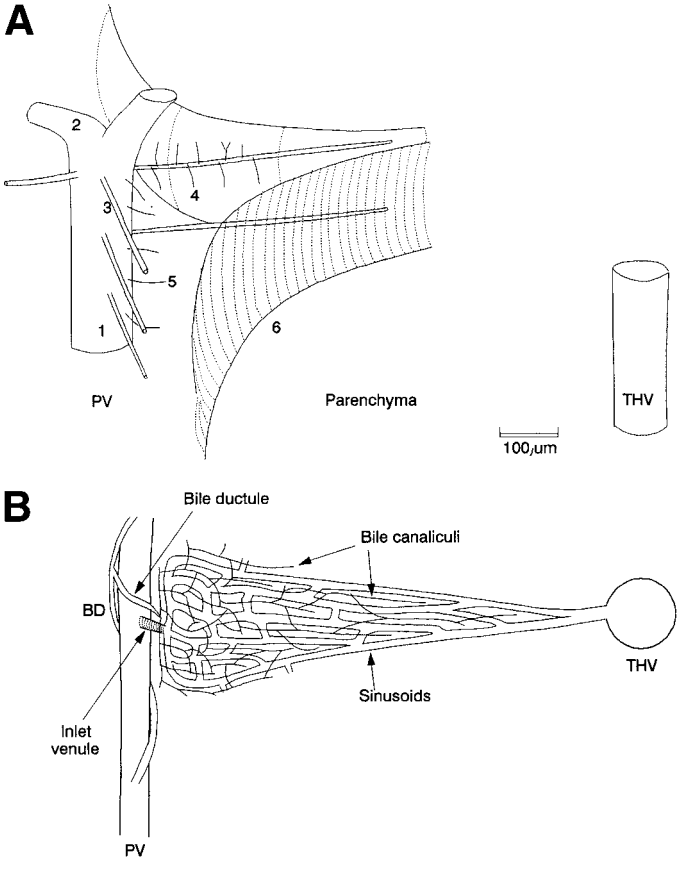


FIG. 1. Schemes of hepatic microarchitecture. (A) Angioarchitecture in the human as described by Matsumoto et al.[12] In this illustration, the conducting PV (1) gives rise to terminal PV branches (2) and to "septal" portal venules (3). These portal venules penetrate the parenchyma and give rise to inlet sinusoids (4). Alternatively, inlet sinusoidal branches may arise directly from the conducting or terminal PV (5). The subsequent sinusoidal channels spread transversely (not shown) before turning inward along a sickle zone (6) and heading radially towards the THV. (B) A unit model of the "cholehepaton" as proposed by Ekataksin et al.[15] using the terminology given in Table 1. The terminal PV gives rise to an inlet sinusoid ("inlet venule") that supplies the sinusoids. The bile ductule connects the terminal BD to a canal of Hering (not shown) and then bile canaliculi (partly illustrated); the embraced hepatocytes are not shown.

tion, corrosion casts with scanning electron microscopy, and *in vivo* microscopy with fluorescent dyes, Ekataksin et al.[14,15] showed in all species they studied a pyramidal microcirculatory unit with its base at the perimeter of the classic lobule and its apex at the central vein (Fig. 1B). Similar vascular beds supplied by an inlet venule are shown by Matsumoto et al.[12] in the human liver. This pyramidal unit, which was named the hepatic microcirculatory subunit (HMS),[14] was supplied by a single-inlet venule feeding a group of approximately 19 sinusoids. The inlet venule colocalized with a canal of Hering, which drained bile from the same pyramidal area. Tracing the path of a fluorescent dye, they showed a dynamic wave of intensity, the "inflow front" filling up the pyramidal unit through the inlet venule. The fluorescent dye then accumulated in cones of hepatic plates and at a later phase, a diffuse chicken-wire pattern of bile canaliculi was observed. They could not, however, visualize the canals of Hering by this method.[15] These investigators believe that the HMS represents the basic functional unit of the liver and in analogy with the nephron, describe a "countercurrent" flow between the inlet venule and the canal of Hering. Blood is supplied to hepatocytes by the inlet venule and bile is drained by the canal of Hering in opposite directions from the same pyramidal territory. Evidence indicating that periportal hepatocytes are primarily responsible for bile salt uptake and secretion under normal conditions[16] supports the "countercurrent" concept. Ekataksin et al.[15] have further observed the presence of an hepatic arteriole in the HMS, although its relationship to the inlet venule and canal of Hering is not clear.

Thus, the continuous system ("muralium") of anastomosing hepatic plates ("laminae hepatis") and sinusoidal spaces, ("labyrinthus hepatis") of Elias[17] has given way to an organization of well-demarcated areas of hepatic parenchyma associated with supply venules and draining biliary channels that follow a regular pattern of branching. However, we have not seen the end of the debate over the functional unit. The questions are, how small is small, and what structures constitute a functional unit? The HMS described by Ekataksin et al.[15] is certainly the smallest grouping of hepatocytes around a ductule and inlet venule. However, it breaks up the functionally integrated sickle zone described by Matsumoto et al.[12] The primary lobule described by Matsumoto et al.[12] on the other hand is more inclusive, but does not deal with the terminal hepatic vein or terminal hepatic artery (HA). If we believe, as Matsumoto et al.[12] have suggested, that the very regular branching of first-order distributing PVs into 11 second-order distributing PVs without accompanying branching of the hepatic vein is tantamount to juxtaposing 6 portal tracts around one central vein, the functional unit begins to resemble the classic lobule as originally described,[1] this time with added functional significance. The 3-D spatial reconstruction of primary lobules of the human liver, as has been done in the rat liver[18] remains to be done.

An important observation from Ekataksin's[14] studies is the variation in shape of the HMS. In peripheral portions of the liver, the inlet venule-inlet venule distance was shorter, sometimes as little as 20 to 25 μm, spanning 1 hepatocyte, so that the inlet venule supplied hepatocytes in 1 plane only. In subcapsular locations, therefore, the generally conical HMS assumed a flattened appearance ("premature" HMS) and "lacked the third dimension."[14] Fusion of adjacent HMS at the periphery created various profiles resembling the triangular portal lobule, the rhomboidal liver acinus, and the kite shaped primary lobule. This observation reflects 2 facts: first, the architectural features vary in various parts of the liver, possibly accounting for observed variations between studies. Second, although there is a regular architectural configuration to the lobule, this does not always translate into a regular geometrical shape, but rather conforms to the local shape of the liver. Thus, what may be true in the peripheral region of the liver may not be assumed to be true in the more central regions.

Because the lobular architecture of the liver depends on the relationship of the branching pattern of the portal venous system with that of the hepatic venous system, these 2 systems will be discussed in that order followed-up by the hepatic arterial system, whose role in parenchymal perfusion is not as well-defined.

PORTAL VENOUS SYSTEM

The portal venous system in humans consists of the conducting and distributing systems.[12] As the name suggests, the conducting system is responsible for getting blood to the farthest corner of the liver. To accomplish this, the conducting system shows frequent branching in the perihilar and subcapsular zones and shows great versatility in branch length, number of branching orders, and number of branches, accommodating to the shape of the organ to ensure even supply of the parenchyma and maintain architectural relationships. The distributing system is responsible for the actual exchange of substances between blood and hepatic elements, and therefore, the maintenance of microarchitectural interfaces. To accomplish this, the distributing system follows a strict pattern of branching. The terminal branches of the conducting system give rise to first-order branches of the distributing system in an orderly, but directionally randomized sequence: each of these supplies a definite mass of human liver parenchyma measuring approximately $1.6 \times 1.2 \times 0.8$ mm. Each first-order branch gives rise to successive, 11 second-step branches at about right angles. The second-order branches correspond to the PVs seen in terminal portal tracts on light microscopy. Third-order branches are the portal venules, which arise from the second-order branches and correspond to the "septal" branches seen in interlobular vascular septa.[12]

HEPATIC VENOUS SYSTEM

The hepatic venous system, long viewed as a passive drainage compartment for both the portal venous and hepatic arterial system,[4] has not received as much attention in literature as the portal venous system. Blood drains from hepatic sinusoids into terminal hepatic veins (THVs) and then, into sublobular veins that ultimately join the hepatic veins. The significance of the pattern of hepatic venous branching in lobular architecture again comes from detailed reconstructions by Matsumoto et al.[12] of the liver angioarchitecture. They observed that the hepatic venous system followed the branching order of the portal venous sytem right up to the first-order branches of the distributing PVs. Thereafter, whereas the first-order branches of the distributing veins gave rise to 11 second-order branches, the hepatic veins did not follow suit. This resulted in the positioning of 6 PVs around 1 central vein and the formation of the classic hexagonal lobule.[12] Similarly, 3-D reconstruction of lobules in the rat liver showed the orientation of the lobules to be

based on centrally located hepatic veins and their relationship with the branching patterns of the portal venous system.[18]

THE HEPATIC ARTERIAL SYSTEM

The HA is responsible for 25% of the hepatic blood flow, yet its role in perfusion of the liver parenchyma is not agreed on. Debate also revolves around the mode of termination of the hepatic arterial system. Do the terminal hepatic arteries terminate into sinusoids via hepatic arterioles, an inlet venule, or a terminal PV? Are there arterio-portal anastomoses and if arterioles do terminate in sinusoids, do they do so in the vicinity of the portal tract or do they travel deep into lobules as intralobular arterioles?

The observations in numerous publications on the subject are complicated by species variation in anatomy,[19] blood flow at the time of observation, dynamics of sphincters, and type of anesthesia.[20] Sphincters have been described at 3 sites in the sinusoidal bed: at the entry of the inlet venule with the sinusoids, the "inlet" or afferent sphincters; at the entry of sinusoids into the central vein, the "outlet" or efferent sphincters; and at the junction of intersinusoidal sinusoids with radial sinusoids. Precapillary sphincters consisting of smooth muscle are also identified in hepatic arterioles.[21]

Studies of the arterial supply in the human liver are very few.[12,13,19,22] Olds and Stafford[13] in 1930 performed double injections in the human liver, injecting India ink into the HA and carmine gelatin into the PV. In 1953, Elias and Petty[22] studied the arterial supply in the liver of a healthy young man who died an accidental death, by injection of India ink. In 1985, Yamamoto et al.[19] injected 2 different colored resins into the HA and PV of a 13-year-old adolescent without liver disease and studied the vasculature by scanning electron microscopy and stereo-pairs of micrographs. Matsumoto et al.[12] in 1979, studied the vasculature in the livers of 2 young men who had no evidence of liver disease. Of the 4 studies, only Elias and Petty[22] observed what they called an "intralobular arteriole," that traveled as far as the inner half of the classic lobule and connected to a sinusoid midway between the portal tract and the THV (Fig. 2A). They concluded that this is to be expected as the oxygen content of the portal blood diminishes as it approaches the pericentral third and intralobular arterioles provide arterial "boosters" to the pericentral third of the lobule. They further speculated that perivenular ischemic necrosis is caused by simultaneous spasm of all sphincters of arterial capillaries supplying a particular zone.[22]

The other 3 studies did not confirm the presence of intralobular arterioles in the human liver and noted that the hepatic arterioles opened into sinusoids in the periportal zone. However, Ekataksin and Wake[23] believe that the HA is a blood vessel directed towards the portal tract stroma and not towards the parenchyma (Fig. 2B). In this capacity, the HA would supply the peribilary vascular plexus, the portal tract interstitium, portal and hepatic vein vasa vasorum, and the hepatic capsule, secondarily draining into PVs, sinusoids, lymphatics, or even sublobular veins.[23] They maintain that the presence of "bypass" pathways in the form of arterio-portal anastomosis is in support of their theory. However, all 4 studies in the normal human liver failed to show arterio-portal anastomoses.[12,13,19,22] In contrast, numerous arterio-portal anastomoses are seen in the rat liver, one of the most frequent models of study, especially in terminal portal tracts where the peribiliary plexus is not well formed[19] (Fig. 2D). At the very least, we conclude that not only is there enormous variation between species, but also between different parts of the liver. We also note the extreme importance of further elucidating these relationships for understanding not only normal liver function, but also how the damaged liver might respond to injury.

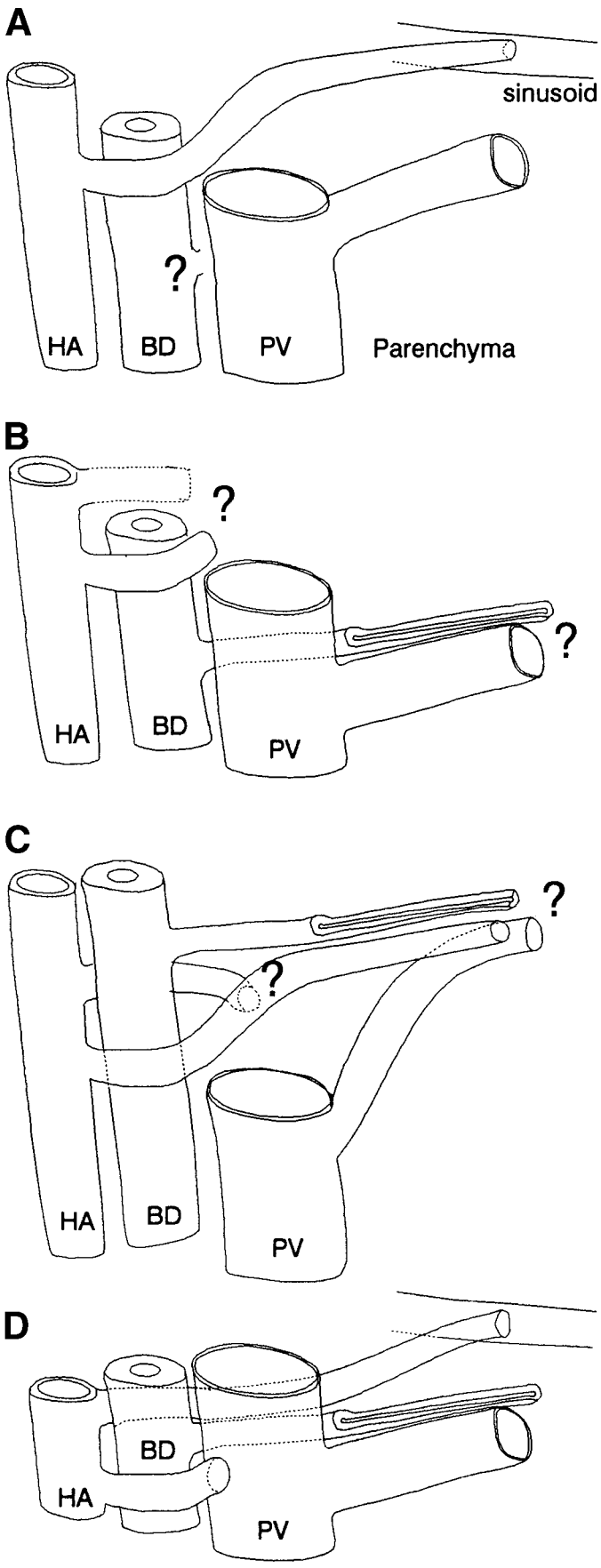
Lastly consideration needs to be given as to whether hepatic arterioles maintain some relationship to penetrating bile ductular channels (leading to canals of Hering, discussed later) (Fig. 2C). Such an arrangement might help preserve blood supply to an important proliferative cell zone in severely damaged livers.

THE BILIARY SYTEM

In all species studied, the intralobular biliary channels begin as bile canaliculi, which are seen on electron microscopy as 1- to 2-μm wide tissue spaces between adjacent hepatocytes, bounded only by the apical membranes of these cells.[24-26] Bile canaliculi form a complex pattern of branching and anastomosing channels with tortuous courses that ultimately link to the terminal bile duct (BD) through bile ductules, variously termed cholangioles, or canals of Hering.[24-28] It is the exact nature of their pathways through lobules to the periphery that has, so far, remained a mystery. Stated differently the question is: Is there an intralobular cholangiole? Or yet: Does the canal of Hering extend into the lobule or does it stop in the vicinity of the limiting plate? Even recently, canals of Hering have been thought to be largely inapparent within the hepatic parenchyma.[29] Furthermore, as Steiner and Carruthers queried in 1961, "Does each duct of Hering correspond to one canaliculus or do canaliculi form a confluence prior to entering the confines of the canals of Hering?"[26] By definition, the canals of Hering are narrow tubular channels present in the vicinity of the portal tract that are partially lined by hepatocytes on one aspect and small cuboidal or pyramidal cells, cholangiocytes, on the other[30] (Fig 3A).

We stained sequential sections of normal human liver for CK19 (cytokeratin 19) to identify biliary epithelium and found, as have others before us,[31] isolated CK19 positive cells in the liver parenchyma. However, on reconstruction of these serial sections, the CK19 positive cells lined up with each other to form linear arrays and narrow tubular channels that ultimately linked up to the interlobular BDs.[32] These structures extended mostly to the periportal or middle third of the lobule with very few present in the perivenular areas. At certain points, these arrays seemed to branch. Studies of normal human liver biopsies have similarly identified "cuboidal strings" of cells within the parenchyma.[33] We believe that these CK19 positive cells represent the cholangiocytic lining of the canals of Hering, the elusive intralobular component of the biliary passages, partially lined by cholangiocytes (corresponding to the linear arrays of CK 19 positive cells) and partially by hepatocytes.[26] They may also represent the final branches of the intrahepatic biliary tree alluded to in a recent study of normal human liver.[34]

Therefore, we postulate that the intralobular biliary system consists of bile canaliculi between hepatocytes throughout the lobule, and that they enter into penetrating canals of Hering in the periportal third of the lobule. As such, the canal of Hering serves as a "trough" for collecting parenchymal bile and conveying it to the portal tract interface (Fig. 3B). Here, the biliary channels acquire a circumferential lining of cholangiocytes and are clearly seen on routine stains or with



anti-CK19 staining as fully "circular" structures. As noted in Figs. 2 and 3 and in Table 1, we choose to refer to these channels as "bile ductules," spanning the mesenchyme from parenchymal interface to the terminal BD. These are normal structures.

Intravital microscopy with a fluorescent dye in various species of animals shows a chicken-wire pattern of bile canaliculi in the inner lobule that then converges into a single canal of Hering.[15] Although convergence of many canaliculi into a single canal is often seen,[15,28] a calculation of the number of canals of Hering to the number of bile canaliculi has never been attempted. In our 3-D reconstruction, an average of 1 parenchymal canal of Hering was found per 10-μm length of BD. By inference, there must be 1 bile ductular branch off the terminal BD per longitudinal layer of cholangiocytes. A similar reconstruction in Matsumoto et al.'s[12] work shows very frequent entry points of the canal of Hering. Therefore, evidence suggests that the canals of Hering are far more frequent than previously appreciated, although a 1:1 ratio of canals of Hering to bile ductules or portal venules has not been shown conclusively. Nevertheless, leaf-like arrays of bile canaliculi draining into canals of Hering may constitute the effluent component of the "hepatic microcirculatory subunit" proposed by Ekataksin et al.[15] (Fig. 3C).

LYMPHATIC SYSTEM

The lymphatic system has been studied in the experimental models of rats, rabbits, and pigs.[35-37] The most accepted view is that lymph is formed in the liver by filtration of plasma into the spaces of Disse as blood passes through the sinusoids.[38] This has been challenged in a rat model and origin from within portal tracts suggested.[36] From the space of Disse, the lymph travels to the space of Mall in the portal tract and from there into the lymphatic vessels which themselves begin as

FIG. 2. Postulated architectural relationships of portal tract structures in the human and rat, with speculations denoted by (?). (A) Scheme showing terminal HA giving rise to a hepatic arteriole, which penetrates the parenchyma to supply a sinusoid deep within the lobule, as proposed by Elias.[22] The terminal PV supplies a portal venule. The terminal BD connection is not addressed. (B) Scheme showing the concept of a "cholehepaton" after Ekataksin et al.,[15] in which the terminal BD supplies first a bile ductule and then the trough-like canal of Hering, accompanied by a portal venule from the terminal PV. Although the terminal HA may simply supply the portal tract structures and mesenchyme,[23] its final destinations are not known. (C) Scheme showing the possibility that the hepatic arteriole arising from the HA may accompany a bile ductule and canal of Hering arising from the terminal BD. Although speculative, this pairing would explain the "dyads" observed by Crawford et al.,[33] because portal venules from the terminal PV may simply appear as sinusoidal channels of greater width, and thence will be largely inapparent without 3-D reconstruction. Moreover, while the peribiliary vascular plexus arising from the terminal HA is thought to supply the terminal BD, it remains unclear whether these vascular channels become hepatic arterioles or, instead, empty into the terminal PV (not shown). (D) Architectural relationships deduced from the rat. The terminal HA gives rise to a peribiliary vascular plexus, which may empty directly into the terminal PV or give rise to a hepatic arteriole supplying a sinusoid deep within the parenchyma. The terminal BD gives rise to a bile ductule and a canal of Hering. The terminal PV gives rise to a portal venule, which accompanies the canal of Hering. Note that the vertical distance is expanded for the purposes of illustration (especially in C). Based on 3-D reconstructions in the human,[12,32] portal venules and canals of Hering are radially arranged around the supplying structures and may arise as frequently as every 10 μm. This is equivalent to a canal of Hering branch for every longitudinal layer of epithelial cells in the BD.

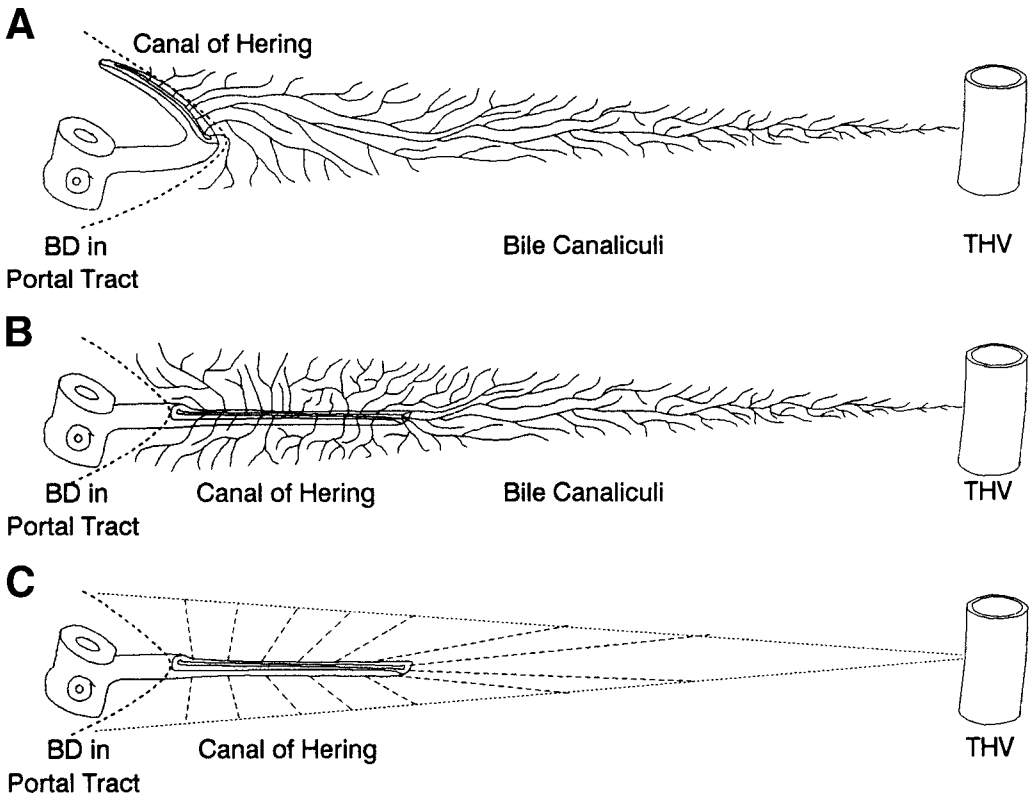


FIG. 3. Proposed relationships of the canal of Hering to the hepatic parenchyma. (A) The terminal BD in the portal tract may give rise to a bile ductule and then a canal of Hering, which connects with hepatocytes (not shown) and their bile canaliculi only at the interface between the portal tract and parenchyma (*dotted line*). (B) The terminal BD in the portal tract may give rise to a bile ductule, and then a canal of Hering that penetrates directly into the parenchyma and extends on average one third of the way to the THV. Because the canal of Hering is by definition made up partially by BD epithelial cells and hepatocytes (not shown), it can act as a "trough" for the collection of bile from hepatocellular bile canaliculi. This is the model supported by 3-D reconstruction of normal human liver.[32] (C) The microarchitecture of (B) is depicted as a layer of the parenchyma in which bile from each segment of the lobule collects into the canal of Hering for drainage into the bile ductule and terminal BD in the portal tract. To the extent that a portal venule from the terminal PV accompanies the canal of Hering, the concept of "cholehepaton" advanced by Ekataksin[15] would be fulfilled.

blind-ended channels within portal tracts.[36] It is believed that lymph percolates through collagen fibers that appear to be continuous between the space of Disse and the portal tracts, making a submicroscopic channel for lymph flow. Lymph is also believed to flow in the matrix investing the portal inlet venules and arterioles that penetrate the limiting plate.[38] In rabbits studied by corrosion casts, the lymphatics formed rich networks around portal vessels and BDs and extend as far distally as the terminal portal tracts. Communication between portal lymphatics and those accompanying the hepatic veins has not been observed.[35]

NERVES IN THE LIVER

The human liver has rich sympathetic and parasympathetic innervation, the former predominating. Sympathetic or adrenergic nerve fibers form a rich perivascular plexus around blood vessels, from which branches are given off. These branches travel through sinusoids to supply the lobules, where terminal branches surround perisinusoidal cells and hepatocytes.[39,40] Parasympathetic or cholinergic nerve fibers have been shown to innervate extrahepatic and intrahepatic branches of the HA, PV, and hepatic vein. Cholinergic innervation of hepatocytes and sinusoids is however sparse.[39,41] An inverse relationship has been shown between the number of nerve fibers and the density of gap junctions. It has been suggested that gap junctions provide light electrical coupling so that the need for nerves to act as communication lines is obviated.[39] Interestingly, with the advent of liver transplantation, our long-held views on the role of hepatic innervation in metabolism and vascular and biliary physiology are being challenged by the effectively functioning denervated liver allograft.[42,43]

DEVELOPMENTAL CONSIDERATIONS

The development of the liver parenchyma, biliary system, and vascular supply reflects the anatomical relationships of these structures in the adult organ. In fetal life, organization of the hepatic epithelial cords occurs around the developing PV, which itself arises from the vitelline and umbilical veins; the HA playing an insignificant role in this process.[44] On the other hand, the development of the HA closely parallels the development of the BDs in both rats and humans.[45,46] In both species, the arterial system, just like the biliary system, is immature at birth. Development of arterial vessels and peri-biliary plexuses begins at the hilum and spreads to the periphery mimicking the development of ducts in that order. Similarly, the peri-biliary arterial plexuses in large BDs begin as a loose network of capillaries and differentiate into the inner and outer layers as the walls of the ducts begin to develop. The hepatic arterial system in humans continues to proliferate and grows to reach an adult form only at 15 years of age, suggesting that, unlike the portal supply, the arterial supply is not crucial to lobular architecture.[46]

An interesting microscopic vestige of liver development is

the intersinusoidal fiber. Ekataksin et al.[14] observed that sinusoids freely anastomosed in the neonatal period in rats, mice, and hamsters by intersinusoidal sinusoids that linked 2 adjacent radial sinusoids. However, with development, the intersinusoidal sinusoids in the perivenular region became attenuated and eventually nonfunctional, existing as slender intersinusoidal bridges of fibers.[14] The "drop-out" of sinusoids in the perivenular region enhances the structure of the HMS and promotes the formation of the sickle and midseptal zones of the lobule.

SPECIES VARIATIONS

Extensive data exist on liver anatomy in various species of animals, but a few examples of species variations will suffice to prove that caution is warranted in the extrapolation of this data to humans. The presence of connective tissue septa between portal tracts is well-known in the porcine liver. In humans and other mammals, sinusoids drain only into the THVs whereas in the rat, one of the most popular experimental models, sinusoids enter the hepatic venous system at all levels of the hepatic venous tree, including the sublobular and collecting veins.[47] In rats, unlike humans, the sinusoids are supplied not only by the terminal PVs but also directly from larger venous branches.[18] In addition, rat livers lack the septal vein branches, which are present both in humans and pigs.[18] The presence of arterio-portal anastomosis is very frequent in rats but evidently not in hamsters and humans.[19] The rat is unique in possessing a perihilar biliary plexus, which consists of a network of anastomosing BDs arising from the BDs and surrounding portal tracts.[48,49] This plexus is present from the large hilar portal tracts to smaller portal tracts containing BDs only 30 μm in size. An equivalent, less developed structure exists in humans only in large portal tracts. The biliary system in pigs lacks this plexus altogether, but contains numerous side pouches throughout the course of the BDs.[49] Thus, the ultimate arbiter of human liver anatomy must be studies conducted in human liver tissue.

CONCLUSION

The apparent histological simplicity of the liver belies a precision-based interplay at the microanatomical interfaces that appears crucial to function and pathophysiology. There have been significant breakthroughs in delineating the terminations of the biliary tree and the portal venous tree. Much more needs to be understood about the terminal branches of the HA. The architectural reconstruction of the human liver, and the relevance of the branching patterns of the hepatic and portal venous systems to lobular organization, truly constitute a quantum leap in our understanding of liver anatomy. This is in no small measure a tribute to the availability of modern imaging techniques that enable 3-D reconstruction of biological structures.

We stand on the threshold of a new era in understanding liver pathobiology as it relates to the function of these interfaces and their responses to injury. In this review, emphasis has been placed on critical and recent insights into the relationships between the vascular tree, the biliary system, and the hepatic parenchyma. It would be fair to say that these aspects of hepatic structure represent, along with the brain, one of the "last frontiers" of microanatomy. As this frontier is traversed, we expect to see significant conceptual and practical advances that are likely to transform the field of hepatology.

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