

## Now You See It, Now You Don't

Fascination with the liver as a structure and with the structure of the liver as an organ probably dates back more than 30 millennia, to a time when our hunter-gatherer forebears first eviscerated speared bison, ibex, or aurochs on the banks of the Vézère. There in southern France, in Lascaux, on September 12, 1940, 4 teenage boys and a dog discovered the now famous caves where Paleolithic artists once painted scenes of contemporary life that indicated, among a multitude of other insights, their familiarity with internal anatomy.<sup>1</sup> One can only imagine the spiritual impact on those cave dwellers of encountering, in the upper abdomen, an imposing large solid organ colored deep red by the blood, the life-force that filled it. Little wonder that in the ancient world the liver was regarded as the seat of the soul—regrettably a guardianship long since relinquished. The ancient Egyptians posthumously entrusted into the care of the human-headed god Imseti, in urns or Canopic jars of limestone, the livers of their recently departed aristocracy for use in the hereafter, leaving the stomach, intestines, and lungs to the safe-keeping of the remaining 3 sons of Horus, the jackal Duamutef, the falcon Quebehsenuf, and the baboon Hapi, respectively. Not so for the peoples of Mesopotamia (especially in Babylon), as Plato noted,<sup>2</sup> or the Hittites, Israelites, Etruscans, Greeks, and Romans, who all practiced *haruspicy* or divination of the future by scrutinizing the liver. The preferred sacrificial animal was a

young sheep or lamb, but almost any creature would do. Not surprisingly, ancestors of modern day luminal gastroenterologists relied on the appearance of the animal's entrails,

*extispicy*, for much the same purpose. *Haruspicy* gave the Ancients great familiarity with the configuration of the liver even though their descriptive terms for its different parts, such as *porta* (gate) *hepatis*, were more those of the urban builder than the anatomist.

Egyptian physicians gave the first explicit written descriptions of the hepatic vasculature<sup>3</sup> but, like Diogenes, Hippocrates, Aristotle, and Galen after them, they got it wrong. The Greco-Roman notions that the parenchyma of the liver is derived from the blood, much as silt from a river builds up on its banks (*parenchyma* “something poured in beside”—*παρά*—beside + *ἔγχυμα* infusion), and that the liver is not only the factory that makes the blood but is also the source of the veins of the body, seem ludicrous now. Nonetheless, lurking among bizarre ancient propositions, like Diogenes' idea that the veins of the right arm connect with the liver while those of the left connect with the spleen (permitting phlebotomy via a peripheral vein for the ill humors of those respective organs), was Erasistratos'

provocative hypothesis of the existence of an intrahepatic capillary bed. Erasistratos reasoned that unpurified blood entering the gateway of the liver through the portal vein on its concave side, could pass to the bile and to the branches of the vena cava on the convex surface. Erasistratos

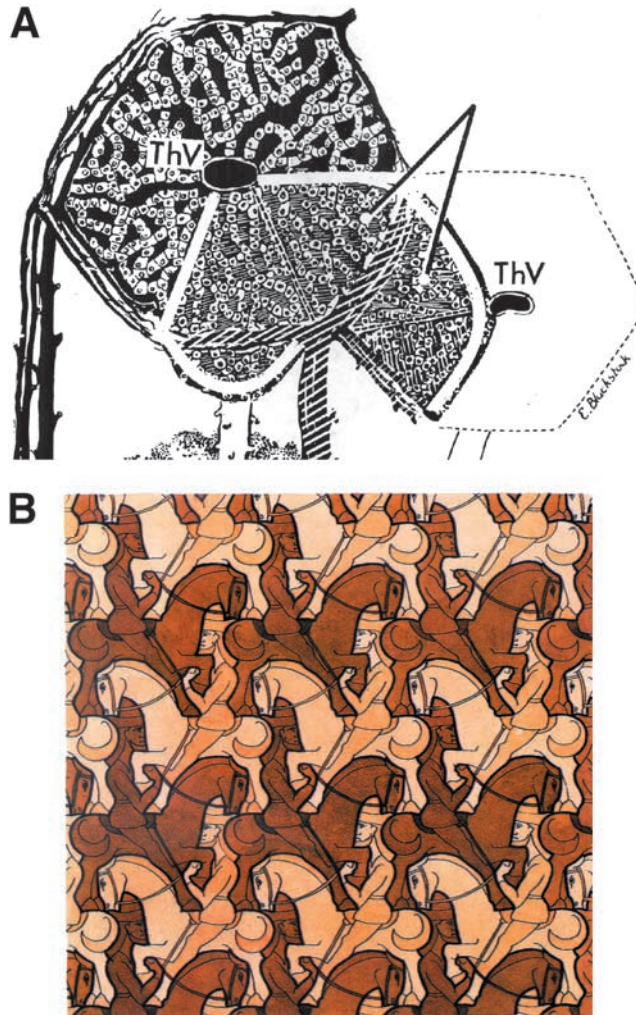


Fig. 1. Above, horizontal section through a crosshatched Rappaport microvascular acinar unit of the liver (light grey, as indicated by the diverging lines), situated between two terminal hepatic venules (ThV), i.e., central veins, overlapping with the classic hepatic lobule shown in black. Copyright © 1954 *The Anatomical Record*.<sup>21</sup> Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. Below, Horsemen: Woodcut in three colours by M.C. Escher July 1946. M.C. Escher's "Horsemen" © 2003 Cordon Art B.V.—Baarn—Holland. All rights reserved.

tratos had clearly predicted the presence of the sinusoids and, although Galen was a bitter critic of this Alexandrian scientist, even he accepted that there must be a system of tiny “labyrinthine” channels connecting the portal vein to the vena cava, a concept that was crucial 1,400 years later to Harvey’s theory of the circulation of the blood. A mere 1,900 years were to pass, however, before Erasistratos’ hypothesis of transhepatic blood flow was vindicated conclusively by Francis Glisson (1597-1677), Regius Professor of Physic at Cambridge (1637-1677), cofounder of the Royal Society, and President of the Royal College of Physicians (1667-1669), which he also helped to establish.

Glisson the discoverer of rickets, whose name will forever be encapsulated with the liver’s investing membranous covering (that he discovered, too), pioneered the technique of dye injection/perfusion to show macroscopic and even microscopic vascular anatomy. With ox bladder and cannula in hand, with which he might have administered an enema in those days, Glisson injected “warm water, coloured with a little milk” into the portal vein of a human cadaver, and found that the liver blanched when all the blood in it was expelled.<sup>4</sup> At the same time, Glisson’s experiment also provided convincing evidence in support of Harvey’s theory of the circulation of the blood, as the milky contrast not only passed through the liver (as Erasistratos would have predicted) but ran through the right heart, the lungs, and the left heart into the systemic arterial circulation, too. Glisson produced exquisite drawings of the hepatic vasculature and biliary tree, which structures became visible after cooking the liver and scraping the parenchyma away. Without a microscope, however, he could not imagine the fine structure of the liver parenchyma itself, namely hepatic lobulation, or *hepatic architecture* as we often call it, perpetuating the tool-language of the urban builder. Defining the fundamental hepatic unit has been the bailiwick of microscopists ever since Marcello Malpighi (1628-1694) ushered in the epoch of histology in 1661 by identifying capillaries for the first time in the lung of a living frog.<sup>5</sup> Malpighi thereby added structural proof of a connection between arteries and veins, as Harvey’s theory demanded.

Born in Crevalcore in the province of Bologna, as fate would have it in the year that Harvey published his theory of the circulation of the blood, the physician and anatomist Malpighi was exiled for 5 years from his position at the University of Bologna, to Messina in Sicily, because his discoveries under the microscope were too large for the small-minded but influential contemporary local followers of Galen. Despite his many critics, Malpighi’s reputation was such that he was soon reinstated in Bologna and,

just a few years before his death from apoplexy, he was appointed as personal physician to Pope Innocent XII. Although preempted a few years earlier by Wepfer’s discovery of lobules or *acini* in the liver of the pig,<sup>6</sup> Malpighi concluded from his extensive microscopic observations on the livers of diverse species such as the lizard, ferret, mouse, squirrel, ox, and human (discussed by Kiernan<sup>7</sup>) and even on an analogous organ in the snail, that the lobule or acinus was the fundamental hepatic unit,<sup>8</sup> acting for the most part as a hollow biliary secretory unit. Approximately 150 years were to elapse before Kiernan<sup>7</sup> published in great detail (and Muller, too,<sup>9</sup> in lesser detail) the now familiar hexagonal liver lobule, based on a central draining sublobular hepatic vein and not a bile ductule. In his experiments, conducted with a hand lens and a quick-silver injection technique, Kiernan scrupulously cleared various animal livers of blood before opacifying the vessels, thereby permitting optimal visualization of the injected structures, which also led to his recognition of triangular spaces containing minute branches of the hepatic artery, portal vein, and bile duct at the periphery of the lobules. Thus, Kiernan had identified the portal tracts or triads.

Once Kiernan’s view of the hepatic lobule was established, the way was seemingly clear to move on and define its intimate circulatory anatomy and physiology (as has been so painstakingly reviewed by the Chens<sup>10</sup>) because microscopy techniques were advancing technologically, experience with sophisticated dye injection and cast methodology was growing, and the functions of the liver were being teased out by biochemists and physiologists. The question of whether hepatocytes radiate out from the central draining vein of the lobule in two-cell thick cords (or trabeculae) that enclose a biliary space<sup>11</sup> or one-cell thick plates<sup>12</sup> was answered by Elias in favor of Hering and his monolayers, using elegant 3-dimensional microscopic liver reconstructions.<sup>13</sup> Hering’s achievement is celebrated to this day by naming after him the biliary canals he described, which convey bile from hepatocyte canaliculi to ductules that then drain into branches of the bile ducts in Kiernan’s portal spaces. Hering’s conceptual breakthrough in linking the hepatocyte canalicular system to the biliary tree has recently assumed even more significance, for it appears very likely that Hering’s canals provide the stem cells that can proliferate and differentiate into both hepatocytes and cholangiocytes.<sup>14</sup>

The thorny question of what constitutes the fundamental liver unit still hangs in the balance, not so much because there have not been valiant attempts to address it but rather because the answer depends on the limitations of the experimental technique used and the perspective of the respondents, *e.g.*, anatomist, physiologist, patholo-

gist, or even clinician, although we can be grateful that nowadays neither the philosopher nor the cleric is given a voice. In most cases the quest for the ultimate liver unit has involved fastidious microscopic sectioning, reexamination of the intrahepatic circulation including dye injection and transillumination to view the vessels *in vivo*, and possibly functional measures that tell which hepatocytes and other liver structures are grouped together in a special arrangement. Saxena et al. have defined the functional unit of an organ as the smallest structurally distinct "self-sufficient" unit that can subservise all the known functions of that organ.<sup>15</sup> For the liver, such a unit would have to account for the dual vascular supply and the vascular and biliary outflow pathways, as well as the myriad of liver functions; and therein lies the challenge.

Over the years, competing models have been put forward to rival Kiernan's classic central hepatic vein-based lobule, whose hexagonal shape is really only defined well by connective tissue boundaries in the pig and a few other animal species like the camel and the polar bear.<sup>16</sup> Mall described a "portal lobule" with a portal tract at its center,<sup>17</sup> whereas for the so-called "biliary lobule" of Brissaud and Sabourin<sup>18</sup> the portal tract also forms the axis but here the bile drainage component is considered dominant. Functional liver lobules have also been defined according to metabolic characteristics of the hepatocytes<sup>19</sup> or the regulatory effects of oxygen gradients.<sup>20</sup> However, compared with all of these and several other models, the landmark acinar concept of the fundamental liver unit in animals and humans devised by Rappaport<sup>21,22</sup> and developed out of his university thesis presentation,<sup>23</sup> has attracted the most attention and captured the imagination of hepatologists and pathologists alike for more than 40 years, at least until recently. The incentive to search for yet another alternative to Kiernan's classic central vein-based hexagon was in part the theoretical dissatisfaction at the apparent lack of microvascular unity that exists when a lobule is supplied from its periphery by portal and arterial vessels that also supply blood to parts of adjacent hexagonal fields.<sup>24</sup> In addition, Rappaport noted that microcirculatory blood flow patterns *in vivo* and liver lesions caused by ischemia and other localized injury, did not follow a classic lobular configuration as expected.<sup>25</sup> Using simultaneous injections of different colored gelatins into two branches of the portal vein or two branches of the hepatic artery or into the bile ducts, Rappaport used serial sectioning and studied the 3-dimensional patterns of distribution of colored gelatins to devise *his* model of a liver acinus that, in its simplest form, expands outwards in zones away from the central core of branches of the portal vein, hepatic artery, and bile duct, and extends from one

terminal hepatic venule to another (Fig. 1). Since all proposals for the fundamental liver unit must draw on the same microanatomic elements (*i.e.*, liver cells, blood spaces, bile ducts, etc.) in the liver, it has often been difficult to conceptualize a 3-dimensional model from a figure drawn in one plane while, at the same time, imagining a second construction drawn in another plane, rather like trying to appreciate simultaneously the conflicting patterns in an Escher optical illusion. The latter is especially true of the series of drawings that Escher used to illustrate his concept of "The Regular Division of The Plane,"<sup>26</sup> which is essentially a jigsaw of identical interlocking pieces that evoke visual conflict and confusion (Fig. 1). In other words, one cannot see both pictures in an optical illusion at the same time, which has often been the case in trying to visualize Rappaport's acinus. As magicians say when performing optical illusions by sleight of hand, "Now you see it, now you don't."

For decades the Rappaport microvascular acinar unit was the model preferred by anatomists, physiologists, and especially pathologists,<sup>27</sup> but recently its popularity has slipped (especially for pathologists<sup>27</sup>) in favor of the *hepatic microcirculatory unit*, described by Ekataksin et al.,<sup>28</sup> yet again based on a reexamination of the angioarchitecture of the liver<sup>29</sup> and the distribution of hepatocyte biochemical activity.<sup>19</sup> The *avant-garde* hepatic microvascular unit has been defined as an elongated pyramidal or conical radial sector of the classic lobule, fed at its base on the periphery by the smallest branches of the portal veins (inlet venules) that colocalize with the canals of Hering. This newly appreciated structure is currently thought to qualify best for the title of *choleohépaton*, the elementary morphofunctional unit of the liver. However, nothing is immutable in hepatic circulatory anatomy, as we have learnt over the past 2 to 3 thousand years, for after all, "now you see it, now you don't."

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## References

1. Coon CS. The history of man. London: Cape, 1955.
2. Plato. Timeus. In: The Dialogues of Plato. Translated by B. Jowett. New York: Random, 1937;II:3-68.
3. Steuer RO, Saunders JB de CM. Ancient Egyptians and Cnidian Medicine: the Relationship of the Ætiological Concepts of Disease. Berkeley: University of California Press, 1959.
4. Glisson F. Anatomia Hepatis. London: O. Pullein, 1654.

5. Malpighi M. De viscerum structura exercitatio anatomica. London, 1666.
6. Wepfer JJ. De Dubiis Anatomicis. Epistola ad Jacob Henricum Paulli. In: Paulli JH, ed. Anatomiae Bilsianae Anatome Occupata Imprimis Circa Vasa Mesereraica et Labyrinthum in Ductu Orifero, 93-100. Simonem Paulli: Argentorati, 1665.
7. Kiernan F. The anatomy and physiology of the livers. Philos Trans R Soc Lond B Biol Sci 1833;123:711-770.
8. Malpighi M. Discours Anatomiques Sur La Structure Des Visceres Sçavoir du Foye, du Cerveau, des Reins, de La Ratte, du Polype du Coeur et de Poulmons. 2nd Edition. Paris: D' Houry, 1687.
9. Müller J. De Glandularum Secernentium Structura Penitiori Earumque Prima Formatione in Homine Atque in Animalibus. Lipsiae: Vossii, 1830:82-84.
10. Chen TS, Chen PS. Understanding the Liver. Chapter 1: Anatomic foundation. Westport, CT: Greenwood Publishing Group, 1984:36-44.
11. Gerlach L. Beiträge zur structurlehre der leber. Mainz: E. Janitsch, 1849.
12. Hering E. The liver. In: Stricker S, ed. Manual of Human and Comparative Histology. Translated by H. Power 1612. London: New Sydenham Society, 1870-1873:1-33.
13. Elias H. A re-examination of the structure of the mammalian liver. 1. Parenchymal architecture. Amer J Anat 1949;84:311-334.
14. Thiese ND, Saxena R, Portmann BC, Thung SN, Yee H, Chiriboga L, Kumar A, et al. The canals of Hering and hepatic stem cells. HEPATOLOGY 1999;30:1425-1433.
15. Saxena R, Thiese ND, Crawford JM. Microanatomy of the human liver—exploring the hidden interfaces. HEPATOLOGY 1999;30:1339-1346.
16. Beresford WA, Henniger JM. A tabular comparative histology of the liver. Arch Histol Jpn 1986;49:267-281.
17. Mall FP. A study of the structural unit of the liver. Am J Anat 1906;5:227-308.
18. Brissaud E, Sabourin C. Sur la constitution lobulaire du foie et les voies de la circulation sanguine intra-hepatique. Compt Rend Soc Biol Année 1888;8:757-776.
19. Lamers WH, Hilberts A, Furt E, Smith J, Jonges GN, van Noorden CJF, Janzen JWG, et al. Hepatic enzyme zonation: a re-evaluation of the concept of the liver acinus. HEPATOLOGY 1989;10:72-76.
20. Jungerman K, Kietzmann T. Oxygen: modulator of metabolic zonation and liver disease. HEPATOLOGY 2000;31:255-260.
21. Rappaport AM, Borowy ZJ, Longheed WM, Lotto WN. Subdivision of hexagonal liver lobules into a structural and functional unit. Role in hepatic physiology and pathology. Anat Record 1954;119:11-33.
22. Rappaport AM. The structural and functional unit in the human liver. Anat Record 1958;130:673-689.
23. Rappaport AM. Circulatory aspects of liver physiology. Thesis. University of Toronto 1952.
24. Rappaport AM. The microcirculatory unit. Microvasc Res 1973;6:212-228.
25. Rappaport AM, Hiraki GY. The anatomical pattern of lesions in the liver. Acta Anat 1958;32:126-140.
26. Bool FH, Kist JR, Locher JL, Wierda F. MC Escher. His life and complete graphic work. New York: Abradal Press. Harry N. Abrams, Inc., 2000: 141-143. (3. The regular division of the plane.)
27. MacSween RNM, Desmet VJ, Roskams T, Scothorne RJ. Developmental anatomy and normal structure. In: MacSween RNM, Burt AD, Portmann BC, Ishak KG, Scheuer PJ, Anthony PP, eds. Pathology of the Liver. 4th Edition. London: Churchill Livingstone, 2002:1-61.
28. Ekataksin W, Zou Z, Wake K, Chunhabundit P, Somana R, Nishida J, M'Cuskey RS. The hepatic microcirculatory sub units: an over-three-century-long search for the missing link between an exocrine unit and an endocrine unit in mammalian liver lobules. In: Motta PM, ed. Recent Advances in Microscopy of Cells, Tissues and Organs. Rome: Antonio Delfino Editore, 1997:407-412.
29. Matsumoto R, Kawakami M. The unit-concept of hepatic parenchyma—a re-examination based on angioarchitectural studies. Acta Pathol Jpn 1982; 32:285-314.