

# Nomenclature of the Finer Branches of the Biliary Tree: Canals, Ductules, and Ductular Reactions in Human Livers

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**The work of liver stem cell biologists, largely carried out in rodent models, has now started to manifest in human investigations and applications. We can now recognize complex regenerative processes in tissue specimens that had only been suspected for decades, but we also struggle to describe what we see in human tissues in a way that takes into account the findings from the animal investigations, using a language derived from species not, in fact, so much like our own. This international group of liver pathologists and hepatologists, most of whom are actively engaged in both clinical work and scientific research, seeks to arrive at a consensus on nomenclature for normal human livers and human reactive lesions that can facilitate more rapid advancement of our field. (HEPATOLOGY 2004;39:1739–1745.)**

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The fine detail of normal liver microanatomy is not well understood.<sup>1,2</sup> This is true whether discussing hepatic vasculature, bile ducts, stroma and matrix, innervation, or lymphatics. Some points are known, but gaps remain. The distal branches of the biliary tree are reasonably well defined: the common bile duct arises from confluence of the right and left hepatic ducts, which arise from segmental ducts, which arise from septal ducts arising from interlobular ducts.<sup>3</sup> It is known that these interlobular ducts arise from still smaller cholangio-cyte-lined structures and that the lumina of these in turn are in structural continuity with the lumen of hepatocellular bile canaliculi. But the terms used for these smallest, most proximal structures have been confusing.

## Structure of Normal Biliary Tree

The structure that came to be known as the canal of Hering was first incompletely described by researchers injecting dye in various mammals. Ewald Hering, reporting the results of Berlin Blue excretion studies, definitively identified a link between the hepatocyte canalicular system (then referred to as "hepatic capillaries") and bile ducts in 1867.<sup>4</sup> Within this publication he included a drawing of bile channels that were partly lined by hepa-

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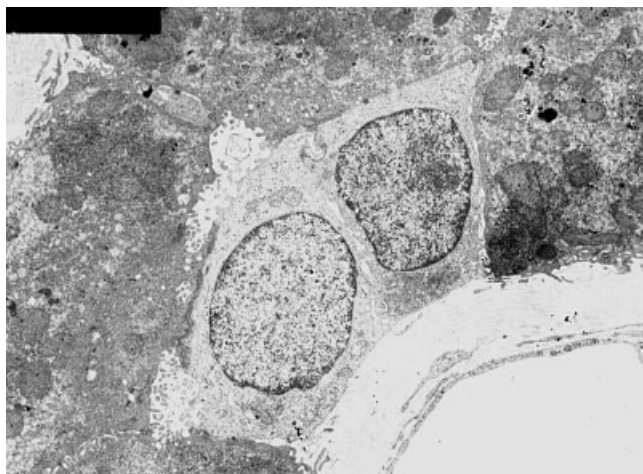


Fig. 1. The canal of Hering, lined by two cuboidal, cholangiocyte-like cells with scant cytoplasmic organelles and by hepatocytes with abundant mitochondria. It is likely that these small cells are functional both as cholangiocytes, involved with bile flow and processing, and as facultative hepatic progenitor cells (electron microscopy).

toocytes and partly by cholangiocytes. This structure led in turn to a channel completely lined by cholangiocytes. Descriptions and terms for these structures varied widely over the course of the next century and a half, based on light microscopy studies using different staining methods.<sup>5-8</sup> One hundred years or so after Hering, with the introduction of the electron microscope,<sup>9-12</sup> a standard description of the interface of the biliary tree and the hepatocyte canalicular system took this form: "Transition from bile canaliculi into bile ducts occurs at the edge of the portal tract. They are connected by the duct of Hering (cholangiole, canalicular-ductular junction, connecting duct, intermediate piece). The ducts of Hering are formed by hepatocytes from the limiting plate and by biliary cells. The lumen of the duct of Hering is slightly wider than that of the bile canaliculi and microvilli from both biliary cells and hepatocytes project into the lumen. Hepatocytes and biliary cells are attached by junctional complexes. The biliary cells have a basement membrane (basal lamina)."<sup>13</sup> (Fig. 1).

This description remains incomplete. Several authors wrote that the channels linking the interlobular bile duct with the canaliculi (the bile ductules), contact the liver parenchyma either at the limiting plate,<sup>8,9,14</sup> or inside the hepatic lobule.<sup>6,7,14-17</sup> Thus, one can distinguish ductules that have only a portal segment and ductules that have a portal and intralobular segment. The intralobular ductules are invested by a thin layer of mesenchymal components, usually only a basement membrane, but also sometimes a few collagen fibers,<sup>6,15</sup> and are accompanied by a microvasculature.<sup>15,18</sup>

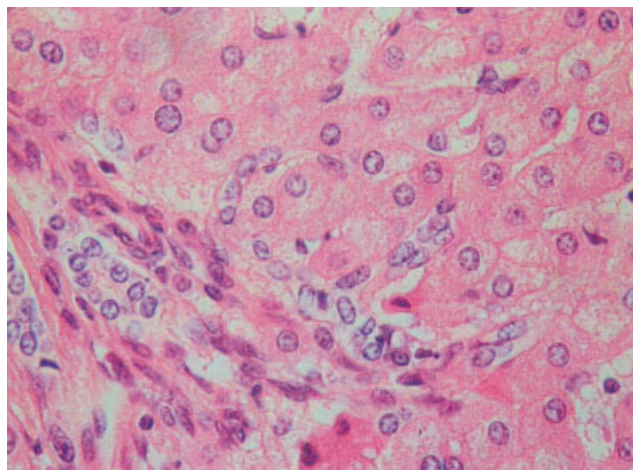


Fig. 2. Canals of Hering and intralobular ductules in normal liver tissue. These are small cuboidal strings of cells up and to the right of the portal tract (HE, original magnification,  $\times 400$ ).

The ductular-hepatocellular junction, lined partly by hepatocytes, partly by cholangiocytes (canal of Hering), apparently varies in length. Recent work using immunohistochemical staining of the biliary tree and three-dimensional analyses of serial sections of normal liver definitively established that the canal of Hering often does not stop at the limiting plate of hepatocytes surrounding the portal tract, but, rather, extends through it, linking to the hepatocyte canalicular system within the lobule, usually in the periportal region<sup>1,19</sup> (Fig. 2). In two-dimensional sections, immunohistochemical staining for biliary markers reveals cholangiocytes, singly or in small clusters, at variable distances from the portal tract (Fig. 3). But these cells prove not to be always isolated; instead, they often represent cross-sections of these most proximal

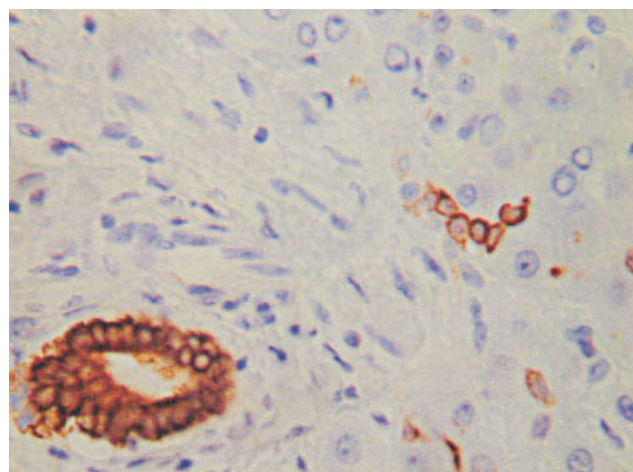


Fig. 3. Canals of Hering immunohistochemically stained for cytokeratin 19. They appear as isolated small cuboidal cells or strings of such cells (immunohistochemistry, Mayer's hematoxylin counterstain, original magnification,  $\times 400$ ).

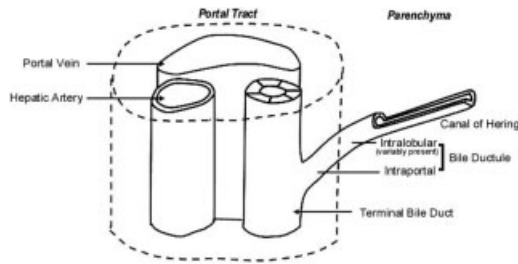


Fig. 4. Schematic diagram of a normal branching of the biliary tree. Note: the ductule segment may only extend to the limiting plate and no further, in which case there would be no intralobular portion.

branches of the biliary tree. Immunophenotyping studies further support this description.<sup>20,21</sup>

The recent interest in these seemingly isolated cells—usually representing cross-sections of the smallest biliary branches—particularly arises from their identification as an intra-organ stem or progenitor cell compartment of the liver.<sup>19,21–23</sup> Such identification guarantees that they will be an increasing focus of research in the coming years. A consensus on terminology so that such investigations can be performed, reviewed, and published in an expedient and generally comprehensible fashion is thus important.

### Terminology of Normal Structures

The terminology for these small structures has been highly variable and therefore confusing. Here we present

an updated view of these structures and names for the component parts (Fig. 4). We have retained well-known terms to avoid confusion through unnecessary change, but define them to reflect our current understanding of anatomy (Table 1):

- The *canal of Hering* is a channel partly lined by hepatocytes and partly by cholangiocytes. It represents the anatomic and physiological link between the intralobular canalicular system and the biliary tree. Cells of morphology and immunophenotype intermediate between hepatocytes and cholangiocytes (“intermediate cells,” see below) are not recognized in normal tissue. A corollary is that the true interface of hepatocytes and biliary tree does not reside, as has been assumed, at the “limiting plate,” but rather along an array of sites that project star-like from the portal tracts, along the canals of Hering.

- The canal of Hering continues into a channel lined entirely by cholangiocytes, which is termed the *ductule*. Ductules may or may not traverse the limiting plate, and thus may have an intralobular segment in addition to their intraportal course. The ductules in turn link to the smallest *interlobular bile ducts*.

Both these structures may branch at any point along their continuum, although their names still depend on whether they are partially or completely lined by cholangiocytes. It should be recognized that the cholangiocytes are a heterogeneous group of cells.<sup>24</sup> They perform a va-

**Table 1. Suggested Terms for Descriptions of Human Tissues, With Definitions, and Terms to be Discontinued**

	Suggested Terminology	Definition	Discouraged Terms
Normal liver	Canal of Hering	Physiologic link between hepatocyte canaliculi and the biliary tree. Partially lined by hepatocytes and by cholangiocytes (not by cells of intermediate morphology, which are not identified in normal livers).	
	Bile ductule	Link between canals of Hering and the interlobular bile ducts. Lined entirely by cholangiocytes, may begin at the edge of portal tract stroma, or may traverse the limiting plate, in which case it will have an “intralobular” as well as an “intraportal” segment.	
	“Isolated” cholangiocytes or progenitor cells in 2-dimensional tissue sections*	These cells are often, if not always, cross sections of canals of Hering and intralobular bile ductules and, therefore, not necessarily isolated. They may be referred to by the immunomarkers used to define them (e.g. CK19+, CK7+, NCAM+) and by the function which is under investigation (e.g. “cholangiocytes”, “progenitor cells”), always recognizing, however that they have multiple functions.	Oval cells Oval-like cells Stem cells
Diseased liver	Ductular reaction*	A reaction of ductular phenotype, possibly but not necessarily of ductular origin, in acute and chronic liver disease. May arise from: 1) proliferation of pre-existing cholangiocytes; 2) progenitor cells (local and/or circulating cells probably bone marrow-derived); 3) rarely, biliary metaplasia of hepatocytes.	Ductular proliferation, “typical” or “atypical” Biliary piecemeal necrosis Oval cells Oval-like cells
	Intermediate hepatobiliary cells*	Cells in ductular reactions that are morphologically or phenotypically intermediate between hepatocytes and cholangiocytes: >6 microns, <40 microns, dual phenotyping by immunostaining or by electron microscopy.	Ductular proliferation Biliary piecemeal necrosis Oval cells Oval-like cells

\*The isolated cholangiocytes/progenitor cells, ductular reaction and intermediate hepatobiliary cells in human liver are the EQUIVALENT of oval cells and oval cell reaction in rodent models. We discourage the term oval cells in human liver, because rodent models are not exactly comparable with human liver diseases.

riety of functional and physiological roles: they are a conduction system for bile,<sup>1,25</sup> they process and modify bile as it flows by,<sup>25</sup> they serve as an intra-organ facultative progenitor cell compartment,<sup>19,21–23</sup> and they may be involved in maintenance of matrix as well as in fibrogenesis.<sup>26–28</sup> They may well have other functions not yet identified. It remains unclear how many of these functions can be performed simultaneously.

When specialized studies (*e.g.*, immunohistochemical staining or electron microscopy) identify isolated cells in sections of typical thickness (<6 microns), most of these structures will be sampled in cross section. These cross sections will have the appearance of individual cells, small cell clusters, or “cuboidal strings.” Even though current research suggests that some of these cells are entering the liver from the circulation, they seem most often to do so in a site-specific, receptor-ligand dependent fashion. Thus, even when coming in from outside, they may still be found in contiguity with preexisting structures. Detailed three-dimensional studies to assess this possibility have not, as yet, been reported. Such cells may be referred to by the markers which highlight them (usually biliary-type markers or others, such as NCAM-1/CD56) and/or by reference to the predominant activity being studied (*e.g.*, “cholangiocytes,” “progenitor cells”). Thus, we would emphasize that: 1) the appearance as isolated cells is often an artifact of sectioning and visualization in a near two-dimensional plane; 2) that these cells are often engaged in multiple physiological tasks, not simply the one under investigation in a given study.

## Terminology of Reactive Lesions

In disease states, a commonly used term for the expanded population of epithelial cells at the interface of the biliary tree and the hepatocytes is “ductular proliferation.” This pair of words is problematic because the reactive lesions may not simply arise from proliferation of preexisting bile ductular cells, as they may also originate from activated and differentiated progenitor cells, from cells which entered from the circulation and differentiate towards liver cells,<sup>29,30</sup> or, more rarely, from biliary metaplasia of hepatocytes.<sup>31</sup>

The term *ductular reaction* was coined by Popper et al. in 1957<sup>32</sup> and has subsequently been used in the hepatology literature.<sup>28,35</sup> We prefer this to “ductular proliferation” for the reasons given in the preceding paragraph. “Ductular reaction” implies *a reaction of ductular phenotype, possibly but not necessarily of ductular origin*. This is in keeping with current practice in oncologic pathology, where the names of neoplasms reflect phenotype, as the supposed cell of origin is not always known. As noted, the epithelial component of the ductular reaction may actu-

ally derive from several sources: not only from the proximal branches of the biliary tree, but also from the circulation (often if not always from bone marrow), and from biliary metaplasia of hepatocytes. “Reaction” encompasses the complex of stroma, inflammatory cells, and other structures of diverse systems, all of which participate in the reactive lesion.

It was in the epithelial components of the ductular reaction that investigators first began to see features that suggested a correspondence with the “oval cells” of rodent models of carcinogenesis and stem/progenitor lineages.<sup>33–36</sup> Thus, investigators studying human tissues have often been tempted or required to use corresponding terminology: oval cells or, worse yet, “oval-like cells.” While similarities exist between the progenitor cell compartments of human and rodent livers, the different rodent models are not entirely comparable with the human situation, and use of the same term has created confusion as to what characteristics may be expected in the human ductular reaction. For example, a defining feature of oval cells in many rodent models of injury is production of alpha-fetoprotein, whereas ductular reactions in human livers rarely display such expression. That more fundamental differences may exist between human and rodent regenerative phenomena is highlighted by a recent study of partial hepatectomy in nonhuman primates.<sup>37</sup> Therefore, we suggest that “oval cell” and “oval-like cell” no longer be used in descriptions of human tissue.

Currently, the progenitor functioning of the ductular reaction attracts much attention. In particular, cells of intermediate morphology and intermediate immunophenotyping are of interest.<sup>38–43</sup> We suggest that these cells be referred to as just that: *intermediate hepatobiliary cells*, defined as larger than 6 microns in diameter (the approximate size of the normal canal of Hering cell, *i.e.*, the smallest cholangiocytes), but less than 40 microns (the typical size of a hepatocyte), with other features suggesting dual characteristics of both hepatocytes and cholangiocytes. These include, but are not limited to: simultaneous expression of biliary antigens (*e.g.*, cytokeratins 19, 7, OV-6) and hepatocyte antigens (*e.g.*, HepPar1, albumin, alpha-1-antitrypsin, biliary glycoprotein-1 detected by canalicular staining with polyclonal anti-CEA, and, occasionally, alpha-fetoprotein), other markers such as NCAM-1/CD56, and structural features such as basement membrane formation typical of cholangiocytes and canalicular membranes typical of hepatocytes (Figs. 5, 6).

Finally, it must be emphasized that we do not suggest that “oval cell” be discarded in describing rodent investigations. Its long history of use in those settings, with agreement between diverse investigators on its appropriateness, is not lessened by clarification of a different no-



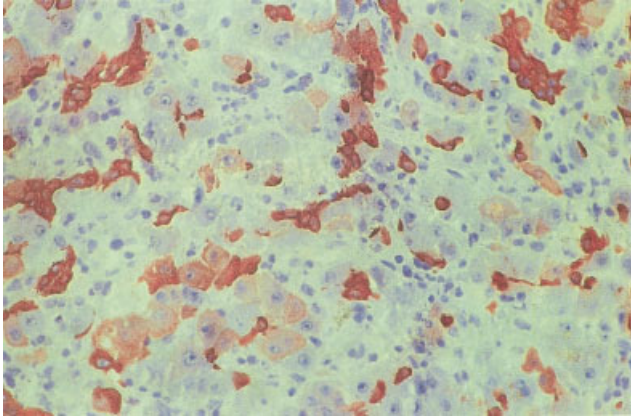


Fig. 5. Ductular reaction in a case of massive hepatic necrosis due to acetaminophen toxicity, immunohistochemically stained for cytokeratin 7 (on formalin-fixed, paraffin-embedded tissue). Arrowheads highlight some of the intermediate cells with biliary-type staining pattern, but morphology midway between cholangiocyte and hepatocyte (immunohistochemistry, Mayer's hematoxylin counterstain, original magnification,  $\times 250$ ).

menclature for human lesions. Rather, we recognize that the oval cells in rodents and the intermediate hepatobiliary cells in humans share important physiological roles, although morphologic and phenotypic differences are also prominent. Studies that move forward in both systems should contribute to knowledge of both. Moreover, differences may be as enlightening as similarities and focusing on these will serve the ends of both groups of investigators as well.

We also discourage the use of the phrase "stem cell" in labeling cells in histologic sections. The phrase, as used by cell biologists, has a particular connotation and definition, namely, that a cell is multipotent, if not totipotent, *and* that it is capable of self-renewal while also giving rise to other differentiated lineages (perhaps via asymmetric division). In humans, at this time, these criteria are difficult to demonstrate. Therefore, indiscriminate use of the term is to be avoided. It should be reserved for experimental settings, in animals or in humans, where both of these features can be demonstrated. "Stem cell-ness" of populations of cells can certainly be discussed, we obviously do not exclude that option, but investigators, at least at this time, cannot point to a cell in the liver and say "that is a true stem cell."

"Typical" and "atypical ductular proliferation" are problematic terms that perhaps are best avoided. These terms arose from attempts to histologically differentiate between extrahepatic and intrahepatic cholestasis in man.<sup>44</sup> Typical ductules allegedly have a recognizable lumen lined by cuboidal cells and are the result of proliferation of preexisting ductules, in analogy with the ductular reaction after ligation of the common bile duct in the

rat.<sup>45</sup> This type of ductular reaction is seen in an acute and total extrahepatic obstruction, like in the case of impaction of a gall stone or when the extrahepatic bile duct is totally occluded by a tumor.<sup>45</sup> Atypical ductules are described as thin, elongated structures that extend irregularly into the lobules, are lined by flattened cells, and lack easily discernible lumina. Atypical ductular reaction is closely related to progenitor cell activation (reminiscent of oval cell reaction in rodent models) (for review, see Refs. 21, 27, 45). This type of ductular reaction is seen in regeneration after necrosis, in extrahepatic subobstruction, and in vanishing bile duct diseases.<sup>45</sup> However, distinction between these two types of ductular reaction is not easy, as emphasized many years ago.<sup>46</sup> Furthermore, the advent of better imaging of the liver and biliary tree in the 1970s decreased the need for histological differentiation between intra- and extrahepatic cholestasis, whereas early endoscopic removal of impacted gall stones and stenting of obstructed bile ducts have rendered the histological observation of complete extrahepatic obstruction in man an uncommon occurrence. On the other hand, in incomplete extrahepatic obstruction and in vanishing bile duct diseases, the histological appearance is complicated by both hepatocellular and cholangiocytic damage, resulting in not only biliary obstructive lesions but also aspects of regeneration of progenitor cells which differentiate towards the most damaged cell type.<sup>37</sup> Since the term "atypical" is further burdened by a connotation of (pre)malignancy in diagnostic histopathology in general, we suggest that these terms are best avoided.

The ductular reaction is not to be confused with *ductal plate malformations*. Ductal plate malformations are abnormal bile duct-like structures: malformations, not reac-

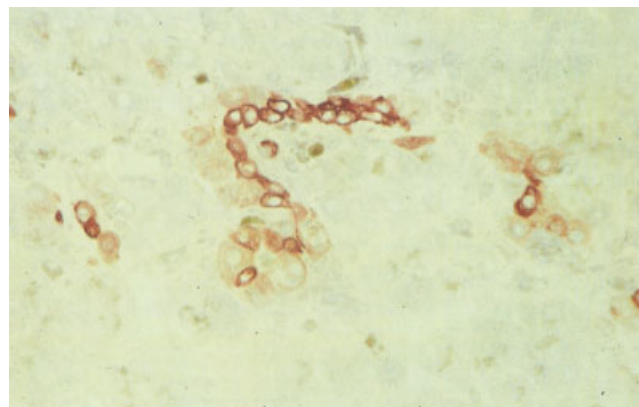


Fig. 6. Ductular reaction in a case of primary biliary cirrhosis, immunohistochemically stained for chromogranin A (on frozen tissue). Arrowheads highlight some of the intermediate cells with biliary-type staining pattern, but morphology midway between cholangiocyte and hepatocyte (immunohistochemistry, Mayer's hematoxylin counterstain, original magnification,  $\times 250$ ).

tive lesions.<sup>47</sup> The ductal plate malformation is observed in fibropolycystic liver diseases and in a subgroup of patients with extrahepatic bile duct atresia.

## Conclusion

This nomenclature is intended to be flexible enough so that it can be useful throughout the coming years, and perhaps even for decades of future investigation. Raising the topic also highlights how much remains to be investigated. Our knowledge of the structure of the canals of Hering and ductules has to be refined. Apart from the peribiliary plexus, the structure of the microvasculature of the liver and how it relates to ductular structures needs further elucidation,<sup>18</sup> as does the role played by innervation in development of ductular reactions.<sup>48</sup> Knowledge about matrix surrounding the ductules, canals of Hering, and ductular reactions<sup>49</sup> is fragmentary at best; still less is known of how it and the epithelial components participate in trafficking of inflammatory cells,<sup>50</sup> progenitor cells, and other cell types.

What has become clear is that full description and analysis of the ductules/canals of Hering in normal and in diseased liver requires three-dimensional analysis and/or serial sampling over time. Staining techniques that can highlight multiple antigens and structures simultaneously will also prove useful. This standardized nomenclature is adaptable for such new activities and approaches in human tissues and can facilitate reporting of future investigations with a degree of clarity that has until now been elusive.

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