The Canals of Hering and Hepatic Stem Cells in Humans

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Small, extraportal, hepatic parenchymal cells, positive for biliary-type cytokeratins, may represent hepatic stem cells, canals of Hering (CoH), and/or ductal plate remnants. We evaluated these cells 3 dimensionally in normal human liver and massive necrosis. Tissues from normal human livers and from 1 liver with acetaminophen-induced massive necrosis were serially sectioned, immunostained for cytokeratin 19 (CK19), and sequentially photographed. Images were examined to determine 3-dimensional relationships among CK19–positive cells. Immunostains for other hepatocyte and progenitor cell markers were examined. In normal livers, intraparenchymal CK19–positive cells lined up as linear arrays in sequential levels. One hundred of 106 (94.3%) defined, complete arrays within levels examined, most having 1 terminus at a bile duct, the other in the lobule, beyond the limiting plate. In massive necrosis, there were 767 individual CK19–positive cells or clusters around a single portal tract, 747 (97.4%) of which were spatially related forming arborizing networks connected to the interlobular bile duct by single tributaries. C-kit was positive in normal CoH. CK19 co-expressed with HepPar1, c-kit, and α-fetoprotein (AFP) in parenchymal cells in massive necrosis. Small, extraportal, biliary-type parenchymal cells represent cross-sections of the CoH that radiate from the portal tract, usually extending past the limiting plate into the proximate third of the hepatic lobule. The 3-dimensional structure of ductular reactions in massive necrosis suggests that these reactions are proliferations of the cells lining the CoH. Therefore, the CoH consist of, or harbor, facultative hepatic stem cells in humans. (Hepatology 1999;30:1425-1433.)

Facultative hepatic bipotent progenitor or stem cells have long been recognized in animal models of liver regeneration and hepatocarcinogenesis[1-4]; they are both facultative and bipotent because they are able to differentiate into mature hepatocytes or cholangiocytes in response to various types of stress or injury. In rodents, these cells have been referred to as oval cells in recognition of their cytologic appearance: oval cells containing oval nuclei with a high nuclear-to-cytoplasmic ratio.

Whether hepatic stem cells exist in human livers has been a topic of increasing interest and intense debate.[4-9] The most commonly recognized tissue reaction in support of such facultative stem cells in humans is the appearance of ductular reactions, often called "ductular hepatocytes," in massive hepatic necrosis.[10,11] These proliferating duct-like structures often have a biliary appearance at one end and a hepatocytic appearance at the other, with a range of morphological and immunophenotypical intermediates between them.[4,10,12-14] The appearance of these structures suggests to many investigators that regenerating hepatocytes in this setting arise from a cholangiocytic, or hepatic stem cell, compartment. However, others suggest that they are merely cholestatic, damaged hepatocytes undergoing biliary metaplasia in response to injury.[11,14] Which of these two interpretations of the ductular reaction to massive hepatic necrosis is correct has heretofore remained an unresolvable question.

If they do exist, the location of hepatic stem cells is also unclear. Because these cells are theoretically bipotent, important for regeneration of both hepatic parenchyma and biliary epithelium, they should lie somewhere in proximity to the juncture of these compartments. The canal of Hering (CoH), the link between the hepatocyte canalicular system and the biliary tree, is the obvious candidate. However, careful review of the literature regarding the location and structure of the CoH reveals a remarkable imprecision, even inconsistency, in conceptions of how the architecture of these structures look, where they are located, and how they may be identified.

Finally, some investigators have previously noted that occasional, small hepatic parenchymal cells, highlighted by immunohistochemical staining for biliary-specific cytokeratins, are consistently present at a small distance from the portal tract.[8] These investigators speculated that these isolated cells might represent ductal plate remnants from fetal development, the CoH, or the elusive hepatic stem cells. In response to these suggestions, we sought to systematically evaluate the relationship of these cells to the CoH in normal adult liver tissue and to the ductular reactions following massive hepatic necrosis.

MATERIALS AND METHODS

Examination of Normal Tissues. We selected 4 formalin-fixed, paraffin embedded normal tissue specimens for study from 4 patients, 2 men and 2 women. One was from a 64-year-old woman undergoing exploratory laparotomy for ovarian carcinoma, without metastatic tumor in the liver (specimen 1). The other 3 resections (specimens

Abbreviations: CK19, cytokeratin 19; CoH, canal of Hering; AFP, α-fetoprotein.

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2, 3, and 4, respectively) were for lesions of metastatic colon carcinoma, patient ages 42, 53, and 66 years. These 3 specimens were distant from the mass lesion for which the resection was performed. All 4 specimens were histologically normal by routine light microscopy, without apparent hepatitic, cholangitic, or vascular injury, *i.e.*, there was no acute or chronic cholestasis, periportal ductular reaction, portal edema or scarring, sinusoidal dilatation or congestion, or hepatitis.

These 4 specimens were serially sectioned at 4-μm intervals, with the order of sections being carefully maintained and labelled, 1 section per slide. Sixty-three serial levels were obtained from specimen 1 and 20 serial levels from each of specimens 2, 3, and 4. Immunohistochemical staining was then performed on each slide using a mouse monoclonal antibody specific for cytokeratin 19 (CK19) (DAKO Corp, Carpinteria CA). In brief, slides were hydrated through graded alcohols and xylene. Following quenching of endogenous peroxidase with hydrogen peroxide and blocking of nonspecific binding with bovine serum albumin, the primary antibody was applied at a dilution of 1:100 for 2 hours at room temperature. Goat antimouse antibodies were then applied followed by ABC complex (Vector Laboratories, Burlingame CA). Diaminobenzidene was used as the colorizing agent with Mayer's hematoxylin as the counterstain. Interlobular bile ducts served as an internal positive control. Substitution of bovine serum albumin for the primary antibody served as a negative control.

Two representative portal tracts were selected on the first level of each sectioned specimen. Portal tracts were selected for examination on the basis that they and all their branches in deeper levels fulfilled 2 criteria: (1) they were present throughout all sampled levels and did not approach the liver capsule, the original margins of resection, or the edge of the tissue as sampled on the slide; (2) they were present predominantly in cross-section throughout all levels, assessed by seeing that the majority of bile ducts in the portal tract were present in circular or slightly oval cross-sectional profile. The selected portal tracts were then serially photographed, level by level, at 100× magnification. Color prints (5×7 inches) were obtained. Each print image was checked against the corresponding slide and its section level labelled on the print. Individual and small clusters of CK19–positive cells, at any distance from the portal tract, were identified (Fig. 1).

Once these images were labelled, organized, and oriented, the relationship of these CK19-positive cells to each other through the subsequent levels of tissue was assessed by careful comparison of 1 level to the next (Fig. 2). Notations of the relationships of these cells from level to level were made as shown in Fig. 3. When cells lined up along sequential levels indicating a continuous string, that string was indexed with a number. Notations were also made as to whether the terminal ends of the string were located in the lobule away from the portal tract, at the limiting plate, or at a well-formed bile duct lying within the portal tract.

Examination of Liver With Massive Hepatic Necrosis. One archival, formalin-fixed, paraffin-embedded block of tissue was selected from a 45-year-old woman who had undergone orthotopic liver transplantation for massive hepatic necrosis secondary to acetaminophen toxicity. This block was serially sectioned at 2-μm intervals, yielding 20 sequential levels. Immunohistochemical staining for CK19 was performed, and a representative portal area with surrounding parenchyma was chosen for study as the normal portal areas had been (Fig. 4). High power (100×) photographs were taken and processed as 8×10-inch prints. Acetate sheets were laid over these images and all CK19–positive duct-like structures (including "ductular hepatocytes") were traced, as were portal blood vessels. These acetate sheets were then put in sequence, with the blood vessels of the portal tract acting as guides for proper rotational alignment.

Once the transparencies were labelled, organized, and oriented, an arbitrarily selected CK19 duct-like structure in the tracing from 1 level was then colored in. This duct was then seen to overlay neighboring duct structures on adjacent levels, which were therefore assumed to be contiguous with this first duct in 3 dimensional space and were also colored in. Moving up and down through the images, additional spacially related CK19–positive duct structures were further shaded in the same color until no further spatially related structures could be identified. Then, another arbitrary, unshaded CK19–positive duct-like structure was colored in and the process repeated. This procedure was carried out with different colors until all duct-like structures surrounding the studied portal area were shaded or were found to be unrelated to any adjacent structures and therefore left unshaded (examples in Fig. 5).

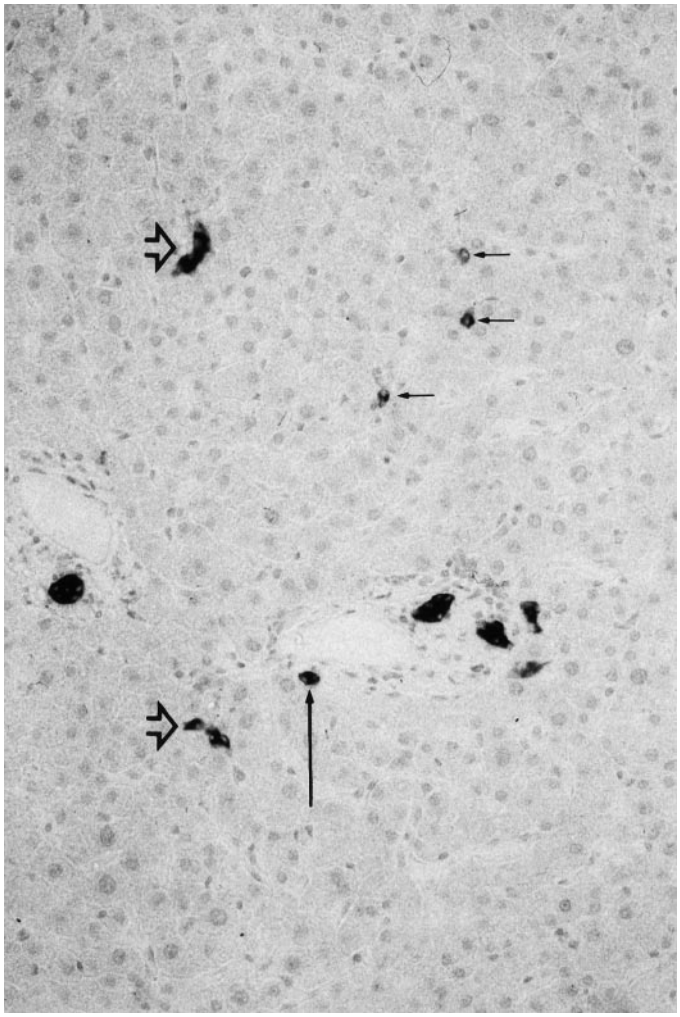

FIG. 1. Individual CK19–positive cells (*smallest arrows*) and strings of cells (*open arrows*) in the hepatic parenchyma, away from the portal tract. One CK19–positive profile (*long arrow*) represents a cross section of the CoH which is traced, in its entirety, in Fig. 2. (100×, immuhonistochemistry, DAB, Mayer's hematoxylin.)

Other Immunohistochemical Studies. Other potential markers of hepatic progenitor cells were also studied in individual sections of the normal liver tissues and in the case of massive hepatic necrosis. These markers included α-fetoprotein (AFP; Nova Castra, Newcastle, UK), chromogranin A (Dako), HepPar1 (Dako), and c-kit (rabbit polyclonal antibodies, Novacastra). Amplified double staining for each of these markers with CK19 was performed with a Catalyzed Amplification System (Dako), Horseradish Peroxidase System. Slides were deparaffinized and rehydrated as described earlier, then heat-induced epitope retrieval was performed by 1,000-watt microwaving in 0.01 mol/L citric acid buffer pH 6.0. Tissues were quenched of endogenous peroxidase with hydrogen peroxide and nonspecific protein binding was blocked with serum-

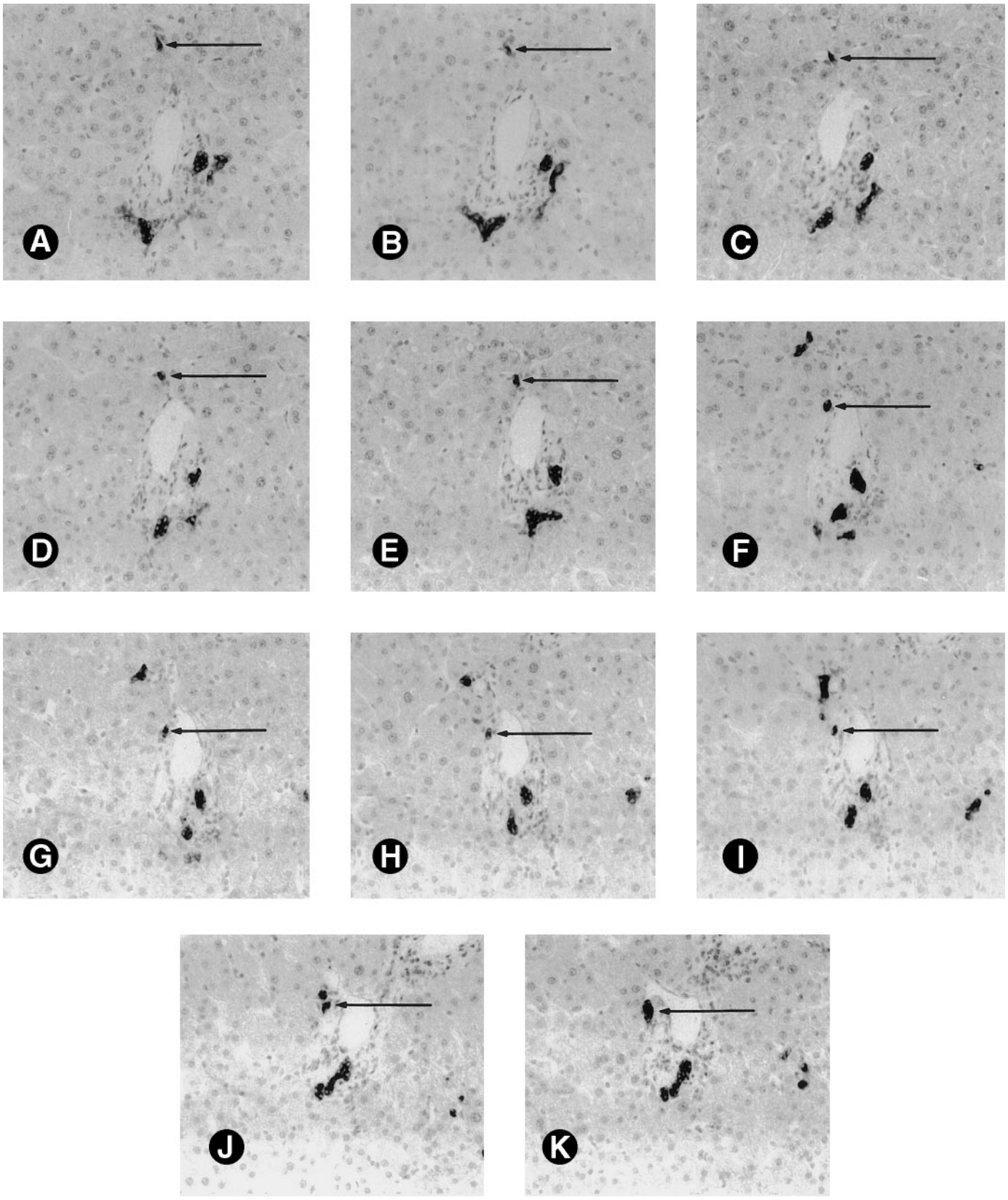

FIG. 2. Sample sequential images from a single portal tract in a normal liver. The tissue was immunohistochemically stained for CK19, highlighting the bile ducts and CoH profiles distant from the portal tract. Here we follow 1 CoH (*arrows*) from its lobular terminus (A) to its bile duct terminus (K). Note that other CoH can be seen in these levels. (40× immunohistochemistry, DAB, Mayer's hematoxylin.)

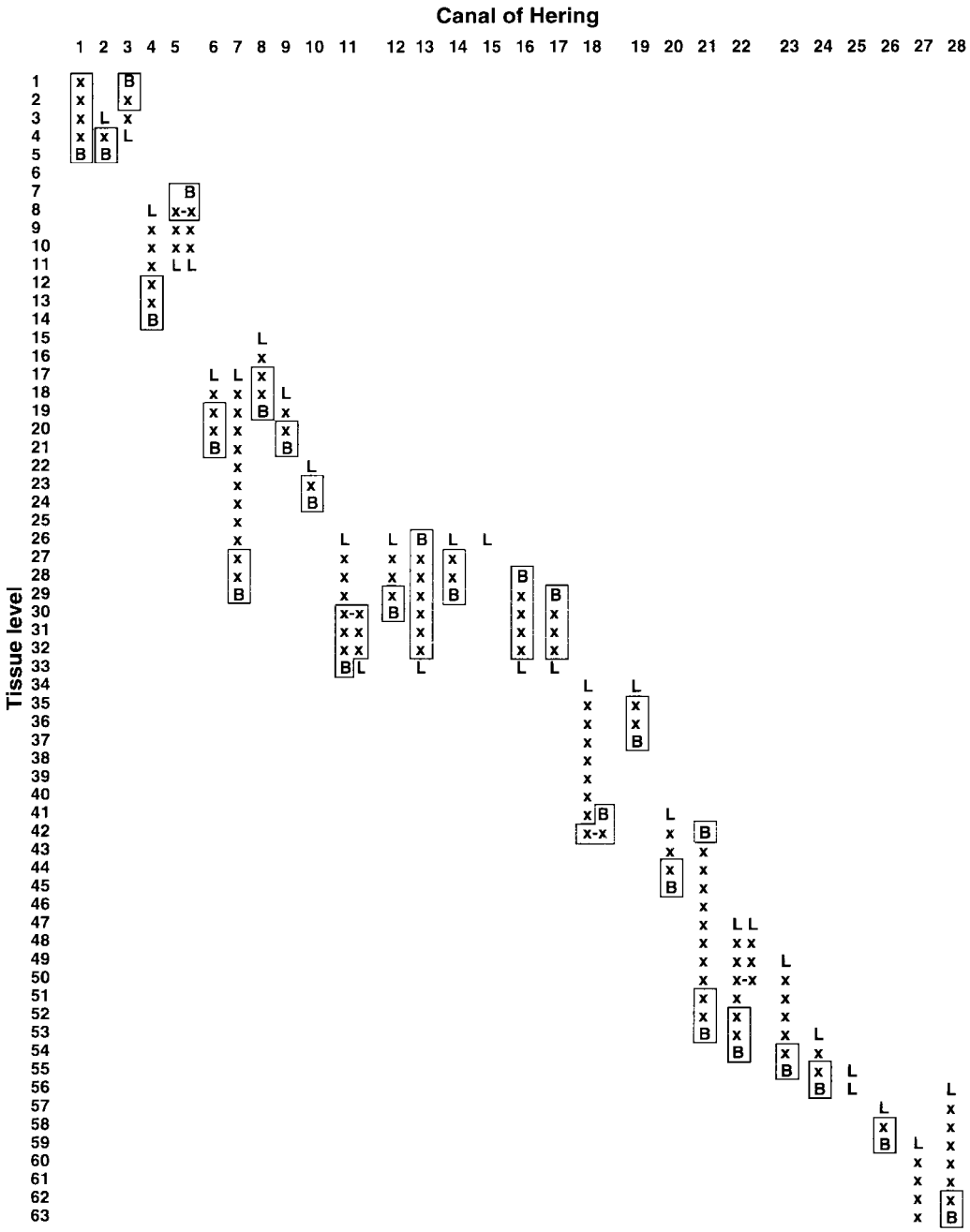

FIG. 3. A sample tabulation of the CK19–positive CoH duct profiles around a single portal tract, followed through 63 sequential sections. L, terminus in the hepatic lobule; B, terminus at interlobular bile duct; x, Canal of Hering profile. The *boxes* indicate profiles that are located within the mesenchyme of the portal tract.

free protein. The primary antibodies were detected using biotinylated antimouse (for HepPar1, AFP, chromogranin A) or antirabbit (for c-kit) secondary antibodies followed by streptavidin-biotin-horseradish-peroxidase conjugate. The conjugate system was then amplified (biotnyl tyramide) and additional streptavidin-horseradish-peroxidase was added. The complex was visualized with DAB. Nonamplified co-staining for the CK19 was then performed on a Ventana Nexus automated immunohistochemical instrument using Ventana's detection systems and buffers (Tuscon, AZ). Slides were incubated with the mouse anti-CK19 primary antibody followed by a biotinylated antimouse secondary antibody followed by the addition of strepavidin-alkaline phosphatase conjugate. Enhancer was then added (0.1% levamisole). The complex was visualized with NBT-BCIP (nitro-blue tetrazolium, 5-bromo-4-chloro-3-indolyl phosphate) substrate/chromogen. Slides were then counterstained with nuclear fast red.

Because of the density of staining of both c-kit and CK19 when co-stained in normal tissues, cells with double staining were difficult to identify in these tissues. Therefore, to determine if the CoH were c-kit–positive, 3 serial sections of normal tissues were obtained, the first and the third stained for CK19 and the intermediate level stained for c-kit alone (again using the Catalyzed Amplification System technique). By examination of serial sections, c-kit staining of the CoH could be assessed.

RESULTS

Examples of tabulation of CK19–positive cells and strings in normal tissue are given in Fig. 3, in which 1 portal tract is followed from specimen 1. The compiled descriptive data for the normal tissues is summarized in Table 1. Seven hundred and forty-six CK19–positive profiles, either individual cells or strings of cells, were identified in proximity to the portal tracts examined. All of these profiles were seen to correspond to additional profiles on at least 1 adjacent tissue level, comprising 144 linear arrays, 106 of which could be traced

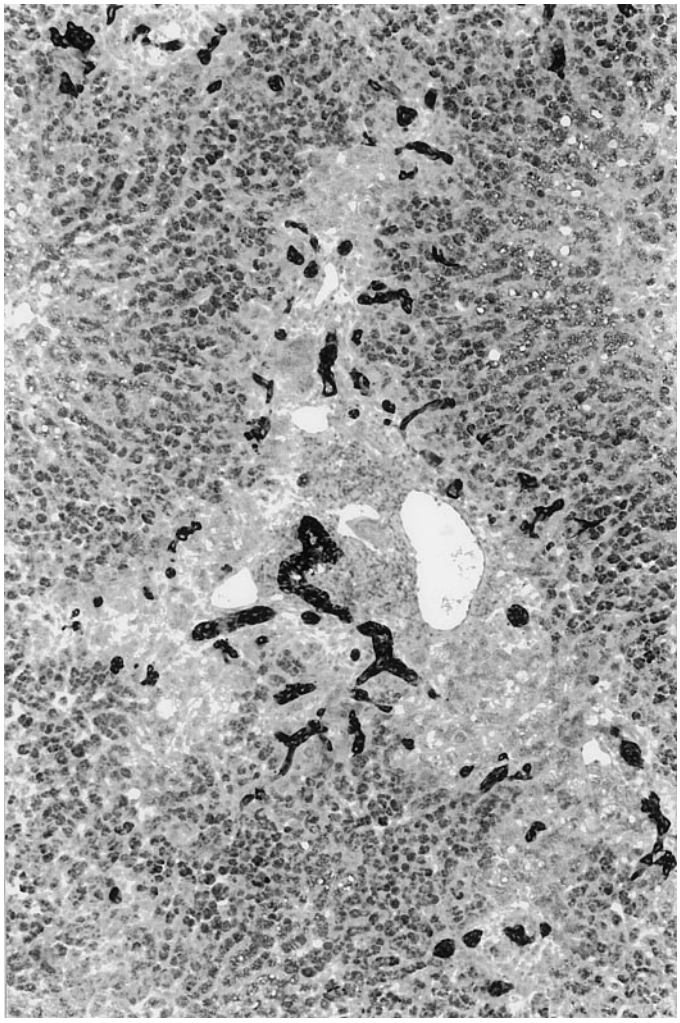

FIG. 4. Immunohistochemistry for CK19 highlights the ductular reaction around a single portal tract in massive hepatic necrosis secondary to acetaminophen toxicity. The dark grey staining cells at the periphery of the image are necrotic hepatocytes with nonspecific staining. This image corresponds to level 12 in Fig. 5. (100×, immunohistochemistry, DAB, Mayer's hematoxylin.)

completely to a terminus at each end, within the tissue levels examined. An example is shown in Fig. 2. The remaining 38 arrays appeared to extend beyond the tissue levels examined and thus could not be fully characterized.

One hundred of these 106 linear arrays of CK19–positive cells (94.3%, "B-B" or "L-B", Table 1) connected to a bile duct at 1 end, only rare arrays being located entirely within the hepatic lobule away from the portal tract (5.7%, "L-L"). The majority of arrays (97/106, 91.5%, "L-B") had 1 terminus in the lobule or at the limiting plate and the other at a bile duct. Only a few arrays (3/106, 2.8%, "B-B") began at a bile duct and ended, after traversing into the lobule beyond the limiting plate, at another bile duct. Eighty-five of 106 arrays (80.2%) traversed beyond the limiting plate for some part of their length (not shown in Table 1). Most linear arrays were directionally monotonic, though some followed more complicated paths. Some arrays, rather than being simply linear, displayed branch points (17/106, 16.0%, "Branched"). The number of CK19–positive profiles per portal tract per level ranged from 0 to 13 (mean 3.5 ± 2.4, median 3). The number of CK19–positive profiles encountered per 100 μm longitudinal length of portal tract was 6 to 15 (mean 10.8 ± 3.0, median 10), or 1 CK19–positive profile originating from the interlobular bile duct approximately every 10 μm.

For purposes of roughly evaluating the distance these arrays extended beyond the limiting plate, the hepatic lobule was divided into 3 concentric regions centered on the examined portal tract and extending to the nearest terminal hepatic veins. None of the 85 linear arrays present in the lobule extended to within the region nearest the central vein. A few strings extended into the middle region (13/85, 15.0%). The majority extended no further than the periportal region (72/85, 85.0%). Careful examination at high power revealed that some of these linear arrays formed complete duct-like circles, but without a lumen visible by light microscopy, before entering the stroma of the portal tract, although most did so on passing through the limiting plate.

A sample CK19–stained level of the ductular reaction in acetaminophen-related massive hepatic necrosis is given in Fig. 4. Examples of the schematicized diagrams obtained by tracing these ductular reactions over several levels are given in Fig. 5. Seven hundred and forty-seven of 767 CK19–positive profiles (97.4%) were spatially associated with neighboring structures on adjacent levels, whereas 20 were not (2.6%). The data for all levels examined is summarized as a histogram, from tissue level 1 to 20, with the number of CK19–positive profiles recorded by color (Fig. 6).

As can be seen in Fig. 5, CK19–positive profiles within well-defined regions around a portal tract shared the same color showing that, on any 2-dimensional cross section, what appeared to be isolated profiles were in fact cross sections of a complex, arborizing network of CK19–positive cells. Moreover, 4 of these networks could be seen to arise from a single chain of cells that in turn linked up to a bile duct, corresponding to the pink, blue, green, and red networks in the histogram (Fig. 6). This is shown in Fig. 5, in levels 11 and 12 where the red network links via such a longitudinally sampled profile to a bile duct.

Other Immunohistochemical Studies. In normal liver tissue, CoH were diffusely and strongly positive for c-kit (Fig. 7), but were negative for chromogranin A, HepPar1, and AFP. Bile ducts occasionally showed a fine granular stain for c-kit, though they were also negative for all other markers. Hepatocytes were negative for c-kit, AFP, and chromogranin A, but showed strong, coarsely granular cytoplasmic staining for HepPar1.

In massive hepatic necrosis, c-kit cytoplasmic staining of CK19–positive cells was present in the ductular reaction and focally in occasional bile ducts, though much more faintly than in the normal CoH, with a diffuse, fine granularity (Fig. 8). Hepatocytes were negative for c-kit. Some of the CK19–positive cells of the ductular reaction showed co-staining for HepPar1 and AFP. Bile ducts were negative for both these antibodies; hepatocytes were focally positive for AFP and diffusely positive for HepPar1. No staining for chromogranin A was identified in any cells.

DISCUSSION

The CoH were named for the anatomist who first described them in 1866 as the link between the hepatocyte canalicular system and the biliary tree.[15] Electron microscopic examination of the canals revealed that they are partially lined by hepatocytes on 1 aspect and by small cuboidal or pyramidal

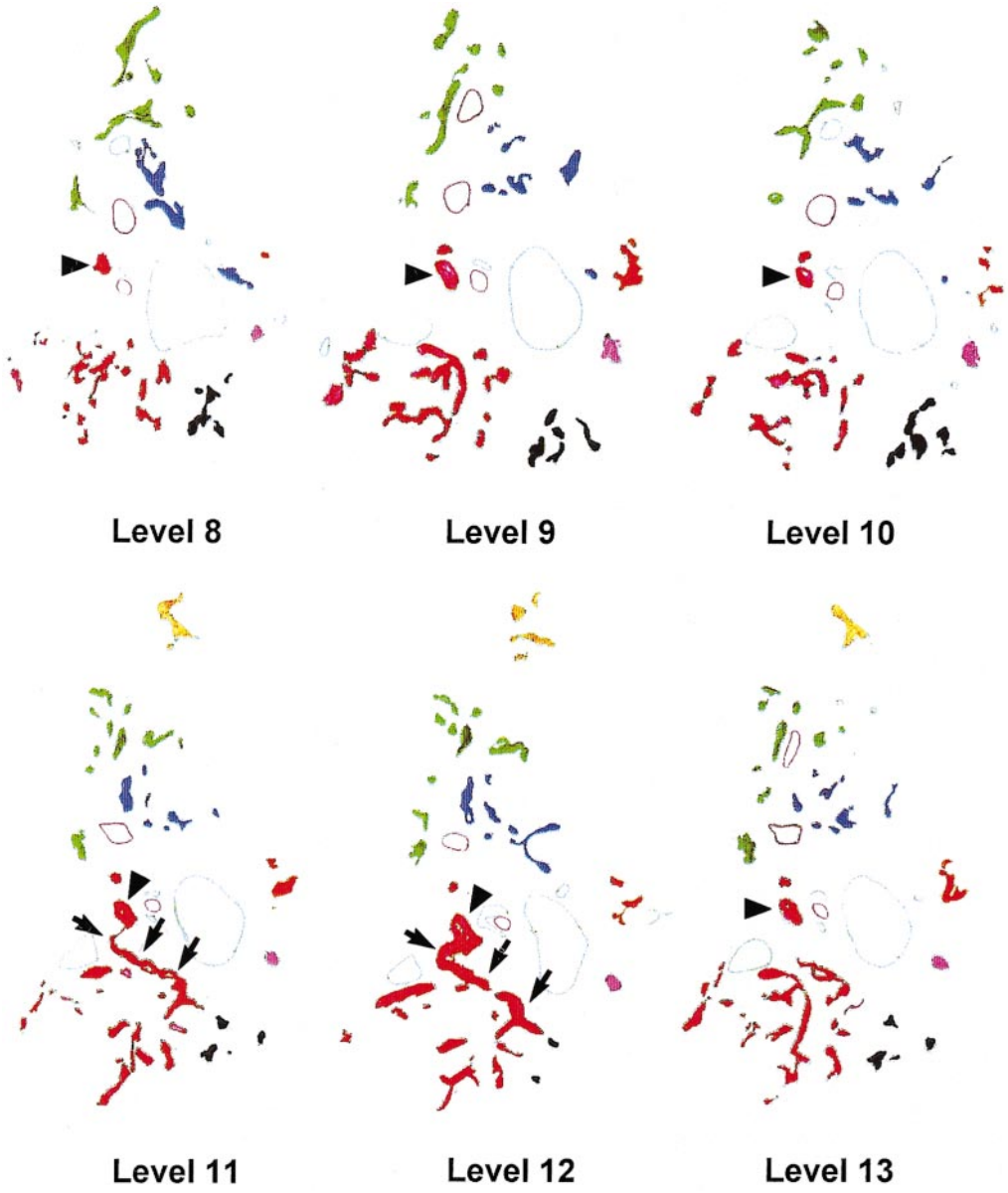

FIG. 5. Six sample tracings of CK19–positive ductular reactions in sequential levels around a single portal tract. Colors are assigned to indicate contiguity of structures when analyzed in 3 dimensions. On each level 1 bile duct is marked by an *arrowhead*. Note in levels 11 and 12 where the red arborizing structure connects via a single branch to this bile duct (*arrows*).

cells with scant cytoplasm on the other.[16] These small cells have basement membrane like more distal portions of the biliary tree, but an apical surface, which, by electron microscopy, looks similar to an hepatocytic canalicular membrane.

In this study we find that the CK19–positive parenchymal cells under investigation only appear isolated from each other when a single histologic section is examined. When sequential sections are examined yielding a 3-dimensional view, they are not, in fact, isolated from each other. Rather, they are cross-sectional profiles of linear structures that extend from the interlobular bile ducts to the limiting plate of hepatocytes and beyond. These profiles appear to be completely circular duct-like structures as they pass through the portal stroma and sometimes into the lobule, but, for at least some portion or all of their extra-portal extent, they are a row of cells lying adjacent to hepatocytes. This corresponds to our understanding of the anatomy of the CoH.

Rare descriptions of the CoH explicitly[16] or implicitly[17] suggested that they extend, at least focally, beyond the limiting plate into the lobule. However, this concept has not

TABLE 1. Summary of Features of CK19–Positive Linear Arrays Associated With 8 Examined Portal Tracts in Normal Livers

Specimen	Portal Tract	# of Levels Examined	Total # of CoH	Termini			Complexity	
				B-B	L-B	L-L	Branched	Linear
1	A	63	25	1	23	1	3	22
	B	63	29	0	26	3	6	23
2	A	20	6	0	6	0	2	4
	B	20	10	1	9	0	2	8
3	A	20	8	0	7	1	1	7
	B	20	12	0	12	0	2	10
4	A	20	5	1	3	1	1	4
	B	20	11	0	11	0	0	11
Total			106	3	97	6	17	89
%				2.8	91.5	5.7	16.0	84.0

Abbreviations: B-B, linear array which runs from 1 bile duct, through the lobule, to another bile duct; L-B, linear array which has 1 terminus in the lobule or at the limiting plate and the other terminus at a bile duct; L-L, linear array located entirely within the lobule without a component entering the portal tract or linking to a bile duct.

been universally acknowledged: most descriptions are vague on the matter[18-20] or explicitly, albeit inaccurately, characterize the CoH as invariably stopping at the limiting plate.[21-24] Our data from normal livers, however, confirms the notion of interlobular extension of the CoH, correcting a common misconception.

Although difficult to identify without the use of immunohistochemical stains, these CoH profiles have been described using more routine histochemical techniques. Recently, a study of normal liver using Masson trichrome by Crawford et al.[25] identified cuboidal strings, which probably correspond to at least some of the CK19–positive cells identified as the CoH here. The only discrepancy between that study and ours is in the quantitation of CoH profiles identified per portal tract. That study only identified 5 ± 5 (range 0-16) cuboidal strings per biopsy for a corresponding 21 ± 10 (range 5-38) portal tracts. That would seem to be a considerable undercount by our results, but our use of immunohistochemical staining for CK19 is in all likelihood more sensitive than the Masson trichrome used in that study. We also counted single cells, which the earlier study did not.

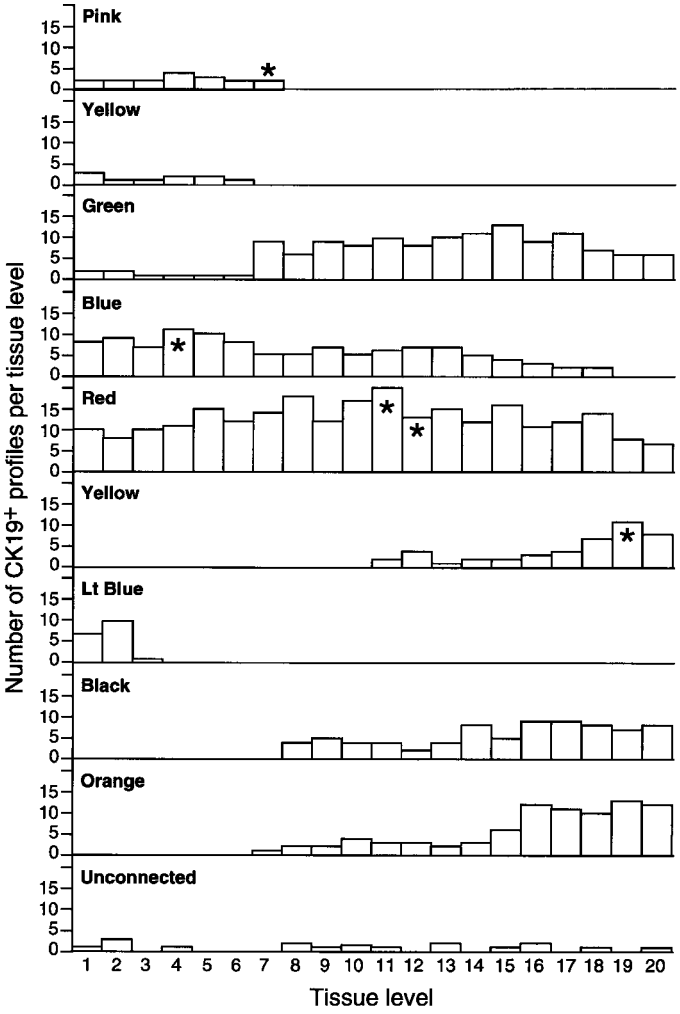

FIG. 6. Histogram summary of the number of interrelated CK19–positive cross-sectional profiles over 20 levels around a single portal tract. Colors indicate structures that were seen to be connected by examination in 3 dimensions, from level to level. *Asterisks* indicate levels where these complex arborizing structures arose from a single branch, which in turn connected to a bile duct.

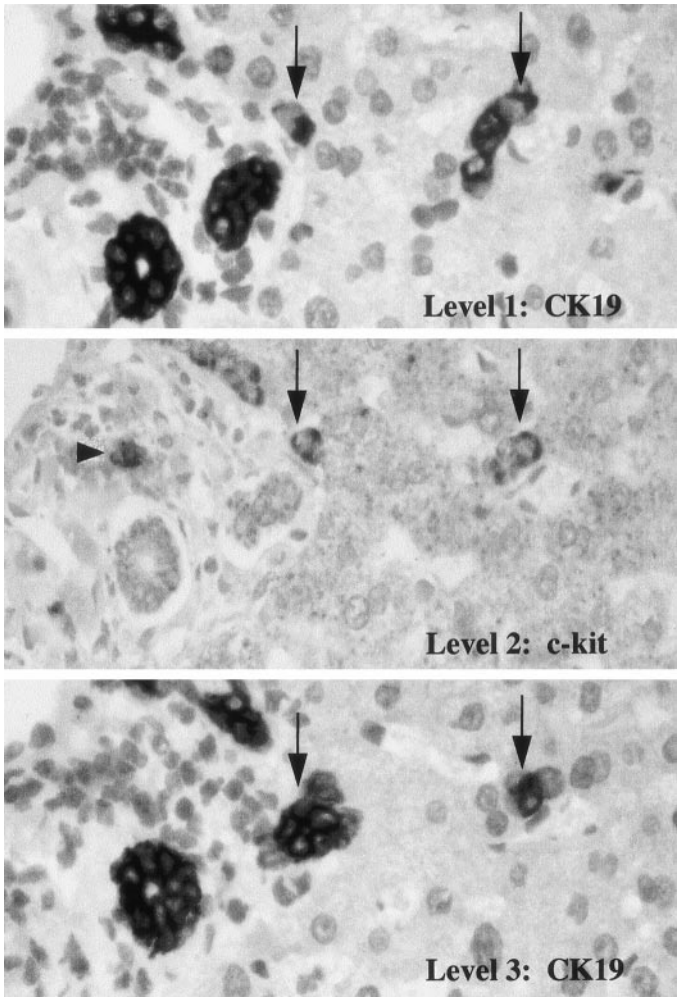

FIG. 7. Three serial sections of normal liver, immunohistochemically stained for CK19 (levels 1 and 3) and c-kit (level 2). The bracketing of the intermediate level by CK19 stained levels on either side allows for accurate identification of the CoH cross-sections in all 3 levels (*arrows*). The cells lining the CoH are positive for c-kit, bile ducts and hepatocytes were negative. In all normal tissues examined this distribution of staining was consistently seen. A single isolated c-kit positive cell in level 2 (*arrowhead*) is probably a mast cell. (100×, immunohistochemistry, DAB, Mayer's hematoxylin.)

The actual structure of the CoH becomes significant when we look at how it changes in the setting of a disease state, in this case that of massive hepatic necrosis secondary to acetaminophen toxicity. Our analysis of sequential levels of such a diseased liver show that the overwhelming majority of CK19–positive cells link up to each other in large, complex, arborizing structures branching from single linear arrays, which then connect to the bile ducts within the portal tracts. Only rare CK19–positive cells remain isolated in the hepatic parenchyma, apart from the biliary tree. Thus, to explain the appearance of ductular hepatocytes in massive hepatic necrosis on the basis of biliary metaplasia of hepatocytes, we would have to accept the unlikely conclusion that such metaplastic hepatocytes, damaged and cholestatic, could find their way topographically to link with existing bile ducts. Moreover, that link would consist of a single linear array of cells.

We suggest that this single array is the pre-existent CoH, which has undergone a reactive proliferation and arborization. Support for this interpretation is twofold: (1) from our

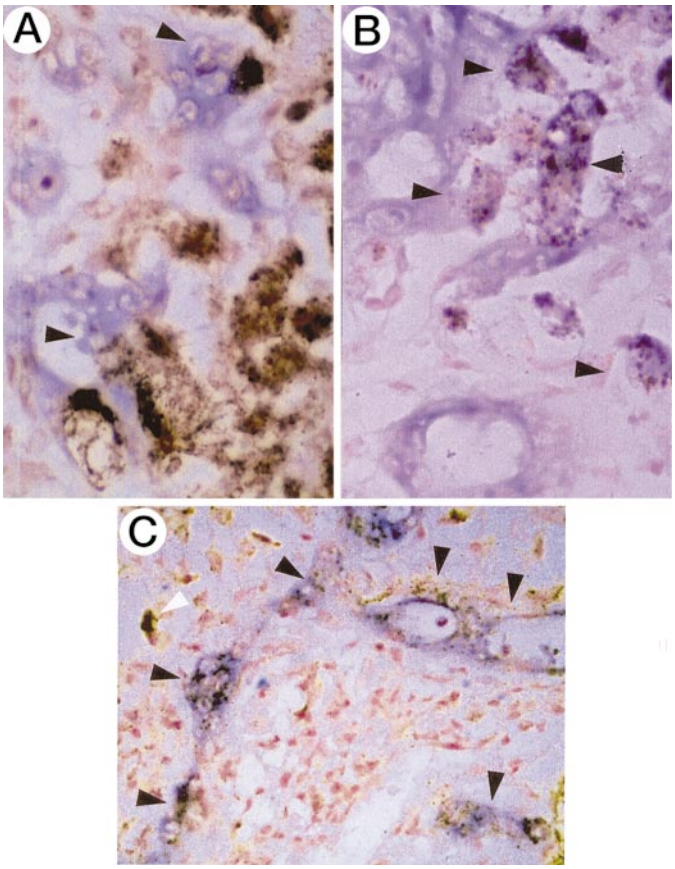

FIG. 8. Double immunohistochemistry for hepatic progenitor cell markers (*brown*) and CK19 (*blue*) in the ductular reaction of massive hepatic necrosis: (A) Co-staining for HepPar1 and CK19 shows cells with either 1 or the other marker, but some intermediate cells (*arrowheads*) show staining for both. (B) Co-staining of AFP and CK19 shows cells with either 1 or the other marker, but some intermediate cells (*arrowheads*) show staining for both. (C) Staining for c-kit shows a diffuse, fine granularity through the cytoplasm of most CK19–positive cells (*dark arrowheads*). A c-kit–positive mast cell is identified on the left (*white arrowhead*). Note: extended digestion for double staining yields less distinct morphology compared with single label immunohistochemistry. (200×, double immunohistochemistry, DAB, NBT-BCIP, Nuclear Fast Red.)

analysis of normal liver tissue, one would expect approximately 4 CoH to branch from a 40-μm length of bile duct. Precisely that number of links between CK19–positive networks of cells are identified in that 40-μm length of portal tract studied in the case of massive necrosis; (2) the case of acetaminophen toxicity used in this study has previously been used in a study of proliferation rates in ductular reactions and these CK19–positive cells are in fact highly proliferative measured by immunohistochemical staining for Ki-67.[26] Therefore, the 3-dimensional examination of these structures seems to convey directionality on the morphological transformation: we conclude that the small cells of the CoH can regenerate hepatocytes in massive hepatic necrosis and thus represent facultative hepatic stem cells, echoing findings in the experimental literature.[27]

Previous studies[28,29] employing immunohistochemistry and electron microscopy identified small chromogranin A–positive epithelial cells that lay directly adjacent to the terminal branches of the biliary tree. These were speculated to be hepatic progenitor cells. We failed to identify chromogranin A–positive cells by immunohistochemistry in our normal or diseased tissue, which may relate to our use of formalin fixed, paraffin embedded tissues as opposed to the frozen tissue used in the other study. The scale of our light microscopic examination of tissues also precludes our ability to conclusively determine if all the CK19–positive cells lie within the CoH, as opposed to some of them perhaps lying directly adjacent to it. Thus our data, neither confirming nor contradicting the earlier studies, remains compatible with their findings.

Regarding the other immunohistochemical staining performed, we found that stem-cell–factor receptor c-kit is positive diffusely through the CoH and the intralobular bile ducts, both in normal liver and in our case of massive hepatic necrosis. This distribution is more widespread than that reported by Baumann et al.,[30] which may relate to our use of a more sensitive detection system than was used in that study. It is tempting to speculate that the presence of c-kit in these cells is related to the origin of hepatic progenitor cells from bone marrow-derived precursors, which has recently been described.[31,32] The difference between the dense staining for c-kit in normal CoH cells compared with the more scattered staining in the ductular reaction may relate to proliferation and growth of the cells; if *de novo* c-kit production does not take place then the existing antigen would be more diffusely localized. We also failed to identify staining for HepPar1 and AFP in the normal CoH indicating, if all these cells can act as progenitor cells, that they do not preserve the fetal phenotype as might have been expected.[8] The development in massive hepatic necrosis of HepPar1 and AFP–positive cells, which co-express CK19 not only confirms the hepatocyte regenerating potential of these cells, but also that it is only during a regenerative process following injury that this fetal phenotype is recapitulated.

As far as the structure of the CoH is concerned, this reconceptualization of their normal anatomy may help to explain the bimodal hepatic response to varying degrees of injury in acute hepatitis. Regenerative responses to a mild degree of acute hepatitic injury are evidenced by mitoses of mature appearing hepatocytes and thickening of the liver cell plates as the liver heals. A ductular reaction is seen only in more severe cases, as in the one studied here. So 2 modes of regeneration are evident depending on severity of the necrosis.[33] The structure of the CoH may account for these differences: perhaps only when injury is severe enough for the necrosis to extend from the central vein to the CoH, then cells comprising the canals begin to proliferate and to regenerate hepatocytes. Thus, by extending out into the lobule, the CoH may act like a trip-wire, the facultative progenitor cells they harbor being stimulated to proliferate and mature into hepatocytes when the necrosis reaches them spatially.

In conclusion, our data suggests that a revision of the common conception of the architecture of the CoH is now required. This revision may call for additional changes or refinements regarding our understanding of hepatic physiology and of hepatic response to injury. The actual interface of hepatic parenchyma and the biliary tree is not the limiting plate, but rather the zone of hepatocytes adjoining the CoH, radiating from the portal tract. With such an extra-portal location, the CoH can now be seen as a likely source for many of the ductular reactions characteristic of a variety of acute and chronic liver diseases occurring at that interface. The canals may even represent primary sites of injury in some

disease processes.[34,35] Finally, in examining a case of massive hepatic necrosis, our data support the idea that the typical ductular reaction in this setting is topographically associated with and derives from the CoH. The CoH therefore must be comprised of, or at least harbor, facultative hepatic stem cells.

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