

PROLIFERATIVE AND SELECTED OTHER LESIONS IN THE LIVER OF RATS

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INTRODUCTION

The liver is not a homogeneous organ. It is composed of two types of epithelial cells, hepatocytes and bile duct cells as well as sinusoidal lining cells of several types and other stromal supporting cells. Each of these cell types can be associated with proliferative lesions. However, this classification scheme focuses on lesions unique to the liver, primarily proliferative lesions of the epithelial components of the liver, i.e. hepatocellular proliferative lesions, cholangiocellular proliferative lesions, and combinations thereof. Hepatoblastomas are not included as they are rare in rats. Neoplasms of the specialized types of sinusoidal lining cells i.e. Kupffer's cells and fat storage (Ito) cells likewise are not described. A few nonproliferative lesions of the liver which sometimes affect interpretation of proliferative lesions also are included. The lesions described occur as spontaneous and/or induced lesions in laboratory rats, primarily F344 and Sprague-Dawley rats. ^{3,12,15,18,22-25,31,37}

MORPHOLOGY

A. HEPATOCELLULAR LESIONS

Foci of Cellular Alteration

Gross appearance: Foci of cellular alteration usually are not observed grossly. Occasionally, large foci may be seen as pinpoint or 1-2 mm pale spots on the surface of the liver.

Microscopic appearance: Focal lesions of hepatocytes characterized primarily by altered cytoplasmic tinctorial properties are classified as foci of cellular alteration. ³² Based upon cytoplasmic staining with hematoxylin and eosin, they have been classified previously as: basophilic, eosinophilic (acidophilic, ground glass), clear cell, or mixed type. ^{1,18,37} More recently, amphophilic cell foci, diffusely basophilic cell foci, and vacuolated cell foci also have been described. ¹⁶

In foci of cellular alteration, the cells may be smaller or larger than unaffected hepatocytes. Cell size along with tinctorial properties of the cytoplasm are two of the key features distinguishing the various types of foci. Many foci of cellular alteration contain more than one cell type (see Discussion). Such foci should be diagnosed by the predominant cell type present. Nuclear criteria also can help differentiate types of foci. Cellular atypia is generally absent. Foci of cellular alteration vary in size from a few cells to lesions that occupy

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multiple hepatic lobules, and there is generally sharp demarcation between foci and surrounding liver. There is little or no alteration of the hepatic lobular architecture within foci, but hepatic cords within foci may not be contiguous with the hepatic cords of adjacent normal liver, *i.e.* the focus may have different pattern of hepatic cords. Very large lesions or those with hypertrophied cells may distort or compress surrounding hepatic plates around a small portion of the periphery. Such compression is not a prominent feature as it usually is with hepatocellular neoplasms, and this, as well as the preservation of hepatic lobular architecture are the main morphologic differences between foci and hepatocellular adenomas.

Focal fatty change without a specific lobular distribution also has been referred to as vacuolated foci of cellular alteration. Focal fatty change is commonly seen under a variety of circumstances. It is not considered to be a type of focus of cellular alteration. Fatty change can be seen within foci of cellular alteration and also within hepatocellular neoplasms and is considered a degenerative change within these lesions.

Several investigative methods have been applied to rat liver foci, including histochemical, biochemical, stereologic and ultrastructural studies.^{2,29,32} There are some correlations that can be made between these techniques and H&E stained sections, but the correlations are very incomplete since H&E stains have not been consistently included. Table I summarizes cytomorphological and metabolic changes which can be correlated with the tinctorial changes characterizing the different types of foci on H&E stains.

Numerous enzyme alterations have been observed in foci which have prompted the term *enzyme altered foci* as a synonym for these lesions. Some commonly observed enzyme alterations have included changes in GGTP, GST, G6P and ATPase levels. Glycogen retention, increased RNA levels, increased DNA synthesis, and iron exclusion also have been reported in foci of cellular alteration.²

Foci of cellular alteration in two-year carcinogenicity studies which are commonly observed as both spontaneous and induced lesions include tigroid basophilic foci, eosinophilic cell foci, and clear cell foci. Homogeneous (diffusely) basophilic foci and amphophilic foci are less frequently observed or are seen only in specific experimental protocols.

BASOPHILIC CELL FOCI

TIGROID BASOPHILIC CELL FOCI (FIGURES 1,2)

The cells in tigroid basophilic foci may be the same size or smaller than unaffected hepatocytes and hepatic cords may be slightly tortuous. The cytoplasmic basophilia generally appears in clumps or in linear arrangements with relatively paler intervening cyto-

plasm. The term "tigroid cell foci" has been applied to these lesions and the clumps of basophilic material represent cisternae of rough endoplasmic reticulum (Bannasch et al., 1985).

HOMOGENEOUS BASOPHILIC CELL FOCI (FIGURES 3,4)

The cells in homogeneous basophilic foci often are enlarged and the cytoplasm is diffusely or uniformly basophilic.^{9,16} Nuclei also are usually enlarged and vesiculate with prominent nucleoli. Often, there is a single prominent central nucleolus with a clear space surrounding it, thus resulting in a "bull's eye" appearance. The hepatic cords may be slightly disrupted with cells in a disassociated, jumbled pattern. These foci also have been called atypical or diffusely basophilic foci. Ultrastructurally, the cells have been shown to contain increased free ribosomes.¹

EOSINOPHILIC CELL (ACIDOPHILIC, GROUND GLASS) FOCI (Figure 5)

The predominant cells in eosinophilic foci often are enlarged because of an increased amount of cytoplasm. The cytoplasm is eosinophilic, pale, and usually has a ground-glass or fibrillar appearance with an H&E stain. These cells contain excess glycogen and smooth endoplasmic reticulum, and are deficient in glucose-6-phosphatase. Clear cells (see below) also may be present in small numbers in eosinophilic foci. Both eosinophilic cells and clear cell foci have been referred to as glycogen storage foci.²

CLEAR CELL FOCI (Figure 6)

The predominant cells in clear cell foci may be slightly or greatly enlarged and they have mostly unstained cytoplasm. The cell membranes are prominent and often have a frayed appearance on the inner surface. Distinct vacuoles such as appear in fatty change are not present. The unstained cytoplasm has been shown to contain excess glycogen which is dissolved out by aqueous fixatives. Eosinophilic cells (see above) also may be present in small numbers in clear cell foci.

AMPHOPHILIC CELL FOCI (Figures 7,8)

The cells in amphophilic cell foci are enlarged. The cytoplasm stains diffusely eosinophilic (acidophilic) with a definite basophilic tint resulting in an amphophilic appearance in well-stained H&E sections. The cytoplasm is dense and homogeneous. The cells have a decreased glycogen content. The nuclei often are slightly enlarged with margined chromatin. Amphophilic foci also have been referred to as atypical eosinophilic foci.¹⁶

Regenerative Hyperplasia (Figures 9,10)

Gross Appearance: The liver may be enlarged or reduced in size with multiple nodules present with regenerative hyperplasia. In severe cases, there is distortion of the shape of the lobes. The nodules are sharply demarcated from adjacent parenchyma and may be the same color as the adjacent liver or tan or pale depending on lipid and glycogen content of the hepatocytes or the amount of blood present in the lesion.

Microscopic Appearance: Two salient characteristics are essential for diagnosis of regenerative hyperplasia. First, one or more discrete nodular lesions of hepatocytes are present which lack the cytologic or histologic features of neoplasia. Normal hepatic plates generally are present although the lobular architecture may be severely distorted or missing in some nodules. Second, there is evidence of prior or ongoing hepatocyte damage (e.g., cytotoxicity, necrosis, atrophy, degeneration, fibrosis, inflammation) within the liver parenchyma. In most instances, evidence of hepatocyte damage will be evident in the same histologic sections containing regenerative hyperplasia.

A number of other phenotypic and structural features may be observed in regenerative hyperplasia. Lesions are discrete and may be comprised of hypertrophic hepatocytes. There often is compression of adjacent hepatic parenchyma. The expansile nature of focal hyperplastic lesions typically distorts the lobular architecture so that central veins are partly collapsed and portal areas may be missing in a given 6 micron section because they lie in a different plane. The degree of hepatocyte hypertrophy may be influenced by specific chemical treatment. In some instances of extensive hyperplasia, evidence of chronic inflammation, oval cell proliferation, hepatic degeneration, and bile duct hyperplasia may be present in portal areas and around the periphery of the nodules of hyperplasia. This pattern morphologically resembles cirrhosis in other species. Focal hyperplastic lesions generally appear round in a two-dimensional view. Within the hyperplastic lesion, there may be an increased number of mitoses, some degenerative changes in hepatocytes, and sometimes microgranulomas are present. However, clear evidence of necrosis or cytotoxicity often is not present or is substantially reduced in the nodule, which suggests regenerating hepatocytes may be relatively resistant to toxic insult by the initiating stimulus.

Hepatocellular Adenoma (Figures 11–13)

Gross Appearance: Hepatocellular adenomas vary in size and color. They range from a few millimeters to several centimeters in diameter. Adenomas may be the same color as the adjacent liver, darker or paler, tan or pale yellow. They may be round to spherical or

have a somewhat irregular border. Adenomas are usually solitary but may be multiple, particularly when induced. When occupying a lobe, adenomas can become pedunculated and occasionally may twist on the pedicle and become infarcted.

Microscopic Appearance: Hepatocellular adenomas are generally circular lesions usually occupying an area greater than one liver lobule.^{7,22} They may protrude above the surface of the liver. At least a portion of the circumference is usually sharply demarcated from the surrounding liver tissue by compression of normal liver and lack of continuity between the cords of the nodule and those of the adjacent unaffected liver. The cords of the adenoma often are perpendicular to or impinge obliquely on the cords of the adjacent liver. Within the tumor, there is a loss of normal hepatic plate and lobular architecture. The cords, when discernible, may be arranged haphazardly and may be more than one cell layer thick. Sinusoids may be compressed or ectatic. Hepatocytes within the adenoma may show variation in size and tinctorial characteristics. The cells may be eosinophilic, basophilic, or an admixture of both. Clear cells are variably present although adenomas composed primarily of clear cells are rarely observed. Except for occasional cellular atypia, the cells in adenomas are comparable morphologically to those described for foci of cellular alteration. Cytologic features which may be present include cytoplasmic vacuolation, nuclear atypia, an increased nuclear/cytoplasmic ratio, and an increased mitotic index. Fatty change and other degenerative changes also may be present. Necrosis within an adenoma is uncommon. Oval cell proliferation or cystic degeneration (spongiosus hepatitis) also may be observed. These need not be diagnosed as separate lesions when present within a tumor. Adenomas usually do not contain portal triads although, occasionally, triads are enveloped as the adenoma grows by expansion.

Hepatocellular Carcinoma (Figures 14–18)

Gross Appearance: Hepatocellular carcinomas may be found in any lobe of the liver including the smaller right and caudate lobes. Hepatocellular carcinomas are extremely variable in size usually ranging from 1 cm to over 10 cm in diameter. They tend to be roughly spherical although the border is frequently irregular. When the lesion protrudes above the liver surface, the capsular surface of the lesion is usually irregular and may be traversed by prominent blood vessels. The capsule may be thickened over the lesion. Hepatocellular carcinomas are variable in color from one lesion to the next. They are most frequently mahogany, resembling normal liver. However, the lesion may be various shades of yellow to tan depending

on the lipid or glycogen content. Within a single tumor, hepatocellular carcinomas also may be variable in color having dark red areas interspersed with areas of mahogany or various shades of tan. In some cases, the hepatocellular carcinoma will have one or more dark red areas due to hemorrhage. The consistency of the hepatocellular carcinoma is similar to normal liver. If there is an excessive amount of fluid draining from the cut surface, either hemorrhage or necrosis usually is present.

Microscopic Appearance: Hepatocellular carcinomas generally have characteristic histologic features readily distinguishing them from other primary and secondary liver tumors.³⁰ Except in anaplastic tumors, the individual cells generally approximate the appearance of normal hepatocytes. They are typically cuboidal with abundant cytoplasm and contain a centrally placed nucleus. The tinctorial appearance of the cytoplasm may be eosinophilic, basophilic, clear or a mixture of types. Cells often resemble cells found in foci of cellular alteration or adenomas. In some cases, the cytoplasm may be vacuolated due to variable amounts of lipid. Nuclei are frequently variable in size with some nuclei being both large and hyperchromatic. Nuclei generally have slightly margined chromatin, making the centrally located nucleoli particularly prominent. Nucleoli generally are distinct and larger than those found in normal hepatocytes.

Neoplastic hepatocytes may form a variety of patterns within the neoplasm. The trabecular pattern is most common and is characterized by neoplastic hepatocytes forming multiple irregular trabeculae that may be several cells thick. Where the trabeculae are unusually thick, the central cells are frequently necrotic, in some cases mimicking a glandular pattern due to a loss of central necrotic cells. The trabecular pattern generally constitutes a major portion of the lesion although it is often readily noticed in only that portion of the tumor where dilated vascular spaces separate the trabeculae. When such spaces are not present, a hepatocellular carcinoma with a trabecular pattern generally has the appearance of packets or sheets of neoplastic cells. A less common pattern for hepatocellular carcinomas is the acinar or glandular form. As the name implies, a central clear space is surrounded by neoplastic hepatocytes that are generally only a single layer thick. If viewed by electron microscopy, the central space is a dilated bile canaliculus. An adenomatous pattern is rarely, if ever, uniform throughout a hepatocellular carcinoma. The non-adenomatous areas are generally composed of fields of neoplastic hepatocytes although some trabeculae may be observed. A third pattern noted in hepatocellular carcinomas is a solid pattern. In this form, the neoplastic cells form large fields of neoplastic cells that fail to form a recognized pattern of organiza-

tion. Cells in solid hepatocellular carcinomas are more likely to be anaplastic compared to cells in other forms of the tumor.

Hepatocholangiocellular carcinomas are composed of neoplastic hepatocytes and neoplastic bile duct epithelial cells. It is important to note that this term should be used only when *both* cell types show evidence of malignancy. This tumor should not be confused with trabecular carcinomas, adenocarcinomas, or solid carcinomas that simply contain a number of normal or hyperplastic bile ducts. Hepatocholangiocellular neoplasms are discussed in a subsequent section (See Hepatocholangiocellular Carcinoma).

Other than normal endothelial cells lining the vascular channels, hepatocellular carcinomas are relatively free of other cell types. Connective tissue including collagen is rare other than in areas of previous necrosis. Bile ducts may be found within hepatocellular carcinomas. The bile ducts may simply be remnants of previous hepatic tissue or may be newly proliferated non-neoplastic ducts. "Oval cells" may occur in the neoplasm but are usually less numerous within the neoplasm compared to the surrounding liver.

Hepatocellular carcinomas generally have an irregular border with the adjacent liver primarily due to pockets of local infiltration and compression. Infiltration is characterized by neoplastic cells moving between normal hepatocytes followed by proliferation of the neoplastic cells to form packets. True invasion into lymph and blood vessels may be observed but is relatively uncommon. Metastasis may occur with hepatocellular carcinomas but appears to be the exception rather than the rule. Metastasis takes one of two forms. Metastatic spread through the lymph channels results in numerous small plaques of neoplastic cells on the peritoneal surface, most notably on the abdominal surface of the diaphragm. When metastatic spread occurs via the blood vessels, metastatic tumors are found in the lung.

B. INTRAHEPATIC CHOLANGIOCELLULAR LESIONS

Bile Duct Hyperplasia (Figure 19)

Gross Appearance: Bile duct hyperplasia usually is not observed grossly.

Microscopic Appearance: Bile duct hyperplasia consists of several small bile ducts occurring in a portal area with variable amounts of periductular fibrosis and mononuclear cell infiltrates. The biliary epithelium in these lesions usually resembles that found in bile ducts of the portal areas. There may be variable amounts of mucoid material present and the ducts may be normal in size or dilated.

Biliary Cysts

SIMPLE BILIARY CYSTS (Figure 20)

Gross Appearance: Simple biliary cysts are usually microscopic. However, they may be observed grossly as a pale or translucent oval or spherical mass bulging above the surface of the liver. When incised, these lesions usually contain clear, viscous, or pale yellow fluid. Similar lesions also may be found within the hepatic parenchyma.

Microscopic Appearance: These lesions consist of a single cystic structure lined by flattened epithelium with occasional oval to cuboidal cells along some aspects of the limiting wall. The lumens generally appear empty. Small amounts of intraluminal eosinophilic material may be present. Compression usually is not present. Cysts may be present with or without biliary hyperplasia elsewhere in the liver.

MULTILOCULATED BILIARY CYSTS (Figures 21,22)

Gross Appearance: Multiloculated biliary cysts are similar in appearance to simple biliary cysts. They may vary in size from microscopic to several centimeters in diameter. On cross section, larger lesions may have septa dividing the lesion into compartments.

Microscopic Appearance: Multilocular cysts are cystic structures lined by flattened or low cuboidal cells separated by loose fibrous stroma. The cystic structures are divided into variable-sized compartments by delicate fibrous connective tissue septa lined by a single layer of flattened epithelium with occasional focal areas of cuboidal epithelium. Septa between smaller cysts within the lesion may be incomplete. Slight compression of surrounding hepatic parenchyma may occur. These lesions also have been referred to as bile duct ectasia. They may be present with or without accompanying diffuse biliary hyperplasia.

Oval Cell Hyperplasia (Figures 23,24)

Gross Appearance: Oval cell hyperplasia per se is not observed grossly. However, it often is found in livers with other evidence of hepatotoxicity. Thus, the livers may have a nodular or granular appearance.

Microscopic Appearance: Oval cell hyperplasia is comprised of bile duct-like cells which stream outward from the periportal zone, along hepatic sinusoids toward the midzonal area of the lobule. These cells have delicate, vesicular oval nuclei and scant, indistinct cytoplasm. They are generally uniform in size and shape and tend to form incomplete duct-like structures. Oval cell hyperplasia is multifocal and usually widespread throughout the liver. It usually is seen in association with other evidence of hepatic toxicity and in

hepatocellular tumors.

Cholangiofibrosis (Figures 25,26)

Gross Appearance: Cholangiofibrosis varies in size from microscopic foci to grossly visible lesions up to 5 cm in diameter. Larger lesions are visible grossly as firm, pearly white areas. When on the surface, the lesions are depressed. The lesions are usually multifocal.

Microscopic Appearance: This lesion is comprised of atypical glandular structures lined by hyperbasophilic, sometimes dysplastic epithelium, which may range from flattened to large cuboidal cells along with goblet cells and occasionally Paneth's cells within the same acinus. The glands are often crescent-shaped as a result of high columnar epithelium in one portion of the gland and attenuated epithelium on the other side. Mitotic figures and necrotic cells often are present in the epithelium. The lumen of the gland usually is filled with mucin and/or necrotic debris derived from epithelial cells and white blood cells. Mucin production often is pronounced. Connective tissue in which these glandular structures are embedded often is dense. Sclerosis often occurs in the more central areas of the lesion. In the more peripheral areas, the connective tissue often is arranged concentrically around the glandular structures. Multifocal areas of cholangiofibrosis often are continuous with one another.

These lesions are compatible with descriptions of chemically induced alterations previously called cholangiohepatitis, toxic hepatitis, toxic cholangitis, cholangiofibrosis, adenofibrosis, and cholangiofibroma.^{1,13,14,18,20,26,27,35,38,39}

Cholangioma (Figures 27,28)

Gross Appearance: Cholangiomas vary from firm, grey-white nodules to lesions with a spongy texture when cystic structures are present. In the solid forms, margins are generally smooth, whereas, in the multilocular cystic forms, the surface is more irregular.

Microscopic Appearance: Cholangiomas are uniform neoplasms that are well-circumscribed and expansile, often compressing adjacent parenchyma. They are comprised of acini of generally uniform size, lined by a single layer of cuboidal cells. The round oval nuclei are located near the base of the cell similar to normal bile duct epithelium. Occasionally, cystic structures may be present. There is very little vascular stroma and mitotic figures are rare. In larger lesions, up to a few centimeters in diameter, the lining epithelium occasionally may be multi-layered. Cystic tumors are distinguished from biliary cysts by their greater cellularity.

Cholangiocarcinoma (Figures 29,30)

Gross Appearance: Cholangiocarcinomas usually are firm, white to grey masses with irregular borders. They may protrude from the surface of the liver. In cystic areas, they may have a spongy texture and exude clear or yellow fluid from a cut surface.

Microscopic Appearance: These tumors may have glandular, solid, or papillary patterns. They are comprised largely of cuboidal to columnar cells with basophilic cytoplasm and prominent hyperchromatic nuclei. Cellular atypia is common and there is often a high mitotic index. Epithelium lining dilated glands occasionally is piled up. In some dilated glands, the lining epithelium may be partially or completely missing. Mucin production is highly variable. The tumors typically contain abundant scirrhous stroma. They usually exhibit microinvasion and can invade surrounding tissues, blood vessels, and may metastasize.

C. MIXED HEPATOCHOLANGIOCELLULAR LESIONS

Hepatocholangiocellular Adenoma

Gross Appearance: Hepatocholangiocellular adenomas are indistinguishable grossly from hepatocellular adenomas (See Hepatocellular Adenoma).

Microscopic Appearance: Hepatocholangiocellular adenomas are morphologically similar to hepatocellular adenomas; however, they contain areas of neoplastic bile duct epithelium. The neoplastic biliary epithelium usually forms slightly dilated acini lined by cuboidal epithelium similar to normal bile duct epithelium. The biliary component appears to infiltrate areas of the hepatocellular adenoma.

The criteria for diagnosing a hepatocellular adenoma are used in evaluating these lesions (See Hepatocellular Adenoma) as well as identifying the presence of neoplastic biliary elements. Hepatocholangiocellular adenomas must be distinguished from hepatocellular adenomas with bile duct hyperplasia. Both elements, hepatocellular and biliary, must be neoplastic.

Hepatocholangiocellular Carcinoma

Gross Appearance: Hepatocholangiocellular carcinomas are indistinguishable from hepatocellular carcinomas grossly (See Hepatocellular Carcinoma).

Microscopic Appearance: Hepatocholangiocellular carcinomas are composed of neoplastic hepatocytes and neoplastic bile duct epithelial cells. It is important to note that in order to make this diagnosis, both cell types must show evidence of malignancy. The

presence of bile ducts or glandular differentiation within an hepatocellular carcinoma are not sufficient to warrant this diagnosis. Hepatocholangiocellular carcinomas are composed of areas within the tumor which meet the criteria described for hepatocellular carcinomas (See Hepatocellular Carcinoma) and cholangiocellular carcinomas (See Cholangiocarcinoma). The biliary component can form acini or small nests without distinct lumens while the hepatocellular component can form a trabecular, solid, or glandular pattern. On occasion, neoplastic hepatocytes and neoplastic biliary epithelial cells can line the same glandular structure.

D. MISCELLANEOUS LESIONS OF THE LIVER

Cystic Degeneration (Figures 31,32)

Gross Appearance: Cystic degeneration usually is not observed grossly. When seen grossly, cystic degeneration appears as pinpoint or 1-2 mm pale or red spots in the liver parenchyma.

Microscopic Appearance: Cystic degeneration of the liver consists of large vacuoles or cystic spaces between hepatocytes. These spaces are variably filled with erythrocytes, degenerating erythrocytes, eosinophilic flocculent or fibrillar material or eosinophilic proteinaceous fluid. The spaces are not lined or are only partially lined by endothelium. Cystic degeneration may be found as an independent lesion in the liver parenchyma or within foci of cellular alteration or hepatocellular neoplasms. When present within foci or tumors, it should not be diagnosed separately.

Angiectasis (Figures 33,34)

Gross Appearance: Angiectasis usually is not observed grossly. When seen grossly, angiectasis appears as pinpoint or 1-2 mm dark red spots in the liver parenchyma.

Microscopic Appearance: Angiectasis of the liver consists of dilated vascular spaces filled with erythrocytes. The spaces are lined by endothelial cells. The dilatation of the vascular spaces separates the hepatic cords which often take on a tortuous appearance. The cells within these cords may become slightly enlarged or appear more eosinophilic. There may be slight compression of the adjacent hepatic parenchyma due to the vascular dilatation. This lesion often is seen as a component of foci of cellular alteration or hepatocellular neoplasms but can also be found as a unique lesion. When found in association with foci or tumors, the angiectasis is found in only a part of the lesion and should not be diagnosed as a separate lesion.

Focal Fatty Change (Figure 35)

Gross Appearance: Focal fatty change usually is not observed grossly. However, larger lesions may appear as minute pale or yellow foci on the surface of the liver.

Microscopic Appearance: Discrete foci of fat without a specific lobular distribution occur in the liver. The cytoplasm of the cells in these foci contain clear spherical vacuoles which may be small and multiple or large and solitary. The vacuoles are filled with lipid (fat), and the spherical nature of the vacuole is a result of the lipid/water surface tension interface. The nuclei may be centrally located or pushed to one side by the cytoplasmic vacuoles. When a single, large vacuole displaces the nucleus, a signet ring appearance to the cell may result. These lesions have been mistaken for clear cell foci on H&E stained sections. Special stains for lipid and glycogen can be used to distinguish the two lesions. Focal fatty change also can be found in the various types of foci of cellular alteration and in hepatocellular neoplasms.

DISCUSSION

Foci of cellular alteration occur spontaneously in aging rats, and certain types may reach close to 100% incidence in certain strains even though the incidence of hepatocellular neoplasms is very low, i.e. F344 females.^{30,40} The incidence, size, and/or multiplicity of foci usually are increased, and the time to development usually is decreased by the administration of hepatocarcinogens. Moreover, foci generally precede the development of tumors, and they have been categorized as preneoplastic lesions.⁴¹ However, they are reversible lesions and there is conflicting evidence regarding the biological nature of rat liver foci. Some studies indicate foci represent an early stage in neoplastic development and that at least some have the capacity to progress to tumors. Other studies indicate that, although induced by carcinogens, foci may be nonneoplastic end-stage lesions.²⁸ Evidence recently has been presented which suggests that not all foci may be related to carcinogenesis.^{16,36} The latter study was a review of F344 rat livers from a limited number of carcinogenicity tests in the National Toxicology Program (NTP) Archives. It was reported that hepatocarcinogenesis was associated with an increase in the homogeneous (diffusely, atypical) basophilic or the amphophilic (atypical eosinophilic) foci. It also was reported that an increase in clear cell foci may occur with hepatocarcinogens. However, the common spontaneous eosinophilic (acidophilic) or tigroid basophilic foci were not associ-

ated with exposure to hepatocarcinogens. The induction of homogeneous basophilic foci has been reported following exposure to peroxisome proliferating agents.⁹ In this study, tigroid basophilic foci were not affected by treatment. Whether these observations will withstand more comprehensive analysis with other chemicals or will apply to other rat strains is not certain. However, the committee believes the classification of foci must allow for different morphological features that may reflect different biological potential and, therefore, recommends a classification to include these recent findings.

Mixed cell foci have been defined in several different ways.^{1,16,18,37} One definition is that mixed cell foci contain basophilic cells in combination with eosinophilic and/or clear cells. Such foci are rare except with certain experimental protocols. Another definition states that mixed cell foci are those which contain two or more cell types. This definition allows many foci to be diagnosed as mixed cell foci since it is not uncommon for a focus of predominantly one cell type to have a small number of cells of a different type. A modification of this definition uses a percentage of the types of cells present to determine whether it is a mixed cell focus rather than a specific cell type. This definition is used most frequently to categorize foci containing both clear cells and eosinophilic cells, the most common mixture of cell types encountered. Because several different definitions have been used for mixed cell foci in the past, the committee decided not to include the mixed cell focus category in the present classification and, instead recommends that foci of cellular alteration be classified by the predominant cell type.

Several lesions are sometimes confused with foci of cellular alteration. These lesions include angiectasis and focal fatty change. Focal fatty change has been mistaken for clear cell foci in H&E stained sections. Special stains for lipid and glycogen can be used to distinguish the two lesions. Focal fatty change also can be found in the various types of foci of cellular alteration and in hepatocellular neoplasms. Angiectasis may be present within a focus of cellular alteration or present in the liver parenchyma as a unique lesion. This distinction is not always easy to make. The presence of dilated vascular spaces, tortuous cords, slightly enlarged cells within the cords, and slight compression do not meet the criteria for foci of cellular alteration.

Foci of cellular alteration must also be distinguished from focal regenerative hyperplasia and hepatocellular adenomas.

Regenerative hyperplasia is defined as a focal or multifocal, regenerative, or compensatory proliferation of hepatocytes secondary to repeated hepatic injury resulting in hepatocyte necrosis. Hepatocyte necrosis may be due to several factors (e.g., cytotoxicity from

chemical exposure, hepatic necrosis from arrest of bile flow, progressive anemia and secondary hepatocyte atrophy, diminution of hepatic blood flow secondary to periportal inflammation). Leukemia infiltrates, such as those often seen with mononuclear cell leukemia often are associated with regenerative hyperplasia. Evidence of hepatocyte necrosis should be present in the section or, with chemical toxicity, known to have occurred earlier in the time-course of chemical treatment.

The diagnosis of hepatocellular adenoma has been the center of controversy for over fifteen years. Originally, there was a great deal of confusion with the terms hyperplastic nodule, nodular hyperplasia, and hepatocellular adenoma. Following a workshop in 1975³⁷, the term neoplastic nodule was introduced to designate lesions considered to be neoplastic but which did not meet the criteria for hepatocellular carcinoma. For many pathologists, the term neoplastic nodule was considered synonymous with hepatocellular adenoma, i.e. a benign hepatocellular neoplasm. For others, the criteria for neoplastic nodule were too vague and did not adequately distinguish benign tumors from non-neoplastic proliferative lesions.

More recently, a return to the use of the term hepatocellular adenoma has been proposed²², including a redefining of the criteria. The committee recommends that the term hepatocellular adenoma be used for benign hepatocellular neoplasms rather than the term neoplastic nodule.

The differential diagnosis between foci of cellular alteration and hepatocellular adenoma and between regenerative hyperplasia and hepatocellular adenoma are the most controversial.

In distinguishing between foci of cellular alteration and hepatocellular adenomas, compression of the surrounding liver and loss of lobular architecture are the main features to consider. With foci, there is generally sharp demarcation from the adjacent liver but little, if any, compression. Very large lesions or those with hypertrophied cells or vascular ectatic lesions sometimes distort or compress surrounding hepatic cords. Such compression is not a prominent feature as it usually is with hepatocellular adenomas. Significant compression, disruption of the normal architecture of the hepatic lobules and plates, and/or presence of cellular atypia distinguish benign hepatocellular neoplasms from foci of cellular alteration.

In distinguishing between hepatocellular adenomas and regenerative hyperplasia, the presence or absence of lobular architecture is important. In hepatocellular adenomas, there is a loss of the lobular and plate architecture although portal triads may be present. With regenerative hyperplasia, the lobular architecture generally is maintained although it may be distorted and pseudolobules may occur. Distinct compression of the

adjacent hepatic parenchyma and the presence of cellular atypia also help distinguish adenomas from hyperplasia. Most importantly, hyperplasia is observed as one or more discrete nodules occurring in livers with evidence of multifocal or diffuse hepatic damage. Care must be taken in evaluating such livers as hepatocellular neoplasms and foci of cellular alteration also can be found in association with toxicity.

Hepatocellular carcinomas generally have identifiable hepatocellular characteristics making the cell of origin of the neoplasm readily identifiable. While various morphologic patterns of the lesion have been identified, the significance of distinguishing between these lesions is less clear. The anaplastic carcinomas probably have a greater propensity to metastasize than the other forms but still have a rather low metastatic rate.

Differential diagnosis between benign and malignant neoplasms of hepatocellular origin is the major diagnostic issue for these neoplasms. In general, marked cellular or histologic atypia is the most important criterion for classifying a tumor as malignant. Hepatocellular carcinomas have greater heterogeneity in cell size, shape, and staining patterns than hepatocellular adenomas. Hepatocellular carcinomas frequently form trabeculae multiple cell layers thick whereas hepatocellular adenomas rarely have trabeculae several cells in thickness. Invasion and metastasis are obvious hallmarks of malignancy; however, these criteria are frequently not helpful with rat liver tumors since metastasis is infrequent and invasion is difficult to confirm in the liver unless vascular structures are penetrated.

Bile duct hyperplasia is a common aging lesion in rats. Simple biliary cysts may destroy parenchymal cells by pressure necrosis, coalesce, and form cysts of varying size and shape, most of which are lined by flattened, benign epithelium. Multiloculated biliary cysts are considered a degenerative lesion by some investigators, formed in the manner just described. Other investigators have described these lesions as cholangiomas or cystic cholangiomas. In the literature, the morphologic distinctions between biliary cysts and cholangioma are not clear.^{5,6,8,18,19,34,35} Similar morphologic lesions are designated as either neoplasms or proliferative non-neoplastic lesions in different reports, often without criteria being described. Two key factors in differentiating biliary cysts and cholangiomas are the appearance of the epithelium (flattened to low cuboidal versus cuboidal to multi-layered respectively) and whether or not the lesion is well-circumscribed and expansile in nature, causing compression of adjacent parenchyma.

Oval cell hyperplasia is seen with many hepatotoxins. Oval cells are generally thought to be

undifferentiated cholangiolar cells or hepatic stem cells which are present although inconspicuous in the normal liver. Oval cells, unlike hepatocytes, are associated with a basement membrane which can be demonstrated with special stains.^{10,17}

Cholangiofibrosis is an unusual lesion occurring in animals treated with certain carcinogens. The biologic nature of this lesion has been questioned. Some authors consider it to be a pre-malignant lesion or a malignant neoplasm while others do not. Metastasis appears to be absent. Cholangiofibrosis has been induced by furan.²¹ In this study, cholangiofibrosis progressed to cholangiocarcinomas three months after stopping treatment. These lesions, as well as similar lesions seen

in continuously exposed rats did metastasize in a few cases and a few grew upon transplantation.

Cholangiofibrosis has been reported to represent small intestinal metaplasia and the lesions may progress to intestinal type adenocarcinoma.¹¹

Cholangiomas and cholangiocarcinomas are uncommon spontaneous tumors in rats, although they have been induced. A benign neoplasm termed cholangiofibroma has been reported although it appears to be rare.¹ Hepatocholangiocellular adenomas and carcinomas are likewise rare tumors, both as spontaneous and induced lesions. There is some argument as to whether hepatocholangiocellular tumors actually exist or whether they are variants of hepatocellular tumors.

Table I.

CHARACTERISTICS OF FOCI OF CELLULAR ALTERATION			
SUBJECTIVE DESCRIPTION	CELL SIZE	CYTOPLASMIC STAINING CHARACTERISTICS	ULTRASTRUCTURAL/CHEMICAL CHARACTERISTICS
clear cell focus*	normal to enlarged cells	clear non-spherical vacuoles (no lipid/water surface tension interface)	increased glycogen
eosinophilic (acidophilic) focus*	enlarged cells	acidophilic	increased glycogen and SER
amphophilic focus	enlarged cells	acidophilic with interspersed basophilic (densely amphophilic)	decreased glycogen
basophilic focus tigroid	normal to small cells	basophilic, granular	increased RER glycogen decreased
basophilic focus homogeneous	enlarged cells	basophilic, homogeneous, uniform, diffuse	increased free ribosomes
focal fatty change **	enlarged cells	clear spherical vacuoles, small and multiple or signet rings (lipid/water surface tension interface)	increased lipid

*Combinations of clear cell foci and acidophilic foci are both glycogen rich (glycogen storage) foci. Use the name of the predominant cell type.

** Lipid, whether in a pure focus or mixed in other types of foci, is incidental.

RECOMMENDED NOMENCLATURE WITH DIAGNOSTIC CRITERIA

A. HEPATOCELLULAR PROLIFERATIVE LESIONS

1. *Foci of Cellular Alteration*

- preservation of lobular architecture although hepatic plates may be altered
- clear demarcation between foci and surrounding liver
- slight or no compression of surrounding liver
- variable in size from a few cells to several lobules
- general absence of cellular atypia
- size of cells and cytoplasmic tinctorial properties dependent on type of focus
- fatty change may be present

a. BASOPHILIC CELL FOCI

1. TIGROID BASOPHILIC CELL FOCI

- cells same size or smaller than normal hepatocytes
- frequently tortuous hepatic cords due to increased number of cells
- cytoplasmic basophilia in clumps or linear arrangements, often marginated, with relatively clear intervening cytoplasm
- increase in rough endoplasmic reticulum (RER) and decreased glycogen content

2. HOMOGENEOUS BASOPHILIC CELL FOCI

- cells usually enlarged to a slight or moderate degree
- cytoplasm stains diffusely and homogeneously basophilic
- cells may be disassociated with a jumbled pattern
- enlarged vesiculate nuclei with prominent nucleoli
- increase in free ribosomes

b. EOSINOPHILIC CELL FOCI (ACIDOPHILIC CELL)

- cells moderately to greatly enlarged with increased amounts of cytoplasm
- cytoplasm eosinophilic, pale with a ground glass or fibrillar appearance
- predominant cell type is the acidophilic cell, some clear cells may be present -increase in glycogen content and smooth endoplasmic reticulum (SER)

c. CLEAR CELL FOCI

- cells may be slightly to greatly enlarged
- cytoplasm appears empty, unstained
- distinguished from focal fatty change by absence of discrete cytoplasmic vacuoles(s)
- predominant cell type is the clear cell, some acidophilic cells may be present -increase in glycogen content

d. AMPHOPHILIC CELL FOCI

- cells moderately to greatly enlarged
- cytoplasm intensely eosinophilic with scattered basophilia, resulting in an amphophilic appearance
- nuclei slightly enlarged with marginated chromatin
- decrease in glycogen content

2. *Regenerative Hyperplasia*

- evidence of multifocal or diffuse hepatic damage
- one or more discrete nodules of hepatocytes

- hepatic plate architecture present but often distorted in nodules
- slight compression of adjacent parenchyma may be present
- cells often enlarged
- may have increased mitotic index

3. Hepatocellular Adenoma

- usually larger than one liver lobule
- distinct compression of surrounding parenchyma around greater portion of lesion
- plates of nodule often discontinuous with surrounding liver; may be perpendicular to or impinge obliquely on adjacent plates
- loss of lobular and plate architecture although portal triads may be present
- cellular atypia may be present
- may have increased mitotic index
- types of cells seen are similar to those found in foci

4. Hepatocellular Carcinoma

- border of lesion can be distinct with compression or indistinct and irregular
- loss of lobular and plate architecture; may have a variety of patterns, including trabecular, glandular, and solid
- cells may resemble any of the types of cells found in foci
- nuclei enlarged but often variable in size
- marked cellular or histologic atypia, invasion or metastases are present
- may have increased mitotic index

B. INTRAHEPATIC CHOLANGIOCELLULAR PROLIFERATIVE LESIONS

1. Bile Duct Hyperplasia

- proliferation of small bile ducts in portal area
- lined by normal to hyperplastic bile duct epithelium
- mucin production occasionally present
- periductular fibrosis variably present
- periductular mononuclear cell infiltrates variably present

2. Biliary Cysts

a. SIMPLE BILIARY CYST

- simple cystic structure
- lined by single layer of flattened bile duct epithelium
- cyst empty or contains eosinophilic material
- compression generally not present

b. MULTILOCULATED BILIARY CYST

- cystic structure divided into multiple variable- sized compartments by delicate connective tissue septa
- lined by single layer of flattened or low cuboidal bile duct epithelium
- compression generally not present

3. Oval Cell Hyperplasia

- cholangiolar-like cells with oval nuclei and scant, indistinct cytoplasm
- cells generally uniform in size and shape
- incomplete duct-like structures often formed
- multifocal and widespread through liver

4. Cholangiofibrosis

- multifocal and often interconnecting areas of biliary epithelial hyperplasia with surrounding fibrosis

- proliferating biliary epithelium forms mucus-producing, crescent-shaped, elongated, branched or anastomized glandular structures surrounded by concentric layers of connective tissue
- glands lined by a single layer of cells
- cells usually cuboidal but may be flattened or lost in some glands
- magnitude of fibrosis may exceed that of the biliary epithelium
- tendency for glandular proliferation to occur at the periphery of the lesion with central portions becoming atrophic, collagenized and less vascularized
- edges of lesions may infiltrate surrounding tissue
- chronic, multifocal inflammation of the proliferating glands may be present
- intestinal metaplasia of the biliary epithelium (cuboidal, tall columnar, goblet cells, Paneth's cells)
- glands may be cystic and lining epithelium may be partly lost
- may resemble contracted area of scar tissue in which proliferating biliary glands are entrapped

5. Cholangioma

- lesion well-circumscribed and expansile
- composed of small uniform acini
- lined by single or occasionally multiple layers of cuboidal bile duct epithelium
- mitotic figures rare
- stroma is scant

6. Cholangiocarcinoma

- glandular structures lined by one to multiple cell layers
- cuboidal to columnar cells with basophilic cytoplasm
- nuclei are prominent and hyperchromatic
- mucin production variable, when abundant, often associated with glandular dilatation
- often have high mitotic index
- cellular atypia common
- abundant scirrhous stroma may be present
- microinvasion often present
- invasion or metastases may be present

C. MIXED HEPATOCHOLANGIOCELLULAR LESIONS

1. Hepatocholangiocellular Adenoma

- usually larger than one liver lobule
- distinct compression of surrounding parenchyma around greater portion of lesion
- both neoplastic hepatocytes and neoplastic bile duct epithelium present
- plates of liver cells within the nodule often discontinuous with surrounding liver; may be perpendicular to or impinge obliquely on adjacent hepatocellular plates
- loss of hepatocellular lobular and plate architecture although portal triads may be present
- types of neoplastic hepatocytes are seen similar to those found in foci of cellular alteration
- neoplastic bile duct epithelium form slightly dilated acini within areas hepatocellular adenoma
- cellular atypia may be present
- may have increased mitotic index

2. Hepatocholangiocellular Carcinoma

- border of lesion can be distinct with compression or indistinct and irregular
- both malignant hepatocytes and malignant bile duct epithelium present
- loss of hepatocyte lobular and plate architecture; may have a variety of patterns, including trabecular, glandular, and solid
- malignant hepatocytes may resemble any of the types of cells found in foci of cellular alteration
- nuclei of malignant hepatocytes enlarged but often variable in size
- malignant biliary epithelium forms acini or small nests without lumens
- cellular atypia is usually present in both cell types

- both cell types may have an increased mitotic index
- invasion or metastases may be present

D. MISCELLANEOUS LESIONS OF THE LIVER

1. Cystic Degeneration

- cystic spaces or large vacuoles between hepatocytes
- incompletely or not lined by endothelium
- spaces filled with erythrocytes, eosinophilic flocculent or fibrillar material or eosinophilic proteinaceous fluid

2. Angiectasis

- dilated vascular space separating hepatic cords
- spaces lined by endothelium
- spaces filled with erythrocytes
- hepatic cords within lesion may be slightly tortuous
- cells within hepatic cords in the lesion may be slightly enlarged and eosinophilic

3. Focal Fatty Change

- discrete foci of fat containing hepatocytes
- cytoplasm contains clear spherical vacuoles which may be small and multiple or large and solitary
- nucleus may be central or eccentrically placed
- cytoplasmic vacuoles filled with fat

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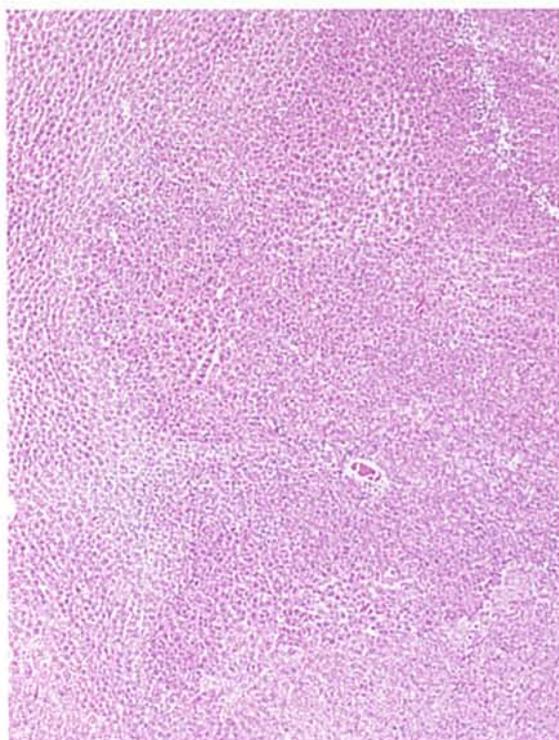


Fig. 1 – Large basophilic focus of cellular alteration. (H&E, 20x)

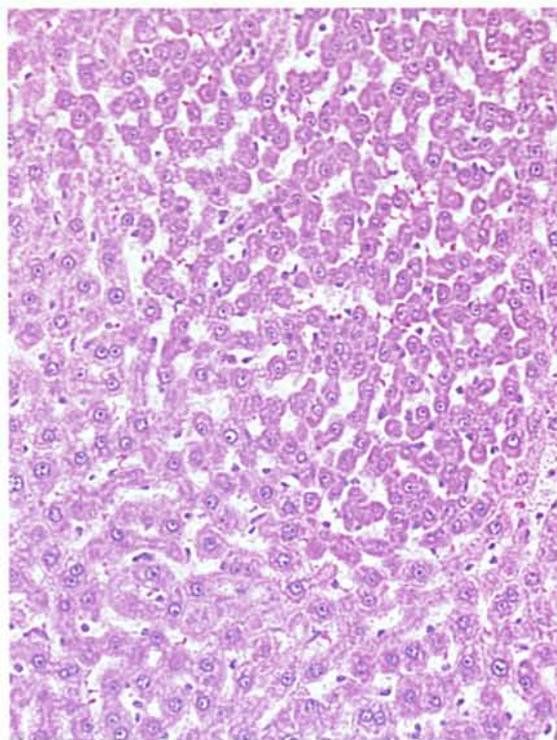


Fig. 2 – Tigroid basophilic focus of cellular alteration. (H&E, 70x)

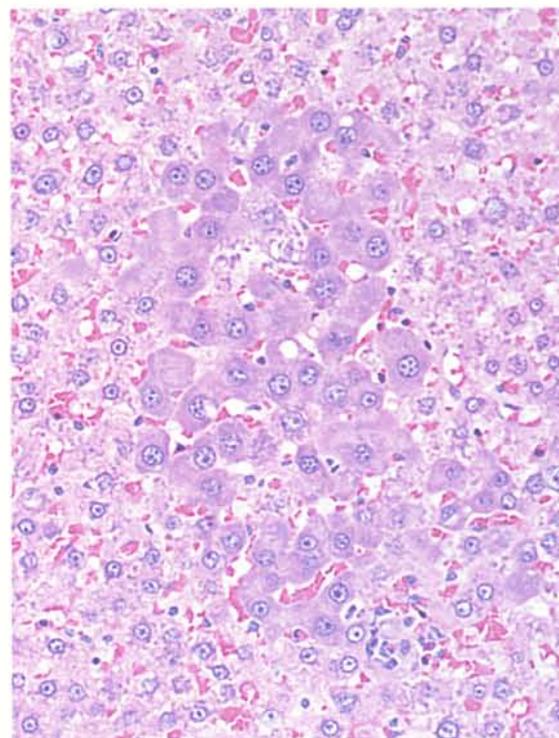


Fig. 3 – Small homogeneous basophilic focus of cellular alteration. Contributed by T. Harada. (H&E, 80x)

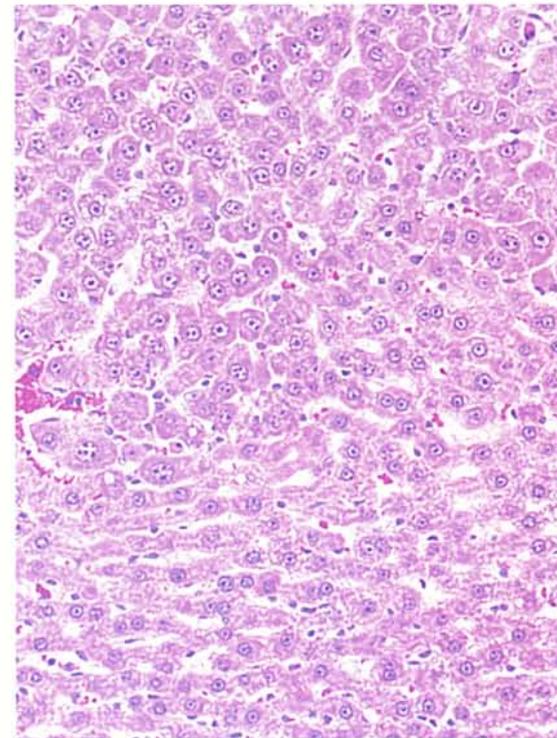


Fig. 4 – Homogeneous basophilic focus of cellular alteration. (H&E, 80x)

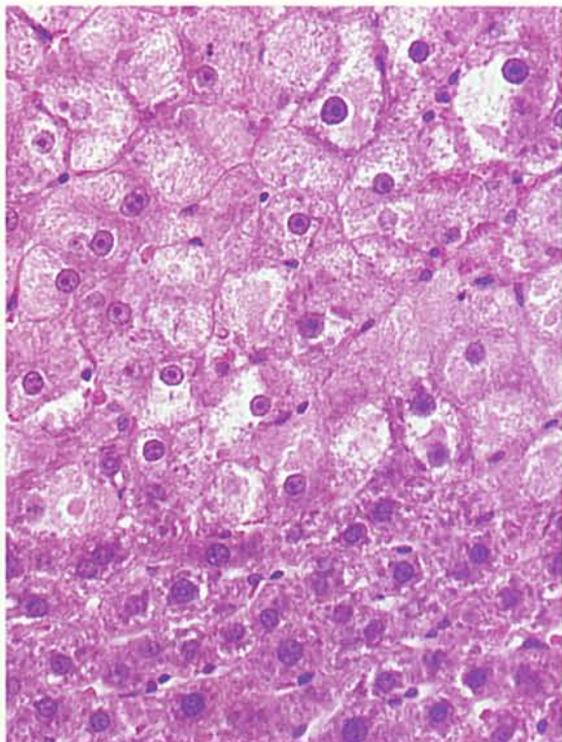


Fig. 5 – Eosinophilic focus of cellular alteration. (H&E, 100x)

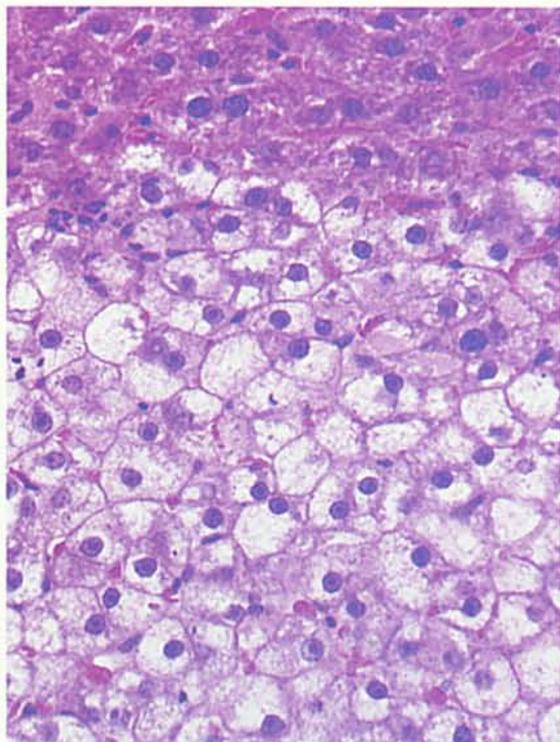


Fig. 6 – Clear cell focus of cellular alteration. (H&E, 100x)

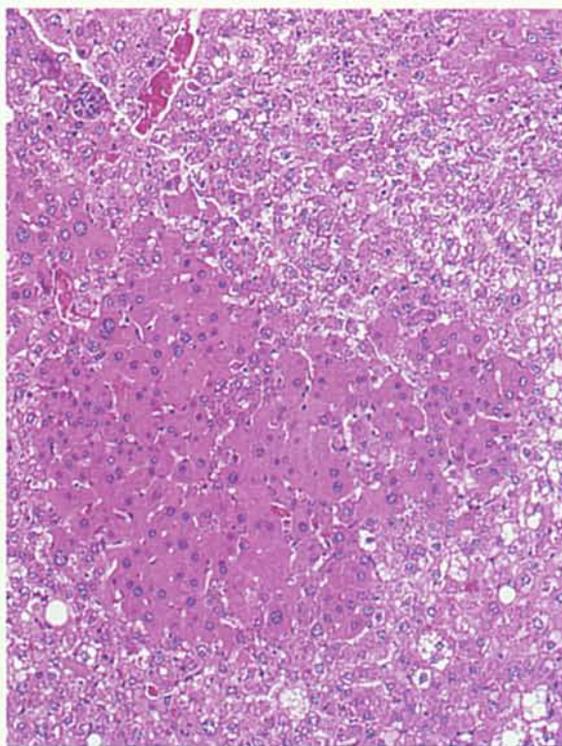


Fig. 7 – Amphiphilic focus of cellular alteration. (H&E, 40x)

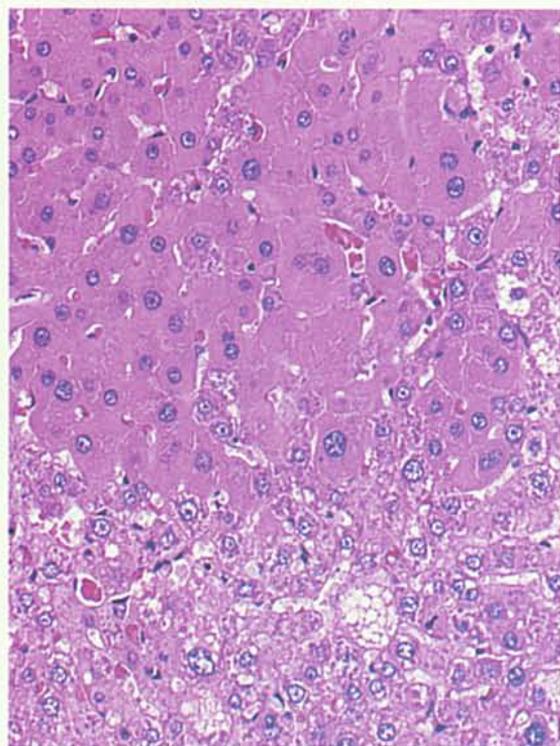


Fig. 8 – Amphiphilic focus of cellular alteration. (H&E, 90x)

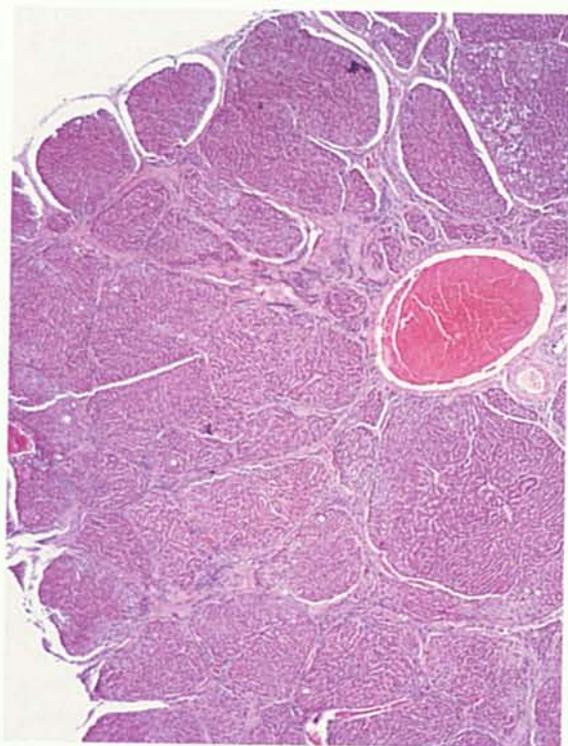


Fig. 9 – Regenerative hyperplasia. (H&E, 5x)

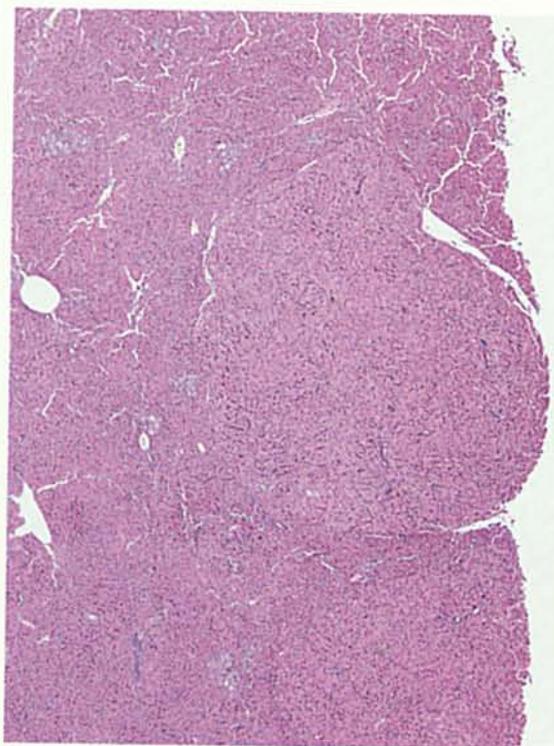


Fig. 10 – Regenerative hyperplasia. (H&E, 10x)



Fig. 11 – Hepatocellular adenoma. (H&E, 4x)

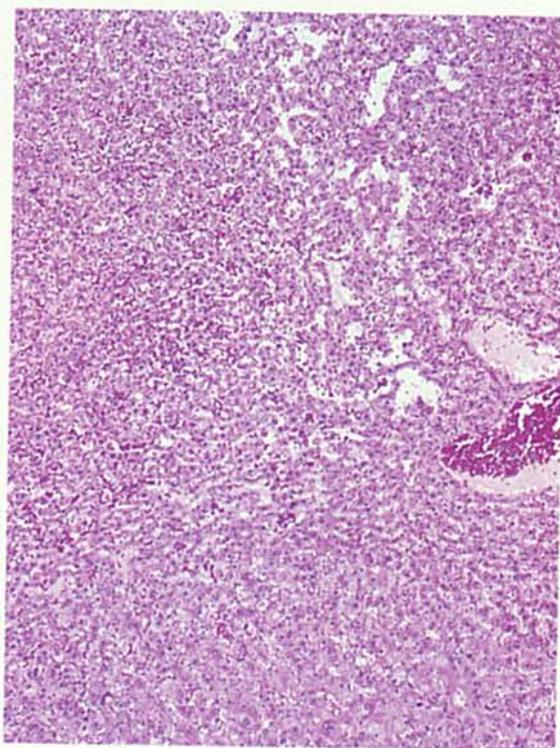


Fig. 12 – Hepatocellular adenoma. (H&E, 30x)

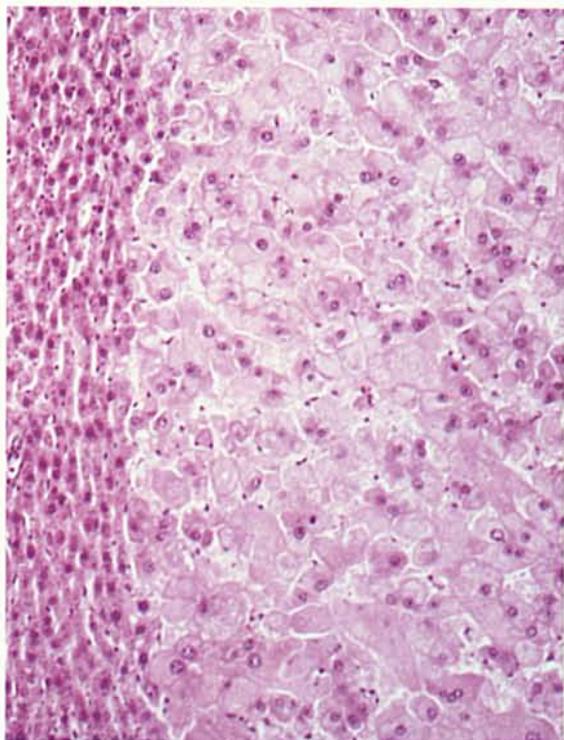


Fig. 13 – Hepatocellular adenoma. (H&E, 80x)

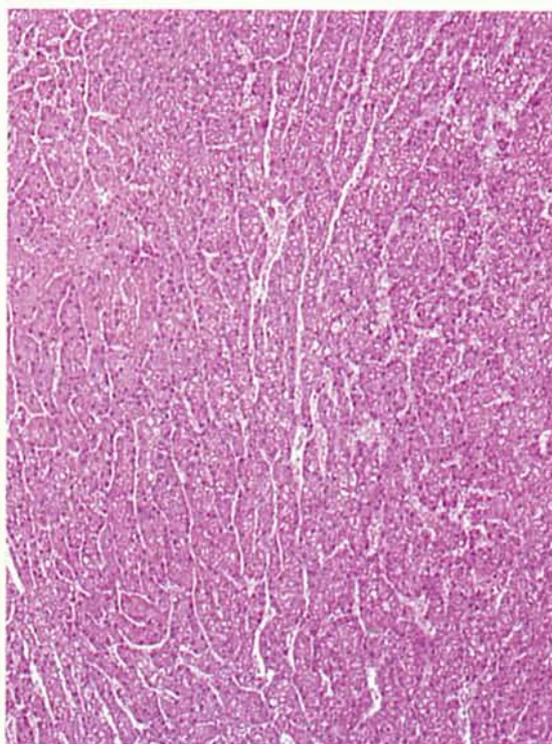


Fig. 14 – Hepatocellular carcinoma. (H&E, 20x)

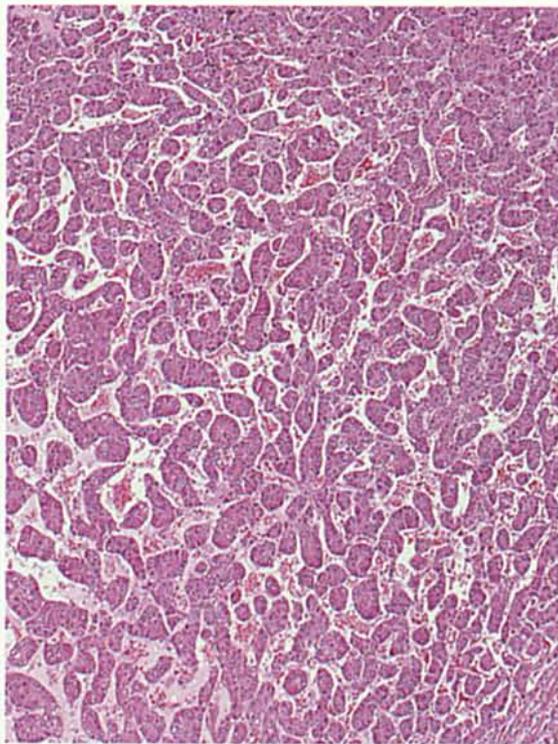


Fig 15 – Hepatocellular carcinoma. (H&E, 25x)

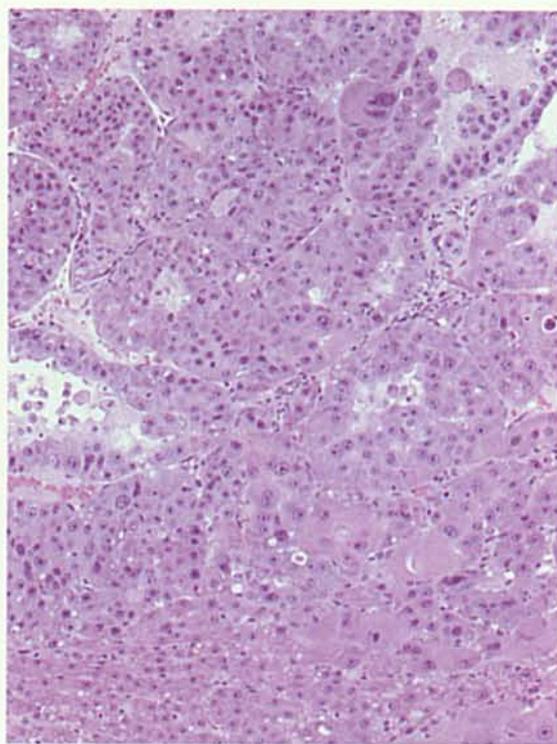


Fig 16 – Hepatocellular carcinoma. (H&E, 33x)

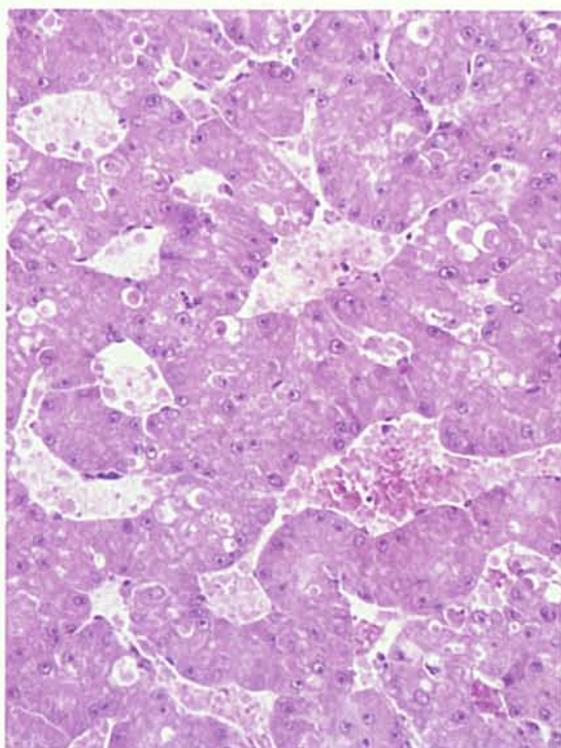


Fig. 17 – Hepatocellular carcinoma. (H&E, 120x)

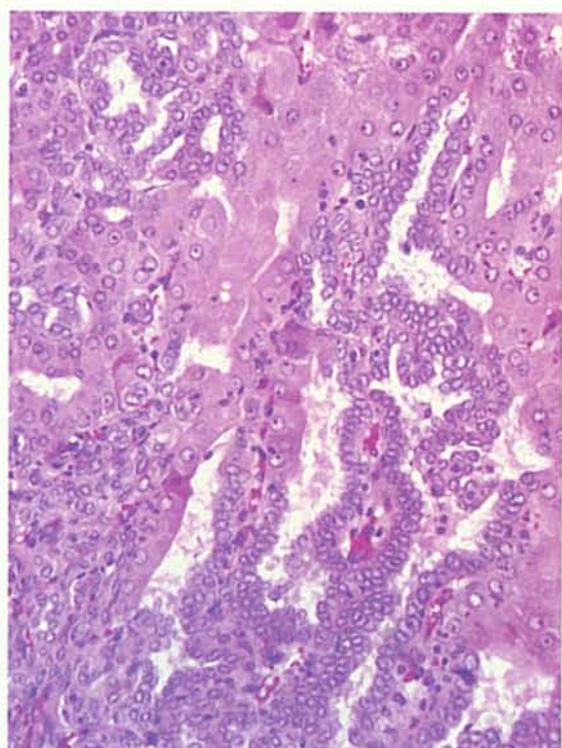


Fig. 18 – Hepatocellular carcinoma with glandular pattern. (H&E, 120x)

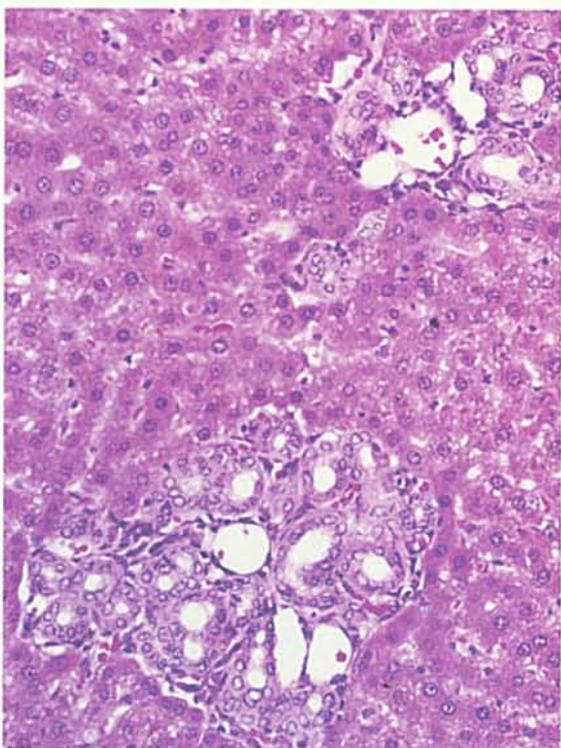


Fig. 19 – Bile duct hyperplasia. (H&E, 70x)

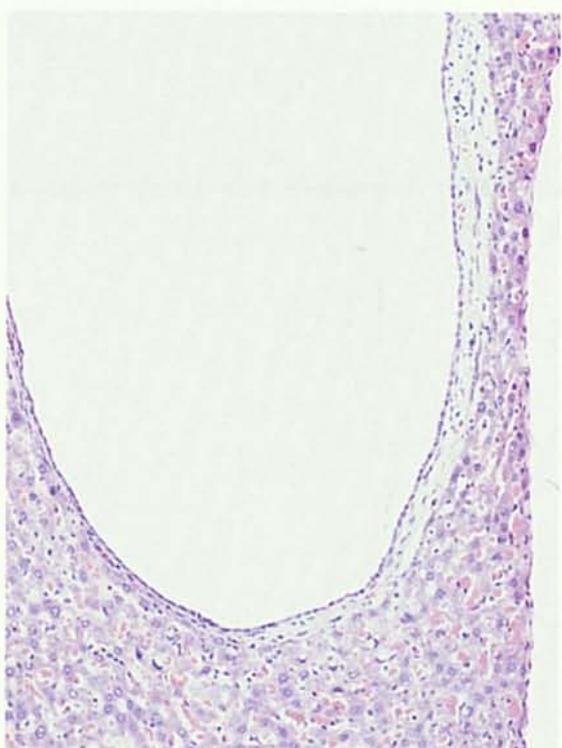


Fig. 20 – Simple biliary cyst. (H&E, 45x)



Fig. 21 – Multiloculated biliary cyst. (H&E, 4.1x)

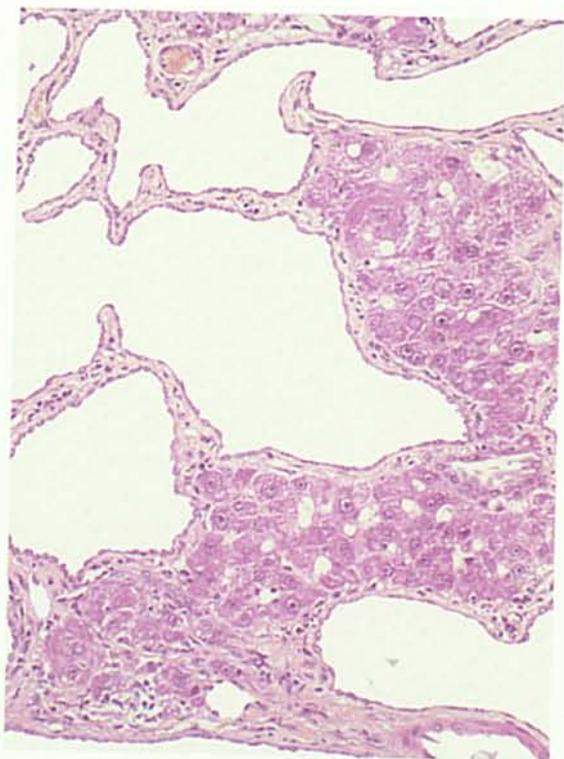


Fig. 22 – Multiloculated biliary cyst. (H&E, 41x)

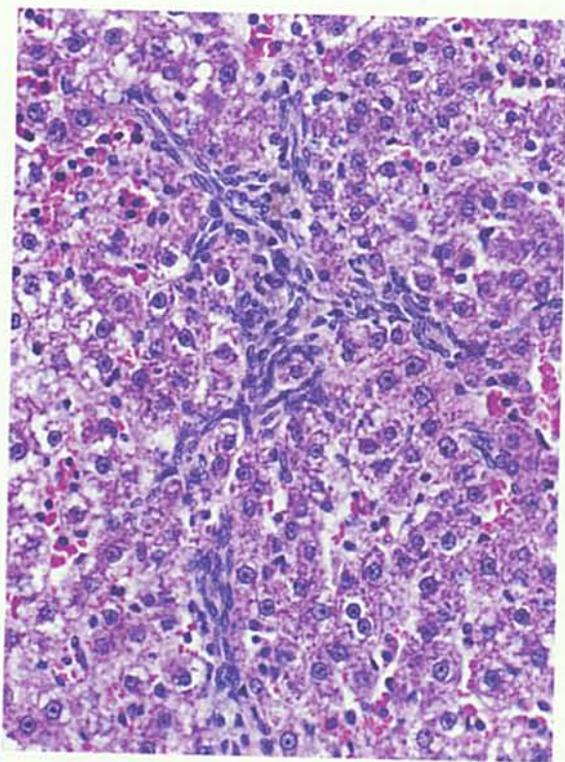


Fig. 23 – Oval cell hyperplasia. (H&E, 82x)

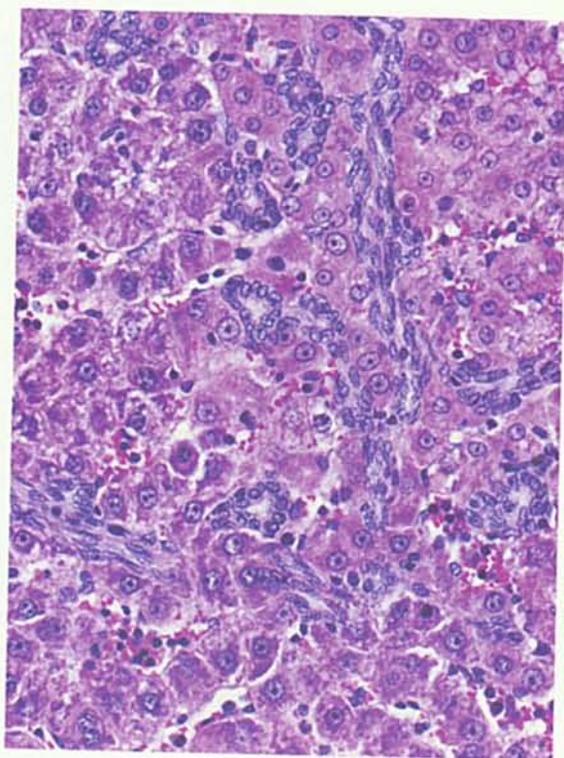


Fig. 24 – Oval cell hyperplasia. (H&E, 82x)

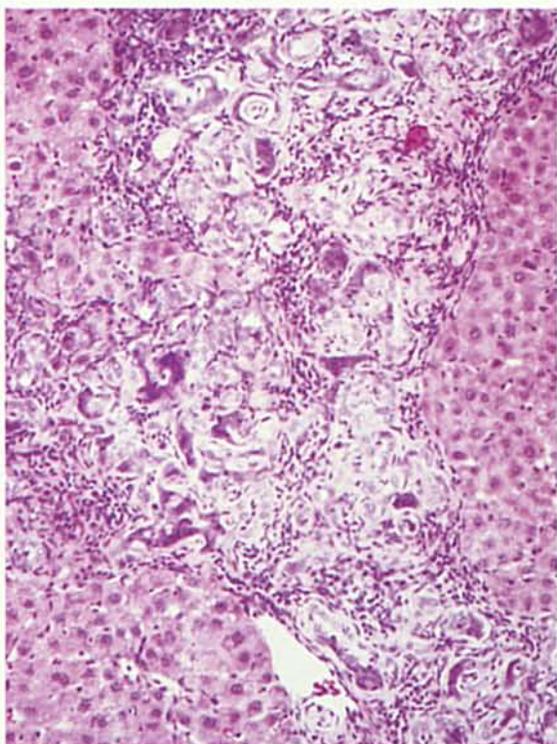


Fig. 25 – Cholangiofibrosis. (H&E, 60x)

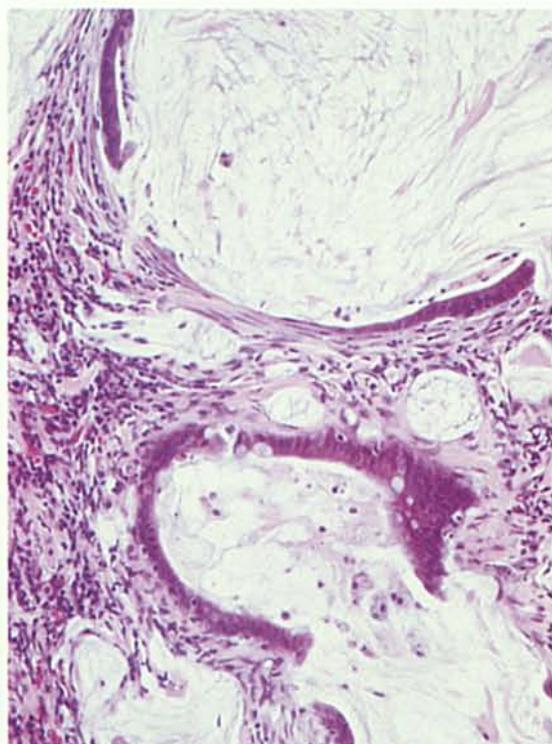


Fig. 26 – Cholangiofibrosis. (H&E, 50x)

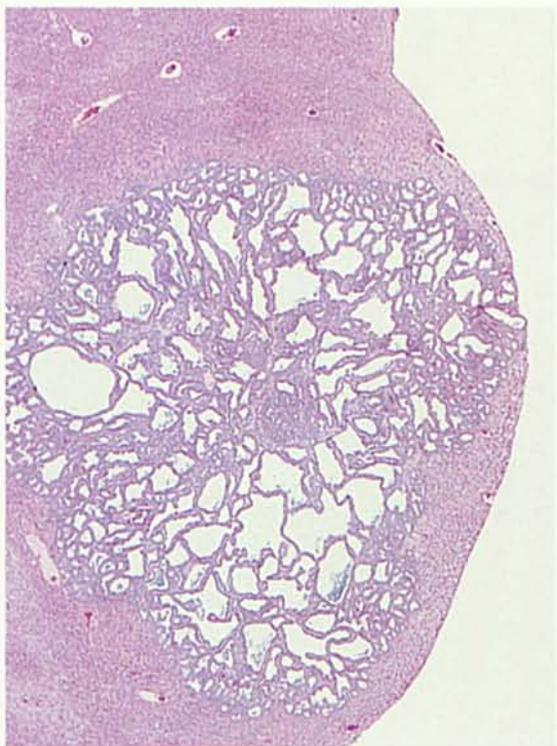


Fig. 27 – Cholangioma. (H&E, 10x)

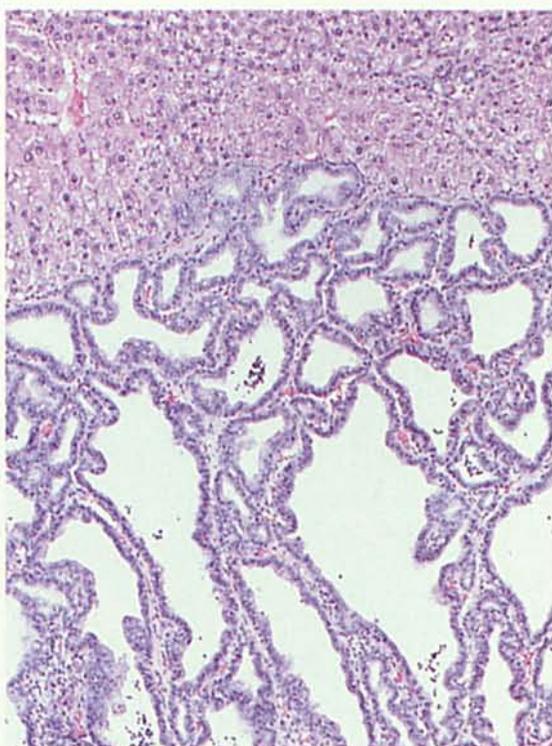


Fig. 28 – Cholangioma. (H&E, 33x)

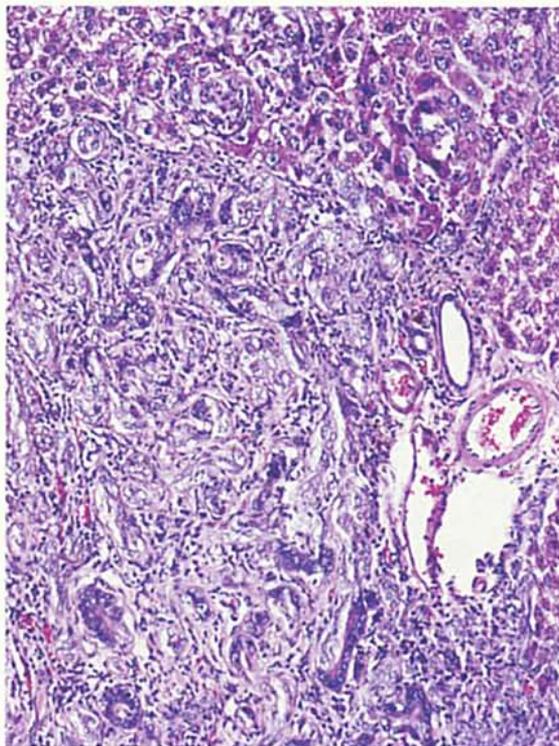


Fig. 29 – Cholangiocarcinoma. (H&E, 41x)

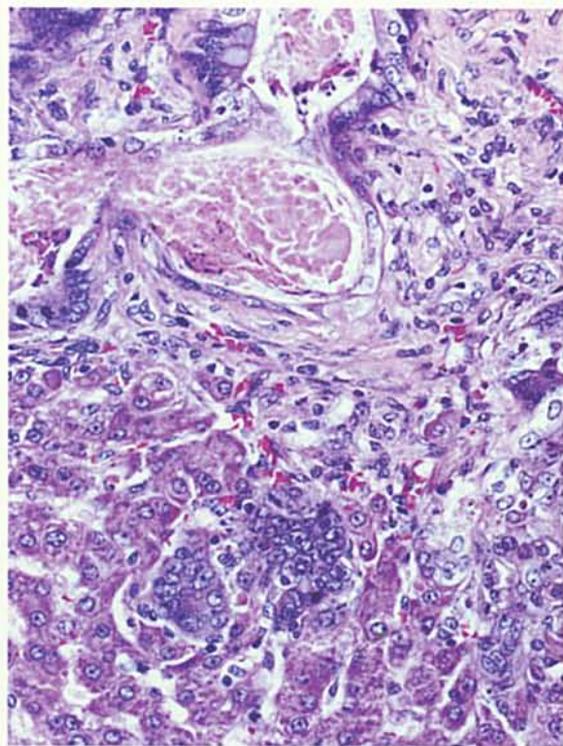


Fig. 30 – Cholangiocarcinoma. (H&E, 82x)

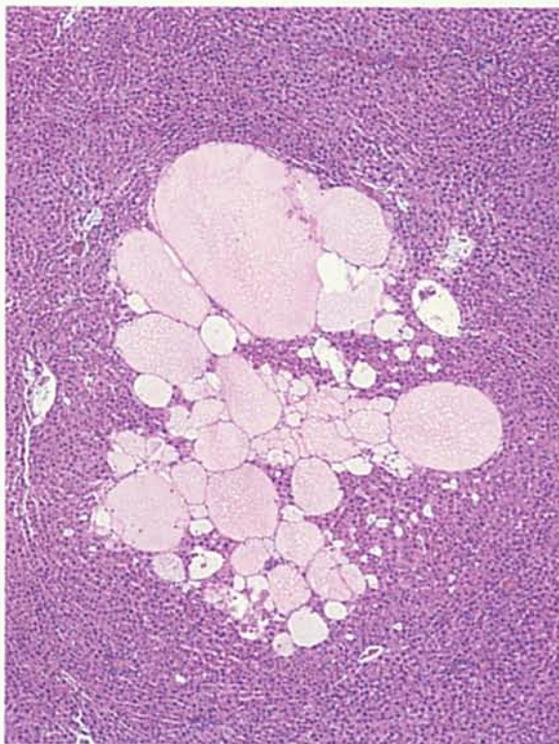


Fig. 31 – Cystic degeneration. (H&E, 10x)

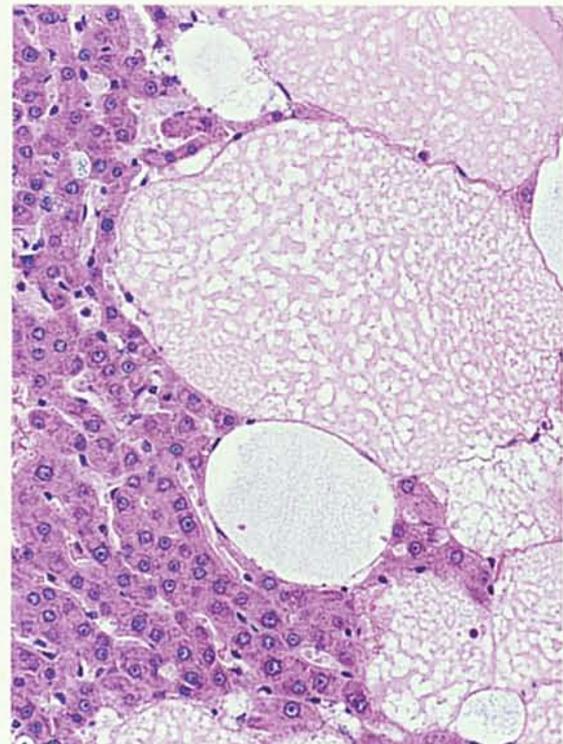


Fig. 32 – Cystic degeneration. (H&E, 70x)

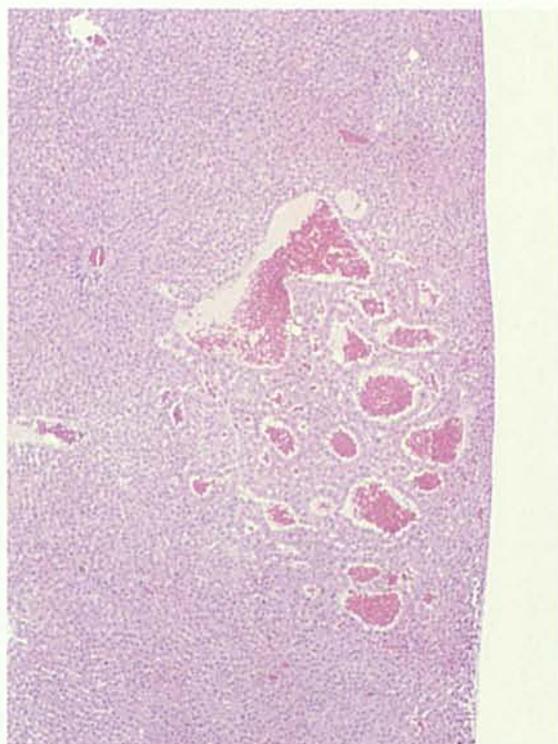


Fig. 33 – Angiectasis. (H&E, 7.5x)

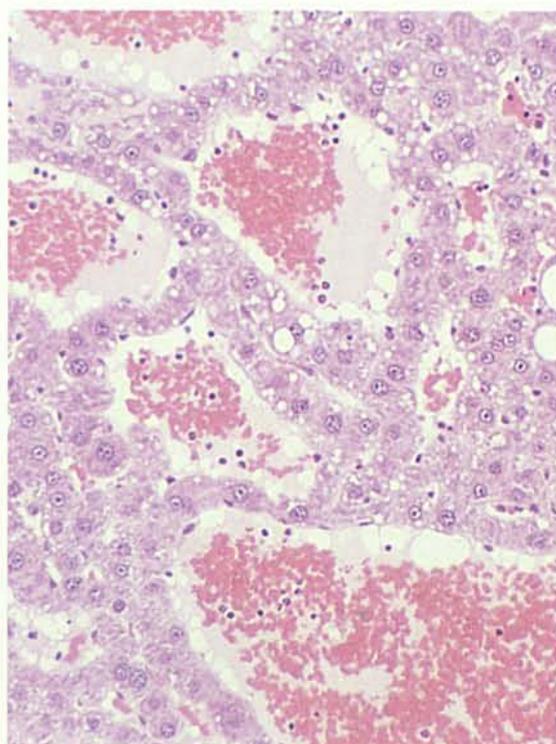


Fig. 34 – Angiectasis. (H&E, 77x)

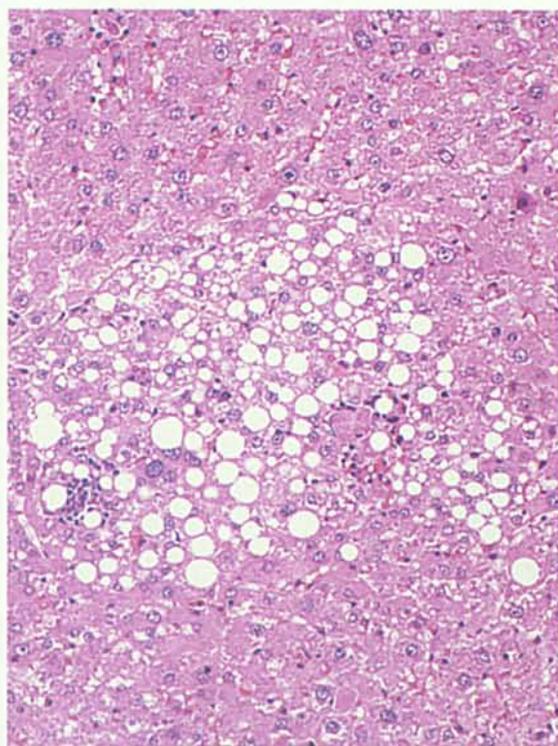


Fig. 35 – Focal fatty change. (H&E, 50x)