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Digestive System

Edited by
T. C. Jones U. Mohr R. D. Hunt

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Hepatocellular Adenoma, Liver, Rat

Paul N. Brooks and Francis J. C. Roe

Synonyms. Benign liver cell tumor; liver parenchymal-cell adenoma.

Gross Appearance

Hepatocellular adenomas vary in size and multiplicity. Most are nonfatal and are discovered incidentally when animals are killed or die for other reasons. If a liver tumor is located close to the ventral body wall, it may be detectable by palpation in a nonobese animal. However, this is not a reliable way to detect liver tumors in living animals. Moreover, since heavy palpation may cause a tumor to bleed, this method of detecting liver tumors in living animals is not recommended. If an adenoma arises near the liver surface, it may be noticed at necropsy even though it has a mean diameter of less than a millimeter. Otherwise the presence of small adenomas may not be suspected until tissues are trimmed after fixation or until sections are examined under the microscope. Even the presence of a very larger tumor that replaces and expands a whole lobe of the liver may not be suspected until necropsy unless it gives rise to abdominal distension. In general, tumors discovered at necropsy in young animals tend to be smaller and more nearly spherical than those found in older animals. Larger tumors are often molded by the shape of the liver lobe in which they arise.

Neoplasms discovered during tissue trimming or microscopically have a different status from macroscopically evident lesions from a statistical viewpoint in the interpretation of findings in a carcinogenicity test. The lesions may be the same color as that of the surrounding liver, or may be darker or lighter depending on the relative degrees of congestion and steatosis in the tumor and in the surrounding liver tissue.

Tumors may be solitary or multiple with an increasing tendency to multiplicity with age. Where there are multiple liver tumors they may be of the same or different histologic type, degree of malignancy, size, and appearance.

Occasionally liver cell adenomas become pedunculated with the risk that the pedicle will become twisted. Infarction can also occur in nonpedunculated tumors located at the edges of the lobes. Such tumors tend to be red or pale depending on how long before death the infarction occurred. Another rare event is that an infarcted tumor may lose all contact with the liver and end up as a free body floating around the peritoneal cavity.

Although the vast majority of liver cell adenomas are without obvious effect on health, occasionally even a relatively small lesion of this kind may cause death from intraperitoneal hemorrhage.

Size, or for that matter any other macroscopically observable characteristic, is not a reliable indicator of malignancy and cannot, therefore, be of specific value in the diagnosis of hepatocellular adenoma.

Microscopic Features

Small adenomas, greater in diameter than one liver lobule, are generally spherical and well circumscribed, but progressive proliferation of the lesion may result in a nodular neoplasm with an irregular boundary (Fig. 35). During this process, the normal hepatic architecture is lost, although central veins and portal tracts are not necessarily always absent from the edges of the lesion (Fig. 36). Structures resembling central veins can be produced within adenomas and portal tracts can become engulfed during parenchymal proliferation and remain within the lesion (Fig. 37). However, the architecture within the adenoma is always atypical in that the normal topography is not maintained and consequently there is no lobular arrangement.

The cytology of hepatocellular adenomas varies considerably. They commonly consist of cells with eosinophilic, basophilic, clear, or vacuolated cytoplasm or various combinations of the different cell types, sometimes with islands of one cell type within a lesion consisting mainly of another cell type. Adenoma cells commonly have slightly enlarged clear nuclei with prominent nucleoli. The trabecular arrangement of cords, which are usually one to two cells thick, is maintained with the formation of discontinuous plates at the boundary; adenoma cords being perpendicular or oblique to those in the normal parenchyma. Adjacent normal liver cords are often compressed and there is sharp demarcation of the neoplastic lesion from the surrounding parenchyma (Figs. 35, 36). Sinusoids within the adenoma have a variable width, depending upon whether or not the ne-

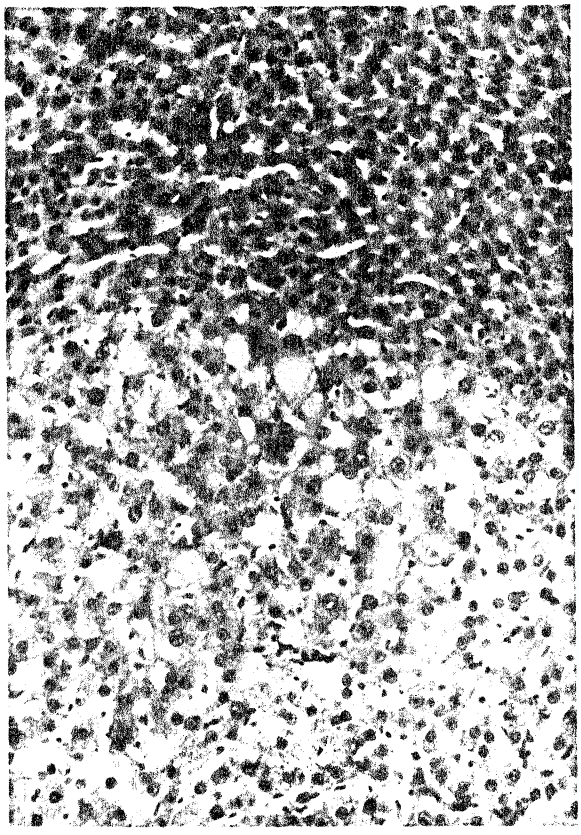


Fig. 35. Hepatocellular adenoma, rat. Note the boundary of the neoplasm with adjacent normal liver parenchyma *(above)*. In general, neoplastic cells are larger than normal and have more eosinophilic cytoplasm. There are no clearly defined sinusoids, and slight compression of adjacent normal liver cords is evident. There is no invasive growth. H and E, ×430

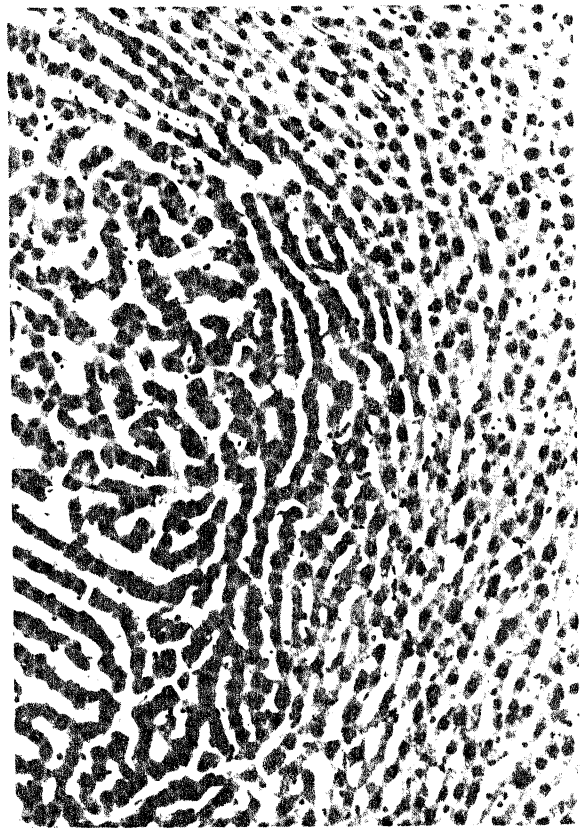


Fig. 36. Hepatocellular adenoma, rat. The cytology of neoplastic cells is very similar to that of normal hepatocytes except that the former have slightly more basophilic cytoplasm. Cords one cell thick form clear sinusoids. There is no evidence of invasive growth. H and E, ×430

oplastic cells are enlarged, and any increase in mitotic rate of neoplastic cells may not be obvious compared with the surrounding parenchyma. Invasive growth of hepatocellular adenomas is not observed.

In some instances changes, which some pathologists regard as regressive, may occur within hepatocellular adenomas. These are more commonly cystic or fatty changes. Neither of these changes necessarily constitutes evidence of regression per se, although spontaneous regression undoubtedly can occur. Cystic and fatty changes can lead to considerable distortion of the microscopic appearance of adenomas and make diagnosis difficult (Figs. 38–40).

Differential Diagnosis

During the past decade the criteria for the diagnosis of benign hepatocellular tumor (adenoma) have been subject to considerable debate and disagreement. Classification schemes have been proposed for the rat (Squire and Levitt 1975; Stewart et al. 1980) and for the mouse (Frith and Ward 1980). In theory the adenoma lies between "hyperplastic lesion" and "hepatocellular carcinoma." In practice there are few clear-cut criteria for distinguishing between adenoma and either hyperplasia or adenocarcinoma. The situation has been further confused by the introduction of the concept of "foci and areas of hepatocellular alteration" since there is no clear demarcation line between some forms of hepatocellular alteration

Fig. 38 *(left)*. An area of fatty degenerative change (steatosis) of the cytoplasm of neoplastic cells. H and E, ×430 ▷

Fig. 39 *(right)*. Steatosis of the cytoplasm of neoplastic cells and the formation of a pseudocapsule by compression of portal tracts. H and E, ×430

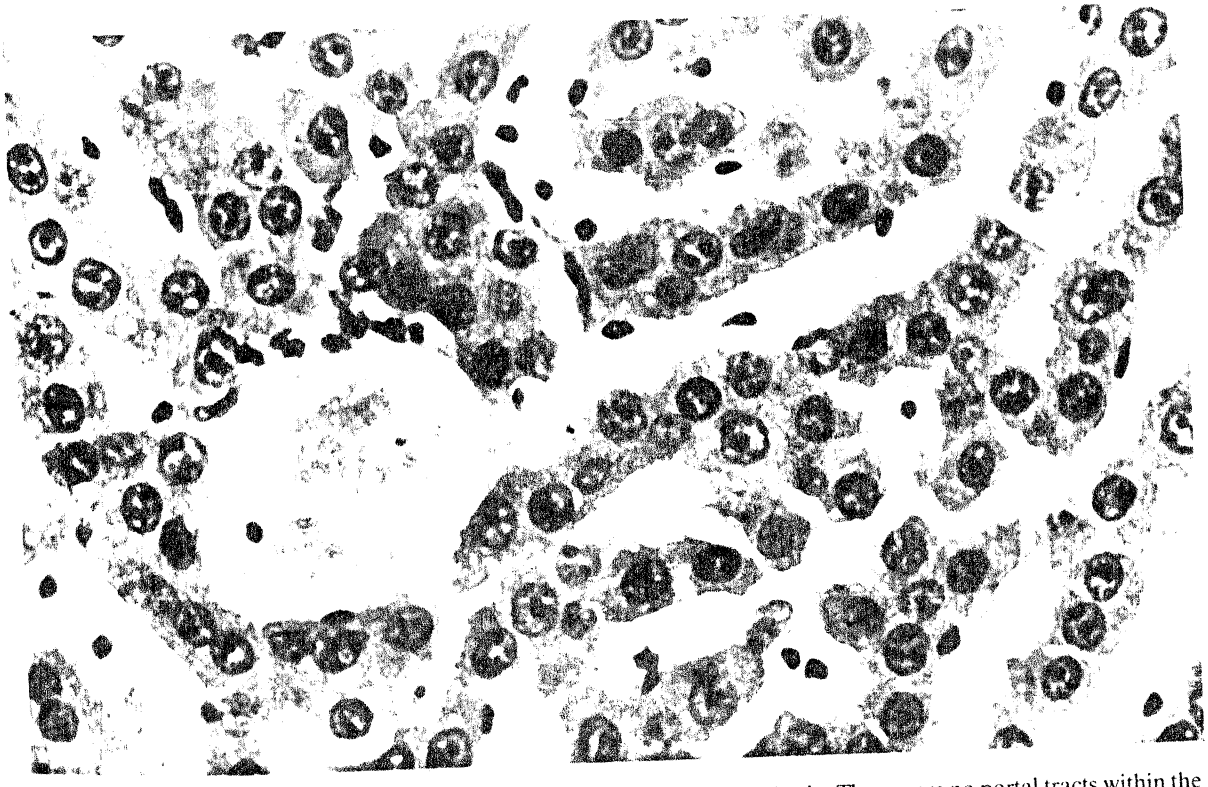
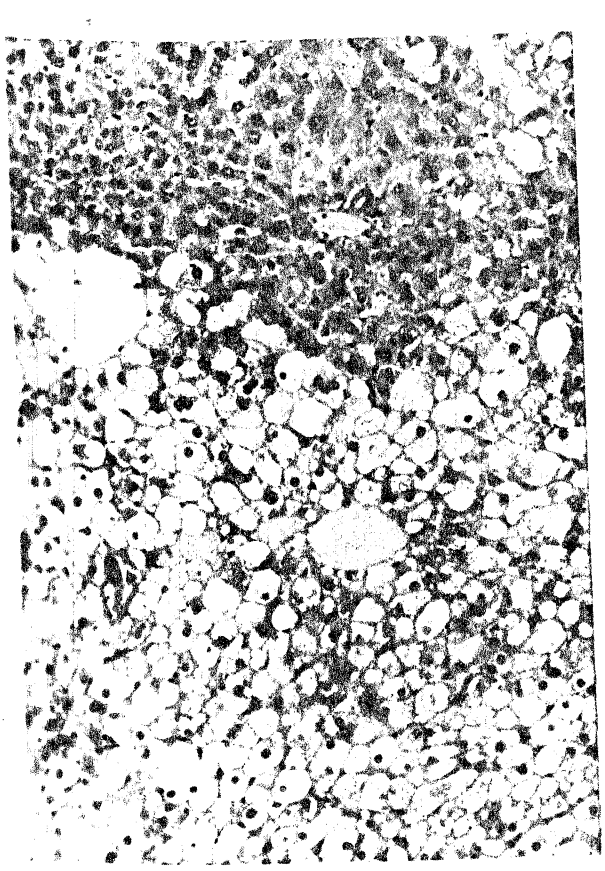
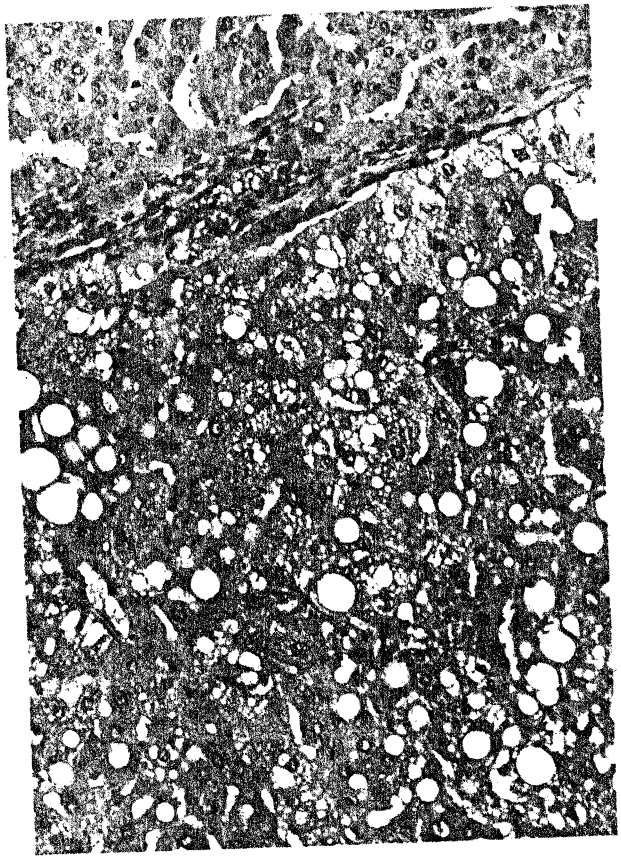


Fig. 37. A higher magnification of the same lesion as Fig. 36, illustrating the formation of a structure resembling that of a central vein. There were no portal tracts within the neoplasm. H and E, × 1720





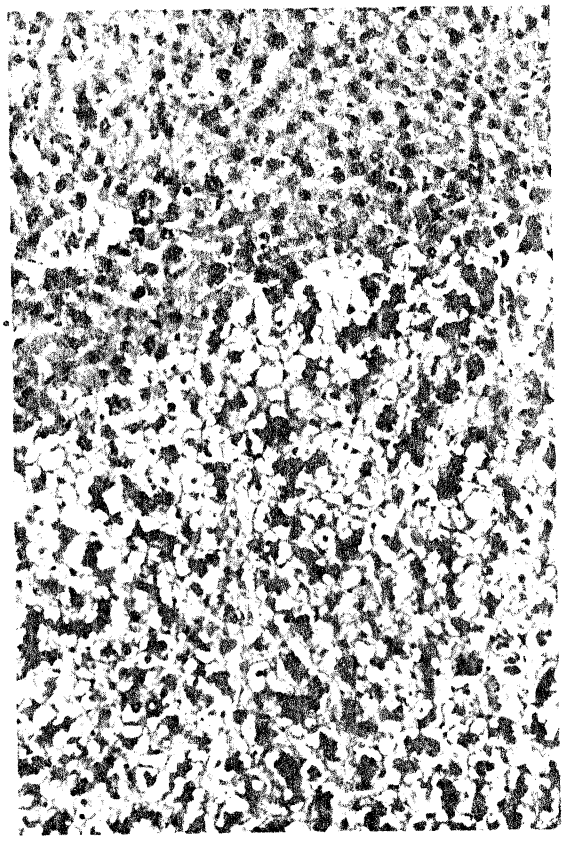


Fig. 40. Vacuolated neoplastic cells associated with cytoplasmic accumulation of glycogen. H and E, × 172

and hyperplasia or even adenoma. A few pathologists faced with these difficulties have decided to regard all the above lesions as hepatocellular carcinomas. To do this, however, is to waste data, to exaggerate the incidence of malignant neoplasia, and, in fact, to risk getting both false-positive and false-negative results in carcinogenicity tests.

It is better practice, therefore, to attempt to distinguish between the various kinds of lesions in the best way possible, knowing that the borderlines between them are indistinct. In the interpretation of chronic toxicity and carcinogenicity tests, the precise diagnosis of each proliferative liver lesion is often of less importance than the avoidance of drift in the use of criteria such that comparisons of the incidences of lesions in treated and control groups of animals are biased.

For a lesion to be regarded as hyperplastic or neoplastic there has to be evidence of proliferative changes (e.g., an expansive lesion with higher mitotic activity than in the surrounding liver). Expansion associated with steatosis or degeneration in the absence of evidence of cellular proliferation would lead toward a diagnosis of *focus of hepatocellular alteration.*

If a lesion shows expansion with compression of surrounding liver tissue, evidence of cellular proliferation, and loss of lobular structure, it must be regarded as neoplastic. Preservation of lobular structure with the presence of portal traids within a lesion suggests it to be hyperplastic rather than neoplastic. For proliferative, expanisve lesions that are less than one lobule in size, there is no sure way of distinguishing between hyperplasia and neoplasia.

In animals where there has been previous liver injury, the whole organ or whole lobes of it may consist of regenerative nodules separated by bands of scar tissue. The term *regenerative nodular hyperplasia* is applicable to this condition, although it is recognized that it may coexist with hepatocellular neoplasia.

At the other end of the spectrum, there is no difficulty in identifying a lesion as a *hepatocellular carcinoma* if it has clearly invaded through some definable barrier, e.g., through the liver capsule or into a walled blood vessel, or if it has metastasized to the lungs or other tissues. However, the interpretation of lesser signs of possible malignancy such as irregular extension into surrounding liver tissue without penetration of a definable barrier leads to disagreements between pathologists. It is particularly in this situation that great attention should be given to cytologic changes suggestive of malignancy (see p. 39) and to variations from normal hepatocellular arrangement.

Because of the difficulties of distinguishing between hyperplasia, benign neoplasia, and malignant neoplasia, there is often a case for blind rereading of sets of liver sections. This may not result in more accurate diagnosis but does serve to eliminate between group biases.

Lesions of endothelial and bile duct origin also must be considered during diagnosis, but these lesions are usually more of a problem in the differential diagnosis of hepatocellular carcinoma than that of adenoma.

Biologic Features

Hepatocellular adenomas generally do not result in the death of animals, although expansive growth may ultimately lead to functional anomalies. Of greater significance is the possibility for progression of hepatocellular adenomas to carcinoma. The earliest indication of localized functional disturbances in response to hepatic carci-

nogens is focal hepatocellular alteration (Williams 1980), and it is possible that such foci of proliferating cells are clones that developed from scattered isolated liver cells (see p. 10, this volume).

Foci and areas of hepatocellular alteration have a variety of enzymatic and other changes that facilitate their demonstration by histochemical techniques. These changes include: loss of adenosine triphosphatase (Hirota and Williams 1979a) and glucose-6-phosphate activity (Hirota and Williams 1979a), accumulation of glycogen (Williams et al. 1976; Williams and Watanabe 1978), increased gamma-glutamyl transpeptidase activity (Hirota and Williams 1979a), resistance to iron accumlation (Hirota and Williams 1979a), and hyperbasophilic staining with toluidine blue (Hirota and Williams 1979b). According to Williams and Yamamoto (1972) and Williams et al. (1976) nodules (hepatocellular adenomas) occur only after the appearance of foci in the process of carcinogenesis, and this view is supported by the recent work of Reznik-Schüller and Gregg (1983) (see also p. 19, this volume).

Many of these indicators of functional abnormalities are also shared by hepatocellular adenomas, although they do not usually exhibit alpha-fetoprotein secretion (Kroes et al. 1972; Tchipysheva et al. 1977; Kuhlmann 1978), which is a feature of some hepatocellular carcinomas.

Both adenomas and foci and areas of hepatocellular alteration are resistant to the cytotoxic effects of the inducing carcinogen and those of other toxic carcinogens requiring metabolic activation. Reduction of cytochrome P-450 within the lesions is probably the basis for this diminished metabolic capability (Gravela et al. 1975).

Since some foci of hepatocellular alteration are able to persist after removal of the chemical stimulus, these must be regarded as irreversible (Schauer and Kunze 1976; Williams 1980). In the rat, foci of areas of clear, eosinophilic or basophilic hepatocellular alteration have been demonstrated to be stages in the development of neoplasia (Bannasch 1968) and this is supported to some extent by the cytologic similarity between lesions of hepatocellular alteration and hepatocellular adenoma.

Adenomas, as might be expected, have been found to persist and increase in size after removal of carcinogen (Hirota and Williams 1979b), but many that persist for 1 year after removal of carcinogen undergo extensive cystic change. This cystic change may not necessarily be a regressive alteration, but closely resembles the lesion referred to as spongiosis hepatis by Bannasch et al. (1981) (see also p. 116, this volume), which probably results from the extracellular accumulation of acid mucopolysaccharides and/or proteins such as collagen. Such accumulation could result from an overproduction or impaired degradation of these substances within the neoplastic lesion.

In transplantation studies, hepatocellular adenomas persisted for months at the transplantation site, but did not exhibit continued growth, although cell division was maintained (Williams et al. 1977). This would suggest that rodent hepatocellular adenomas require the environment of the intact liver for progressive growth (Williams 1980).

Much of the work undertaken in elucidating the morphological identification and behavior of proliferative parenchymal lesions of the rodent liver has been based on experiments employing known carcinogens. Although the morphology and presumably also the behavior and progression of spontaneous and induced neoplastic lesions is generally similar, it is quite possible that carcinogens, particularly if there is continuous exposure to them, to some extent modify the natural progression and behavior of such lesions, thereby providing a somewhat different basis for classification.

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Cholangiofibroma and Cholangiocarcinoma, Liver, Rat

Peter Bannasch, Ulger Benner, and Heide Zerban

Synonyms. Nodules of cholangiofibrosis; cholangiocellular carcinoma; cholangiolar adenocarcinoma; bile duct carcinoma; malignant cholangioma; adenocarcinoma.

Gross Appearance

Both cholangiofibroma and cholangiocarcinoma appear macroscopically as firm nodules frequently distributed in the liver in a multinodular fashion (Fig. 41). The tumor tissue usually has a grayish-white color and may also have yellow areas. The macroscopic picture may become very complex and colorful when the cholangiocellular tumors are combined with hepatocellular carcinomas or malignant mesenchymal tumors, such as angiosarcomas.

Microscopic Features

Although transitions between cholangiofibromas and cholangiocarcinomas have been observed, it appears to be appropriate to give a separate description of their histologic and histochemical characteristics, since they basically differ in their biologic behavior. Whereas metastases of cholangiofibromas have not been described up to now, it is beyond doubt that cholangiocarcinoma may metastasize (Bannasch and Massner 1976; Schauer and Kunze 1976).

Cholangiofibroma (Figs. 41–44). The cholangiofibroma is composed of atypical ductules and large amounts of collagen-rich connective tissue (Bannasch and Massner 1976). As a rule, the epithelium of the neoplastic ductular structures is composed of one cell layer (Fig. 42) which contains many goblet cells storing and secreting abundant mucous substances (Fig. 42b). The remaining epithelia are usually intensely basophilic and may appear rather atypical. Mitotic figures (which are frequently pathologically altered), and also necrotic cells, are often present within the epithelium of the glandular structures. The lumina of the ductules are filled with mucous substances which may be mixed with shedded, lipid-laden, or necrotic epithelial cells and polymorphonuclear leukocytes (Fig. 42a). The mucous substances contain both acid and neutral mucopolysaccharides, as demonstrated histochemically by staining with alcian blue and by the Hale- or periodic-acid-Schiff reaction (Fig. 42b, 43b). Glycogen particles