Pathology of the Liver and Biliary Tract

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Preface

Our aim has been to provide a practical, problem-oriented approach to the pathologic diagnosis of hepatic and biliary disorders. We hope that it will be useful in the day-to-day practice of pathologists and that it will also be consulted by physicians and surgeons when they have to care for patients with such ailments.

This book begins with a discussion of the indications for obtaining different types of hepatic specimens and the process by which a pathologic diagnosis is made. Most chapters are oriented toward clinicopathologic entities, such as hepatitis, alcoholic liver disease, fatty liver, granulomas, cholestasis, space-occupying lesions, and drug-induced liver disease. Pediatric hepatic pathology, including storage disorders, is also discussed, and chapters on the gallbladder and biliary tract are included. The pathologic features of the various conditions are described, often with tables included for differential diagnosis. Our approach has not been encyclopedic. References are, therefore, provided for more detailed discussions of individual topics. Frequently, a disorder is referred to in more than one chapter. To minimize duplication we have inserted cross references to the more detailed descriptions wherever necessary. Careful consideration has been given to the roles of electron microscopy, immunopathology, and chemistry in reaching a diagnosis. In selecting illustrations, our aim has been to depict those morphologic lesions that are seen most frequently, or that have particular diagnostic importance.

We are most grateful to those of our colleagues in this country and abroad who provided us with material for illustrations. We believe that their generosity has greatly enhanced the usefulness of this book. Several of our colleagues, particularly S. W. French, M.D., Liisa Russell, M.D., and Rick Baier, M.D., have read the manuscript and provided constructive criticisms. Ms. Eileen Ginsberg of John Wiley was most helpful during the production stage. Any comments from readers will be most welcome.

B.H.R.
C.K.M.

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A General Approach to Hepatic Pathology

DIFFERENT TYPES OF SPECIMEN

The amount of information that can be obtained from a liver biopsy and the clinical value of this information depend to a considerable extent on the specimen itself. Significant considerations are choice of the most suitable type of specimen (needle or wedge), the manner in which it is obtained (percutaneously, at laparoscopy or laparotomy), and the anatomic relationship of the specimen to the lesions. Most specimens of liver received in the surgical pathology laboratory are obtained by blind percutaneous aspiration needle biopsy, although needle biopsy specimens can also be obtained through a laparoscope. A combination of laparoscopy with biopsy permits experienced operators to describe the surface of the liver and to select the specimen from area(s) they consider most likely to provide maximum information. The relative usefulness of needle biopsy specimens of hepatic lesions encountered at laparotomy compared with the more common surgical wedge biopsy specimen has been debated. Inspection and palpation of the liver assist the surgeon in selecting the most appropriate specimen; either type of material has its advantages and disadvantages, which will be discussed below. Larger samples are obtained surgically when space-occupying lesions are resected or at debridement for trauma.

Needle Biopsy

Percutaneous needle biopsy of the liver is a well-established diagnostic tool that, in most cases, should be employed as the first step in morphologic diagnosis (1). Wider use of percutaneous neecle biopsy by surgeons as well as other physicians sometimes obviates the need for surgical intervention (2). The selection of patients and the technique of aspiration have been clearly described by Sherlock (3), Menghini (4), and Walters and Paton (1), among others. Fine needle aspiration produces cytologic smears that can be employed more frequently in the future (5,6a,6b). Brushing of tumors at laparoscopy has also been recommended (6b).

The site of biopsy deserves some consideration before puncture. A standard intercostal biopsy in the right anterior axillary line is indicated if a patient suspected of having a hepatic disorder does not have a focal lesion. The tissue obtained by an adequate needle biopsy weighs 50–200 mg, compared with an

adult liver weight of approximately 1500 g (3). This method, therefore, samples only a very small proportion of the liver. Nevertheless, there is little doubt that in diffuse hepatic diseases, such as acute viral hepatitis, alcoholic hepatitis, or chronic passive congestion, needle biopsy specimens measuring 15 mm or longer are representative of the principal histopathologic changes in the entire liver (7,8). In fact, specimens measuring 5 mm may be adequate (8). The length of the cylindrical needle biopsy specimen ensures that most of the tissue is not from the vicinity of the capsule, thereby avoiding one of the problems of wedge biopsy. By contrast, the narrowness of the tissue obtained sometimes makes it difficult histologically to identify complete lobules, which can pose a disadvantage in histologic interpretation. Needle biopsy, especially if less than 15 mm in length, is not as reliable in the diagnosis of patchy hepatic lesions, such as chronic active hepatitis, cirrhosis, neoplasms, or granulomas (7,8). Stepsections are mandatory if such lesions are suspected but not found on the first cuts. If a space-occupying lesion is suspected, the biopsy needle should be directed to the site most likely to be positive (9). If there is a large palpable nodule, this should be the target. Similarly, for a lesion on a scan, multiple scintiscans or CT scans should be used to localize the lesion and determine the direction of approach. The same is true if there appears to be a lesion involving one hepatic duct or one hepatic vein. It is in such situations that a combination of laparoscopy with biopsy should be seriously considered if an experienced operator is available. The recently introduced method of obtaining a biopsy through a hepatic vein catheter permits the use of needle biopsies, even in patients with a hemorrhagic tendency.

Wedge Biopsy

Surgical wedge biopsies are undertaken for diagnosis if a previous needle biopsy has not given adequate information, particularly if primary biliary cirrhosis, macronodular cirrhosis, or malignancy are suspected, but not confirmed. In some cases, preliminary needle biopsy should be omitted, especially if the patient has a hemorrhagic diathesis and hepatic vein biopsy is not available. Wedge biopsies can also be obtained if laparotomy is performed for diagnostic purposes, as in fever of unknown origin and in jaundice when the biliary tract is explored for possible obstruction. Another indication for wedge biopsy is the elucidation of unexpected focal or diffuse hepatic abnormalities encountered during abdominal surgery. Diffuse pallor and nodularity, for instance, suggest fatty change and cirrhosis, respectively. Adequate wedge biopsy specimens weigh 0.5–1 g, making it possible to obtain a microscopic sample of a much larger proportion of the liver than is possible with needle biopsies. The likelihood of finding focal lesions such as granulomas or metastatic tumors, is thus increased considerably. The subcapsular location of such specimens can give rise to difficulties in interpretation because of variation in the structure of the liver immediately beneath the capsule. A considerable amount of fibrous tissue may be present in this zone in otherwise normal livers. Islands of parenchyma that appear to be isolated and superficially suggestive of cirrhosis are occasionally seen in the immediate subcapsular zone. Some pathologists have been so disturbed by such changes that they prefer to interpret needle biopsy specimens taken from the liver at surgery to the more usual wedge specimens. Our obser-

vations agree with those of Petrelli and Scheuer (10), who found these changes to be limited to a depth of 2 mm below the capsule and did not consider these appearances a serious source of difficulty. Certainly, their observations reinforce the need for the surgeon to take wedge biopsy specimens of adequate size, that is, at least to a depth of 1 cm. Wedge biopsies permit better appreciation of certain microscopic features, for example, lobular architecture and lesions of the larger portal bile ducts. Wedge biopsy specimens also provide a larger sample for biochemical estimations than can be obtained by needle biopsy. Needle biopsy specimens may be taken during laparotomy in addition to wedge biopsies in order to obtain sampling from areas more remote from the capsule than can be obtained by a wedge biopsy. Needle biopsies at laparotomy can also provide a sampling of more widely distributed areas than can be obtained by a single wedge biopsy. Routine needle biopsy of the liver in all patients undergoing upper abdominal operations has been recommended (11).

Resection

Larger specimens are resected surgically for the diagnosis and treatment of space-occupying hepatic lesions and lacerations. The latter range from specimens only slightly larger than diagnostic wedge specimens to resections of hepatic segments, lobes (12), or even entire livers, before hepatic transplantation.

INSPECTION AND DESCRIPTION OF THE SPECIMEN

General Description

For a needle biopsy, it is usually sufficient to record the length of the biopsy cylinder. Whereas it is desirable to record the weight of needle biopsy specimens, it is an essential step in the case of wedge and larger biopsy specimens. Careful examination of every specimen is mandatory. Although examination can be accomplished with the naked eye, a dissecting microscope is helpful. Good lighting of the dissecting surface is essential. Specimens should be viewed not only by direct, but by transmitted light as well.

The capsular surface is easily identified in wedge biopsy specimens and in surgically resected specimens. Normally smooth and glistening, granularity or nodularity suggests cirrhosis. The capsule usually cannot be identified in needle biopsy specimens. Cirrhosis can sometimes be recognized, however, in such specimens by the observation of brownish cirrhotic nodules held together by paler fibrous connective tissue. Generally, though, the hepatic tissue obtained by needle biopsy from cirrhotic patients tends to crumble into irregular fragments. A fragmented needle biopsy specimen, therefore, should always raise the suspicion of cirrhosis.

The color of the cut surface of surgically resected and needle biopsy specimens is normally a uniform brown or tan. The regular arrangement of normal portal triads and central veins is generally recognizable on careful inspection. A pale, yellowish, greasy appearance indicates a fatty liver, which can be confirmed if the specimen floats in watery fixatives, such as buffered neutral formalin. A greenish

appearance indicates cholestasis, either extra- or intrahepatic. The green coloration becomes more striking after exposure of the tissue to air or to formalin-containing fixatives. Under both conditions, bilirubin is oxidized to biliverdin, which is more intensely green. The green staining of cholestasis often has a finely punctate appearance, because the cholestasis is generally most pronounced in centrilobular zones. The dark red appearance of congestive cardiac failure and of the Budd-Chiari syndrome is also more pronounced in the centrilobular zones, forming the so-called nutmeg pattern. A diffuse rusty brown color suggests hemosiderosis. In hemochromatosis, cirrhosis may be present in addition to the brown pigmentation. Diffuse chocolate or black coloration suggests the Dubin-Johnson syndrome. We recommend that every fresh specimen received in the surgical pathology laboratory be examined under ultraviolet light so as not to overlook a diagnosis of porphyria cutanea tarda, which causes the liver to emit a purple fluorescence. A Woods light can be used, or alternatively, frozen sections or imprints of the fresh unfixed biopsy specimen can be viewed microscopically under an ultraviolet microscope. This procedure must be done promptly in order to protect the specimen from undue exposure to ambient light (13). Fluorescence is also found in protoporphyria and in some cases of porphyria variegata (Chapter 9). The liver in amyloidosis is often said to appear abnormally pale and translucent. Unfortunately, we have never found this helpful in making a gross diagnosis.

Focal Lesions

Multiple small diffusely scattered foci of white or yellowish discoloration measuring approximately 1–2 mm in diameter suggest neoplastic lesions, particularly leukemia or lymphoma, granulomas, or necrosis. Lymphomatous infiltration tends to be whitish and may have a regular distribution throughout the specimen because of localization in the portal areas. This pattern is particularly true of lymphatic leukemia. Granulomas usually appear as irregularly distributed small white or gray infiltrates. They are generally seen best by transillumination. Irregular yellowish foci suggest hepatic necrosis, particularly of viral origin, such as herpes simplex infection.

Larger space-occupying lesions may almost completely replace the normal hepatic parenchyma in a needle biopsy specimen. Such specimens are quite unlike normal liver in both color and consistency. The tissue obtained may vary from whitish and firm in the case of a scirrhous carcinoma to purple and soft in the case of vascular lesions. Because of the contrast between the lesion and the uninvolved part of the liver, space-occupying lesions are easier to describe in wedge specimens and in hepatic resection specimens. Firm, homogeneous, white lesions are suggestive of neoplasia, particularly metastases or primary bile duct carcinoma. Hepatocarcinomas may be hemorrhagic but are not uncommonly brown or green because of cholestasis. A variegated hemorrhagic appearance is suggestive of necrosis in a malignant tumor. The border between the lesion and the rest of the hepatic parenchyma should always be examined carefully. An irregular border with infiltration into the parenchyma is suggestive of malignancy, whereas the presence of a capsule at the periphery of the lesion favors a benign process. Penetration of the lesion to the serosa is of serious significance,

because it suggests peritoneal involvement. A shaggy periphery with a necrotic center, often containing pus, suggests an abscess. Such lesions are sometimes encapsulated. Granulomas are whitish-gray with an irregular border, fibrotic or calcified. Yellowish liquid material in the center of such lesions is characteristic of caseation. Caseation is rare in hepatic granulomas. However, if present, caseation strongly suggests a diagnosis of tuberculosis or fungal infection. Caseation has also been described in brucellosis (14). Pale wedge-shaped lesions with a hemorrhagic border suggest infarcts, whereas lesions that are diffusely hemorrhagic are suggestive of hepatocellular or vascular neoplasms, trauma, or peliosis hepatis. Tears in the hepatic parenchyma with or without adherent thrombi are indicative of trauma. Cystic lesions are suggestive of a congenital or parasitic etiology. The hepatic parenchyma surrounding space-occupying lesions is frequently compressed and may show centrilobular congestion or cholestasis. Although such changes can be striking in a biopsy close to a lesion, it should not necessarily be assumed that the liver away from the lesion will show the same changes.

Anatomic Relationships

Larger specimens excised for diagnosis or treatment of space-occupying lesions should be described with an emphasis on the appearance and measurements of the lesions and on the anatomic relationships of the lesions to the hepatic landmarks. In order to describe such specimens correctly and obtain the appropriate blocks, an understanding of the surgical anatomy of the liver is required. The true anatomic division of the liver into lobes and lobules is more closely related to the internal distribution of vessels and ducts than to any external landmark, such as the falciform ligament, conventionally taken to divide the liver into right and left lobes. According to more recent views (14a), the true left lobe of the liver is much larger than conventionally described (Fig. 1). As they enter the hilum, the hepatic artery, portal vein, and the common hepatic duct divide into two separate systems serving the true right and left lobes. The line of division between the two lobes, the principal plane, extends in an oblique anteroposterior direction from the bed of the gallbladder to the hepatic fossa of the inferior vena cava. This plane bisects the caudate lobe. About equal in mass, the right and left lobes function almost like separate paired organs, and there is essentially no intrahepatic communication between the two systems. The division of the right lobe into segments has little surgical importance. However, the left lobe is divided into a medial and a lateral segment by the plane of the falciform ligament. The medial segment includes the quadrate lobe and part of the caudate lobe. Riedel's lobe is a downward prolongation of the right lobe often adherent to the mesocolon. Hypoplasia of entire lobes may occur (15).

Occasionally, ectopic hepatic tissue is found entirely detached from the liver in the abdominal (16) or thoracic (17,18) cavities. Such ectopia can be congenital (16) or the result of trauma sustained several years earlier (19). Ectopic hepatic tissue may be fibrotic when the rest of the liver is normal, but it can also be involved in hepatic diseases (20).

The level at which the right and left hepatic ducts join to form the common hepatic duct varies from just outside the liver to a distance of 3 cm or more. The

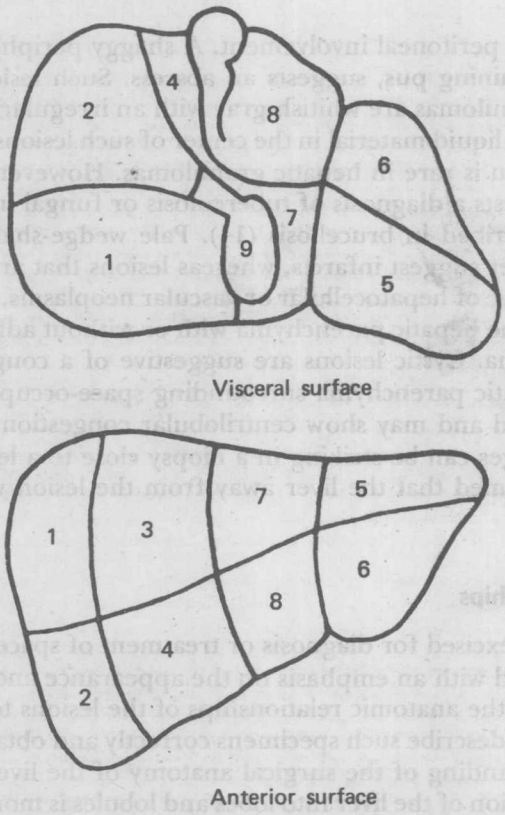


Figure 1. Diagrammatic representation of the division of the liver into subsegments. The relative sizes of the subsegments shown are those most frequently found. The right lobe is composed of subsegments 1, superior posterior; 2, inferior posterior; 3, superior anterior; and 4, inferior anterior. The left lobe is composed of subsegments 5, superior lateral; 6, inferior lateral; 7, superior medial; and 8, inferior medial (quadrate lobe). Number 9 is the caudate lobe. (Modified from Gupta, ref. 14.)

junction can even be intrahepatic, particularly in the presence of hepatomegaly (21). The cystic duct usually joins the common duct distal to the junction, but can join the right hepatic duct. Another anomaly involves a major segmental duct, most frequently from the posterior segment of the right lobe, joining the common hepatic duct. Anomalous divisions of the portal vein also occur (22). The hepatic arterial system is quite variable. Origin of the right hepatic artery from the superior mesenteric artery instead of the celiac artery is the commonest vascular anomaly. Biliary vascular bundles composed of portal vein, hepatic artery, and bile duct supply each of the segments described in the preceding discussion. The caudate lobe is unique in that it can receive branches from either or both right and left lobar systems.

There are three major hepatic veins—the left, the middle, and the right—located in the interlobar and intersegmental planes. The left and middle hepatic veins usually join to form a short stalk before entering the inferior vena cava. The right vein enters the inferior vena cava separately. The left hepatic vein drains

the left lateral segment only. The middle vein lies in the interlobar plane and drains approximately one-third of the liver, that is, the medial segment of the left lobe and most of the anterior segment of the right lobe. The right hepatic vein drains the posterior segment and part of the anterior segment of the right lobe. A series of four or five small, but surgically important, paired hepatic veins pass directly from the inferior or posterior surface of the liver into the vena cava. One pair drains from the right and one from the left. Multiple small veins also drain from the caudate lobe into the diaphragmatic portion of the inferior vena cava. These small hepatic veins tend to remain patent in patients who have the Budd-Chiari syndrome, in whom occlusions of the major hepatic veins are generally found. It is for this reason that the caudate lobe tends to be somewhat protected from the pathologic changes in the Budd-Chiari syndrome. The caudate lobe can actually undergo hypertrophy in such patients (23).

After partial hepatectomy, an inflow and an outflow supply must be preserved for the remaining hepatic tissue. Thus, if a right hepatectomy is performed, the liver is transected in a plane to the right of the middle hepatic vein (23a). For an extended right hepatectomy, the liver is transected in a plane to the right of the falciform ligament, thereby removing the right lobe and the medial segment of the left lobe. For a left hepatectomy, the liver is transected in a plant to the left of the middle hepatic vein. For an extended left hepatectomy, the liver is transected on the right side of the principal plane, and the entire left lobe and a medial portion of the right lobe are removed. If a left lateral segmental resection is performed, the liver is transected in a plane to the left of the falciform ligament, thereby preserving the umbilical fissure and with it the left branches of the portal vein, hepatic artery, and bile duct that supply or drain the medial segment of the left lobe (12,24). A middle wedge resection or central hepatic resection consists of removing part of the liver located anterior to the hilum and including part of the anterior portion of the right lobe and part of the medial segment of the left lobe as well as the gallbladder.

GENERAL PRINCIPLES OF SPECIMEN HANDLING

In deciding to perform a liver biopsy, the physician should also decide whether routine handling is expected to be adequate. Routine handling consists of the production of paraffin sections and can include fixation of blocks for possible electron microscopic examination. If the operator believes that special methods might be indicated, this possibility should be discussed with the pathologist. A decision to employ special fixatives or methods, such as immunopathology, enzyme histochemistry, electron microscopy, biochemistry, or microbiology, should be made jointly by the operator and the pathologist. If this decision has been arrived at before obtaining the biopsy specimen, the pathologist will have made available to the operator the fixatives, other material, and technical help required. If the appearance of the specimen suggests special handling to the operator, the specimen should be taken immediately to the pathologist in a clean, and preferably sterile, container without fixative. After discussion of diagnostic possibilities, the pathologist can then arrange for appropriate processing. There is no doubt

that there is no single correct way in which to handle a specimen. Ultimately, each laboratory has to use the methods best adapted to its needs and resources. We present here the methods we have found to work in our own laboratories.

Processing for Light Microscopy

Fixation and Preparation of Paraffin Blocks

Needle biopsy specimens are obtained most commonly using either a Menghini needle (4) or one of its modifications. Menghini's method is both quick and safe. Its main disadvantage is that in patients with fibrosis, the failure rate is high and the specimen obtained generally small or fragmented (3). With the Vim-Silverman needle, a larger specimen is obtained. This method is more successful in cirrhosis. Distortion and compression along the edges of the specimen are often severe, however. Menghini specimens are aspirated into a syringe containing normal saline. The tip of the needle is then placed into saline in a flat-bottomed glass receptacle, and only then is aspiration stopped and the specimen expelled. The Vim-Silverman needle is also attached to a syringe during aspiration, but the syringe contains no fluid.

A satisfactory needle biopsy specimen has been defined as 1–4 cm in length and weighing 50–200 mg (3). Depending on the length of the specimen, up to 0.5 cm of the liver biopsy cylinder can be cut off with a sharp razor blade, diced into small 1-mm^3 cubes, and put into modified Karnovsky's or McDowell's fixative (p. 13). If indicated, this portion can be processed for electron microscopic examination. The rest of the specimen is placed on a strip of thin paper before fixation, a practice that tends to keep the specimen flattened and in one piece during processing. The paper strip and the attached biopsy specimen are placed into 10% phosphate buffered formalin (25) for 3–10 hours. The biopsy is then dehydrated in graded alcohols, cleared in benzene, taken off its paper strip, and embedded in paraffin. Other investigators prefer different fixatives, such as Carnoy's. We employ special fixatives if storage diseases, particularly the glycogenoses or mucopolysaccharidoses, are suspected and for histochemistry of some enzymes. The advantage in using phosphate-buffered formaldehyde glutaraldehyde for routine fixation is that this fixative allows acceptable ultrastructural preservation, even after prolonged storage of tissue in the fixative as well as after routine processing with paraffin embedment. If electron microscopy is desirable, tissue can be excised from the paraffin block, rehydrated, and prepared for ultrastructural study. The fixative is dependably stable at room temperature for 1 week and for 2–3 months if stored at 4°C.

Wedge biopsy specimens of the liver should be cut at right angles to the capsule such that the capsule forms the base of a triangular block, each side of which measures approximately 1 cm. Blocks are selected to include both lesions and grossly uninvolved parenchyma. They are cut to a thickness of 1–2 mm. For electron microscopic study, cubes of approximately 1-mm thickness are cut and placed in fixative as described for needle biopsies. Tissue for light microscopic analysis is processed in the same way as material obtained by needle biopsy, but fixation should be somewhat longer. Care is taken to embed the blocks flat with the capsule along one edge.

Larger specimens should be cut into slices 0.5–1 cm in width. Several blocks should be taken to include grossly visible lesions and their relationship to the surrounding uninvolved liver and capsule. Blocks through apparently uninvolved liver should also be taken, both at the margin of resection and, if possible, some distance from the lesions. Blocks should also be obtained through any thickened, dilated, or obstructed ducts, and through any abnormal appearing vessels. One should sample ducts and vessels draining a lesion. Fixation is as described above for wedge and needle specimens. Sections should also be taken through any lymph nodes, such as those of the hilum (26), supplied with the specimen. If the gallbladder is included in the specimen, it should be described and processed as outlined in the discussion of the biliary tract (Chapter 16).

Microtomy and Staining

Paraffin sections are cut on a microtome at 5μm and stained by hematoxylin and eosin, by a silver method for reticulum, by Gomori's trichrome for collagen, and by Perl's stain for hemosiderin. Two sections may be left unstained for special stains. For example, if granulomas are found, Ziehl-Nielson's stain for acid-fast bacilli and a silver methenamine stain for fungi are used. Diastase-labile material, positive by the periodic acid-Schiff (PAS) reaction, is specific for glycogen. Unfortunately, the preservation of glycogen in formalin-fixed material is erratic. Preservation is better in paraffin sections of alcohol-fixed tissue or in fresh frozen sections. Diastase-resistant PAS-positive material and a positive mucicarmine stain indicate a mucin.

Recuts of the block may be required if more than two additional special stains are required, or if focal lesions such as granulomas or tumor are suspected on clinical or histopathologic grounds. If this is the case, serial sections are cut through the entire block and retained. Only a proportion, such as every fifth or tenth section, are mounted and stained with hematoxylin and eosin. If lesions are found, special stains may be indicated and can be done on adjacent sections. Recuts are also indicated in chronic persistent hepatitis to exclude focal piecemeal necrosis. If the latter is found, a diagnosis of chronic active hepatitis should be considered. If alpha-1-antitrypsin deficiency is suspected clinically or in neonatal hepatitis, in cirrhosis without a definite etiology, and when hyaline inclusions are found in hematoxylin and eosin sections, a PAS stain with diastase digestion should be done to demonstrate the PAS-positive inclusions characteristic of alpha-1-antitrypsin deficiency. If the patient's serum is positive for hepatitis B antigen, if ground-glass cells are seen by light microscopy, or if the histology suggests acute or chronic hepatitis, one of the empirical stains for HB_sAg should be done (27,28). We find the orcein method stain most satisfactory. Orcein stains elastic fibers (p. 120) and copper-binding protein (p. 120) as well. Immunohistologic staining with peroxidase-labeled antibody (see below) is desirable to confirm the diagnosis of alpha-1-antitrypsin deficiency or hepatitis B infection. The orcein staining method used in our laboratory is as follows:

1. Fix tissue in buffered formalin, embed it in paraffin, and cut 5μm sections.
2. Place deparaffinized sections in potassium permanganate for 15 minutes. (it is important to have good oxidation for contrast).
   Potassium permanganate, 0.5 g
   Distilled water, 95 ml
   3% H_2SO_4, 5 ml

3. Place in 2.0 oxalic acid until colorless (about 10 minutes).
4. Wash in tap water.
5. Wash in distilled water.
6. Stain in orcein for 4–6 hours at room temperature (6 hours preferred).
   Orcein (British Drug Houses or National Aniline), 1 g
   70% alcohol, 100 ml
   Concentrated HCl, 1 ml
   Adjust pH to 1–2
7. Differentiate in 1% HCl in 70% ethanol.
8. Dehydrate; then mount.

Plastic Sections

One or 2-μm-thick sections embedded in a water-soluble resin have become more popular in recent years (29,29a). Such sections provide better optical resolution and faster processing at somewhat higher cost.

Processing of Frozen Sections for Histologic Diagnosis and Histochemistry

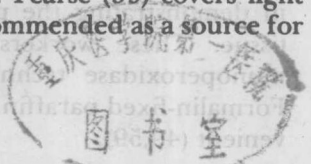
Rapid diagnosis continues to be the principal purpose of frozen section examination of liver specimens in the surgical pathology laboratory. However, frozen sections can be used for many other purposes, such as fat stains, immunohistochemistry, and enzyme histochemistry. Frozen tissue is also required for chemical investigations and virologic studies. It is, therefore, most desirable to deep-freeze part of any liver specimen other than needle biopsy specimens received in the surgical pathology laboratory, on a routine basis. Of course, this is possible only when unfixed specimens are sent to the surgical pathology laboratory. Specimens should be sent immediately from the operating room to the laboratory in a clean, preferably sterile, container.

Blocks of fresh, unfixed tissue can be frozen and cut immediately in a cryostat at 5–10 μm. For immunohistochemistry and enzyme histochemistry, better results are obtained if the tissue is quick-frozen in liquid nitrogen or in isopentane cooled with dry ice and cut immediately, or at most, within 48 hours. Sections are placed on glass slides, to which they generally adhere well. For some of the more complicated stains, we have found gelatin coating of slides useful. Gelatin acts as an adhesive and tends to prevent sections from becoming detached from the slides (30). Sections are stained with hematoxylin and eosin in the same way as other rush-frozen sections. We have noted that bile pigment is frequently better preserved in rush-frozen hematoxylin and eosin sections than in permanent paraffin sections. Glycogen also tends to be better preserved in frozen sections stained with the PAS reaction than in formalin-fixed paraffin sections stained by the same method.

For fat stains, formalin-fixed tissue is cut on a cryostat. Fixed sections adhere less well than do unfixed sections. Therefore, gelatin coating of glass slides before staining is recommended to prevent sections from getting detached during staining (30). Oil-red-O and Sudan IV are generally reliable. Positive and negative controls are particularly desirable with fat stains. Osmium tetroxide fixation also results in the staining of most lipids (30A). Such tissue can be paraffin

embedded, cut, and stained with hematoxylin and eosin without extraction of the lipids. If Reye's syndrome is suspected, it is recommended that frozen sections of unfixed tissue be stained with undiluted Giemsa stain for 30 seconds and be mounted in water (31).

Enzyme histochemistry, widely employed in the study of experimental liver injury, has, thus far, found relatively little application in diagnostic histopathology (32–34). However, this may change, particularly with respect to the identification of hepatic neoplasms, and perhaps of premalignant changes, as well as for diagnosis of certain storage diseases. Oxidative enzymes, such as succinic dehydrogenase, TPNH diaphorase, DPNH diaphorase, and glucose-6-dehydrogenase, are demonstrated by incubation with tetrazolium salts, such as nitro-BT or tetranitro-BT (35,36). Glucose-6-phosphatase is demonstrated on similar material by incubation with lead phosphate (35). For adenosine triphosphatase, cryostat sections are fixed in buffered neutral formalin. Alternatively, blocks fixed overnight in Baker's neutral formalin can be employed. Sections are stained by a modified Gomori lead salt method (35). For acid and alkaline phosphatase (33,34), simultaneous azo dye coupling methods using naphthol AS-TR and AS-BI, respectively, can be used after fixation of cryostat slides in acid-buffered acetone (37). Alternatively, a modified Gomori lead salt technique can be used on formalin-fixed cryostat sections or on sections obtained from blocks fixed overnight in Baker's neutral formalin (33,34). For electron histochemistry, the tissue is minced into small fragments and then briefly fixed in 2% buffered glutaraldehyde. After washing in buffer, the tissue is incubated with lead salts as described earlier for glucose-6-phosphatase, acid phosphatase, alkaline phosphatase, and ATPase. For details on light microscopic histochemistry, we have found Barka and Anderson's description (38) useful. Pearse (39) covers light microscopic histochemistry in greater detail and is recommended as a source for electron microscopic histochemical methods.



Processing for Immunohistology

Hepatitis B. Conventionally, demonstration of hepatitis antigens and antibodies in tissues requires the use of unfixed frozen sections and fluorescence microscopy. A study employing unfixed cryostat sections of rapidly frozen liver and indirect immunofluorescence for the Australia or hepatitis B surface (HB_sAg) and core (HB_cAg) antigens was that of Gudat et al. (40). Arnold et al. (41) used frozen sections and direct immunofluorescence to localize the hepatitis B e-antigen (HB_eAg). Another recent study (42) employed frozen sections and antibodies labeled with peroxidase. The hepatitis B antigens have been shown to be relatively resistant to routine fixatives and are preserved quite well in paraffin sections (43–45), clearly a great advantage in routine practice because tissue blocks can be easily stored, the size of the blocks can be larger, and there is no hazard of laboratory infection. Good histologic quality is easy to achieve, and retrospective studies are possible. Freezing equipment and procedures are not required. In paraffin sections fluorescence is almost as intense as in frozen sections, and localization of the antigen is more precise (45). Instead of labeling antibodies with fluorescein, they can be labeled with peroxidase, which does not require a fluorescent microscope. Of these two immunologic staining reac-

tions, some workers prefer the fluorescein tracer at the light microscopic level (43). Background fluorescence interfering with immunofluorescence can be reduced by digestion of the sections with pronase (43). However, peroxidase is rapidly becoming the method of choice (44, 46–48). Indirect immunostaining is generally preferred for the peroxidase method (49). Fixation in buffered formalin is generally adequate, if restricted to the time required for optimum fixation (49). There is little doubt that these immunologic methods are more sensitive and more specific in demonstrating the hepatitis B antigens than are Shikata's empirical methods (27). However, both free horseradish peroxidase and peroxidase conjugated to an antibody other than hepatitis B have an affinity for HB_sAg, which can give false-positive reactions for such antigens (50).

In chronic hepatitis B, intranuclear IgG capable of fixing complement in vitro has been demonstrated by direct immunofluorescence (51). This type of complement fixation appears to indicate a poor prognosis (52). Immunoglobulin G (IgG) has also been demonstrated by immunofluorescence on the cell membranes of hepatocytes isolated from biopsies of patients with chronic hepatitis (53–55). In demonstrating sections of immunoglobulin by the peroxidase method, Zenker's fixative or commercial B5 fixative (preferably at half-strength) may be preferable to buffered formalin (49).

Alpha-fetoprotein. Just as in the case of hepatitis B antigens, a variety of methods have been employed in demonstrating the presence of alpha-fetoprotein (AFP). Purtilo and Yunis (56) used fresh-frozen tissue sectioned at 5 μm and fixed in 95% ethanol. Indirect immunofluorescence was used. Husby et al. (57) used the same method, except for cold acetone fixation. Nayak et al. (58) were successful in demonstrating the presence of AFP in paraffin sections of ethanol-fixed tissue. These workers used both indirect immunofluorescence and immunoperoxidase techniques, preferring the immunoperoxidase technique. Formalin-fixed paraffin embedded tissue is adequate, and, therefore, most convenient (49,59).

Alpha-1-Antitrypsin. The PAS-positive droplets found in the livers of patients with alpha-1-antitrypsin deficiency have been shown to contain alpha-1-antitrypsin. As in the case of hepatitis B and of alpha-fetoprotein, the indirect immunoperoxidase method is rapidly becoming the method of choice in confirming the identity of these droplets (49,59,60).

PROCESSING FOR TRANSMISSION ELECTRON MICROSCOPY

Scanning electron microscopy was introduced into hepatology only very recently, in particular to study the bile canaliculi. However, its usefulness for diagnosis has not yet been established. It is most desirable to fix a part of every specimen for transmission electron microscopy as soon as the biopsy has been performed. It is important to use a new, or at least a very sharp, razor blade to prepare blocks of the appropriate size for electron microscopy. Blocks should be cubes approximately 1 mm in diameter modified. Karnovsky's fixative is widely