Biopsy Pathology of the Liver

R.S. Patrick & J.O'D. McGee

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R. S. PATRICK
M.D., F.R.C. Path.
Professor of Pathology, University of Glasgow and Honorary Consultant Pathologist, Royal Infirmary, Glasgow

and

J. O'D. McGEE
M.D., Ph.D., M.R.C. Path., M.A. (Oxon)
Professor of Morbid Anatomy, University of Oxford and Honorary Consultant Pathologist, John Radcliffe Hospital, Oxford

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Preface

With the growing awareness of the value of histopathology in the diagnosis of human hepatic disease, liver biopsy interpretation has become an important component of the work of most general hospital pathology departments. It follows that many histopathologists must now become proficient in the assessment of these specimens and this book has been written to assist them in this task. It is by no means a textbook of liver pathology, its purpose being the provision of illustrated descriptions of most human liver diseases which may be encountered in hospital practice. For those with limited experience of the subject it includes an early chapter on definitions and a terminal chapter which draws together some clinical and pathological correlations.

The great majority of photographs have been produced from our own collections of biopsies supplemented with a few autopsy specimens. We are indebted to our colleagues for their kindness in providing microscopic slides for the following illustrations: Figs 2.7, 12.47, 12.48 (Dr A. A. M. Gibson, Royal Hospital for Sick Children, Glasgow); Fig. 12.49 (Dr Jean Keeling, John Radcliffe Hospital, Oxford); Figs 4.20, 5.10, 5.13, 5.14, 6.18 (Dr F. D. Lee, Royal Infirmary, Glasgow); Figs 12.20, 12.21, 12.22 (Dr W. G. S. Spilg, Victoria Infirmary, Glasgow). We must record our thanks also to our secretaries, Mrs M. Thomson, Glasgow, and Mrs V. Macintosh, Oxford, for their work on the typescript. The photomicrographs were prepared by Mr T. Parker, University of Glasgow, Department of Pathology, Royal Infirmary, Glasgow, and by Dr T. Parry and Mr R. Holton, University of Oxford, Department of Pathology, John Radcliffe Hospital, Oxford.

Contents

1 Introduction

Liver biopsy plays a most important role in the investigation of human hepatic disease and is indeed necessary for the correct assessment of the majority of cases. Apart from its diagnostic value in individual patients it has contributed considerably to our knowledge of liver pathology in general and many recent advances in this subject have followed the introduction of the operation as a routine investigative procedure; there can be little doubt that future developments will continue to depend, to a large extent, on the study of fresh hepatic tissue obtained in this way.

Liver biopsy by itself may be sufficient for a final diagnosis although in many cases it is essential not to treat the result in isolation but to take into account the clinical findings and reports of laboratory tests of liver function. Thus, the detailed history of drug administration is often necessary in assessing cholestatic hepatitis, and the results of non-specific serum antibody tests can be helpful in differentiating some cases of primary biliary cirrhosis from chronic active hepatitis. Repetition of liver biopsy after a few weeks or months is sometimes justified, for example in the differential diagnosis between persistent viral hepatitis and chronic active hepatitis, and in assessing a particular form of therapy.

Aspiration needle biopsy is a safe procedure when undertaken by an experienced operator. It should, of course, be avoided when there is any risk of serious internal bleeding as in patients with a coagulation defect or Weil's disease, and also in obstructive jaundice with the possibility of biliary peritonitis following leakage from dilated ducts. In the latter instance fine needle transhepatic cholangiography is often sufficient for the recognition of conditions which require surgical intervention, and a wedge biopsy can always be obtained more safely during subsequent laparotomy.

With the introduction of needles of the 'Trucut' variety aspiration biopsy specimens are usually very adequate for assessing any diffuse hepatic condition. Fragmentation results from faulty technique, unusual friability of tissue such as tumour, and is also common in cirrhosis. Focal

lesions may, of course, be missed but it is often possible to direct the needle to particular targets revealed by radiological and isotope screening investigations or in combination with peritoneoscopy. At laparotomy, a wedge biopsy is usually taken and this can, with advantage, be supplemented at the time by single or multiple needle biopsies, as the subcapsular tissue in the former type of specimen may not be typical of the liver as a whole.

Light microscopy of paraffin sections prepared in conventional ways continues to be the main method for examination, while increasing use is being made of immunohistochemistry, electron-microscopy, microchemical analysis and other specialized techniques. These will be referred to only where they provide important additional information or are essential for diagnosis. Accordingly, there is little reference in this book to electron microscopy except where ultrastructural changes have diagnostic value.

1.1 Laboratory handling of biopsies

1.1.1 Record of gross appearance

This is rarely of much value and in most cases hardly necessary when the entire specimen will be processed for histological examination. Cirrhosis or tumour may be observed, especially in open wedge biopsy material. Some preliminary information can be obtained from the colour: pallor in severe fatty change (in extreme cases the tissue may float in fixative); green in cholestasis (especially after fixation); brown in haemochromatosis; slate grey in Dubin–Johnson syndrome.

The gross appearance of partial hepatectomy specimens is, by contrast, of considerable importance. Usually there is a tumour, when adequacy of resection, vascular invasion, encapsulation and changes in surrounding liver should all be noted, and blocks of tissue taken for the further investigations of these points. The gross appearance of the tumour itself may point to the correct diagnosis, such as bile staining in some hepatocellular adenomas or cancers and a central stellate scar in focal nodular hyperplasia.

1.1.2 Fixation for light microscopy

Kinks in needle biopsies are prevented by arranging the specimens on filter paper prior to fixation. If unusually long they may be cut into a few segments with a sharp razor and the parts laid out parallel to each other. Slices about 2 mm thick are prepared from wedge biopsies, cut at right angles to the capsule. All are fixed in 10 per cent neutral formalin for at

least six hours and preferably over-night, when they can be passed to paraffin wax in the usual way.

1.1.3 Staining

Six-micron sections should be stained routinely by haematoxylin and eosin, and for reticulin fibres and haemosiderin. A section stained by Masson's trichrome or Van Gieson's method is often useful in assessing the relationship between developing fibrous tissue and adjacent parenchyma. Periodic acid-Schiff-diastase sections may give prominence to macrophage activity which can be the only remaining evidence of recent liver injury, and would, incidentally, be the best method for revealing α–1 antitrypsin inclusions. It is useful to retain a few unstained sections for special investigations, e.g. the orcein or immunoperoxidase staining of hepatitis B surface antigen, rubeanic acid and rhodanine demonstration of copper, autoradiography for the detection of thorotrast, etc.

1.1.4 Serial sections

These are routinely indicated if the specimen is unusually small or if it is important to uncover some suspected small focal lesion, such as a granuloma in sarcoidosis or in primary biliary cirrhosis.

1.1.5 Frozen sections

Hepatocyte cytological detail which is so often important in diagnosis is frequently distorted or lost completely by freezing. Accordingly, it is wise to resist requests for rapid frozen sections of liver biopsies unless to confirm the presence of tumour or other gross lesion or unless, as with wedge biopsies, sufficient material is also available for the preparation of paraffin sections.

1.1.6 Fixation for electron microscopy

For the liver especially, meaningful electron microscopy depends on good fixation which can be achieved only by the pathologist or his technician being present at the operation, so that appropriate blocks are placed in chilled 2.5 per cent buffered glutaraldehyde as soon as possible, preferably within one minute of removal from the patient. The specimen should be laid on dental wax and a sharp scalpel or razor blade used to obtain small cubes not more than 1 mm thick, and preferably thinner than this, for rapid transfer to fixative. A very thin slice of tissue wedge can be placed immediately in a drop of fixative and then diced into tiny cubes.

Further embedding, cutting and staining are by conventional methods but if this is delayed it is preferable to store the tissue in cacodylate or phosphate buffer after one to three hours' fixation. It follows that the pathologist must be notified in advance of cases which might require electron microscopy to facilitate the diagnosis, for example in halothane hepatitis and certain metabolic disorders.

1.2 Normal liver morphology

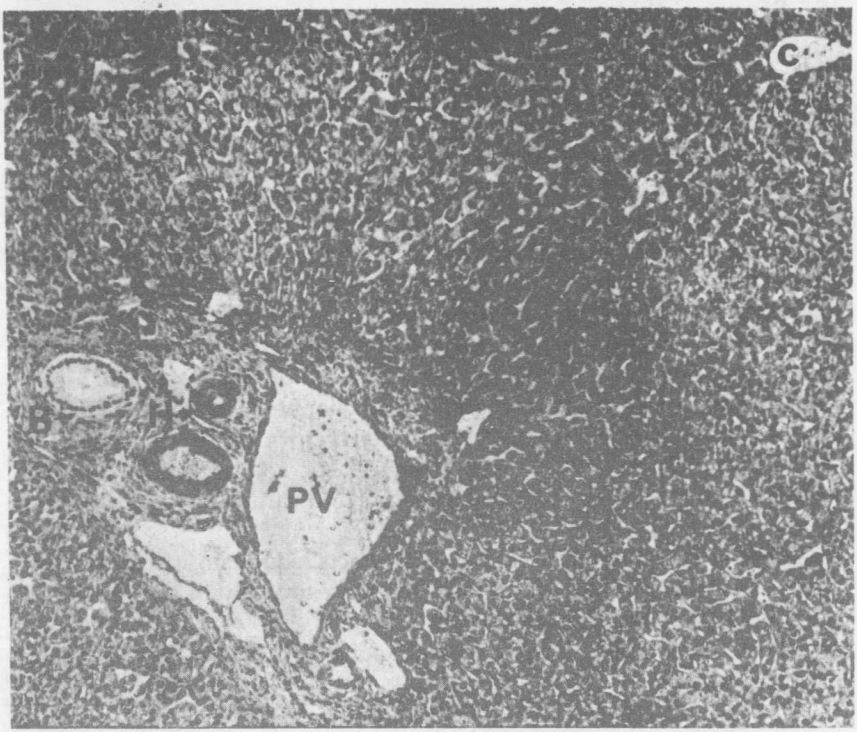
1.2.1 Microanatomy

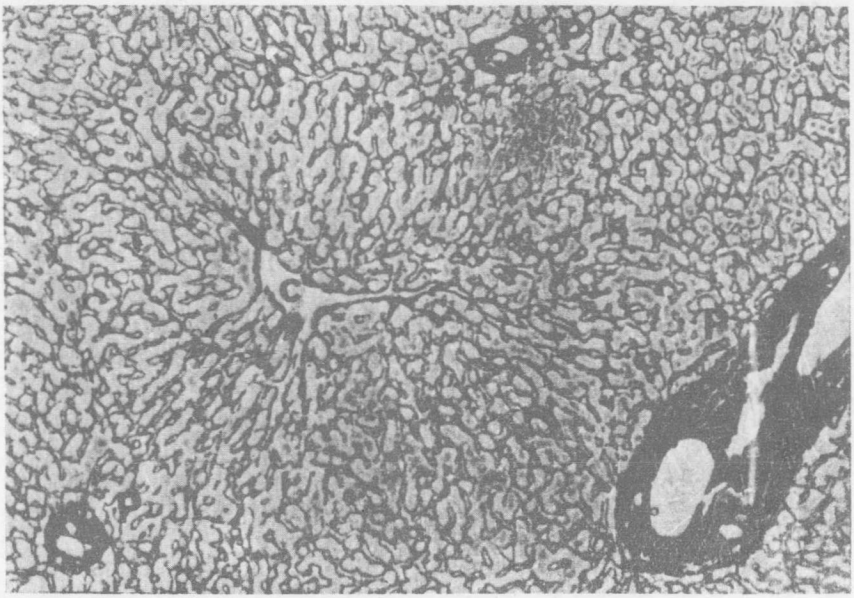
Judged by histochemical studies of normal hepatic parenchyma and the distribution of ischaemic and toxic liver damage, the acinus is the functional unit of the liver, each consisting of plates of hepatocytes radiating from portal tracts which contain terminal branches of the portal vein, hepatic artery and bile ducts. As seen in three dimensional reconstructions the peripheral part of the acinus interdigitates with its neighbours and is in contact with hepatic venous radicles. For the assessment of two dimensional sections it is more convenient to hold to the long established lobular concept of the liver by which each unit consists of cords of cells radiating from the central vein to peripheral portal tracts; it will be retained in this book as a basis for our histopathological descriptions (Fig. 1.1).

Each radiating plate consists of hepatocytes one cell thick and merges with similar limiting plates which border upon portal tracts and hepatic veins. Separating these plates are the liver sinusoids along which blood flows from portal tracts to central veins. Sinusoids are lined by cells which have indistinct cytoplasm and small elongated and darkly staining nuclei. Between this lining and the hepatocytes there is a space or potential space (the space of Disse) in which tissue fluid drains peripherally to lymphatics in the portal tracts. Disse's space also contains reticulin fibres which constitute the normal framework of the liver and scanty perisinusoidal cells (Ito cells; fat-storage cells) which are probably modified fibroblasts. The reticulin framework can be demonstrated readily by standard silver impregnation methods (Fig. 1.2) but a basement membrane is not present in the sinusoidal walls of normal human liver.

Between the adjacent surfaces of hepatocytes are tiny capillary chan-

Fig. 1.1 Normal liver showing centrilobular vein (C) and large portal tract with interlobular bile duct (B), hepatic artery branches (H) and portal vein (PV). Smaller branches of these entities are also present. Haematoxylin and eosin × 90.
Fig. 1.2 Reticulin framework of normal liver. There is a central vein (C) together with one large and two small portal tracts (P) situated peripherally. Reticulin stain × 90.


C
B
PV


C
P
P
P

nels or canaliculi into which bile is excreted and along which it flows peripherally towards the portal tracts. These channels are not readily recognized in paraffin sections unless distended in intrahepatic cholestasis, but are seen readily in one micron Epon preparations. They communicate with small acinar structures or ductules lined either partly by hepatocytes and partly by cuboidal (ductular) cells, or entirely by the latter type. These canals of Hering, which have no accompanying blood vessels, are very scanty in the liver lobules but may be seen adjacent to some portal tracts. They in turn drain into other more conspicuous interlobular ducts lying within the portal tracts and lined by cuboidal epithelium (Fig. 1.3). This epithelium rests on a distinct basement membrane. Septal bile ducts are still larger channels lined by columnar epithelium with basal nuclei which is often thrown into folds, and in the walls of which there is a distinct fibro-elastic coat (Fig. 1.4); smooth muscle fibres may be included especially towards the hilum of the liver.

The vascular components of the portal tracts require no special description. Small autonomic nerves may be seen especially in the septa and larger portal tracts close to the porta hepatis. They permeate into the smallest tracts and can be demonstrated also in the walls of hepatic venous radicles.

1.2.2 *Cytology*

Hepatocytes are polygonal cells, each with a clearly defined round nucleus containing one or more prominent nucleoli. Binucleate forms are not very uncommon but mitotic activity is rare. Some nuclei are larger than others indicating polyploidy, the normal cell being diploid. Hepatocyte cytoplasm is also distinct and stains readily with eosin. Indistinct basophilic granules may be seen, an appearance which is accentuated in sections treated with toluidine blue, thionin or Romanowsky stains. These granules represent aggregates of rough endoplasmic reticulum and disappear quickly in liver damage associated with dislocation of ribosomes from membranes. Scanty droplets of lipid especially in a few periportal hepatocytes are usually unimportant. Cytoplasmic glycogen is seen readily in appropriately stained sections of fresh tissue, particularly in the centrilobular zones. A fine dusting of brown pigment in centrilobular hepatocyte cytoplasm indicates lipofuscin in lysosomes but has little significance.

Compared with hepatocytes, bile ductular epithelium consists of much smaller cuboidal cells with scanty cytoplasm and distinct round nuclei.

Reference has already been made to sinusoidal and perisinusoidal cells

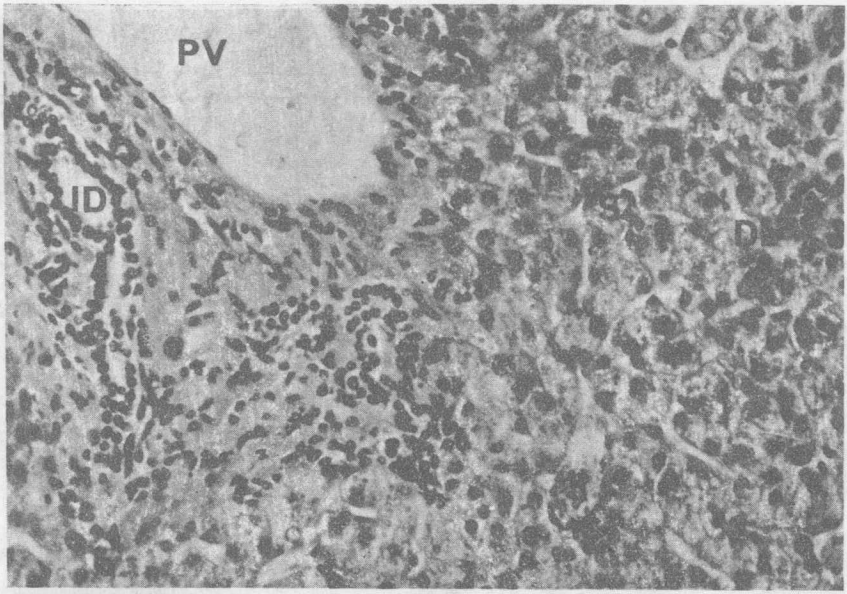


Fig. 1.3 Part of portal tract (top left) with portal vein (PV), interlobular bile duct (ID) and a number of small bile ductules at the periphery. Note the small round nuclei of ductular cells including those within the liver lobule (DL) and compare with the more elongated nuclei of sinusoidal lining cells (S). H. and E. × 280.

which are readily differentiated only in electron microscopic preparations. However, the perisinusoidal variety can be detected with the light microscope when there is accentuation of their lipid storage capacity (Fig. 1.5), as in hypervitaminosis A. Larger Kupffer cells are evident as scanty histiocytes on sinusoidal surfaces. Small numbers of similar macrophages may be seen in the stroma of otherwise normal portal tracts together with a few lymphocytes. Prominence of Kupffer cells is a common feature of hepatic injury and may occur also in generalized infections and other conditions associated with histiocytic activity unrelated to primary liver disease (Fig. 1.6).

1.2.3 *Normal histological variations*

In normal infant liver and as a regenerative phenomenon after partial hepatectomy or destruction by disease, the parenchymal plates may be several cells thick. In biopsies from elderly patients hepatocyte pleomorphism with hyperchromatic nuclei due to increased polyploidy may be conspicuous and should not be misinterpreted as regenerative

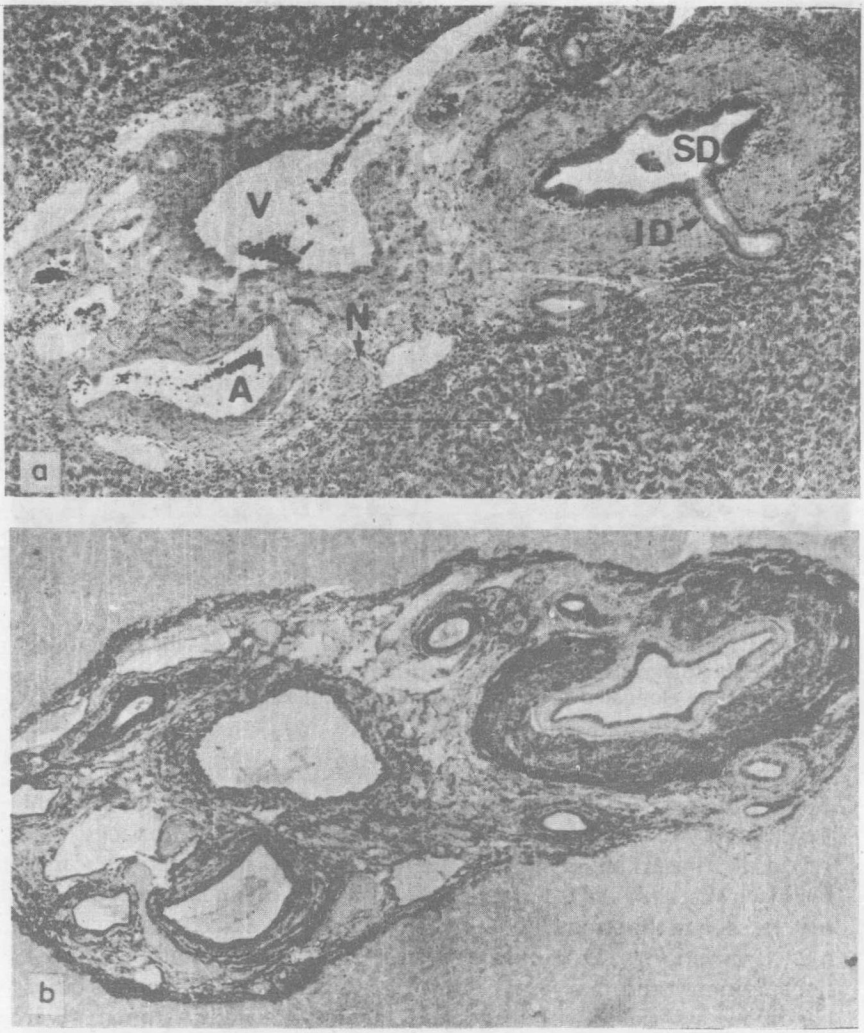
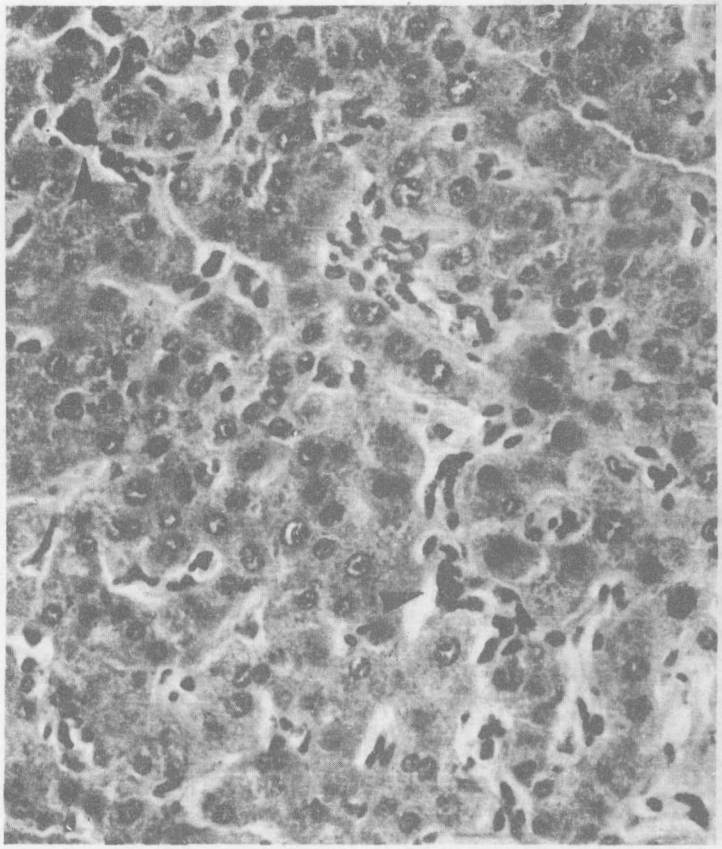
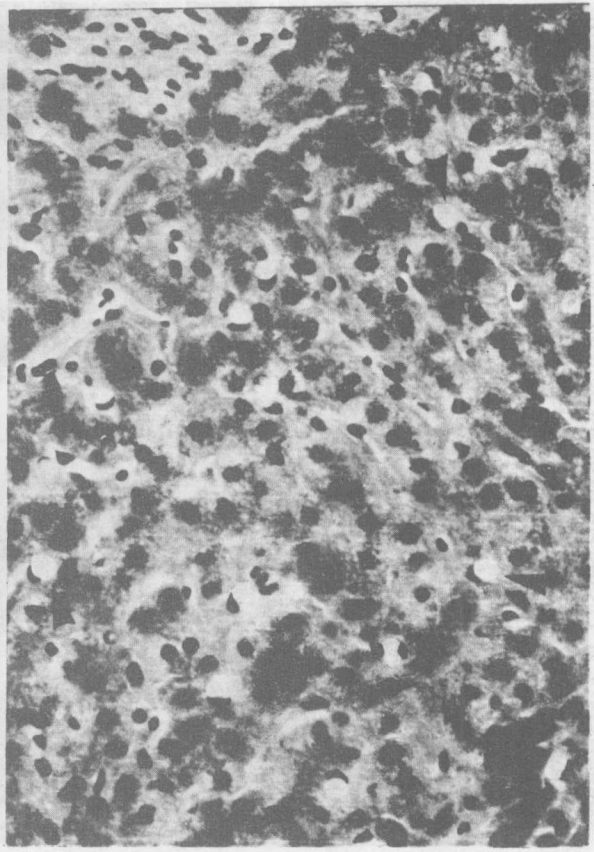


Fig. 1.4 (a) Hepatic septum containing nerve (N) and several blood vessels including a prominent hepatic artery (A) and portal vein (V). On the right an interlobular bile duct (ID) enters a much larger septal duct (SD). (b) Fibroelastic tissue is present in the wall of the septal duct but not in smaller interlobular ducts. (a) H. and E., (b) Orcein; both × 56.

Fig. 1.5 Unusual prominence of perisinusoidal cells which contain small droplets of fat (arrows). H. and E. × 375.

Fig. 1.6 Unusual prominence of Kupffer cells (arrows). From a case of coliform bacillary septicaemia. H. and E. × 450.





activity (Fig. 1.7). Lipofuscin pigment may also be excessive especially in elderly subjects (Fig. 2.18); this again is of little significance provided that Dubin–Johnson syndrome be kept in mind.

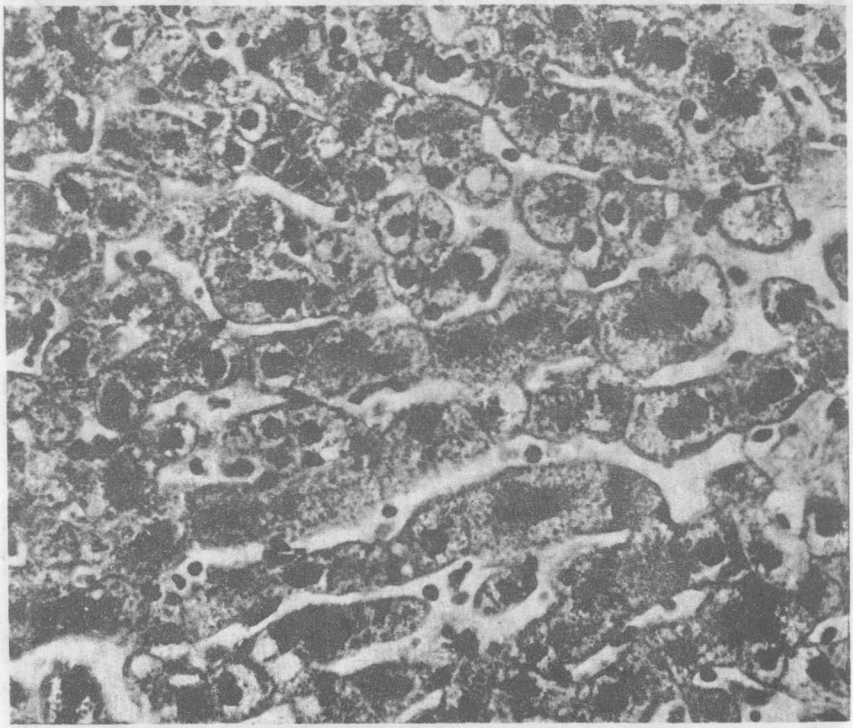


Fig. 1.7 Pleomorphic, hyperchromatic and binucleate hepatocytes in otherwise normal liver from an elderly patient. H. and E. × 450.

Golden-brown granules of haemosiderin pigment are abundant in the hepatocyte cytoplasm of infants by the first week of life (Fig. 1.8). This is gradually reduced in amount and should be absent in normal liver after six to nine months. In small amounts it is most conspicuous in periportal cells. It may reappear in association with high iron intake or excessive blood destruction. Foci of extramedullary haemopoiesis are a normal feature of the newborn infant liver which persists for a few weeks and which also may reappear in older patients associated with various blood disorders (Fig. 1.9). In the neonatal liver granulocyte differentiation occurs preferentially in portal tracts.

Strands of fibrous tissue may be seen in wedge biopsies linking