The uses of liver biopsy

I. D. ANSELL
M.B., B.Chir. (Cantab.)

LIVER biopsy may be performed at operation or peritoneoscopy, or by using one of the needle techniques. Operative biopsy allows assessment of the macroscopic appearance of the liver and the selection of any abnormal area; it is less liable to sampling error but the biopsies obtained often contain histological artefacts as a result of the anaesthetic and operative trauma. The livers of more than half the population have some degree of fibrosis around portal tracts immediately beneath the capsule (Petrelli & Scheuer, 1967) and in one-quarter there may be extension of fibrous tissue from portal tracts to central veins such that cirrhosis may be diagnosed by the unwary. However, these changes are superficial and inspection of the deeper, less fibrous tissue will enable the correct interpretation to be made. Surgeons should, nevertheless, be requested to take biopsies from the surface of the liver rather than the inferior edge. Peritoneoscopy combined with needle biopsy, like operative biopsy, allows visualization of the surface of the liver and biopsy of selected sites (Caroli & Ricardeau, 1961). The Menghini aspiration is the most widely used of the needle techniques (Menghini, 1958) as it usually produces an adequate sample for histological appraisal; it may produce a fragmented biopsy in patients with cirrhosis and in such cases the Vim-Silverman needle, which has a cutting edge, is to be preferred.

Culture of part of the biopsy may yield valuable information in cases such as suspected tuberculosis, brucellosis, histoplasmosis, and in suspected cholangitis or abscess, but this must be considered before all the biopsy is placed into fixative. The causative organism may occasionally be demonstrated in the fixed biopsy by the use of such special staining techniques as Ziehl-Neelson for tubercle bacilli and PAS (periodic acid-Schiff) for such fungi as *Histoplasma capsulatum*.

The mortality of needle liver biopsy in two series, each of over 20,000 cases, has been reported as 0.17% (Zamcheck & Klausenstock, 1953) and 0.017% (Thaler, 1964), the lower figure being associated with the exclusive use of the Menghini technique. Complications of biopsy include transient pleurisy and perihepatitis, haemorrhage, biliary peritonitis and puncture of adjacent organs; but it

must be stressed that in experienced hands such complications are extremely uncommon.

The main indications can be grouped under the following headings:

1. Cirrhosis

There have been many definitions of cirrhosis in the past but that employed now is that it is a disease affecting the whole of the liver, although not necessarily affecting every lobule, in which cell necrosis occurs at some time with resulting fibrosis and nodular regeneration. It is the fibrosis and especially the nodule formation which by pressure on the branches of the portal vein leads to portal hypertension and ascites and it is these two histological features on which the pathological diagnosis of the condition is made. It must be distinguished from:

(a) Congenital hepatic fibrosis, in which there is much fibrosis but no nodule formation (Kerr *et al.*, 1961), and which has a characteristic appearance (Fig. 1). The fibrous tissue and scarcity of portal vein radicles may produce portal hypertension and cases have been described in which cirrhosis has developed (Williams, Scheuer & Heard, 1964).

(b) Partial nodular transformation (Sherlock *et al.*, 1966) in which areas of the liver show nodule formation but no fibrosis. Such nodules may produce portal hypertension if they obstruct large branches of the portal vein.

Older ideas of the classification of cirrhosis have recently been reassessed in the light of the knowledge that many aetiological factors may give the same pathological picture (Popper *et al.*, 1960). Cirrhosis is now classified according to the size of the regeneration nodules. In macronodular cirrhosis corresponding to the older post-necrotic, cryptogenic or post-hepatic cirrhosis the nodules are large and of variable size with wide bands of dense fibrous tissue separating them. Micronodular cirrhosis in contrast, corresponding to the older portal or Laennec's cirrhosis, is composed of uniform small nodules, and is that often seen in alcoholic cirrhosis. Pictures intermediate between these two are often seen. It is thought that in micronodular cirrhosis the factors damaging the liver are still active and prevent the growth of the nodules beyond a certain size. Release

FIG. 1. Congenital hepatic fibrosis. Note the dense fibrous tissue containing many bile ducts but few branches of the portal vein. H & E, $\times 100$.

FIG. 2. Active chronic hepatitis showing ballooned cells forming rosettes. H & E, $\times 135$.

from such restraining influences may be responsible for the higher incidence of hepato-cellular carcinoma in those alcoholics who have stopped drinking and developed a macronodular cirrhosis than in those who continue to drink and retain a micronodular cirrhosis, 55% and 15%, respectively, in one series (Lee, 1966). Stone, Islam & Paton (1968) using a clinical classification of cirrhosis found a rate of hepatocellular carcinoma complicating 33% of alcoholic cirrhotics and 12% of cryptogenic cirrhotics.

Biopsy is useful to confirm the diagnosis especially in those cases in which the clinical signs are not obvious. Although cirrhosis is by definition a diffuse disease an apparently normal histological picture may be seen if needle biopsy obtains tissue from a large nodule only in a case of macronodular cirrhosis; such cases may require operative biopsy to establish the diagnosis. Sampling errors in needle biopsies may also lead to difficulties when attempting to assess the effect of treatment by the examination of sequential biopsies.

A final group includes those fibrotic conditions of the liver which only lead to a true cirrhosis with nodule formation late in the natural history of the disease, as in secondary biliary cirrhosis and cardiac cirrhosis.

In some types of cirrhosis autoimmune mechanisms are thought to perpetuate the hepato-cellular damage. This is seen at its most florid in active chronic hepatitis (Read, Sherlock & Harrison, 1963) which has also been labelled juvenile cirrhosis and lupoid hepatitis, a positive LE cell test being found in approximately 15% of cases. Patients with this condition have evidence of probable autoimmune

disease with a raised ESR, positive antinuclear factor, antibodies to smooth muscle and raised serum γ -globulins. There is also evidence of cell destruction with raised serum transaminase levels. A recent computer-analysis of this syndrome (Goldstein & Mackay, 1967) has defined an earlier hepatitis-like illness which progresses to a true cirrhosis. The histological features of this condition are marked swelling of liver cells which are grouped together to form 'rosettes' and a dense infiltrate of both portal tracts and liver parenchyma by lymphocytes and plasma cells (Fig. 2).

Specific histological features

Biopsy may also yield information as to the aetiology of the cirrhosis. In alcoholic cirrhosis fatty infiltration is almost invariably present. Mallory's alcoholic hyaline, amorphous eosinophilic material in the cytoplasm of the hepatocytes, is often seen during the acute phase, although this is not specific for alcoholism, occurring occasionally in Wilson's disease and Indian childhood cirrhosis. Focal toxic necrosis of hepatic cells associated with collections of neutrophil polymorphs is often seen in those who have recently taken excess alcohol, giving the picture of acute alcoholic hepatitis (Leiber, 1965) (Fig. 3).

Haemochromatosis is diagnosed by finding cirrhosis with a Grade 4 deposition of iron within hepatocytes (Scheuer, Williams & Muir, 1962). The distinction between haemochromatosis and secondary siderosis in alcoholic cirrhosis may present difficulties and require other investigations (Williams, 1967). In cirrhosis in children and young adults galactosaemia is suggested by diffuse fatty changes (Smetana & Olen, 1962), fibrocystic disease by

FIG. 3. Alcoholic cirrhosis and hepatitis showing fat and foci of neutrophil polymorphs. H & E, $\times 165$.

FIG. 4. Large duct obstruction showing oedema of portal tract, neutrophil polymorph infiltrate and bile duct reduplication. H & E, $\times 135$.

finding dilated bile ducts containing amorphous eosinophilic material (Bodian, 1952) and Wilson's disease by the presence of nuclear vacuolation and ballooning of the periphery of the hepatic cells with fat (Sass-Kortsak, 1965).

In a large percentage (50% in Great Britain) of cases, however, no indication of aetiology is seen or obtained from the history. Such cases are labelled cryptogenic and show the features of a macronodular cirrhosis.

Primary biliary cirrhosis can sometimes only be diagnosed by operative wedge biopsy as the diagnostic lymphoid follicles situated around the damaged larger interlobular bile ducts (Scheuer, 1967) may not be sampled in a needle biopsy. In the late stages of the disease the picture is that of a cirrhosis indistinguishable from any other mixed or macronodular cirrhosis.

2. Jaundice

In jaundice liver biopsy is indicated in only a proportion of cases, as the clinical history and biochemical observations will usually suffice to make a diagnosis. Especial vigilance is needed in patients with extrahepatic obstructive jaundice as complications such as biliary peritonitis following leakage from a dilated bile duct, or haemorrhage as a result of an increased prothrombin time may occur. Biopsy should never be performed on patients whose prothrombin time is more than two seconds in excess of that of the normal control after the administration of parenteral vitamin K. Such reversion only occurs in those patients whose jaundice is obstructive rather than hepato-cellular in origin.

Cholestasis has been defined as the syndrome associated with failure of bile to reach the duodenum

(Sherlock, 1968). The most usual indication for biopsy is in those cases where there is disagreement between the laboratory and clinical findings in order to differentiate between extra-hepatic cholestasis resulting from carcinoma, stone or stricture, and intra-hepatic cholestasis due usually to either drugs or viral hepatitis (Williams, 1968).

Obstructive jaundice

In large duct obstruction features of the ascending cholangitis secondary to the blockage are seen with oedema and neutrophil polymorph infiltration of the portal tracts (Fig. 4); bile duct reduplication is often present to some degree also. Bile lakes (Fig. 5), often also named bile infarcts or bile necroses, resulting from the toxic effect of retained bile on liver cells are diagnostic of this condition, but unfortunately are only seen in a small number of cases.

Hepatocellular jaundice

The actions of drugs on the liver and the variable functional and morphological effects that they produce have to date defied comprehensive classification. The C_{17} alkyl-substituted steroids such as methyl testosterone produce cholestasis only, with plugging of the biliary canaliculi in the majority of patients if sufficient of the drug is taken. The other drugs likely to lead to problems in diagnosis and in which biopsy may be performed are those which affect the liver by a hypersensitivity reaction. This may be apparent clinically by the presence of arthralgia or a skin rash. The phenothiazines, such as chlorpromazine, give cholestasis and a mononuclear infiltrate of the portal tracts whilst the monoamine-oxidase inhibitors, such as phenelzine,

FIG. 5. Bile lake. H & E, $\times 150$.

FIG. 6. Granuloma with central Langhans' giant cell. H & E, $\times 150$.

produce a hepatitis which may be indistinguishable both clinically and pathologically from viral hepatitis. Occasionally in cholestasis due to hypersensitivity reactions an excess of eosinophils may be seen in the liver biopsy.

In viral hepatitis focal necrosis of hepatocytes is present which produces condensation of the reticulin framework of the liver in the areas of necrosis. The hepatocytes themselves show degenerative changes with swelling of their cytoplasm, which may also contain areas of dense eosinophilic material. With complete necrosis of the hepatocyte the cell is extruded as a dense rounded mass into the sinusoid as the so-called eosinophil body. Another constant feature is the presence of a mononuclear cell infiltrate of the portal tracts. Following viral hepatitis histological abnormalities may persist for up to 1 year and this should always be borne in mind when following up patients with previous viral hepatitis and positive flocculation tests as the final demonstration of a normal liver will prevent the production of unnecessarily medically-induced invalidism.

Other forms of jaundice

Biopsy is also indicated in cases of persistent low-grade jaundice such as may occur in the congenital hyperbilirubinaemias, Gilbert's disease and the Dubin-Johnson syndrome. In the latter condition macroscopic examination of the biopsy will suggest the diagnosis as the liver is black, the responsible pigment being histochemically indistinguishable from the 'wear-and-tear' pigment lipofuscin although being present in larger amounts and in larger granules.

3. Systemic disease

Liver biopsy is often useful in cases of pyrexia of unknown origin and in this way the systemic

granulomata may be detected. They are recognized by a central area of Langhans' giant cells surrounded by epithelioid macrophages and lymphocytes (Fig. 6). Such granulomata can be produced by a large number of conditions, such as miliary tuberculosis, sarcoidosis, brucellosis, berylliosis, tularaemia and histoplasmosis. It is usually impossible to distinguish one from another, unless special stains or culture methods reveal the causative organism as mentioned earlier.

4. Hepatomegaly

The malignant lymphomas such as Hodgkin's disease, lymphosarcoma and reticulum-cell sarcoma may present with hepatomegaly or unexplained jaundice only and biopsy may obtain diagnostic histological material.

Metastatic carcinoma of the liver is detected in 78% of cases in which it enters the differential diagnosis (Fenster & Klatskin, 1961) and in this way an unnecessary laparotomy may be prevented. The success rate is improved by biopsy of such selected sites as palpable nodules or cold areas seen on liver scan which may indicate a subcostal approach rather than the conventional lateral. In negative cases a non-specific reactive hepatitis may be seen with oedema and inflammatory cell infiltrate of portal tracts, these changes being attributed to blockage of larger interlobular bile ducts by a nearby space-occupying lesion.

Biopsy in a number of other diseases with hepatomegaly may yield diagnostic material. In Gaucher's disease and Niemann-Pick disease the characteristic cells laden with kersin and sphingomyelin, respectively, are seen. Amyloid can often be identified on needle biopsy (either within the sinusoids or around blood vessels) and in Kala-Azar, Leishman-Donovan

bodies are seen within the Kupffer cells. Needling of an enlarged liver suspected of containing an abscess has been performed for many years and in this way confirmation of the diagnosis of pyogenic or amoebic abscess can be made. It should be noted that needle biopsy is contraindicated if hydatid disease is suspected as fatal anaphylaxis may result from dissemination of cyst fluid into the peritoneal cavity.

Pre-symptomatic diagnosis

Liver biopsy has also been used in the pre-symptomatic diagnosis of Wilson's disease (Levi *et al.*, 1967) and haemochromatosis (Scheuer *et al.*, 1962), an increased copper content being found in the former and increased iron storage in the latter. Since both diseases are thought to be inherited it has been argued that siblings of patients with either disease should be biopsied, so that treatment can be commenced before irreparable damage has occurred. A recent paper on the assessment of the treatment of pre-symptomatic Wilson's disease (Sternleib & Schneiberg, 1968) uses the histories of previous patients from their clinic as controls. This lack of paired controls has resulted in the criticism that the validity of the diagnostic criteria employed remains unproven. This highlights the dangers of misdiagnosis in a condition which requires lifelong therapy with penicillamine, a drug of not inconsequential side-effects and which costs approximately £125 per year. It must be difficult, however, for a clinician to deny treatment to children they consider to have Wilson's disease, who will become mentally retarded and cirrhotic if the disease does develop.

Electron microscopy

The advent of the electron microscope in the study of human liver biopsy material might have been thought to result in increased diagnostic acumen as it has in the field of renal disease. This has not proved to be so both for intrinsic reasons and because of the enormous sampling error. In glycogenosis type 2 and in Hurler's syndrome electron microscopy may help in diagnosis (Popper, 1967) but it is of little value in other conditions. Electron microscopy has resulted in a better understanding of the manner in which hepatocytes are damaged (Schaffner & Popper, 1967), and changes seen reflect the severity and stage of the disease process. Viral hepatitis produces disruption of the endoplasmic reticulum initially whilst in alcoholic hepatitis it is the mitochondria which first show signs of damage. Virus particles have not yet been confidently demonstrated in biopsies from patients with viral hepatitis. With electron microscopy both extra-hepatic and intra-hepatic cholestasis are seen

to have the same defect in the intra-cellular biliary excretory apparatus with damage to microvilli lining the canaliculi and changes in the cellular organelles nearby. In primary biliary cirrhosis there are no changes confirming the opinion gained by light microscopy that cholestasis in this condition results from regurgitation of bile into the blood stream through damaged bile ducts.

Acknowledgments

I should like to thank Mr Peter Kirby for his help in the preparation of the photomicrographs and both Mrs J. M. Johnson and Mrs L. E. Bailey for typing the manuscript.

References

- BODIAN, M. (1952) *Fibrocystic Disease of the Pancreas*, p. 104. Heinemann, London.
- CAROLI, J. & RICARDEAU, P. (1961) Value of peritoneoscopy and peritoneoscopic photography in colour and of scintillography in the diagnosis of liver disease. *Progress in Liver Diseases* (Ed. by H. Popper and F. Schaffner), Vol. I. Heinemann, London.
- FENSTER, L.F. & KLATSKIN, G. (1961) Manifestations of metastatic tumours of liver. *Amer. J. Med.* **31**, 238.
- GOLDSTEIN, G. & MACKAY, I.R. (1967) Lupoid hepatitis: Computer analysis defining 'hepatitis' and 'cirrhosis' phases and relationship between hepatocellular damage and immune reactions in the liver. *Aust. Ann. Med.* **16**, 62.
- KERR, D.N.S., HARRISON, C.V., SHERLOCK, S. & WALKER, R.M. (1961) Congenital hepatic fibrosis. *Quart. J. Med.* **n.s.** **30**, 91.
- LEE, F.I. (1966) Cirrhosis and hepatoma in alcoholics. *Gut* **7**, 77.
- LEIBER, C.S. (1965) Alcohol and the liver. *Progress in Liver Diseases* (Ed. by H. Popper and F. Schaffner), Vol. II, p. 134. Heinemann, London.
- LEVI, A.J., SHERLOCK, S., SCHEUER, P.J. & CUMMINGS, J.N. (1967) Presymptomatic Wilson's disease. *Lancet*, **ii**, 575.
- MENGHINI, G. (1958) One second needle biopsy of the liver. *Gastroenterology*, **35**, 190.
- PETRELLI, M. & SCHEUER, P.J. (1967) Variations in sub-capsular liver structure. *J. clin. Path.* **20**, 743.
- POPPER, H. (1967) Electron Microscopy in the diagnosis of liver disease. *The Liver* (Ed. by A. E. Read), p. 47. Butterworth's, London.
- POPPER, H., RUBIN, E., KRUS, S. & SCHAFFNER, F. (1960) Postnecrotic cirrhosis in alcoholics. *Gastroenterology*, **39**, 669.
- READ, A.E., SHERLOCK, S. & HARRISON, C.V. (1963) Active 'juvenile' cirrhosis considered as part of a systemic disease and the effect of corticosteroid therapy. *Gut*, **4**, 378.
- SASS-KORTSAK, A. (1965) Copper metabolism. *Advanc. clin. Chem.* **8**, 1.
- SCHAFFNER, F. & POPPER, H. (1967) Electron microscopy of human liver disease. *Modern Trends in Pathology* (Ed. by T. Crawford), p. 252. Butterworth's, London.
- SCHEUER, P.J. (1967) Primary biliary cirrhosis. *Proc. roy. Soc. Med.* **60**, 1257.
- SCHEUER, P.J., WILLIAMS, R. & MUIR, A.R. (1962) Hepatic pathology in relatives of patients with haemochromatosis. *J. Path. Bact.* **84**, 53.
- SHERLOCK, S. (1968) *Diseases of the Liver and Biliary System*, 4th edn, p. 277. Blackwell Scientific Publications, Oxford.
- SHERLOCK, S., FELDMAN, C.A., MORAN, B. & SCHEUER, P.J. (1966) Partial nodular transformation of the liver with portal hypertension. *Amer. J. Med.* **50**, 195.
- SMETANA, H.F. & OLEN, E. (1962) Hereditary galactose disease. *Amer. J. clin. Path.* **38**, 3.

- STERNLEIB, I. & SCHNEIBERG, I.H. (1968) Prevention of Wilson's disease in asymptomatic patients. *New Engl. J. Med.* **278**, 352.
- STONE, W.D., ISLAM, N.K.R. & PATON, A. (1968) The natural history of cirrhosis. *Quart. J. Med.* n.s. **37**, 119.
- THALER, H. (1964) Ueber vorteil und Risiko der leberbiopsie methode nach Menghini. *Wien. Klin. Wchschr.* **29**, 533.
- WILLIAMS, R. (1967) Cirrhosis with secondary siderosis or primary haemochromatosis? *Proc. roy Soc. Med.* **60**, 1255.
- WILLIAMS, R. (1968) Jaundice. *Advances in Medicine*. Pittman, London.
- WILLIAMS, R., SCHEUER, P.J. & HEARD, B.E. (1964) Congenital hepatic fibrosis with an unusual pulmonary lesion. *J. clin. Path.* **17**, 135.
- ZAMCHECK, N. & KLAUSENSTOCK, O. (1953) Liver biopsy. II. The risk of needle biopsy. *New Engl. J. Med.* **249**, 1062.