This chapter looks at the chemical pathology of liver and gall bladder disorders. These are common in clinical practice, and liver function tests constitute one of the most frequently requested clinical biochemistry laboratory profiles.

FUNCTIONS OF THE LIVER

The liver has essential synthetic and excretory functions and can be thought of as a large ‘metabolic factory’. It also detoxifies and, like the kidneys, excretes the end products of metabolism. The main blood supply to the liver is via the portal vein. The liver is made up of hexagonal lobules of cells (Fig. 17.1). Rows of hepatocytes radiate from the central hepatic vein and are separated by sinusoidal spaces, along the walls of which are interspersed hepatic macrophages, the Kupffer cells. These phagocytic cells are part of the reticuloendothelial system and have an important detoxifying function. At the corners of each lobule are the portal tracts that contain branches of the hepatic artery, the portal vein and bile ducts. Blood flows from the portal tracts towards the central hepatic vein. Therefore:

- Hypoxia and toxins that are metabolized in the liver cause damage to the centrilobular area first.
- Toxins that do not depend on hepatic metabolism primarily affect the periphery of the lobule.

Almost all nutrients from the gastrointestinal tract pass through the sinusoidal spaces prior to entering the systemic circulation. The hepatic architecture may be disturbed in cirrhosis (fibrosis).

General metabolic functions

When the glucose concentration is high in the portal vein, it is converted to glycogen and the carbon skeletons of fatty acids, which are transported to adipose tissue as very low-density lipoprotein (VLDL). During fasting, the systemic plasma glucose concentration is maintained by the breakdown of glycogen (glycogenolysis) or by the synthesis of glucose from substrates such as glycerol, lactate and amino acids (gluconeogenesis). Fatty acids reaching the liver from fat stores may be metabolized in the tricarboxylic acid cycle, converted to ketones or incorporated into triglycerides (see Chapter 13).

Synthetic functions

Hepatocytes synthesize:

- plasma proteins, excluding immunoglobulins and complement,
- most coagulation factors, including fibrinogen and factors II (prothrombin), V, VII, IX, X, XI, XII and XIII – of these, prothrombin (II) and factors VII, IX and X cannot be synthesized without vitamin K,
- primary bile acids,
- the lipoproteins, such as VLDL and high-density lipoprotein (HDL) (see Chapter 13).
The liver has a very large functional reserve. Deficiencies in synthetic function can be detected only if liver disease is extensive. Before a fall in plasma albumin concentration is attributed to advanced liver disease, extrahepatic causes must be excluded, such as the loss of protein through the kidney, gut or skin, or across capillary membranes into the interstitial space, as in even mild inflammation or infection (see Chapter 19).

Prothrombin levels, assessed by measuring the prothrombin time, may be reduced because of impaired hepatic synthesis, whether due to failure to absorb vitamin K or to hepatocellular damage. If hepatocellular function is adequate, parenteral administration of vitamin K may reverse the abnormality.

Excretion and detoxification

The excretion of bilirubin is considered in more detail below. Other substances that are inactivated and excreted by the liver include the following:

- **Cholesterol** – excreted in the bile either unchanged or after conversion to bile acids.
- **Amino acids** – which are deaminated in the liver. Amino groups, and the ammonia produced by intestinal bacterial action and absorbed into the portal vein, are converted to urea.
- **Steroid hormones** – which are metabolized and inactivated by conjugation with glucuronate and sulphate and excreted in the urine in these water-soluble forms.
- **Many drugs** – which are metabolized and inactivated by enzymes of the endoplasmic reticulum system; some are excreted in the bile.
- **Toxins** – the reticuloendothelial Kupffer cells in the hepatic sinusoids are well placed to extract toxic substances that have been absorbed from the gastrointestinal tract.

Efficient excretion of the end products of metabolism and of bilirubin depends on:

- normally functioning liver cells,
- normal blood flow through the liver,
- patent biliary ducts.

**Formation and excretion of bilirubin (Fig. 17.2)**

At the end of their lifespan, red blood cells are broken down by the reticuloendothelial system, mainly in the spleen. The released haemoglobin is split into globin, which enters the general protein pool, and haem, which is converted to bilirubin after the removal of iron, which is reused (see Chapter 21).

About 80 per cent of bilirubin is derived from haem within the reticuloendothelial system. Other sources include the breakdown of immature red cells in the bone marrow and of compounds chemically related to haemoglobin, such as myoglobin and the cytochromes. Less than 300 µmol of bilirubin is produced daily from the breakdown of erythrocytes, while the normal liver is able to conjugate up to about 1 mmol/day, and
therefore hyperbilirubinaemia is an insensitive index of parenchymal hepatic disease.

Bilirubin is normally transported to the liver bound to albumin. In this form it is called unconjugated bilirubin, which is lipid soluble and therefore, if not protein bound, can cross cell membranes, including those forming the blood–brain barrier. In this form it is potentially toxic; however, at physiological concentrations it is all protein bound.

In the adult, about 300 µmol per day of bilirubin reaches the liver, where it is transferred from plasma albumin, through the permeable vascular sinusoidal membrane. The hepatocytes can process a much greater load than this. Bilirubin is bound to ligandin (Y protein). From there it is actively transported to the smooth endoplasmic reticulum, where it is conjugated with glucuronate by a process catalysed by uridine diphosphate glucuronyl transferase.

Bilirubin monoglucuronide passes to the canalicular surfaces of the hepatocytes, where, after the addition of a second glucuronate molecule, it is secreted by active processes into the bile canaliculi. This process is largely dependent on the active secretion of bile acids from hepatocytes. These energy-dependent steps are the ones most likely to be impaired by liver damage (hypoxia and sepsicaemia) and by increased pressure in the biliary tract. Other anions, including drugs, may compete for binding to ligandin, thus impairing bilirubin conjugation and excretion. Novobiocin inhibits glucuronyl transferase, thus exacerbating unconjugated hyperbilirubinaemia. Bilirubin is often assayed by the Van den Bergh reaction, which allows conjugated (direct-reacting) and unconjugated (indirect-reacting) bilirubin to be distinguished.

Bilirubin metabolism and jaundice

Jaundice usually becomes clinically apparent when the plasma bilirubin concentration reaches about 50 µmol/L (hyperbilirubinaemia), about twice the upper reference limit. It occurs when bilirubin production exceeds the hepatic capacity to excrete it. This may be because:

- An increased rate of bilirubin production exceeds normal excretory capacity of the liver (prehepatic jaundice).
- The normal load of bilirubin cannot be conjugated and/or excreted by damaged liver cells (hepatic jaundice).
- The biliary flow is obstructed, so that conjugated bilirubin cannot be excreted into the intestine and is regurgitated into the systemic circulation (post-hepatic jaundice).

Retention of bilirubin in plasma: jaundice

Unconjugated hyperbilirubinaemia occurs if there is:

- a marked increase in the bilirubin load as a result of haemolysis, or of the breakdown of large amounts of blood after haemorrhage into the gastrointestinal tract or, for example, under the skin due to extensive bruising; in cases of haemolysis, plasma bilirubin rarely exceeds 75 µmol/L,
- impaired binding of bilirubin to ligandin or impaired conjugation with glucuronate in the liver.

In some pathological conditions, plasma unconjugated bilirubin levels may increase so much that they exceed the protein-binding capacity. The lipid-soluble, unbound bilirubin damages brain cells (kernicterus). This is most likely to occur in newborn, particularly premature, infants in whom the hepatic conjugating mechanisms are immature. In addition, the proportion of unbound, unconjugated bilirubin, and therefore the risk of cerebral damage, increases if:

- plasma albumin concentration is low,
- unconjugated bilirubin is displaced from binding sites, for example by high levels of free fatty acids or drugs such as salicylates or sulphonamides.

Unconjugated bilirubin is normally all protein bound and is not water soluble and therefore cannot be excreted in the urine. Patients with unconjugated hyperbilirubinaemia do not have bilirubinuria (‘acholuric jaundice’) such as Gilbert’s syndrome. Conjugated bilirubinaemia is one of the earliest signs of impaired hepatic excretion. In most cases of jaundice in adults, both conjugated and unconjugated fractions of bilirubin are increased in plasma but conjugated bilirubin predominates. Conjugated bilirubin is water soluble and is less strongly protein bound than the unconjugated form, and therefore can be excreted in the urine. Bilirubinuria is always pathological. Dark urine may be an early sign of some forms of hepatobiliary disease.

Conjugated bilirubin enters the gut lumen in bile; it is broken down by bacteria in the distal ileum and the colon into a group of products known as stercobilinogen (faecal urobilinogen). Some is absorbed into the portal circulation, most of which is re-excreted in bile (enterohepatic circulation). A small amount enters the systemic circulation and is excreted in the urine as urobilinogen, which can be oxidized to a coloured pigment, urobilin.
Urobilinogen
Urobilinogen, unlike bilirubin, is often detectable in the urine of normal people by testing with commercial strip tests, particularly if the urine, and therefore the urobilinogen, is concentrated. Urinary urobilinogen concentration is increased in the following situations.

- When haemolysis is very severe: large amounts of bilirubin enter the intestinal lumen and are converted to stercobilinogen. An increased amount of urobilinogen is formed and absorbed. If the hepatic capacity to re-secrete it is exceeded, it is passed in the urine.
- When liver damage impairs re-excretion of normal amounts of urobilinogen into the bile.

The colourless, unabsorbed stercobilinogen is oxidized to stercobilin, a pigment that contributes to the brown colour of faeces. Pale stools may, therefore, suggest biliary obstruction associated with an absence of urinary urobilinogen.

BIOCHEMICAL TESTS FOR LIVER DISEASE
Several biochemical tests constitute what are called the 'liver function tests'. Different tests can give different information about hepatic dysfunction.

Hepatocyte damage
Strictly speaking, changes in plasma enzyme activity generally indicate liver cell membrane damage rather than hepatic function capacity. Because these enzymes are also present in other tissues, changes in plasma activities may reflect damage to those tissues rather than to the liver (see Chapter 18).

Aminotransferases (alanine and aspartate)
A rise in plasma aminotransferase activities is a sensitive indicator of damage to cytoplasmic and/or mitochondrial membranes. Plasma enzyme activities rise when the membranes of only very few cells are damaged. Liver cells contain more aspartate aminotransferase (AST) than alanine aminotransferase (ALT), but ALT is confined to the cytoplasm, in which its concentration is higher than that of AST. Raised plasma transaminase concentrations are indicative of hepatocyte damage, but do not necessarily reveal its mechanism.

In inflammatory or infective conditions, such as viral hepatitis, the cytoplasmic membrane sustains the main damage; leakage of cytoplasmic contents causes a relatively greater increase in plasma ALT than AST activities.

In infiltrative disorders in which there is damage to both mitochondrial and cytoplasmic membranes, there is a proportionally greater increase in plasma AST than ALT activity.

The relative plasma activities of ALT and AST may help to indicate the type of cell damage. The former is more specific for hepatic disease; AST may be present in skeletal muscle and is more sensitive than ALT. A plasma AST:ALT ratio of > 2 is suggestive but not diagnostic of alcoholic liver disease and a ratio < 1 suggests chronic viral hepatitis or hepatic steatosis (see Chapter 18).

Hepatic synthetic function
The measurement of plasma albumin and prothrombin time may be used to assess function. The hepatic synthetic and secretory capacities are large; only severe and usually prolonged liver disease, for example cirrhosis, demonstrably impairs albumin and prothrombin synthesis.

Albumin
Hypoalbuminaemia is such a common finding in many severe illnesses that it is a less specific indicator of impaired synthetic capacity than a prolonged prothrombin time. A plasma albumin concentration below the lower reference limit may imply hepatic disease chronicity. However, there are many other causes of a low plasma albumin concentration that are not due to hepatic disease (see Chapter 19).

Prothrombin time
The prothrombin time may be prolonged by cholestasis: fat-soluble vitamin K cannot be absorbed normally if fat absorption is impaired due to intestinal bile salt deficiency. The abnormality is then corrected by parenteral administration of the vitamin. A prolonged prothrombin time may also result from severe impairment of synthetic ability if the liver cell mass is greatly reduced; in such cases it is not corrected by parenteral administration of vitamin K.

Hepatic excretory function
A high plasma conjugated bilirubin concentration indicates impaired hepatic excretory function but as this is also raised in hepatocellular disease it is not specific for cholestasis. This may be accompanied by a high plasma alkaline phosphatase (ALP) activity, as we will now see. Jaundice has been described above.
Liver disorders and gallstones

Other tests for liver disease

Alkaline phosphatase

Alkaline phosphatase is derived from a number of different tissues, including the liver, the osteoblasts in bone and the placenta. Plasma activities rise in cholestatic liver disease because ALP synthesis is increased and the enzyme within the biliary tract is regurgitated into plasma. A raised ALP concentration in the presence of a raised γ-glutamyl transferase (GGT) concentration implies that the ALP is of hepatic origin.

γ-Glutamyl transferase

γ-Glutamyl transferase is an enzyme derived from the endoplasmic reticulum of the cells of the hepatobiliary tract. As this reticulum proliferates, for example in response to the prolonged intake of alcohol and of drugs such as phenobarbital and phenytoin, synthesis of the enzyme is induced and plasma GGT activity increases. Therefore, raised plasma activities do not necessarily indicate hepatocellular damage, but may reflect enzyme induction or cholestasis.

Biochemical tests can be used to investigate hepatic disorders, the mechanisms underlying which can be divided into three main groups; these often coexist, but one usually predominates in any particular condition (Table 17.1).

- Liver-cell damage is characterized by the release of enzymes (AST and ALT) from damaged hepatocytes. Plasma ALT and AST activities are increased.
- Cholestasis is characterized by retention of conjugated bilirubin and of ALP, and by increased ALP synthesis at the sinusoidal surface. Plasma conjugated bilirubin levels and ALP activities are increased.
- Reduced mass of hepatocytes, if considerable, is characterized by a reduction in albumin and prothrombin synthesis. The plasma albumin concentration is reduced and the prothrombin time is prolonged.

Urine tests useful in suspected hepatic disease

Fresh urine analysis may confirm the presence of bilirubin (conjugated hyperbilirubinaemia) and urobilinogen

Urine Multistix include stabilized, diazotized 2,4-dichloraniline, which reacts with bilirubin to form azobilirubin. The test will detect about 3 µmol/L of bilirubin. Drugs, such as large doses of chlorpromazine, may give false-positive results.

Urine Multistix also include paradimethylaminobenzaldehyde, which reacts with urobilinogen. Urine Multistix do not react with porphobilinogen. This test will detect urobilinogen in urine from some normal individuals. False-positive results may occur after taking drugs such as para-aminosalicylic acid and some sulphonamides.

Bile acid measurement in obstetric cholestasis

Raised total plasma bile acid concentrations in the third trimester of pregnancy associated with pruritus are suggestive of obstetric cholestasis, which can lead to both maternal and fetal morbidity and mortality. Elevation of plasma ALT concentration may follow the increase in the concentration of plasma bile salts (see Chapter 10).

New hepatic function tests

Owing to the very large hepatic reserve, tests for impairment of metabolic (including synthetic and

Table 17.1 Typical biochemical features of certain hepatic disorders

<table>
<thead>
<tr>
<th></th>
<th>Plasma albumin</th>
<th>Bilirubin</th>
<th>ALT</th>
<th>ALP</th>
<th>GGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute alcoholic hepatitis</td>
<td>–</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Acute viral hepatitis</td>
<td>–</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Chronic viral hepatitis</td>
<td>– or ↓</td>
<td>↑ or –</td>
<td>↑</td>
<td>↑ or –</td>
<td>↑</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>↓</td>
<td>↑ or –</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Gilbert’s syndrome</td>
<td>–</td>
<td>↑</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>– or ↓</td>
<td>↑↑</td>
<td>↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Tumour secondaries</td>
<td>–</td>
<td>↑ or –</td>
<td>↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
</tbody>
</table>

↑, raised; –, normal; ↓, reduced.

ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyl transferase.
secretory) function are relatively insensitive indicators of liver disease. There are a number of new tests that are being devised to improve the accuracy of the diagnosis of hepatic disorders. Tests of hepatocellular activity have been proposed, such as galactose elimination capacity, the aminopyrine breath test, indocyanine green clearance, and monoethylglycinexylidide (MEGX) production. All these tests are indirect measures of hepatic activity that rely on measuring compounds or their metabolites after they have been acted on by the liver. As yet, they do not have a place in routine clinical diagnosis.

DISEASES OF THE LIVER

Cholestasis

Cholestasis may be either:

- **intrahepatic**, in which bile secretion from the hepatocytes into the canaliculi is impaired, due to:
  - viral hepatitis,
  - drugs such as chlorpromazine or toxins such as alcohol,
  - inflammation of the biliary tract (cholangitis),
  - autoimmune disease (primary biliary cirrhosis),
  - cystic fibrosis,
- **extrahepatic**, due to obstruction to the flow of bile through the biliary tract by:
  - biliary stones,
  - inflammation of the biliary tract,
  - pressure on the tract from outside by malignant tissue, usually of the head of the pancreas,
  - biliary atresia (rare).

It is essential to distinguish between intrahepatic and extrahepatic causes of cholestasis, as surgery may be indicated for the latter but is usually contraindicated for intrahepatic lesions. The biochemical findings may be similar:

- Bilirubin concentrations in plasma may be normal if only part of the biliary system is involved by intrahepatic lesions such as cholangitis, early primary biliary cirrhosis or primary or secondary tumours. The unaffected areas can secrete bilirubin.
- Alkaline phosphatase activity is a sensitive test for cholestasis. Increased synthesis of ALP in the affected ducts increases the activity of this enzyme in plasma. If this is the only abnormal finding, it must be shown to be of hepatic origin before it is assumed to indicate liver disease.

Patients with prolonged and more widespread cholestasis may present with severe jaundice and pruritus due to the deposition of retained bile salts in the skin; the plasma bilirubin concentration may be more than 800 µmol/L. More rarely, there is bleeding due to malabsorption of vitamin K, with consequent prothrombin deficiency. Cholesterol retention may cause hypercholesterolaemia. Dark urine and pale stools suggest biliary retention of conjugated bilirubin.

The jaundice caused by extrahepatic obstruction due to malignant tissue is typically painless and progressive, but there may be a history of vague persistent back pain and weight loss. By contrast, intrahepinal obstruction by a gallstone may cause severe pain, which, like the jaundice, is often intermittent. Gallstones may not always cause such symptoms. If a large stone lodges in the lower end of the common bile duct, the picture may be indistinguishable from that of malignant obstruction.

Although most of the findings are directly attributable to cholestasis, biliary back pressure may damage hepatocytes, and plasma aminotransferase activities may increase. Unless the cause is clinically obvious, evidence of dilated ducts due to extrahepatic obstruction should be sought using tests such as ultrasound, computerized tomography (CT) scanning or cholangiography.

Primary biliary cirrhosis

This is a rare autoimmune disorder that occurs most commonly in middle-aged women. Destruction and proliferation of the bile ducts produce a predominantly cholestatic picture, with pruritus and a plasma ALP activity that may be very high. Jaundice develops late in most patients. Mitochondrial antibodies are detectable in the plasma of more than 90 per cent of cases; the plasma immunoglobulin M (IgM) concentration is usually raised. Patients may also manifest hypercholesterolaemia, xanthelasma (see Chapter 13), other autoimmune disorders and osteoporosis.

Parenteral nutrition

Prolonged parenteral nutrition may be associated with a progressive increase in plasma ALP activity and a subsequent rise in the plasma aminotransferase concentrations. The cause is not known, although biliary sludging may occur and the biochemical changes may return to normal when the parenteral feeding is discontinued. Cyclical parenteral nutrition may help the situation when patients are fed intermittently (see Chapter 14).
Liver disorders and gallstones

Acute hepatitis

The biochemical findings in acute hepatitis are predominantly those of cell membrane damage with an increase in plasma ALT activity greater than that of AST. There may be a superimposed cholestatic picture and, in very severe cases, impaired prothrombin synthesis.

Viral hepatitis

Viral hepatitis may be associated with many viral infections, such as infectious mononucleosis (Epstein-Barr virus), rubella and cytomegalovirus. However, the term is most commonly used to describe three principal types of viral infection in which the clinical features of the acute illness are very similar, although they have a different incubation period:

- **Hepatitis A** (‘infectious hepatitis’), transmitted by the faecal–oral route as a food-borne infection, is relatively common in schools and other institutions and has an incubation period of between 15 and 45 days. Relapses may occur, but it rarely progresses to chronic hepatitis.
- **Hepatitis B** (‘serum hepatitis’) is transmitted by blood products and other body fluids; it occurs more sporadically than hepatitis A. It has a longer incubation period, of between 40 and 180 days. Some patients may be anicteric; some may develop fulminant hepatitis or chronic active hepatitis and later cirrhosis and hepatocarcinoma. They may become asymptomatic carriers of the disease.
- **Hepatitis C** (non-A, non-B hepatitis), which may be the result of sexual transmission or the transfusion of blood products, has an incubation period of between 15 and 50 days. It may progress to cirrhosis.

In all types there may be a 3- to 4-day history of anorexia, nausea and tenderness or discomfort over the liver before the onset of jaundice. Some patients remain anicteric. Plasma aminotransferase activities are very high from the onset of symptoms; they peak about 4 days later, when jaundice becomes detectable, but may remain elevated for several months. Once jaundice appears, some of the initial symptoms improve.

In the early stages there is often a cholestatic element, with pale stools due to reduced intestinal bilirubin, and dark urine due to a rise in plasma conjugated bilirubin concentration; unconjugated bilirubin concentrations also increase due to impaired hepatocellular conjugation.

Plasma bilirubin concentrations rarely exceed 350 μmol/L, and the plasma ALP activity is only

CASE 1

A 52-year-old woman was referred to the hepatology clinic because of jaundice, pruritus, hepatomegaly, xanthelasma and the following abnormal liver test results:

**Plasma**

- Bilirubin 93 μmol/L (< 20)
- Alanine aminotransferase 111 U/L (< 42)
- Alkaline phosphatase (ALP) 826 U/L (< 250)
- Albumin 34 g/L (35–45)
- γ-Glutamyl transferase (GGT) 764 U/L (< 55)

She had a positive test result for anti-mitochondrial antibodies.

**DISCUSSION**

Subsequent studies, including liver biopsy, showed the patient to have primary biliary cirrhosis. Note the predominant cholestatic biochemical picture with raised plasma ALP and GGT activities. This condition is associated with hyperlipidaemia, hence the xanthelasma, and is more common in middle-aged women with other autoimmune disorders. There may also be raised plasma IgM concentration and osteoporosis and osteomalacia.

CASE 2

A 22-year-old woman who was an intravenous drug addict was referred to the hepatology clinic because of the following abnormal liver test results:

**Plasma**

- Bilirubin 93 μmol/L (< 20)
- Alanine aminotransferase 761 U/L (< 42)
- Alkaline phosphatase 306 U/L (< 250)
- Albumin 44 g/L (35–45)
- γ-Glutamyl transferase 324 U/L (< 55)

Urinary bilirubin positive.

**DISCUSSION**

Note the grossly elevated plasma aminotransferase activities, indicative of extensive hepatocyte damage. Intravenous drug addicts are at increased risk of hepatitis B infection. The urinary bilirubin is positive, as the hyperbilirubinaemia is predominantly conjugated, that is, water soluble.
moderately raised, or even normal. If hepatocellular damage is severe and extensive, the prothrombin time may be increased and, in patients with cholestasis, malabsorption of vitamin K may be a contributory factor.

Serological findings

Testing for viral antigens, or for antibodies synthesized in response to the virus, can be used to diagnose viral hepatitis.

Hepatitis A viral (HAV) antibodies of the IgM class are detectable in the plasma of patients at the onset of symptoms. The presence of an IgG anti-HAV antibody is suggestive of previous infection. Most cases of hepatitis A recover completely.

Hepatitis B viral (HBV) infection, during the prodromal illness, can be diagnosed by the presence in the plasma of a viral surface antigen (HBsAg) and a core antigen (HBeAg), an internal component of the virus. These antigens are short lived. During the next few weeks, an antibody response occurs, with the appearance of plasma antibodies to the viral core (anti-HBc), to HBsAg, and finally to the surface antibody (anti-HBs), which may be used to document previous infection (Fig. 17.3).

The presence of the HBeAg antigen correlates with infectivity, and its disappearance is a good prognostic sign; HBsAg may persist, especially in patients with an impaired immune response, and indicates chronicity associated with raised plasma aminotransferase activities.

Hepatitis C viral (HCV; non-A, non-B hepatitis) infection is often diagnosed by exclusion. Anti-HCV antibodies may be detected in plasma about 12 weeks after exposure to the virus in about 50 per cent of patients.

It should be noted that there are other possible viruses that can cause liver dysfunction such as hepatitis D and E. Hepatitis D is a ribonucleic acid (RNA) subviral satellite and needs hepatitis B to propagate, while hepatitis E is a RNA virus spread more commonly by the oral–faecal route.

Alcoholic hepatitis

Alcoholic hepatitis occurs in heavy drinkers, often after a period of increased alcohol intake. Although the clinical features may mimic acute viral hepatitis, the plasma aminotransferase activities and bilirubin concentration are not usually as markedly elevated, although GGT may be.

There is no perfect laboratory marker of alcohol abuse. A raised mean corpuscular red cell volume (MCV), hypertriglyceridaemia, hyperuricaemia and elevated plasma GGT are clues, but are not specific. Increased plasma desialylated or carbohydrate-deficient transferrin of greater than 2 per cent or raised plasma sialic acid levels have been proposed as markers of alcohol abuse, as sialic acid metabolism may be perturbed in the presence of high concentrations of alcohol. Note that the consumption of more than 80 g a day of alcohol may raise GGT concentrations, not necessarily by hepatic damage, but by enzyme induction.

Drugs and other toxins

Various drugs and other toxins are hepatotoxic, sometimes directly and sometimes due to a hypersensitivity reaction; in the latter case, the damage is not dose related. The clinical picture may resemble that of acute viral hepatitis or cholestasis. A drug history is an essential part of the assessment of a patient presenting with liver disease. Table 17.2 lists some of these agents.

Chronic hepatitis

The finding of persistent, (usually only slightly) raised plasma aminotransferase activities, sometimes with chronic or recurrent symptoms suggesting liver disease, may be due to several disorders. It may be the only abnormal biochemical finding.

Chronic persistent hepatitis

This is a term used to describe the finding of raised plasma aminotransferase activities without clinical
Liver disorders and gallstones

Signs or symptoms and without a significant change in activity over many years. The activities rarely exceed three times the upper reference limits. Jaundice is unusual.

Chronic active hepatitis

This is caused by active hepatocellular destruction with episodes of relapses and remissions. It may progress to cirrhosis. It occurs at any age, but is most common in women. It may:

- be associated with, or a consequence of, viral infections such as HBV or HCV, or may be drug induced,
- be part of an autoimmune process that sometimes involves more than one organ,
- have no obvious cause.

The earliest findings that differentiate it from chronic persistent hepatitis are an increasing plasma IgG concentration, perhaps detected by a rising plasma γ-globulin concentration, and the presence of smooth muscle and antinuclear antibodies. As the disease progresses, more cells are destroyed and the plasma AST activity may rise to or exceed that of ALT; slight jaundice may develop. If there is significant hepatocellular destruction, the plasma albumin concentration falls.

Cirrhosis

Cirrhosis is the end result of many inflammatory and metabolic diseases involving the liver, including prolonged toxic damage, usually due to alcohol. In ‘cryptogenic cirrhosis’, the cause is unknown. The fibrous scar tissue distorts the hepatic architecture, and regenerating nodules of hepatocytes disrupt the blood supply, sometimes increasing the pressure in the portal vein, causing portal hypertension. Blood may be shunted from the portal into the hepatic vein, bypassing the liver.

In the early stages there may be no abnormal biochemical findings. During phases of active cellular destruction, the plasma AST, and sometimes ALT, activities rise. In advanced cases, the biochemical

Table 17.2 Some drug effects on the liver

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hepatic necrosis</th>
<th>Acute hepatitis-like reaction</th>
<th>Chronic hepatitis</th>
<th>Cholestasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
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<tr>
<td>Chlorpromazine</td>
<td></td>
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<td>+</td>
<td></td>
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<tr>
<td>Cytotoxic drugs</td>
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</tr>
<tr>
<td>Erythromycin</td>
<td></td>
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<td>+</td>
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<tr>
<td>Ferrous sulphate</td>
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<td>Halothane</td>
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<td>Isoniazid</td>
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<td>Methylldopa</td>
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<td>Nitrofurantoin</td>
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<tr>
<td>Phenothiazines</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Phenybutazone</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Phenytoin</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Salicylate (aspirin)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-α-alkylated steroids (oral contraceptives)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins, e.g. simvastatin</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valproate</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

*Indicates that the damage is dose dependent and predictable.
findings are mostly associated with a reduced functioning cell mass. The vascular shunting allows antigenic substances, which have been absorbed from the intestine, to bypass the normal hepatic sinusoidal filtering process, and to stimulate increased synthesis of IgG and IgA, producing the typical serum protein electrophoretic pattern of β–γ fusion (see Chapter 19).

Portal hypertension and impaired lymphatic drainage lead to the accumulation of fluid in the peritoneal cavity (ascites). This may be aggravated by hypoalbuminaemia, which may also cause peripheral oedema. In advanced cirrhosis, the findings of hepatocellular failure develop.

There are a number of causes of ascites including cirrhosis, malignancy or infection, nephrotic syndrome, hypothyroidism, pancreatitis and cardiac failure. Primary hepatocellular carcinoma may develop in a cirrhotic liver.

The Child–Pugh classification system is a way of grading the severity of cirrhosis in the face of portal hypertension (Table 17.3). Survival for patients with grade C cirrhosis is usually less than 1 year.

### Table 17.3 Child–Pugh scores for severity of cirrhosis

<table>
<thead>
<tr>
<th>Grade</th>
<th>Plasma bilirubin (µmol/L)</th>
<th>Plasma albumin (g/L)</th>
<th>Ascites/encephalopathy</th>
<th>INR</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal</td>
<td>&gt; 35</td>
<td>None</td>
<td>&lt; 1.7</td>
</tr>
<tr>
<td>B</td>
<td>34–50</td>
<td>28–35</td>
<td>Mild</td>
<td>1.7–2.3</td>
</tr>
<tr>
<td>C</td>
<td>&gt; 50</td>
<td>&lt; 28</td>
<td>Severe</td>
<td>&gt; 2.3</td>
</tr>
</tbody>
</table>

INR, international normalized ratio.

follow an overdose of a liver toxin such as paracetamol (acetaminophen). The biochemical findings may include any or all of those of acute hepatitis. Jaundice is progressive. In the final stage, the number of hepatocytes, and so the total amount of aminotransferases released, may be so reduced that plasma activities fall despite continuing damage to the remaining cells. This finding should not be interpreted as a sign of recovery. Other features may include the following:

- Hypovolaemia and hypotension, which are due to loss of circulating fluid in ascites and in the oedema fluid formed because of hypoalbuminaemia, and which may be aggravated by vomiting. The resultant low renal blood flow may have two consequences:
  - increased antidiuretic hormone (ADH) and secondary hyperaldosteronism, causing electrolyte disturbances, especially hypokalaemia, and sometimes dilutional hyponatraemia (see Chapter 2),
  - renal circulatory insufficiency, causing oliguria, a high plasma creatinine concentration and ureaemia despite reduced urea synthesis.

- Impaired hepatic deamination of amino acids, causing accumulation of amino acids in plasma with overflow amino aciduria and sometimes hyperammonaemia. If the reduced formation of urea from amino acids is not balanced by renal retention due to the decrease in glomerular filtration rate (GFR), the plasma urea concentration may be low.

- Impairment of hepatic gluconeogenesis may cause hypoglycaemia.

### Treatment of end-stage liver disease

Liver transplantation may be the only possible treatment for end-stage liver disease. Complications include graft failure, hepatic artery thrombosis, infection and acute and chronic rejection. Both acute rejection (which occurs in up to 80 per cent of recipients) and chronic rejection (occurring in about 10 per cent of cases) are

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**CASE 3**

A 50-year-old known alcoholic man attended the general medical clinic because of ascites and the following abnormal liver test results:

**Plasma**

- Bilirubin 52 µmol/L (< 20)
- Alanine aminotransferase 76 U/L (< 42)
- Alkaline phosphatase 271 U/L (< 250)
- Albumin 18 g/L (35–45)
- γ-Glutamyl transferase 324 U/L (< 55)

**DISCUSSION**

The abnormal liver test results and hypoalbuminaemia together with ascites supported the diagnosis of cirrhosis, secondary to his alcohol problem. Hypoalbuminaemia may be due to many disorders, such as gross proteinuria, but in the presence of hepatic disease suggests a reduction in hepatic synthetic capacity typical of cirrhosis.

Liver damage severe enough to cause obvious clinical signs of impaired hepatocellular function may be caused by severe hepatitis or advanced cirrhosis, or may
associated with a rise in plasma bilirubin concentration and ALP activity; chronic rejection may be irreversible. The indications for hepatic transplantation may include prolonged prothrombin time and plasma bilirubin more than 300 µmol/L.

**Hepatic infiltration and malignant disease**

Invasion of the liver by secondary carcinoma, or infiltration by lymphoma or granulomas such as sarcoidosis, may be associated with abnormal biochemical tests. Sometimes the only abnormal finding is raised plasma AST activity; the ALT activity may also be raised to a lesser extent or there may be GGT elevation. The picture may reflect cholestasis, with or without jaundice.

Metabolic function is rarely demonstrably impaired. If a primary hepatocellular carcinoma develops, either in a cirrhotic liver or de novo, the plasma aminotransferase and ALP activities usually rise rapidly, and plasma α-fetoprotein concentrations are often very high; this latter finding is not diagnostic of primary hepatic malignancy (see Chapter 24).

**Metabolic liver disease**

A group of rare metabolic disorders, most of which are inherited, is associated with liver disease, especially cirrhosis.

**Haemochromatosis**

Idiopathic haemochromatosis is a genetically determined disorder in which slightly increased intestinal absorption of iron over many years produces large iron deposits of parenchymal distribution, including the liver (see Chapter 21).

**α₁-Antitrypsin deficiency**

α₁-Antitrypsin deficiency (see Chapter 19) is associated with neonatal hepatitis in individuals with the PiZZZ phenotype, which progresses to cirrhosis in childhood. The condition can also present in adulthood, and often there is basal emphysema particularly in smokers (Fig. 17.4).

**Galactosaemia**

This autosomal recessive disorder, due most commonly to a deficiency of galactose-1-phosphate uridylytransferase, may cause cirrhosis of the liver if untreated. Liver transplantation may be indicated if hepatocellular carcinoma, a complication of cirrhosis, develops (see Chapter 27).

---

**CASE 4**

A 70-year-old man with known colonic carcinoma, operated on 2 years previously, was found by his general practitioner to have the following liver test results:

- Plasma Bilirubin 33 µmol/L (< 20)
- Alanine aminotransferase 62 U/L (< 42)
- Alkaline phosphatase (ALP) 408 U/L (< 250)
- Albumin 35 g/L (35–45)
- γ-Glutamyl transferase (GGT) 527 U/L (< 55)

An abdominal ultrasound scan showed multiple space-occupying lesions within the liver.

**DISCUSSION**

The patient was eventually found to have widespread hepatic secondaries from his colonic tumour and died a few months later. The combination of raised plasma ALP and GGT activities is suggestive of hepatic space-occupying lesions, particularly in the absence of major cholestasis.

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**CASE 5**

A healthy 43-year-old man, on no medication, had the following results from tests performed during a private healthcare screening programme:

- Plasma Bilirubin 43 µmol/L (< 20)
- Unconjugated bilirubin 36 µmol/L (< 5)
- Alanine aminotransferase 21 U/L (< 42)
- Alkaline phosphatase 126 U/L (< 250)
- Albumin 40 g/L (35–45)
- γ-Glutamyl transferase 24 U/L (< 55)
- Urinary bilirubin negative.
- Normal full blood count, reticulocytes, plasma haptoglobin concentration and blood film.

**DISCUSSION**

The raised concentration of plasma bilirubin is predominantly unconjugated. There is no evidence of haemolysis and the other liver function tests are normal. A likely diagnosis is of Gilbert’s syndrome. This is a common condition and its diagnosis is based on the exclusion of liver disease and haemolysis in the presence of a modest concentration of unconjugated hyperbilirubinaemia.
Wilson’s disease

This is a rare, recessively inherited disorder caused by reduced biliary excretion of copper and by impaired hepatic incorporation of copper into caeruloplasmin. The symptoms are due to the excessive accumulation of copper in the liver, brain and kidneys and present with evidence of acute liver failure, chronic hepatitis or cirrhosis in children or in young adults (see also Chapter 14).

Reye’s syndrome

This rare disorder presents as acute hepatitis, associated with marked encephalopathy, severe metabolic acidosis and hypoglycaemia in children typically between the ages of 3 and about 12 years. There is acute fatty infiltration of the liver. The plasma aminotransferase activities are high, but plasma bilirubin levels are only slightly raised.

The aetiology is uncertain, but the condition may be precipitated by viral infections, such as influenza A or B, drugs such as salicylates and sodium valproate, and certain toxins; it has been recommended that children should not be given aspirin. One possible mechanism is that there is uncoupling of mitochondrial oxidative phosphorylation. A number of inherited metabolic disorders, particularly those involving fatty acid oxidation, may present with a Reye-like syndrome in children under the age of about 3 years.

Non-alcoholic steatotic hepatitis or fatty liver

Non-alcoholic fatty liver disease (NAFLD) refers to a spectrum of liver disease ranging from fatty liver (steatosis) to non-alcoholic steatotic hepatitis (NASH) through to cirrhosis (irreversible, fibrosis and scarring). This is associated with insulin resistance (see Chapter 12). It is one of the most common causes of abnormal liver function tests. Plasma aminotransferases are usually raised and may relate to body mass index. It is important to exclude other liver disorders, and hepatic ultrasound may show increased echogenicity of the liver. The biochemical features of NAFLD may improve with dietary measures and treatment of hyperlipidaemia (see Chapter 13).

Hepatorenal syndrome

This syndrome occurs when cirrhosis and often portal hypertension presents in conjunction with renal dysfunction. It is thought to be due to impaired renal perfusion due to vasoconstriction of renal arteries. Usually the creatinine clearance is less than 40 mL/min and plasma creatinine is greater than about 130 µmol/L, with a urine volume of less than 500 mL/day and urinary sodium less than 10 mmol/L.

Drugs and liver fibrosis

Some drugs such as methotrexate can cause hepatic fibrosis. Conventional liver function tests are insensitive to liver fibrosis. Increased serum procollagen-3 N-terminal peptide (P3NP) is a marker of fibrosis and may reduce the need for liver biopsy.

JAUNDICE

Haemolytic jaundice

There are many causes of haemolysis, including sickle-cell anaemia, thalassaemia and spherocytosis, and it can also be drug or autoimmune induced. In adults, unconjugated hyperbilirubinaemia is usually mild because of the large reserve of hepatic secretory capacity. The plasma bilirubin concentration is usually less than 70 µmol/L. Erythrocytes contain high amounts of AST and lactate dehydrogenase (LDH1 and LDH2) (see Chapter 18). Blood reticulocytes may be raised, with abnormal blood film and a low plasma haptoglobin concentration. A haemolytic component may be seen in alcoholic hepatitis known as Zieve’s syndrome. Urinary bilirubin concentration is usually not raised in haemolysis (acholuric jaundice).
Liver disorders and gallstones

Jaundice in the newborn infant

Red cell destruction, together with immature hepatic processing of bilirubin, may cause a high plasma level of unconjugated bilirubin in the newborn infant; so-called physiological jaundice is common. Normal full-term babies may show jaundice between days 2 and 8 of life.

Physiological jaundice rarely exceeds 100 µmol/L. Jaundice on the first day of life is invariably pathological, as are levels of bilirubin exceeding 100 µmol/L or if the hyperbilirubinemia is conjugated. As a result of haemolytic disease, the plasma concentration of unconjugated bilirubin may be as high as 500 µmol/L and may exceed the plasma protein-binding capacity; free unconjugated bilirubin may be deposited in the brain, causing kernicterus. Neonatal jaundice and its treatment are discussed more fully in Chapter 26.

The inherited hyperbilirubinemia

There is a group of inherited disorders in which either unconjugated or conjugated hyperbilirubinemia is the only detectable abnormality.

Unconjugated hyperbilirubinemia

Gilbert’s syndrome

This is a relatively common (3–7 per cent of the population) familial condition, which may be present at any age but usually develops after the second decade. Plasma unconjugated bilirubin concentrations are usually between 20 µmol/L and 40 µmol/L and rarely exceed 80 µmol/L. They fluctuate, and may rise during intercurrent illness, dehydration, menstruation and fasting. The condition is probably harmless but must be differentiated from haemolytic hepatitis and liver disease. It often becomes evident when plasma bilirubin concentrations fail to return to normal after an attack of hepatitis, or during any mild illness, which, because of the jaundice, may be misdiagnosed as hepatitis. Conjugated bilirubin is less than 20 per cent of the total bilirubin.

Mutations in the hepatic uridine diphosphate glucuronyl transferase (UGT) gene are present, but not all patients with the polymorphism develop the phenotype. Hepatic UGT activity is decreased to approximately 30 per cent of normal in individuals with Gilbert’s syndrome. Decreased activity has been attributed to an expansion of thymine–adenine repeats in the promoter region of the UGT1A1 gene, the principal gene encoding this enzyme.

Diagnosis of Gilbert’s syndrome is by exclusion of haemolysis and other hepatic disorders. It should be remembered that sometimes thyrotoxicosis can cause raised unconjugated bilirubin due to reduced UGT activity and so can reabsorption of a large haematoma due to haemoglobin breakdown. Prolonged fasting (48 h with calorie intake restricted to 300 kcal/day) may result in a rise, predominantly in the unconjugated bilirubin fraction, of around 100 per cent if Gilbert’s syndrome is present. Increases are also seen after the administration of 50 mg intravenous nicotinic acid. However, these dynamic tests are not without side effects and are rarely used.

Crigler–Najjar syndrome

This is due to a rare deficiency of hepatic UGT, and is a more serious condition. It usually presents at birth. The plasma unconjugated bilirubin may increase to concentrations that exceed the binding capacity of albumin and so cause kernicterus. The defect may be complete (type I), and inherited as an autosomal recessive condition, or partial (type II), and inherited as an autosomal dominant condition.

In type II drugs that induce enzyme synthesis, such as phenobarbital, may reduce plasma bilirubin concentration.

Conjugated hyperbilirubinemia

Dubin–Johnson syndrome

This is probably harmless and is due to defective excretion of conjugated bilirubin, but not of bile acids. It is characterized by slightly raised plasma conjugated bilirubin levels that tend to fluctuate. Because the bilirubin is conjugated, it may be detectable in the urine. Plasma ALP activities are normal. There may be hepatomegaly, and the liver is dark brown in appearance due to the presence of a pigment with the staining properties of lipofuscin. The diagnosis may be confirmed by the characteristic staining of a specimen obtained by liver biopsy.

Rotor’s syndrome

Rotor’s syndrome is similar in most respects to Dubin–Johnson syndrome, but the liver cells are not pigmented (Box 17.1).

Bile and gallstones

Bile acids and bile salts

Four bile acids are produced in humans. Two of these, cholic acid and chenodeoxycholic acid, are synthesized
in the liver from cholesterol and are called primary bile acids. They are secreted in bile as sodium salts, conjugated with the amino acid glycine or taurine (primary bile salts). These are converted by bacteria within the intestinal lumen to the secondary bile salts, deoxycholate and lithocholate, respectively (Fig. 17.5).

Secondary bile salts are partly absorbed from the terminal ileum and colon and are re-excreted by the liver (enterohepatic circulation of bile salts). Therefore, bile contains a mixture of primary and secondary bile salts.

Deficiency of bile salts in the intestinal lumen leads to impaired micelle formation and malabsorption of fat (see Chapter 13). Such deficiency may be caused by cholestatic liver disease (failure of bile salts to reach the intestinal lumen) or by ileal resection or disease (failure of reabsorption causing a reduced bile salt pool). Bile salts are also important biochemical modulators and activate various nuclear receptors.

Formation of bile

Between 1L and 2L of bile is produced daily by the liver. This hepatic bile contains bilirubin, bile salts, phospholipids and cholesterol, as well as electrolytes in concentrations similar to those in plasma. Small amounts of protein are also present.

In the gall bladder there is active reabsorption of sodium, chloride and bicarbonate, together with an isosmotic amount of water. Consequently, gall bladder bile is 10 times more concentrated than hepatic bile; sodium is the major cation and bile salts the major anions. The concentrations of other non-absorbable molecules, such as conjugated bilirubin, cholesterol and phospholipids, also increase.

Gallstones

Although most gallstones contain all biliary constituents, they consist predominantly of one. Only about 10 per cent contain enough calcium to be radio-opaque and in this way they differ from renal calculi. They can be shown on gall bladder ultrasound (Fig. 17.6).

Conjugated hyperbilirubinaemia

Drugs, e.g. see Table 17.2
Infections, e.g. hepatitis, cytomegalovirus, Epstein–Barr virus, sepsis
Damage to bile ducts, e.g. primary biliary cirrhosis, sclerosing cholangitis
Alcohol, haemochromatosis, Wilson’s disease
Gallstones, cholangiocarcinoma, bile duct strictures, pancreatic tumours
Metabolic disorders, e.g. α1-antitrypsin, Reye’s syndrome, fatty liver, intrahepatic cholestasis of pregnancy
Diffusion infiltration, e.g. sarcoïd, lymphoma, amyloid
Inborn errors, e.g. Dubin–Johnson syndrome, Rotor’s syndrome

Unconjugated hyperbilirubinaemia

Physiological jaundice of the newborn
Gilbert’s syndrome
Crigler–Najjar syndrome
Haemolysis
Rarely thyrotoxicosis

Box 17.1 Some of the causes of jaundice
Liver disorders and gallstones

Pigment stones

Pigment stones are found in such chronic haemolytic states as hereditary spherocytosis. Increased breakdown of haemoglobin increases bilirubin formation and therefore biliary secretion. The stones consist mostly of bile pigments, with variable amounts of calcium. They are small, hard and dark green or black, and are usually multiple. Rarely, they contain enough calcium to be radio-opaque.

Cholesterol gallstones

Cholesterol is most likely to precipitate if bile is supersaturated with it; further precipitation on a nucleus of crystals causes progressive enlargement. Not all patients with a high biliary cholesterol concentration suffer from bile stones. Changes in the relative concentrations of different bile salts may favour precipitation. The stones may be single or multiple. They are described as mulberry-like and are either white or yellowish; the cut surface appears crystalline.

There is no clear association between hypercholesterolaemia and the formation of cholesterol gallstones, although both may be more common in obese individuals. However, there may be an increased incidence in patients taking some lipid-lowering drugs, such as the fibric acid derivatives.

Mixed stones

Most gallstones contain a mixture of bile constituents, usually with a cholesterol nucleus as a starting point. They are multiple-faceted, dark-brown stones with a hard shell and a softer centre and may contain enough calcium to be radio-opaque.

Consequences of gallstones

Gallstones may remain silent for an indefinite length of time and be discovered only at laparotomy for an unrelated condition. They may, however, lead to various clinical consequences.

- Biliary colic.
- Acute cholecystitis: obstruction of the cystic duct by a gallstone causes chemical irritation of the gall bladder mucosa by trapped bile and secondary bacterial infection.
- Chronic cholecystitis may also be associated with gallstones.
- Obstruction of the common bile duct occurs if a stone lodges in it. The patient may present with biliary colic, obstructive jaundice (Table 17.4), which is usually intermittent, or acute pancreatitis if the pancreatic duct is also occluded.
- Rarely, gallstones may be associated with gallstone ileus or carcinoma of the gall bladder.

Treatment of gallstones

The most common treatment for symptomatic gallstones is cholecystectomy, either open or laparoscopic. The following are some alternative approaches:

- Dissolving the gallstone: the bile acid ursodeoxycholic acid reduces the relative saturation of bile with cholesterol and so facilitates dissolving cholesterol stones, particularly small, uncalcified ones.
- Shock-wave lithotripsy: the stone is shattered into tiny fragments and can then pass through the cystic duct.

INVESTIGATION OF SUSPECTED LIVER DISEASE

The commonly available biochemical laboratory tests for the diagnosis of liver disease involve the measurement of plasma levels of:

- bilirubin – excretory function,
- aminotransferases (ALT and/or AST) – hepatocellular damage,
- alkaline phosphatase – cholestasis,
- albumin and/or prothrombin time – synthetic function,
- γ-glutamyl transferase – enzyme induction, cholestasis or hepatocellular damage.

The initial selection of investigations depends on the age of the patient, the history and clinical features.

Jaundice as a presenting feature (Fig. 17.7)

Whereas a patient with chronic liver disease may present with jaundice, the differential diagnosis of jaundice with bilirubinuria (due to conjugated bilirubin) in a previously Table 17.4 Some causes of obstructive jaundice or cholestasis

<table>
<thead>
<tr>
<th>Dilated ducts/mechanical obstruction (extrahepatic)</th>
<th>Undilated ducts/non-mechanical obstruction (intrahepatic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallstones</td>
<td>Primary biliary cirrhosis</td>
</tr>
<tr>
<td>Pancreatic carcinoma</td>
<td>Primary sclerosing cholangitis</td>
</tr>
<tr>
<td>Pancreatic duct stricture</td>
<td>Cholestatic drug reaction</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>Parasite infections</td>
</tr>
<tr>
<td>Parasite infections</td>
<td></td>
</tr>
</tbody>
</table>
Investigation of suspected liver disease

well patient is usually between acute hepatocellular damage and cholestasis. A suggested scheme is as follows (for neonatal jaundice, see Chapter 26):

- When taking the history pay special attention to:
  - recent exposure to hepatitis or infectious mononucleosis, including a sexual and occupational history,
  - recent administration of blood or blood products,
  - medication and alcohol intake (see Table 17.2),
  - intravenous drug abuse or tattoos,
  - associated symptoms such as abdominal pain, pruritus, weight loss or anorexia and nausea,
  - recent changes in the colour of the urine or stools,
  - recent foreign travel.
- On clinical examination, look for:
  - severity of jaundice,
  - hepatomegaly and splenomegaly,
  - signs of liver decompensation such as liver flap, ‘liver palms’, spider naevi, ascites etc.
- Measure plasma bilirubin and unconjugated/conjugated bilirubin fractions:
  - Predominantly unconjugated hyperbilirubinaemia with plasma conjugated bilirubin levels less than about 10 per cent of the total, and with little or no bilirubinuria, may suggest haemolysis as a cause. Haemolysis is supported by a raised reticulocyte count and diagnostic blood film, reduced plasma haptoglobin and raised plasma lactate dehydrogenase concentrations.
  - If haemolysis is excluded, consider Gilbert’s syndrome provided other liver tests are normal and other hepatic disorders have been excluded.
- A fresh urine sample should be examined. This test may show the presence of bilirubin if conjugated hyperbilirubinaemia is present. Dark-yellow or brown urine suggests biliary obstruction. An absence

Figure 17.7 Algorithm for the investigation of jaundice in an adult.
of urinary urobilinogen is seen in biliary obstruction. Reagent strips are available for testing for bilirubin and urobilinogen in urine.

- Pale stools suggest biliary obstruction as a cause of jaundice.
- Whatever the results of the urine and stool inspection and testing, request plasma aminotransferase, ALP and GGT assays:
  - In hepatitis, there is a predominant increase in the concentrations of plasma aminotransferases; usually the plasma ALT activity is higher than the AST activity. If hepatitis or infectious mononucleosis is suggested by the history, request serological tests.
  - In cholestasis, there is predominant elevation of the plasma ALP and GGT activities. Bile duct dilatation should be sought using ultrasound or other radiological tests:
    - if the bile ducts are dilated, there is obstruction that may require surgery,
    - if the plasma ALP activity is high but dilated ducts are not demonstrated, there is probably intrahepatic cholestasis.
- Other tests, such as ferritin and iron saturation (haemochromatosis), and autoantibody (such as smooth muscle, p-anti-neutrophil cytoplasmic antigen, mitochondrial, nuclear antibodies) and immunoglobulin levels, may also be indicated.
- If acute alcoholic hepatitis is suspected, contributory evidence may be the finding of a disproportionately high plasma GGT activity compared with those of the aminotransferases. There may also be macrocytosis, hypertriglyceridaemia and hyperuricaemia.
- In obstructive jaundice (biliary obstruction), the plasma ALP is usually more than four to five times and GGT more than 10 times normal. Liver/biliary ultrasound is useful to distinguish between obstructive jaundice with dilated biliary ducts or undilated ducts. Endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous cholangiography may be indicated.
- If the diagnosis is in doubt, a liver biopsy may be indicated, although there may be a risk of bleeding and therefore the prothrombin time should be measured beforehand.

Suspected liver disease showing abnormal plasma hepatic enzymes

- Relevant points in the clinical evaluation are:
  - a previous history of hepatitis, intravenous drug use, occupational and sexual history,
  - alcohol intake and medication history, for example paracetamol,
  - the presence, or history, of other autoimmune disorders,
  - jaundice, pruritus or features of malabsorption,
  - family history of liver disease.
- Request tests for plasma bilirubin, aminotransferases (ALT and AST), albumin, GGT and ALP:
  - A raised plasma ALT activity higher than that of the AST may be due to reversible alcoholic hepatitis, to chronic persistent hepatitis, or to early chronic active hepatitis.
  - A raised plasma AST activity higher than that of ALT may be due to cirrhosis or severe chronic active hepatitis.
  - A high plasma ALP activity with raised GGT concentration suggests cholestasis.
  - Aminotransferase levels of more than 10 times normal suggest primary hepatocyte damage, as in viral hepatitis or caused by drugs/toxins.
- An alcohol-related aetiology is supported by a macrocytosis and plasma GGT concentration, but neither test is fully diagnostic. See above.
- Check hepatitis serology, for example A, B and C.
- Detectable plasma mitochondrial or smooth-muscle antibodies are suggestive of primary biliary cirrhosis or chronic active hepatitis, respectively.
- A raised plasma ferritin concentration with high iron saturation (see Chapter 21) may reveal haemochromatosis.
- Plasma protein electrophoresis and immunoglobulin assay may help in the diagnosis of:
  - cirrhosis – high plasma IgG and IgA concentrations causing β-γ fusion on the electrophoretic strip,
  - alcoholic cirrhosis – may present with raised IgA concentration,
  - chronic active hepatitis – a high plasma IgG concentration and normal IgA,
  - primary biliary cirrhosis – a high plasma IgM concentration.

A low plasma albumin concentration (hypoalbuminaemia) in the face of abnormal liver function tests can be seen in cirrhosis implying chronicity.

- A prolonged prothrombin time implies poor hepatic synthetic capacity, for example clotting factors, and is prognostic in paracetamol overdose. It is also important if a liver biopsy is considered.
- Significant infiltration of the liver by tumour cells, or by granulomas such as sarcoidosis
Investigation of suspected liver disease

(possibly with raised plasma angiotensin-converting enzyme activity), may occur. In this situation raised plasma AST activity may be the most sensitive test, despite a normal plasma ALT activity. Hepatic space-occupying lesions may additionally present with raised GGT and ALP concentrations, and a liver ultrasound is useful to detect these.

- A fatty liver may be revealed by liver ultrasound as this may show increased echogenicity. This may be associated with hypertriglyceridaemia, obesity and type 2 diabetes mellitus or impaired glucose regulation.
- If primary hepatocellular carcinoma is suspected, the plasma α-fetoprotein level may also be high.
- Low plasma copper and caeruloplasmin concentrations may suggest Wilson's disease (see Chapter 15), and plasma α1-antitrypsin deficiency can result in cirrhosis (see Chapter 19).
- Radionuclide scans or other imaging procedures (CT or MRI) may be useful. A liver biopsy may be indicated to clarify a histological diagnosis.

SUMMARY

- The liver has an enormous synthetic capacity and is involved in numerous metabolic pathways, including vitamin storage, amino acid deamination, bile salt and cholesterol synthesis, and the production of various proteins such as clotting factors and hormones.
- Compounds ‘released’ from the liver into the plasma can be used as markers of liver damage, including bilirubin, ALT, AST, GGT and ALP.
- Hyperbilirubinaemia can be due to raised unconjugated or conjugated bilirubin concentration. The former may be due to haemolysis and the latter to hepatic or extrahepatic causes.
- Raised plasma aminotransferase activities suggest hepatocyte damage and raised hepatic ALP concentration is associated with cholestasis.
- Plasma GGT is a sensitive marker of hepatic damage but its activity can also be raised as a result of drug enzyme induction, hypertriglyceridaemia and increased alcohol intake.
- A prolonged prothrombin time and low plasma albumin concentration may both reflect reduced hepatic synthetic capacity.
This chapter discusses the principles of clinical enzymology, which have already been encountered in some of the preceding chapters. Enzymology can be defined as the assay of an enzyme(s) in body fluids, usually blood, that can be used diagnostically or to monitor a clinical condition.

An enzyme is a protein that catalyses one or more specific biochemical reactions. It is usually easier to measure enzyme activity in body fluids, by monitoring changes in either substrate or product concentrations, than to measure enzyme protein concentration directly, although this is sometimes done. However, measurement of the enzyme protein concentration is more specific and less prone to analytical variation.

Generally, enzymes are present in cells at much higher concentrations than in plasma. Some occur predominantly in cells of certain tissues, where they may be located in different cellular compartments such as the cytoplasm or the mitochondria. ‘Normal’ plasma enzyme concentrations reflect the balance between the rate of synthesis and release into plasma during cell turnover, and the rate of clearance from the circulation.

The enzyme activity in plasma may be:

- higher than normal, due to the proliferation of cells, an increase in the rate of cell turnover or damage or in enzyme synthesis (induction), or to reduced clearance from plasma,
- lower than normal, due to reduced synthesis, congenital deficiency or the presence of inherited variants of relatively low biological activity – examples of the latter are the cholinesterase variants.

Sometimes macroenzymes are found, that is to say, a high-molecular-weight form of a native enzyme. Often these are enzymes [such as lactate dehydrogenase (LDH), creatine kinase (CK) and alkaline phosphatase (ALP)] complexed with immunoglobulins and are more common in individuals with autoimmune disease. It is important to recognize macroenzymes as they can sometimes cause diagnostic confusion. As we will now see, changes in plasma enzyme activities may be useful to detect and localize tissue cell damage or proliferation, or to monitor the treatment and progress of disease.

**ASSESSMENT OF CELL DAMAGE AND PROLIFERATION**

Plasma enzyme levels depend on the extent of cell damage and the rate of release from damaged cells, which, in turn, depends on the rate at which damage is occurring.

In the absence of cell damage, the rate of release depends on the degree of induction of enzyme synthesis and the rate of cell proliferation.

These factors are balanced by the rate of enzyme clearance from the circulation.

Acute cell damage, for example in viral hepatitis, may cause very high plasma aminotransferase activities that reduce as the condition resolves. By contrast, the liver may be much more extensively involved in advanced cirrhosis but the rate of cell damage is often low, and consequently plasma enzyme activities may be only slightly raised or within the reference range. In very severe liver disease, plasma enzyme activities may even fall terminally when the number of hepatocytes is grossly reduced (see Chapter 17).

Relatively small enzymes, such as amylase, can be cleared by the kidneys. Thus, plasma amylase activity may be high as a result of renal glomerular impairment rather than pancreatic damage. However, most enzymes are large proteins and may be catabolized by plasma proteases before being taken up by the reticuloendothelial system.

In healthy individuals, each enzyme has a fairly constant and characteristic biological half-life, a fact that may be used to assess the time since the onset of an acute illness. After a myocardial infarction, for example, plasma levels of CK and aspartate aminotransferase (AST) fall to normal before those of LDH, which has a longer half-life (see Chapter 22).