LECTURE-17

Clinical Enzymology

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 .

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 .

Assessment Of Cell Damage And Proliferation

Localization of damage

Most of the enzymes commonly measured to assess tissue damage are present in nearly all body cells, although their relative concentrations in certain tissues may differ. Measurement of the plasma activity of an enzyme known to be in high concentration within cells of a particular tissue may indicate an abnormality of those cells, but the results will rarely enable a specific diagnosis to be made. For example, if there is circulatory failure after a cardiac arrest, very high plasma concentrations of enzymes originating from many tissues may occur because of hypoxic damage to cells and reduced rates of clearance.

The distribution of enzymes within cells may differ. Alanine aminotransferase (ALT) and LDH are predominantly located in cytoplasm, and glutamate dehydrogenase (although this is not usually measured clinically) in mitochondria,

whereas AST occurs in both these cellular compartments. Different disease processes in the same tissue may affect the cell in different ways, causing alteration in the relative plasma enzyme activities.

The diagnostic precision of plasma enzyme analysis may be improved by the following:

_ *Serial enzyme estimations* The rate of change of plasma enzyme activity is related to a balance between the rate of entry and the rate of removal from the circulation. A persistently raised plasma enzyme activity is suggestive of a chronic disorder or, occasionally, impaired clearance.

_ *Isoenzyme determination* Some enzymes exist in more than one form; these isoenzymes may be separated by their different physical or chemical properties. If they originate in different tissues, such identification will give more information than the measurement of plasma total enzyme activity; for example, CK may be derived from skeletal or cardiac muscle, but one of its isoenzymes is found predominantly in the myocardium.

_ *Estimation of more than one enzyme* Many enzymes are widely distributed, but their relative concentrations may vary in different tissues. For example, although both ALT and AST are abundant in the liver, the concentration of AST is much greater than that of ALT in heart muscle.

Non-specific causes of raised plasma enzyme activities

Before attributing a change in plasma enzyme activity to a specific disease process, it is important to exclude the presence of factitious or non-specific causes.

Slight rises in plasma ALT and AST activities are common, non-specific findings in many illnesses. Moderate exercise, or a large intramuscular injection, may lead to a rise in plasma CK activity; isoenzyme determination may identify skeletal muscle as the tissue of origin.

Some drugs, such as the anticonvulsants phenytoin and phenobarbital, may induce the synthesis of the microsomal enzyme g-glutamyl transferase (GGT), and so increase its plasma activity in the absence of disease.

Plasma enzyme activities may be raised if the rate of clearance from the circulation is reduced. In the absence of hepatic or renal disease, this may occur if, for example, the plasma enzyme forms complexes with immunoglobulins, known as a macroenzyme. Various enzymes can form clinically significant macroenzymes including amylase, LDH, ALP and CK.

FACTORS AFFECTING RESULTS OF PLASMA ENZYME ASSAYS

Analytical factors

The total concentration of all plasma enzyme proteins is less than 1 g/L. The results of enzyme assays are not usually expressed as concentrations, but as activities. Changes in concentration may give rise to proportional changes in catalytic activity, but the results of such measurements depend on many analytical factors, including:

_ substrate concentration,
_ product concentration,
_ enzyme concentration,
_ reaction temperature,
_ reaction pH,
_ presence of activators or inhibitors.

The definition of 'international units' does not takethese factors into account, and the results from different laboratories, which are apparently expressed in the

same units, may not be directly comparable. Therefore ,plasma enzyme activities must be interpreted in relation to the reference ranges from the issuing laboratory. In some countries such as the UK there are plans to harmonize or converge laboratory reference ranges for certain analytes.

Non-disease factors

Examples of non-disease factors affecting enzyme activities include the following

Age

Plasma AST activity is moderately higher during the neonatal period than in adults. Plasma ALP activity of bony origin is higher in children than in adults and peaks during the pubertal bone growth spurt before falling to adult levels. A second peak occurs in the elderly.

Sex

Plasma GGT activity is higher in men than in women. Plasma CK activity is also higher in males, probably in part due to their increased muscle bulk.

Race/ethnicity

Plasma CK activity is higher in black people and Afro-Caribbeans than in white people.

Physiological conditions

Plasma ALP activity rises during the last trimester of pregnancy because of the presence of the placental isoenzyme. Several enzymes, such as AST and CK, rise moderately in plasma during and immediately after

labour or strenuous exercise.

Plasma enzyme activities should therefore be interpreted in relation to the sex-, race-/ethnicity- and age-matched reference ranges of the issuing laboratory.

LECTURE -18

NORMAL PLASMA ENZYME ACTIVITIES

Aminotransferases

The aminotransferases (ALT and AST) are enzymes involved in the transfer of an amino group from a

2-amino acid to a 2-oxoacid; they need the cofactor pyridoxal phosphate for optimal activity. They are widely distributed in the body. The aminotransferases are used as part of the biochemical liver profile.

Aspartate aminotransferase (AST)

Aspartate aminotransferase (glutamate oxaloacetate aminotransferase, GOT) is present in high concentrations in cells of cardiac and skeletal muscle,

liver, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST levels.

Causes of raised plasma aspartate aminotransferase activities

- Artefactual: due to in vitro release from erythrocytes if there is haemolysis or if separation of plasma from cells is delayed.

- Physiological: during the neonatal period (about 1.5 times the upper adult reference limit).

- Marked increase (may be greater than 5–10 times the upper reference limit or URL):

– circulatory failure with ‘shock’ and hypoxia,

– myocardial infarction,

– acute viral or toxic hepatitis.

- Moderate to slight increase (usually less than five times URL):

– Hepatic steatosis [fatty liver or non-alcoholic fatty liver disease (NAFLD)],

– cirrhosis (may be normal sometimes),

– infectious mononucleosis (due to liver involvement),

– cholestatic jaundice,

– malignant infi ltration of the liver (may be normal),

– skeletal muscle disease,

– after trauma or surgery (especially after cardiac surgery),

– severe haemolytic episodes (of erythrocyte origin),

– certain drugs.

Note that AST is not specific for hepatic disease.

Alanine aminotransferase(ALT)

Alanine aminotransferase (glutamate pyruvate aminotransferase, GPT) is present in high concentrations in liver and, to a lesser extent, in skeletal muscle, kidney and heart.

Causes of raised plasma alanine aminotransferase activities

- Marked increase (may be greater than 5–10 times URL):

– circulatory failure with ‘shock’ and hypoxia,

– acute viral or toxic hepatitis.

- Moderate to slight increase (usually less than five times URL):

– Hepatic steatosis (fatty liver or NAFLD),

– cirrhosis (may be normal sometimes),

– infectious mononucleosis (due to liver involvement),

– liver congestion secondary to congestive cardiac failure,

– cholestatic jaundice,

– coeliac disease,

– surgery or extensive trauma and skeletal muscle

disease (much less affected than AST),

– certain drugs.

Note that ALT is more specific for hepatic disease than AST.

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Lactate dehydrogenase (LD,LDH)

Lactate dehydrogenase catalyses the reversible interconversion of lactate and pyruvate. The enzyme is widely distributed in the body, with high concentrations

in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes; measurement of plasma total LDH activity is therefore a non-specific marker of cell damage.

Causes of raised plasma total lactate dehydrogenase activity

- Artefactual: due to in vitro haemolysis or delayed

separation of plasma from whole blood.

- Marked increase (may be greater than 5–10 times URL):

– circulatory failure with ‘shock’ and hypoxia,

– myocardial infarction

– some haematological disorders: in blood diseases such as megaloblastic anaemia, acute leukaemias and lymphomas,

very high levels (up to 20 times the URL in adults) may be found. In cases of lymphoma LDH can be used as a tumour marker. Smaller increases occur

in other disorders of erythropoiesis, such as thalassaemia, myelofibrosis and haemolytic anaemias, renal infarction or, occasionally, during rejection of a renal transplant.

- Moderate to slight increase (usually less than five times URL):

– viral hepatitis,

– malignancy of any tissue,

– skeletal muscle disease,

– pulmonary embolism,

– infectious mononucleosis,

– certain drugs.

Isoenzymes Of Lactate Dehydrogenase

Five main isoenzymes can be detected by electrophoresis and are referred to as LDH1 to LDH5. LDH1, the fraction that migrates fastest towards the anode, predominates in cells of cardiac muscle, erythrocytes and kidney. The

slowest moving isoenzyme, LDH5, is the most abundant form in the liver and in skeletal muscle. Whereas in many conditions there is an increase in all fractions, the finding of certain patterns is of diagnostic value:

- Predominant elevation of LDH1 and LDH2 (LDH1 more than LDH2) occurs after myocardial infarction, in megaloblastic anaemia and after renal infarction.

- Predominant elevation of LDH2 and LDH3 occurs in acute leukaemia; LDH3 is the main isoenzyme elevated as a result of malignancy of many tissues.

- Elevation of LDH5 occurs after damage to the liver or skeletal muscle.

A rise in LDH1 is most significant in the diagnosis of myocardial infarction.

Lactate dehydrogenase was used in the delayed diagnosis of a myocardial infarct (troponin has largely taken over this role from LDH) and also as a marker for

certain tumours, for example lymphomas, and to help determine haemolysis.

Creatine kinase

Creatine kinase is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle. Isoenzymes of creatine kinase Creatine kinase consists of two protein subunits, M and B, which combine to form three isoenzymes, BB (CK-1), MB (CK-2) and MM (CK-3).

- CK-MM is the predominant isoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal subjects.

- CK-MB accounts for about 35 per cent of the total CK activity in cardiac muscle and less than 5 per cent in skeletal muscle; its plasma activity is always high after myocardial infarction. It may be detectable in the plasma of patients with a variety of other disorders in whom the total CK activity is raised, but this accounts for less than 6 per cent of the total CK activity.

- CK-BB is present in high concentrations in the brain and in the smooth muscle of the gastrointestinal and genital tracts. Increased plasma activities may occur during parturition. Although they have also been reported after brain damage, for example trauma or cerebrovascular accident, and in association with malignant tumours of the bronchus, prostate and breast, measurement is not of proven value for diagnosing these conditions. In malignant disease, plasma total CK activity is usually normal.

There are also other forms of CK. One is a mitochondrial form seen in hepatic disease, certain tumours and critically ill patients. There are also type 1 and type 2 macroenzyme forms of CK. Type 1 macroenzyme is associated with autoimmune

disease such as rheumatoid arthritis and is thought to be CK complexed with IgG; type 2 macroenzyme is an oligomer of mitochondrial CK.

Causes of raised plasma creatine kinase activities

- Artefactual: due to in vitro haemolysis, using most methods.

- Physiological:

– neonatal period (slightly raised above the adult URL),

– during and for a few days after parturition,

– plasma CK is generally higher in Africans than in Caucasians.

- Marked increase (may be greater than 5–10 times URL):

– dermatomyositis and polymyositis,

– 'shock' and circulatory failure,

– myocardial infarction,

– muscular dystrophies,

– rhabdomyolysis (the breakdown of skeletal muscle),

– necrotizing fasciitis.

- Moderate to slight increase (usually less than five times URL):

– muscle injury,

– infections, for example viral,

– after surgery (for about a week),

– physical exertion – there may be a significant rise in plasma activity after only moderate exercise, muscle cramp or following an epileptic fit,

– after an intramuscular injection,

– hypothyroidism

– alcoholism (possibly partly due to alcoholic myositis),

– some cases of cerebrovascular accident and head injury,

– malignant hyperpyrexia,

– certain drugs, for example statins, ciclosporin, cocaine,

– glycogen storage diseases,

– carnitine palmityl transferase deficiency.

Plasma CK activity is raised in all types of muscular dystrophy, but not usually in neurogenic muscle diseases such as poliomyelitis, myasthenia gravis,

multiple sclerosis or Parkinson's disease. Rhabdomyolysis can be defined as an acute increase in plasma CK concentration greater than 10 times the upper limit of normal. Severe muscle breakdown results in grossly elevated plasma CK concentrations, sometimes up to 100 000 U/L. This can be due to trauma,

severe exertion, alcohol, heat, electrolyte disturbances and drugs such as statins. Plasma myoglobin is elevated and, being of low molecular weight, is filtered through the renal glomeruli and can precipitate out in the renal tubules, resulting in acute kidney injury .Urinary myoglobin can be inferred by the red brown colour and positive urine dipstick test for haem in the absence of erythrocytes as judged

by microscopy. Intravascular volume expansion with saline and sometimes mannitol–alkaline diuresis my help reduce the risk of rhabdomyolysis.

Rhabdomyolysis is associated with hyperkalaemia and hyperphosphataemia due to the release of intracellular ions from myocytes, and calcium can be sequestered, resulting in hypocalcaemia.

Causes of low plasma creatine kinase activity

This is unusual but may include cachetic states associated with reduced muscle mass, for example alcoholism, undernutrition and patients in intensive care.

Amylase

Amylase (molecular weight 45 kDa) breaks down starch and glycogen to maltose. It is present at a high concentration in pancreatic juice and in saliva and may be extracted from other tissues, such as the gonads, Fallopian tubes, skeletal muscle and adipose tissue. Being of relatively low molecular weight, it is excreted in the urine.

Estimation of plasma amylase activity is mainly requested to help in the diagnosis of acute pancreatitis,in which the plasma activity may be very high. However, it may also be raised in association with other intraabdominal

Plasma activity may be near normal in chronic or haemorrhagic pancreatitis.

Causes of raised plasma amylase activity - Marked increase (may be greater than 5–10 times URL):

– acute pancreatitis,

– severe glomerular impairment,

– diabetic ketoacidosis,

– perforated peptic ulcer, especially if there is perforation into the lesser sac.

- Moderate to slight increase (usually less than five times URL).

– Other acute abdominal disorders:

• perforated peptic ulcer,

• acute cholecystitis,

• intestinal obstruction,

• abdominal trauma,

• ruptured ectopic pregnancy.

– Salivary gland disorders:

• mumps,

• salivary calculi,

• Sjögren's syndrome,

• after injection of contrast medium into

salivary ducts for sialography.

– Miscellaneous causes:

• morphine administration (spasm of the sphincter of Oddi),

• myocardial infarction (occasionally),

• acute alcoholic intoxication,

• macroamylasaemia,

• ectopic production from tumour.

Macroamylasaemia

In some patients, high plasma amylase activity is due to low renal excretion of a macroenzyme form, despite normal glomerular function. The condition is symptomless and it is thought that the enzyme is bound to IgA, giving a complex of molecular weight about 270 kDa. This harmless condition may be confused with other causes of hyperamylasaemia. If amylase and creatinine are assayed in simultaneous plasma and urine samples, an amylase clearance to creatinine clearance ratio can be calculated. If the result is multiplied by 100, a ratio of less than 0.02 is suggestive of macroamylasaemia. Electrophoretic techniques can also be used to determine the presence of macroamylasaemia.

Isoenzymes of amylase . Plasma amylase is derived from the pancreas and salivary glands. It is rarely necessary to identify the isoenzyme components in plasma, but they can be distinguished by electrophoretic techniques, or by using an inhibitor derived from wheat germ. Possible indications for isoenzyme determination include:

- the coexistence of mumps or renal failure, which complicate the interpretation of high activities due to acute pancreatitis;

- the possibility of chronic pancreatic disease, in which low activities may be found.

Some laboratories now measure plasma-specific ‘pancreatic’ amylase activity.

Alkaline phosphatase

The ALPs are a group of enzymes that hydrolyse organic phosphates at high pH. They are present in most tissues but are in particularly high concentration

in the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta.

In adults, plasma ALP is derived mainly from bone and liver in approximately equal proportions; the proportion due to the bone fraction is increased when

there is increased osteoblastic activity that may be physiological .

Causes of raised plasma alkaline phosphatase activity

- Physiological:

– During the last trimester of pregnancy, the plasma total ALP activity rises due to the contribution of the placental isoenzyme.

Plasma ALP concentration may increase by up to five times and usually returns to normal levels by 1 month postpartum.

– In preterm infants, plasma total ALP activity is up to five times the URL in adults, and consists predominantly of the bone isoenzyme.

– In children, the total activity increases by about two to five times during the pubertal bone growth spurt. There is a gradual increase in the proportion of liver ALP with age.

– In the elderly, the plasma bone isoenzyme activity may increase slightly.

- Bone disease:

– rickets and osteomalacia

– Paget's disease of bone (may be very high),

– secondary malignant deposits in bone,

– osteogenic sarcoma (only if very extensive),

– primary hyperparathyroidism with extensive bone disease (usually normal but may be slightly elevated),

– secondary hyperparathyroidism.

- Liver disease:

– intrahepatic or extrahepatic cholestasis

– space-occupying lesions, tumours, granulomas and other causes of hepatic infiltration.

- Inflammatory bowel disease: the gut ALP isoenzyme can be increased in ulcerative colitis.

- Malignancy: bone or liver involvement or direct tumour production.

A placental-like, so-called 'Regan' isoenzyme may occasionally be identified in plasma in patients with malignant disease, especially carcinoma of the bronchus. There is also a Nago isoenzyme released by certain tumours.

Transient very high levels of ALP (up to 30 times URL) have been recorded in children, but the clinical significance of this finding is unknown. This has been called transient hyperphosphatasaemia and may be either the bone or the liver isoenzyme. It may be associated with abdominal symptoms and usually resolves within a few months.

Possible causes of low plasma alkaline phosphatase Isoenzymes of alkaline phosphatase

Bone disease with increased osteoblastic activity and liver disease with involvement of the biliary tracts are the most common causes of an increased total ALP activity.

The isoenzymes originating from the cells of bone, liver, intestine and placenta may be separated by electrophoresis, but interpretation may be difficult if the total activity is only marginally raised.The placental and 'Regan' isoenzymes are more stable at 65°C than the bone, liver and intestinal isoenzymes, and heat inactivation may help to differentiate the heatstable from the heat-labile fraction.

The placental isoenzyme does not cross the placenta and is therefore not detectable in the plasma of the newborn infant .

Acid phosphatase

Acid phosphatase (ACP) is found in cells of the prostate,liver, erythrocytes, platelets and bone. The main indication for estimation was to help diagnose prostatic carcinoma and to monitor its treatment. Estimation has

been largely replaced by the measurement of plasma prostate-specific antigen (PSA), a protein derived from the prostate . This test is more specific

and sensitive for diagnosis and monitoring treatment and has essentially rendered the plasma ACP assay obsolete in the diagnosis and management of prostatic

carcinoma.

Haemolysed blood samples should also be avoided,as ACP is found in erythrocytes. Normally, ACP drains from the prostate, through the prostatic ducts and intothe urethra, and very little can be detected in plasma.

In extensive prostatic carcinoma, particularly if it has spread extensively or has metastasized, plasma ACP activity rises, probably because of the increased number of prostatic ACP-containing cells. Another problem with plasma ACP is that its concentration can increase after rectal examination.

Isoenzymes of acid phosphatase

The release of ACP from blood cells in vitro may occur even in unhaemolysed samples and many methods have been devised in an attempt to measure only the prostatic fraction, without complete success. One method makes use of the fact that L-tartrate inhibits prostatic ACP; the assay is performed with and without the addition of L-tartrate; the difference in activity between the results (the tartrate-labile fraction) is mainly prostatic ACP.

Other methods measure the prostatic enzyme protein concentration directly by immunoassay.

Causes of raised plasma acid phosphatase activity

_ *Tartrate labile*:

– artefactually raised following rectal examination, acute retention of urine or passage of a urinary catheter, due to pressure on prostatic cells,

– disseminated carcinoma of the prostate.

_ *Total*:

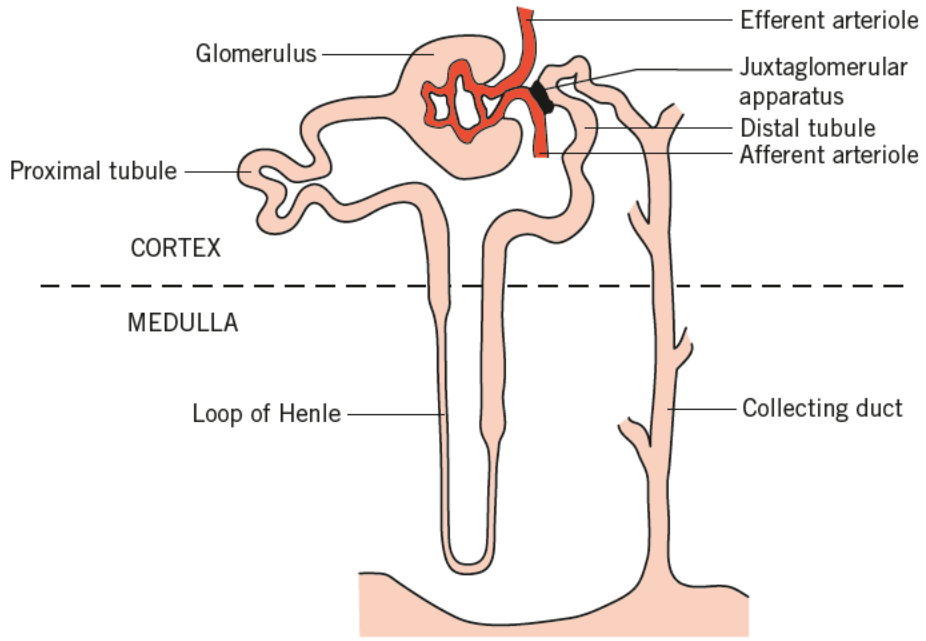
– artefactually in a haemolysed specimen, or following rectal examination, acute retention of urine or passage of a catheter,

– disseminated carcinoma of the prostate,

– Paget's disease of bone,

– some cases of metastatic bone disease, especially
with osteosclerotic lesions,
– Gaucher's disease (probably from Gaucher cells),
– occasionally in thrombocythaemia or polycythaemia.
The assay is not of major diagnostic value in these conditions, although there is current research looking at the potential use of bone-derived ACP as a marker of osteoclastic activity in osteoporosis .

LECTURE -8



ıical relation between the nephron and the juxtaglomerular apparatus.

Renal Function Tests

The kidneys excrete metabolic waste products ,and have an essential homeostatic function in that they control the body solute and water status and the acid–base balance. There are about one million nephrons per kidney.

The glomeruli, in the cortex of the kidney, are invaginated and surround a capillary network of blood vessels derived from the afferent, and draining into the efferent, arterioles. Small molecules and water are passively filtered during the passage of blood through these capillaries, the ultrafiltrate passing through the vessel walls and the glomerular membranes into the glomerular spaces (Bowman's capsules).

Normal function of the kidneys depends on the following:

- an adequate blood supply, which under normal circumstances is about 20 per cent of the cardiac output, flowing through the kidneys,

- normal secretion and feedback control of hormones acting on the kidney,

- the integrity of the glomeruli and the tubular cells.

In addition to the excretory function and acid– base control, the kidneys have important endocrine functions, including:

1- production of 1,25-dihydroxyvitamin D, the active metabolite of vitamin D, which is produced following hepatic hydroxylation of 25-hydroxyvitamin by the renal enzyme 1-hydroxylase,

2- production of erythropoietin, which stimulates erythropoiesis.

Renal Glomerular Function

About 200 L of plasma ultrafiltrate usually enter the tubular lumina daily, mainly by glomerular filtration into glomerular capsules but also through the spaces between cells lining the tubules (tight junctions).

Production of ultrafiltrate depends on the blood flow through normal glomeruli and on the difference between the hydrostatic pressure gradient and the plasma effective colloid osmotic (oncotic) pressure gradient across the membranes and tight junctions. The colloid osmotic effect is weak relative to the hydrostatic gradient but does facilitate some reabsorption of fluid from the proximal renal tubules.

The huge volume of filtrate allows adequate elimination of waste products such as urea; death from water and electrolyte depletion would occur within a few hours were the bulk of this water containing essential solutes not reclaimed.

Renal Tubular Function

Changes in filtration rate alter the total amount of water and solute filtered, but not the composition of the filtrate. From the 200 L of plasma filtered daily, only about 2 L of urine are formed. The composition of urine differs markedly from that of plasma, and therefore of the filtrate. The tubular cells use adenosine triphosphate-dependent active transport, sometimes selectively, against physicochemical gradients. Transport of charged ions tends to produce an electrochemical gradient that inhibits further transport. This is minimized by two processes.

Isosmotic transport :This occurs mainly in the proximal tubules and reclaims the bulk of filtered essential constituents.

Active transport of one ion leads to passive movement of an ion of the opposite charge in the same direction, along the electrochemical gradient. The movement of sodium (Na^+) depends on the availability of diffusible negatively charged ions, such as chloride (Cl^-). The process is 'isosmotic' because the active transport of solute causes equivalent movement of water reabsorption in the same direction. Isosmotic transport also occurs to a lesser extent in the distal part of the nephron. **Ion exchange** : occurs mainly in the more distal parts of the nephrons and is important for fine adjustment after bulk reabsorption has taken place. Ions of the same charge, usually cations, are exchanged and neither electrochemical nor osmotic gradients are created. Therefore, during cation exchange there is insignificant net movement of anions or water. For example, Na^+ may be reabsorbed in exchange for potassium (K^+) or hydrogen (H^+)

ions. Na+ and H+ exchange also occurs proximally, but at that site it is more important for bicarbonate reclamation than for fine adjustment of solute reabsorption .

Other substances, such as phosphate and urate, are secreted into, as well as reabsorbed from, the tubular lumen. The tubular cells do not deal actively with waste products such as urea and creatinine to any significant degree. Most filtered urea is passed in urine (which accounts for most of the urine's osmolality), but some diffuses back passively from the collecting ducts with water; by contrast, some creatinine is secreted into the tubular lumen.

BIOCHEMISTRY OF RENAL DISORDERS

Renal dysfunction of any kind affects all parts of the nephrons to some extent, although sometimes either glomerular or tubular dysfunction is predominant. The net effect of renal disease on plasma and urine depends on the proportion of glomeruli to tubules affected and on the number of nephrons involved.

To understand the consequences of renal disease it may be useful to consider the hypothetical individual nephrons, first with a low glomerular filtration rate

(GFR) and normal tubular function, and then with tubular damage but a normal GFR. It should be emphasized that these are hypothetical examples, as

in clinical reality a combination of varying degree may exist.

Uraemia is the term used to describe a raised plasma urea concentration and is almost always accompanied by an elevated creatinine concentration also referred to as azotaemia (a raised nitrogen concentration).

Reduced Glomerular Filtration Rate with Normal Tubular Function

The total amounts of urea and creatinine excreted are affected by the GFR. If the rate of filtration fails to balance that of production, plasma concentrations

will rise. Phosphate and urate are released during cell breakdown. Plasma concentrations rise because less than normal is filtered. Most of the reduced amount reaching the proximal tubule can be reabsorbed, and the capacity for secretion is impaired if the filtered volume is too low to accept the ions; these factors further contribute to high plasma concentrations.

A large proportion of the reduced amount of filtered sodium is reabsorbed by isosmotic mechanisms; less than usual is then available for exchange with hydrogen and potassium ions distally. This has two main outcomes:

- reduced hydrogen ion secretion throughout the nephron: bicarbonate can be reclaimed only if hydrogen ions are secreted; plasma bicarbonate

concentrations will fall,

- reduced potassium secretion in the distal tubule, with potassium retention (potassium can still be reabsorbed proximally).

If there is a low GFR accompanied by a low renal blood flow:

- Systemic aldosterone secretion will be maximal: in such cases, any sodium reaching the distal tubule will be almost completely reabsorbed in exchange for H+ and K+, and the urinary sodium concentration will be low.

- ADH secretion will be increased: ADH acting on the collecting ducts allows water to be reabsorbed in excess of solute, further reducing urinary volume and increasing urinary osmolality well above that of plasma and reducing plasma sodium concentration.

This high urinary osmolality is mainly due to substances not actively dealt with by the tubules. For example, the urinary urea concentration will be well above that of plasma. This distal response will occur only in the presence of ADH; in its absence, normal nephrons will form a dilute urine. If the capacity of the proximal tubular cells to reabsorb solute, and therefore water, is normal, a larger proportion than usual of the reduced filtered volume will be reclaimed by isosmotic processes, thus further reducing urinary volume.

In summary, the findings in venous plasma and urine from the affected nephrons will be as follows.

Plasma

- High urea (uraemia) and creatinine concentrations.

- Low bicarbonate concentration, with low pH (acidosis).

- Hyperkalaemia.

- Hyperuricaemia and hyperphosphataemia.

Urine

- Reduced volume (oliguria).

- Low (appropriate) sodium concentration – only if renal blood flow is low, stimulating aldosterone secretion.

- High (appropriate) urea concentration and therefore a high osmolality – only if ADH secretion is stimulated.

LECTURE -9

Box 3.1 Some causes of acute kidney injury (AKI)

Pre-renal

Hypotension
Hypovolaemia
Decreased cardiac output
Renal artery stenosis + angiotensin-converting enzyme inhibitor
Hepatorenal syndrome

Renal or intrinsic renal disease

Acute tubular necrosis, e.g. hypotension, toxins, contrast media, myoglobinuria, sepsis, drugs, sustained pre-renal oliguria
Vasculitis
Glomerulonephritis
Drugs that are nephrotoxic, e.g non-steroidal anti-inflammatory drugs
Sepsis
Thrombotic microangiopathy or thromboembolism
Atheroembolism
Bence Jones proteinuria
Interstitial nephritis
Infiltration, e.g. lymphoma
Severe hypercalcaemia
Severe hyperuricaemia

Post-renal

Calculi
Retroperitoneal fibrosis
Prostate hypertrophy/malignancy
Carcinoma of cervix or bladder

Acute Kidney Injury

This was previously known as acute renal failure. In adults, oliguria is defined as a urine output of less than 400 mL/day, or less than 15 mL/h; it usually indicates

a low GFR and a rapid decline in renal function over hours to weeks, with retention of creatinine and nitrogenous waste products.

Acute oliguria with reduced GFR (pre-renal)

This is caused by factors that reduce the hydrostatic pressure gradient between the renal capillaries and the tubular lumen. A low intracapillary pressure is the most common cause. It is known as renal circulatory insufficiency (‘pre-renal uraemia’) and may be due to:

- intravascular depletion of whole blood (haemorrhage) or plasma volume (usually due to gastrointestinal loss), or reduced intake,

- reduced pressure as a result of the vascular dilatation caused by ‘shock’, causes of which include myocardial infarction, cardiac failure and intravascular haemolysis,

including that due to mismatched blood transfusion.

The patient is usually hypotensive and clinically volume depleted. If renal blood flow is restored within a few hours, the condition is reversible, but, the longer it

persists, the greater the danger of intrinsic renal damage. As most glomeruli are involved and tubular function is relatively normal, the biochemical findings in plasma and urine are those described earlier.

Uraemia due to renal dysfunction may be aggravated if there is increased protein breakdown as a result of tissue damage, a large haematoma or the presence of blood in the gastrointestinal lumen.

Intravenous amino acid infusion may have the same effect because the urea is derived, by hepatic metabolism, from the amino groups of amino acids. Increased tissue breakdown may also aggravate hyperkalaemia, hyperuricaemia and hyperphosphataemia.

Acute oliguria due to intrinsic renal damage , which may be due to:

- prolonged renal circulatory insufficiency,

- acute glomerulonephritis, usually in children – the history of a sore throat and the finding of red cells in the urine usually make the diagnosis obvious,

- septicaemia, which should be considered when the cause of oliguria is obscure,

- ingestion of a variety of poisons or drugs,

- myoglobulinuria .

- Bence Jones proteinuria .

One problem in the differential diagnosis of acute oliguria is distinguishing between renal circulatory insufficiency and intrinsic renal damage that may have followed it. Acute oliguric renal dysfunction often follows a period of reduced GFR and renal circulatory insufficiency.The oliguria is due to reduced cortical blood flow with glomerular damage, aggravated by back-pressure on the glomeruli due to obstruction to tubular flow by oedema. At this stage, the concentrations of

many constituents in plasma, such as urea and creatinine, are raised with hyperkalaemia; tubular damage results in an inappropriately dilute urine for the degree of hypovolaemia. Fluid must be given with caution, and only until volume depletion has been corrected; there is a danger of overloading the circulation.

During recovery, oliguria is followed by polyuria. When cortical blood flow increases, and as tubular oedema resolves, glomerular function recovers before that of the tubules. The biochemical findings gradually progress to those of tubular dysfunction until they approximate those for 'pure' tubular lesions. Urinary output is further increased by the osmotic diuretic effect of the high load of urea. The polyuria may cause water and electrolyte depletion. The initial hyperkalaemia may be followed by hypokalaemia. Mild acidosis (common to both glomerular and tubular disorders) persists until late.

Acute oliguria due to renal outflow obstruction (postrenal) .Oliguria or anuria (absence of urine) may occur in post-renal failure. The cause is usually, but not always, clinically obvious and may be due to the following:

- Intrarenal obstruction, with blockage of the tubular lumina by haemoglobin, myoglobin and, very rarely,urate or calcium. Obstruction caused by casts and oedema of tubular cells is usually the result of true renal damage.

- Extrarenal obstruction, due to calculi, neoplasms, for example prostate or cervix, urethral strictures or prostatic hypertrophy, any of which may cause sudden obstruction. The finding of a palpable bladder indicates urethral obstruction, and in males is most likely to be due to prostatic hypertrophy, although there are other, rarer, causes. Early correction of outflow obstruction may rapidly

increase the urine output. The longer it remains untreated, the greater the danger of ischaemic or pressure damage to renal tissue. Imaging studies such

as renal tract ultrasound may be useful to confirm post-renal obstruction .

Chronic kidney disease

Chronic renal dysfunction [defined as being reduced eGFR (estimated GFR), proteinuria, haematuria and/or renal structural abnormalities of more than 90 days' duration] is usually the end result of conditions such as diabetes

mellitus, hypertension, primary glomerulonephritis, autoimmune disease, obstructive uropathy, polycystic disease, renal artery stenosis, infections and tubular dysfunction and the use of nephrotoxic drugs .It is common, perhaps affecting about 13 per cent of the population. Acute or chronic renal dysfunction

can occur when angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) are given to patients with renal artery stenosis; a clue to this is an increase in plasma creatinine of about 20 per cent and/or a decrease in eGFR of about 15 per cent soon after initiation of the drug.

In most cases of acute oliguric renal disease there is diffuse damage involving the majority of nephrons. A patient who survives long enough to develop

chronic renal disease must have some functioning nephrons. Histological examination shows that not all nephrons are equally affected: some may be

completely destroyed and others almost normal.

Chronic renal dysfunction may pass through two main phases:

- an initially polyuric phase,

- subsequent oliguria or anuria, sometimes needing dialysis or renal transplantation.

Summary of stages through which CKD passes :

Stage Description GFR (mL/min per 1.73 m2) Metabolic features

1– Presence of kidney damage with normal or raised GFRb >90 Usually normal

2 Presence of kidney damage with mildly reduced GFR 60–89 Plasma urea and creatinine rise,PTH starts to rise

3– Moderately reduced GFR 30–59 Calcium absorption decreased

Lipoprotein lipase decreased Anaemia – erythropoietin decreased

4– Severely reduced GFR (pre–end stage) 15–29 Dyslipidaemia

,Hyperphosphataemia,Metabolic acidosis ,Hyperkalaemia and hyperuricaemia

5– End–stage kidney disease (may need dialysis or transplant) <15 Marked elevation of urea (uraemia) and creatinine

Table 3.2 Stages of renal dysfunction (chronic kidney disease)[a]

Stage	Description	GFR (mL/min per 1.73 m^2)	Metabolic features
1	Presence of kidney damage with normal or raised GFR[b]	>90	Usually normal
2	Presence of kidney damage with mildly reduced GFR	60–89	Plasma urea and creatinine rise
			PTH starts to rise
3	Moderately reduced GFR	30–59	Calcium absorption decreased
			Lipoprotein lipase decreased
			Anaemia – erythropoietin decreased
4	Severely reduced GFR (pre-end stage)	15–29	Dyslipidaemia
			Hyperphosphataemia
			Metabolic acidosis
			Hyperkalaemia and hyperuricaemia
5	End-stage kidney disease (may need dialysis or transplant)	<15	Marked elevation of urea (uraemia) and creatinine

Note that a suffix of 'p' with staging can be used if proteinuria is present.
[a]National Kidney Foundation.
[b]Such as proteinuria or haematuria.
GFR, glomerular filtration rate; PTH, parathyroid hormone.

Box 3.2 Some causes of chronic kidney disease

Diabetes mellitus
Nephrotoxic drugs
Hypertension
Glomerulonephritis
Chronic pyelonephritis
Polycystic kidneys
Urinary tract obstruction
Severe urinary infections
Amyloid and paraproteins
Progression from acute kidney injury
Severe hypothyroidism (rare)

Proteinuria

TABLE 25-1 Characterization of Proteinuria

Type of Proteinuria	Causes	Examples of Proteins Seen
Glomerular	Increased glomerular permeability	Progressively increasing excretion of higher molecular weight proteins as permeability increases (e.g., albumin, IgG)
Overflow	Increased plasma concentration of relatively freely filtered protein	Bence Jones protein Lysozyme Myoglobin
Tubular	Proximal tubular damage: decreased tubular reabsorptive capacity and/or release of intracellular components (e.g., due to nephrotoxic drugs)	α_1-Microglobulin β_2-Microglobulin Retinol binding protein Enzymuria (e.g., *N*-acetyl-β-D-glucosaminidase, alkaline phosphatase, α-glutathione-S-transferase)
	Decreased nephron number: increased filtered load per nephron	As above
	Distal tubular damage	Tamm-Horsfall glycoprotein π-Glutathione-S-transferase

NEPHRITIC SYNDROME

Nephrotic-range proteinuria is the loss of 3 grams or more per day of protein into the urine or, on a single spot urine collection, the presence of 2 g of protein per gram of urine creatinine. Nephrotic syndrome is the combination of nephrotic-range proteinuria with a low serum albumin level and edema. This comprises reduced eGFR, oedema, hypertension and proteinuria with significant haematuria

Nephrotic syndrome has many causes, including primary kidney diseases such as minimal-change disease, focal segmental glomerulosclerosis, and membranous glomerulonephritis. Nephrotic syndrome can also result from systemic diseases that affect other organs in addition to the kidneys, such as diabetes, amyloidosis,

and lupus erythematosus, in addition to postinfectious glomerulonephritis, e.g. post-streptococcal or immunoglobulin A (IgA) nephropathy..

DIAGNOSIS OF RENAL DYSFUNCTION

Glomerular function tests

As glomerular function deteriorates, substances that are normally cleared by the kidneys, such as urea and creatinine, accumulate in plasma.

For a substance (S) that is filtered by the glomerulus, but not reabsorbed from or secreted into the tubules,

$$\text{GFR} \times \text{plasma}[S] = \text{urinary}[S] \times \text{urine volume per unit time}$$

Thus, rearranging gives:

$$\text{GFR} = \frac{\text{urinary}[S] \times \text{urine volume per unit time}}{\text{plasma}[S]}$$

For endogenously produced substances such as creatinine, with its relatively constant production, the following equation can be used to calculate a clearance that acts as an approximation for GFR:

$$\text{Creatinine clearance (mL/min)} = \frac{\text{urinary [creatinine]} \times \text{urine volume (mL)}}{\text{plasma [creatinine]} \times \text{urine collection period (min)}}$$

The modification of diet in renal disease (MDRD) formula can be used to estimate GFR (eGFR) and has generally superseded the need to use creatinine clearances in clinical practice and is also used to titrate drug dosing in patients with renal impairment . It is suitable for use in black African-Americans, and there is no requirement for patient weight. It originally used age, gender, race (black or white), serum creatinine.

$$\text{GFR}\,(\text{mL/min}/1.73\,\text{m}^2) = 186 \times [\text{serum creatinine}\,(\text{mg/dL})]^{-1.154} \times (\text{age})^{-0.203} \times (1.210 \text{ if patient is black}) \times (0.742 \text{ if patient is female})$$

Cystatin C

Another endogenous substance that can be used as a marker of GFR is plasma cystatin C (Cys C), and its use may alleviate some of the problems associated with creatinine clearance determinations. This is a 13-kDa protein that is a member of the family of cystine proteinase inhibitors. Unlike other endogenous compounds such as creatinine, Cys C is not secreted by the renal tubules and does not return to the bloodstream after glomerular filtration. It has been suggested that plasma Cys C may approximate to the 'ideal' endogenous marker for GFR, as blood concentrations are independent of patient age and sex, although currently this test is not routinely available in most laboratories.

GFR = 84.69 ∗ Cystatin C –1.680 (0.948 if female)

Several proteins with molecular weights of less than 30 kDa are primarily cleared from the circulation by renal filtration and can be considered to be relatively freely filtered at the glomerulus. These include (1) α2-microglobulin, (2) RBP,

(3) α1-microglobulin, (4) β-trace protein, and (5) cystatin C. These proteins are filtered at the glomerulus, then are reabsorbed (and metabolized) in the proximal tubule or excreted into the urine; thus they are entirely eliminated from the circulation. Therefore they have the potential to meet the criteria for use as a marker of GFR. However, most of these have been shown to have serum concentrations that are influenced by other, non renal factors such as inflammation (α2-microglobulin) and liver disease (RBP, α1- microglobulin).

The relationship between circulating concentrations of these proteins shows the same curvilinear form as serum creatinine, but several groups have demonstrated

that cystatin C measurement may offer a more sensitive and specific means of monitoring changes in GFR than serum creatinine.

LECTURE- ONE

CHO Metabolism & Related Disorders

CHEMISTRY of CHO

The main monosaccharide hexoses are reducing sugars. Naturally occurring polysaccharides are long-chaincarbohydrates composed of glucose subunits.

_ *Starch*, found in plants, is a mixture of amylose (straight chains) and amylopectin (branched chains).

_ *Glycogen*, found in animal tissue, is a highly branched polysaccharide.

Functions of extracellular glucose

The main function of glucose is as a major tissue energy source. The simplified pathways of glycolysis and the Krebs cycle [tricarboxylic acid (TCA) cycle] .

The brain is highly dependent upon the extracellular glucose concentration for its energy supply; indeed, hypoglycaemia is likely to impair cerebral function or even lead to irreversible neuronal damage. This is because the brain cannot:

_ synthesize glucose,

_ store glucose in significant amounts,

_ metabolize substrates other than glucose and ketones

– plasma ketone concentrations are usually very low and ketones are of little importance as an energy source under physiological conditions,

_ **extract enough glucose from the extracellular fluid (ECF) at low concentrations for its metabolic needs ,because entry into brain cells is not facilitated by insulin.**

Normally the plasma glucose concentration remains between about 4 mmol/L and 10 mmol/L, despite the intermittent load entering the body from the diet. Themaintenance of plasma glucose concentrations below about 10 mmol/L minimizes loss from the body as well as providing the optimal supply to the tissues. Renal tubular cells reabsorb almost all the glucose filtered by the glomeruli, and urinary glucose concentration is normally too low to be detected by the usual tests.

Control of plasma glucose concentration

During normal metabolism, little glucose is lost unchanged from the body. Maintenance of plasma glucose concentrations within the relatively narrow range of 4–10 mmol/L, despite the widely varying input from the diet, depends on the balance between the glucose entering cells from the ECF and that leaving them into this compartment.

Hormones concerned with glucose homeostasis

Insulin

Insulin is the most important hormone controlling plasma glucose concentrations. A plasma glucose concentration of greater than about 5 mmol/L acting via the glucose transporter 2 stimulates insulin release from the pancreas β-cell. Human insulin [molecular weight (MW) 5808 Da] consists of 51 amino acids in two chains (A and B) joined by two disulfide bridges, with a third disulfide bridge within the A chain. The basal

insulin secretory rate is about 1 U (43 μg)/h, with total daily secretion of about 40 U. The half-life of insulin in the circulation is between 4 and 5 minutes.

Insulin synthesis:

Preproinsulin, a protein of about 100 amino acids (MW 12,000 Da), is formed by ribosomes in the rough endoplasmic reticulum of the pancreatic β-cells

Preproinsulin is not detectable in the circulation under normal conditions because it is rapidly converted by cleaving enzymes to proinsulin (MW 9000 Da), an 86 amino acid polypeptide.

This is stored in secretory granules in the Golgi complex of the β-cells, where proteolytic cleavage to insulin and connecting peptide (C-peptide) occurs.

Cleavage of proinsulin is catalyzed by two Ca2+-regulated endopeptidases: prohormone convertases 1 and 2 (PC1 and PC2).

Release

Glucose, amino acids, pancreatic and gastrointestinal hormones (e.g., glucagon, gastrin, secretin, pancreozymin, gastrointestinal polypeptide), and some medications (e.g., sulfonylureas, β-adrenergic agonists) stimulate insulin secretion.

Insulin release is inhibited by hypoglycemia, somatostatin (produced in the pancreatic δ-cells), and various drugs(e.g., α-adrenergic agonists, β-adrenergic blockers, diazoxide, phenytoin, phenothiazines, nicotinic acid).184 In healthy individuals, insulin is secreted in a pulsatile fashion, with glucose and insulin the main signals in the feedback loop.

Glucose elicits the release of insulin from the pancreas in two phases. The first phase begins 1 to 2 minutes after intravenous injection of glucose and ends within 10 minutes. This phase, illustrated by the sharp spike in Figure 46-4, A, represents

the rapid release of stored insulin. The second phase, beginning at the point where the first phase ends, depends on continuing insulin synthesis and release and lasts until normoglycemia has been restored, usually within 60 to 120 minutes. With progressive failure of β-cell function, the first phase insulin response to glucose is lost, but other stimuli such as glucagon or amino acids may be able to elicit this response. Although the second-phase insulin response is preserved in most patients with type 2 diabetes mellitus, both the first-phase response (Figure 46-4, B) and normal pulsatile insulin secretion174 are lost. In contrast, patients with type 1 diabetes mellitus exhibit minimal or no insulin response (Figure 46-4, C).

Insulin binds to specific cell surface receptors on muscle and adipose tissue, thus enhancing the rate of glucose entry into these cells. Insulin-induced activation of enzymes stimulates glucose incorporation into glycogen (glycogenesis) in liver and muscle . Insulin also inhibits the production of glucose (gluconeogenesis) from fats and amino acids, partly by inhibiting fat and protein breakdown (lipolysis and proteolysis). The transport of glucose into liver cells is insulin independent but, by reducing the intracellular glucose concentration, insulin does indirectly promote the passive diffusion of glucose into them. Insulin also directly increases the transport of amino acids, potassium and phosphate into cells, especially muscle; these processes are independent of glucose transport. In the longer term, insulin regulates growth and development and the expression of certain genes.

Glucagon

Glucagon is a single-chain polypeptide synthesized by the α-cells of the pancreatic islets. Its secretion is stimulated by hypoglycaemia. Glucagon enhances hepatic glycogenolysis (glycogen breakdown) and gluconeogenesis .

Somatostatin

Somatostatin, also called growth hormone–inhibiting hormone,is a 14 amino acid peptide found in the gastrointestinal tract, the hypothalamus, and the δ-cells of the pancreatic islets . Although somatostatin does not appear to have a direct effect on carbohydrate metabolism, it inhibits the release of growth hormone from the pituitary. In addition, somatostatin inhibits secretion of glucagon and insulin by the pancreas, thus modulating the reciprocal relationship between these two hormones .This peptide hormone is released from the D cells of the pancreas and inhibits insulin and growth hormone release.

Other hormones

When plasma insulin concentrations are low, for example during fasting, the hyperglycaemic actions of hormones, such as growth hormone (GH), glucocorticoids, adrenaline (epinephrine) and glucagon, become apparent, even if there is

no increase in secretion rates. Secretion of these so-called counterregulatory - hormones may increase during stress and in patients with acromegaly , Cushing's syndrome or in phaeochromocytoma and thus oppose the normal action of insulin.

Lecture 2

Role of liver in glucose homeostasis

The liver is the most important organ maintaining a constant glucose supply for other tissues, including the brain. It is also

of importance in controlling the postprandial plasma glucose concentration.

Portal venous blood leaving the absorptive area of the intestinal wall reaches the liver first, and consequently the hepatic cells are in a key position to buffer the hyperglycaemic effect of a high-carbohydrate meal .The entry of glucose into liver and cerebral cells is not directly affected by insulin, but depends on the extracellular glucose concentration. The conversion of glucose to glucose-6-phosphate (G6P), the first step in glucose metabolism in all cells, is catalysed in the liver by the enzyme glucokinase, which has a low affinity for glucose compared with that of hexokinase, which is found in most other tissues. Glucokinase activity is induced by insulin.

Therefore, hepatic cells extract proportionally less glucose during fasting, when concentrations in portal venous plasma are low, than after carbohydrate ingestion. This helps to maintain a fasting supply of glucose to vulnerable tissues such as the brain.

The liver cells can store some of the excess glucose as glycogen. The rate of glycogen synthesis (glycogenesis) from G6P may be increased by insulin secreted by the β-cells of the pancreas in response to systemic hyperglycaemia. The liver can convert some of the excess glucose to fatty acids, which are ultimately transported as triglyceride in very low-density lipoprotein (VLDL) and stored in adipose tissue.

The liver contains the enzyme glucose-6-phosphatase, which by hydrolysing G6P derived from either glycogenolysis or gluconeogenesis, releases glucose and helps tomaintain extracellular fasting concentrations. Hepatic glycogenolysis is stimulated by the hormone glucagon, secreted by the a-cells of the pancreas in response to a fall in the plasma glucose concentration, and by catecholamines such as adrenaline or noradrenaline.

During feeding state the liver modifies the potential hyperglycaemic effect of a high-carbohydrate meal by extracting relatively more glucose than in the fasting state from the portal plasma. However, some glucose does pass through the liver and the rise in the systemic concentration stimulates the b-cells of the pancreas to secrete insulin.

Insulin may further enhance hepatic and muscle glycogenesis. More importantly, entry of glucose into adipose tissue and muscle cells, unlike that into liver and brain, is stimulated by insulin and, under physiological conditions, the plasma glucose concentration falls to near fasting levels. Conversion of intracellular glucose to G6P in adipose and muscle cells is catalysed by the enzyme hexokinase, which, because its affinity for glucose is greater than that of hepatic glucokinase, ensures that glucose enters the metabolic pathways in these tissues at lower extracellular concentrations than those in the liver. The relatively high insulin activity after a meal also inhibits the breakdown of triglyceride (lipolysis) and protein

(proteolysis). If there is relative or absolute insulin deficiency, as in diabetes mellitus, these actions are impaired.

Role of other organs in glucose homeostasis

The renal cortex is the only other tissue capable of gluconeogenesis, and of converting G6P to glucose. The gluconeogenic capacity of the kidney is particularly important in hydrogen ion homeostasis and during prolonged fasting.

Other tissues, such as muscle, can store glycogen but, because they do not contain glucose-6-phosphatase,they cannot release glucose from cells and so can only use it locally; this glycogen plays no part in maintaining the plasma glucose concentration.

Ketosis

During fasting, when exogenous glucose is unavailable and the plasma insulin concentration is therefore low, endogenous triglycerides are reconverted to free non-esterifi ed fatty acids (NEFAs) and glycerol by lipolysis . Both are transported to the liver in plasma, the NEFA being protein bound, predominantly to albumin. Glycerol enters the hepatic gluconeogenic pathway at the triose phosphate stage; the glucose synthesized can be released from these cells, thus minimizing the fall in glucose concentrations . Ketosis occurs when fat stores are the main energy source and may result from fasting or from reduced nutrient absorption, for example due to vomiting. Mild ketosis may occur after as little as 12 h of fasting. After short fasts, metabolic acidosis is not usually

detectable, but, after longer periods, more hydrogen ions may be produced than can be dealt with by homeostatic buffering mechanisms, depleting the plasma bicarbonate concentration, which therefore falls .

The plasma glucose concentration is maintained principally by hepatic gluconeogenesis, but during prolonged starvation, such as that in anorexia nervosa or during childhood, ketotic hypoglycaemia may occur.

lactic acidosis

Lactic acid, produced by anaerobic glycolysis, may either be oxidized to CO2 and water in the TCA cycle or be reconverted to glucose by gluconeogenesis in the liver.

Both the TCA cycle and gluconeogenesis need oxygen; anaerobic glycolysis is a non-oxygen-requiring pathway. Pathological accumulation of lactate may occur because:

1-production is increased by an increased rate of anaerobic glycolysis,

2- use is decreased by impairment of the TCA cycle or impairment of gluconeogenesis.

Tissue hypoxia due to the poor tissue perfusion of the 'shock' syndrome is usually the most common cause of lactic acidosis. Hypoxia increases plasma lactate concentrations because:

_ the TCA cycle cannot function anaerobically and oxidation of pyruvate and lactate to CO2 and water is impaired,

_ hepatic and renal gluconeogenesis from lactate cannot occur anaerobically,

_ anaerobic glycolysis is stimulated because the falling adenosine triphosphate (ATP) levels cannot be regenerated by the TCA cycle under anaerobic conditions. The combination of impaired gluconeogenesis and increased anaerobic glycolysis converts the liver from an organ that consumes lactate and H^+ to one that generates large amounts of lactic acid. Severe hypoxia, for example following a cardiac arrest, causes marked lactic acidosis. If diabetic ketoacidosis is associated with significant volume depletion, this hypoxic syndrome may aggravate the acidosis.

Glucose Transport

The transport of glucose into cells is modulated by two families of proteins. The sodium-dependent glucose transporters (SGLTs) use the electrochemical sodium gradient to transport glucose against its concentration gradient. SGLTs promote the uptake of glucose and galactose from the lumen of the small bowel and their reabsorption from urine in the kidney. Members of the second family of glucose carriers arecalled *facilitative glucose transporters* (GLUT) . These transporters are designated GLUT1 to GLUT14, based on the order in which they were identified. Eleven have been shown to catalyze sugar transport. They can be divided into three classes, based on sequence similarities and characteristics.

The best characterized are class I. Less is known about those in classes II and III. GLUT1 is widely expressed and provides many cells with their basal glucose requirement.

GLUT1 in the blood-brain barrier and GLUT3 in neuronal cells provide the constant high concentrations of glucose

required by the brain. GLUT2 is expressed in hepatocytes, β-cells of the pancreas, and basolateral membranes of intestinal and renal epithelial cells. It is a low-affinity, high-capacity transport system that allows non–rate-limiting movement of glucose into and out of these cells. GLUT4 catalyzes the rate limiting step for glucose uptake and metabolism in skeletal muscle, the major organ of glucose consumption. GLUT4 is also present in adipose tissue.

When circulating insulin concentrations are low, most of the GLUT4 is localized in intracellular compartments and is inactive. After eating, the pancreas releases insulin, which stimulates the translocation of GLUT4 to the plasma membrane, thereby promoting glucose uptake into skeletal muscle and fat. Insulin-stimulated glucose transport into skeletal muscle is defective in type 2 diabetes mellitus, but the mechanism has not been established.

TABLE 46-1 Facilitative Human Glucose Transporters

Name	Class	Tissue	Function
GLUT1	I	Wide distribution, especially brain, kidney, colon, and fetal tissues	Basal glucose transport
GLUT2	I	Liver, β-cells of pancreas, small intestine, and kidney	Non–rate-limiting glucose transport
GLUT3	I	Wide distribution, especially neurons, placenta, and testis	Glucose transport in neurons
GLUT4	I	Skeletal muscle, cardiac muscle, adipose tissue	Insulin-stimulated glucose transport
GLUT5	II	Small intestine, kidney, skeletal muscle, brain,	Transports fructose (not

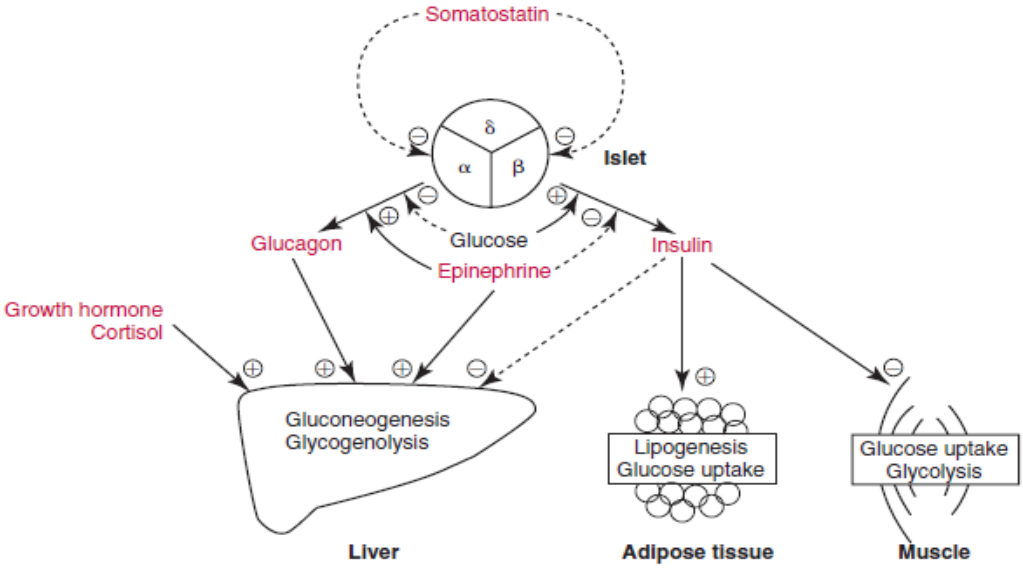


Figure 46-1 Hormonal regulation of blood glucose. Key: +, stimulation; –, inhibition. Cortisol, growth hormone, and epinephrine antagonize the effects of insulin.

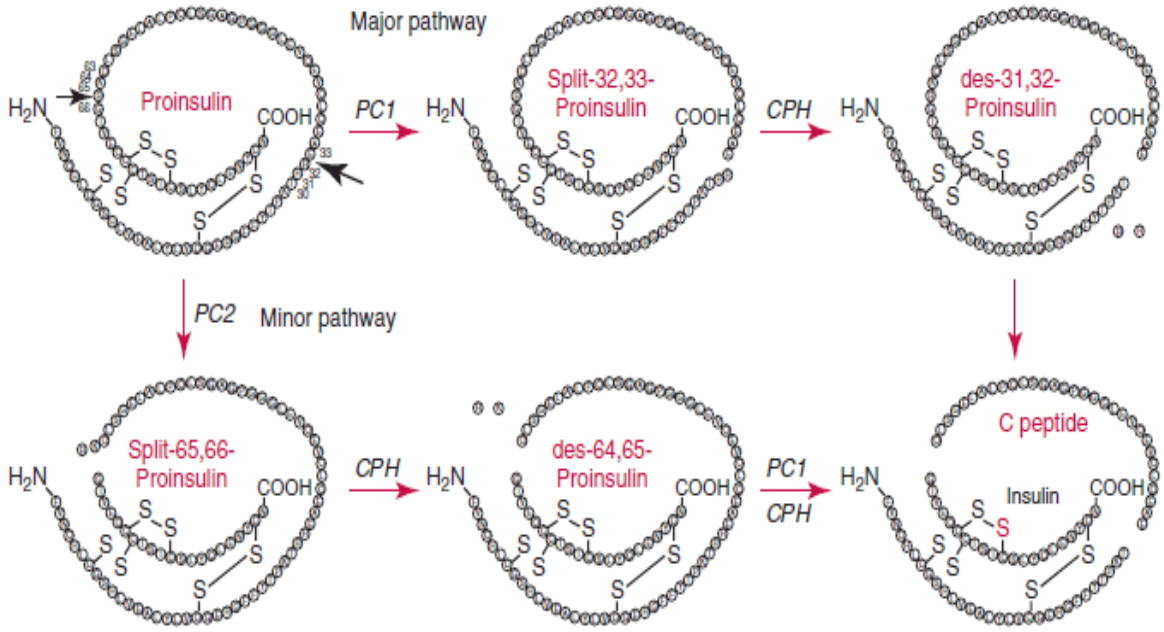


Figure 46-3 Processing of proinsulin. The enzymes prohormone convertase 1 and 2 (PC1 and PC2) act on proinsulin to form the appropriate split proinsulins. Carboxypeptidase-H (CPH) removes the two exposed basic amino acid residues *(circles)*.

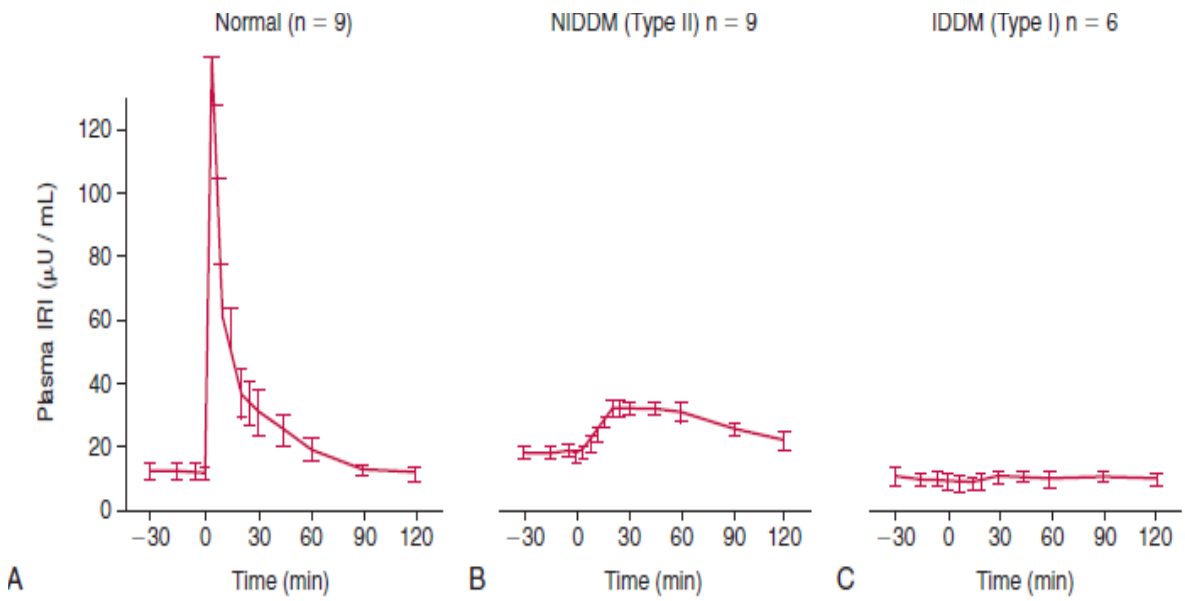


Figure 46-4 **Response of plasma insulin to glucose stimulation. A 20 g glucose pulse is given intravenously at time 0. A, Healthy subjects. B, Patients with type 2 diabetes mellitus (NIDDM). C, Patients with type 1 diabetes mellitus (IDDM). *IRI,* Immunoreactive insulin. Values before time 0 represent baseline.** *(From Pfeifer MA, Halter JB, Porte D Jr. Insulin secretion in diabetes mellitus. Am J Med 1981;70:579-88.)*

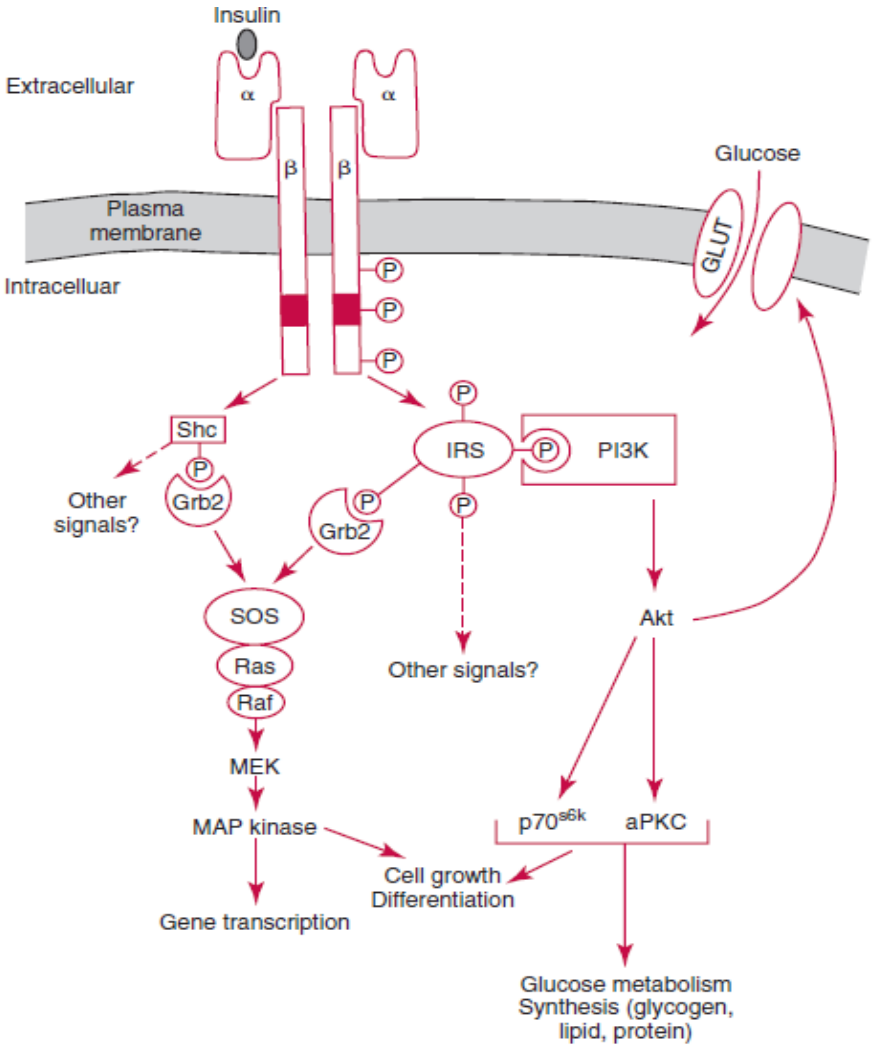


Figure 46-5 **Mechanism of insulin action. Binding of insulin to the extracellular α-subunit of the insulin receptor induces autophosphorylation of the β-subunit of the receptor and phosphorylation of selected intracellular proteins, such as Shc and the insulin-receptor substrate (IRS) family. These latter phosphoproteins interact with other targets, thereby activating phosphorylation cascades, which result in glucose uptake (in adipose tissue and skeletal muscle), glucose metabolism, synthesis (of glycogen, lipid, and proteins), enhanced gene expression, cell growth, and differentiation. *aPKC*, Atypical protein kinase C; *p*, protein phosphorylation. See text for details.**

LECTURE -3

Insulin-like Growth Factors (IGFs)

Insulin-like growth factors 1 and 2 (IGF-1 and IGF-2) are polypeptides structurally related to insulin. These hormones (previously referred to as *non-suppressible insulin-like activity* or *somatomedin*) exhibit metabolic and growth promoting effects similar to those of insulin. Accumulating evidence implicates the IGF axis in the development of several common cancers. IGF-1 (previously known as somatomedin C) is an important mediator of growth hormone action and is one of the major regulators of cell growth and differentiation.

The physiologic role of IGF-2 is not known. Synthesis of IGF-1 depends on growth hormone and occurs predominantly in the liver. In addition, many other cells produce IGF-1 that does not enter the circulation but acts locally. Circulating IGF concentrations are approximately 1000-fold higher than insulin concentrations, and the hormone is kept inactive by binding to a family of at least six specific binding proteins. These proteins regulate IGF by protecting the ligands in the circulation and delivering them to their target tissue. In contrast to insulin, which is unbound in the circulation, less than 10% of total serum IGF-1 is free. The biological actions of IGF are exerted through specific IGF receptors or the insulin receptor. The IGF-1 receptor is closely related to the insulin receptor in structure and biochemical properties. While , the IGF-2 receptor is quite different; it lacks tyrosine

kinase activity, and its physiologic relevance is not understood. The IGF-1 receptor has a high affinity for both IGF-1 and IGF-2, but a low affinity for insulin. The IGF-2 receptor has high, low, and no affinity for IGF-2, IGF-1, and insulin, respectively. The insulin receptor binds insulin with high affinity and IGF-1 and IGF-2 with low affinity .

HYPERGLYCAEMIA AND DIABETES MELLITUS

Hyperglycaemia may be due to:

_ intravenous infusion of glucose-containing fluids,

_ severe stress (usually a transient effect) such as trauma, myocardial infarction or cerebrovascular accidents,

_ diabetes mellitus or impaired glucose regulation

Diabetes mellitus

Diabetes mellitus is caused by an absolute or relative insulin deficiency. It has been defined by the World Health Organization (WHO), on the basis of laboratory findings, as a fasting venous plasma glucose concentration of 7.0 mmol/L or more (≥126 mg/dl) ,on more than one occasion or once in the presence of diabetes symptoms, or a random venous plasma glucose concentration of 11.1 mmol/L or more (≥200 mg/dl). Or HbA1C values ≥ 6.5 % .

Sometimes an oral glucose tolerance test (OGTT) may be required to establish the diagnosis in equivocal cases.

BOX 46-3 Criteria for the Diagnosis of Diabetes Mellitus

Any one of the following is diagnostic:

A. Glucose
   1. Fasting plasma glucose (FPG) ≥7.0 mmol/L (126 mg/dL)*
   OR
   2. Symptoms of hyperglycemia and casual plasma glucose ≥11.1 mmol/L (200 mg/dL)†
   OR
   3. 2 hour plasma glucose ≥11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (OGTT)‡

B. Hemoglobin A1c (HbA_{1c})§
   $HbA_{1c} \geq 6.5\%$

In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeating the same test on a different day. Mixing different methods to diagnose diabetes should be avoided.

*Fasting is defined as no calorie intake for at least 8 hours.

†Casual is defined as any time of day without regard to time since last meal. The classic symptoms of hyperglycemia include polyuria, polydipsia, and unexplained weight loss.

‡The OGTT should be performed as described by the World Health Organization (WHO), using a glucose load containing the equivalent of 75 g of anhydrous glucose dissolved in water.

§The test should be performed in a laboratory that is NGSP-certified and standardized to the DCCT assay. Point-of-care assays should not be used for diagnosis.

From the American Diabetes Association. Standards of medical care in diabetes—2010. Diabetes Care 2010;33(Suppl 1):S11-61.

Diabetes mellitus can be classified into the following categories :

1-Type 1 diabetes mellitus (T1DM)

Previously called insulin-dependent diabetes mellitus, this is the term used to describe the condition in patients for whom insulin therapy is essential because they are prone to develop ketoacidosis. It usually presents during childhood or adolescence. Most of these cases are due to immune-mediated processes and may be associated with other autoimmune disorders such as Addison's disease, vitiligo and Hashimoto's thyroiditis.

The most practical markers of β-cell autoimmunity are circulating antibodies, which have been detected in the serum years before the onset of hyperglycemia. The best characterized antibodies are as follows :

1. *Islet cell cytoplasmic antibodies* (ICAs) These antibodies are detected in the serum of 0.5% of normal subjects and 75 to 85% of patients with newly diagnosed type 1 diabetes.
2. *Insulin autoantibodies* (IAAs) are present in more than 90% of children who develop type 1 diabetes before age 5, but in less than 40% of individuals who develop diabetes after age 12.

3. *Antibodies to the 65 kDa isoform of glutamic acid decarboxylase*(GAD65) have been found up to 10 years before the onset of clinical type 1 diabetes and are present in ≈60% of patients with newly diagnosed diabetes. GAD65 antibodies may be used to identify patients with apparent type 2 diabetes who will subsequently progress to type 1 diabetes.
4. *Insulinoma-associated antigens* (IA-2A and IA-2βA),detected in more than 50% of newly diagnosed type 1 diabetes patients.

5. *Zinc transporter ZnT8* was identified recently as a major autoantigen in type 1 diabetes, ZnT8 in 60 to 80% of patients with new-onset type 1 diabetes ,compared with less than 3% of individuals with type 2 diabetes.

2-Type 2 Diabetes Mellitus (T2DM)

Previously called non-insulin-dependent diabetes mellitus, this is the most common variety worldwide (about 90 per cent of all diabetes mellitus cases). Patients are much less likely to develop ketoacidosis than those with type 1 diabetes, there is a familial tendency and an association with obesity. There is a spectrum of disorders ranging from mainly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance.

3-Other Specific Types Of Diabetes Mellitus

A variety of inherited disorders may be responsible for the syndrome, either by reducing insulin secretion or by causing relative insulin deficiency because of resistance to its action or of insulin receptor defects, despite high plasma insulin concentrations.

a-Genetic defects of b*-cell function*

_ Maturity-onset diabetes of the young (MODY):

– MODY 1: mutation of the hepatocyte nuclear factor (*HNF4A*) gene,

– MODY 2: mutation of the glucokinase gene,

– MODY 3: mutation of the *HNF1A* gene.

Some cases are thought to be point mutations in mitochondrial deoxyribonucleic acid (DNA) associated with diabetes mellitus and deafness and are usually autosomal dominant.

b-Genetic defects of insulin action

_ Type A insulin resistance (insulin receptor defect),for example leprechaunism, lipoatrophy and Rabson–Mendenhall syndrome.

c-Insulin deficiency due to pancreatic disease

_ Chronic pancreatitis.

_ Pancreatectomy.

_ Haemochromatosis.

_ Cystic fibrosis.

d-Endocrinopathies

_ Relative insulin deficiency, due to excessive GH (acromegaly), phaeochromocytoma, glucocorticoid secretion (Cushing's syndrome).

e-Drugs

_ Thiazide diuretics.

_ Interferon-a.

_ Glucocorticoids.

f-Infections

_ Septicaemia.

_ Congenital rubella.

_ Cytomegalovirus.

g-Rare forms of autoimmune-mediated diabetes

_ Anti-insulin receptor antibodies.

_ Stiff man syndrome, with high levels of GAD autoantibodies.

h-Genetic syndromes associated with diabetes

_ Down's syndrome.

_ Turner's syndrome.

_ Klinefelter's syndrome.

_ Myotonic dystrophy.

4- Gestational Diabetes Mellitus (GDM)

It is first to be diagnosed during gestation ,(i.e., diabetic women who become pregnant are not included in this category).Estimates of the frequency of abnormal glucose tolerance during pregnancy range from 1 to 14%, depending on the population studied and the diagnostic tests employed. Women with GDM are at significantly increased risk for the subsequent development of type 2 diabetes mellitus , who are obese, and those whose GDM was diagnosed before 24 weeks′ gestation.

GDM is associated with increased fetal abnormalities, for example high birth weight, cardiac defects and polyhydramnios. In addition, birth complications , maternal hypertension and the need for caesarean section may occur. If maternal diet/lifestyle factors fail to restore glucose levels, insulin is usually required to try to reduce the risk of these complications.

Women at high risk for GDM include those who have had GDM before, have previously given birth to a high-birthweight baby, are obese, have a family history of diabetes mellitus .These women should be screened at the earliest opportunity and, if normal, retested at about 24–28 weeks, as glucose tolerance progressively deteriorates throughout pregnancy. Where 50 g oral glucose is

used and the blood glucose is sampled at 1 h – plasma glucose of more than or equal to 7.8 mmol/L being diagnostic (O'Sullivan's screening test for gestational diabetes). If fasting venous plasma glucose is 7.0 mmol/L or more and/or the random measurement gives a concentration of 11.1 mmol/L or more (some doctors prefer to use a lower cut-off of about 9.0 mmol/L in pregnancy), the woman has GDM.

Impaired glucose tolerance

The WHO definition of impaired glucose tolerance (IGT) is a fasting venous plasma glucose concentration of less than 7.0 mmol/L and a plasma glucose concentration between 7.8 mmol/L and 11.1 mmol/L 2 h after an OGTT.

Some patients with IGT develop diabetes mellitus later and may require an annual OGTT to monitor for this. However, because of the increased risk of vascular complications, secondary causes of IGT should be sought, dietary advice given, if necessary, and the patient followed up. In pregnancy IGT is treated as GDM because of the risks to the fetus.

Impaired fasting glucose

Impaired fasting glucose (IFG), like IGT, refers to a metabolic stage intermediate between normal glucose homeostasis and diabetes mellitus. The definition is that the fasting venous plasma glucose is 6.1 mmol/L or more but less than 7.0 mmol/L, and less than 7.8 mmol/L 2 h after an OGTT.

BOX 46-3 Criteria for the Diagnosis of Diabetes Mellitus

Any one of the following is diagnostic:

A. Glucose
   1. Fasting plasma glucose (FPG) ≥7.0 mmol/L (126 mg/dL)*
   OR
   2. Symptoms of hyperglycemia and casual plasma glucose ≥11.1 mmol/L (200 mg/dL)†
   OR
   3. 2 hour plasma glucose ≥11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (OGTT)‡

B. Hemoglobin A1c (HbA_{1c})§
   HbA_{1c} ≥ 6.5%

In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeating the same test on a different day. Mixing different methods to diagnose diabetes should be avoided.

*Fasting is defined as no calorie intake for at least 8 hours.

†Casual is defined as any time of day without regard to time since last meal. The classic symptoms of hyperglycemia include polyuria, polydipsia, and unexplained weight loss.

‡The OGTT should be performed as described by the World Health Organization (WHO), using a glucose load containing the equivalent of 75 g of anhydrous glucose dissolved in water.

§The test should be performed in a laboratory that is NGSP-certified and standardized to the DCCT assay. Point-of-care assays should not be used for diagnosis.

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LECTURE-4

Insulin Resistance Syndrome (Metabolic Syndrome)

It has been recognized that certain coronary heart disease risk factors occur together. There is an aggregation of lipid and non-lipid risk factors of metabolic origin. A particular cluster is known as the metabolic syndrome, syndrome X or Reaven's syndrome and is closely linked to insulin resistance. One definition is the presence of three or more of the following features:

_ Abdominal obesity (waist circumference):

- male more than 102 cm (40 in),
- female more than 88 cm (35 in).

_ Fasting plasma triglycerides more than 1.7 mmol/L.

_ Fasting plasma high-density lipoprotein (HDL) cholesterol:

– male less than 1.0 mmol/L,

– female less than 1.3 mmol/L,

_ Blood pressure more than or equal to 130/85 mmHg.

_ Fasting blood glucose more than 5.5 mmol/L.

Plasma levels of insulin would be expected to be raised, that is, hyperinsulinaemia. Other associated features may include polycystic ovary syndrome, fatty liver, raised fibrinogen and plasminogen activator inhibitor 1 concentrations, renal sodium retention, hyperuricaemia and dense low-density lipoprotein (LDL) particles .

Metabolic Features of Diabetes Mellitus

I -Hyperglycaemia

If plasma glucose concentration exceeds about 10 mmol/L, glycosuria would be expected. High urinary glucose concentrations produce an osmotic diuresis and therefore polyuria. Cerebral cellular dehydration due to hyperosmolality, secondary to hyperglycaemia, causes thirst (polydipsia). A prolonged osmotic diuresis may cause excessive urinary electrolyte loss. These 'classic' symptoms are suggestive of diabetes mellitus. Diabetic patients on insulin may show the following conditions. The 'dawn' phenomenon is the physiological response of the elevation of blood glucose concentration in the early morning prior to breakfast due to nocturnal spikes in GH concentration and a rise in plasma cortisol concentration that increase hepatic gluconeogenesis. Conversely, in some diabetic patients nocturnal hypoglycaemia may evoke a rebound counter-regulatory hyperglycaemia called the Somogyi phenomenon. Patient blood glucose checking at 02.00– 04.00 h, or continuous glucose monitoring if available, may distinguish these conditions, as the Somogyi phenomenon reveals hypoglycaemia.

2-Abnormalities in lipid metabolism

These may be secondary to insulin deficiency. Lipolysis is enhanced and plasma NEFA concentrations rise.In the liver, NEFAs are converted to acetyl CoA and ketones, or are re-esterified to form endogenous triglycerides and incorporated into VLDLs; the latter accumulate in plasma because lipoprotein lipase, which Is necessary for VLDL catabolism, requires insulin for optimal activity.

High-density lipoprotein cholesterol concentration tends to be low in type 2 diabetes. If insulin deficiency is very severe, there may also be chylomicronaemia. The rate of cholesterol synthesis is also increased, with an associated increase in plasma LDL concentrations. Consequently, patients with diabetes may show high plasma triglyceride, raised cholesterol and low HDL cholesterol concentrations.

Pathogenesis OF **CHRONIC COMPLICATIONS OF DIABETES MELLITUS**

Patients with both type 1 and type 2 diabetes are at high risk for the development of chronic complications. Diabetes-specific microvascular pathology in the retina, renal glomerulus, and peripheral nerve produces retinopathy, nephropathy, and neuropathy. As a result of these microvascular complications, diabetes is the most frequent cause of new cases of blindness in the industrialized world in persons between 25 and 74 years and the leading cause of end-stage renal disease. Diabetes is also associated with a marked increase in atherosclerotic macrovascular disease involving cardiac, cerebral, and peripheral large vessels. The consequence is that patients with diabetes have a high rate of myocardial infarction (the major cause of mortality in diabetes), stroke, and limb amputation. Prospective clinical studies document a strong relationship between hyperglycemia and the development of microvascular complications.

Both hyperglycemia and insulin resistance appear to be important in the pathogenesis of macrovascular complications. Progress has been made in our understanding of the molecular mechanisms underlying derangements produced by hyperglycemia.

Four main hypotheses have been proposed to explain how hyperglycemia causes the neural and vascular pathology. These include:

1- **increased aldose reductase (or polyol pathway) flux;**
2- **enhanced formation of advanced glycation end products (AGE);**
3- **activation of protein kinase C;**
4- **increased hexosamine pathway flux.**

Inhibitors of each of these have been shown to ameliorate diabetes-induced abnormalities in cell culture and animal models. Overproduction of superoxide by the mitochondrial electron transport chain integrates these four apparently disparate mechanisms. Clinical trials are under way using novel therapies specifically directed at the signaling molecules (such as protein kinase C) or employing antioxidants to neutralize the effects of the oxidants.

Monitoring of Diabetes Mellitus

Glycosuria

Glycosuria can be defined as a concentration of urinary glucose detectable using relatively insensitive, but specific, screening tests. These tests often depend on the action of an enzyme, such as glucose oxidase, incorporated into a diagnostic strip. Glycosuria, occurs only when the plasma, and therefore glomerular filtrate, concentrations exceed the tubular reabsorptive capacity. This may be because the plasma and glomerular filtrate concentrations are more than about 10 mmol/L, and therefore the normal tubular reabsorptive capacity is significantly exceeded.

Very rarely, if the glomerular filtration rate is much reduced, there may be no glycosuria despite plasma glucose concentrations more than 10 mmol/L. A diagnosis of diabetes mellitus should *never* be made on the basis of glycosuria.

Blood glucose

Blood glucose concentrations may be measured using glucose testing reagent strips. The colour change ofthe strip can be assessed visually or by using a portable glucose meter and the reaction often involves an enzyme determination of glucose, for example glucose oxidase.

Glycated haemoglobin

Glycated haemoglobin (HbA1c) is formed by nonenzymatic glycation of haemoglobin and is dependent on the mean plasma glucose concentrations and on the lifespan of the red cell; falsely low values may be found in patients with haemolytic disease. Measurement of blood HbA1c may not reveal potentially dangerous short-term swings and nor does HbA1c detect hypoglycaemic episodes and thus plasma glucose estimations may also be useful.

This was expressed as a percentage of total blood haemoglobin concentration and gives a retrospective assessment of the mean plasma glucose concentration during the preceding 6–8 weeks. The higher the glycated haemoglobin, the poorer the mean diabetic or glycaemic control. Glycated haemoglobin used to be expressed in percentage units but now is expressed as mmol/mol .

Intervention trials for type 1 and type 2 diabetes have shown that trying to optimize glycaemic control, as judged by HbA1c < 6.5 % reduces the risk of microvascular diabetic complications.

Fructosamine

The measurement of plasma fructosamine concentrations may be used to assess glucose control over a shorter time course than that of HbA1c (about 2–4 weeks), but the assay has methodological limitations. Fructosamine reflects glucose bound to plasma proteins, predominantly albumin, which has a plasma half-life of about 20 days but is problematic in patients with hypoalbuminaemia, for example due to severe proteinuria. This assay may sometimes be useful in pregnancy and also if haemoglobin variants, for example HbS or HbC, exist that may interfere with certain HbA1c assays.

Acute metabolic complications of diabetes mellitus

Patients with diabetes mellitus may develop various metabolic complications that require emergency treatment,including coma, and these include the following.

Hypoglycaemia

This is probably the most common cause of coma seen in diabetic patients. Hypoglycaemia is most commonly caused by accidental overadministration of insulin or sulphonylureas or meglitinides. Precipitating causes include too high a dose of insulin or hypoglycaemicdrug; conversely, the patient may have missed a meal or taken excessive exercise after the usual dose of insulin or

oral hypoglycaemic drugs. Hypoglycaemia is particularly dangerous, and some patients lack awareness of this; that is to say, they lose warning signs such as sweating, dizziness and headaches. Driving is a major hazard under such circumstances. Patients should monitor their own .blood glucose closely, carry glucose preparations .

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Diabetic ketoacidosis

Diabetic ketoacidosis may be precipitated by infection, acute myocardial infarction or vomiting. The patient who reasons 'no food, therefore no insulin' could mistakenly withhold insulin. In the absence of insulin, there is increased lipid and protein breakdown, enhanced hepatic gluconeogenesis and impaired glucose entry into cells. The clinical consequences of diabetic ketoacidosis are due to:

_ hyperglycaemia causing plasma hyperosmolality,

_ metabolic acidosis,

_ glycosuria.

Plasma glucose concentrations are usually in the range 20–40 mmol/L, but may be considerably higher, although euglycaemic diabetic ketoacidosis has been described when plasma glucose concentrations are only slightly elevated. Hyperglycaemia causes glycosuria and hence an osmotic diuresis. Water and electrolyte loss due to vomiting, which is common in this syndrome, increases fluid depletion. There may be haemoconcentration and reduction of the glomerular filtration rate enough to cause uraemia due to renal circulatory insufficiency.

The extracellular hyperosmolality causes a shift of water out of the cellular compartment and severe cellular dehydration occurs. Loss of water from cerebral cells is probably the reason for the confusion and coma. Thus there is both cellular and extracellular volume depletion. The rate of lipolysis is increased because of decreased insulin activity; more free fatty acids are produced than can be metabolized by peripheral tissues. The free fatty acids are either converted to ketones by the liver or, of less immediate clinical importance, incorporated as endogenous triglycerides into VLDL, sometimes causing severe hypertriglyceridaemia.

Hydrogen ions, produced with ketones other than acetone, are buffered by plasma bicarbonate. However,when their rate of production exceeds the rate of bicarbonate generation, the plasma bicarbonate falls. Hydrogen ion secretion causes a fall in urinary pH. The deep, sighing respiration (Kussmaul's respiration) and the odour of acetone on the breath are classic features of diabetic ketoacidosis. Plasma potassium concentrations may be raised, secondarily to the metabolic acidosis, before treatment is started. This is due to failure of glucose entry into cells in the absence of insulin and because of the low glomerular filtration rate. Despite hyperkalaemia, there is a total body deficit due to increased urinary potassium loss in the presence of an osmotic diuresis.

During treatment, plasma potassium concentrations may fall as potassium re-enters cells, sometimes causing severe hypokalaemia unless potassium is prescribed. Plasma sodium concentrations may be low (hyponatraemia) or low-normal at presentation, partly because of the osmotic effect of the high extracellular glucose

concentration, which draws water from the cells and dilutes the sodium. In the presence of a very high plasma glucose concentration, a normal or raised plasma sodium concentration is suggestive of significant water depletion.

Hyperosmolal Non-Ketotic Coma

In diabetic ketoacidosis there is always plasma hyperosmolality due to the hyperglycaemia, and many of the symptoms, including those of confusion and coma, are related to it. However, the term 'hyperosmolal' coma or 'pre-coma' is usually confined to a condition in which there is marked hyperglycaemia but no detectable ketoacidosis. The reason for these different presentations is not clear. It has been suggested that insulin activity is sufficient to suppress lipolysis but insufficient to suppress hepatic gluconeogenesis or to facilitate glucose transport into cells. Hyperosmolal non-ketotic (HONK) coma now may be referred to as hyperosmolar hyperglycaemic state (HHS) and may be of sudden onset. It is more common in older patients. Plasma glucose concentrations may exceed 50 mmol/L. The effects of glycosuria are as described above, but hypernatraemia due to predominant water loss is more commonly found than in ketoacidosis and aggravates the plasma hyperosmolality. Cerebral cellular dehydration, which contributes to the coma, may also cause hyperventilation, and a respiratory alkalosis, although sometimes plasma lactic acid may rise, evoking a metabolic acidosis and hus a mixed acid–base disturbance may occur. There may also be an increased risk of thrombosis.

Lactic acidosis

Lactic acidosis can cause a high anion gap metabolic acidosis and coma. It may be due to the use of metformin in certain situations, such as high doses in elderly people. Lactic acid, produced by anaerobic glycolysis, may either be oxidized to CO2 and water in the TCA cycle or be reconverted to glucose by gluconeogenesis in the liver. Both the TCA cycle and gluconeogenesis need oxygen; anaerobic glycolysis is a non-oxygen-requiring pathway. Pathological accumulation of lactate may occur because:

_ production is increased by an increased rate of anaerobic glycolysis,

_ use is decreased by impairment of the TCA cycle or impairment of gluconeogenesis.

Tissue hypoxia due to the poor tissue perfusion of the 'shock' syndrome is usually the most common cause of lactic acidosis. Hypoxia increases plasma lactate concentrations because:

_ the TCA cycle cannot function anaerobically and oxidation of pyruvate and lactate to CO2 and water is impaired

The combination of impaired gluconeogenesis and increased anaerobic glycolysis converts the liver from an organ that consumes lactate and H+ to one that generates large amounts of lactic acid. Severe hypoxia, for example following a cardiac arrest, causes marked lactic acidosis. If diabetic ketoacidosis is associated with significant volume depletion, this hypoxic syndrome may aggravate the acidosis.

LECTURE -5

HYPOGLYCAEMIA

By definition, hypoglycaemia is present if the plasma glucose concentration is less than 2.5 mmol/L in a specimen collected into a tube containing an inhibitor of glycolysis, for example fluoride oxalate. Blood cells continue to metabolize glucose in vitro, and low concentrations found in a specimen collected without such an inhibitor can be dangerously misleading (pseudohypoglycaemia). Symptoms of hypoglycaemia may develop at higher concentrations if there has been a rapid fall from a previously raised value, when adrenaline secretion is stimulated and may cause sweating, tachycardia and agitation.

Cerebral metabolism depends on an adequate supply of glucose from ECF, and the symptoms of hypoglycaemia may resemble those of cerebral hypoxia (neuroglycopenia). Faintness, dizziness or lethargy may progress rapidly to coma and, if untreated, permanent cerebral damage or death may occur. Existing cerebral or cerebrovascular disease may aggravate the clinical picture. Whipple's triad is defined as hypoglycaemia, neuroglycopenic symptoms, and relief of these symptoms on raising the blood glucose.

Hypoglycaemia is a disease manifestation and not a diagnosis. There is no completely satisfactory classifi cation of its causes. However, one useful approach is to divide hypoglycaemia into (inappropriate) hyperinsulinaemia, (appropriate) hypoinsulinaemia and reactive hypoglycaemia

Hypoinsulinaemic hypoglycaemia

Non-pancreatic tumours (non-islet cell tumours)

Although carcinomas (especially of the liver) and sarcomas have been reported to cause hypoglycaemia, this occurs most commonly in association with

etroperitoneal tumours of mesenchymal origin, but also with lymphomas, haemangiopericytomas, liver carcinoma and leukaemia.

Hypoglycaemia may be the presenting feature. The mechanism is not always clear, but may sometimes be due to the secretion of insulin-like growth factor 2 (IGF-2) or abnormal glycosylated big IGF-2. The IGF-2 suppresses GH and IGF-1. Tumours secreting IGF-2 are characterized by an increased plasma total IGF-2:IGF-1 ratio and low plasma insulin concentration.

Endocrine causes

Hypoglycaemia may occur in hypothyroidism, pituitary or adrenal insufficiency. However, it is rarely the presenting manifestation of these conditions.

Impaired liver function

The functional reserve of the liver is so great that, despite its central role in the maintenance of plasma glucose concentrations, hypoglycaemia is a rare complication of liver disease. It may complicate very severe hepatitis, hypoxic liver disease associated with congestive cardiac failure or liver necrosis if the whole liver is affected. Plasma IGF-1 concentration may be low.

Renal failure

Renal failure can result in hypoglycaemia as the kidney, like the liver, is a gluconeogenic organ.

Hyperinsulinaemic hypoglycaemia

Insulin or other drugs are probably the most common causes. It is most important to take a careful drug history. Unless the facts are deliberately concealed by the patient, the offending drug should be easily identifiable. Hypoglycaemia in a diabetic patient may be caused by accidental insulin overdosage, by changing insulin requirements, or by failure to eat after insulin has been given.

Sulphonylureas or meglitinides may also induce hypoglycaemia, especially in the elderly.

Hypoglycaemia due to exogenous insulin suppresses insulin and C-peptide secretion. Measurement of plasma C-peptide concentrations may help to differentiate exogenous insulin administration, when C-peptide secretion is inhibited, from endogenous insulin secretion, when plasma C-peptide is raised, whether it is from an insulinoma or following pancreatic stimulation by sulphonylurea drugs.

An insulinoma is usually a small, histologically benign primary tumour of the islet cells of the pancreas .

Reactive (functional) hypoglycaemia

Some people develop symptomatic hypoglycaemia between 2 and 4 h after a meal or a glucose load. Loss of consciousness is very rare. Similar symptoms may follow a gastrectomy or bariatric gastric banding, when rapid passage of glucose into the intestine, and rapid absorption, may stimulate excessive insulin secretion (‘late dumping syndrome’). Reactive hypoglycaemia is uncommon.

Alcohol-induced hypoglycaemia

Hypoglycaemia may develop between 2 and 10 h after the ingestion of large amounts of alcohol. It is found most often in undernourished subjects and chronic alcoholics but may occur in young subjects when they first drink alcohol. Hypoglycaemia is probably caused by the suppression of gluconeogenesis during the metabolism of alcohol.

Investigation of adult hypoglycaemia

Some of the causes of hypoglycaemia can be divided into hyperinsulinaemic and hypoinsulinaemic groups. The following scheme may be useful in investigating hypoglycaemia. It is important to exclude pseudohypoglycaemia due to in vitro glucose metabolism, for example an old blood sample or one not collected into fluoride oxalate anticoagulant.

Sometimes a cause may be evident from the medical and drug histories and clinical examination. One of the most important tests in a patient with proven hypoglycaemia is to measure the plasma insulin and C-peptide concentrations when the plasma glucose concentration is low. Plasma for these assays should be separated from cells immediately and the plasma stored at –20°C until hypoglycaemia has been proven.

PLASMA LIPOPROTEINS & HYPERLIPIDEMIA

Importance:

Lipids are ubiquitous in the body tissue and play a vital role in virtually all aspects of life.

(1) hormones or hormone precursors, (2) aiding in digestion,

(3) providing a source of metabolic fuel and energy storage,

(4) acting as functional and structural components in cell membranes, and

(5) forming insulation to allow nerve conduction or to prevent heat loss.

BASIC BIOCHEMISTRY

1-FATTY ACIDS

These are straight-chain carbon compounds of varying lengths. They may be saturated, containing no double bonds, monounsaturated, with one double bond, or polyunsaturated, with more than one double bond.

Fatty acids can esterify with glycerol to form triglycerides or be non-esterified (NEFAs) or free.

Plasma NEFAs liberated from adipose tissue by lipase activity are transported to the liver and muscle mainly bound to albumin. The NEFAs provide a significant proportion of the energy requirements of the body.

Triglycerides are transported from the intestine to various tissues, including the liver and adipose tissue, as lipoproteins. Following hydrolysis, fatty acids are taken up, re-esterified and stored as triglycerides.

2- CHOLESTEROL

Cholesterol is a steroid alcohol found exclusively in animals and present in virtually all cells and body fluids. It is a precursor of numerous physiologically important steroids, including bile acids and steroid hormones.

The rate-limiting enzyme is 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), which is controlled by negative feedback by the intracellular cholesterol concentration. About two-thirds of the plasma cholesterol is esterified with fatty acids to form cholesterol esters.

3-LIPOPROTEINS

Because lipids are relatively insoluble in aqueous media, they are transported in body fluids as, often spherical soluble protein complexes called lipoproteins. Lipids can be derived from food (exogenous) or synthesized in the body (endogenous). The water-soluble (polar) groups of proteins, phospholipids and free cholesterol face outwards and surround an inner insoluble (nonpolar) core of triglyceride and cholesterol esters.

Lipoproteins are classified by their density, which inversely reflects their size. The greater the lipid to protein ratio, the larger their size and the lower the density. Lipoproteins can be classified into five main groups.

The first three are triglyceride rich and, because of their large size, they scatter light, which can give plasma *a turbid appearance* (lipaemic) if present in high concentrations:

i- Chylomicrons are the largest and least dense lipoproteins and transport exogenous lipid from the intestine to all cells.

ii-- Very low-density lipoproteins (VLDLs) transport endogenous lipid from the liver to cells.

iii- Intermediate-density lipoproteins (IDLs), which are transient and formed during the conversion of VLDL to low-density lipoprotein (LDL), are not normally present in plasma.

The other two lipoprotein classes contain mainly cholesterol and are smaller in size:

iv- Low-density lipoproteins (LDL) are formed from VLDLs and carry cholesterol to cells.

v- High-density lipoproteins (HDLs) are the most dense lipoproteins and are involved in the transport of cholesterol from cells back to the liver (*reverse cholesterol transport*). These lipoproteins can be further divided by density into HDL2 and HDL3.

In cases of hyperlipidaemia, the lipoprotein patterns have been classified (Fredrickson's classification) according to their electrophoretic mobility. Four principal bands are formed, based on their relative positions, by protein electrophoresis, namely α (HDL), pre-β (VLDL), β (LDL)and chylomicron (origin) .

Ultracentrifugation (separation based upon particle buoyant density) or electrophoretic techniques are rarely used in routine clinical practice as these may require completed

apparatus and experienced operators. Instead, the lipoprotein composition of plasma may be inferred from standard clinical laboratory lipid assays.

As fasting plasma does not normally contain chylomicrons, the triglyceride content reflects VLDL. Furthermore, generally about 70 per cent of plasma cholesterol is incorporated as LDL and 20 per cent as HDL. The latter particles, because of their high density, can be quantified by precipitation techniques that can assay their cholesterol content by subtraction, although direct HDL assays are now often used.

The Friedewald equation enables plasma LDL cholesterol concentration to be calculated and is often used in clinical laboratories:

LDL cholesterol = total cholesterol – HDL cholesterol- [triglyceride]/2.2

This equation makes certain assumptions, namely that the patient is fasting and the plasma triglyceride concentration does not exceed 4.5 mmol/L (otherwise chylomicrons make the equation inaccurate).

There has been recent interest in the subdivision of LDL particles into small dense LDL2 and LDL3,which appear to be more atherogenic and more easily oxidized than the larger LDL1 particles. Additionally another lipoprotein called lipoprotein (a), or Lp(a), has been found. This is similar in lipid composition to LDL but has a higher protein content. One of its proteins, called apolipoprotein (a), shows homology to plasminogen and may disrupt fibrinolysis, thus evoking a thrombotic tendency. The plasma concentration of Lp(a) is normally less than 0.30 g/L and it is thought to be an independent cardiovascular risk factor.

The proteins associated with lipoproteins are called *apolipoproteins (apo)*. ApoA (mainly apoA1 and apoA2) is the major group associated with HDL particles. The apoB series (apoB100) is predominantly found with LDL particles and is the ligand for the LDL receptor. Low-density lipoprotein has one molecule of apoB100 per particle. Some reports have suggested that the plasma apoA1 to apoB ratio may be a useful measure of cardiovascular risk (increased if the ratio is less than 1) and it is not significantly influenced by the fasting status of the patient. The apoC series is particularly important in triglyceride metabolism and, with the apoE series , freely interchanges between various lipoproteins.

DISORDERS OF LIPID METABOLISM

The study of hyperlipidaemias is of considerable importance, mainly because of the involvement of lipids in cardiovascular disease. Fredrickson, Levy and Lees first defined the hyperlipidaemias in a classification system based on which plasma lipoprotein concentrations were increased .Although this so-called Fredrickson's classification helped to put lipidology on the clinical map, it was not a diagnostic classification. It gives little clue as to the aetiology of the disorder ;indeed, all of the phenotypes can be either primary or secondary.

Nowadays, a more descriptive classification is used for the primary hyperlipidaemias, as follows :-

1-Chylomicron syndrome

This can be due to familial lipoprotein lipase deficiency ,an autosomal recessive disorder affecting about 1 in 1 000 000 people. The gene for lipoprotein lipase is found on chromosome 8, and genetic studies have shown

insertions or deletions within the gene. Lipoprotein lipase is involved in the exogenous lipoprotein pathway by hydrolysing chylomicrons to form chylomicron remnants, and also in the endogenous pathway by converting VLDL to IDL particles. Presentation as a child with abdominal pain (often with acute pancreatitis) is typical. There is probably no increased risk of coronary artery disease. Gross elevation of plasma triglycerides due to the accumulation of un cleared chylomicron particles occurs. Lipid stigmata include eruptive xanthomata, hepatosplenomegaly and lipaemia retinalis .

Other variants of the chylomicron syndrome include circulating inhibitors of lipoprotein lipase and deficiency of its physiological activator apoC2. Apolipoprotein C2 deficiency is also inherited as an autosomal recessive condition affecting about 1 in1 000 000 people. The gene for apoC2 is located on chromosome 19 and mutations resulting in low plasma concentrations have been found.

2-Familial hypercholesterolaemia (FH)

This condition is usually inherited as an autosomal dominant trait .The inheritance of one mutant gene that encodes for the LDL receptor affects about 1 in every 500 , resulting in impaired LDL catabolism and hypercholesterolaemia.

At least five types of mutation of the LDL receptor have been described, resulting in reduced synthesis, failure of transport of the synthesized receptor to the Golgi complex within the cell, defective LDL binding or inadequate expression or defective recycling of the LDL receptor at the cell surface.

Definite familial hypercholesterolaemia (FH) is defined as a plasma cholesterol concentration of more than 7.5 mmol/L in an adult (more than 6.7 mmol/L in children

under 16 years) or a plasma LDL cholesterol concentration of more than 4.9 mmol/L in an adult in the presence of tendon xanthoma. plus a family history of either an elevated plasma cholesterol concentration of more than 7.5 mmol/L in a first-degree or second-degree relative or myocardial infarction below the age of 50 years in a first-degree relative or below the age of 60 years in a second-degree relative. Typically, patients manifest severe hypercholesterolaemia, with a relatively normal plasma triglyceride concentration in conjunction with xanthomata, which can affect the back of the hands, elbows, Achilles tendons or the insertion of the patellar tendon into the pretibial tuberosity . Premature cardiovascular disease is often observed, along with premature corneal arci .Using the Fredrickson's classification, this condition has also been termed familial type IIa.

The diagnosis of FH is usually obvious from the markedly elevated plasma cholesterol concentration and the presence of tendon xanthomata in the patient or first-degree relation.

Homozygous FH can be very severe. There is a considerable risk of coronary artery disease, aortic stenosis and early fatal myocardial infarction before the age of 20 years.

3-Familial Combined Hyperlipidaemia

In familial combined hyperlipidaemia (FCH), the plasma lipids may elevated, plasma cholesterol concentrations often being between 6 mmol/L and 9 mmol/L and plasma triglyceride between 2 mmol/L and 6 mmol/L. The Fredrickson's phenotypes seen in this condition include IIa, IIb and IV. Familial combined hyperlipidaemia may be inherited as an autosomal dominant trait (although others suggest that there may be co-segregation of more than one gene). About 0.5 per cent of the European population is affected, and there is an increased incidence of coronary artery disease in family members.

The metabolic defect is unclear, although plasma apoB is often elevated due to increased synthesis; LDL and VLDL apoB concentration is increased. The synthesis of VLDL triglyceride is increased in FCH and there may also be a relationship with insulin resistance.

The diagnosis of FCH is suspected if there is a family history of hyperlipidaemia, particularly if family members show different lipoprotein phenotypes. There is often a family history of cardiovascular disease.

However, the diagnosis can be difficult and it sometimes needs to be distinguished from FH (xanthomata are not usually present in FCH) and familial hypertriglyceridaemia (the IIa and IIb phenotypes are not usually found in familial hypertriglyceridaemia,
Unlike familial hypertriglyceridaemia, plasma VLDL particles are usually smaller in FCH. Dietary measures and, if indicated, either a statin or a fibrate are sometimes used.

4-Familial hypertriglyceridaemia

Familial hypertriglyceridaemia is often observed with low HDL cholesterol concentration. The condition usually develops after puberty and is rare in childhood. The exact metabolic defect is unclear, although overproduction of VLDL or a decrease in VLDL conversion to LDL is likely.

There may be an increased risk of cardiovascular disease. Acute pancreatitis may also occur, and is more likely when the concentration of plasma triglycerides is more than 10 mmol/L. Some patients show hyperinsulinaemia and insulin resistance. Dietary measures, and sometimes lipid-lowering drugs such as the fibrates or ω-3 fatty acids, are used to treat the condition.

5-Type III hyperlipoproteinaemia

This condition is also called familial dysbetalipoproteinaemia or broad β-hyperlipidaemia. The underlying biochemical defect is one of a reduced clearance of chylomicron and VLDL remnants. The name broad b-hyperlipidaemia is sometimes used because of the characteristic plasma lipoprotein electrophoretic pattern that is often observed (the broad b-band that is seen being remnant particles).

An association with type III/broad b-hyperlipidaemia and homozygosity for apoE2 or variants of apoE2 has been described. Apolipoprotein E shows three common alleles, E2, E3 and E4, coded for on chromosome 19,which are important for the binding of remnant particles to the remnant receptor. The mechanism for the disorder seems to be that apoE2-bearing particles have poor binding to the apoB/E (remnant) receptor and thus are not effectively cleared from the circulation.

The palmar striae (palmar xanthomata) are considered pathognomonic for the disorder, but tuberoeruptive xanthomata, typically on the elbows and knees, xanthelasma and

corneal arcus have also been described in this condition. Peripheral vascular disease is a typical feature of this hyperlipidaemic disorder, as is premature coronary artery disease.

6-Polygenic Hypercholesterolaemia

This is one of the most common causes of a raised plasma cholesterol concentration. This condition is the result of a complex interaction between multiple environmental and genetic factors. In other words, it is not due to a single gene abnormality, and it is likely that it is the result of more than one metabolic defect.

There is usually either an increase in LDL production or a decrease in LDL catabolism. The plasma lipid phenotype is usually either IIa or IIb Fredrickson's phenotype.

The plasma cholesterol concentration is usually either mildly or moderately elevated. An important negative clinical finding is the absence of tendon xanthomata, the presence of which would tend to rule out the diagnosis. Usually less than 10 per cent of first-degree relations have similar lipid abnormalities, compared with FH or FCH in which about 50 per cent of first-degree family members are affected. There may also be a family history of premature coronary artery disease. Treatment involves dietary intervention and sometimes the use of lipid-lowering drugs such as the statins.

7- Hyperalphalipoproteinaemia

Hyperalphalipoproteinaemia results in elevated plasma HDL cholesterol concentration and can be inherited as an autosomal dominant condition or, in some cases, may show polygenic features. The total plasma cholesterol concentration can be elevated, with normal LDL cholesterol concentration. There is no increased prevalence of cardiovascular disease in

this condition; in fact, the contrary probably applies, with some individuals showing longevity. Plasma HDL concentration is thought to be cardioprotective, and individuals displaying this should be reassured. Box 13.1 gives the causes of raised plasma HDL cholesterol concentrations.

Secondary hyperlipidaemias

One should not forget that there are many secondary causes of hyperlipidaemia. These may present alone or sometimes concomitantly with a primary hyperlipidaemia. Some of the causes of secondary hyperlipidaemia are listed in Box 13.2.

Box 13.2 Some important causes of secondary hyperlipidaemia

Predominant hypercholesterolaemia

Hypothyroidism
Nephrotic syndrome
Cholestasis, e.g. primary biliary cirrhosis
Acute intermittent porphyria
Anorexia nervosa/bulimia
Certain drugs or toxins, e.g. ciclosporin and chlorinated hydrocarbons

Predominant hypertriglyceridaemia

Alcohol excess
Obesity
Diabetes mellitus and metabolic syndrome
Certain drugs, e.g. estrogens, β-blockers (without intrinsic sympathomimetic activity), thiazide diuretics, acitretin, protease inhibitors, some neuroleptics and glucocorticoids
Chronic kidney disease
Some glycogen storage diseases, e.g. von Gierke's type I
Systemic lupus erythematosus
Paraproteinaemia

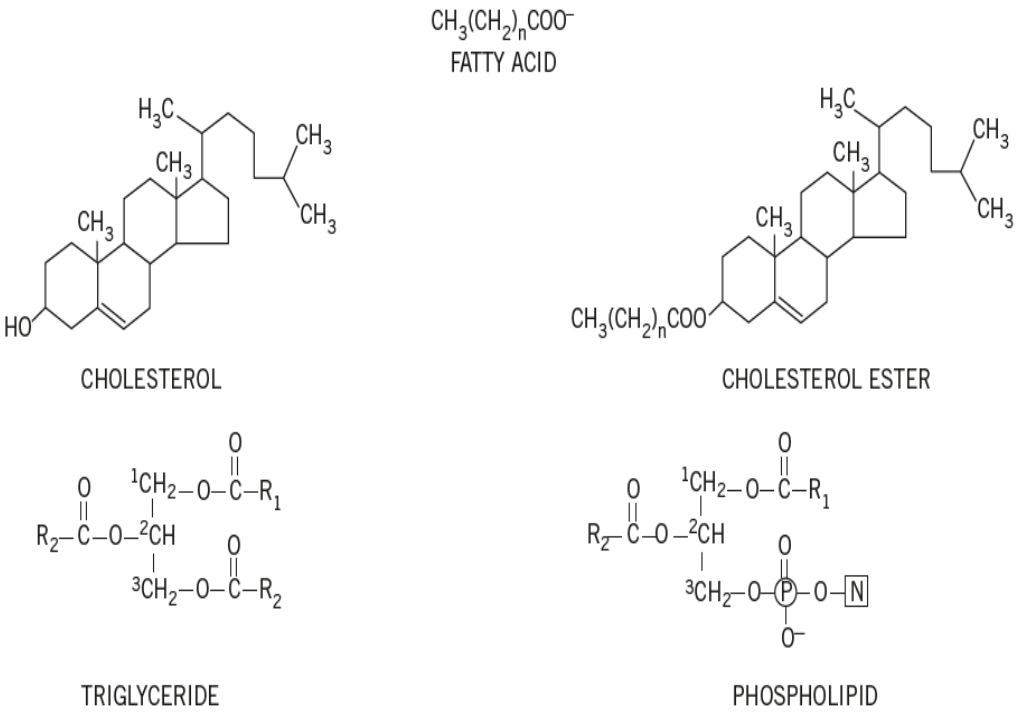
OTHER LIPID ABNORMALITIES:

Inherited disorders of low plasma HDL concentration (hypoalphalipoproteinaemia) occur, and plasma HDL cholesterol concentration should ideally be more than 1.0 mmol/L. A number of such conditions have been described (such as apoA1 deficiency), many of which are associated with premature cardiovascular disease.

In Tangier's disease, individuals have very low levels of HDL, large, yellow tonsils, hepatomegaly and accumulation of cholesterol esters in the reticuloendothelial system. There is a defect in the ABC1 gene involved in HDL transport .Defects of apoB metabolism have also been described.

In abetalipoproteinaemia or LDL deficiency there is impaired chylomicron and VLDL synthesis. This results in a failure of lipid transport from the liver and intestine. Transport of fat-soluble vitamins is impaired and steatorrhoea, progressive ataxia, retinitis pigmentosa and acanthocytosis (abnormal erthyrocyte shape) can result. In hypobetalipoproteinaemia, a less severe syndrome occurs, sometimes due to a truncated form of apoB.

In LCAT deficiency, the accumulation of free un-esterified cholesterol in the tissues results in corneal opacities, renal damage, premature atherosclerosis and haemolytic anaemia. The enzyme LCAT catalyses the esterification of free cholesterol. Another condition that is probably due to a defect of LCAT is fish-eye disease, in which there may be low HDL cholesterol concentrations and eye abnormalities .



13.1 Lipid structures. P, phosphate; N, nitrogenous base; R, fatty acid.

Table 13.2 Characteristics of major lipoproteins

Lipoprotein	Source	Composition (% mass)				Apolipoprotein	Electrophoretic mobility
		Pro	Cho	Tg	PL		
Chylomicrons	Gut	1	4	90	5	A, B, C, E	Origin
VLDL	Liver	8	25	55	12	B, C, E	Pre-β
LDL	VLDL via IDL	20	55	5	20	B	β
HDL	Gut/liver	50	20	5	25	A, C, E	α

Cho, cholesterol; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; PL, phospholipid; Pro, protein; Tg, triglyceride; VLDL, very low-density lipoprotein.

Table 13.4 The main apolipoproteins and their common functions

Apolipoprotein	Associated lipoprotein	Function
A_1	Chylomicrons and HDL	LCAT activator
A_2	Chylomicrons and HDL	LCAT activator
B_{48}	Chylomicrons and VLDL	Secretion of chylomicrons/VLDL
B_{100}	IDL, VLDL, LDL	LDL receptor binding
C_2	Chylomicrons, HDL, VLDL, IDL	Lipoprotein lipase activator
C_3	Chylomicrons, HDL, VLDL, IDL	Lipoprotein lipase inhibitor
E	Chylomicrons, HDL, VLDL, IDL	IDL and remnant particle receptor binding

HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LCAT, lecithin–cholesterol acyltransferase; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

Box 13.1 Some causes of raised plasma high-density lipoprotein (HDL) cholesterol

Primary

Hyperalphalipoproteinaemia
Cholesterol ester transfer protein deficiency

Secondary

High ethanol intake
Exercise
Certain drugs, e.g. estrogens, fibrates, nicotinic acid, statins, phenytoin, rifampicin

Box 13.3 Causes of low plasma high-density lipoprotein (HDL) cholesterol

Primary

Familial hypoalphalipoproteinaemia
$ApoA_1$ abnormalities
Tangier's disease
Lecithin–cholesterol acyltransferase (LCAT) deficiency
Fish-eye disease

Secondary

Tobacco smoking
Obesity
Poorly controlled diabetes mellitus
Insulin resistance and metabolic syndrome
Chronic kidney disease
Certain drugs, e.g. testosterone, probucol, β-blockers (without intrinsic sympathomimetic activity), progestogens, anabolic steroids, bexarotene

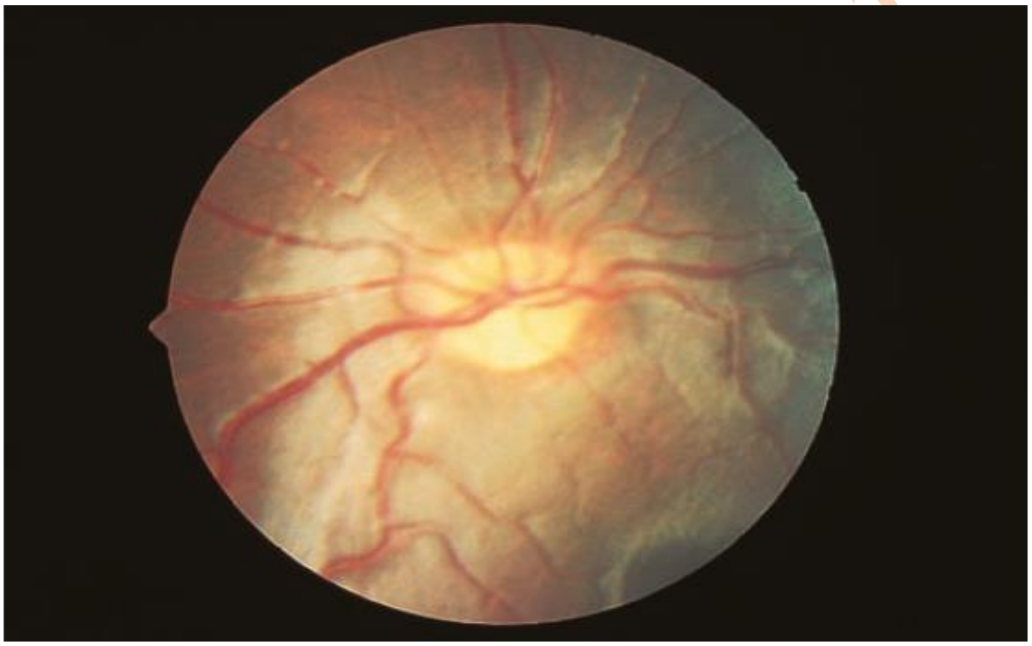


Figure 13.10 Lipaemia retinalis in a patient with lipoprotein lipase deficiency. Reproduced with kind

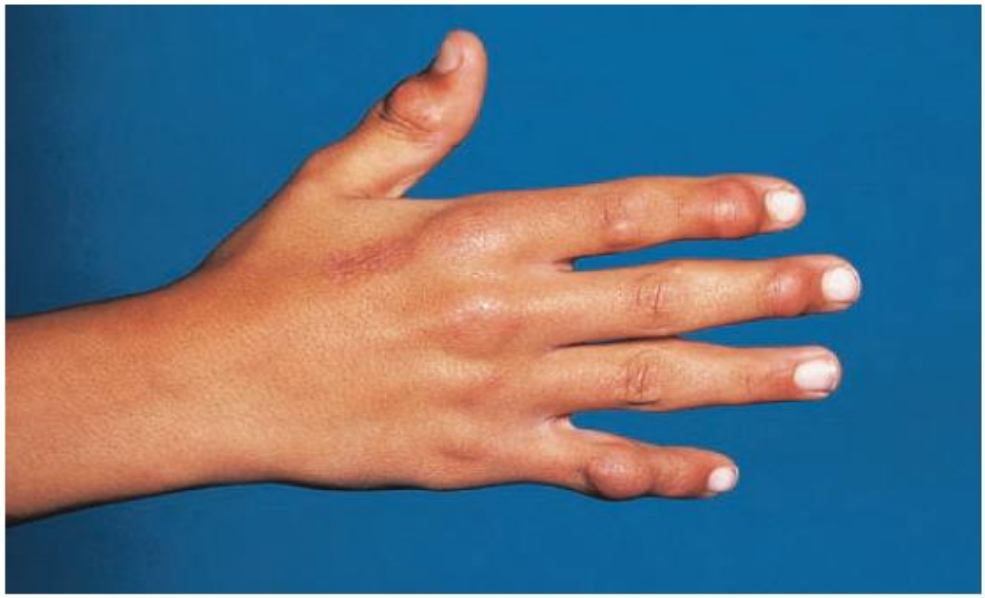


Figure 13.11 Tendinous xanthomas in familial hypercholesterolaemia. Reproduced with kind

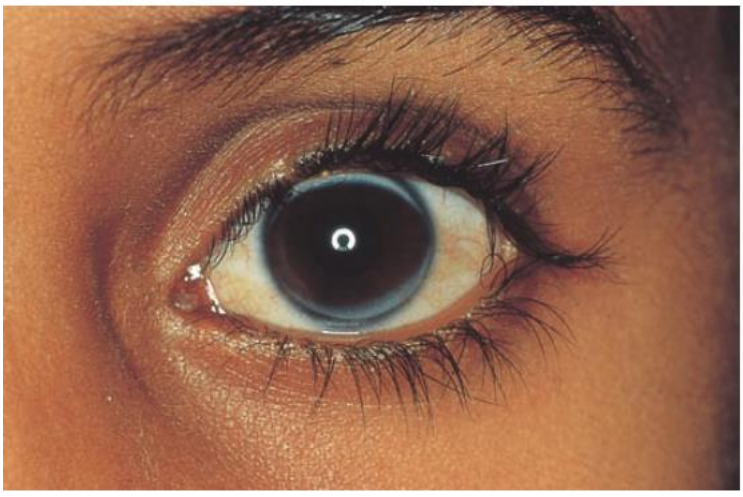


Figure 13.12 Corneal arcus in familial hypercholesterolaemia. Reproduced with kind

LIVER FUNCTION TESTS

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.

Liver have several important functions to the body :-

1-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 .

2-Synthetic functions

Hepatocytes synthesize:

a- plasma proteins, excluding immunoglobulins and complement,

b- 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,

c- primary bile acids,

d- the lipoproteins, such as VLDL and high-density .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, hextrahepatic 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 .

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.

3-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

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

.

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 septicaemia) 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 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).

Jaundice (Retention of Bilirubin In Plasma)

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 75μmol/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 hepaticconjugating 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.

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 .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 .

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 (extra hepatic) of a low plasma albumin concentration that are not due to hepatic disease .

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.

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 g-glutamyl transferase (GGT) concentration implies that the ALP is of hepatic origin.

γ-Glutamyl transferase(GGT)

Gamma-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 .

- 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)

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 .

New hepatic function tests

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

1- 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,

2- 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, intraluminal 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 immmunoglobulin M (IgM) concentration is usually raised. Patients may also manifest hypercholesterolaemia, xanthelasma ,other autoimmune disorders and osteoporosis.

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 slightly increased.

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 is elevated.

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.

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.

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.

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 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 .

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 . Survival for patients with grade C cirrhosis is usually less than 1 year.

Table 17.3 Child–Pugh scores for severity of cirrhosis

Grade	Plasma bilirubin (μmol/L)	Plasma albumin (g/L)	Ascites/ encephalopathy	INR
A	Normal	> 35	None	< 1.7
B	34–50	28–35	Mild	1.7–2.3
C	>50	< 28	Severe	> 2.3

INR, international normalized ratio.

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.

Hepatocellular Failure And Hepatic Encephalopathy

Liver damage severe enough to cause obvious clinical signs of impaired hepatocellular function may be caused by severe hepatitis or advanced cirrhosis, or may 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 ,– renal circulatory insufficiency, causing oliguria, a high plasma creatinine concentration and uraemia 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.

Metabolic Liver Disease

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 .

a1-Antitrypsin deficiency

a1-Antitrypsin deficiency is associated with neonatal hepatitis in individuals with

the PiZZ phenotype, which progresses to cirrhosis in childhood. The condition can also present inadulthood, and often there is basal emphysema particularly in smokers .

Galactosaemia

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

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. ultrasound may show increased echogenicity of the liver. The biochemical features of NAFLD may improve with

dietary measures and treatment of hyperlipidaemia .

Tumor Markers

Neoplastic cells of differentiated tissues sometimes synthesize enough compounds not normally thought of as coming from that tissue to be detectable in body fluids. These substances fall into two principal groups:

_ those that alter metabolism and thus may produce clinical effects, some of which are hormonal syndromes,

_ those that, although biologically inactive, may be analytically detectable in body fluids; these are sometimes used as tumour markers.

Tumor Markers

For the measurement of a tumor marker to be clinically useful, the result should clearly separate those patients with from those without a tumor. Therefore (although in practice this is not the case), a tumor marker should ideally be:

_ 100 per cent *sensitive*: levels should be raised if the tumor is present,

_ 100 per cent *specific*: levels should *not* be raised if the tumor is *not* present

Tumor markers applications in medicine:

_ *To screen for disease:* Very few markers are sufficiently sensitive or specific to be used to screen for the presence of a tumor

_ *To diagnose a tumor :*If a patient presents with clinical signs or symptoms, the measurement of a marker in plasma or urine may very occasionally be used to confirm a diagnosis.

_ *To determine the prognosis :* In some cases the concentration of a specific marker is related to the mass or spread of the tumor.

_ *To monitor the response to treatment :* If a tumour marker is present, the rate of its decrease in concentration may be used to assess the response to treatment such as surgery, chemotherapy or radiotherapy.

_ *To identify the recurrence of a tumour :* If the concentration of the marker was previously raised, intermittent measurement during remission may sometimes be used to identify recurrence.

Occasionally, however, tumours may dedifferentiate and fail to express the marker despite continued growth and spread.

Some examples of tumour markers

Prostate-specific antigen

Prostate-specific antigen (PSA) is a marker for prostatic carcinoma, a common male tumour, and is a 33-kDa protein and is homologous with the protease kallikrein family; it has a plasma half-life of about 3 days. One of its probable functions is to help liquidize semen. Its level is raised in benign prostatic hyperplasia (BPH) and prostatic carcinoma but also in prostate infection, for example prostatitis, and after rectal examination. Levels of PSA increase with age, which is mainly due to the increase in the volume of the prostate that occurs. Therefore age-adjusted reference ranges should be used. There may also be a place for expressing plasma PSA in terms of prostate volume as found on ultrasound examination.

One diagnostic limitation is that the values of PSA overlap in BPH and prostatic carcinoma. After a radical prostatectomy, plasma PSA levels become undetectable at 2–3 weeks. Finasteride, a 5-a-reductase inhibitor that is sometimes used to treat BPH, decreases plasma PSA by up to 50 per cent.

The PSA is bound in the plasma to either α1- antichymotrypsin or α2-macroglobulin. The concentration of bound or complexed PSA is higher in prostate carcinoma, whereas that of free PSA is higher in BPH. The ratio of free to total PSA is lower in men with prostatic carcinoma. The PSA index is expressed as the percentage of the total plasma PSA that is free; an index above about 17 per cent is suggestive of BPH and one of less than 17 per cent of prostate carcinoma.

Plasma PSA concentrations greater than 10 μg/L are strongly suggestive of carcinoma, although carcinoma may be present even if values fall within the reference range. A PSA above 20 μg/L is suggestive of prostatic carcinoma that has spread beyond the prostate gland. Plasma PSA assays in conjunction with digital rectal examination may be used as part of a screening programme for prostatic carcinoma in at-risk males.

There is, however, no universally agreed screening protocol for prostatic carcinoma in the general population. **Prostate cancer antigen 3 (*PCA3*) and** specific **PSA isoforms** may also prove useful markers in conjunction with PSA.

Prostate biopsy is usually necessary if the PSA concentration is above 10 μg/L; however, the decision regarding biopsy is more difficult if PSA levels are 4–10 μg/L, although the PSA index may help.

Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen may be produced by some malignant tumours, especially colorectal carcinomas. If the initial plasma concentration is raised, serial plasma CEA estimations may sometimes help to monitor the effectiveness of, or recurrence after, treatment (Fig. - below). Plasma concentrations correlate poorly with tumour mass, but a very high concentration usually indicates a bad prognosis. Plasma concentrations may also rise in non-malignant disease of the gastrointestinal tract and in smokers. Thus, the test is non-specific and thus lacks value in diagnosis.

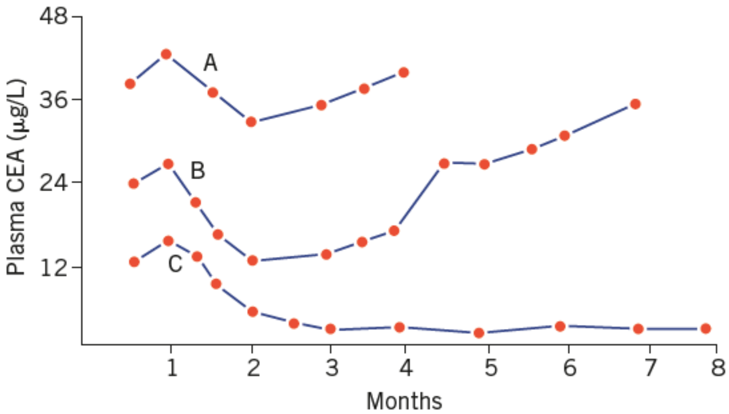


Figure 24.4 The use of the tumour marker carcinoembryonic antigen (CEA) in monitoring disease progression. Patient A had an advanced gastrointestinal tumour and after initial chemotherapy died. Patient B had initial remission as a result of surgery but died after tumour relapse. Patient C had successful surgery with CEA essentially normalizing.

α-Fetoprotein

α-Fetoprotein (AFP) is an oncofetal protein, the synthesis of which is suppressed as the fetus matures. Concentrations may be very high in the plasma of patients with certain tumours such as hepatocellular carcinoma (primary hepatomas and hepatoblastomas) and teratoma. Moderately raised concentrations may be due to non-malignant liver disease.

Human chorionic gonadotrophin

Human chorionic gonadotrophin (hCG) is normally produced by the placenta, but also by trophoblastic cells of gonadal and extragonadal germ cell tumours. Ectopic secretion has been observed in some bronchial carcinomas. The measurement of hCG can be used to screen for choriocarcinoma in women who have had a hydatidiform mole . Plasma concentrations may be raised in patients with malignancy of the gonads such as seminomas, and hCG may be used to monitor the response to treatment and tumour recurrence.

Carbohydrate antigens

Carbohydrate antigens (CAs) are a group of tumour markers, raised plasma concentrations of which may be used to monitor the response to treatment and the recurrence of certain tumours.

_ ***CA-125*** concentration may be raised in the plasma of patients with ovarian carcinoma. It can also be raised in pregnancy, fibroids, liver and pancreatic disease, endometriosis and pelvic inflammatory disease. Additionally, it can also be raised in other malignant diseases such as lung, breast or colon carcinoma. If plasma CA-125 is 35 kU/L or more,an ultrasound scan of the abdomen and pelvis has been proposed as a means of screening for ovarian carcinoma.

_ ***CA-15-3*** concentration may be raised in the plasma of some patients with advanced breast carcinoma, although it can also be raised in cirrhosis, and with ovarian cysts.

_ ***CA-19-9*** concentration may be raised in the plasma of patients with pancreatic or colorectal carcinoma and those with obstructive liver disease.

None of the **CA** tumour markers fulfils the criteria of an ideal marker, as none is sufficiently sensitive or specific to be used to screen for early disease.Furthermore, some advanced tumours differentiate and fail to produce a marker despite continued growth and spread. These tumour markers may, however, be useful in the monitoring of patients with tumours and in the assessment of therapy. High levels are associated with tumour spread and relapse, and low levels are suggestive of tumour remission.

Other tumour markers

_ ***Serum paraprotein and urinary Bence Jones protein***.

_ ***Plasma lactate dehydrogenase*** **(LDH)**: the activity can be raised in certain haematological tumours such as lymphomas.

_ ***Placental alkaline phosphatase***: true placental alkaline phosphatase and placental-like isoenzyme levels are raised in seminoma and dysgerminoma. Levels are not usually raised in teratomata. In conjunction

with AFP and hCG, it is useful in the diagnosis and monitoring of extragonadal and gonadal germ cell tumours. However, plasma levels are also elevated in smokers .

_ ***Thyroglobulin*****:** this high-molecular-weight protein is produced in the follicular cells of the thyroid. Its concentration is raised in follicular or papillary carcinoma of the thyroid. Spuriously low levels may be found in the presence of thyroglobulin antibodies,
which interfere with the assay.

_ ***Neuronal-specific enolase***: plasma levels may be raised in small cell lung carcinoma and neuroblastoma; it is derived from neurodectal tissue.

_ ***Inhibin***: this is secreted by the granulosa cells of the ovary and by the Sertoli cells of the testis. It can be used as a plasma tumour marker of ovarian granulosa cell tumours and testicular Sertoli cell tumours.

_ ***Squamous cell carcinoma antigen*****:** this is a plasma tumour marker of potential use in squamous cell carcinoma of the cervix.

_ ***Chromogranin A*** is released from neuroendocrine cells such as in phaeochromocytoma and carcinoid tumours.

_ ***Protein S100B*** is a calcium-binding protein. It is expressed in brain astrocytes and glial cells and also in melanocytes and may be useful in monitoring therapy in malignant melanoma.

_ ***Human epididymis protein*** **(HE4)** is being used as a tumour marker for ovarian carcinoma.

New tumour markers are likely to be revealed in the future .Genetic tests are also being developed that may be useful to predict those individuals at risk of developing various carcinomas, for example ***BRCA*** gene mutations for breast carcinoma. Tumour-modified deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and nucleic acids also circulate in the blood and may be useful in the diagnosis of certain cancers.