16 Principles of clinical biochemistry

J. FYFFE AND K. WILSON

16.1 PRINCIPLES OF CLINICAL BIOCHEMICAL ANALYSIS

16.1.1 Basis of analysis of body fluids for diagnostic, prognostic and monitoring purposes

Underlying most human diseases is a change in the amount or function of one or more proteins that in turn triggers changes in cellular, tissue or organ function. The dysfunction is commonly characterised by a significant change in the biochemical profile of body fluids. The application of quantitative analytical biochemical tests to a large range of biological analytes in body fluids and tissues is a valuable aid to the diagnosis and management of the prevailing disease state. In this section the general biological and analytical principles underlying these tests will be discussed and related to the general principles of quantitative chemical analysis discussed in Section 1.3.

Body fluids such as blood, cerebrospinal fluid and urine in both healthy and diseased states contain a large number of inorganic ions and organic molecules. Whilst the normal biological function of some of these chemical species lies within that fluid, for the majority it does not. The presence of this latter group of chemical species within the fluid is due to the fact that normal cellular secretory mechanisms and the temporal synthesis and turnover of individual cells and their organelles within the major organs of the body, both result in the release of cell components, particularly those located in the cytoplasm, into the surrounding extracellular fluid and eventually into the blood circulatory system. This in turn transports them to the main excretory organs, namely the liver, kidneys and lungs, so that these cell components and/or their degradation products are eventually excreted in faeces, urine, sweat and expired air. Examples of cell components in this category include enzymes, hormones, intermediary metabolites and small organic and inorganic ions.

The concentration, amount or activity of a given cell component that can be detected in these fluids of a healthy individual at any point in time depends on many factors that can be classified into one of three categories, namely chemical characteristics of the component, endogenous factors characteristic of the individual and exogenous factors that are imposed on the individual.

- *Chemical characteristics*: Some molecules are inherently unstable outside their normal cellular environment. For example, some enzymes are reliant on the presence of their substrate and/or coenzyme for their stability and these may be absent or in too low concentrations in the extracellular fluid. Molecules that can act as substrates of catabolic enzymes found in extracellular fluids, in particular blood, will also be quickly metabolised. Cell components that fall into these two categories therefore have a short half-life outside the cell and are normally present in low concentrations in fluids such as blood.
- *Endogenous factors*: These include age, gender, body mass and pregnancy. For example:
  (a) serum cholesterol concentrations are higher in men than premenopausal women but the differences decreases post-menopause;
  (b) serum alkaline phosphatase activity is higher in children than in adults and is raised in women during pregnancy;
  (c) serum insulin and triglyceride concentrations are higher in obese individuals than in the lean;
  (d) serum creatinine, a metabolic product of creatine important in muscle metabolism, is higher in individuals with a large muscle mass;
  (e) serum sex hormone concentrations differ between males and females and change with age.
- *Exogenous factors*: These include time, exercise, food intake and stress. Several hormones are secreted in a time-related fashion. Thus cortisol and to a lesser extent thyroid stimulating hormone (TSH) and prolactin all show a diurnal rhythm in their secretion. In the case of cortisol, its secretion peaks around 9.00 am and declines during the day reaching a trough between 11.00 pm and 5.00 am. The secretion of female sex hormones varies during a menstrual cycle and that of 25-hydroxycholecalciferol (vitamin D_3) varies with the seasons peaking during the late summer months. The concentrations of glucose, triglycerides and insulin in blood rise shortly after the intake of a meal. Stress, including that imposed by the process of taking a blood sample by puncturing a vein (**venipuncture**), can stimulate the secretion of a number of hormones and neurotransmitters including prolactin, cortisol, adrenocorticotropic hormone (ACTH) and adrenaline.

The influence of these various factors on the extent of release of cell components into extracellular fluids inevitably means that even in healthy individuals there is a considerable **intraindividual variation** (i.e. variation from one occasion to another) in the value of any chosen test analyte of diagnostic importance and an even larger **interindividual variation** (i.e. variation between individuals). More importantly, the superimposition of a disease state onto these causes of intra- and interindividual variation will result in an even greater variability between test occasions.

Many clinical conditions compromise the integrity of cells located in the organs affected by the condition. This may result in the cells becoming more 'leaky' or, in

more severe cases, actually dying (**necrosis**) and releasing their contents into the surrounding extracellular fluid. In the vast majority of cases the extent of release of specific cell components into the extracellular fluid, relative to the healthy reference range, will reflect the extent of organ damage and this relationship forms the basis of diagnostic clinical biochemistry. If the cause of the organ damage continues for a prolonged time and is essentially irreversible (i.e. the organ does not undergo self-repair), as is the case in cirrhosis of the liver for example, then the mass of cells remaining to undergo necrosis will progressively decline so that eventually the release of cell components into the surrounding extracellular fluid will decrease even though organ cells are continuing to be damaged. In such case the measured amounts will not reflect the extent of organ damage.

Clinical biochemical tests have been developed to complement in four main ways a provisional clinical diagnosis based on the patient's medical history and clinical examination:

- *To support or reject a provisional diagnosis* by detecting and quantifying abnormal amounts of test analytes consistent with the diagnosis. For example, serum myoglobin, troponin-I (part of the cardiac contractile muscle), creatine phosphokinase (specifically the CK-MB isoform) and aspartate transaminase all rise following a myocardial infarction (heart attack) that results in cell death in some heart tissue. The released cellular components also cause cell inflammation (**leakiness**) in surrounding cells causing an amplification of total cellular component release. Tests can also help a differential diagnosis, for example in distinguishing the various forms of jaundice (yellowing of the skin due to the presence of the yellow pigment bilirubin, a metabolite of haem) by the measurement of alanine transaminase (ALT) and aspartate transaminase (AST) activities and by determining whether or not the bilirubin is conjugated with β-glucuronic acid.
- *To monitor recovery following treatment* by repeating the tests on a regular basis and monitoring the return of the test values to those within the reference range. Following a myocardial infarction, for example, the raised serum enzyme activities referred to above usually return to reference range values within 10 days (Section 16.3.2, Fig. 16.3). Similarly, the measurement of serum tumour markers such as CA125 can be used to follow recovery or recurrence after treatment for ovarian cancer.
- *To screen for latent disease* in apparently healthy individuals by testing for raised levels of key analytes. For example, measuring serum glucose for diabetes mellitus and immunoreactive trypsin for cystic fibrosis. It is now common for serum cholesterol levels to be used as a measure of the risk of the individual developing heart disease. This is particularly important for individuals with a family history of the disease. An action limit of serum cholesterol >5.2 mM has been set by the British Hyperlipidaemia Association for an individual to be counselled on the importance of a healthy (low fat) diet and regular exercise and a higher action limit of serum cholesterol >6.6 mM for cholesterol-lowering 'statin' drugs to be prescribed (Section 18.2.2) and clinical advice given.
- *To detect toxic side effects of treatment*, for example in patients receiving hepatotoxic drugs, by undertaking regular liver function tests. An extension of this is **therapeutic drug monitoring** in which patients receiving drugs such as phenytoin and

carbamezepine (both of which are used in the treatment of epilepsy) that have a low therapeutic index (ratio of the dose required to produce a toxic effect relative to the dose required to produce a therapeutic effect) are regularly monitored for drug levels and liver function to ensure that they are receiving effective and safe therapy.

16.1.2 Reference ranges

For a biochemical test for a specific analyte to be routinely used as an aid to clinical diagnosis, it is essential that the test has the required performance indicators (Section 16.2), especially **specificity** and **sensitivity**. Sensitivity expresses the proportion of patients with the disease who are correctly identified by the test. Specificity expresses the proportion of patients without the disease who are correctly identified by the test. These two parameters may be expressed mathematically as follows:

$$\text{sensitivity} = \frac{\text{true positive tests} \times 100\%}{\text{total patients with the disease}}$$

$$\text{specificity} = \frac{\text{true negative tests} \times 100\%}{\text{total patients without the disease}}$$

Ideally both of these indicators for a particular test should be 100% but this is not always the case. This problem is most likely to occur in cases where the change in the amount of the test analyte in the clinical sample is small compared with the reference range values found in healthy individuals. Both of these indicators express the performance of the test but it is equally important to be able to quantify the probability that the patient with a positive test has the disease in question. This is best achieved by the **predictive power** of the test. This expresses the proportion of patients with a positive test who are correctly diagnosed as disease positive:

$$\text{positive predictive value} = \frac{\text{true positive patients}}{\text{total positive tests}}$$

$$\text{negative predictive value} = \frac{\text{true negative patients}}{\text{total negative tests}}$$

The concept of predictive power can be illustrated by reference to foetal screening for Down's syndrome and neural tube defects. Preliminary tests for these conditions in unborn children are based on the measurement of α-fetoprotein (AFP), human chorionic gonadotropin (hCG) and unconjugated oestriol (uE_3) in the mother's blood. The presence of these conditions results in an increased hCG and decreased AFP and uE_3 relative to the average in healthy pregnancies. The results of the tests are used in conjunction with the gestational and maternal ages to calculate the risk of the baby suffering from these conditions. If the risk is high, further tests are undertaken including the recovery of some foetal cells for genetic screening from the amniotic fluid surrounding the foetus in the womb by inserting a hollow needle into the womb (**amniocentesis**). The three tests detect two out of three cases (67%) of Down's syndrome and four out of five cases (80%) of neural tube defects. Thus the performance indicators of the tests are not 100% but they are sufficiently high to justify their routine use.

The correct interpretation of all biochemical test data is heavily dependent the use of the correct reference range against which the test data are to be judged. As previously pointed out, the majority of biological analytes of diagnostic importance are subject to considerable inter- and intraindividual variation in healthy adults, and the analytical method chosen for a particular analyte assay will have its own precision, accuracy and selectivity that will influence the analytical results. In view of these biological and analytical factors, individual laboratories must establish their own reference range for each test analyte using their chosen methodology and a large number (hundreds) of 'healthy' individuals. The recruitment of individuals to be included in reference range studies presents a considerable practical and ethical problem due to the difficulty of defining 'normal' and of using invasive procedures, such as venipuncture, to obtain the necessary biological samples. The establishment of reference ranges for children, especially neonates, is a particular problem.

Reference ranges are most commonly expressed as the range that covers the mean ± 1.96 standard deviations of the mean of the experimental population. This range covers 95% of the population. The majority of reference ranges are based on a normal distribution of individual values but in some cases the experimental data are asymmetric often being skewed to the upper limits. In such cases it is normal to use logarithmic data to establish the reference range but even so, the range may overlap with values found in patients with the test disease state. Typical reference ranges are shown in Table 16.1.

16.2 CLINICAL MEASUREMENTS AND QUALITY CONTROL

16.2.1 The operation of clinical biochemistry laboratories

The clinical biochemistry laboratory in a typical general hospital in the UK serves a population of about 400 000 containing approximately 60 General Practitioner (GP) groups depending upon the location in the UK. This population will generate approximately 1200 requests from GPs and hospital doctors each weekday for clinical biochemical tests on their patients. Each patient request will require the laboratory to undertake an average of seven specific analyte tests. The result is that a typical general hospital laboratory will carry out between 2.5 and 3 million tests each year. The majority of clinical biochemistry laboratories offers the local medical community as many as 200 different clinical biochemical tests that can be divided into eight categories as shown in Table 16.2.

Most of the requests for biochemical tests will arise on a routine daily basis but some will arise from emergency medical situations at any time of the day. The large number of daily test samples coupled with the need for a 24-hour 7-day week service dictates that the laboratory must rely heavily on automated analysis to carry out the tests and on information technology to process the data.

Table 16.1 **Typical reference ranges for biochemical analytes**

Analyte	Reference range	Comment
Sodium	133–145 mM	
Potassium	3.5–5.0 mM	Values increased by haemolysis or prolonged contact with cells.
Urea	3.5–6.5 mM	Range varies with sex and age e.g. values up to 12.1 will be found in males over the age of 70.
Creatinine	75–115 mM (males) 58–93 mM (females)	Creatinine (a metabolite of creatine) production relates to muscle mass and is also a reflection of renal function. Values for both sexes increase by 5–20% in the elderly.
Aspartate transaminase (AST)	<40 IU dm^{-3}	Perinatal levels are <80 IU dm^{-3} and fall to adult values by the age of 18. Some slightly increased values up to 60 IU dm^{-3} may be found in females over the age of 50. Results are increased by haemolysis.
Alanine transaminase (ALT)	<40 IU dm^{-3}	Higher values are found in males up to the age of 60.
Alkaline phosphatase (AP)	<122 IU dm^{-3} (adults) <455 IU dm^{-3} (children <12 y)	Significantly raised results of up to two- or three-fold would be experienced during growth spurts through teenage years. Slightly raised levels also seen in the elderly and in women during pregnancy.
Cholesterol	No reference range but recommended value of <5.2 mM	The measurement of cholesterol in an adult 'well' population does not show a Gaussian distribution but a very tailed distribution with relatively few low results. The majority are <10 mM but there is a long tail up to over 20 mM. There is a tendency for males to have higher cholesterol than females of the same age but after the menopause female values revert to those of males. Generally values increase with age.

To achieve an effective service, a clinical biochemistry laboratory has three main functions:

- *to advise* the requesting GP or hospital doctor on the appropriate tests for a particular medical condition and on the collection, storage and transport of the patient samples for analysis;
- *to provide* a quality analytical service for the measurement of biological analytes in an appropriate and timely way;
- *to provide* the requesting doctor with a data interpretation and advice service on the outcome of the biochemical tests and possible further tests.

Table 16.2 **Examples of biochemical analytes used to support clinical diagnosis**

Type of analyte	Examples
Foodstuffs entering the body	Cholesterol, glucose, fatty acids, triglycerides
Waste products	Bilirubin, creatinine, urea
Tissue-specific messengers	Adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), luteinising hormone (LH), thyroid-stimulating hormone (TSH)
General messengers	Cortisol, insulin, thyroxine
Response to messengers	Glucose tolerance test assessing the appropriate secretion of insulin; tests of pituitary function
Organ function	Adrenal function – cortisol, ACTH; renal function – K^+, Na^+, urea, creatinine; thyroid function – free thyroxine (FT_4), free tri-iodothyronine (FT_3), TSH
Organ disease markers	Heart – troponin-I, creatine kinase (CKMB), AST, lactate dehydrogenase (LD); liver – ALT, AP, γ-glutamyl transferase (GGT), bilirubin, albumin
Disease-specific markers	Specific proteins ('tumour markers') secreted from specific organs – prostate-specific antigen (PSA), CA-125 (ovary), calcitonin (thyroid), $\alpha_1$fetoprotein (liver)

The advice given to the clinician is generally supported by a User Handbook, prepared by senior laboratory personnel, that includes a description of each test offered, instructions on sample collection and storage, normal laboratory working hours and the approximate time it will take the laboratory to undertake each test. This turnaround time will vary from less than one hour to several weeks depending upon the speciality of the test. The vast majority of biochemical tests are carried out on serum or plasma derived from a blood sample. Serum is the preferred matrix for biochemical tests but the concentrations of most test analytes are almost the same in the two fluids. Serum is obtained by allowing the blood to clot and removing the clot by centrifugation. To obtain plasma it is necessary to add an anticoagulant to the blood sample and remove red cells by centrifugation. The two most common anticoagulants are heparin and EDTA, the choice depending on the particular biochemical test required. For example, EDTA complexes calcium ions so that calcium in EDTA plasma would be undetectable. For the measurement of glucose, fluoride-oxalate is added to the sample not as an anticoagulant but to inhibit glycolysis during the transport and storage of the sample. Special vacuum collection tubes containing specific anticoagulants or other additives are available for the storage of blood samples. Collection tubes are also available containing clot enhancers to speed the clotting process for serum preparation. Many containers incorporate a gel with a specific gravity designed to float the gel between cells and serum providing a barrier between the two for up to 4 days. During these 4 days, the cellular component will experience lysis, so any subsequent contamination of the serum will include intracellular components. Biochemical tests may also be carried out on whole blood,

urine, cerebrospinal fluid (the fluid surrounding the spinal cord and brain), faeces, sweat, saliva and amniotic fluid. It is essential that the samples are collected in the appropriate container at the correct time (particularly important if the test is for the measurement of hormones such as cortisol subject to diurnal release) and labelled with appropriate patient and biohazard details. Samples submitted to the laboratory for biochemical tests are accompanied by a request form, signed by the requesting clinician, which gives details of the tests required and brief details of the reasons for the request to aid data interpretation and to help identify other appropriate tests.

Laboratory reception

On receipt in the laboratory both the sample and the request form will be assigned an acquisition number usually in an optically readable form but with a bar code. A check is made of the validity of the sample details on both the request form and sample container to ensure that the correct container for the tests required has been used. Samples may be rejected at this stage if details are not in accordance with the set protocol. Correct samples are then split from the request form and prepared for analysis typically by centrifugation to prepare serum or plasma. The request form is processed into the computer system that identifies the patient against the sample acquisition number, and the tests requested by the clinician typed into the database. It is vital at this reception phase that the sample and patient data match and that the correct details are placed in the database. These details must be adequate to uniquely identify the patient bearing in mind the number of potential patients in the catchment area, and will include name, address, date of birth, CHI number (a unique identifier for each individual in the UK for health purposes), hospital or Accident and Emergency number and acquisition number.

16.2.2 Analytical organisation

The analytical organisation of the majority of clinical biochemical laboratories is based on three work areas:

- auto-analyser section,
- immunoassay section,
- manual section.

Auto-analyser section

Auto-analysers dedicated to clinical biochemical analysis are available from many commercial manufacturers. The majority of analysers are fully automated and have carousels for holding the test samples in racks each carrying up to 15 samples, one or two carousels each for up to 60 different reagents that are identified by a unique bar code, carousels for sample washing/preparation and a reaction carousel containing up to 200 cuvettes for initiating and monitoring individual test reactions. The Abbott ARCHITECT c1600 (Fig. 16.1) utilises three methodologies: spectrophotometry, immunoturbidometry and potentiometry. The spectrophotometry system is capable of measuring at 16 wavelengths simultaneously whilst the potentiometric system, based on the use of ion-selective electrodes (ISEs) (Section 1.3.5) that are

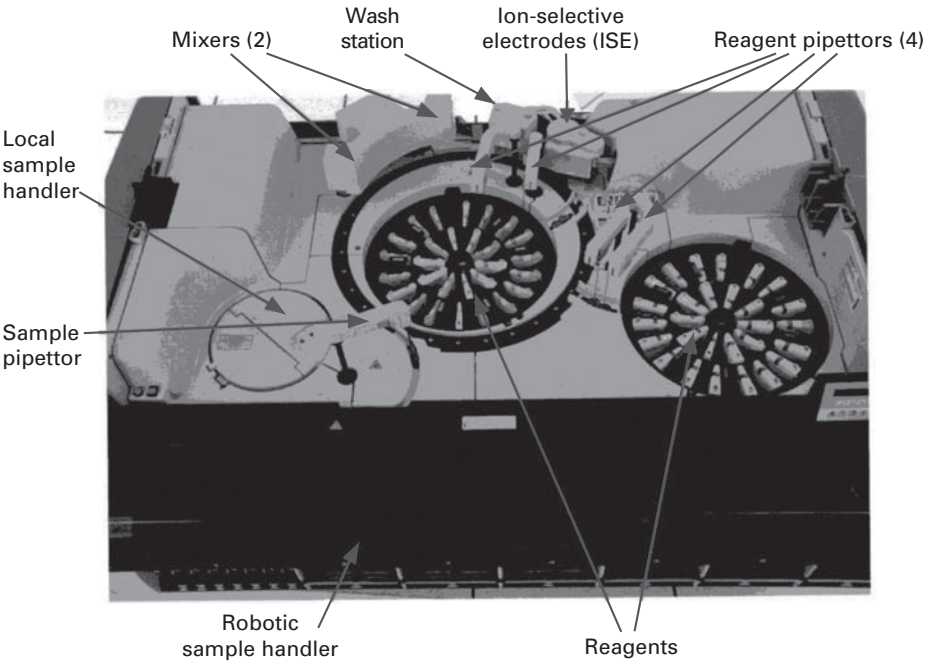

Fig. 16.1 The Abbott ARCHITECT c1600 clinical chemical analyser. (Reproduced by permission of Abbott Diagnostics UK & Northern Ireland, Maidenhead, UK.)

combined into a single unit, is used to measure electrolyte concentrations of Na^+, K^+ and Cl^- simultaneously with an analytical time of less than 4 min on a sample size of only 25 mm^3.

The reaction carousel contains 330 cuvettes and before each assay each cuvette is automatically cleaned and dried using an eight-stage wash mechanism. Supplementary washes are available to reduce carryover when necessary. Reagents for up to 62 assays based on different analytical techniques (Table 16.3) may be loaded at the same time and to maximise throughput, testing is organised in two parallel lines each one of which is resource and analysis-controlled separately. The analyser uses a fixed cycle time. Every 4.5 seconds the reaction carousel moves approximately one-quarter turn and readings are taken each full turn. The total analysis time is dependent upon the assay being performed and is determined by the assay parameters. All tests are complete in less than 10 minutes and results are reported immediately whilst complete results for an individual patient sample are recorded as soon as the final assay is complete. The theoretical throughput for the analyser is 1800 tests per hour. As with the majority of analysers, the c1600 is an 'open system' in that in addition to the programmed tests, 'in house' tests may also be incorporated into the routine by laboratory personnel. These may include therapeutic drug monitoring, drugs of abuse tests and specific protein assays. Each laboratory will have at least two analysers each offering a similar analytical repertoire so that one can back up the other. The analyser reads the bar code acquisition number for each sample and on the basis of the reading interrogates the host computer database to identify the tests to be carried out on the sample. The identified

Table 16.3 **Examples of analytical techniques used to quantify analytes by auto-analysers**

Analytical technique	Examples of analytes
Ion-selective electrodes	K^+, Na^+, Li^+, Cl^-
Visible and UV spectrophotometry	Urea, creatinine, calcium, urate
Turbidimetry	IgG, IgA, IgM, D-dimer (a metabolic product of fibrinogen)
Reaction rate	Enzymes – AST, ALT, GGT, AP, CK, LD,
Enzyme multiplier immunoassay test (EMIT)	Therapeutic drug monitoring – phenytoin, carbamezapine

tests are then automatically prioritorised into the most efficient order and the analyser programmed to take the appropriate volume of sample by means of a sampler that may also be capable of detecting microclots in the sample, add the appropriate volume of reagents in a specified order and to monitor the progress of the reaction. **Internal quality control** samples are also analysed on an identical basis at regular intervals. The analyser automatically monitors the use of all reagents so that it can identify when each will need replenishing. When the test results are calculated, the operator can validate them either on the analyser or on the main computer database. When appropriate, the results can also be checked against previous results on the same patient.

Micro sensor analysers

Recent advances in micro sensor technology have stimulated the development of miniaturised multi-analyte sensor blocks that are widely used in the clinical biochemistry laboratory for the routine measurement of pH, pCO_2, Na^+, K^+, Ca^{2+}, Cl^-, glucose and lactate (Section 1.3.5). The basic component of the block is a 'cartridge' that contains the analyte sensors, a reference electrode, a flow system, wash solution, waste receptacle and a process controller. The block is thermostatically controlled at 37 °C and its surface provides an interface to the analyte sensors (Fig. 16.2a). The sensors are embedded in three layers of plastic, the size and shape of a credit card. Each card may contain up to 24 sensors. A metallic contact under each sensor forms the electrical interface with the cartridge. As the test sample passes over the sensors, a current is generated by mechanisms specific to the individual analyte and recorded. The size of the current is proportional to the concentration of analyte in the fluid in the sample path. Calibration of the sensors with standard solutions of the analyte allows the concentration of the test sample to be evaluated. The sensor card and the sample path are automatically washed after each test sample and can be used for the analysis of up to 750 whole blood samples before being discarded.

The glucose and lactate sensors have a platinum amperometric electrode with a positive potential relative to the reference electrode. In the case of the measurement of glucose, the glucose oxidase reacts with the glucose and oxygen to generate hydrogen

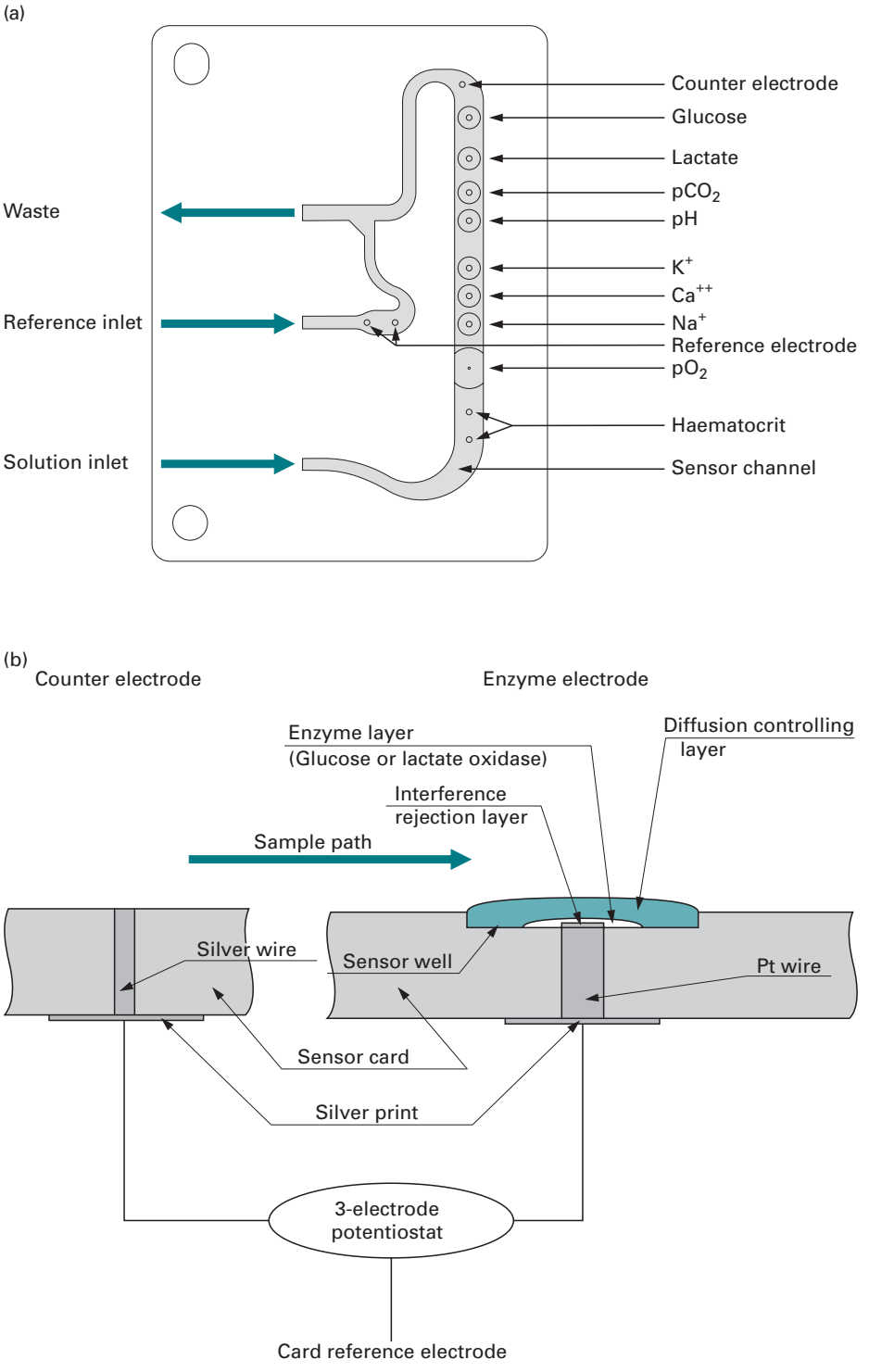

Fig. 16.2 Micro sensor analyte detectors. (a) The GEM 3000 Sensor Card; (b) the Amperometric Glucose/Lactate Analyte Sensors. (Reproduced by permission of IL Critical Care, Lexington, USA.)

peroxide which then diffuses through a controlling layer (Fig. 16.2b) and is oxidised by the platinum electrode to release electrons and create a current flow, the size of which is proportional to the rate of hydrogen peroxide diffusion.

The GEM Premier 4000 system contains an active quality control process controller that monitors the operation of the system, validates the integrity of the cartridge and monitors the electrode response to detect microclots in the test sample that may invalidate the analytical results.

Immunoassay section

Immunoassay procedures undertaken by modern auto-analysers are mostly based on fluorescence or polarised fluorescence techniques. The range of analytes varies from manufacturer to manufacturer but usually involves basic endocrinology (e.g. thyroid function tests), therapeutic drugs (theophylline, digoxin) and drugs of abuse (opiates, cannabis). The operation of auto-analysers in immunoassay mode is similar to that described above and the results are generally reported on the same day, and are generally compared with the previous set of results for the patient.

Manual assays section

This approach to biochemical tests is generally more labour intensive than the other two sections and covers a range of analytical techniques such as acetate or gel electrophoresis, immunoelectrophoresis and some more difficult basic spectrophotometric assays. Examples include the assays for catecholamines (for the diagnosis of phaeochromocytoma), 5-hydroxyindole acetic acid (for the diagnosis of carcinoid syndrome) or HbA_{1c} (for the monitoring of diabetes).

Result reporting

The instrument operator or the section leader initially validates analytical results. This validation process will, in part, be based on the use of internal quality control procedures for individual analytes. Quality control samples are analysed at least twice daily or are included in each batch of test analytes. The analytical results are then subject to an automatic process which identifies results that are either significantly abnormal or require clinical comment or interpretation against rules set by senior laboratory staff.

Neonatal screening

Neonatal or newborn screening is the process of testing newborn babies for certain potentially dangerous disorders. If these conditions are detected early, preventative measures can be adopted that help to protect the child from the disorders. However, such testing is not easy due to the difficulty of obtaining adequate samples of biological fluids for the tests. The development of tandem MS techniques has significantly alleviated this problem. It is possible to screen for a range of metabolic diseases using a single dried bloodspot 3 mm in diameter. There is no need for pre HPLC separation of the sample but the technique is used simply to deliver the sample to the MS. A large number of inherited metabolic diseases can be screened by the technique including **aminoacidopathies** such as phenylketonuria (PKU) caused by a deficiency of the enzyme phenylalanine hydroxylase, **fatty acid oxidation defects** such as medium chain

acyl-CoA dehydrogenase deficiency (MCADD) and organic acidaemias such as propionic acidaemia caused by a deficiency of the enzyme propionyl-CoA carboxylase.

16.2.3 Quality assessment procedures

In order to validate the analytical precision and accuracy of the biochemical tests conducted by a clinical biochemistry department, the department will participate in external quality assessment schemes in addition to routinely carrying out internal quality control procedures that involve the repeated analysis of reference samples covering the full analytical range for the test analyte. In the UK there are two main national clinical biochemistry external quality assessment schemes: the UK National External Quality Assessment Scheme (UK NEQAS: www.ukneqas.org.uk) coordinated at the Queen Elizabeth Medical Centre, Birmingham and the Wales External Quality Assessment Scheme (WEQAS: www.weqas.com) coordinated at the University Hospital of Wales, Cardiff. The majority of UK hospital clinical biochemistry departments subscribe to both schemes. UK NEQAS and WEQAS distribute test samples on a fortnightly basis, the samples being human serum based. In the case of UK NEQAS the samples contain multiple analytes each at an undeclared concentration within the analytical range. The concentration of each analyte is varied from one distribution to the next. In contrast, WEQAS distributes four or five test samples containing the test analytes at a range of concentrations within the analytical range. Both UK NEQAS and WEQAS offer a number of quality assessment schemes in which the distributed test samples contain groups of related analytes such as general chemistry analytes, peptide hormones, steroid hormones and therapeutic drug monitoring analytes. Participating laboratories elect to subscribe to schemes relevant to their analytical services.

The participating laboratories are required to analyse the external quality assessment samples alongside routine clinical samples and to report the results to the organising centre. Each centre undertakes a full statistical analysis of all the submitted results and reports them back to the individual laboratories on a confidential basis. The statistical data record the individual laboratory's data in comparison with all the submitted data and with the compiled data broken down into individual methods (e.g. the glucose oxidase and hexokinase methods for glucose) and for specific manufacturers' systems. Results are presented in tabular, histogram and graphical form and are compared with the results from recent previously submitted samples. This comparison with previous performance data allows longer-term trends in analytical performance for each analyte to be monitored. Laboratory data that are regarded as unsatisfactory are identified and followed up. Selected data from typical UK NEQAS reports are presented in Table 16.4 and Fig. 16.3 and from a WEQAS report in Table 16.5.

16.2.4 Clinical audit and accreditation

In addition to participating in external quality assessment schemes, clinical laboratories are also subject to clinical audit. This is a systematic and critical assessment of the general performance of the laboratory against its own declared standards and procedures

Table 16.4 **Selected UK NEQAS quality assessment data for serum glucose (mM) © The data are reproduced by permission of UK NEQAS, Wolfson EQA Laboratory, Birmingham**

Analytical method	n	Mean	SD	CV (%)
All methods	521	16.04	0.48	3.0
Dry slide	78	15.45	0.51	3.3
OCD (J & J) slides [1JJ]	78	15.45	0.51	3.3
Glucose oxidase electrode	59	15.74	0.32	2.0
Beckman reagents [11BK]	56	15.77	0.28	1.8
Hexokinase + G6PDH	347	16.03	0.44	2.8
Abbott reagents [15AB]	89	16.14	0.43	2.7
Olympus reagents [15OL]	113	16.06	0.40	2.5
Roche Modular reagents [15BO]	70	15.93	0.35	2.2
Glucose oxidase/dehydrogenase	112	16.20	0.47	2.9
Roche Modular reagents [4BO]	60	16.12	0.37	2.3

Notes: The Beckman Glucose Analyser uses the glucose oxidase method and measures oxygen consumption using an oxygen electrode. The Vitros method is a so-called 'dry slide' method that involves placing the sample on a slide, similar to a photographic slide, that has the reagents of the glucose oxidase method impregnated in the emulsion. A blue colour is produced and its intensity measured by reflected light.
These data are for a laboratory that used the hexokinase method and reported a result of 16.0 mM. UK NEQAS calculates a Method Laboratory Trimmed Mean (MLTM) as a target value. It is the mean value of all the results returned by all laboratories using the same method principle with results ±2 SD outside the mean omitted. Its value was 16.03. On the basis of the difference between the MLTM and the laboratory's result UK NEQAS also calculates a score of the specimen accuracy and bias together with a measurement of the laboratory's consistency of bias. This involves aggregating the bias data from all specimens of that analyte submitted by the laboratory within the previous 6 months representing the 12 most recent distributions. This score is an assessment of the tendency of the laboratory to give an over-positive or under-negative estimate of the target MLTM values. The score indicated that the laboratory was performing consistently in agreement with the MLTM.
The results embodied in this table are shown in histogram form in Fig. 16.3.

and against nationally agreed standards. In the context of analytical procedures, the audit evaluates the laboratory performance in terms of the appropriateness of the use of the tests offered by the laboratory, the clinical interpretation of the results and the procedures that operate for the receipt, analysis and reporting of the test samples. Thus whilst it includes the evaluation of analytical data, the audit is primarily concerned with processes leading to the test data with a view to implementing change and improvement. The ultimate objective of the audit is to ensure that the patient receives the best possible care and support in a cost-effective way. The audit is normally undertaken by junior doctors, laboratory staff or CPA (see below) assessors from the hospital, lasts for several days, and involves interaction with all laboratory personnel.

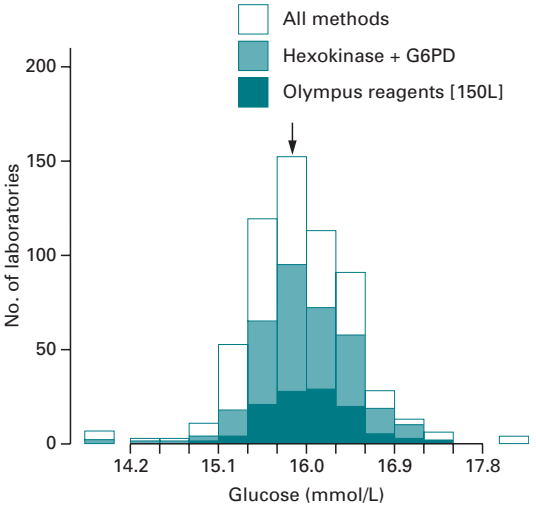

Fig.16.3 Histogram of UK NEQAS quality assessment data for serum glucose based on data in Table 16.2. The arrow indicates the location of the value submitted by the participating laboratory. (Reproduced by permission of UK NEQAS, Wolfson EQA Laboratory, Birmingham, UK.)

Closely allied to the process of clinical audit is that of **accreditation**. However, whereas clinical audit is carried out primarily for the local benefit of the laboratory and its staff and ultimately for the patient, accreditation is a public and national recognition of the professional quality and status of the laboratory and its personnel. The accreditation process and assessment is the responsibility of either a recognised public professional body or a government department or agency. Different models operate in different countries. In the UK, accreditation of clinical biochemistry laboratories is required by government bodies and is carried out by either Clinical Pathology Accreditation (UK) Ltd (CPA) or less commonly the United Kingdom Accreditation Service (UKAS). In the USA accreditation is mandatory and may be carried out by one of a number of 'deemed authorities' such as the College of American Pathologists. Accreditation organisations also exist for non-clinical analytical laboratories. Examples in the UK include the National Measurement Accreditation Service (NAMAS) and the British Standards Institution (BSI). The International Accreditation Co-operation (ILAC), the European Co-operation for Accreditation (EA) and the Asia-Pacific Laboratory Accreditation Co-operation (APLAC) are three of many international fora for the harmonisation of national standards of accreditation for analytical laboratories.

Assessors appointed by the accreditation body assess the compliance by the laboratory with standards set by the accreditation body. The standards cover a wide range of issues such as those of accuracy and precision, timeliness of results, clinical relevance of the tests performed, competence to carry out the tests as judged by the training and qualifications of the laboratory staff, health and safety, the quality of administrative and technical support systems and the quality of the laboratory management systems and document control. The successful outcome of an assessment is the national recognition

Table 16.5 **WEQAS quality assessment data for serum glucose (mM) © The data are reproduced by permission of WEQAS, Directorate of Laboratory Medicine, University Hospital of Wales, Cardiff**

		Sample number			
Analytical method		**1**	**2**	**3**	**4**
Reported result		7.2	3.7	17.2	8.7
Hexokinase	Mean	6.9	3.6	17.0	8.4
	SD	0.2	0.1	0.5	0.3
	Number	220	221	219	219
Aeroset	Mean	7.2	3.8	17.3	8.5
	SD	0.15	0.08	0.045	0.2
	Number	8	8	9	8
Overall	Mean	7.0	3.7	17.2	8.6
	SD	0.28	0.20	0.54	0.33
	Number	388	392	388	389
WEQAS SD		0.26	0.16	0.6	0.3
SDI		1.15	0.63	0.33	1.0

Notes: SD, standard deviation; SDI, standard deviation index.
These data are for a laboratory that used the hexokinase method for glucose using an Aeroset instrument. Accordingly, the WEQAS report includes the results submitted by all laboratories using the hexokinase method and all results for the method using an Aeroset. The overall results refer to all methods irrespective of instrument. All the data are 'trimmed' in that results outside ±2 SD of the mean are rejected which explains why the total number for each test sample varies slightly. WEQAS SD is calculated from the precision profiles for each analyte and the SDI (Standard Deviation Index) is equal to (the laboratory result – method mean result)/WEQAS SD at that level. SDI is a measure of total error and includes components of inaccuracy and imprecision. The four SDIs for the laboratory are used to calculate an overall analyte SDI, in this case 0.78. A value of less than 1 indicates that all estimates were within ±1 SD and is regarded as a good performance. An unacceptable performance would be indicated by a value greater than 2.

that the laboratory is in compliance with the standards and hence provides quality healthcare. The accreditation normally lasts for 4 years.

16.3 EXAMPLES OF BIOCHEMICAL AIDS TO CLINICAL DIAGNOSIS

16.3.1 Principles of diagnostic enzymology

The measurement of the activities or masses of selected enzymes in serum is a long-established aid to clinical diagnosis and prognosis. The enzymes found in serum can be divided into three categories based on the location of their normal physiological function:

- *Serum-specific enzymes*: The normal physiological function of these enzymes is based in serum. Examples include the enzymes associated with lipoprotein metabolism and with the coagulation of blood.
- *Secreted enzymes*: These are closely related to the serum-specific enzymes. Examples include pancreatic lipase, prostatic acid phosphatase and salivary amylase.
- *Non-serum-specific enzymes*: These enzymes have no physiological role in serum. They are released into the extracellular fluid and consequently appear in serum as a result of normal cell turnover or more abundantly as a result of cell membrane damage, cell death or morphological changes to cells such as those in cases of malignancy. Their normal substrates and/or cofactors may be absent or in low concentrations in serum.

Serum enzymes in this third category are of the greatest diagnostic value. When a cell is damaged the contents of the cell are released over a period of several hours with enzymes of the cytoplasm appearing first since their release is dependent only on the impairment of the integrity of the plasma membrane. The release of these enzymes following cell membrane damage is facilitated by their large concentration gradient, in excess of a thousand-fold, across the membrane. The integrity of the cell membrane is particularly sensitive to events that impair energy production, for example by the restriction of supply of oxygen. It is also sensitive to toxic chemicals including some drugs, microorganisms, certain immunological conditions and genetic defects. Enzymes released from cells by such events may not necessarily be found in serum in the same relative amounts as were originally present in the cell. Such variations reflect differences in the rate of their metabolism and excretion from the body and hence of differences in their serum half-lives. This may be as short as a few hours (intestinal alkaline phosphatase, glutathione S-transferase, creatine kinase) or as long as several days (liver alkaline phosphatase, alanine aminotransferase, lactate dehydrogenase).

The clinical exploitation of non-serum-specific enzyme activities is influenced by several factors:

- *Organ specificity*: Few enzymes are unique to one particular organ but fortunately some enzymes are present in much larger amounts in some tissues than in others. As a consequence, the relative proportions (pattern) of a number of enzymes found in serum are often characteristic of the organ of origin.
- *Isoenzymes*: Some clinically important enzymes exist in isoenzyme forms and in many cases the relative proportion of the isoenzymes varies considerably between tissues so that measurement of the serum isoenzymes allows their organ of origin to be deduced.
- *Reference ranges*: The activities of enzymes present in the serum of healthy individuals are invariably smaller than those in the serum of individuals with a diagnosed clinical condition such as liver disease. In many cases, the extent to which the activity of a particular enzyme is raised by the disease state is a direct indicator of the extent of cellular damage to the organ of origin.
- *Variable rate of increase in serum activity*: The rate of increase in the activity of released enzymes in serum following cell damage in a particular organ is a characteristic of each enzyme. Moreover, the rate at which the activity of each enzyme decreases towards the reference range following the event that caused cell damage and the subsequent

treatment of the patient is a valuable indicator of the patient's recovery from the condition.

The practical implication of these various points to the applications of diagnostic enzymology is illustrated by its use in the management of heart disease and liver disease.

16.3.2 Ischaemic heart disease and myocardial infarction

The healthy functioning of the heart is dependent upon the availability of oxygen. This oxygen availability may be compromised by the slow deposition of cholesterol-rich atheromatous plaques in the coronary arteries (Section 18.2.2). As these deposits increase, a point is reached at which the oxygen supply cannot be met at times of peak demand, for example at times of strenuous exercise. As a consequence, the heart becomes temporarily ischaemic ('lacking in oxygen') and the individual experiences severe chest pain, a condition known as **angina pectoris** ('angina of effort'). Although the pain may be severe during such events, the cardiac cells temporarily deprived of oxygen are not damaged and do not release their cellular contents. However, if the arteries become completely blocked either by the plaque or by a small thrombus (clot) that is prevented from flowing through the artery by the plaque, the patient experiences a **myocardial infarction** ('heart attack') characterised by the same severe chest pain but in this case the pain is accompanied by the irreversible damage to the cardiac cells and the release of their cellular contents. This release is not immediate, but occurs over a period of many hours. From the point of view of the clinical management of the patient, it is important for the clinician to establish whether or not the chest pain was accompanied by a myocardial infarction. In about one-fifth of the cases of a myocardial event the patient does not experience the characteristic chest pain ('silent myocardial infarction') but again it is important for the clinician to be aware that the event has occurred. Electrocardiogram (ECG) patterns are a primary indicator of these events but in atypical presentations ECG changes may be ambiguous and additional evidence is sought in the form of changes in serum enzyme activities. The activities of three enzymes are commonly measured:

- *Creatine kinase* (CK): This enzyme converts phosphocreatine (important in muscle metabolism) to creatine. CK is a dimeric protein composed of two monomers, one denoted as M (muscle), the other as B (brain), so that three isoforms exist: CK-MM, CK-MB and CK-BB. The tissue distribution of these isoenzymes is significantly different such that heart muscle consists of 80–85% MM and 15–20% MB, skeletal muscle 99% MM and 1% MB and brain, stomach, intestine and bladder predominantly BB. CK activity is raised in a number of clinical conditions but since the CK-MB form is almost unique to the heart, its raised activity in serum gives unambiguous support for a myocardial infarction even in cases in which the total CK activity remains within the reference range. A rise in total serum CK activity is detectable within 6 hours of the myocardial infarction and the serum activity reaches a peak after 24–36 hours. However, a rise in CK-MB is detectable within 3–4 hours, has 100% sensitivity within 8–12 hours and reaches a peak within 10–24 hours. It remains raised for 2–4 days.

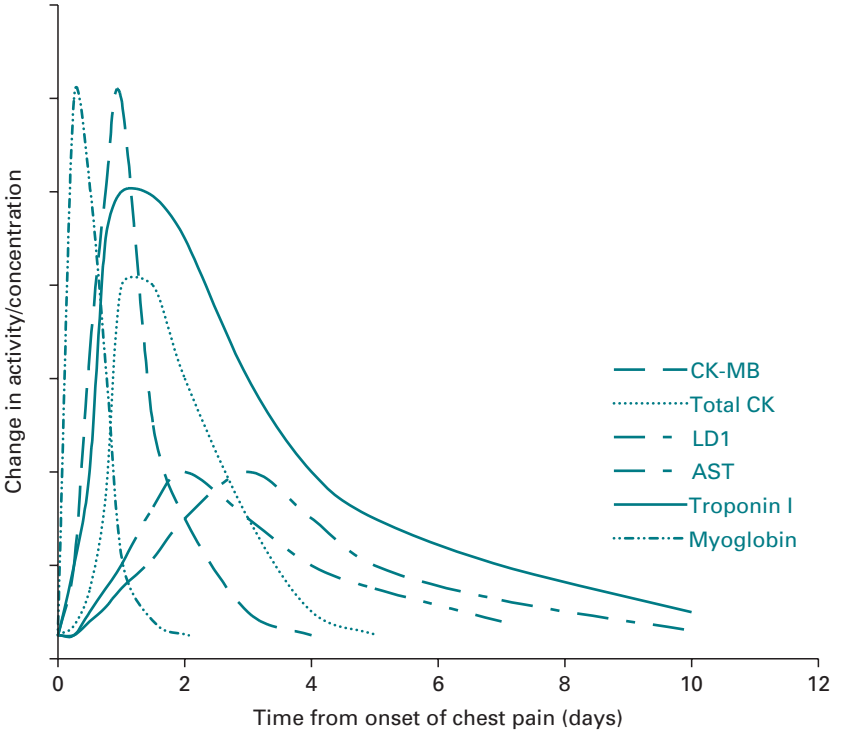

Fig. 16.4 Serum enzyme activity and myoglobin and troponin-I concentration changes following a myocardial infarction. Changes are expressed as a multiple of the upper limit of the reference range. Values vary according to the severity of the event, but the time course of each profile is characteristic of all events.

- *Aspartate aminotransferase* (AST): This is one of a number of transaminases involved in intermediary metabolism. It is found in most tissues but is abundant in heart and liver. Its activity in serum is raised following a myocardial infarction and reaches a peak between 48 and 60 hours. It has little clinical value in the early diagnosis of heart muscle damage but is of use in the case of delayed presentation with chest pain.
- *Lactate dehydrogenase* (LD): This is a tetrameric protein made of two monomers denoted as H (heart) and M (muscle) so that five isoforms exist: LD-1 (H4), LD-2 (H3M), LD-3 (H2M2), LD-4 (HM3) and LD-5 (M4). LD-1 predominates in heart, brain and kidney and LD-5 in skeletal muscle and liver. Total LD activity and LD-1 activity in serum increases following a myocardial infarction and reaches a peak after 48–72 hours. The subsequent decline in activity is much slower than that of CK or AST. The diagnostic value of LD activity measurement is mainly confined to monitoring the patient's recovery from the myocardial infarction event.

Typical changes in the activities of these three enzymes following a myocardial infarction are shown in Fig. 16.4. All three enzymes are assayed by an automated method based on the following reactions.

- *Total CK activity*: This is assessed by coupled reactions (Section 15.3.2) with hexokinase and glucose-6-phosphate dehydrogenase in the presence of *N*-acetylcysteine as

activator, and the measurement of increase in absorbance at 340 nm or by fluorescence polarisation (primary wavelength 340 nm, reference wavelength 378 nm):

$$\text{creatine phosphate} + \text{ADP} \rightleftharpoons \text{creatine} + \text{ATP}$$

$$\text{ATP} + \text{D-glucose} \rightleftharpoons \text{ADP} + \text{D-glucose-6-phosphate}$$

$$\text{D-glucose-6-phosphate} + \text{NAD(P)}^+ \rightleftharpoons \text{D-6-phosphogluconate} + \text{NAD(P)H} + \text{H}^+$$

- *CK-MB activity*: This is assessed by the inhibition of the activity of the M monomer by the addition to the serum sample of an antibody to the M monomer. This inhibits CK-MM and the M unit of CK-MB. The activity of CK-BB is unaffected but is normally undetectable in serum hence the remaining activity in serum is due to the B unit of CK-MB. It is assayed by the above coupled assay procedure and the activity doubled to give an estimate of the CK-MB activity. An alternative assay uses a double antibody technique: CK-MB is bound to anti-CK-MB coated on microparticles, the resulting complex washed to remove non-bound forms of CK and anti-CK-MM conjugated to alkaline phosphatase added. It binds to the antibody–antigen complex, is washed to remove unbound materials and assayed using 4-methylumbelliferone phosphate as substrate, the released 4-methylumbelliferone being measured by its fluorescence and expressed as a concentration (ng cm^{-3}) rather than as activity.
- *Aspartate aminotransferase activity*: This is assessed by a coupled assay with malate dehydrogenase and the measurement of the decrease in absorbance at 340 nm:

$$\text{L-aspartate} + \text{2-oxoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{L-glutamate}$$

$$\text{oxaloacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{malate} + \text{NAD}^+$$

- *Lactate dehydrogenase*: The measurement of total activity is based on the measurement of the increase in absorbance at 340 nm using lactate as substrate. The measurement of LD-1 is based on the use of 2-hydroxybutyrate as substrate since only LD-1 and LD-2 can use it:

$$\text{Total LD:} \quad \text{lactate} + \text{NAD}^+ \rightleftharpoons \text{pyruvate} + \text{NADH} + \text{H}^+$$

$$\text{LD-1 : 2-hydroxybutyrate} + \text{NAD}^+ \rightleftharpoons \text{2-ketobutyrate} + \text{NADH} + \text{H}^+$$

The clinical importance of obtaining early unambiguous evidence of a myocardial infarction has encouraged the development of markers other than enzyme activities and currently two tests are commonly run alongside enzyme activities. These are based on myoglobin and troponin-I:

- *Myoglobin*: Concentrations in serum, assayed by HPLC or immunoassay, increase more rapidly than CK-MB after a myocardial infarction. An increase is detectable within 1–2 hours, has 100% sensitivity and reaches a peak within 4–8 hours and returns to normal within 12–24 hours. However, myoglobin changes are not specific for myocardial infarction since similar changes also occur in other syndromes such as muscle damage or crush injury such as that following a road accident.
- *Troponin-I*: This is one of three proteins (the others being troponin-T and troponin-C) of a complex which regulates the contractility of the myocardial cells. Its activity in

serum increases at the same rate as CK-MB after a myocardial infarction, has a similar time for 100% sensitivity and for peak time, but it remains raised for up to 4 days after the onset of symptoms. Its reference range is less than 1 ng cm^{-3} but its concentration in serum is raised to up to 30–50 ng cm^{-3} within 24 hours of a myocardial infarction event. It is assayed by a 'sandwich' immunological assay in which the antibody is labelled with alkaline phosphatase. Using 4-methylumbelliferone phosphate as substrate, the release of 4-methylumbelliferone is measured by fluorescence. The measurement of serum troponin-I is widely used to exclude cardiac damage in patients with chest pain since it remains raised for several days following a myocardial infarction, but the timing of the test sample is important as a sample taken too early may give a false negative result. A limitation of its use is that its release into serum is not specific to myocardial infarction; an increase in serum mass may occur following a crush injury.

The measurement of enzyme activities and myoglobin and troponin-I concentrations, together with plasma potassium, glucose and arterial blood gases, is routinely used to monitor the recovery of patients following a myocardial infarction. A patient may experience a second myocardial infarction within a few days of the first. In such cases the pattern of serum enzymes shown in Fig. 16.4 is repeated, the pattern being superimposed on the remnants of the first profile. CK-MB is the best initial indicator of a second infarction since the levels of troponin-I may not reflect a secondary event.

The sensitivity and specificity (Section 16.1.2) of ECG and diagnostic enzymology in the management of heart disease are complementary. Thus the specificity of ECG is 100% whilst that of enzyme measurements is 90%, and the sensitivity of ECG is 70% whilst that of enzyme measurements is 95%.

16.3.3 Liver disease

Diagnostic enzymology is routinely used to discriminate between several forms of liver disease including:

- *Hepatitis*: General inflammation of the liver most commonly caused by viral infection but which may also be a consequence of blood poisoning (septicaemia) or glandular fever. It results in only mild necrosis of the hepatic cells and hence of a modest release of cellular enzymes.
- *Cirrhosis*: A general destruction of the liver cells and their replacement by fibrous tissue. It is most commonly caused by excess alcohol intake but is also a result of prolonged hepatitis, various autoimmune diseases and genetic conditions. They all result in extensive cell damage and release of hepatic cell enzymes.
- *Malignancy*: Primary and secondary tumours.
- *Cholestasis*: The prevention of bile from reaching the gut due either to blockage of the bile duct by gallstones or tumours or to liver cell destruction as a result of cirrhosis or prolonged hepatitis. This gives rise to **obstructive jaundice** (presence of bilirubin, a yellow metabolite of haem, in the skin).

Patients with these various liver diseases often present to their doctor with similar symptoms and a differential diagnosis needs to be made on the basis of a range of

investigations including imaging techniques especially ultrasonography (ultrasound), magnetic resonance imaging (MRI), computerised tomography (CT) scanning, microscopic examination of biopsy samples and liver function tests. Four enzymes are routinely assayed to aid differential diagnosis:

- *Aspartate aminotransferase* (*AST*) and *alanine aminotransferase* (*ALT*): As previously stated, these enzymes are widely distributed but their ratios in serum are characteristic of the specific cause of liver cell damage. For example, an AST/ALT ratio of less than 1 is found in acute viral hepatitis and fresh obstructive jaundice, a ratio of about 1 in obstructive jaundice caused by viral hepatitis and a ratio of greater than 1 in cases of cirrhosis.
- *γ-Glutamyl transferase* (*GGT*): This enzyme transfers a γ-glutamyl group between substrates and may be assayed by the use of γ-glutamyl-4-nitroaniline as substrate and monitoring the release of 4-nitroaniline at 400 nm. GGT is widely distributed and is abundant in liver, especially bile canaliculi, kidney, pancreas and prostate but these do not present themselves by contributing to serum levels. Raised activities are found in cirrhosis, secondary hepatic tumours and cholestasis and tend to parallel increases in the activity of alkaline phosphatase especially in cholestasis. Its synthesis is induced by alcohol and some drugs also cause its serum activity to rise.
- *Alkaline phosphatase* (*AP*): This enzyme is found in most tissues but is especially abundant in the bile canaliculi, kidney, bone and placenta. It may be assayed by using 4-nitrophenylphosphate as substrate and monitoring the release of 4-nitrophenol at 400 nm. Its activity is raised in obstructive jaundice and when measured in conjunction with ALT can be used to distinguish between obstructive jaundice and hepatitis since its activity is raised more than that of ALT in obstructive jaundice. Decreasing serum activity of AP is valuable in confirming an end of cholestasis. Raised serum AP levels can also be present in various bone diseases and during growth and pregnancy.

16.3.4 Kidney disease

The kidneys, together with the liver, are the major organs responsible for the removal of waste material from the body. The kidneys also have other specific functions including the control of electrolyte and water homeostasis, and the synthesis of erythropoietin. Each of the two kidneys contains approximately 1 million nephrons that receive the blood flowing to the kidneys. Blood flowing to the kidneys is first presented to the glomerulus of each nephron which filters the plasma water to produce the **ultrafiltrate** or **primary urine** removing all the contents of the plasma except proteins. Each nephron produces approximately 100 mm^3 of primary urine per day giving a total production of primary urine by the two kidneys of approximately 100–140 cm^3 per minute or 200 dm^3 per day in a healthy adult person. This is referred to as the **glomerular filtration rate** (GFR). The primary urine then encounters the tubule of the nephron that is the site of the reabsorption of water and the active and passive reabsorption of lipophilic compounds and cellular nutrients such as sugars and amino acids and the active secretion of others. These two processes in combination result in the production of approximately 2 dm^3 of urine per day that is collected in the urinary bladder.

Glomerular filtration rate is the accepted best indicator of kidney function. Any pathology of the kidneys is reflected in a decreased GFR and this in turn has serious physiological consequences including anaemia and severe cardiovascular disease. Kidney disease is a progressive one, proceeding through subacute or intrinsic renal disease such as glomerular nephritis into chronic kidney disease (CKD). Complete kidney failure leads to the need for kidney dialysis and kidney transplantation. There is evidence that the incidence of CKD is increasing in developed countries and is associated with increasing risk of diabetes and an increasingly elderly population. There is thus a great clinical demand for accurate measurements of GFR in order to detect the onset of kidney disease, to assess its severity and to monitor its subsequent progression.

Measurement of glomerular filtration rate

The measurement of GFR is based on the concept of **renal clearance** which is defined as the volume of serum cleared of a given substance by glomerular filtration in unit time. It therefore has units of $cm^3\ min^{-1}$. In principle any endogenous or exogenous substance that is subject to glomerular filtration and is not reabsorbed could form the basis of the measurement. The polysaccharide inulin meets these criteria and is subject to few variables or interferences but because it is not naturally occurring in the body is inconvenient for routine clinical use but is commonly used as a standard for alternative methods. In practice serum creatinine is the most commonly used marker. It is the end product of creatine metabolism in skeletal muscle and meets the excretion criteria so that its serum concentration is inversely related to GFR. However, it is subject to a number of non-renal variables including:

- *Muscle mass*: Serum values are influenced by extremes of muscle mass as in athletes and in individuals with muscle-wasting disease or malnourished patients.
- *Gender*: Serum creatinine is higher in males than females for a given GFR.
- *Age*: Children under 18 years have a reduced serum creatinine and the elderly have an increased value.
- *Ethnicity*: African–Caribbeans have a higher serum creatinine for a given GFR than have Caucasians.
- *Drugs*: Some commonly used drugs such as cimetidine, trimethoprim and cephalosporins interfere with creatinine excretion and hence give elevated GFR values.
- *Diet:* Recent intake of red meats and oily fish can raise serum creatinine levels.

Routine laboratory estimations of GFR (referred to as *e*GFR) are based on the measurement of serum creatinine concentration and the calculation of *e*GFR from it using an equation that makes corrections for four of the above variables. Serum creatinine is routinely measured by one of two ways:

- *Spectrophotometric method based on the Jaffe reaction*: This involves the use of alkaline picric acid reagent which produces a red-coloured product that is measured at 510 nm. A limitation is that the reagent also reacts with some non-creatinine chromogens such as ketones, ascorbic acid and cephalosporins and as a result gives high values.

- *Coupled enzyme assay*: The method uses either creatinine kinase, pyruvate kinase or lactate dehydrogenase and measuring the change in absorption at 340 nm:

$$\text{creatinine} + H_2O \rightleftharpoons \text{creatine}$$
$$\text{creatine} + \text{ATP} \rightleftharpoons \text{phosphocreatine} + \text{ADP}$$
$$\text{ADP} + \text{phosphoenolpyruvate} \rightleftharpoons \text{ATP} + \text{pyruvate}$$
$$\text{pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{L-lactate} + NAD^+$$

or an alternative coupled assay using creatinine iminohydrolase and glutamate dehydrogenase.

For research, two other methods are available:

- *HPLC or GC/MS:* HPLC uses a C18 column and water/acetonitrile (95:5 v/v) eluent containing 1-octanesulphonic acid as a cation-pairing agent. GC-MS is based on the formation of the *t*-butyldimethylsilyl derivative of creatinine.
- *Isotopic dilution*: This method is coupled with mass spectrometry (ID-MS). This involves the addition of ^{13}C- or ^{15}N-labelled creatinine to the serum sample, isolation of creatinine by ion-exchange chromatography and quantification by mass spectrometry using selective ion monitoring. The lower limit of detection is about 0.5 ng.

The lack of an internationally or even nationally agreed standard assay for creatinine leads to significant inter-laboratory differences in both bias and imprecision so that national external quality assurance schemes, such as UK NEQAS and WEQAS (Section 16.2.3) have important roles in alerting laboratories to assays that stray outside national control values. UK NEQAS provides clinical laboratories that participate in the *e*GFR scheme with an **assay-specific adjustment factor** (F) to correct for methodological variations in estimations of serum creatinine. The factor is obtained using calibration against a GC-MS creatinine assay. It is updated at 6-monthly intervals. A number of equations have been derived to calculate *e*GFR from serum creatinine values but the one currently used throughout the UK is the four-variable **Modification of Diet in Renal Disease** (MDRD) Study equation:

$$e\text{GFR} = F \times 175 \times (\text{serum creatinine}/88.4)^{-1.154} \times \text{age}^{-0.203} \times 0.742(\text{if female}) \times 1.212(\text{if black})$$

Serum creatinine concentrations are expressed in μM to the nearest whole number and are adjusted for variations in body size by normalising using a factor for body surface area (BSA) correcting to a BSA value of 1.73 m^2. The units of *e*GFR are therefore $cm^3\,min^{-1}\,1.73\,m^{-2}$ and values are reported to one decimal place. The equation has been validated in a large-scale study against the most accurate method based on the use of ^{125}I-iothalamate GFR. Alternative equations exist for use with children.

Reference values for *e*GFR are 130 $cm^3\,min^{-1}\,1.73\,m^{-2}$ for males in the age range 20–30 and 125 $cm^3\,min^{-1}\,1.73\,m^{-2}$ for females of the same age. Values decline with increasing age becoming 95 and 85 $cm^3\,min^{-1}\,1.73\,m^{-2}$ for males and females respectively in the age range 50–60 and 70 and 65 $cm^3\,min^{-1}\,1.73\,m^{-2}$ for males and females in the age range 70–80.

Table 16.6 **Stages of chronic kidney disease (CKD)**

CKD stage	eGFR ($cm^3\ min^{-1}$ 1.73 m^{-2})	Clinical relevance
1	>90	Regard as normal unless other symptoms present[a]
2	60–89	Regard as normal unless other symptoms present[a]
3	30–59	Moderate renal impairment
4	15–29	Severe renal impairment
5	<15	Advanced renal failure

Note: [a]Symptoms include persistent proteinuria, haematuria, weight loss, hypertension.

Clinical assessment of renal disease

Acute renal failure (ARF)

Acute renal failure is the failure of renal function over a period of hours or days and is defined by increasing serum creatinine and urea. It is a life-threatening disorder caused by the retention of nitrogenous waste products and salts such as sodium and potassium. The rise in potassium may cause ECG changes and a risk of cardiac arrest. Acute renal failure may be classified into pre-renal, renal and post-renal. Prompt identification of pre- or post-renal factors and appropriate treatment action may allow correction before damage to the kidneys occurs. Pre-renal failure occurs due to a lack of renal perfusion. This can occur in volume loss in haemorrhage, gastrointestinal fluid loss and burns or because of a decrease in cardiac output caused by cardiogenic shock, massive pulmonary embolus or cardiac tamponade (application of pressure) or other causes of hypertension such as sepsis. Post-renal causes include bilateral uretic obstruction because of calculi or tumours or by decreased bladder outflow/urethral obstruction e.g. urethral stricture or prostate enlargement through hypertrophy of carcinoma. Correction of the underlying problem can avoid any kidney damage. Renal causes of acute renal failure include glomerular nephritis, vascular disease, severe hypertension, hypercalcaemia, invasive disorders such as sarcoidosis or lymphoma and nephrotoxins including animal and plant toxins, heavy metals, aminoglycosides, antibiotics and non-steroidal anti-inflammatory drugs.

Chronic kidney disease (CKD)

CKD is a progressive condition characterised by a declining *e*GFR (Table 16.6). All CKD patients are subject to regular clinical and laboratory assessment and once Stage 3 has been reached to additional clinical management. This is aimed at attempting to reverse or arrest the disease by drug therapy saving the patient the inconvenience and the paying authority the cost of dialysis or transplantation.

16.3.5 Endocrine disorders

Endocrine hormones are synthesised in the brain, adrenal, pancreas, testes and ovary, and most importantly in the hypothalamus and pituitary, but they act elsewhere in

Table 16.7 **Examples of hormones of the hypothalamus–pituitary axis**

Secreted hormone	Pituitary effect	Gland effect
Thyrotropin-releasing hormone (TRH)	Release of thyroid-stimulating hormone (TSH)	Release of thyroxine (T4) and triiodothyronine (T3) by thyroid gland
Growth-hormone-releasing hormone (GHRH)	Release of growth hormone (GH)	Stimulates cell and bone growth
Corticotropin-releasing hormone (CRH)	Release of adrenocorticotropic hormone (ACTH)	Stimulates production and secretion of cortisol in adrenal cortex
Gonadotropin-releasing hormone (GnRH)	Release of follicle-stimulating hormone (FSH) and luteinising hormone (LH)	FSH – maturation of follicles/ spermatogenesis; LH – ovulation/ production of testosterone

the body as a result of their release into the circulatory system. The result is a **hormonal cascade** that incorporates an amplification of the amount of successive hormone released into the circulatory system, increasing from micrograms to milligrams, as well as a negative feedback that operates to control the cascade when the level of the 'action' hormone has reached its optimum value. Most signals originate in the central nervous system as a result of an environmental (external) signal, such as trauma or temperature, or an internal signal. The response is a signal to the hypothalamus and the release of a hormone such as corticotropin-releasing hormone (CRH). This travels in the bloodstream to the anterior pituitary gland where it acts on its receptor and results in the release of a second hormone, adrenocorticotropic hormone (ACTH). It circulates in the blood to reach its target gland, the adrenal cortex, where it acts to release the 'action' hormone, cortisol, known as the stress hormone. The released cortisol raises blood pressure and blood glucose and is subject to a natural diurnal variation, peaking in early morning and being lowest around midnight. It has a negative feedback effect on the pituitary and adrenal cortex. Glands linked by the action of successive hormones are referred to as an **axis**, e.g. the hypothalamus–pituitary–adrenal axis. These coordinated cascades regulate the growth and function of many types of cell (Table 16.7). The hormones released act at specific receptors, commonly of the GPCR type discussed in Section 17.4.3, which trigger the release of second messengers such as cAMP, cGMP, inositol triphosphate, Ca^{2+} and protein kinases. Diseases of the endocrine system result in dysregulated hormone release, inappropriate signalling response or, in extreme cases, the destruction of the gland. Examples include diabetes mellitus, Addison's disease, Cushing's syndrome, hyper- and hypothyroidism and obesity. Such medical conditions are characterised by their long-term nature. Laboratory tests are commonly employed to measure hormone levels in order to assist in the diagnosis of the condition and the subsequent care of the patient.

Thyroid function tests

Approximately 1% of the population suffer from some form of thyroid disease although in many cases the symptoms may be non-specific. Even so, over 1 million thyroid function tests are conducted annually in the UK. As shown in Table 16.7, the hypothalamus releases thyrotropin-releasing hormone (TRH) which acts directly on the pituitary to produce thyroid-stimulating hormone (TSH) which in turn stimulates the thyroid gland to produce two thyroid hormones, thyroxine (T4) and triiodothyronine (T3). The gland produces approximately 10% of the circulating T3, the remainder being produced by the metabolism of T4 mainly in the liver and kidney. The majority of T4 and T3 are bound to thyroxine-binding globulin (TBG) but only the free unbound forms (fT4, fT3) are biologically active. Although the concentration of T3 is approximately one-tenth of that of T4, T3 is ten times more active. Both hormones act on nuclear receptors to increase cell metabolism and both have a negative feedback effect on the hypothalamus to switch off the secretion of TRH and on the pituitary to switch off TSH secretion. Hyperthyroidism is a consequence of the overproduction of the two hormones and common causes are thyroiditis, Grave's disease and TSH-producing pituitary tumours. Hypothyroidism, characterised by weakness, fatigue, weight gain and joint or muscle pain, may be primary due to the undersecretion of T4 and T3, possibly due to irradiation or drugs such as lithium, or secondary due to damage to the hypothalamus or pituitary. Normal laboratory tests for these conditions are based on the measurement of TSH and either total (bound and unbound) T4 and total T3 or fT4 and fT3 all by immunoassay.

16.3.6 Hypothalamus–pituitary–gonad axis

In both sexes, the hypothalamus produces gonadotropin-releasing hormone (GRH) that stimulates the pituitary to release luteinising hormone (LH) and follicle-stimulating hormone (FSH). In males, the release of LH and FSH is fairly constant, whereas in females the release is cyclical. In males LH stimulates Leydig cells in the testes to produce testosterone which together with FSH causes the production of sperm. The testosterone has a negative feedback effect on both the hypothalamus and the pituitary thereby controlling the release of GRH. The testosterone acts on various body tissues to give male characteristics. In females, FSH acts on the ovaries to produce both oestradiol and the development of the follicle. The oestradiol and LH then act to stimulate ovulation. Oestradiol has a negative feedback effect on the hypothalamus and the pituitary and acts on body tissues to produce female characteristics.

16.3.7 Diabetes mellitus

Diabetes is the most common metabolic disorder of carbohydrate, fat and protein metabolism, and is primarily due to either a deficiency or complete lack of the secretion of insulin by the β-cells of the islets of Langerhans in the pancreas. It affects 1–2% of Western populations and 5–10% of the population over the age of 40. It is characterised

Case study **HYPOTHYROID CASE**

A 59-year-old woman presented with a history of lethargy, cold intolerance and weight gain. On examination, the doctor noticed that the patient's hair appeared thin and her skin dry. Several tests were requested including thyroid function tests the results of which were:

$\mathrm{TSH} = 46.9\,\mathrm{mU\,dm^{-3}}$ (normal range $0.4 - 4.5\,\mathrm{mU\,dm^{-3}}$)
$\mathrm{fT4} = 5.6\,\mathrm{pM}$ (normal range $9.0 - 25\,\mathrm{pM}$)

These results indicate overt primary hypothyroidism. As the patient suffered from cardiovascular disease the doctor commenced thyroxine replacement therapy at an initial dose of 25 μg daily. After 2 weeks, the tests were repeated:

$\mathrm{TSH} = 37.6\,\mathrm{mU\,dm^{-3}}$
$\mathrm{fT4} = 8.2\,\mathrm{pM}$

These results remain abnormal so it was agreed that the tests should be repeated in 6 weeks' time. At this stage the results were:

$\mathrm{TSH} = 19.1\,\mathrm{mU\,dm^{-3}}$
$\mathrm{fT4} = 11.8\,\mathrm{pM}$

These results confirm that either the thyroxine replacement dosage was inadequate or that compliance was poor. As the patient confirmed that she had been taking the therapy as prescribed, the doctor increased the dose to 50 μg per day. After a further 8 weeks the tests were repeated:

$\mathrm{TSH} = 1.5\,\mathrm{mU\,dm^{-3}}$
$\mathrm{fT4} = 14.8\,\mathrm{pM}$

The patient reported feeling much better and had improved clinically.

Comment The above results are typical of patients with hypothroidism, also referred to as **myxoedema**. Primary hypothyroidism due to thyroid gland dysfunction is by far the most common cause of the condition but secondary (pituitary) and tertiary (hypothalamic) causes also exist. In these latter two cases the main biochemical abnormality is a low fT4. TSH may be low or within the reference range in secondary and tertiary hypothroidism, i.e. it does not respond to low fT4. Patients with primary hypothroidism require lifelong therapy. Patients with cardiovascular disease, as in this case, must be initiated at a lower dose than normal as over-treatment can lead to angina, cardiac arrhythmia and myocardial infarction. Elderly patients are also started at a lower dose for the same reason. Once therapy has been commenced, thyroid function tests should be carried out after 2–3 months to check for steady-state conditions and thereafter repeated on an annual basis.

by **hyperglycaemia** (elevated blood glucose level) leading to long-term complications. Diabetes can be classified into a number of types:

- *Insulin-dependent diabetes* (Type 1) (also called *juvenile diabetes* and *brittle diabetes*) is due to the autoimmune destruction of β-cells in the pancreas. Generally it has a rapid onset with a strong genetic link.

Case study **PREGNANCY**

A 28-year-old female PE teacher presented to her GP with non-specific symptoms of increased tiredness, nausea, stomach cramps and amenorrhoea with a last menstrual period 3 months previously. She was a previously fit, healthy lady who had a normal menstrual history. Having recently moved house, she thought that stress might be the cause of her symptoms. Her GP requested routine biochemistry tests including thyroid function tests, all of which were within normal reference range. A urine pregnancy test was also performed and to the patient's surprise was positive and confirmed by laboratory serum β-human chorionic gonadotropin (β-hCG) of 150 640 IU dm^{-3}.

Comment These results confirm that this lady was approximately 10 weeks pregnant. The serum β-hCG levels during pregnancy are shown in Fig. 16.5. A level >25 IU dm^{-3} is indicative of pregnancy. Implantation of the developing embryo into the endometrial lining of the uterus results in the secretion of β-hCG and as pregnancy continues its synthesis increases at an exponential rate, doubling every 2 days, and reaching a peak of 100 000–200 000 IU dm^{-3} at 60–90 days (1st trimester). Levels then decline to approximately 1000 IU dm^{-3} at around 20 weeks pregnancy (during 2nd trimester) to a stable plateau for the remainder of the pregnancy. Oestradiol, oestrone, oestriol and progesterone all increase in the early stages of pregnancy as a result of the action of β-hCG on the corpus luteum of the ovaries. Unlike β-hCG levels, the levels of the three oestrogens and progesterone continue to rise during pregnancy playing a vital role in the sustenance and maintenance of the foetus. At the end of pregnancy, the placental production of progesterone falls, stimulating contractions leading to birth.

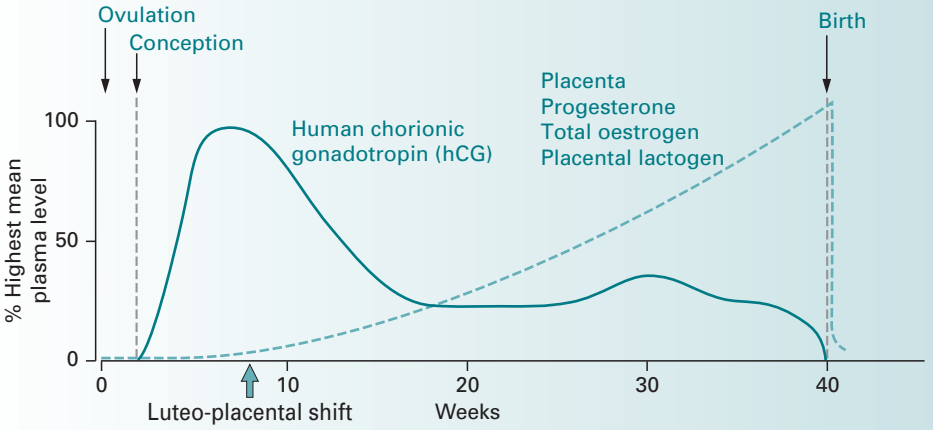

Fig. 16.5 Hormonal profile during pregnancy. (Adapted with permission from Professor Alan S. McNeilly, MRC Human Reproductive Science Unit, Edinburgh, UK.)

- *Non-insulin-dependent diabetes* (Type 2) (also called *adult-onset diabetes* and *maturity-onset diabetes*), is a complex progressive metabolic disorder characterised by β-cell failure and variable insulin resistance. A subtype is *maturity-onset diabetes of the young* (MODY) which usually occurs before the age of 25 years. It is the first form of diabetes for which a genetic cause and molecular consequence have been established. Mutations of the genes for hepatocyte nuclear factor 4α (MODY1), glucokinase (MODY2), HNF1α (MODY3), insulin promotor factor 1 (MODY4), HNF1β (MODY5) and neurogenic differentiation factor 1 (MODY6) have all been characterised.

- *Impaired glucose tolerance* where there is an inability to metabolise glucose in the 'normal' way but not so impaired as to be defined as diabetes.
- *Gestational diabetes* that is any degree of glucose intolerance developed during pregnancy. It is characterised by a decrease in insulin sensitivity and an inability to compensate by increased insulin secretion. The condition is generally reversible after the termination of pregnancy, but up to 50% of women who develop it are prone to develop Type 2 diabetes later in life.
- *Other types* which include certain genetic syndromes, pancreatic disease, endocrine disease and drug or chemical induced diabetes.

Insulin-dependent diabetes (Type 1)

Between 5% and 10% of all diabetics have the insulin-dependent form of diabetes requiring regular treatment with insulin. Type 1 develops in young people with a peak incidence of around 12 years of age. In this type of diabetes the degree of insulin deficiency is so severe that only insulin replacement can avoid the complications of diabetes that are discussed later. Dietary control or oral drugs are not sufficient. The disease is caused by the autoimmune destruction of β-cells in the pancreas thus reducing the ability of the body to produce insulin. Islet cell antibodies (ICA), antibodies IA-2 and IA-2β to transmembrane protein tyrosine phosphatases in islet cells, autoantibodies to glutamic acid decarboxylase (GAD) found in β-cells and insulin autoantibodies (IAA) are all used as diagnostic markers of the disease.

Non-insulin-dependent diabetes (Type 2)

Type 2 accounts for 90% of all cases and develops later in life and can be exacerbated by obesity. MODY versions account for 1–5% of all cases and are not associated with obesity. From population screening studies it is thought that only half of those individuals with Type 2 have been diagnosed. Control of blood glucose levels in this group is normally by a combination of diet and oral drug therapy but occasionally it may require insulin injection. There is growing evidence that the increasing worldwide incidence of Type 2 diabetes may, in part, be linked to the increasing concentration of so-called persistent organic pollutants, such as bisphenol A, DDT and polychlorinated biphenyls (PCBs), in the environment. These compounds suppress adiponectin, a hormone that regulates fatty acid catabolism and glucose metabolism.

Diagnosis and monitoring of control of diabetes

Diabetes is frequently recognised by the symptoms it causes but can be confirmed by clinical biochemical measurements based on World Health Organisation (WHO) recommendations:

- a fasting (12 hours) plasma glucose level greater than 7.0 mM;
- a random plasma glucose level greater than 11.1 mM;
- application of an oral glucose tolerance test in which a 75 g dose of glucose is administered and the plasma level measured after 2 hours. Diabetes is characterised by a value greater than 11.1 mM.

The diagnostic cut-off values of 7.0 and 11.1 mM are based on the level at which retinopathy begins to appear in a population. The clinical aim in the treatment of Type 1

diabetes is to maintain plasma glucose levels in the healthy range of 4–6 mM. This is typically monitored by patients themselves by measuring their blood glucose at predetermined times that are interrelated to their meal times during the day. For example, the lowest blood glucose of the day is likely to be after the longest fast before breakfast and the highest blood glucose of the day is likely to be 1 hour after the main meal. By manipulating treatment around these highs and lows, good glycaemic control is generally maintained. The patients measure their blood glucose using hand-held, portable blood glucose meters based on glucose oxidase using dry stick technology to measure finger-prick blood samples.

Another measure of glycaemic control is by using haemoglobin A1c (Hb_{a1c}) measurements. This testing strategy works on the basis that most proteins (in this case haemoglobin A) will bind glucose dependent on the length of time they are in contact with glucose, the temperature and the concentration of glucose. Hence haemoglobin, having a typical half-life of 120 days and a standardised body temperature of 37 °C, will bind the appropriate amount of glucose depending on the concentration of glucose. Hb_{a1c} is typically measured in the clinic using HPLC to separate the different haemoglobin pigments and is expressed as a percentage of total haemoglobin. In 2009 the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) published recommendations for the standardisation of Hb_{alc} using IFCC standards that allow traceability of the method back to the IFCC reference method. This caused a change in units from % to mM. This was introduced in June 2009 in the UK although most laboratories report results in both units for education purposes. The lower the result the better the control. This test is extremely useful in measuring long-term control of diabetes but is not without its pitfalls. For example, if the patient has very brittle diabetes having equal numbers of hypoglycaemic and hyperglycaemic periods (see below), then the hypos will cancel out the hyper periods and the Hb_{a1c} will appear to show that the patient is in good glycaemic control.

Complications of diabetes

The diabetic patient needs to have regular intake of carbohydrate to maintain their blood glucose level and appropriate levels of insulin treatment. If these are not in balance then hypoglycaemia or hyperglycaemia may take place. In hypoglycaemia the patient will become cold, clammy and sweaty and may become confused or even unconscious. Giving a sweet drink easily treats this complication. The major complication of hyperglycaemia is diabetic ketoacidosis. Almost one-third of insulin-dependent diabetic patients present for the first time with ketoacidosis that is often precipitated by infection. The biochemical features of presentation are a high or very high blood glucose level, glycosuria (glucose in the urine) and ketonuria (ketones in the urine). The patient's breath will often smell of acetone. Treatment consists of administration of fluids and an insulin infusion but this can often lead to precipitate falls in serum potassium and this must be monitored at all times.

All types of diabetes are also associated with several types of long-term complication. These can largely be split into macro-vascular disease, micro-vascular disease and others. Macro-vascular disease involves accelerated atherosclerosis in the large and medium-sized vessels. Macro-vascular disease accounts for most of the excessive

mortality seen in diabetes. Micro-vascular disease leads to diabetic retinopathy and diabetic nephropathy and diabetic foot that may turn to gangrenous ulcers of the feet. The other major complications of diabetes are conditions such as gout, fatty changes in the liver, hypertension and diabetic dyslipidaemias (raised blood lipid levels).

16.3.8 Plasma proteins

Plasma contains a very large number of proteins many of which are present only in trace amounts. The ones that have their main physiological role in plasma have three main functions:

- osmotic regulation;
- transport of ligands such as hormones, metal ions, bilirubin, fatty acids, vitamins and drugs;
- response to infection or foreign bodies entering the body.

All plasma proteins are synthesised in the liver with the exception of the immunoglobulins which are synthesised in the bone marrow. Plasma proteins are readily separated by electrophoresis and this technique forms the basis of several clinical diagnostic tests. The tests are normally recorded subjectively, but a densitometer may be used to get a semi-quantitative result.

Albumin

Albumin is the commonest plasma protein making up some 50% of all plasma protein. Its half-life in plasma is about 20 days and in a good nutritional state the liver produces about 15 g a day to replace this loss. Albumin is the main regulator of the osmotic pressure of plasma but also acts as a transporter of haem, bilirubin (a metabolite of haem), biliverdin (a metabolite of bilirubin), free fatty acids, steroids and metal ions (e.g. Cu^{2+}, Fe^{3+}). It also binds some drugs. Other specialist proteins found in plasma are also involved in transport, for example of steroids e.g. cortisol-binding globulin, sex-hormone-binding globulin (androgens and oestrogens) and metal ions, e.g. ceruloplasmin (Cu^{2+}) and transferrin (Fe^{3+}). Other transport plasma proteins include thyroid-binding globulin (thyroxine T4 and triiodothyronine T3) and haptoglobin (haemoglobin dimers).

Immunoglobulins

Immunoglobulins are synthesised in bone marrow in response to the exposure to a specific foreign body (Chapter 7). Immunoglobulins share a common Y-shaped structure of two heavy and two light chains, the light chains forming the upper arms of the Y. There are two types of light chains, these are either kappa (κ) or lambda (λ), and each are found in all classes of the immunoglobulins. The class of immunoglobulin is determined by the heavy chain that gives rise to five types – IgG, IgA, IgM, IgD and IgE.

IgG accounts for approximately 75% of the immunoglobulins present in the plasma of adults and has a half-life of approximately 22 days. It is present in extracellular fluids and appears to eliminate small proteins through aggregation and the reticuloendothelial system. IgA is the secretory immunoglobulin protecting the mucosal surfaces. IgA is synthesised by mucosal cells and represents approximately 10% of plasma immunoglobulins and has a half-life of 6 days. It is found in bronchial and intestinal secretions

and is a major component of colostrum (the form of milk produced by the mammary gland immediately after giving birth). IgA is the primary immunological barrier against pathogenic invasion of the mucosal membranes. IgM is found in the intravascular space and its role is to eliminate circulating microorganisms and antigens. IgM accounts for about 8% of plasma immunoglobulins and has a half-life of 5 days. IgM is the first antibody to be synthesised after an antigenic challenge. IgD and IgE are minor immunoglobulins whose roles are not clear since a deficiency of either seems to be associated

Case study **MYELOMA**

A 72-year-old woman presented to her GP with a 3 week history of painful hips, chest and shoulders, and with shortness of breath on exertion. She was constipated and had lost 12.5 kg over the last 6 months. She complained that she was very thirsty and had to get up to urinate during the night, something that she had previously never had to do.

Initial laboratory investigations found her to be hypercalcaemic, dehydrated and anaemic. Her biochemistry results showed marked renal impairment, with raised urea and creatinine. Her alkaline phosphatase (AP) was within the reference range. Her serum protein concentration was raised, despite having a low albumin. Serum protein electrophoresis showed a large monoclonal band in the gamma globulin region. By comparison of area under the peak with total protein, the band was quantified as 61 g dm^{-3}. The band was typed as IgAκ by immunofixation, using antisera specific against individual immunoglobulin subclasses to bind the monoclonal protein to the electrophoresis before staining. An early morning urine sample was requested for Bence Jones protein analysis by electrophoresis. This detected a large band of free κ light chains in the urine. Her immunoglobulins were quantified by turbidimetry, a method that measures the refraction of light by antibody complexes. The results indicated that her other immunoglobulins were suppressed, leaving her susceptible to infection. An isotope bone scan using diphosphonates labelled with ^{99}Te demonstrated osteolytic lesions (bone loss) that are characteristic of multiple myeloma.

Comment The two most common causes of hypercalcaemia are primary hyperparathyroidism and malignancy. The signs and symptoms in a person of this age are typical of multiple myeloma, especially in the context of hypercalcaemia with a normal AP, which is raised in primary hyperparathyroidism. Hypercalcaemia results from stimulation of osteoclasts (a type of bone cell) released by the myeloma cells and can cause polyuria, polydipsia (need to drink excessive fluid) and dehydration. The impaired renal function in this lady may be a result of hypercalcaemia and Bence Jones protein as both are nephrotoxic. As the malignant plasma cells proliferate throughout the bone marrow, the bone marrow has a reduced capacity to produce normal cells, causing anaemia and immunosuppression. The difference between the concentration of serum total protein and albumin is attributed to the Bence Jones proteins. These proteins contribute to this fraction and consequently myeloma patients can have a high total protein concentration in the presence of normal or low albumin.

The patient was rehydrated with intravenous saline and started on a bisphosphonate to lower her calcium. Her renal failure resolved over time, although some patients with advanced myeloma will require haemodialysis. A bone marrow aspiration was performed, which showed $>$80% infiltration of plasma cells, confirming the diagnosis of multiple myeloma. A serum β2-microglobulin was requested as a prognostic indicator. Once she had stabilised, she was commenced on a course of dexamethasone, a synthetic glucocorticoid that binds immunoglobulins and hence relieves some of the symptoms of the malignancy. Her response to treatment was monitored by regular quantification of her monoclonal band.

with no obvious pathology. IgE plays a major part in allergy and may be significantly raised in situations of allergic response, for example in hay fever and atopic eczema.

Myeloma

Myeloma, also called **multiple myeloma**, is a malignant pathology of plasma cells in which there is a proliferation of a single β-cell clone in the bone marrow effectively behaving as a tumour. The replication of the cell is unregulated so it proliferates. The cells produce large quantities of a single identical antibody which runs as a single dense band in the gamma globulin region on electrophoresis of a serum sample. The protein is called the **para protein** and has been shown to be an immunoglobulin with two light chains and two heavy chains. Some myelomas produce an excess of light chains that appear in the serum and because they are small they also appear in the urine. They are detected by electrophoresis and are referred to as **Bence Jones proteins**. Their detection is a bad prognosis as they indicate that the cell line may be more aggressive and replicating faster. In rare cases of myeloma, the marrow cells only produce light chains.

Acute phase response

Following a stimulus of tissue injury or infection, the body will respond by producing an **acute phase response** characterised by the release from the liver of a number of **acute phase proteins** which cause a change in the pattern of plasma protein electrophoresis. There will be an increased synthesis of some proteins such as α-1-antitrypsin, a proteinase inhibitor that down regulates inflammation, fibrinogen and prothroylian (coagulation) complement and C-reactive protein (CRP). These are referred to as **positive** acute phase proteins. There will also be a decrease in the production of other proteins such as albumin, transferrin and transcortin. These are known as **negative** acute phase proteins. The clinical measurement of acute phase proteins, particularly CRP, by immunoassay is widely used as a marker of inflammation in a variety of clinical conditions.

16.4 SUGGESTIONS FOR FURTHER READING

Basic principles

Saunders, G. C. and Parkes, H. C. (1999). *Analytical Molecular Biology*. Teddington: LGC. (Contains an excellent chapter on quality in the molecular biology laboratory.)

Clinical biochemistry

Beckett, G. I., Walker, S. W., Rae, P. and Ashby, P. (2005). *Lecture Notes on Clinical Biochemistry*, 6th edn. Oxford: Blackwell Science. (An excellent reference text for all aspects of clinical biochemistry.)

Bruns, D. E. and Ashwood, E. R. (2007). *Tietz Fundementals of Clinical Chemistry*, 6th edn. Philadelphia: W. B. Saunders. (A comprehensive coverage of the principles and practice of clinical biochemistry.)

Data analysis

Jones, R. and Payne, B. (1997). *Clinical Investigation and Statistics in Laboratory Medicine*. London: ACB Ventures. (Written specifically for analytical studies in clinical biochemistry.)

Newborn screening

Blau, N., Duran, M., Blaskovics, M. E. and Gibson, K. M. (eds.) (2003). *Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases*, 2nd edn. Berlin: Springer-Verlag.

Chace, D. H. and Kalas, T. A. (2005). A biochemical perspective on the use of tandem mass spectrometry for newborn screening and clinical testing. *Clinical Biochemistry*, **38**, 296–309.

16.5 ACKNOWLEDGEMENTS

We are grateful to the following colleagues at Yorkhill Hospital and the Southern General Hospital in Glasgow for their help in the preparation of the case studies presented in this chapter: Dr Jane McNeilly (pregnancy), Mr Graeme Chalmers (thyroid) and Mr Neil Squires (myeloma). We would also like to thank Dr Susan Bonham Carter for her advice on neonatal clinical measurements.