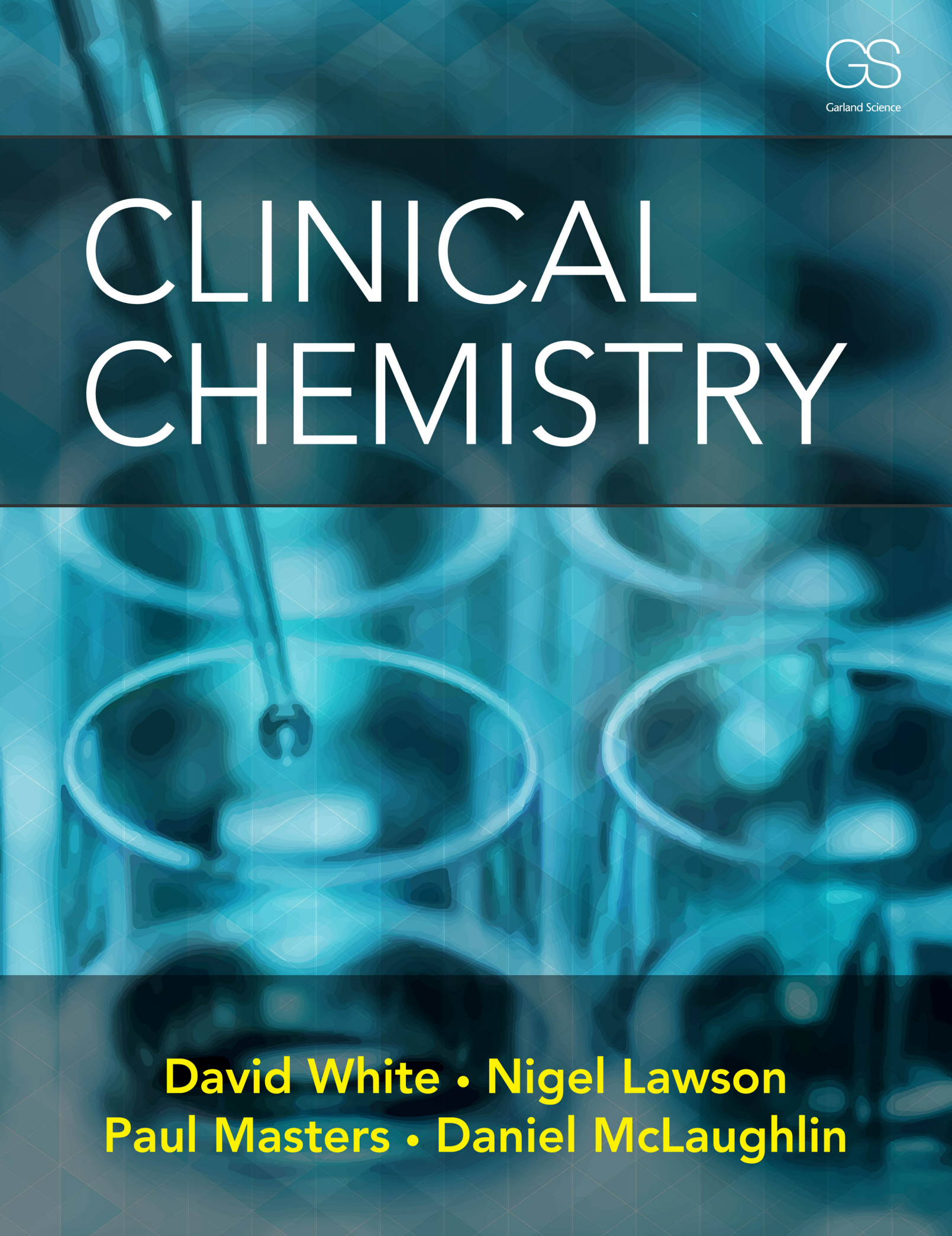
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CLINICAL CHEMISTRY
David White • Nigel Lawson
Paul Masters • Daniel McLaughlin

CLINICAL CHEMISTRY

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David White

Nigel Lawson

Paul Masters

Daniel McLaughlin

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About the authors

David White was awarded a BSc in Chemistry and a PhD in Physiological Chemistry at the University of Birmingham. Following postdoctoral study at The Johns Hopkins Medical School in Baltimore, he pursued an academic career at the University of Nottingham where he was promoted to Associate Professor and Reader in Medical Biochemistry and awarded a DSc in 1998. Dr White has taught undergraduate courses to both bioscience and medical students, supervised many graduate PhD students, and been external examiner in Biochemistry at medical schools in the UK, the Republic of Ireland, and Libya. He was appointed Director of Academic Affairs at the University of Nottingham Graduate Entry Medical School in 2002.

Nigel Lawson graduated in 1976 (BSc Biochemistry, University of Sheffield) and 1980 (PhD, University of Nottingham). In 1982 he became a Clinical Biochemist at Heartlands Hospital, Birmingham. From 1991 to 2005 he worked as a Consultant Clinical Biochemist (Nottingham City Hospital), and from 2005 to 2013 the Royal Derby Hospital as Clinical Director for Pathology. Dr Lawson has taught Clinical Chemistry to undergraduates and postgraduates for many years and is a Fellow of the Royal College of Pathologists, and was an Examiner for the College. He established the Nottingham-Derby NHS Clinical Chemistry training scheme and is an Emeritus Member of the Association of Clinical Biochemistry.

Paul Masters graduated in Medicine from the University of Leeds in 1988, having done an intercalated BSc in Chemical Pathology. After house jobs, he did postgraduate training in Leeds and Nottingham. He took up the post of Consultant in Chemical Pathology and Metabolic Medicine in Chesterfield in 1997, later becoming Clinical Lead for Blood Sciences. He currently works as an NHS hospital consultant in both Chesterfield and Derby. As well as practising and teaching clinical chemistry, Dr Masters also runs outpatient clinics for lipid disorders, diabetes and metabolic bone disease.

Danny McLaughlin earned his BSc in Human Physiology from Glasgow University in 1986 and his PhD from the Council for National Academic Awards in 1991. Since 2003 Professor McLaughlin has taught medical students at the University of Nottingham and Durham University, where he is the Academic Director of Undergraduate Medicine. Professor McLaughlin is a Senior Fellow of the UK Higher Education Academy, a member of the Association for Medical Education in Europe, and a Fellow of Durham University's Wolfson Research Institute for Health and Wellbeing.

PREFACE

The discipline of Clinical Chemistry has many aliases throughout the world, including Chemical Pathology, Medical Biochemistry, and different permutations of the terms clinical, medical, and biochemistry. Even within the departments in which it is a subdiscipline it may be known variously as Clinical Pathology, Blood Sciences, or—increasingly—Laboratory Medicine. Despite this slight identity issue, Clinical Chemistry is a strong discipline that underpins much of the diagnosis and monitoring of patients in modern health care systems. A good understanding of clinical chemistry and an awareness of its use in the management of patients are essential for both users and providers.

This text evolved from our combined experiences in the postgraduate teaching of Clinical Chemistry, using material from courses in biochemistry, physiology, and sub-specialties of medicine. Content has been expanded to include core subjects from the syllabuses of examinations for the Royal Colleges, particularly of the Royal College of Pathologists, and other graduate courses. The book is thus a suitable text for both undergraduate students of medicine and biosciences as well as for postgraduates undergoing professional training.

From the start, we wanted the book to be relevant to clinical practice, but also to include the core knowledge that enables the reader to see the full picture from first principles. Our extensive combined experiences both as full-time academics at the University of Nottingham (David and Danny) and as hospital consultants in Clinical Chemistry at the Chesterfield Royal and Royal Derby Hospitals (Paul and Nigel) have been equally valuable in enabling us to achieve these aims. The book contains many analytical and clinical practice points, distilled as "pearls of wisdom," which are intended to be relevant on a day-to-day basis to people working (or wishing to work) in Clinical Chemistry. In addition, there are many case histories included that help to put the subject into its clinical context. We believe that inclusion of clinical cases in each chapter is essential to illustrate the way in which an understanding of the underlying basic science enables interpretation of laboratory data in real-life clinical practice. Each case is based on genuine data and scenarios that we have encountered in our laboratory and clinical services. These case histories have been found to be very popular with medical students, especially those undertaking case-oriented courses, such as the University of Nottingham's Graduate Entry Medicine program in the UK.

In selecting topics for the book, we have sought to provide a varied yet comprehensive approach to Clinical Chemistry, with a mixture of chapters; some describe individual analytes, while others emphasize tissue integrity and function. In addition, we have included chapters on therapeutic drug monitoring, poisons, pregnancy, inborn errors of metabolism, and, importantly, immediate assessment of the critically ill patient. Clinical Chemistry, like any other discipline, is always developing, and we have tried to ensure that the book reflects current practice.

We have been very fortunate to work with many excellent academic, scientific, and clinical staff from the University of Nottingham and the Royal Derby and Chesterfield Royal Hospitals. Such good working interactions are vital in running successful Clinical Chemistry departments. We believe those working relationships have been reflected in how we have put Clinical Chemistry into context with the rest of medicine. Countless numbers of students have been through

our training courses, and many have gone on to become medical and scientific consultants. We hope that this book will enable a new generation of students to develop their careers, and hopefully some will become Clinical Chemists.

We are forever indebted to various members of staff at Garland Science for commissioning the book. The initial project would never have got off the ground without the continued "gentle" prompting and encouragement of our editor Liz Owen and, latterly, of our assistant editor David Borrowdale, whose tireless work, patience, and humor ensured the eventual delivery of the first set of draft chapters and who oversaw the rewriting following the comments of the reviewers. Matt McClements deserves credit for turning our self-made lecture slides into the full-color pictures presented here. We would also like to thank Georgina Lucas (Senior Production Editor) for typesetting of the book and delivery of the final product.

Thanks also go to many of our close colleagues, especially Julia Forsyth and John Monaghan in the Clinical Chemistry Department of the Royal Derby Hospital.

We would also like to thank Angela White for producing many of the anatomical drawings used in the book. Finally, we wish to thank our wives and families who have given us constant support throughout and have made it possible for us to write this book.

David White, Nigel Lawson, Paul Masters, and Danny McLaughlin

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CHAPTER 1

LABORATORY MEDICINE—AN INTRODUCTION

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QUALITY CONTROL
ACCREDITATION

From a somewhat humble beginning using rudimentary chemical and biological tests and microscope slides, laboratory medicine has progressed to become an essential component in the diagnosis and management of disease. It has evolved into a group of subspecialties which includes Histopathology or Cell Pathology, Hematology, Microbiology, Clinical Biochemistry or Clinical Chemistry, and Immunology and these disciplines have specialized further as new areas of science and technology have been adopted. For example, the techniques of molecular biology and genetics have been embraced by all disciplines leading to the development of separate departments in some institutions. In other institutions, however, such developments have prompted a rethink of working practices and the combination of aspects of hematology, immunology, and biochemistry into all-embracing departments of Blood Sciences or Clinical Laboratory Sciences. Throughout these changes, the principles of clinical biochemistry have remained constant, relating and adapting human biochemistry to the development of diagnosis and the management of disease.

Although terminology may vary between institutions, the terms Chemical Pathology, Clinical Chemistry, Medical Biochemistry, and Clinical Biochemistry are often used interchangeably; the term Clinical Chemistry will be used in this text. The clinical biochemistry of tissues, organs, and molecules is discussed in the context of disease processes and related to the diagnosis, monitoring, and management of disease. This will include outlining the fundamental metabolism of biochemical processes and physiological interrelationships. Some space has been given to descriptions of analytical processes and theory, such as immunoassay, and how these relate to clinical practice. Although the emphasis of this book is clinical biochemistry, some chapters include sections on hematology, radiology, and microbiology as appropriate only where this helps in the understanding of disease processes. However, it is not intended to provide the

detail expected in specialized textbooks on these disciplines. The increasing use of the techniques of molecular biology and genetics in the investigation of disease is acknowledged also by appropriate inclusion of these disciplines in a number of chapters.

1.1 PROCESS AND TERMINOLOGY

Several general aspects of clinical biochemistry process and terminology are first defined to enable the reader to better understand subsequent chapters. Clinical chemistry laboratories are engaged in the analysis of tissue samples and the interpretation of data derived from these analyses. In doing so, they have a number of key functions:

- To help in the diagnosis, monitoring, and management of disease
- To screen populations for disease
- To assess risk factors for disease processes
- To engage in research and development of analytical and diagnostic tools
- To teach and train new staff

While this illustrates the scope of the work of major clinical biochemistry departments and each of the above functions is important per se, the fundamental role of clinical chemistry in most hospitals is in the diagnosis, monitoring, and management of disease.

For this textbook, Standard International (SI) units of measurement have been employed. For some tests, where non-SI units are in common use as well as SI units, both sets of units are quoted.

1.2 HOW DOES THE SYSTEM WORK?

To illustrate the process, consider this simple scenario where a patient attends his family doctor complaining of tiredness (**Figure 1.1**). After first taking a short history, recording symptoms, and carrying out a physical examination, the doctor considers whether the patient might be anemic. The next step is to take a blood sample to determine whether the tiredness and any other symptoms, including looking pale, might be due to anemia; that is, to a low hemoglobin level (**Figure 1.2**). The blood sample is transferred to a (hospital) laboratory for analysis and interpretation, and a report is sent to the requesting doctor. The test for anemia is usually performed by Hematology or Combined Blood Science Departments as part of a full blood count (FBC) which assesses a number of blood cell parameters, including hemoglobin concentration. Usually, such analyses are undertaken using large automated cell counters (instruments). Where appropriate, some of the blood sample is examined under a microscope to determine blood cell morphology. For the patient above, the following results were found (**Figure 1.3**). The hemoglobin concentration of 97 g/L (9.7 g/dL) is clearly below the normal range of 130–180 g/L (13.0–18.0 g/dL) for an adult male and implies that the patient is anemic. A decreased concentration of hemoglobin

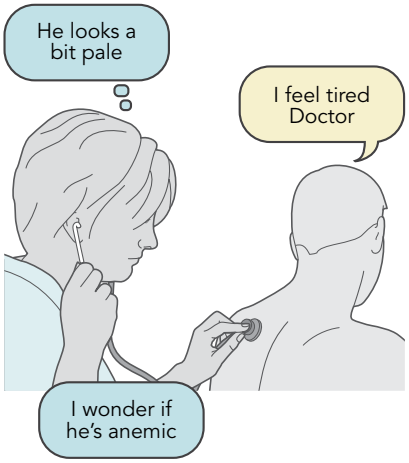


Figure 1.1 Before tests are performed.
Before a doctor considers requesting tests on a patient they will usually take a history, check for symptoms, and consider a possible diagnosis.

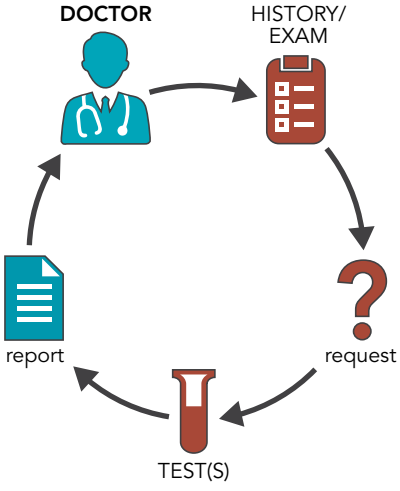
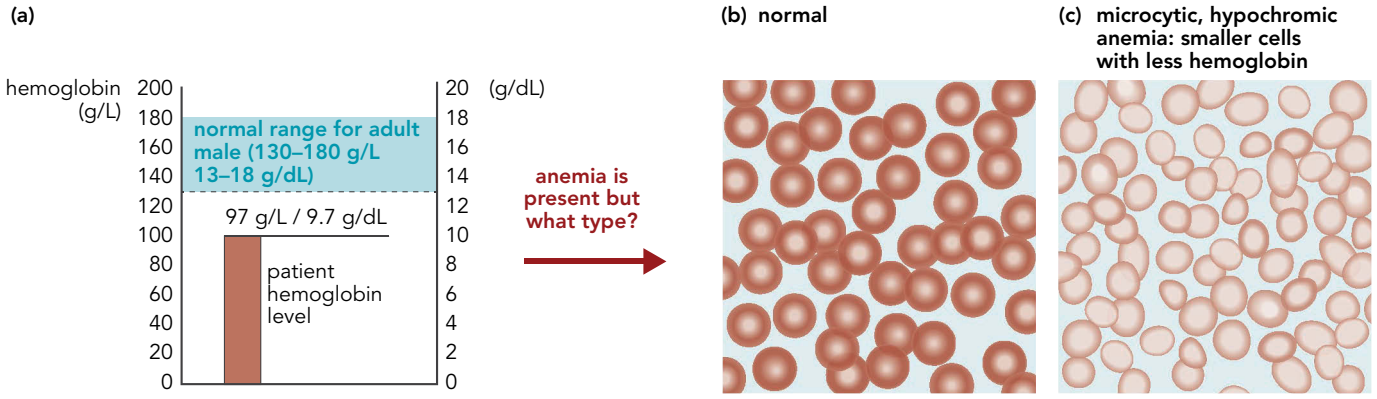


Figure 1.2 The consultation, request, and report cycle.
Once the doctor has made a potential diagnosis, in this case anemia, they will send a blood sample to the laboratory and request the sample is tested for anemia, and the result is reported back to the doctor. Anemia can be defined as having a low blood hemoglobin, which will lead to symptoms such as tiredness. Therefore the laboratory will measure the patient's blood hemoglobin concentration, as well as other red blood cell parameters.



lowers the capacity of the red cells to transport oxygen to the tissues throughout the body, giving rise to tiredness, and explains some of the patient's symptoms. However, there are a number of different types of anemia (see Chapters 10 and 16) and further tests are required to ascertain which is present in this case.

Microscopic examination of the patient's blood film (Figure 1.3c) indicates that the red cells of the patient have less color and are smaller than red cells from a healthy, adult male. In summary, the patient has small red cells lacking in pigmentation and a low hemoglobin content; that is, the patient has microcytic, hypochromic anemia. A common cause of this type of anemia is iron deficiency and further tests in the clinical biochemistry laboratory are required to investigate this.

Although some clinical biochemistry analyses are made on whole blood, most tests have been developed on the supernatant fluid remaining after the blood cells have been separated by centrifugation. If blood is taken into a plain glass or plastic tube and the sample is allowed to clot, the resulting supernatant is termed serum. However, if an anticoagulant is added to the tube to prevent clotting, the supernatant liquid is termed plasma. Measurements assessing iron deficiency may be made on either serum or plasma. In the current scenario, the patient's serum iron is indeed below the normal range for adult males (**Figure 1.4**), suggesting iron deficiency. This may be due to decreased

Figure 1.3 Does the patient have anemia? Hematology results. (a) The patient has a blood hemoglobin result of 97 g/L (9.7 g/dL) of blood, which is low compared to most adult males, who usually have a blood hemoglobin greater than 130 g/L (13.0 g/dL) in health; that is, the patient has anemia. Normal results for adult males are shown in parentheses. In addition, the patient's red blood cells will be examined by microscopy and compared to normal cells (b) to help to define what type of anemia is present. In this case, the patient's red blood cells (c) are smaller (microcytic) and have less pigment (hypochromic) than normal. The patient has a microcytic, hypochromic anemia. This type of anemia is often found in patients with iron deficiency.

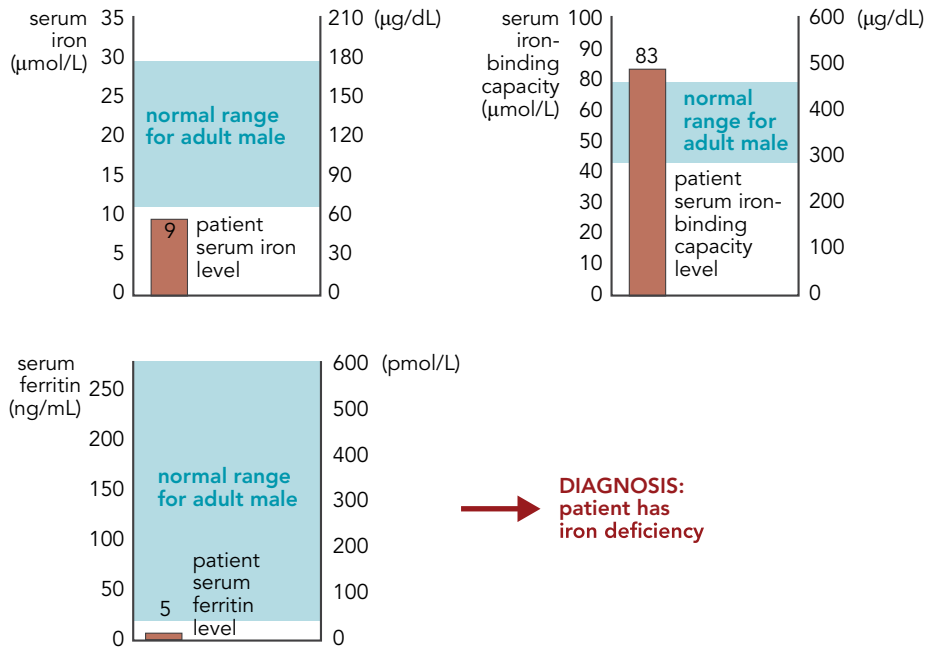


Figure 1.4 Clinical chemistry results. In order to confirm the diagnosis of iron deficiency the doctor will request further tests on the patient. With iron deficiency, a patient's serum iron would be expected to be low, as would an estimate of the patient's total body iron stores (serum ferritin). Conversely, the blood capacity to transport iron is increased in iron deficiency. Comparing this patient's results to normal (see Table 10.1), they have a low iron and ferritin but a high iron-binding capacity. They have iron deficiency.

intake into the body due to low dietary iron or malabsorption, increased loss through bleeding, or a combination of both. Further tests are required to investigate these possibilities. Iron is transported in the circulation bound to a protein, transferrin (see Chapter 10), and the amount of iron that can be bound to transferrin can be measured and expressed as total iron-binding capacity (TIBC). As a patient becomes progressively iron deficient, the liver synthesizes more transferrin, thereby increasing the TIBC, to maximize the amount of iron being absorbed from the gut and transported to the tissues. This response in itself is not specific for iron deficiency, so serum ferritin is routinely measured. Ferritin is a protein that can bind large amounts of iron and functions as an intracellular store of iron, particularly in liver and bone marrow. Small amounts of ferritin are released into the circulation during cell turnover and measurement of serum ferritin gives an indication of the iron stores in these tissues. The lower the serum ferritin level is, the lower the tissue ferritin stores and thus whole body iron stores. The results for the patient shown in Figure 1.4 indicate that all three additional parameters measured are below the normal range for adult males, confirming that the patient has iron deficiency. This is a diagnosis and helps to direct investigations into the cause of iron deficiency (see Chapter 10). Where possible, the underlying cause is treated and the patient is given iron supplements to return his iron and hemoglobin concentrations to within the normal range. Hematology and clinical biochemistry tests are used to monitor the effectiveness of treatment.

1.3 USING FLUIDS TO ASSESS TISSUE DISEASE

Although several diseases are due to abnormalities in the blood, for example abnormal red blood cells, most diseases are characterized by abnormalities in specific tissues. Since it is difficult to routinely take biopsy material from the affected tissue, blood and urine are frequently examined to show whether tissue damage or disease is present. Many compounds are released into the blood by different organs and have a specific function within the bloodstream, for example hormones. These are routinely measured to assess disease. However, there are also many compounds circulating in the bloodstream which have no known function there. They may be being transported to and from other tissues, or alternatively they are present due to the constant turnover of cells. Most of these compounds are transported to various organs, metabolized, and then recycled or excreted. Such compounds can also be measured in urine if they are excreted by the kidneys.

Enzymes are typical examples of such compounds, being used extensively as specific markers of organ damage in clinical biochemistry. The plasma level of an enzyme reflects the general turnover of cells that contain significant concentrations of that particular enzyme. As a cell breaks down, proteins (including enzymes) will appear in the bloodstream, and their level in plasma will be dependent upon the amount of enzyme in the organ or tissue, the rate at which it appears in the plasma, and the rate at which it is cleared from the plasma. This can be seen for many enzymes, for example lactate dehydrogenase (LDH), an enzyme found in the cytoplasm of most human cells. The measured value in the plasma reflects a steady state which will exist in the normal, non-diseased state, where there is a balance between production and elimination. This will be equivalent to the reference range. Changes to this equilibrium can occur due to more enzyme being present in the tissue or organ, which may be simply due to more cells being present; for example, in some lymphoma cases, higher LDH values are seen as the tumor mass increases. Alternatively, higher values can occur due to reduced clearance of the enzyme from the plasma. In the case of many diseases, increased enzyme values occur due to damage to the cells of the tissue or organ (**Figure 1.5**). The increase in the plasma enzyme is related to the

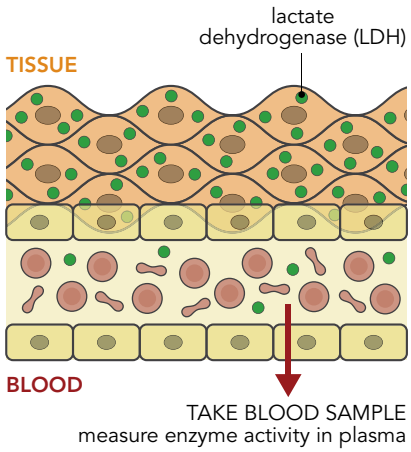


Figure 1.5 Enzymes in plasma. Enzymes, for example lactate dehydrogenase, are frequently measured in plasma or serum to help to diagnose and monitor disease. The plasma level is dependent upon the rate at which the enzyme is released into the plasma from its tissue of origin, and how quickly it is cleared from the plasma by various mechanisms, including removal by the kidneys and liver. Clearly an increased release of enzymes into the plasma, for example by damage to the tissue, will lead to increased levels in the plasma. Similarly, reduced clearance may also lead to raised levels in the plasma. Most of these processes are dynamic, with the enzyme levels rising and falling in disease dependent upon the degree and duration of tissue damage and the rates of clearance.

TABLE 1.1 Enzyme activities in tissues in comparison to plasma

Tissue	Aspartate transaminase (AST) relative to plasma	Alanine transaminase (ALT) relative to plasma
Heart	7800	450
Liver	7100	2850
Skeletal muscle	5000	300
Kidney	4500	1200
Pancreas	1400	130
Spleen	700	80
Lung	500	45
Erythrocytes	15	7
Plasma	1	1

Table shows the relative concentrations of two enzymes, aspartate transaminase (AST) and alanine transaminase (ALT), compared to their concentrations in plasma, where plasma concentration has been defined as 1 arbitrary unit. Clearly, the enzymes have much higher concentrations in tissues than plasma, and there is a degree of tissue specificity, with much higher ALT values in liver compared to other tissues.

degree of damage. More damage means higher plasma enzymes. In addition, the intracellular location of the enzyme could determine how much is seen in plasma. The level of a cytoplasmic enzyme is likely to rise quicker in the bloodstream than is that of a mitochondrial enzyme.

Although LDH is used to monitor certain diseases it is not very organ specific. Other enzymes have more specificity and this is reflected in their clinical use. Both aspartate transaminase (AST) and alanine transaminase (ALT) are found in many tissues as well as liver, but in the case of ALT the liver has the highest concentration. In addition, there is a huge difference in the concentration of ALT in the liver and plasma, allowing small amounts of damage to be reflected in changes in the plasma concentration (**Table 1.1**).

Since the enzyme level reflects production and clearance, raised values are dependent on whether the increased damage is a chronic effect or not, and how quickly the enzyme is cleared from the plasma. In acute tissue damage, the enzyme levels will rise and fall, and the speed and magnitude of these rises depend upon the plasma half-lives of each enzyme (**Table 1.2**).

TABLE 1.2 Half-lives of enzymes in plasma

Enzyme	Half-life
Lactate dehydrogenase	3–7 days
Alkaline phosphatase	3–7 days
Gamma-glutamyl transferase	3–4 days
Alanine transaminase	1.5–2.5 days
Creatine kinase	12–24 hours
Amylase	3–6 hours

1.4 PRE-ANALYTICAL FACTORS

Whilst the examples above illustrate the role of clinical chemistry in diagnosis and monitoring of therapy, there are a number of factors which need to be considered in obtaining appropriate samples for analysis. These are often termed

pre-analytical factors and include an awareness of the patient's medical history and the preparation of a patient for taking samples, as well as the procedures of sample collection, processing, and storage. It is essential that these are carried out correctly to provide valid information for diagnosis and treatment.

Patient preparation

Consider as an example blood glucose measurement, where correct patient preparation has a major influence on the interpretation of results and diagnosis of disease. Patients with poorly controlled type 2 diabetes mellitus have a raised fasting blood glucose concentration of >7.0 mmol/L (126 mg/dL) and this is diagnostic for the condition (see Chapter 2). However, the blood glucose concentration is raised significantly in healthy individuals following a meal, particularly one enriched in carbohydrate. It is essential therefore that subjects be fasted fully prior to taking a blood sample for glucose analysis to ensure a meaningful result. Fully fasted requires that the subject ingests only water for 12 hours prior to sampling. Other pre-analytical factors include dietary and drug histories, consideration of diurnal and seasonal variations, and the effects of stress, exercise, and posture on analysis. These are discussed in context in later chapters where relevant.

Analytical practice point 1.1

Whether an electronic requesting system or handwritten forms are used, it is essential to clearly label all samples with the correct patient identifiers (name, date of birth, and so on). It does not matter how accurate and precise the analysis has been, sending the result out to the wrong patient can have disastrous consequences.

Taking samples

Once a patient has been prepared correctly, samples may be taken. The majority of clinical biochemical analyses are performed on whole blood, serum, or plasma (separated blood), but many other sample types may be used, including:

- Urine
- Cerebrospinal fluid (CSF)
- Sweat
- Saliva
- Feces
- Pleural, ascitic, and other fluids
- Tissue biopsies

Of course, the quality of the sample taken is dependent often on the skill of the health care professional taking the sample, but other samples, such as 24 h urine collection, require an understanding of sample collection by the patient. In such cases, understanding sampling protocols is essential since the discarding of preservative from collection bottles or incomplete urine collection, for example, may lead to incorrect diagnosis despite accurate sample analysis in the laboratory.

Blood sampling tubes

Selection of the appropriate tube for blood collection is extremely important and will depend on whether or not the blood sample may be left to clot prior to measurement of a particular analyte. If the analyte is stable, blood may be collected into a tube containing no anticoagulant and allowed to clot. Since this may take some time, in excess of 30 minutes at ambient temperature, some collection tubes contain clot activators to speed up the process of coagulation. On the other hand, if whole blood or plasma is required, anticoagulant is added to the collection tube prior to collection of blood. The choice of anticoagulant will depend on which parameters are being assayed; potassium-EDTA (ethylenediaminetetraacetic acid) is the anticoagulant of choice for full blood counts. However, potassium-EDTA tubes are obviously inappropriate for measurements of plasma potassium and, since EDTA is a very good chelator of divalent cations, the use of these tubes is also precluded in the measurement of calcium and magnesium. A display of tubes currently available commercially is shown in **Figure 1.6**. Since there is no universal color coding for different tubes, it is

Analytical practice point 1.2

Correct selection of blood tube and anticoagulant is essential. The wrong tube (or contamination by mixing the contents of two different tubes) can give spurious results.

Figure 1.6 Blood collection tubes. Different types of blood tubes are used depending upon what tests are required. The simplest tubes are just straightforward plain tubes to collect clotted samples; that is, there is no anticoagulant or preservative present. Different anticoagulants can be used, usually lithium heparin, EDTA, oxalate, or citrate. Occasionally preservatives are needed, for example fluoride to inhibit glycolysis when measuring glucose.

essential to read the label on tubes before blood sample collection to ensure that the tube chosen is fit for purpose.

Processing and storage of samples

The processing of a blood sample depends on the stability of the analyte to be measured. It should be remembered that red cell metabolism, particularly that of glucose, does not stop when blood is collected into a tube and, unless steps are taken to remove the red cells from the sample by centrifugation or to inhibit this metabolism by the use of metabolic poisons, erroneous results may be obtained. For example, if blood samples are kept at room temperature, anaerobic glycolytic activity in red cells will reduce the blood glucose concentration at a rate of 5–10% per hour with a concomitant rise in lactate concentration. Also, potassium ions leak from red cells when blood is kept at ambient temperature for a few hours giving rise to elevated plasma potassium levels. Paradoxically, potassium also leaks from red cells when blood samples are cooled, due to slowing of the activity of the sodium-potassium pump, and this again produces an elevated plasma potassium level. In both these cases, the measured potassium level is in

excess of the true plasma concentration in the patient. Thus separation of cells from plasma is a key process in sample preparation and storage. Centrifugation is usually carried out at 3000*g* for 5–10 minutes, taking care not to damage cells. Some collection tubes contain a gel that creates a barrier between plasma and blood cells (see Figure 1.6). If the analyte to be assayed is labile, the time between phlebotomy and centrifugation should be minimized. Further knowledge of the stability of the analyte will dictate at what temperature a sample is centrifuged and stored, and whether preservatives and/or metabolic inhibitors such as fluoride (to inhibit red cell glycolysis) are added to the sample. Particular instances of sample processing and storage which impact on results and may affect their clinical interpretation are included in individual chapters.

1.5 WHAT CONSTITUTES AN ABNORMAL RESULT?

The normal range for any given parameter or analyte defines the range of values found in a normal healthy adult population. It is against this range that any value for a given patient is compared. Simplistically, it may be thought that an abnormal result is one that lies outside of the normal range, being lower or higher. To some extent, however, the concept of a normal range is somewhat artificial because in many cases (diseases) there may be considerable overlap between values in the normal and diseased states. Furthermore, because of a number of confounding factors, it is often difficult to decide on what is normal since what may be normal in one population group may be distinctly abnormal in another. Thus, when an analyte is measured, the result is compared with a reference range for that analyte taking into account any potential confounding factors.

1.6 WHAT IS A REFERENCE RANGE?

When the concentration of an analyte in a given population is normally distributed, the reference range is calculated from the mean and standard deviation of results from that population. The reference range is defined as the mean ± two standard deviations (mean ± 2SD); this will include 95% of the results from the population group and exclude the top and bottom 2.5% (**Figure 1.7**). Results outside the reference range (mean ± 2SD) are considered abnormal but do not indicate the presence of disease per se merely because the result lies outside of the reference range. However, the likelihood of disease being present usually increases with the distance of the measured value from the mean.

Analytical practice point 1.3

Any result has a 5% chance of being outside the reference range and the more tests are done, the greater the chance of at least one result being abnormal. The chance is $1 - 0.95^n$, so that if a profile of 10 tests is done, the chance of at least one abnormal result is 40%.

The creation of a reference range needs to take into account potential confounding factors that might influence the concentration of a given analyte in a patient. For example:

Time of day

Measurement of blood glucose may be affected by several factors and the sources of variation must be minimized prior to taking a sample for analysis. Since blood glucose concentration rises after a meal, the reference range of 3.5–5.8 mmol/L (63–104 mg/dL) may be quoted for fasting subjects. Values that are slightly above or below the reference range are not necessarily indicative of disease, and there may be no obvious symptoms or signs in these patients

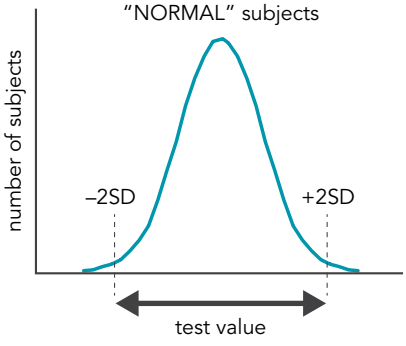


Figure 1.7 The reference range.
The range of results found in the general healthy population when they are distributed normally. This reference range in a normally distributed population would consist of all results which are ±2 standard deviations (SD) of the mean. That refers to 95% of the population.

compared to those whose blood glucose concentrations lie within the reference range. Should the results exceed three standard deviations from the mean, the probability of processes and symptoms of disease being present increases considerably. A blood glucose concentration below 2.5 mmol/L (45 mg/dL), for example, is insufficient to support normal brain function and symptoms of hypoglycemia (tremor and eventually loss of consciousness; see Chapter 2) are manifest.

Gender
Testosterone concentrations would be expected to be much higher in men than in women.

Time of year
Vitamin D concentrations in blood would be higher in summer compared to winter due to exposure of skin to sunlight.

Age of patient
There is usually less lean body mass (muscle) in the young and in the elderly and so blood parameters which are derived from muscle, creatinine for example, will be lower in these two age groups compared with a healthy adult group.

The number of factors that have the potential to influence the reference range makes it difficult if not impossible to generate a generic reference range for all subject groups. It is relatively straightforward for instance to collect blood from, say, 200 healthy medical students and to establish reference ranges for a number of analytes in the student group. These reference ranges are unlikely, however, to be applicable to the majority of patients who are elderly, or to young children and babies. Indeed, the establishment of reference ranges for normal healthy children creates problems of its own because of ethical concerns in collecting blood and CSF, both painful procedures from which the child derives no obvious benefit.

1.7 AN IDEAL DIAGNOSTIC TEST

In terms of usefulness in diagnosis, an ideal test is one where the reference range for a normal population is normally distributed and quite separate from the reference range derived from the disease group, also normally distributed; that is, there is no overlap of the two reference ranges (**Figure 1.8**). This is rarely the case, however, and overlap of the two ranges occurs due to false positives—individuals who have no disease but have a positive result (a value within the disease range)—and false negatives, patients who have disease but whose test result lies within the normal reference range (**Figure 1.9**). Knowledge of

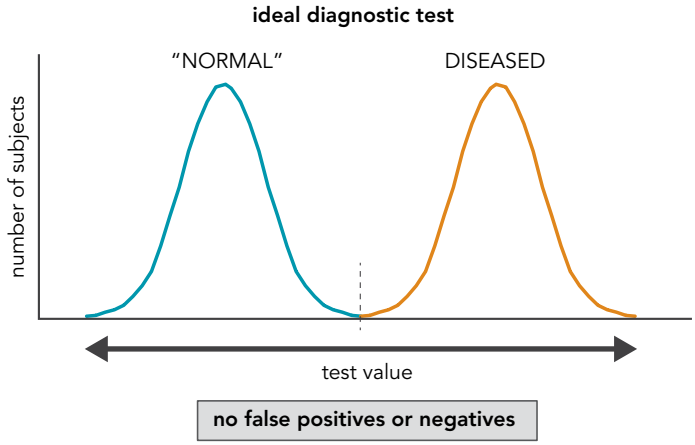


Figure 1.8 Ideal diagnostic test. Hypothetical results from an ideal diagnostic test where the test result is increased in disease (orange line) compared to the non-disease state (blue line). There is no overlap between values found in patients with disease compared to those without. There would be no false positive results or false negative results.

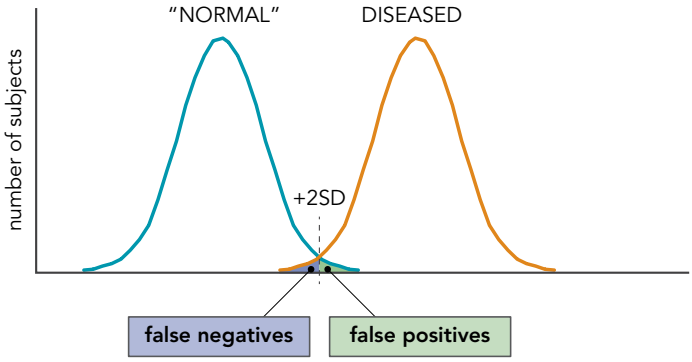


Figure 1.9 Uncertainty of results.
In the vast majority of pathology tests there is overlap between results found in the diseased and non-diseased states. In this hypothetical case, where the diseased state would consist of raised results, an abnormal result would be considered as any value greater than two standard deviations (2SD) above the mean. Of course this would include 2.5% of the normal population without disease; therefore at least 2.5% of a normal population would give a false positive result. Dependent upon the disease and test, patients with a disease may produce results that are within the reference range; that is, false negative results.

$$\text{diagnostic sensitivity} = \frac{\text{true positives}}{\text{true positives} + \text{false negatives}}$$

IDEAL TEST WOULD HAVE
100% DIAGNOSTIC SENSITIVITY

$$\text{diagnostic specificity} = \frac{\text{true negatives}}{\text{true negatives} + \text{false positives}}$$

IDEAL TEST WOULD HAVE
100% DIAGNOSTIC SPECIFICITY

Figure 1.10 Diagnostic sensitivity and specificity.
Simple equations can determine the diagnostic sensitivity and specificity of a test. True positives are defined as people who have disease and produce abnormal results, whilst true negatives are defined as people who do not have disease and have normal results. False positives are defined as people who do not have disease but produce abnormal results, whilst false negatives are defined as people who have disease but produce normal results. Sensitivities and specificities are expressed as percentages.

the numbers of true and false positives and negatives allows the determination of the diagnostic sensitivity and specificity of a test (**Figure 1.10**). A test of high diagnostic sensitivity has a low number of false negative results, while high diagnostic specificity is associated with a low number of false positives. In an ideal test, both sensitivity and specificity are 100%. Unfortunately, in real life this seldom occurs and although some test ranges are normally distributed (for example, blood glucose in normal and diabetic individuals) many are not and the use of parametric statistics is precluded. Many analytes, for example prolactin, have skewed reference ranges (**Figure 1.11**) and the use of the mean ± 2SD would indicate many normal results to be abnormal; thus, nonparametric statistics are used to determine 95% confidence intervals and reference ranges.

1.8 USING REFERENCE RANGES

As mentioned earlier, a number of factors other than disease may impact on the concentration of a particular analyte and these should be borne in mind in determining reference ranges and interpreting test results. However, as is shown in

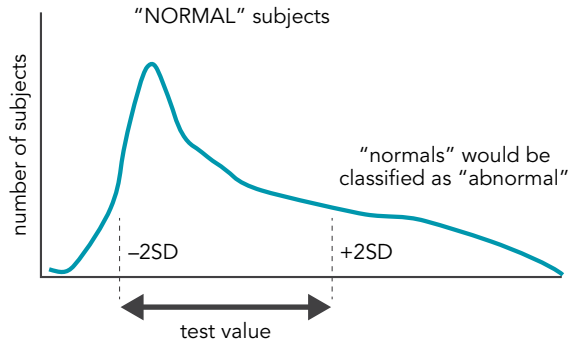


Figure 1.11 Skewed reference ranges.
The diagram shows that when the results in a healthy population have a nonparametric distribution, in this case a positive skew, the reference range cannot be determined using a simple mean ± two standard deviations (2SD). In these cases, the true 95% confidence limits have to be determined to calculate the reference range.

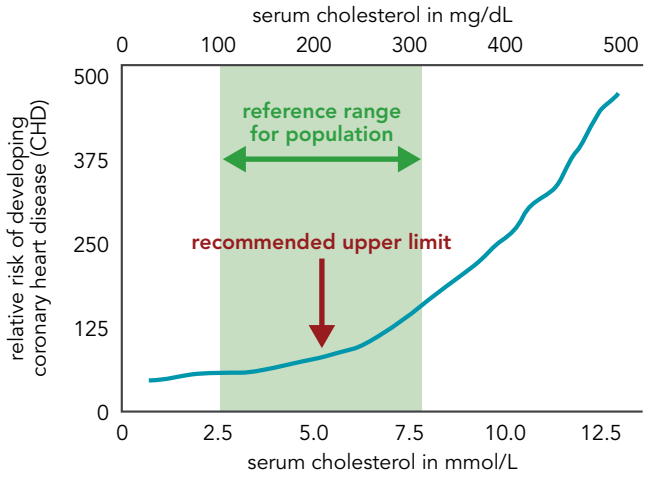


Figure 1.12 Not using the reference range.
The figure shows the relative risk of developing coronary heart disease (CHD; *y* axis) compared to the serum concentration of cholesterol (*x* axis). Statistically, the higher the concentration of serum cholesterol, the greater the risk of developing CHD. Certain populations have reference ranges where the upper 97.5% confidence interval is much higher than the recommended upper level for cholesterol, due to diet and other factors. In this example, an individual with a serum cholesterol at the upper end of the reference range (7.8 mmol/L or 300 mg/dL) would have a much higher risk of developing CHD than with one of the quoted recommended upper levels (5.2 mmol/L or 200 mg/dL). In these situations, quoting a clinical reference range is not clinically useful, and referral to an ideal range or referral to guidelines would be preferable.

later chapters, the use of reference ranges has proved extremely useful in the investigation of disease, particularly using serum samples. Consider, for example, the determination of tissue damage in acute myocardial infarction. Cardiac-specific proteins, troponins, are released from heart cells into the circulation in low concentration as part of the homeostatic process of cell turnover. When cell damage occurs, as during a myocardial infarct, cell proteins are released into the blood and there is a transient abnormal rise in troponins which exceeds the background normal range and is diagnostic of infarction. Perversely, there are also cases where the reference range for an apparently healthy population (one showing no obvious signs or symptoms of disease) may not be ideal and a value in the normal range might indicate a risk factor for disease. For example, the normal range for blood cholesterol concentration in Western societies probably constitutes a risk factor for coronary heart disease and reference ranges for cholesterol are now rarely quoted; rather, ideal target ranges and relative risk factors are proposed (**Figure 1.12**).

1.9 HOW DOES CLINICAL CHEMISTRY OPERATE?

The range of institutions involved in the delivery of clinical chemistry is extremely broad. State-of-the-art research laboratories involved in the development of new assays are quite different from routine hospital laboratories providing a day-to-day service. Even hospital laboratories vary considerably, from large factory-like units with wide analytical repertoires processing millions of tests per year to smaller units processing a few tests per day. In addition of course, some automated tests may be carried out at the bedside, in the ward, or at home by a doctor or nurse or sometimes the patients themselves (so-called point-of-care testing).

A typical large, 1000-bed general hospital in Europe or North America processes over 500,000 samples and carries out over 5,000,000 tests per year. This can be achieved only through the use of large-scale automation, robotics, and sophisticated computing techniques. Many such hospital laboratories are able to perform over 300 different tests using a wide variety of analytical techniques including:

- Photometrics (simple color changes and absorption in the ultraviolet range)
- Enzyme assays
- Ion-selective electrodes (ISE)

- Gas chromatography (GC)
- Mass spectrometry (MS)
- High performance liquid chromatography (HPLC)
- Electrophoresis
- Immunoassays
- Polymerase chain reaction (PCR)
- Atomic absorption

Many of these techniques have been automated using large-scale analyzers, which combine sample processing with analysis to produce total laboratory automation systems.

1.10 QUALITY ASSURANCE

The concept of quality assurance is embodied in two questions related to accuracy and precision: Are you measuring what you think you are measuring? If you measured it again tomorrow, would the result be the same as today? The ability to ensure that the correct result is reported on the correct patient in the right place at the right time is key to the working practice of a clinical chemistry laboratory. Failure to comply with good quality assurance may lead to disastrous consequences including misdiagnosis, mistreatment, and, in the worst-case scenario, death of a patient.

Accuracy

Accuracy concerns the specificity of a test for a given analyte and the reliability of that test when used in different laboratories. For example, in the measurement of blood glucose, a high degree of accuracy implies that the test measures only glucose and not potential interfering molecules such as other reducing sugars of similar structure. Furthermore, when the test is used in different laboratories, the same result is found for a given standard glucose solution. There is a high degree of accuracy in tests for many of the common analytes and reference ranges for these analytes are common across a number of laboratories. However, for more complex assays, particularly immunoassays, methods may vary between laboratories and result in different reference ranges between individual laboratories. In such cases, a result from a patient must be compared with the reference range from the hospital in which the analyte was measured. Occasionally, this may be the case for a common analyte such as creatinine. It could be argued that accuracy might not be critical if a routine method in widespread use generated results for a standard reference sample that were consistent and for which there was good agreement between laboratories. However, comparison of accuracy between laboratories becomes more of a problem for assays for which there is no standard reference material and for which interfering molecules have not been identified, for example assays for some peptides and tumor markers. In these cases, misleading results may be produced and inappropriate treatment may ensue.

Precision

The practice of clinical chemistry involves the measurement of a particular analyte and comparison of the result with a reference range or with a measurement made hours, days, months, or even years earlier. The precision of a test is a measure of the reliability of the test from day to day and can be determined by repetitive measurements of a reference standard assayed with the patient samples. Obviously, for diagnosis and monitoring response to treatment, high precision is of the essence.

1.11 QUALITY CONTROL

Since the production of a wrong result can have serious consequences, it is essential that results are as accurate and precise as possible, and much time and expense is spent on quality control—both internal (IQC) and external quality control (EQC)—to monitor this. Commercial quality control samples of known composition are available for many common analytes, which allows individual laboratories to determine their own accuracy and precision. With less common analytes, such material may not be available and laboratories may obtain reference material and prepare their own in-house quality control standards or reach a consensus on values with other laboratories.

Analytical variation

Most IQC checks on precision are based upon the analytical variation of a test. This is often quoted as an analytical coefficient of variation (CV) which is expressed as

$$\mathrm{CV} = \frac{\mathrm{SD} \times 100}{\mathrm{Mean}} \qquad \text{(Equation 1.1)}$$

where the mean and standard deviation are derived from multiple assays of samples from a reference standard. Traditionally, where the assays are performed on a sample from a single batch, the CV is referred to as an intrabatch CV; when samples are taken from different batches, the CV is referred to as an interbatch CV. With the increasing use of auto-analyzers and other automated techniques, where analyses are performed under constant conditions, it is more relevant to refer to within-day and between-day CVs.

The performance of any individual assay can then be monitored regularly using simple IQC rules. Although there are many excellent quality control packages in use, most work on the basis that essentially batches would be accepted if the IQC samples fell within two standard deviations of the expected mean value. Such a value can be achieved by chance. If the IQC result falls outside of this range the batch can be rejected, and corrective action such as recalibration can be taken. The IQC shows the degree of imprecision and the degree of bias away from the true result.

Many routine assays have a very low CV, less than 1%. So, for example, in the case of calcium where the control value was 2.00 mmol/L (8.00 mg/dL), the batch would be considered to be acceptable if the result for the IQC sample was between 1.96 and 2.04 mmol/L (7.84 and 8.16 mg/dL). This degree of precision is very important for analytes like calcium, where the physiological concentration is kept within a very tight range and where small changes can be important clinically. Many other assays in routine use are more imprecise, with a CV in excess of 5% or even 10%. Imagine trying to use a calcium assay with a CV of 10% with a control value of 2.50 mmol/L (10.00 mg/dL). Acceptance limits of ±2SD would mean that IQC values ranging between 2.00 and 3.00 mmol/L (8.00 and 12.00 mg/dL) could be accepted. Considering the fact that the reference range for calcium is often given as 2.20 to 2.60 mmol/L (8.80 to 10.40 mg/dL), this degree of imprecision is not acceptable.

External quality assurance

Although internal quality control enables laboratories to keep their laboratory tests performing within certain limits of precision and bias, it by no means enables total quality assurance, nor does it enable ready comparison with other laboratories. The majority of clinical laboratories participate in external quality assurance (EQA) schemes for most of the tests that they undertake. Essentially, these schemes send samples to participating laboratories; these samples have

predetermined concentrations of specific analytes, but the participating laboratories do not know the values. After the samples have been analyzed, the quantitative and/or qualitative results, along with interpretations in some cases, are sent back to the coordinating center for the scheme. Laboratories can then be scored for accuracy and precision, and ranked for performance. Laboratories that perform poorly over time can thus be identified and action taken to improve performance. This process, when performed correctly, should assure the quality of a laboratory and should give reassurance to the physician and patient that the correct results and interpretative information are being produced.

1.12 ACCREDITATION

As pathology services have developed in general, there has been an increasing awareness of the need for formal assessment of laboratories to ensure they are operating appropriately. Early schemes for pathology were Clinical Laboratory Improvement Amendments (CLIA) and Clinical Pathology Accreditation (CPA), which both were operating along the lines of International Organization for Standardization (ISO), Good Laboratory Practice, and Food and Drug Administration (FDA) guidelines.

GLUCOSE

CHAPTER 2

IN THIS CHAPTER

HORMONAL REGULATION OF BLOOD GLUCOSE CONCENTRATION

GLUCOSE STORAGE AND METABOLISM

INSULIN

GLUCAGON

MAINTENANCE OF GLUCOSE HOMEOSTASIS IN THE FED AND FASTING STATES

GLYCATION OF PROTEINS

HYPERGLYCEMIA AND DIABETES MELLITUS

CLINICAL CHEMISTRY MARKERS OF GLYCEMIC CONTROL

HYPOGLYCEMIA

METABOLISM OF FRUCTOSE AND GALACTOSE

INBORN ERRORS OF CARBOHYDRATE METABOLISM

Glucose is the single common substrate that can be used by all tissues as an energy source. Although most tissues can also use fatty acids for energy, nervous tissue, red cells (erythrocytes), kidney, and, in normal circumstances, brain have an obligatory requirement for glucose (**Table 2.1**). It is for this reason that the maintenance of plasma glucose within a narrow concentration range (**Table 2.2**) is fundamental to health. The normal distribution range of blood glucose in the UK population is 4.1–5.9 mmol/L (74–106 mg/dL) with a slightly higher range for adults of 60 years and above of 4.4–6.4 mmol/L (80–115 mg/dL). Pathological consequences ensue when the glucose concentration lies

TABLE 2.1 Major fuel sources consumed by tissues after a meal

Tissue	Immediately postprandial	2–5 hours postprandial
Brain	Glucose	Glucose
Red blood cells	Glucose	Glucose
Nervous tissue	Glucose	Glucose
Kidney	Glucose	Glucose (fatty acids)
Skeletal muscle	Glucose	Fatty acids (glucose)
Liver	Glucose	Fatty acids (glucose)
Heart	Glucose	Fatty acids (glucose)
Lung	Glucose	Fatty acids (glucose)
Gastrointestinal tract	Glucose	Fatty acids, glucose
Adipose	Glucose	Fatty acids (glucose)

Substrates in parentheses are secondary and minor fuel sources 2–5 hours postprandial. During longer-term fasting, all tissues except liver, kidney, adipose, and red blood cells adapt to using ketone bodies as the major metabolic fuel for energy production.

TABLE 2.2 Normal ranges for glucose, HbA1c, and associated parameters

		SI units	Conventional units
Plasma	Glucose (fasting)	3.9–6.0 mmol/L	70–110 mg/dL
	Insulin (fasting)	14–140 pmol/L	2.0–20 μIU/mL
	C-peptide (fasting)	0.17–0.90 nmol/L	0.5–2.7 ng/mL
	3-Hydroxybutyrate	<0.1 mmol/L	<1 mg/dL
	Acetoacetate	<0.1 mmol/L	<1 mg/dL
	Free fatty acids	0.3–0.9 mmol/L	8–25 mg/dL
Whole blood	HbA_{1c}	<48 mmol/mol Hb	<6.5% of Hb

HbA_{1c}, glycated hemoglobin (Hb).

outside of this range for prolonged periods; hypoglycemia is characterized by impaired mental function and severe hypoglycemia by coma, while hyperglycemia, particularly chronic hyperglycemia, promotes the insidious pathology of diabetes mellitus.

2.1 HORMONAL REGULATION OF BLOOD GLUCOSE CONCENTRATION

Blood glucose concentration is maintained within the normal range (homeostasis) by the opposing actions of anabolic hormones, mainly insulin, which promote the removal of glucose from the circulation, and catabolic hormones, mainly glucagon and, in times of stress, also epinephrine, growth hormone, and cortisol, which promote the input of glucose into blood. For the most part, glucose homeostasis can be thought of as a balance between the actions of insulin and glucagon (insulin–glucagon ratio; **Table 2.3**). The effectiveness of the acute control by these hormones can be seen from the observation that while blood glucose may reach >10 mmol/L (>180 mg/dL) after a carbohydrate-rich meal, blood glucose concentration falls to less than 6.7 mmol/L (121 mg/dL) within two hours as seen in the oral glucose tolerance test. Furthermore, blood glucose does not fall much below 4 mmol/L (72 mg/dL) in the absence of dietary glucose, such as is seen after an overnight fast.

TABLE 2.3 Actions of insulin and glucagon on carbohydrate metabolism

Tissue	Metabolic pathway	Insulin effect	Glucagon effect
Liver	Glycolysis	Stimulatory	Stimulatory
	Glycogen synthesis	Stimulatory	Inhibitory
	Gluconeogenesis	Inhibitory	Stimulatory
	Lipolysis	Inhibitory	Stimulatory
	Lipogenesis	Stimulatory	Inhibitory
	Ketogenesis	Inhibitory	Stimulatory
Muscle	Glucose uptake	Stimulatory	Limited or no action
	Glucose metabolism	Stimulatory	Limited or no action
	Glycogen synthesis	Stimulatory	Limited or no action
	Amino acid uptake	Stimulatory	Limited or no action
	Protein synthesis	Stimulatory	Limited or no action

2.2 GLUCOSE STORAGE AND METABOLISM

Glucose is stored as glycogen, a macromolecular branched polymer, primarily in liver and muscle. Storing glucose units as a macromolecule eliminates the problem of increased osmotic pressure that would arise if glucose were stored as individual molecules in the cell. The highly branched glycogen molecule consists of chains of glucose monomers linked α1:4, with branch points linked α1:6, and a single reducing terminal glucose (**Figure 2.1**). Such a structure presents a multitude of substrate sites for the enzymes that are involved in both its hydrolysis (by glycogen phosphorylase) and its synthesis (by glycogen synthase). The amount of glycogen present in the tissue at any one time represents a balance between the activities of these two enzymes, both of which are under hormonal control.

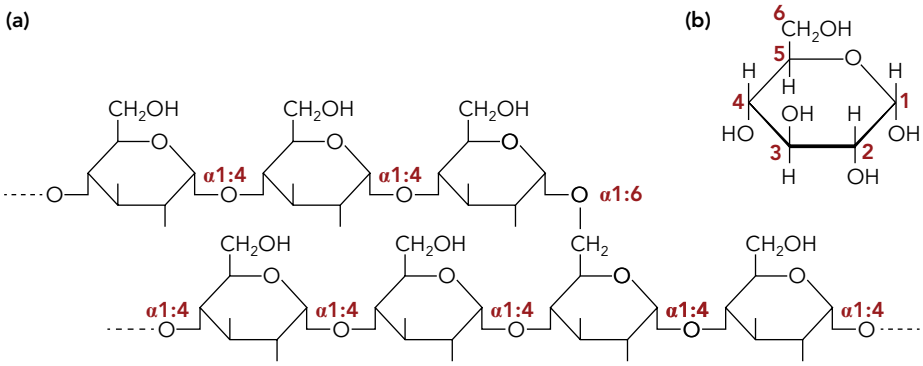


Figure 2.1 Partial structure of glycogen showing a branch point. (a) Glucose monomers are linked α1:4 intrachain and α1:6 interchain (branch point). (b) Structure of glucose with carbon numbering in red; the reducing carbon is C1. Hydrogen atoms are included in the monomeric structure but have been omitted from the polymer for clarity.

Quantitatively, muscle stores more glycogen *in toto* than liver—approximately 250 g in muscle and approximately 75 g in liver—reflecting the relative tissue masses, but the concentration is greater in liver (0.5–1% in muscle, 3–5% in liver). However, only liver can generate free glucose from the breakdown of stored glycogen; muscle lacks the key enzyme, glucose 6-phosphatase, required for this. Thus liver alone is capable of releasing glucose into the circulation for use by other tissues. Its glycogen content will thus fluctuate throughout the day as it releases glucose to maintain homeostasis and replenishes this lost glucose from dietary intake. Glucose enters and leaves the liver via a high-capacity, low-affinity transporter, GLUT2. In contrast, muscle glycogen is used solely to provide energy for contraction and its concentration will only fall during exercise and again be replenished from dietary glucose during the recovery period.

The five metabolic pathways involving glucose are described in **Table 2.4** and shown diagrammatically in **Figure 2.2**. The two pathways regulating the

TABLE 2.4 Pathways involving the metabolism of glucose

Metabolic pathway	Role of pathway in metabolism
Glycogenesis	Storage of glucose as glycogen in liver and muscle; glucose is first activated to UDP-glucose
Glycogenolysis	Hydrolysis of glycogen producing glucose 1-phosphate (G1P). G1P is converted to glucose 6-phosphate (muscle and liver) and then glucose (liver)
Glycolysis	Metabolism of six-carbon glucose to three-carbon pyruvate (oxidized in the mitochondrion to CO_2 and water)
Pentose phosphate pathway	Metabolism of glucose to five-carbon sugars (for nucleic acids) plus generation of reducing equivalents (NADPH) for anabolism
Gluconeogenesis	Synthesis of glucose from non-carbohydrate precursors: glycerol, lactate, and amino acid carbon skeletons

UDP-glucose, uridine diphosphate glucose.

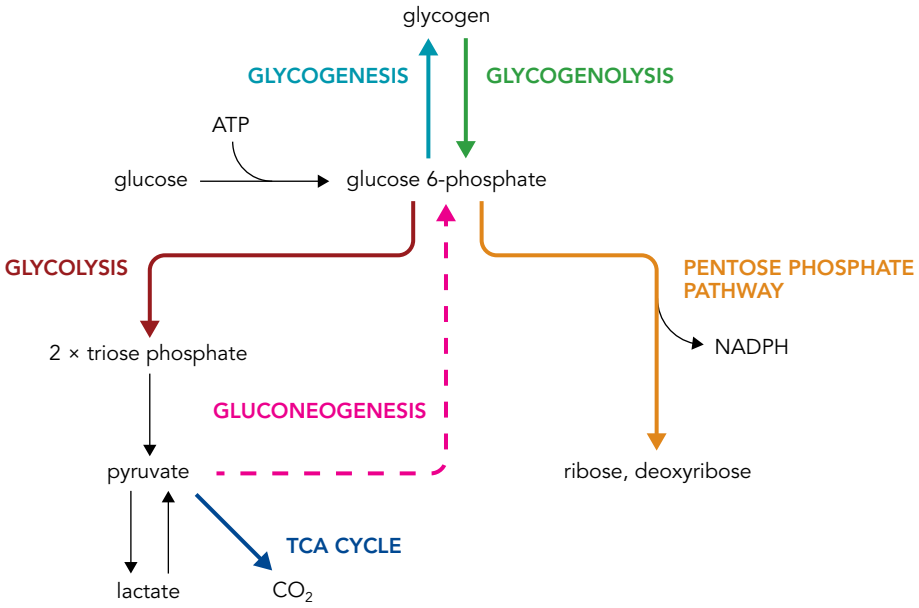


Figure 2.2 Pathways of glucose metabolism in liver.
Free glucose entering liver from blood is phosphorylated to glucose 6-phosphate by hexokinase before further metabolism via one of three pathways to: (i) pyruvate (by glycolysis), which is reduced to lactate anaerobically or to carbon dioxide (CO_2) aerobically via the tricarboxylic acid (TCA) cycle; (ii) the five-carbon sugars ribose and deoxyribose (by the pentose phosphate pathway), which are essential for nucleotide synthesis; or (iii) glycogen (by glycogenesis). Glucose 6-phosphate may also be synthesized from three-carbon precursors (gluconeogenesis) and from stored glycogen (glycogenolysis).

amount of glucose stored as glycogen in liver and muscle, glycogenesis and glycogenolysis, exhibit a reciprocal relationship which is controlled mainly by insulin and to a lesser extent by glucagon in liver and epinephrine in muscle. Thus the plasma insulin–glucagon ratio is a key factor in controlling the two processes.

Glycogenesis

The pathway of glycogenesis (**Figure 2.3**) is prominent following ingestion of a carbohydrate-rich meal. Diet-derived glucose and amino acids and also gastrointestinal peptides stimulate the release of insulin from the β cells of the pancreas, and insulin promotes the synthesis of energy stores including glycogen and triacylglycerol. Glycogenesis begins with the phosphorylation of free glucose by the enzymes glucokinase and hexokinase in liver and hexokinase in muscle, using adenosine triphosphate (ATP) as the phosphate donor. The product of this reaction is glucose 6-phosphate, which inhibits hexokinase but not glucokinase, and this difference has implications in the resynthesis of glycogen from dietary glucose following the depletion of hepatic glycogen stores brought about by fasting. Glucokinase (K_m 5–7 mmol/L) is a glucose-specific, inducible enzyme whose activity increases when the portal blood glucose concentration rises above 5 mmol/L (90 mg/dL).

Glucose 6-phosphate is converted to glucose 1-phosphate by the enzyme phosphoglucomutase before being activated to uridine diphosphate glucose (UDP-glucose) by uridine diphosphate pyrophosphatase. UDP-glucose is the donor of glucose to the growing glycogen molecule in a reaction catalyzed by glycogen synthase. The ability of liver to store glucose as glycogen is primarily due to the rapid activation of this enzyme. Glycogen synthase adds glucose in

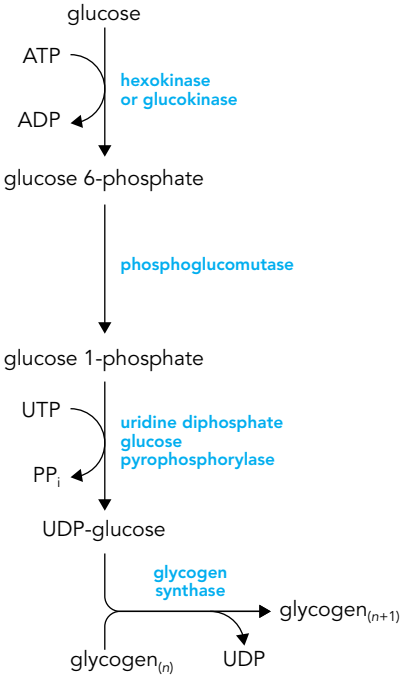


Figure 2.3 Glycogenesis in liver.
Glucose is activated to glucose 6-phosphate which undergoes isomerization to glucose 1-phosphate. The donor of glucose to the growing glycogen chain is uridine diphosphate glucose (UDP-glucose) formed from UTP and glucose 1-phosphate. PP_i, pyrophosphate; *n*, the number of glucose monomers in the polymeric glycogen molecule.

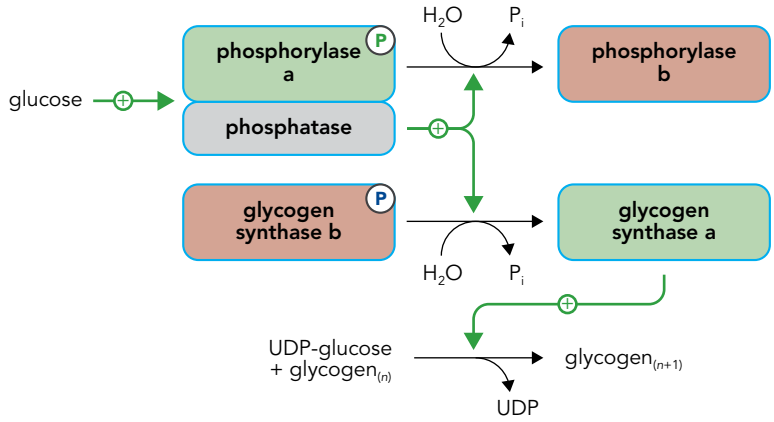


Figure 2.4 Stimulation of hepatic glycogen synthase by glucose. After a meal, the hepatic portal vein carries a high concentration of glucose to the liver. This glucose is rapidly incorporated into glycogen through activation of glycogen synthase. Glycogen synthase, which is inactive in the phosphorylated form (glycogen synthase b), is activated by the same phosphatase that inactivates glycogen phosphorylase a by converting it to inactive phosphorylase b. The phosphatase itself is inactive when complexed with phosphorylase a, but at intracellular concentrations of glucose >5 mmol/L (>90 mg/dL), glucose binds to the phosphorylase a–phosphatase complex and allows the phosphatase to dephosphorylate phosphorylase a, forming inactive phosphorylase b; this also releases the phosphatase in order to activate glycogen synthase b to glycogen synthase a. Free glucose can thus stimulate its own incorporation into glycogen. P_i, inorganic phosphate; *n*, the number of glucose monomers in the polymeric glycogen molecule.

an α1:4 linkage and gradually builds a chain of α1:4-linked glucose units. When the chain length reaches eight glucose units, a branching enzyme, a transglycosylase, transfers some of the chain to form an α1:6 linkage, now providing two chains for subsequent addition of glucose. In this way, the multibranched nature of the mature glycogen molecule is achieved. Glycogen synthase is the key regulatory enzyme of glycogenesis and its activity is increased by insulin released from the pancreas in response to a rise in blood glucose. Insulin increases the expression of genes coding for enzymes of carbohydrate storage as well as protein tyrosine phosphorylation which, by activation of GTPase, phosphodiesterase, and phosphoprotein phosphatases, inhibits the cyclic adenosine monophosphate (cAMP)-activated pathway of glycogenolysis. Insulin also inhibits the pancreatic secretion of glucagon, the hormone that activates glycogen phosphorylase.

Glycogen synthase exists in an inactive phosphorylated form, glycogen synthase b, and activation involves dephosphorylation to glycogen synthase a by a specific phosphatase, the same enzyme that inactivates glycogen phosphorylase a by a similar dephosphorylation. In the liver, the phosphatase forms an inactive complex with phosphorylase a; as the intracellular concentration of glucose rises above 5 mmol/L (90 mg/dL), glucose binds to phosphorylase a which acts as an intracellular glucose receptor, changing its shape such that it becomes susceptible to the action of the phosphatase (**Figure 2.4**). Dephosphorylation of phosphorylase a to inactive phosphorylase b releases the phosphatase, which can now activate glycogen synthase b by converting it to glycogen synthase a. Free glucose in the liver is thus able to stimulate its own incorporation into glycogen. This is enhanced by raised insulin and the stimulatory action of glucose on hepatic glucokinase. A similar cAMP-independent mechanism involving activation of the phosphatase that is common to the glycogen synthase–phosphorylase system exists in muscle. Insulin also stimulates the uptake of glucose into muscle and adipose tissue via the GLUT4 transporter system. It has no effect on glucose uptake into liver, however, where the rate of uptake reflects the glucose concentration gradient across the plasma membrane. Interestingly, the concentration of insulin required to stimulate glycogenesis is lower in muscle than in liver.

Glycogenolysis

Glycogenolysis (**Figure 2.5**) involves the release from glycogen of terminal glucose (nonreducing) units as glucose 1-phosphate; this is termed phosphorolysis and is catalyzed by the action of glycogen phosphorylase a. The phosphate donor is inorganic phosphate present in the cytosol. Different phosphorylases are present in liver, kidney, and muscle. Under catabolic physiological conditions where plasma epinephrine is raised, glucose is mobilized rapidly

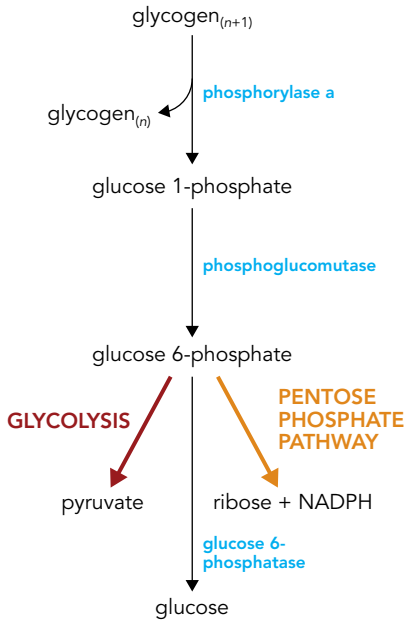


Figure 2.5 Glycogenolysis and the possible fates of glucose 6-phosphate in liver.
Glycogen phosphorylase hydrolyzes glucose monomers as glucose 1-phosphate from the nonreducing ends of the glycogen macromolecule. Glucose 1-phosphate is converted to glucose 6-phosphate before metabolism via glycolysis or the pentose phosphate pathway. Only liver contains glucose 6-phosphatase which hydrolyzes glucose 6-phosphate to free glucose for the circulation in times of decreased intake of dietary glucose. *n* is the number of glucose monomers in the polymeric glycogen molecule.

from glycogen in skeletal muscle and also in the liver when epinephrine levels are very high, whilst raised glucagon stimulates glycogenolysis only in liver (**Table 2.5**). The molecular mechanism of the stimulation of glycogen phosphorylase by epinephrine in muscle has been studied in detail and a similar mechanism obtained for the action of glucagon in liver. A rise in the plasma concentration of glucagon increases the binding of the hormone to its specific receptor on the plasma membrane of the target tissue (liver) causing activation of the intracellular synthesis of cAMP via the adenylate cyclase system, shown in outline in **Figure 2.6**. Raised intracellular cAMP initiates a phosphorylation cascade resulting eventually in the phosphorylation of the less active form of phosphorylase, phosphorylase b, to the more active phosphorylase a. Thus, an increase in the intracellular concentration of cAMP simultaneously increases glycogen breakdown and decreases glycogen synthesis (by inactivating glycogen synthase).

The effect of epinephrine on glycogenolysis in liver is not mediated by cAMP but via a rise in intracellular free calcium concentration which effects a direct activation of phosphorylase b kinase, the enzyme that phosphorylates inactive phosphorylase b to active phosphorylase a. A similar mechanism is found in skeletal muscle, where a rise in cytosolic calcium as a result of neural stimulation can activate phosphorylase b kinase and promote glycogen breakdown independently of stimulation by epinephrine. In this case, Ca^{2+}-induced glycogenolysis depends on calcium binding to the calmodulin subunit of the kinase and is linked to contraction. The simultaneous phosphorylation of glycogen synthase under these conditions causes its inactivation and minimizes the futile cycling of glucose. The active conformation of phosphorylase is stabilized by phosphorylation even in the absence of an allosteric activator and only glucose is a significant inhibitor of the phosphorylated form.

Glucose 1-phosphate released from glycogen by the action of glycogen phosphorylase a then isomerizes to glucose 6-phosphate under the action of

TABLE 2.5 Actions of hormonal changes on glycogenolysis and glycolysis in liver and muscle

Tissue	Hormone change	Glycogen to glucose	Glucose to pyruvate
Liver	Insulin decrease	No effect	No effect
	Glucagon increase	Major stimulus	Inhibition
	Epinephrine increase	Some effect at high concentration of hormone	No effect
Muscle	Insulin decrease	No effect	No effect
	Glucagon increase	No effect	No effect
	Epinephrine increase	Major stimulus	Major stimulus

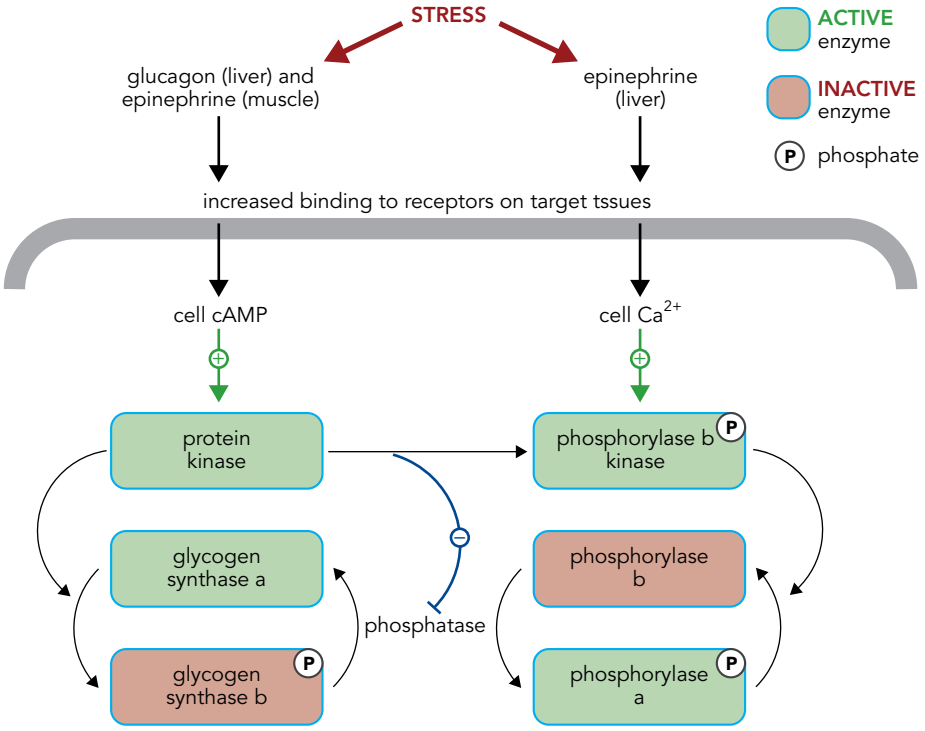


Figure 2.6 Activation of glycogen phosphorylase and inhibition of glycogen synthase by glucagon (liver) or epinephrine (muscle). A rise in the plasma concentration of glucagon during stress increases its binding to receptors on target tissues, activating the synthesis of intracellular cAMP. This molecule initiates a phosphorylation cascade that eventually results in the net phosphorylation of less active glycogen phosphorylase b to more active glycogen phosphorylase a, and of active glycogen synthase a to less active glycogen synthase b. Epinephrine has similar actions in skeletal muscle. The action of epinephrine in liver, however, is not mediated via cAMP but via a rise in intracellular Ca^{2+}.

phosphoglucomutase (see Figure 2.5). This enzyme is present predominantly in liver, kidney, and muscle. Release of free glucose from glucose 6-phosphate is mediated by the enzyme glucose 6-phosphatase, which is present only in liver and kidney. The lack of this enzyme in muscle prevents release of free glucose from the tissue. Phosphorylase a hydrolytic activity is specific for α1:4 links and the enzyme does not hydrolyze glucose monomers linked α1:6. It will hydrolyze α1:4-linked glucose residues along a glycogen branch to within 3–4 residues from an α1:6 branch point, where it is unable to reach the bond to be cleaved. A debranching enzyme (transglycosylase) cleaves the short sequence at the α1:6 site and transfers it to the end of a near-neighbor α1:4 chain, leaving a single glucose at the branch. This residue is cleaved as free glucose by an α1:6-glucosidase, leaving another α1:4 chain for phosphorylase activity. The transglycosylase and glucosidase activities, which release small amounts of free glucose from exposed α1:6 branch points during glycogen breakdown, are not regulated directly by hormone action. Approximately 90% of glucose stored in glycogen is released as glucose 1-phosphate with the remainder as free glucose.

Glycolysis

Glycolysis (**Figure 2.7**), in which six-carbon glucose is metabolized to two molecules of three-carbon pyruvate, occurs in all tissues and can be thought of as anabolic for lipid synthesis in liver and white adipose tissue or catabolic for energy production in tissues such as exercising muscle, brain, and red cells (erythrocytes). The activity of the glycolytic pathway is high in those tissues that depend on glucose as their major energy source (for example brain, erythrocytes, and kidney) although most tissues will also metabolize glucose via this route after a carbohydrate-rich meal. Only small amounts of ATP can be generated from glycolysis but for mature erythrocytes, which lack mitochondria, this represents their only means of generating ATP. In other tissues, much more energy is generated by subsequent metabolism of pyruvate in mitochondria, as shown in the overview of oxidation in **Figure 2.8**. The entry of glucose into resting muscle and also white adipose tissue is insulin dependent, but glucose

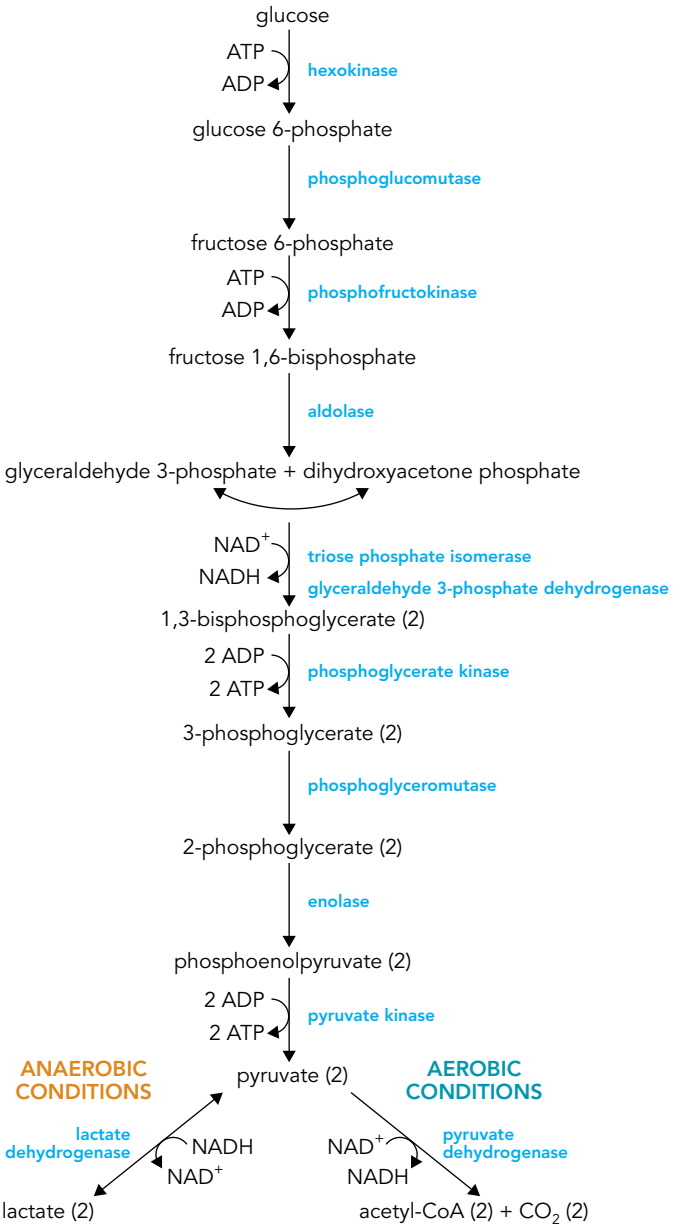


Figure 2.7 The glycolytic pathway. The metabolism of six-carbon glucose to three-carbon pyruvate occurs in the cell cytosol. Two molecules of the intermediates from 1,3-bisphosphoglycerate to lactate are formed per molecule of glucose entering the pathway. Under aerobic conditions pyruvate enters the mitochondrion and is oxidized to CO_2 whilst under anaerobic conditions it is reduced to lactate.

entry into working muscle is less dependent on insulin and glycolysis can proceed from exogenous glucose during exercise, even under catabolic conditions.

The major regulatory step of the glycolytic pathway is at the level of phosphofructokinase (PFK; **Figure 2.9**), which catalyzes the step prior to the cleavage of six-carbon sugar to two three-carbon molecules. This enzyme is inhibited by allosteric effectors that reflect the energy charge ratio (ATP–AMP ratio) of the cell. For example, with a plentiful supply of energy substrate, the level of ATP will rise relative to AMP leading to an inhibition of PFK and, as a consequence, inhibition of glycolysis. Raised levels of ATP also slow the tricarboxylic acid (TCA) cycle, raising the cytosolic concentration of citrate, another allosteric inhibitor of PFK. Inhibition of PFK will in turn lead to an increase in glucose 6-phosphate, an inhibitor of hexokinase, and this will decrease the rate of glucose entry into the glycolytic pathway.

The most potent activator of glycolysis in the liver is fructose 2,6-bisphosphate, a molecule formed when circulating glucose is abundant, after a meal for example. This molecule also acts allosterically to activate PFK by antagonizing

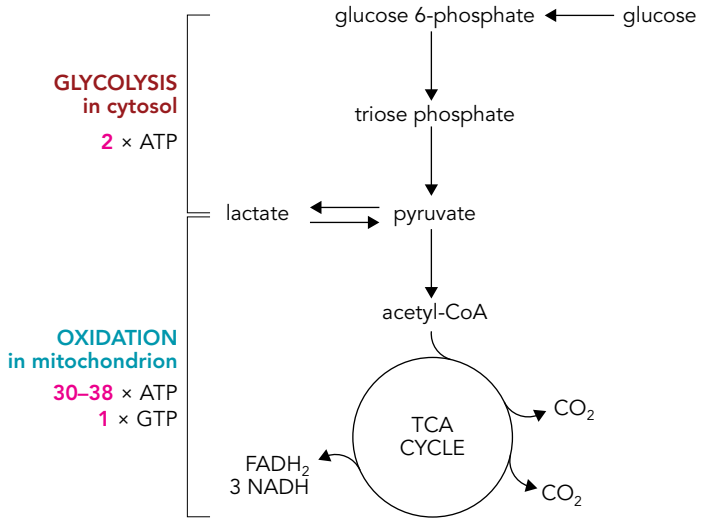


Figure 2.8 Overview of glucose oxidation.
There is a net formation of two molecules of ATP per molecule of glucose entering the glycolytic pathway. ATP is formed in two reactions—catalyzed by phosphoglycerate kinase and pyruvate kinase (four ATP in total per molecule of glucose)—while one molecule of ATP is consumed by each of the hexokinase and phosphofructokinase steps. On entering the mitochondrion, pyruvate is oxidized to acetyl-CoA which in turn is oxidized to CO_2 in the TCA cycle with the formation of reducing equivalents in the form of reduced co-factors NADH and $FADH_2$. These molecules, NADH and $FADH_2$, donate electrons to the electron transport chain on the inner mitochondrial membrane and drive the synthesis of ATP by oxidative phosphorylation: three molecules of ATP are generated per pair of electrons from NADH and two molecules per pair of electrons from $FADH_2$. Red numbers indicate the yield of ATP molecules per molecule of glucose metabolized: two in the cytosol versus 30–38 via mitochondrial oxidation of pyruvate. A single molecule of GTP is generated by substrate-level phosphorylation at the succinyl CoA synthase step of the cycle.

the inhibitory effects of ATP and citrate. Hepatic glycolysis is inhibited at two points in the pathway by the raised circulating glucagon concentration of the catabolic state: (i) at pyruvate kinase and (ii) at PFK. Glucagon action decreases the intracellular concentration of fructose 2,6-bisphosphate thereby decreasing PFK activity; glucagon also stimulates cAMP-activated phosphorylation of pyruvate kinase, producing an enzyme which is inhibited by the prevailing concentration of phosphoenolpyruvate.

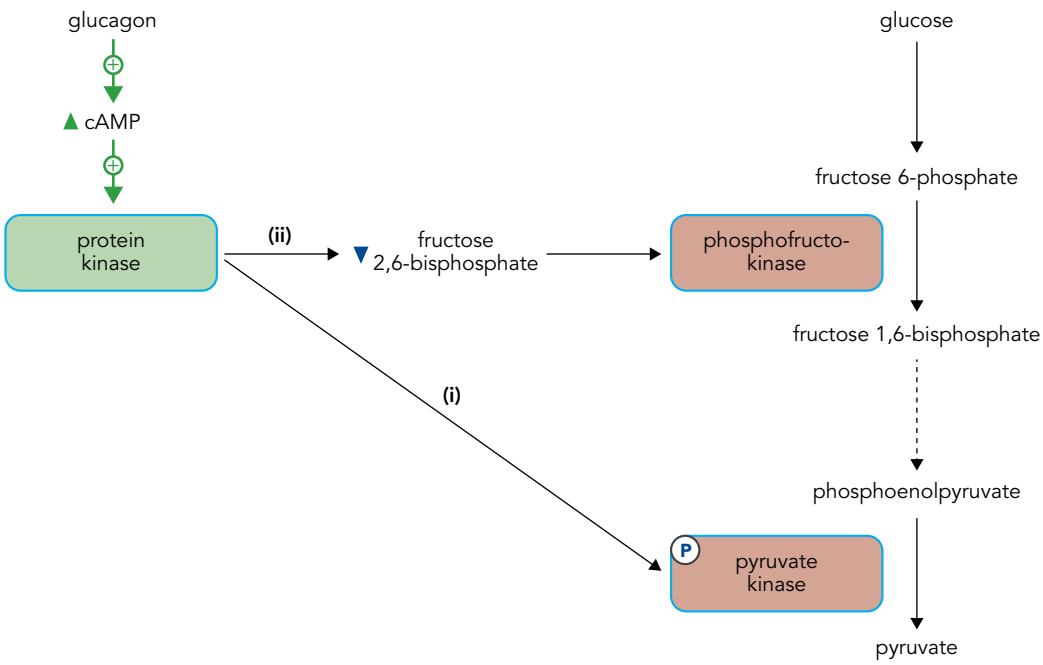


Figure 2.9 Inhibition of hepatic glycolysis by glucagon.
The glucagon-stimulated elevation of cAMP in liver activates protein kinases which are responsible for the inhibition of glycolysis at two sites in the pathway: (i) by phosphorylating pyruvate kinase so that it is inhibited by the prevailing concentration of phosphoenolpyruvate and (ii) by decreasing the intracellular concentration of fructose 2,6-bisphosphate. Phosphofructokinase is a major regulatory enzyme of the glycolytic pathway and fructose 2,6-bisphosphate is the most potent activator of this enzyme, antagonizing the allosteric inhibition exerted by ATP and citrate. A decreased fructose 2,6-bisphosphate concentration reduces the activity of phosphofructokinase.

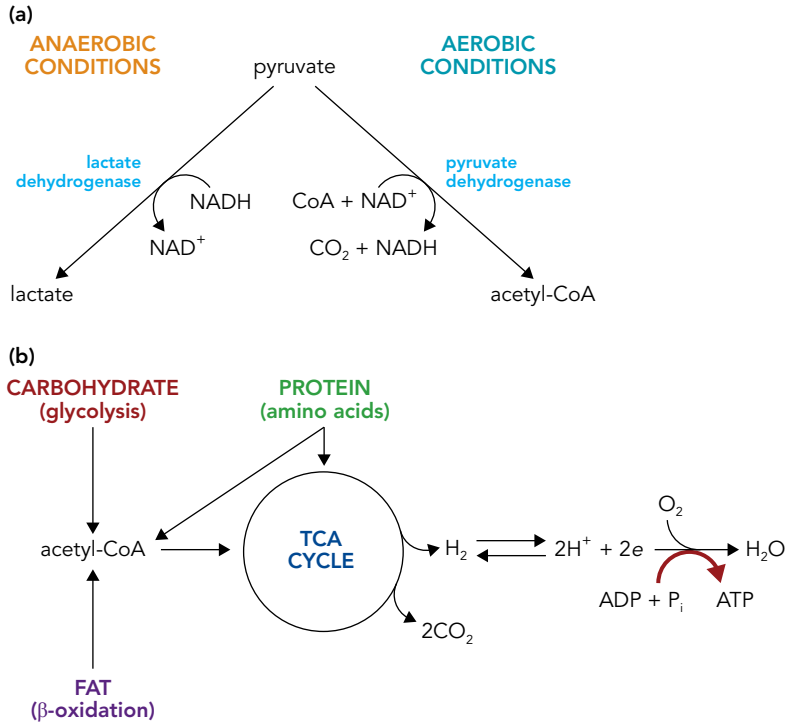


Figure 2.10 Metabolism of pyruvate under aerobic and anaerobic conditions and oxidation of acetyl-CoA in the TCA cycle. (a) Pyruvate is oxidized to acetyl-CoA and CO_2 aerobically and reduced to lactate anaerobically. NADH is derived from glycolysis. Pyruvate dehydrogenase is inhibited by acetyl-CoA. (b) Acetyl-CoA formed from oxidation of pyruvate is further oxidized to CO_2 and H_2O in the TCA cycle in mitochondria. Some of the energy released from this exothermic process drives the synthesis of ATP.

Further metabolism of pyruvate

Under aerobic conditions, where the oxygen supply to tissues is not limited, pyruvate enters the mitochondrion and is eventually oxidized via the TCA cycle to carbon dioxide and water (**Figure 2.10**). The initial step in this process, the oxidative decarboxylation of pyruvate to acetyl-CoA, is irreversible and, because human cells cannot synthesize glucose from two-carbon molecules such as acetate, this represents a net loss to the body of potential carbohydrate reserves. Conservation of three-carbon molecules is essential when the exogenous supply of glucose is limited and under such conditions, fasting for example, the oxidation of pyruvate is tightly regulated. The enzyme that catalyzes this step, pyruvate dehydrogenase (PDH), undergoes a cAMP-independent phosphorylation-dephosphorylation cycle catalyzed by a specific kinase and phosphatase, respectively. In the fed state, raised plasma insulin and raised cytosolic calcium stimulate the phosphatase, thereby increasing the amount of active enzyme and pyruvate oxidation. On the other hand, the kinase that phosphorylates and inactivates PDH is activated by acetyl-CoA and NADH which are produced when tissues, particularly liver, switch to metabolism of fatty acids for energy. In this way, pyruvate oxidation is inhibited by both the decreased insulin concentration and by the increased β-oxidation activity of the catabolic state.

Under anaerobic conditions where the oxygen supply is limited, such as in muscle during high-intensity exercise, or where no mitochondria are present, as in mature erythrocytes, pyruvate is reduced to lactate by the enzyme lactate dehydrogenase. Lactic acid is a waste end product in muscle and erythrocytes and, being a strong acid, is toxic to these tissues. It diffuses out of muscle and erythrocytes and is carried in the blood to the liver. The liver is well-oxygenated through its blood supply via the hepatic artery (see Chapter 3) and converts the lactate back to pyruvate. Pyruvate is either oxidized to CO_2 and water in mitochondria or used as a substrate for glucose synthesis via gluconeogenesis. The process whereby glucose from muscle glycogen is metabolized to lactate and this lactate is used for glucose synthesis in liver is known as the Cori cycle (**Figure 2.11**), named for the husband and wife team who first described it and who were awarded the Nobel Prize in Medicine in 1947 for their work on glycogen metabolism.

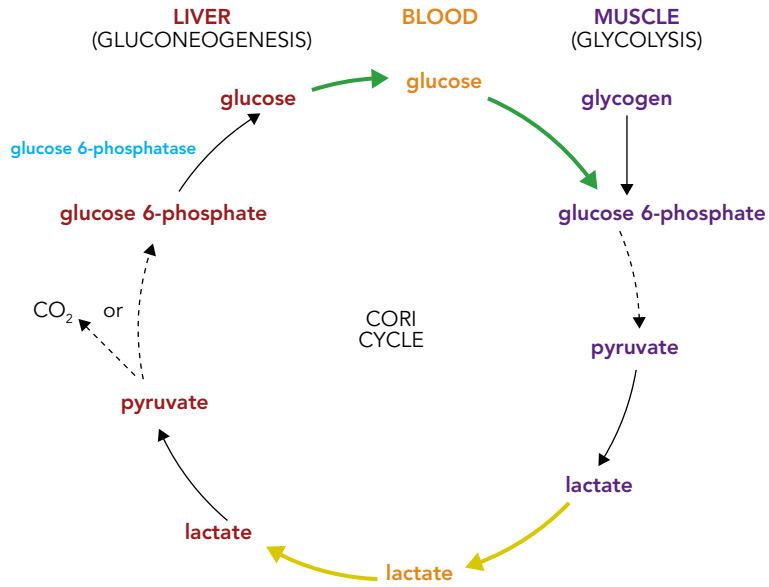


Figure 2.11 The Cori cycle: recycling of muscle glucose. Lactate produced via anaerobic metabolism of glucose derived from muscle glycogen breakdown during exercise, for example, is transported via the blood to liver. Liver, being well vascularized, oxidizes lactate to pyruvate, which can be oxidized to CO_2 or used as a substrate for gluconeogenesis through production of glucose 6-phosphate. Liver also possesses the enzyme glucose 6-phosphatase and can thus release free glucose to the circulation. Glucose can then enter the circulation and return to muscle.

Pentose phosphate pathway

The pentose phosphate pathway (**Figure 2.12**), also known as the hexose monophosphate shunt, is particularly active in rapidly dividing tissues, including activated lymphocytes, and in tissues engaged in reductive biosynthesis, particularly of fatty acids, such as lactating mammary gland and adipose tissue. As its name implies, in this pathway glucose 6-phosphate is metabolized to five-carbon sugars, which may be used as precursors of the ribose and 2-deoxyribose components of nucleic acids. The pathway is also a major source of reducing equivalents in the form of NADPH (reduced nicotinamide adenine dinucleotide phosphate) which is required for the reductive biosynthesis of macromolecules such as fatty acids and cholesterol. NADPH is also the electron donor to the

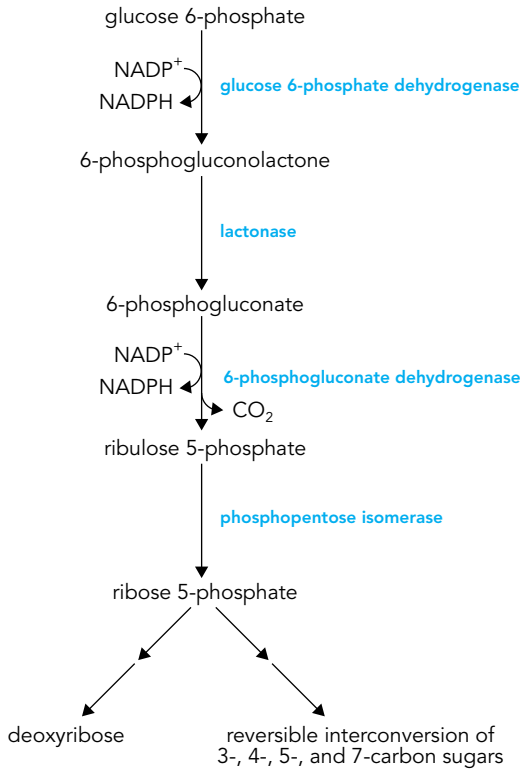


Figure 2.12 The pentose phosphate pathway. In this pathway, also known as the hexose monophosphate shunt or phosphogluconate pathway, six-carbon glucose is converted to five-carbon sugars such as ribose with the release of CO_2. The pathway is also an important source of NADPH (at steps 1 and 3) required for the reductive biosynthesis of fatty acids. Five-carbon sugars are components of ribonucleotides and deoxyribonucleotides.

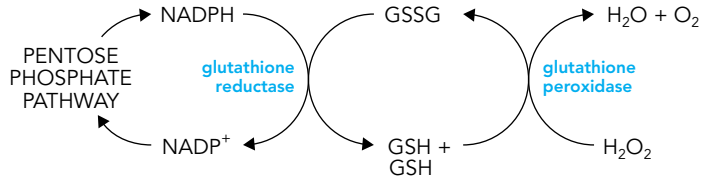


Figure 2.13 The role of the pentose phosphate pathway and glutathione in the reduction of oxidative stress in erythrocytes.
The presence of oxygen and reactive oxygen species such as peroxide in erythrocytes causes a stress that promotes the denaturation of membrane proteins and other proteins. Protection against this stress is afforded by the alternative oxidation of reduced glutathione (GSH) to its oxidized form (GSSG). With only a finite amount of glutathione in the cell, this protection is lost when all the glutathione is oxidized. Glutathione reductase, with its co-factor NADPH derived from the pentose phosphate pathway, converts oxidized glutathione to its reduced form to allow continued protection from oxidative stress. Glutathione peroxidase reduces peroxide to water.

hepatic drug-metabolizing system described in Chapter 3. Importantly, high activity of the pentose phosphate pathway is found in activated macrophages where NADPH provides electrons required for the generation of reactive oxygen species, such as superoxide and hydroxyl radical, which are involved in killing bacteria and degradation of macromolecules. In erythrocytes, an important role of NADPH produced from pentose phosphate pathway activity is the maintenance of glutathione in its reduced form to counter the oxidative stress put upon the cells (**Figure 2.13**).

Gluconeogenesis

Gluconeogenesis (**Figure 2.14**) describes the synthesis of new glucose from non-carbohydrate precursors such as lactate (via the Cori cycle), glycerol, and amino acids. Its name suggests that it is an anabolic process but it occurs only when the body is in an overall catabolic state. Gluconeogenesis is stimulated by raised plasma concentrations of glucagon (acutely) and glucocorticoid (chronically) and inhibited by raised plasma insulin. The minimal structural requirement for a molecule to serve as a precursor for gluconeogenesis is three carbons. This precludes fatty acids since they are degraded into two-carbon acetyl-CoA and, unlike plants, humans lack the enzymes required to synthesize glucose from acetate. The major gluconeogenic tissue is liver although kidney and, to some extent, small intestine also possess gluconeogenic activity. Gluconeogenesis in the liver provides a mechanism for synthesis of glucose from lactate, derived from exercising muscle or anaerobic glycolysis in

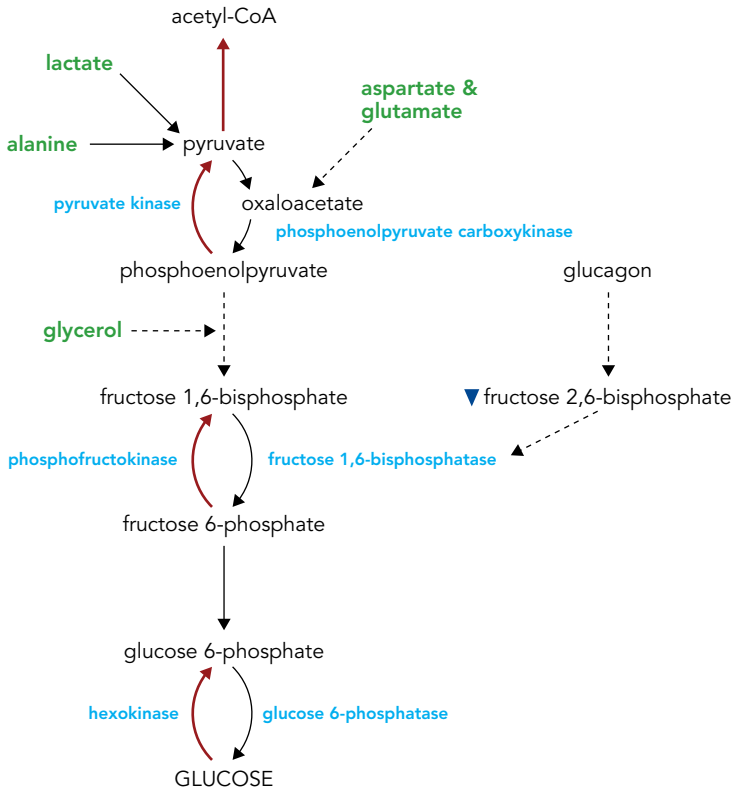


Figure 2.14 Gluconeogenesis: synthesis of new glucose.
Gluconeogenesis, the synthesis of glucose from non-carbohydrate precursors (lactate, glycerol, and amino acids), occurs mainly in the liver but also in kidney. It is essentially the reverse of the glycolytic pathway apart from at three key, energetically unfavorable steps: conversion of phosphoenolpyruvate to pyruvate (catalyzed by pyruvate kinase); phosphorylation of fructose 6-phosphate (by phosphofructokinase); and phosphorylation of glucose (by hexokinase). These steps are overcome by the actions of phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase, and glucose 6-phosphatase, respectively. A decrease in fructose 2,6-bisphosphate stimulates the action of fructose 1,6-bisphosphatase. The minimum structural requirement for a substrate for gluconeogenesis is three carbons. Gluconeogenic precursors are shown in green. Red arrows show reactions inhibited by the catabolic state. Dashed arrows indicate intermediate steps that are not shown.

erythrocytes, and from glycerol released from hydrolysis of adipose tissue triglyceride. It is particularly important during the early stages of fasting when, in the absence of dietary glucose, hepatic glycogen levels become depleted and the liver synthesizes glucose to satisfy the needs of tissues that have an obligatory requirement for glucose as an energy source, particularly brain and erythrocytes. In this case, the carbon skeletons of amino acids derived from hydrolysis of muscle protein are the precursors for hepatic glucose synthesis. The acute stimulation of gluconeogenesis by glucagon is due to inhibition of synthesis of fructose 2,6-bisphosphate, which thereby inhibits the metabolism of glucose via glycolysis (by reducing PFK activity), and stimulation of fructose-1,6-bisphosphatase activity, a key step on the gluconeogenic pathway. The lowered plasma insulin level seen in the fasting state also promotes the phosphorylation and thus the inhibition of pyruvate dehydrogenase, which conserves three-carbon pyruvate for gluconeogenesis. Chronic hormonal changes in the prolonged catabolic state, particularly raised glucocorticoid, lead to increased synthesis of key enzymes of the gluconeogenic pathway.

The rate of gluconeogenesis is governed by substrate supply and is inhibited by high redox states (high NADH–NAD^+ ratios) in the hepatocyte, such as is seen in excess alcohol ingestion (see Chapter 22). Even under optimal conditions of substrate supply, however, the liver is incapable of meeting the needs of glucose-dependent tissues and the adaptation of the brain to the use of fatty acid-derived ketone bodies is described in Chapter 3. Indeed, the rate of hepatic gluconeogenesis falls during prolonged fasting as the tissue engages in ketone body synthesis and, under this condition, kidney becomes the major gluconeogenic tissue.

2.3 INSULIN

The actions of insulin are key to the whole-body metabolism of glucose and lack of insulin action results in the hyperglycemia of diabetes mellitus and severe, pathological consequences. The insulin gene is located on the short arm of chromosome 11 and encodes an initial translation product, a 109-amino-acid peptide called preproinsulin, from which an N-terminal signal peptide sequence of 23 amino acids is cleaved co-translationally to yield proinsulin (86 amino acids). Proinsulin undergoes post-translational cleavage involving excision of a C-peptide to yield A and B chains, which are linked via disulfide bridges to form the parent hormone comprising 51 amino acids. Insulin is thus a dimeric protein linked through both intra- and interchain disulfide bridges (**Figure 2.15**). The

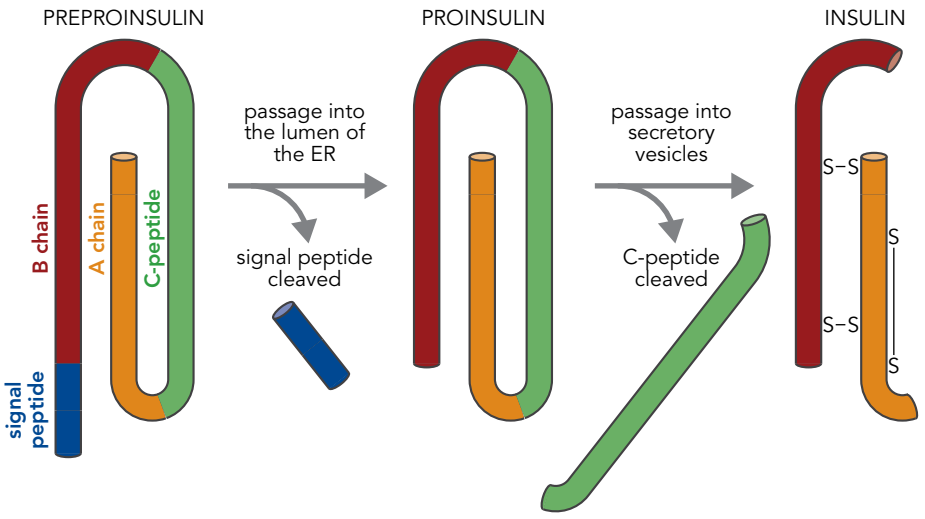


Figure 2.15 Structure of insulin. The nascent insulin polypeptide (preproinsulin, 109 amino acids) is translated as a monomeric species from its messenger RNA on the rough endoplasmic reticulum (ER) of pancreatic β cells. Its passage into the lumen of the ER is directed by an N-terminal signal peptide (23 amino acids) and this is cleaved co-translationally. The signal peptide is not found in the mature hormone. Further post-translational processing of the insulin polypeptide occurs during its passage through the secretory pathway into secretory vesicles. This involves excision of the connecting C-peptide and production of the A and B chains. Disulfide bond formation, both intra- and interchain, then occurs to form the dimeric parent hormone. The C-peptide is released with the parent hormone from secretory vesicles.

parent hormone and the C-peptide are stored in secretory vesicles complexed with zinc and both are released into the circulation by a hyperglycemic signal in the portal circulation or in response to an intestinal peptide that promotes insulin secretion (see Section 2.4 below). Thus, when the plasma glucose rises above the homeostatic concentration of about 4.5 mmol/L (81 mg/dL), glucose enters the pancreatic β cell via the GLUT2 transport protein and is metabolized via glycolysis; this initiates a signal transduction system that closes ATP-sensitive potassium channels thereby depolarizing the cell and stimulating calcium entry into the cytoplasm. The subsequent rise in cytoplasmic calcium triggers fusion of insulin-containing secretory vesicles and exocytosis of vesicle contents into the bloodstream. The magnitude of insulin secretion is dependent on the plasma glucose concentration. Both insulin and C-peptide are released into the portal circulation and approximately 50% of the parent hormone is metabolized in the first pass through the liver, such that the concentration of insulin in the portal vein is two- to fourfold greater than in the peripheral circulation. The C-peptide, however, does not undergo a first-pass effect and measurement of C-peptide rather than of insulin itself is a more precise marker of endogenous insulin secretion.

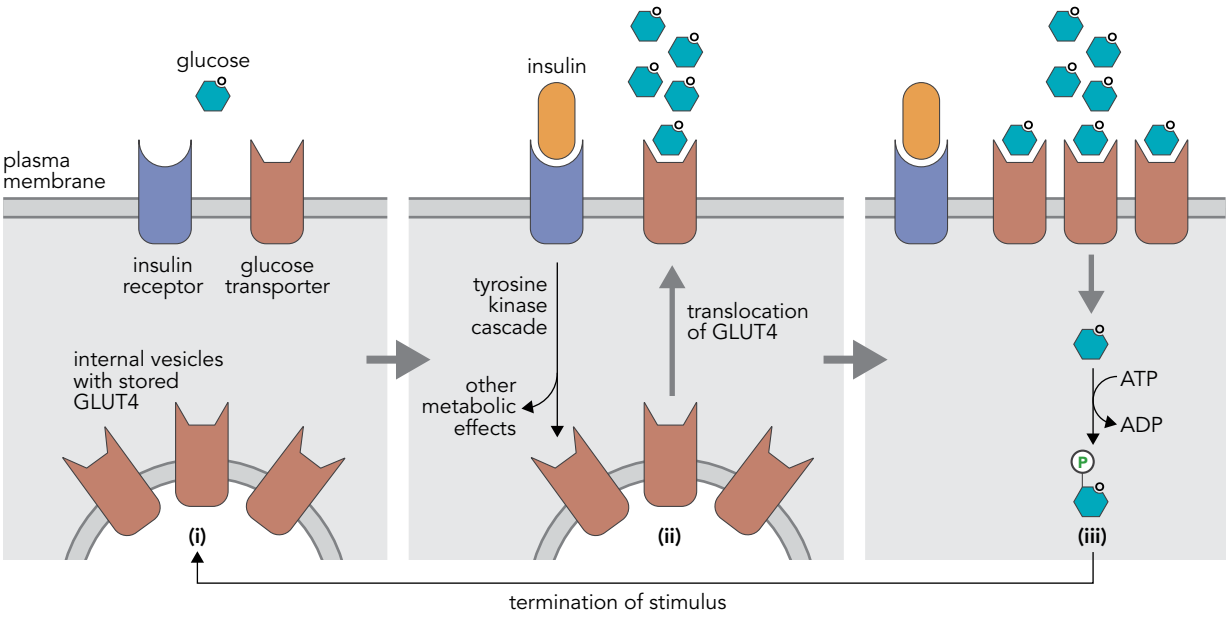
Metabolic actions of insulin

Insulin has both acute anabolic effects on metabolism and, via somewhat complex signaling to the nucleus, more chronic effects on protein synthesis, cell growth, and replication. Only the acute effects are relevant to the current text.

Following a rise in plasma glucose, after a meal for example, the primary actions of insulin are to promote glucose uptake into liver, muscle, and adipose tissue and glucose storage as glycogen in liver thereby reducing the rise in plasma glucose concentration.

Insulin acts by binding to its specific receptors on target cells (**Figure 2.16**). The receptor is heterodimeric, consisting of two surface-bound α-subunits and two membrane-spanning β-subunits linked via disulfide bonds. The β-subunits have intrinsic tyrosine kinase activity and can autophosphorylate on serine, threonine, and tyrosine residues. Insulin binding to the α-subunits situated on the extracellular surface of the plasma membrane promotes autophosphorylation of the β-subunits and initiation of signal transduction pathways that promote the short- and longer-term effects of insulin mentioned above. One such pathway involving 1-phosphatidylinositol 3′-kinase leads to translocation of the

Figure 2.16 Overview of insulin action on glucose transport into muscle and adipose tissue. Insulin binding to its receptor stimulates receptor dimerization and activates a protein tyrosine kinase cascade. This in turn promotes the translocation of GLUT4 (glucose transporter 4) from intracellular sites on the endoplasmic reticulum to the cell surface thereby increasing glucose uptake into the tissues. In the unstimulated, basal state, shown as (i), greater than 95% of the GLUT4 glucose transporters are in intracellular vesicles with only a few in the plasma membrane. Dimerization of the insulin receptor effected by the binding of insulin initiates a tyrosine kinase-mediated signaling cascade, which ultimately leads to the translocation of the intracellular GLUT4 molecules to the plasma membrane (ii), thereby increasing glucose transport into the cell by facilitated diffusion. Transport of glucose out of the cell is prevented by its rapid phosphorylation to glucose 6-phosphate (iii). The translocation of GLUT4 is readily reversible and the basal state (i) is re-attained within an hour of removal of the insulin stimulus.



glucose transport protein GLUT4 to the cell surface and facilitation of glucose uptake into muscle and adipose tissue. Uptake of glucose into liver, however, is not facilitated by GLUT4 and is not stimulated by insulin; glucose enters the liver down a concentration gradient. The process of glycogenesis, however, is stimulated by insulin and this is responsible for increased glucose uptake by the liver. Approximately 30% of the glucose ingested during a meal is stored in the liver as glycogen, replacing that lost during the previous fasting period. Insulin also stimulates triglyceride synthesis in liver and, as a consequence, promotes very-low-density lipoprotein (VLDL) synthesis and secretion. The insulin-stimulated uptake of glucose into muscle promotes glycogen synthesis and storage by the tissue, and increased glucose uptake into adipose tissue leads to storage of triglyceride.

Hepatic actions of insulin

The overall action of insulin in the liver is anabolic and it regulates the supply of fuels, such as glucose and ketone bodies, to the systemic circulation. Under normal dietary conditions of moderate glucose consumption, the major hepatic actions of insulin are to decrease glucose production and promote glucose storage by inhibiting glycogenolysis and gluconeogenesis and promoting glycogenesis, processes which are sensitive to small increases in insulin concentration. It also stimulates glucose metabolism via glycolysis and the TCA cycle to supply energy to the tissue and inhibits β-oxidation of fatty acids and ketogenesis. The liver hydrolyzes 80% of insulin arriving from the portal circulation such that, under normal conditions, the concentration of insulin in the systemic circulation is considerably lower than that of the portal system.

Extrahepatic actions of insulin

The principal sites of insulin action in the systemic circulation are skeletal muscle and adipose tissue. Here, its major action is to increase the recruitment of insulin-sensitive glucose transport proteins (GLUT4) to the plasma membrane of both tissues and thereby increase the rate of glucose uptake and supply of metabolic substrate. In skeletal muscle, the increased supply of glucose and elevated insulin concentration promote glucose storage as glycogen as a result of activation of glycogen synthase and inhibition of glycogenolysis. This storage is an important energy source for muscle not only for acute exercise but also during long-term fasting; its mobilization is considerably slower than for hepatic glycogen reserves. In addition, insulin stimulates amino acid uptake into muscle and, by also inhibiting proteolysis, it promotes protein accumulation. An important anabolic action of insulin is to promote the storage of triglyceride in adipose tissue. Dietary fatty acids are transported in the systemic circulation as the triglyceride component of chylomicrons, and activation by insulin of lipoprotein lipase in adipose tissue results in hydrolysis of the triglyceride and uptake of free fatty acids into the tissue. Activation of glycolysis in the tissue also provides energy and glycerol phosphate for triglyceride synthesis. In addition to this action, insulin inhibits the hormone-sensitive lipase of adipose tissue required for mobilization of fatty acids from adipose-tissue triglyceride, such that the overall effect of the hormone is to promote fat storage.

When dietary carbohydrate exceeds total daily energy expenditure, a raised insulin concentration stimulates the conversion of glucose to fatty acids in liver and formation of triglyceride. This is exported as a component of very-low-density lipoprotein and can also donate its fatty acids to adipose tissue by the same route as for chylomicrons. In this way, excess dietary glucose is converted to stored fat.

Two final important actions of insulin are (i) inhibition of ketogenesis in liver and (ii) promotion of the uptake of K^+ ions by cells through stimulation of the plasma membrane Na^+/K^+-ATPase; insulin may be used to correct hyperkalemia, as described later. Hypokalemia may also be present in patients with certain types of pancreatic tumors (ectopic insulin).

Thus insulin is primarily an anabolic hormone that regulates the supply of glucose into the systemic circulation, facilitates the synthesis of energy stores in the form of glycogen and triglyceride, and promotes protein deposition.

2.4 GLUCAGON

Glucagon may be considered metabolically as an insulin antagonist, its actions for the most part being the opposite of those of insulin. The gene for glucagon is located on the long arm of chromosome 2 and encodes a large precursor molecule, preproglucagon, which is expressed mainly in the α cells of the pancreas and to a lesser extent in the duodenum and brain. The glucagon gene is a member of a multigene superfamily that includes secretin, vasoactive peptide, and gastric inhibitory peptide (GIP). In the pancreatic α cells, the initial translation product, preproglucagon, undergoes intracellular processing to yield the parent 29-amino-acid hormone and two further peptides, glucagon-like peptides 1 and 2 (GLP-1, GLP-2; **Figure 2.17**). Proteolytic processing of preproglucagon in the L-cells of the intestine yields peptides that serve to promote insulin secretion by pancreatic β cells (GLP-1 and glicentin) and which may suppress appetite following a meal (oxyntomodulin). For the purposes of this text, only the metabolic actions of glucagon are considered.

Glucagon secretion increases rapidly in response to a fall in plasma glucose and glucagon acts acutely to provide glucose to the circulation. It has a very short half-life of about 5 minutes and is destroyed rapidly when carbohydrate is consumed. The level of glucagon in the blood rises between meals as the glucose concentration falls and is raised chronically during fasting or on consumption of a diet low in carbohydrate. Glucagon is also a stress-response hormone and is released through direct sympathetic stimulation of the pancreas during times of acute psychological or physical stress.

Metabolic actions of glucagon

Glucagon acts by binding to its trimeric, G-protein-linked receptor on the surface of the target tissue, mainly liver and adipose tissue, and signaling via a cAMP-activated phosphorylation cascade to activate glycogen phosphorylase and inhibit glycogen synthase in liver, and activate hormone-sensitive lipase in adipose tissue. In this way, it mobilizes readily metabolizable fuels—glucose and fatty acids—for use by tissues when the dietary supply of these fuels is reduced or at zero. Activation by glucagon of the hormone-sensitive lipase in adipose tissue results in the release of free fatty acids and glycerol. The free fatty acids are transported in the circulation bound to albumin and provide fuel to most tissues. Glycerol is returned to the liver and used as a substrate for gluconeogenesis. In

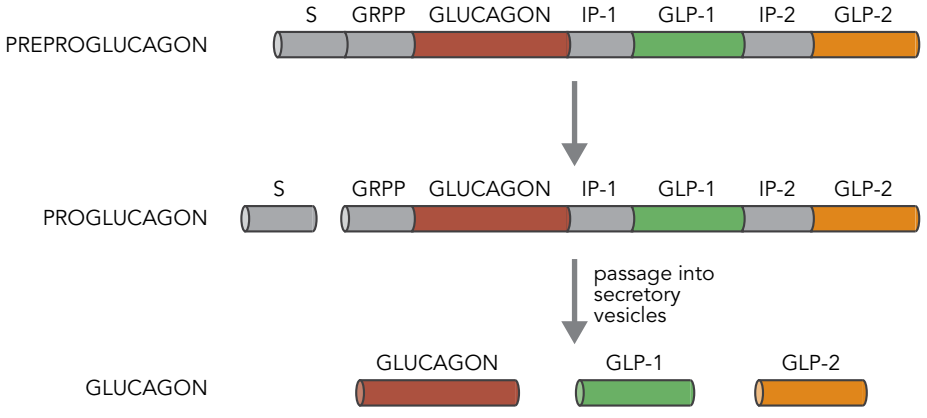


Figure 2.17 Structure of glucagon. Glucagon is formed from the translation product of the GCG gene, preproglucagon, which is translated as a monomeric polypeptide on the rough endoplasmic reticulum (ER). An N-terminal signal peptide (S) directs the passage of the nascent polypeptide into the lumen of the ER, after which it is cleaved by a signal peptidase. Further tissue-specific processing in the secretory pathway yields other biologically active peptides including, in pancreatic α-cells, glucagon. GLP, glucagon-like peptide; GRPP, glicentin-related peptide; IP, intervening peptide.

the longer term, glucagon also promotes the process of ketogenesis, key to the provision of ketone bodies as an alternative fuel for the brain during prolonged fasting (see Table 2.3).

2.5 MAINTENANCE OF GLUCOSE HOMEOSTASIS IN THE FED AND FASTING STATES

Blood glucose concentration is maintained through the opposing actions of insulin and counter-insulin hormones—glucagon, epinephrine, cortisol, and growth hormone—which combine to ensure that the amount of glucose entering and leaving the bloodstream is balanced in both the fed and fasting states. The glucose supply to the circulation during a 24-hour period is shown in **Figure 2.18**. Insulin concentration will rise as the blood glucose level rises after each meal, while levels of the counter-insulin hormones rise as glucose concentration falls. Of paramount importance is the supply of glucose to the brain, other nervous tissue, and erythrocytes.

The rise in blood glucose following digestion of a carbohydrate-enriched meal is detected by glucose receptors in the β cells of the pancreas and triggers the release of insulin. At the same time, glucagon release from the pancreatic α cells is suppressed. By increasing the number of glucose-specific transporters (GLUT4) on the cell surface in peripheral tissues, especially muscle and adipose tissue but not liver and brain, insulin promotes the uptake of glucose into these tissues. In addition, a raised concentration of glucose arrives at the liver via the hepatic portal circulation and glucose enters the tissue via insulin-independent, low-affinity, high-capacity transporters (GLUT2). The increase in the insulin-to-glucagon ratio promotes hepatic glycogen synthesis and inhibits glycogenolysis and gluconeogenesis such that there is an increase in glucose uptake by the liver. The condition of raised insulin and intracellular glucose concentrations promotes the synthesis and activity of glucokinase, which converts glucose to glucose 6-phosphate. Since this enzyme is not product-inhibited, the hepatic concentration of glucose 6-phosphate rises and the flux of glucose 6-phosphate into glycolysis, the pentose phosphate pathway, and glycogenesis is increased. Thus, enhanced hepatic metabolism of glucose also contributes to the clearance of blood glucose following a meal. The rise in blood glucose will be maintained until the clearance of glucose into liver and peripheral tissues is greater than the rate of glucose released from the splanchnic bed (arising from ingested food and endogenous production). At this point, the glucose concentration begins

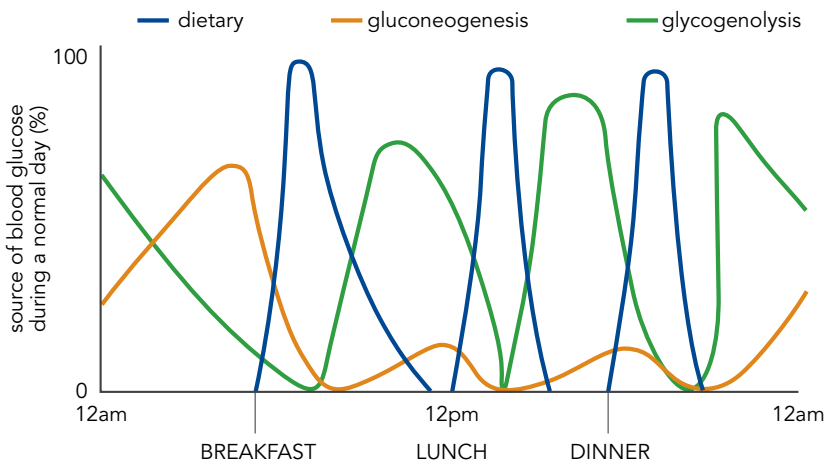


Figure 2.18 Contributions to the maintenance of blood glucose through a period of 24 hours. The figure assumes consumption of three meals (breakfast, lunch, and dinner) during the period. Sources of glucose are diet (exogenous), hepatic gluconeogenesis (endogenous), and hepatic glycogenolysis (endogenous). Between meals, hepatic glycogenolysis becomes the major contributor. Only overnight with a longer interval between meals (just prior to the next breakfast) does hepatic gluconeogenesis play any significant role.

to fall toward its preprandial level, with a concomitant fall in insulin and a rise in glucagon, such that the basal level is reached 4–6 hours after the meal. The various interacting factors that limit the rise in glucose concentration following a meal and give rise to a smooth return to homeostasis are:

- The rate of glucose absorption from the gut
- The relative timing and amounts of insulin and glucagon secreted by the pancreas
- The ability of the liver to store and release glucose
- The responses of the liver, muscle, and adipose tissue to insulin and counter-insulin hormones

The fasted state

As the blood glucose falls slightly overnight, glucose is released from the liver (**Figure 2.19**), initially from glycogen breakdown and later through gluconeogenesis from glycerol, lactate, and amino acids, which occurs mainly in liver but also a little in kidney. The overall rate of glucose production at this stage is about 2 mg/kg/min (11 μmol/kg/min).

In the absence of ingested carbohydrate, hepatic gluconeogenesis becomes a progressively more and more important source of blood glucose as the fast continues and hepatic glycogen is used up. This is to meet the needs of the glucose-dependent tissues. Obviously, the rate of hepatic glycogen depletion will depend on the previous nutritional state of the subject (that is, having a full store of glycogen to start with) and factors such as exercise. The major need now is to reduce the demand for glucose, particularly by the major consumer, the brain. Most tissues, including liver, muscle, and adipose tissue, will already be using fatty acids as their major respiratory substrate and the brain will also gradually adapt to using fatty acid-derived substrates—ketone bodies (acetoacetate and 3-hydroxybutyrate)—for energy. A reduction in blood glucose concentration reduces insulin and increases glucagon, cortisol, and growth hormone, and the swing in favor of the action of the counter-insulin hormones increases lipolysis in adipose tissue and ketogenesis in liver. The use of ketone bodies by the brain reduces the overall glucose demand and thereby the rate of hepatic gluconeogenesis from carbon skeletons of amino acids; in this way, vital protein is conserved. This is extremely important since even at its maximal rate (approximately 40 g/day), hepatic gluconeogenesis cannot supply sufficient glucose to

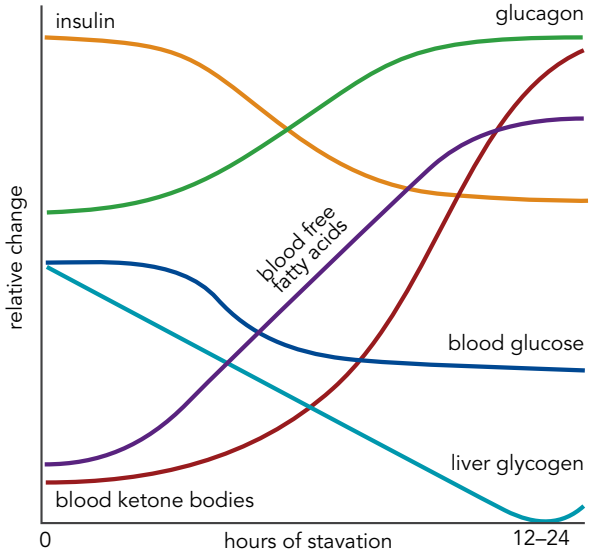


Figure 2.19 The effect of fasting over a period of 24 hours on blood insulin and glucagon concentrations and consequent changes in metabolic parameters.
The change from an anabolic to a catabolic state results in a total loss of liver glycogen and a marked reduction in blood glucose as tissues apart from brain, other nervous tissue, and kidney switch to the use of fatty acids and ketone bodies for metabolic fuel. The concentrations of free fatty acids and ketone bodies rise dramatically over the time period.

satisfy the needs of the brain. The overall switch of the body from glucose-based metabolism to fatty acid-based metabolism allows the glucose concentration to fall to 2–3 mmol/L (36–54 mg/dL) without inducing the symptoms commonly seen in hypoglycemia.

As may be deduced from this discussion, defects in enzymes of the glycogenesis, glycogenolysis, or gluconeogenesis pathways, lack of substrates, or abnormal levels of insulin or counter-insulin hormones will prevent the maintenance of normoglycemia.

2.6 GLYCATION OF PROTEINS

Glucose is an aldohexose, a six-carbon sugar with a functional aldehyde group at carbon 1 as illustrated in **Figure 2.20**, which depicts both the Haworth ring and Fischer open-chain representations of the molecule. The aldehyde group allows it to react nonenzymatically with a free amino group, of a polypeptide for example, to form a Schiff base (an aldimine), which can undergo an Amadori rearrangement to a ketimine (**Figure 2.21**) where the double bond at carbon 1 moves to carbon 2 and forms a fructosamine derivative. The formation of the Schiff base is reversible but the rearrangement to the stable ketimine is irreversible. As with all chemical reactions, the rate of formation of product is proportional to the concentration of reactants and so Schiff base formation is proportional to the concentration of glucose and/or the amino group. Glycation of proteins occurs even at concentrations of glucose within the normal physiological range and assumes importance pathologically as the concentration of plasma glucose rises. The extent of glycation in plasma can be measured as glycated hemoglobin (HbA_{1c}); in this case, the free amino group of the N-terminal valine of the β-chain of hemoglobin reacts with glucose to form a Schiff base initially and this undergoes rearrangement to form the stable fructosamine conjugate. Measurement of HbA_{1c} is used to assess glycemic control in diabetic patients.

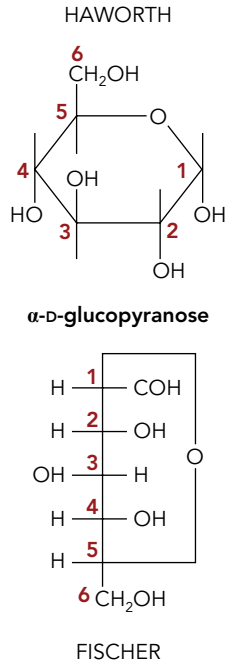


Figure 2.20 Haworth and Fischer projection formulas of glucose (α-D-glucopyranose).
The aldehyde reducing carbon is C1 on both structures, which are shown in the lactone form. Brown numbers show the numbering of carbons.

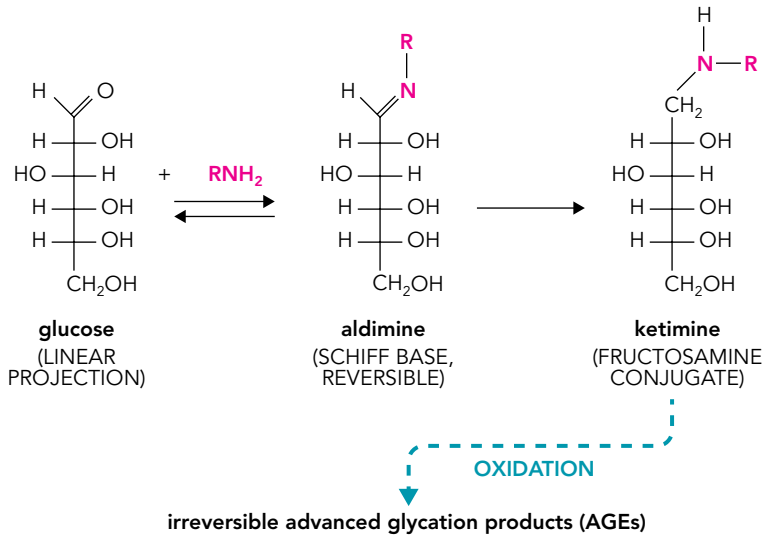


Figure 2.21 Glycation of proteins leading to formation of advanced glycation end products.
Glucose is shown here in the open chain form to illustrate the reversible formation of a Schiff base between the aldehyde moiety on C1 and a free amino group on proteins, RNH_2 (internal lysine residues and N-terminal amino acids). The further formation of the fructosamine conjugate (ketimine) and subsequent oxidation to advanced glycation end products (AGEs) are irreversible.

TABLE 2.6 Definitions of various glycated hemoglobins

Hemoglobin	Definition
Glycated hemoglobin	Carbohydrate, usually glucose, is bound to any free amino group (of the N-terminal amino acid or internal lysine) in the hemoglobin chains
HbA_1	Carbohydrate is bound to the N-terminal valine of hemoglobin β-chains
HbA_{1a1}	Fructose 1,6-bisphosphate is bound to N-terminal valine
HbA_{1a2}	Glucose 6-phosphate is bound to N-terminal valine
HbA_{1b}	Unknown carbohydrate is bound to N-terminal valine
HbA_{1c}	Glucose is bound to N-terminal valine

In each hemoglobin subspecies, the carbohydrate is covalently bound to the hemoglobin chain via a Schiff base linkage to a free amino group.

While other sugars, including fructose 1,6-bisphosphate and glucose 6-phosphate, with a functional keto group can also form glycated proteins with free amino groups on hemoglobin (**Table 2.6**), only HbA_{1c} is measured in clinical practice.

The ε-amino groups of internal lysine residues on proteins also present opportunities for Schiff base formation and eventual formation of glycated proteins. Thus, virtually any protein can undergo glycation, including albumin and plasma proteins, and measurement of plasma fructosamine (glycated plasma proteins, mainly glycated albumin) provides an index of short-term (2–3 weeks) glycemic control in individuals.

Glycation leads to changes in the three-dimensional shape of a protein such that it exhibits an altered antigenic profile and may no longer be recognized by its receptor. For example, glycation of apoprotein B100, the only protein of low-density lipoprotein (LDL), yields a product that is no longer recognized by the LDL receptor and is cleared by unregulated scavenger receptors, leading to cholesterol deposition in extrahepatic tissues. In addition, in the longer term, oxidation of the fructosamine conjugates can lead to further cross-linking of proteins, including collagen, and the formation of advanced glycation end products (AGEs; see Figure 2.21) with subsequent changes in protein function and turnover. In the case of collagen and other structural proteins, this increases the rigidity of the cytoskeletal network and probably contributes to circulatory, joint, and vision problems, particularly in patients with diabetes.

2.7 HYPERGLYCEMIA AND DIABETES MELLITUS

Transient hyperglycemia may be seen in normal individuals after ingestion of a carbohydrate-enriched meal and poses no clinical problems. However, chronic hyperglycemia gives rise to disease, the treatment of which is a major cost to national health budgets.

Diabetes mellitus

Diabetes mellitus was defined by the World Health Organization in 2000 as a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Thus, although the diagnosis of diabetes depends upon demonstration of hyperglycemia, it is important to remember that other metabolic processes are also affected.

Diabetes mellitus is a chronic, noncommunicable condition which, if untreated, proceeds to micro- and macrovascular disease. The trademark hyperglycemia of diabetes mellitus arises from an inability to clear glucose into insulin-sensitive tissues, particularly skeletal muscle and adipose tissue, due to

either (i) decreased insulin production by the β cells of the pancreas (type 1; juvenile-onset, insulin-dependent diabetes mellitus) or (ii) decreased sensitivity of tissues to the prevailing insulin concentration (type 2; adult-onset, non-insulin-dependent diabetes mellitus).

Diabetes was recognized as a metabolic disease by the ancient Greeks and the term diabetes (Greek for siphon) was used in the second century by Aretaeus of Capodocia, who observed that patients with the disease "suffered liquefaction of the flesh and bones into urine such that kidneys and bladder do not cease emitting urine." Avicenna (980–1037) observed that the urine of diabetic patients was "wonderfully sweet" and the Latin term mellitus ("honey sweet") was added by Thomas Willis (1621–1676) who noted that diabetic patients "piss a great deal" whilst "suffering from a persistent thirst." It is now evident that the diuresis and sweet-tasting urine are caused by glycosuria.

The early descriptions of the wasting disease and associated thirst, polyuria, and early death clearly refer to the type 1, insulin-dependent form. This insulin-dependent form was shown in the 1950s to be an autoimmune condition that gives rise to the highly selective destruction of the insulin-producing β cells of the pancreas. It was in the 1880s that the French physiologist Lanceaux differentiated between this maigre (thin) presentation and the gras (fat) presentation characterized by corpulence and stupor, which is now associated with type 2, non-insulin-dependent diabetes mellitus.

Since the discovery of insulin by Banting and Best in Canada in the early 1920s, early death from acute insulin deficiency has become avoidable, and insulin-dependent diabetic patients can lead relatively normal lives with daily injections of the hormone. However, this increase in life-expectancy is associated with long-term complications of diabetes which have significant effects on the overall morbidity and mortality of the disease. Such complications, described later, are also associated with the later-onset type 2 form of the disease. This form accounts for about 95% of patients presenting with diabetes in Western societies and is becoming an increasing problem in developing countries. Such patients present with variable combinations of insulin resistance and β-cell dysfunction leading to defects in insulin secretion. It is now apparent that type 2 diabetes mellitus is a syndrome with many different causes including a more sedentary lifestyle, obesity, and dietary factors imposing upon an innate genetic susceptibility.

Diagnosis of diabetes

Until 2009, diagnosis of diabetes was based entirely on blood or plasma glucose concentrations exceeding thresholds specified by the World Health Organization. These concentrations are different for samples taken in the fasting state (**Table 2.7**), at random times during the day, and after a standard 75 g oral glucose load (oral glucose tolerance test [OGTT]; **Figure 2.22** and **Table 2.8**). The use of HbA_{1c} for monitoring of long-term glucose control has been well established for almost two decades, but its use as a diagnostic test for diabetes is controversial. The reasons for this include differences in standardization of measurement, lack of availability in poorer countries, variable correlation with average blood glucose, and the effect of variant hemoglobins and states which alter the red cell survival time. Nevertheless, a number of diabetes organizations have recently agreed that a confirmed HbA_{1c} value of ≥6.5% (48 mmol/mol) is

TABLE 2.7 Ranges of fasting blood glucose concentration in normal, glucose-intolerant, and diabetic subjects

Subject	Range (mmol/L)	Range (mg/dL)
Normal	Up to 6	Up to 108
Impaired glucose tolerance	6–7	108–126
Diabetic	7 and above	126 and above

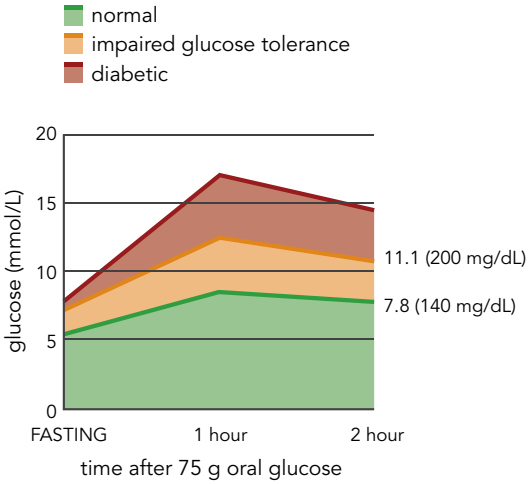


Figure 2.22 Oral glucose tolerance tests in normal subjects and patients with diabetes mellitus types 1 and 2.
An oral glucose tolerance test involves ingestion of 75 g of glucose dissolved in 100 mL of water and measurement of blood glucose over the following two hours. In normal, non-diabetic subjects, blood glucose rises during the first hour after ingestion, peaking at about 7.8 mmol/L (140 mg/dL), and then falls as glucose is cleared by liver, adipose tissue, and muscle in response to a rise in insulin. This contrasts with the situation in patients with diabetes, where insulin is either absent (type 1) or tissues are less responsive to the prevailing insulin concentration (type 2); in these patients, glucose may rise to concentrations >15 mmol/L (270 mg/dL) and fall slowly subsequently, remaining at >11.1 mmol/L (200 mg/dL) after two hours. Blood glucose levels tend to be higher in untreated type 1 than in untreated type 2 diabetes because patients with type 2 diabetes retain some insulin activity. Some subjects with impaired glucose tolerance have raised glucose levels but these do not exceed values used to diagnose the presence of diabetes mellitus.

sufficient evidence to diagnose diabetes without glucose testing. This approach has not been universally accepted and it remains to be seen whether misdiagnosis is a significant problem.

Analytical practice point 2.1

Glucose decreases rapidly in whole blood samples, due to glycolysis, and must be collected into tubes containing fluoride/oxalate unless analyzed immediately.

TABLE 2.8 World Health Organization (WHO) criteria for the diagnosis of diabetes mellitus following an oral glucose tolerance test

Subject	Fasting glucose		Blood glucose after 2 h	
	(mmol/L)	(mg/dL)	(mmol/L)	(mg/dL)
Non-diabetic	<6.0	<108	<7.8	<140.4
Impaired glucose tolerance (IGT)	<6.7	<120.6	7.9–11.0	142.2–198
Impaired fasting glucose	6.1–6.9	109.8–124.2	>7.8	>140.4
Diabetic	>7.0	>126	>11.1	>199.8

Subjects are given 75 g of glucose in water (100 mL) and the glucose concentration in capillary whole blood is measured over a period of two hours.

Types of diabetes

Diabetes is classified into several Types (**Table 2.9**) with the largest numbers of patients having either type 1 or type 2. About 90% of diabetes worldwide is type 2. Diabetes of other types should not be overlooked but make up a very small fraction of cases.

TABLE 2.9 Classification of diabetes mellitus and probable causal mechanisms

Classification	Causal mechanism
Type 1	Insulin deficiency
Type 2	Relative insulin deficiency/peripheral insulin resistance
Gestational diabetes	Increased insulin resistance during pregnancy
Maturity-onset diabetes of the young	Single gene defect affecting glucose metabolism, for example, glucokinase
Secondary diabetes due to	
• endocrine disease	Increased concentration of hormones antagonistic to insulin*
• pancreatic disease	Relative to absolute insulin deficiency
• malnutrition	Relative to absolute insulin deficiency
• drugs	Impaired insulin secretion or increased insulin resistance

*For example, increased thyroid hormones in thyrotoxicosis.

TABLE 2.10 Major clinical differences between patients with type 1 and type 2 diabetes mellitus

Clinical feature	Type 1	Type 2
Usual age at onset	<40 years	>40 years
Obesity	Uncommon	Usual
Prone to ketosis	Yes	No
Insulin secretion	Absent	Present
Insulin resistance	Rare (unless obese)	Usual
Genetic factors*	+	++
HLA association	Yes	No
Islet cell antibodies	Yes	No

*There appear to be genetic influences on both disorders which are more pronounced in the case of non-insulin-dependent diabetes mellitus.

Pathogenesis and clinical aspects of diabetes mellitus type 1 and type 2

The major differences between diabetes mellitus types 1 and 2 are shown in **Table 2.10** and the clinical presentations of each in **Table 2.11**. The most striking difference is the inability to synthesize and secrete insulin in type 1 diabetes. Polyuria, thirst, and polyphagia are features common to both types and their causes are explained in **Table 2.12**. In type 1 diabetes there is destruction of pancreatic β cells, due to an autoimmune process, leading to insulin deficiency. This usually presents as an acute illness with weight loss, symptoms of hyperglycemia, and sometimes ketoacidosis. Type 2 diabetes is a more slowly progressive condition, often asymptomatic for many years, characterized by peripheral resistance to insulin and a compensatory increase in β-cell secretion of insulin. As the disease progresses there is a gradual failure of β cells superimposed on the insulin resistance, but this is partial rather than total, and ketoacidosis rarely occurs. Insulin resistance is strongly correlated with obesity and lack of physical activity and its prevalence is increasing across the globe in parallel with the growth in unhealthy lifestyle. The development of hyperglycemia is often the final step in the progression of insulin resistance, which may exist for many years in association with cardiovascular risk factors including dyslipidemia and hypertension. This combination has been termed the metabolic syndrome and is considered to be a major contributor to cardiovascular disease.

Whilst most patients with type 1 or type 2 diabetes conform to the expected phenotypes listed in Table 2.10, it should be realized that exceptions occur and the conditions are not mutually exclusive. Thus, increasing obesity amongst the

TABLE 2.11 Clinical presentation of diabetes mellitus

Diabetes mellitus type	Clinical signs and symptoms
Type 1 (usually acute onset)	Polyuria
	Thirst
	Acute visual changes
	Weight loss
	Ketosis
Type 2 (usually slow onset)	Tiredness, mood changes, fungal infections
	Incidental finding in routine examination for other diseases
	Polyuria, thirst, visual changes if severe hyperglycemia
	Late presentation may be with complications, for example sight loss, foot ulcer

TABLE 2.12 Clinical symptoms and their causes in diabetes mellitus

Symptom	Cause
Polyuria	Retention of glucose in renal tubule as glucose load exceeds the reabsorptive capacity of the kidney. Glucose acts as an osmotic diuretic causing the production of large volumes of urine
Thirst	CNS-driven response to dehydration; may be mediated by angiotensin secreted in response to hypovolemia
Polyphagia	Hunger stimulated by nonutilization of dietary glucose
Weight loss	Increased catabolism of all stored metabolic fuels; muscle glycogen and protein, and adipose tissue triglyceride
Tiredness	Muscular weakness due to (i) proteolysis and mobilization of muscle protein and (ii) reduced availability to muscle of metabolic substrate (glucose)
Blurred vision	Systemic dehydration of the lens and aqueous and vitreous humors, thereby reducing visual acuity
Vomiting	CNS-driven response to ketones stimulating the area postrema in the floor of the fourth ventricle
Hyperventilation (Kussmaul breathing)	Respiratory compensation to metabolic acidosis due to raised concentrations of lactic acid and keto acids in plasma
Itching	Impaired humoral immunity leading to increased risk of skin infections

CNS, central nervous system.

young is increasing the prevalence of type 2 diabetes amongst the under 40s, even affecting children. As people with type 1 diabetes conform to the population trend in obesity, they may become insulin resistant as well as deficient. Equally, there is no age beyond which β-cell destruction cannot occur and cases of type 1 diabetes appearing in the ninth decade of life have been described. Slow onset of type 1 diabetes is also seen on occasion with patients initially classified as type 2. It is more accurate to consider diabetes as a spectrum with pure insulin deficiency at one end and insulin resistance at the other. The acute effects of insulin deficiency are shown in **Figure 2.23**.

Clinical practice point 2.1

Type 1 and type 2 diabetes are not mutually exclusive conditions, and elements of both can occur in the same individual.

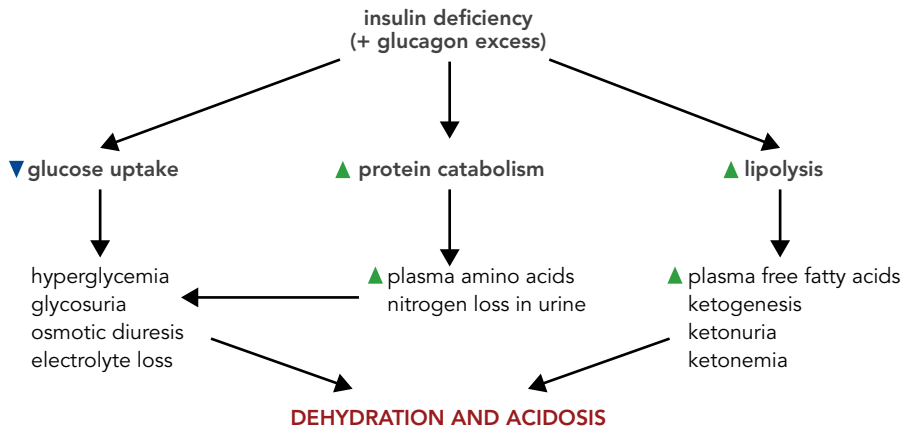


Figure 2.23 Acute metabolic effects of insulin deficiency. Decreased glucose uptake by tissues leads to hyperglycemia and increased excretion of glucose in urine (glycosuria) accompanied by osmotic diuresis and dehydration. The catabolic state, stress for example, increases the blood concentrations of amino acids and fatty acids, which are mobilized by hydrolysis of proteins and stored fat, and is responsible for increased nitrogen loss in urine and formation of ketone bodies and acidosis.

Diabetes mellitus type 1

The biochemical signs and their causes in type 1 diabetes are listed in **Table 2.13**.

The propensity to develop ketosis is one of the most important distinguishing features of insulin deficiency and its presence usually means that lifelong insulin treatment is mandatory. However, some patients with type 2 diabetes may develop ketosis when ill but may not always need long-term insulin therapy. The major clinical aspects of ketoacidosis are as follows:

- It generally occurs in known diabetic patients
- It is often precipitated by intercurrent illness, especially infections

TABLE 2.13 Biochemical signs and their causes in insulin-dependent diabetes mellitus

Biochemical sign	Cause
Hyperglycemia	(1) Decreased uptake of glucose into peripheral tissues (2) Increased mobilization of hepatic glycogen (3) Increased hepatic gluconeogenesis
Glycosuria	Glucose load exceeds capacity for reabsorption in renal tubule
Ketoacidosis	Increased β-oxidation of adipose tissue-derived fatty acids in liver; leads to raised hepatic acetyl-CoA concentration and ketone body synthesis
Ketonuria	Loss of ketone bodies to urine via renal tubule
Hyperlactatemia	Mobilization and metabolism of muscle glycogen to lactate, a precursor for hepatic gluconeogenesis (Cori cycle)
Hyperlipidemia	Free fatty acids derived from increased lipolysis in adipose tissue
Hypertriglyceridemia	Increased synthesis of triglycerides in liver, secreted as component of VLDL
Hypovolemia/hyperosmolarity	Excessive loss of body water as urine due to glucose acting as an osmotic diuretic
Hyponatremia	Loss of body sodium due to glucose-induced osmotic diuresis

- It develops relatively slowly compared to hypoglycemia, which is immediate
- Anorexia, nausea, vomiting, polyuria, and thirst are associated symptoms
- Untreated patients present with stupor progressing to coma; dehydration, shock, air hunger, and acetone in breath are features
- Urine is typically markedly positive for glucose and ketones

Pathogenesis of diabetic ketoacidosis

The pathogenesis of the diabetic ketoacidosis seen in poorly controlled type 1 diabetes mellitus is shown in **Figure 2.24** and may be deduced from the lack of action of insulin (see Figure 2.23).

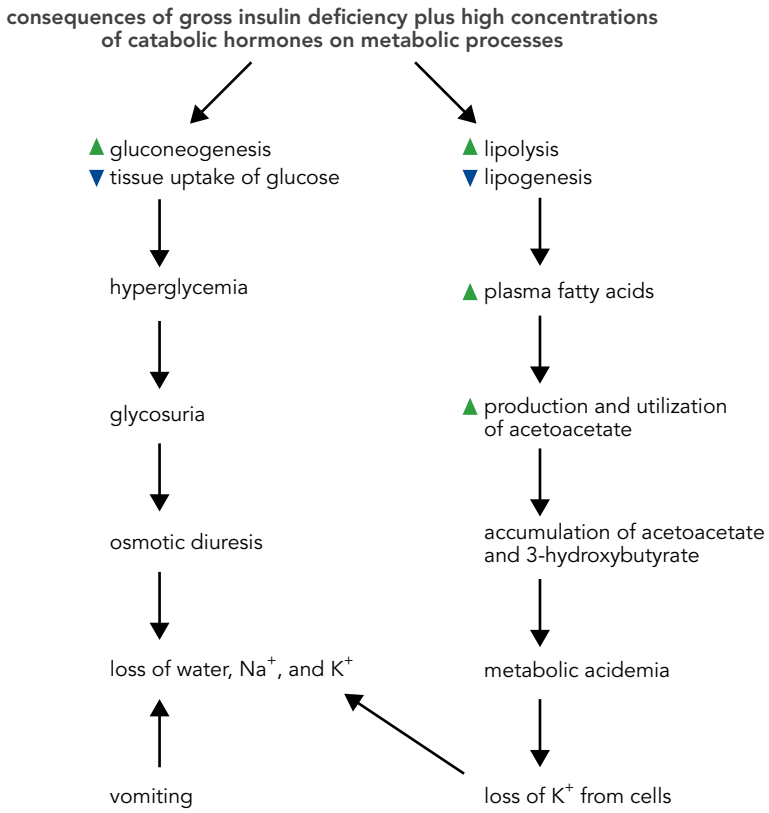


Figure 2.24 Pathogenesis of diabetic ketoacidosis. Diabetic ketoacidosis is seen only in patients with poorly controlled diabetes mellitus type I where insulin is absent. The metabolic sequelae are similar to those seen in Figure 2.23 and can only be prevented/reversed by administration of insulin to the patient.

Hyperglycemia

With no insulin to support glycogen synthesis and inhibit gluconeogenesis, the partition between the portal and systemic glucose concentrations no longer exists and so all glucose derived from the diet moves directly to the systemic circulation, producing a marked hyperglycemia. With a total lack of insulin, hepatic carbohydrate metabolism is responsible for glucose production from the breakdown of stored glycogen (glycogenolysis) and glucose synthesis (gluconeogenesis) and this glucose is released directly to the systemic circulation, contributing to the hyperglycemic state. The hyperglycemia is also exacerbated to some extent by the reduced uptake of glucose by adipose tissue and skeletal muscle. Even during starvation—the major therapy for type 1 diabetic patients prior to the discovery of insulin in 1923—the high production of glucose and reduced utilization of glucose is sufficiently high to maintain a state of hyperglycemia. The presence of a raised blood glucose concentration has a number of physiological sequelae including glycosuria and dehydration.

Glycosuria and dehydration

Glucose is normally completely reabsorbed in the proximal renal tubule such that it is undetected in urine. However, once the capacity for reabsorption is exceeded (10mmol/L; 180mg/dL), glucose is lost into the urine. The presence of glucose in the loop of Henle and the distal tubule acts as an osmotic diuretic giving rise to the production of large volumes of dilute urine (polyuria). Such increased production of urine shortens dramatically the tubular transit time of urine and decreases the ability of the kidneys to reabsorb water. The combined effects of these processes can lead to gross diuresis and severe dehydration.

Sequelae of dehydration

Dehydration and an increased serum osmolarity activate the thirst center located in the hypothalamus and promote polydipsia, a marked drinking response. Failure to compensate adequately for dehydration results in depletion of intravascular volume, hypotension, and reduced peripheral circulation. Furthermore, a reduction in peripheral perfusion reduces the oxygen supply to tissues leading to increased anaerobic metabolism of glucose and production of lactic acid, thereby creating a metabolic acidosis.

Catabolism in skeletal muscle and adipose tissue

The absence of insulin in the systemic circulation promotes catabolism in muscle and adipose tissue. In muscle, gluconeogenic precursors are released into the circulation following the catabolism of stored glycogen to pyruvate and lactate, and of protein to amino acids, mainly alanine and glutamine. These gluconeogenic precursors are transported to the liver where they are converted, somewhat inappropriately, to glucose and serve to perpetuate the hyperglycemic state. In adipose tissue, the breakdown of stored triglyceride to free fatty acids and glycerol leads to an increase in plasma free fatty acid and glycerol concentrations. Fatty acids bind to albumin in the blood and are transported to the liver where they undergo mitochondrial β-oxidation to acetyl-CoA. With no insulin to control catabolism, the rate of production of acetyl-CoA exceeds the capacity of the normal route of metabolism to CO_2 and water via the TCA cycle and acetyl-CoA is converted to ketone bodies (acetoacetate and 3-hydroxybutyrate). This process of ketogenesis is normally inhibited by even low levels of insulin. Both ketone bodies are strong acids and their release into the circulation contributes to the worsening metabolic acidosis. The condition of diabetic ketoacidosis now exists. Free fatty acids are toxic to the liver and if the rate of ketogenesis is insufficient to reduce their concentration in the liver, the tissue responds by converting them to triglycerides and exporting them as part of very-low-density lipoproteins, giving rise to hypertriglyceridemia.

Acid–base balance

A fall in blood pH as a consequence of metabolic acidosis causes an efflux of potassium ions from cells via the K^+/H^+ antiport system in their plasma membranes in an attempt to maintain acid–base balance. Much of this potassium is lost due to the severe diuresis and significant depletion of the total body pool of potassium ensues. Paradoxically, however, the initial rapid efflux of potassium from the tissues can lead to a potentially dangerous hyperkalemia. Another characteristic of patients with diabetic ketoacidosis is Kussmaul breathing, or air hunger. In this situation, the respiratory drive is increased, giving rise to rapid, shallow breathing, to blow off CO_2 in a further attempt to alleviate the metabolic acidosis.

The pathological changes described above are self-perpetuating and, unless the patient is treated, will continue to deteriorate, progressing to coma and eventually death of the patient.

Laboratory investigations in ketoacidosis

The biochemical hallmarks of diabetic ketoacidosis (**Table 2.14**) are hyperglycemia (plasma glucose typically 20–40 mmol/L [360–720 mg/dL]) plus ketones detectable in either serum or urine and evidence of systemic acidosis. Acidosis may be diagnosed by a venous blood gas analysis showing decreased pH (increased hydrogen ion concentration) with low CO_2 content due to compensatory hyperventilation. Blood gas analysis is not always essential, however, if the clinical picture is sufficiently convincing. A low bicarbonate or total CO_2 in the urea and electrolyte profile is also used as evidence of a metabolic acidosis. Serum potassium is often elevated due to the effects of insulin deficiency and acidosis allowing movement of potassium from the intracellular to the extracellular space. However, total body potassium tends to be low due to increased renal losses. This results in a risk of hypokalemia when insulin therapy is started, and so potassium is added to the intravenous fluid until serum potassium reaches the reference range. Serum sodium may be low, normal, or high in ketoacidosis. This is because several factors operate in different directions. Ketones are anions and require a balancing cation when excreted in urine. This may be sodium or potassium. The inability to reabsorb water in the distal nephron, despite high levels of antidiuretic hormone, is due to unabsorbed glucose reducing the transmembrane osmotic gradient (osmotic diuresis). This net loss of free water tends to cause hypernatremia. Finally, the hyperglycemia itself has an osmotic effect across cell membranes, pulling water out and diluting the sodium. The net effect on serum sodium thus depends on the relative magnitudes of these different effects.

Serum urea and creatinine are elevated, reflecting decreased glomerular filtration due to intravascular fluid loss. Urea may be disproportionately higher as it is able to diffuse back from the tubular fluid when the flow rate falls, whereas creatinine cannot. In some assays for creatinine, ketones may positively interfere, giving falsely high results.

Analytical practice point 2.2

Blood gas analysis can be performed on venous blood in diabetic ketoacidosis. Arterial samples are unnecessary.

TABLE 2.14 Clinical chemistry of diabetic ketoacidosis

Patient group	Typically in patients with diabetes mellitus type 1
Biochemical basis of disorder	Diminished glucose utilization and excessive lipolysis with ketogenesis
Clinical chemistry features	Hyperglycemia; plasma glucose usually >40 mmol/L (720 mg/dL)
	Plasma and urine positive for ketones
	Low plasma bicarbonate
	Plasma sodium usually low but may be normal or high
	Plasma potassium high or high-normal

There are situations where detection of ketones in urine is unreliable. Dipsticks detect acetoacetate rather than 3-hydroxybutyrate. In situations of tissue hypoxia, relatively more 3-hydroxybutyrate is formed than acetoacetate, thus potentially giving false negative results when using dipsticks. In addition, excretion of ketones requires an adequate glomerular filtration rate (GFR). As this rate diminishes, the renal threshold rises and ketonuria ceases. The ketones are still being produced and retained in the plasma, however. Hence it may be wrongly deduced that the patient is getting better because the ketones are no longer detected in urine, when in fact this just reflects deteriorating renal function.

Other findings in these patients include hypertriglyceridemia, due to increased synthesis of VLDL, and sometimes raised plasma amylase. When associated with abdominal pain, which is not uncommon in ketoacidosis, this may lead to an incorrect diagnosis of acute pancreatitis.

Analytical practice point 2.3

Ketones are not detected in urine if renal function is significantly reduced.

Diabetes mellitus type 2

The pathogenesis of grossly uncontrolled non-ketotic diabetes mellitus is shown in **Figure 2.25**. In contrast to the situation described above for type 1 patients, hepatic function is relatively normal in non-insulin-dependent diabetic patients and this has marked consequences for the presentation and progression of the disease. For example, the synthesis of ketone bodies is extremely sensitive to insulin and even low levels of insulin in the portal circulation will inhibit ketogenesis and protect against ketoacidosis. Thus, type 2 diabetic patients do not develop ketosis. In these patients, the major metabolic consequences arise from effects on muscle and adipose tissue, particularly those resulting from insulin resistance and the increased action of catabolic hormones. Decreased glucose uptake into both tissues leads to hyperglycemia, and increased lipolysis in adipose tissue causes a raised plasma free fatty acid concentration. In the absence of ketogenesis, fatty acids arriving at the liver must be oxidized or converted to triglyceride for export as VLDL and contribute to the hyperlipidemia. The reduced peripheral response to the prevailing insulin concentration will also decrease the activity of lipoprotein lipase and slow the clearance of VLDL. The hyperglycemia in type 2 patients may go undetected for months or even years and, without intervention, plasma glucose concentrations *in extremis* can often reach very high levels of >40 mmol/L (>720 mg/dL). Even though such patients do not develop ketoacidosis, they are still at risk from severe dehydration and increased serum osmolarity and have a marked increase in the risk of developing a major vascular (arterial and venous) thrombosis. If untreated, these patients can enter a diabetic, hyperglycemic, hyperosmolar, non-ketotic coma and die. The clinical features of grossly uncontrolled diabetes mellitus are:

- Patients are usually elderly and often not previously known to be diabetic.
- It is often precipitated by intercurrent illness, especially infection.
- It is often associated with arterial and venous thrombosis.
- The condition develops over several days.
- Features include polyuria, thirst, and dehydration; stupor progressing to coma; no air hunger.
- High mortality related to age of patient and arterial thrombosis.
- It may be diagnosed from glucose in CSF and serum osmolarity.
- There are occasionally trace of ketones in urine.

The major therapeutic requirement for patients is rehydration.

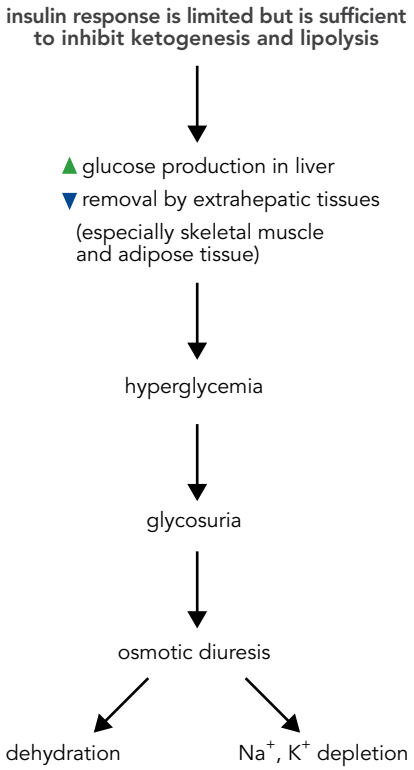


Figure 2.25 Pathogenesis of grossly uncontrolled non-ketotic diabetes mellitus. Lipolysis and ketogenesis are very sensitive to insulin and in non-ketotic diabetes mellitus (type 2), although the insulin response is blunted, it is sufficient to inhibit these catabolic processes.

The hyperglycemic, non-ketotic, hyperosmolar state

In type 2 diabetes, severe, decompensated hyperglycemia is not usually associated with significant ketone production (**Table 2.15**). If acidosis is present it is

TABLE 2.15 Clinical chemistry of hyperosmolar non-ketotic crisis	
Patient group	Typically seen in older patients with diabetes mellitus type 2
Biochemical basis of disorder	Diminished glucose utilization, severe dehydration, and lactic acidosis
Clinical chemistry features	Severe hyperglycemia; plasma glucose often >40 mmol/L (720 mg/dL)
	Plasma and urine negative for ketones
	Plasma bicarbonate normal or slightly low
	Plasma sodium high
	Plasma potassium normal or high

usually due to lactic acid, reflecting the tissue hypoxia caused by the reduced circulating volume. There is polyuria and polydipsia, which may continue for several days and be exacerbated by the patient having sugary drinks. As a result of free water loss, hypernatremia develops, despite the osmotic draw of water from the intracellular compartment. When insulin treatment is initiated, the fall in glucose may cause the hypernatremia to worsen, as water moves back into cells.

Complications of diabetes

Diabetic complications may be divided into short-term (acute) and long-term (chronic) problems. The acute complications are hypo- and hyperglycemic crises due to short-term metabolic decompensation. The chronic complications (**Figure 2.26**) are manifestations of vascular disease and may be subdivided into microvascular and macrovascular. Microvascular complications are unique to diabetes and are caused to a large extent by hyperglycemia itself. The small vessels supplying blood to the retina, nerves, and kidney tissues are affected causing retinopathy, neuropathy, and nephropathy, respectively. Diabetic nephropathy is the most common cause of renal failure. Tight control of blood glucose (as shown by low HbA_{1c}) has been shown to reduce these complications significantly. Macrovascular complications are ischemic heart disease (angina and myocardial infarction), stroke, and peripheral vascular disease. These occur in the general population as well as in diabetic patients, but hyperglycemia is an additional risk factor and these complications often affect younger individuals.

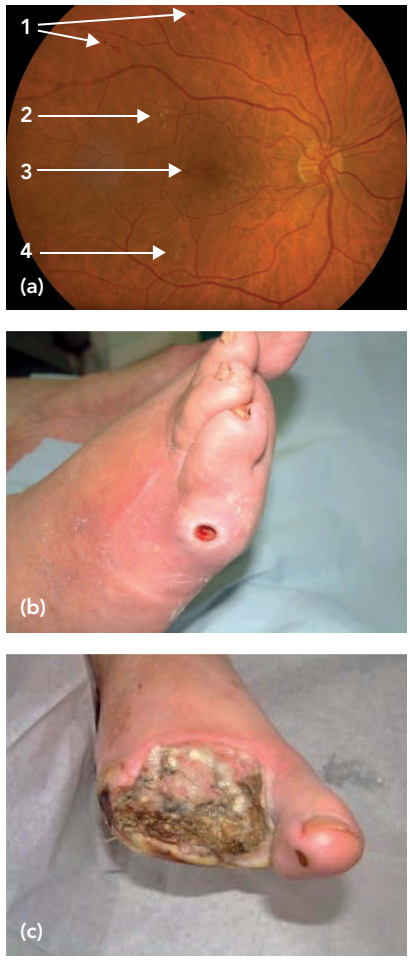


Figure 2.26 Long-term clinical complications of diabetes mellitus. (a) Diabetic retinopathy and diabetic maculopathy. Indicated by arrows are: (1) dot blot hemorrhages in retinal periphery; (2) hard semicircinate exudates superior to macula; (3) diabetic maculopathy with hemorrhages; and (4) inferior cotton wool spot indicating retinal inflammation. (b) A small, clean, innocuous-looking foot ulcer must be treated aggressively to prevent progression to amputation. (c) Ulceration 8 weeks after initial presentation as a small area of redness. No treatment was sought during this period. Amputation is now essential. (a, courtesy of David Bennett. b and c, courtesy of Vinod Patel, Warwick Medical School.)

2.8 CLINICAL CHEMISTRY MARKERS OF GLYCEMIC CONTROL

Self-monitoring of blood glucose

The explosion in the prevalence of diabetes has created a huge market for patient-held blood glucose meters**.** These have become progressively smaller and more convenient to use, producing accurate results within seconds from as little as 1 μL of capillary blood. Whilst self-monitoring is extremely useful in many patients, it may be unnecessary and a waste of resources in others. The key is whether the patient is able to act on the results obtained, and if they are not then collecting the data is not worthwhile. Where patients are on an insulin regime requiring variable doses or where hypoglycemic awareness is impaired, self-monitoring of glucose is mandatory. However, for patients who manage their diabetes with dietary restriction alone, self-monitoring is rarely necessary.

Glycated hemoglobin: HbA_{1c}

The proportion of hemoglobin that is glycated during its circulation in the vascular system has been used as a surrogate marker of average blood glucose since

TABLE 2.16 Assumptions and pitfalls in the measurement of HbA_{1c}	
Assumptions	Blood glucose is the only variable
	Red cell survival time is constant
Pitfalls	Shortened red-cell survival due to iron deficiency, hemolytic anemias, or venesection
	Prolonged red-cell survival due to drugs such as dapsone or erythropoietin
	Interference in assay due to hemoglobin variants such as HbS, HbD, or HbC (>900 variants identified)

the 1980s. Its clinical value has been shown by two landmark studies published during the 1990s, which demonstrated the beneficial effects of improved glycemic control as measured by HbA_{1c}. The Diabetes Control and Complications Trial (DCCT) looked at type 1 diabetes, whilst the United Kingdom Prospective Diabetes Study (UKPDS) was a study of type 2. Techniques for measuring HbA_{1c} have become better standardized following the DCCT and UKPDS, allowing clinicians and patients to aim for the same target values.

There are a number of factors to be considered in the interpretation of HbA_{1c} measurements (**Table 2.16**). As hemoglobin glycation is nonenzymatic, the two main factors determining $\%HbA_{1c}$ are the prevailing concentration of glucose and the length of time the red cells (and hence the hemoglobin) are present in the circulatory system. Most clinicians focus on the first factor and overlook the second. In the majority of cases this does not matter, but in conditions where red-cell survival is longer or shorter than average, the HbA_{1c} will be higher or lower, respectively, for the same average blood glucose concentration. In addition, the presence of hemoglobin variants may affect the way HbA_{1c} is calculated, and this depends on the exact laboratory method used. Most commonly, a chromatographic technique is used to separate hemoglobin types and the relative proportions can then be calculated. If a variant co-migrates with either the HbA_{1c} (numerator) or the HbA_0 (denominator), the HbA_{1c} expressed as a percentage will be falsely high or low.

An international initiative to improve further the standardization of HbA_{1c} resulted in a change of the units in which it is reported (**Table 2.17**). Since 2011, HbA_{1c} has been reported as mmol HbA_{1c} per mole of total Hb (mmol/mol). The numerical results thus appear very different from those that had become familiar and so, for a period of two years from 2009, dual results in both % and mmol/mol were reported.

Universal HbA_{1c} targets, however, are controversial. A comparison of diagnostic criteria for diabetes mellitus using HbA_{1c} measured as % or concentration is shown in **Table 2.18**. Nevertheless, there are clinical situations in which these figures do not apply:

- Conditions with abnormal red-cell turnover such as anemias from hemolysis, spherocytosis, or iron deficiency (for example, in pregnancy)
- Hemoglobinopathies; HbS, HbC, HbF, and HbE, for example, may interfere with the measurement of HbA_{1c}, depending on the method used.
- In rapid-onset diabetes, such as in type 1 diabetes, the HbA_{1c} can be within the normal range despite marked hyperglycemia
- Near-patient testing using current HbA_{1c} tests is not deemed to be sufficiently accurate for diagnosis

In these and other cases where there is doubt as to the use of HbA_{1c}, the glucose criteria shown in Table 2.8 must be used. Concerns regarding renal failure can be overcome if specific assays are used.

Although it is accepted that good glycemic control reduces complications, this comes at the expense of more frequent hypoglycemia, which is unpleasant

Analytical practice point 2.4

Glycation of hemoglobin is affected by red blood cell survival time as well as average blood glucose concentration. HbA_{1c} is lowered when red-cell turnover is high.

TABLE 2.17 Changes in the units used in reporting HbA_{1c}	
HbA_{1c} %	HbA_{1c} mmol/mol total Hb
6.0	42
7.0	53
8.0	64
9.0	75

HbA_{1c} has been reported as mmol/mol total Hb since June 1st 2009. The new target range for diabetic patients on treatment is 48–59 mmol HbA_{1c} /mol total Hb. New units and old were reported together until 2011.