3RD EDITION

Metabolic Regulation

A Human Perspective

Keith N. Frayn

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Third Edition

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Preface

When I prepared the second edition of this book, published in 2003, it seemed that there had been quite a revolution in metabolism since the first edition. A major discovery had been the hormone leptin, and with it recognition of the role of adipose tissue as an endocrine organ. As I started work on this third edition, I had the feeling that not so much had changed. However, I soon came to realize that this was not true. In part through the increasing use of genetically-engineered mice, especially those that lack a particular gene, we have been led to a new appreciation of several metabolic pathways that we thought were well understood. Adipose tissue again provides a perfect example. Fat mobilization, when I wrote the second edition, was thought to depend on the enzyme hormone-sensitive lipase (HSL). Then several researchers produced mice lacking HSL: and, to everyone's surprise, they were relatively normal. This led to the discovery of another enzyme involved in fat mobilization, adipose triglyceride lipase. Bernard Thorens (University of Lausanne) has challenged my understanding of liver metabolism by producing a mouse lacking the glucose transporter GLUT2. Glucose release from the hepatocytes is relatively normal. There are many more examples, mostly now also shown to translate into human metabolism.

As with previous editions, this book is not a conventional textbook of biochemistry. Indeed, I have always recognized that students would probably need to consult a standard biochemical tome for details of molecular structures and all the intermediates in pathways. What I have tried to do is to convey a sense of how metabolic pathways interact in a dynamic sense, in the whole organism. The kind comments that I have received on previous editions suggest that I am not the only person who enjoys thinking about metabolism in this way. I hope this "holistic" view of metabolism will be interesting, and perhaps more memorable than standard biochemistry teaching, especially to those studying the more applied aspects of biochemistry, under which heading I would include medicine, nutrition, exercise physiology, and many other disciplines.

Several people have written to me with helpful comments on the text, including Hugo van den Berg, Remigio Giacomel from Argentina, Robert Horn, John Wells, and Jack Salway. I have also received helpful advice from many experts: Bernard Thorens, who prompted me to re-read some of the older literature on what happens to dietary glucose; Jan Glatz who helped me with fatty acid binding proteins and transporters; my friends from the adipose tissue world, Peter Arner, Dominique Langin, and Max Lafontan, who each wrote me an essay on adrenergic receptors on the fat cell; Claude Forest and Richard Hanson, who helped me to understand glyceroneogenesis and the role of PEPCK. Jack Salway and Geoff Gibbons have been of considerable help to me in understanding some of the pathways less well covered in conventional texts. Many of my colleagues have also spotted errors or made useful comments: Ingrid Mostad, Andrew Nesbit, Matt Neville, Katherine Pinnick, Siobhán McQuaid. Siobhán McQuaid, Fredrik Karpe, and Leanne Hodson have also kindly allowed me to use some of our unpublished data in Chapter 11. Anne Clark and Rachel Roberts provided me with photomicrographs. I am also extremely grateful to my colleagues who have read and commented on the chapters: Barbara Fielding, Geoff Gibbons, Leanne Hodson, Fredrik Karpe, and Sara Suliman. Any errors that remain are my responsibility.

Nigel Balmforth at Blackwell Publishing, now Wiley-Blackwell, has been a constant source of help and encouragement, and I am grateful to Kate Nuttall and Catriona Dixon who have seen this edition through the production process. Once again, I also thank my wife Theresa for her support, and for not minding that I spent so many Sunday afternoons at my computer.

Abbreviations

Note: some abbreviations for compounds that are used just once within a figure or box are defined in the corresponding legend and not listed here.

ABC	ATP-binding cassette (defines a family of proteins)
ACAT	acyl-CoA: cholesterol acyltransferase
ACC	acetyl-CoA carboxylase
ACS	acyl-CoA synthase
ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone
ADP	adenosine 5′-diphosphate
AGE	advanced glycation end-product
AIB	α-amino-isobutyric acid
AMP	adenosine 5′-monophosphate
ASP	acylation stimulating protein
ATGL	adipose triglyceride lipase
ATP	adenosine 5′-trisphosphate
ATPase	enzyme hydrolyzing ATP
BCAA	branched-chain amino acids
BFE	bifunctional enzyme (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase)
BMI	body mass index
BMR	basal metabolic rate
cAMP	cyclic adenosine 3′,5′-monophosphate (cyclic AMP)
CCK	cholecystokinin
CE	cholesteryl ester
CETP	cholesteryl ester transfer protein
cGMP	cyclic guanosine 3′,5′-monophosphate (cyclic GMP)

ChRE(BP)	carbohydrate response element (binding protein)
CNS	central nervous system
CoA	coenzyme A (esterified form)
CoASH	coenzyme A (free form)
CPT	carnitine O-palmitoyltransferase
CRE	cyclic AMP response element
CREB	cAMP-responsive element binding protein
DAG	diacylglycerol
DHA	docosahexaenoic acid (22:6 *n*-3)
DIT	diet-induced thermogenesis
DNA	deoxyribonucleic acid
eNOS	endothelial nitric oxide synthase
*F*1, 6-P_2	fructose 1,6-bisphosphate
*F*2, 6-P_2	fructose 2,6-bisphosphate
*F*6-*P*	fructose 6-phosphate
FABP	fatty acid binding protein
FAT	fatty acid translocase
FATP	fatty acid transport protein
FBPase	fructose-1,6-bisphosphatase
FC	free (unesterified) cholesterol
FH	familial hypercholesterolemia
FOXO1	forkhead transcription factor
FQ	food quotient
FSH	follicle-stimulating hormone
G6-P	glucose 6-phosphate
G-6-Pase	glucose-6-phosphatase
GDP	guanosine 5′-diphosphate
GH	growth hormone
GIP	gastric inhibitory polypeptide (also known as glucose-dependent insulinotrophic polypeptide)
GK	glucokinase
GLP(1,2)	glucagon-like peptide(-1,2)
GLUT	glucose transporter
glycerol 3-P	glycerol 3-phosphate
G-proteins (G_i, G_s, G_q)	GTP-binding proteins (inhibitory and stimulatory forms with respect to adenylyl cyclase; stimulatory form with respect to phospholipase C)
GSK	glycogen synthase kinase
GTP	guanosine 5′-trisphosphate
HDL	high-density lipoprotein
HIF	hypoxia-inducible factor
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
HSL	hormone-sensitive lipase
IDDM	insulin-dependent diabetes mellitus (Type 1 diabetes mellitus)

IGF	insulin-like growth factor
IMP	inosine 5′-monophosphate
IP_3	inositol (1′,4′,5′)-trisphosphate
IRS	insulin receptor substrate
K_a	dissociation constant for an acid
LCAT	lecithin-cholesterol acyltransferase
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
LH	luteinising hormone
LPL	lipoprotein lipase
MAG	monoacyglycerol
MET	multiple of resting metabolic rate (unit for whole-body energy expenditure)
M_r	relative molecular mass (molecular weight)
mRNA	messenger-RNA (ribonucleic acid)
NAD^+, *NADH*	nicotinamide adenine dinucleotide ($^+$, oxidized form; H, reduced form)
$NADP^+$, *NADPH*	nicotinamide adenine dinucleotide phosphate ($^+$, oxidised form; H, reduced form)
NAFLD	non-alcoholic fatty liver disease
NEFA	non-esterified fatty acid(s) (also called free fatty acids)
NIDDM	non-insulin-dependent diabetes mellitus (Type 2 diabetes mellitus)
PCr	phosphocreatinine
PDH	pyruvate dehydrogenase
PDX1	pancreatic-duodenal homeobox factor-1 (a transcription factor controlling expression of the preproinsulin gene)
PEPT	peptide transporter
PFK	phosphofructokinase
P_i	inorganic phosphate
PI3K	phosphatidylinositol-3-kinase
PIP_2	phosphatidylinositol (4′,5′)-bisphosphate
PIP_3	phosphatidylinositol (3′,4′,5′)-trisphosphate
PKA,B,C,G	protein kinase A,B,C,G
PL	phospholipid
PLC	phospholipase C
POMC	pro-opiomelanocortin
PPAR	peroxisome proliferator-activated receptor
PP_i	inorganic pyrophosphate
RAGE	receptor for advanced glycation end-products
RER	respiratory exchange ratio
RQ	respiratory quotient
SCAP	SREBP cleavage activating protein
SGLT	sodium-glucose co-transporter
SR(-BI)	scavenger receptor(-BI)

SREBP	sterol regulatory element binding protein
T_3	triiodothyronine
T_4	thyroxine
TAG or TG	triacylglycerol (triglyceride in some literature)
TCA cycle	tricarboxylic acid cycle
TORC2	CREB-regulated transcription coactivator 2
TRL	triacylglycerol-rich lipoprotein
TSH	thyroid-stimulating hormone
TZD	thiazolidinedione
UCP	uncoupling protein
UTP	uridine 5′-trisphosphate
VLDL	very-low-density lipoprotein
VO_2max	maximal rate of oxygen consumption for an individual

1 The Underlying Principles of Human Metabolism

Key learning points

- We eat food. We expend energy doing exercise, sleeping, just being. What happens to the food between it entering out mouths and its being used for energy? That's what metabolic regulation (at least, so far as this book is concerned) is all about.
- In order to cover the periods when we are not eating, we need to store metabolic fuels. We store fuel as fat (triacylglycerol) and as carbohydrate (glycogen). Fat provides considerably more energy per gram stored.
- Molecules involved in metabolism differ in an important property: polarity. Polar molecules (those with some degree of electrical charge) mix with water (which is also polar); non-polar molecules, which include most lipids (fatty substances), usually don't mix with water. This has profound implications for the way they are handled in the body.
- Some molecules have both polar and non-polar aspects: they are said to be amphipathic. They can form a bridge between polar and non-polar regions. Amphipathic phospholipid molecules can group together to form membranes, such as cell membranes.
- The different organs in the body have their own characteristic patterns of metabolism. Substrates flow between them in the bloodstream (circulation). Larger blood vessels divide into fine vessels (capillaries) within the tissues, so that the distances that molecules have to diffuse to or from the cells are relatively small.

1.1 Metabolic Regulation in Perspective

To many students, metabolism sounds a dull subject. It involves learning pathways with intermediates with difficult names and even more difficult formulae. Metabolic regulation may sound even worse. It involves not just remembering the pathways, but remembering what the enzymes are called, what affects them and how. This book is not simply a repetition of the molecular details of metabolic pathways. Rather, it is an attempt to put metabolism and metabolic regulation into a physiological context, to help the reader to see the relevance of these subjects. Once their relevance to everyday life becomes apparent, then the details will become easier, and more interesting, to grasp.

This book is written from a human perspective because, as humans, it is natural for us to find our own metabolism interesting – and very important for understanding human health and disease. Nevertheless, many of the principal regulatory mechanisms that are discussed are common to other mammals. Some mammals, such as ruminants, have rather specialized patterns of digestion and absorption of energy; such aspects will not be covered in this book.

A consideration of metabolic regulation might begin with the question: why is it necessary? An analogy here is with mechanical devices, which require an input of energy, and convert this energy to a different and more useful form. The waterwheel is a simple example. This device takes the potential energy of water in a reservoir – the mill-pond – and converts it into mechanical energy which can be used for turning machinery, for instance, to grind corn. As long as the water flows, its energy is extracted, and useful work is done. If the water stops, the wheel stops. A motor vehicle has a different pattern of energy intake and energy output (Figure 1.1). Energy is taken in very spasmodically – only when the driver stops at a filling station. Energy is converted into useful work (acceleration and motion) with an entirely different pattern. A long journey might be undertaken without any energy intake. Clearly, the difference from the waterwheel lies in the presence of a storage device – the fuel tank. But the fuel tank alone is not sufficient: there must also be a control mechanism to regulate the flow of energy from the store to the useful-work-producing device (i.e., the engine). In this case, the regulator is in part a human brain deciding when to move, and in part a mechanical system controlling the flow of fuel.

What does this have to do with metabolism? The human body is also a device for taking in energy (chemical energy, in the form of food) and converting it to other forms. Most obviously, this is in the form of physical work, such as lifting heavy objects. However, it can also be in more subtle forms, such as producing and nurturing offspring. Any activity requires energy. Again, this is most obvious if we think about performing mechanical work: lifting a heavy object from the floor onto a shelf requires conversion of chemical energy (ultimately derived from food) into potential energy of the object. But even maintaining life involves work: the work of breathing, of pumping blood around the vascular system, of chewing food and digesting it. At a cellular level, there is constant work performed in the pumping of ions across membranes, and the synthesis and breakdown of the chemical constituents of cells.

What is your pattern of energy intake in relation to energy output? For most of us, the majority of energy intake occurs in three relatively short periods during each 24 hours, whereas energy expenditure is largely continuous (the *resting metabolism*)

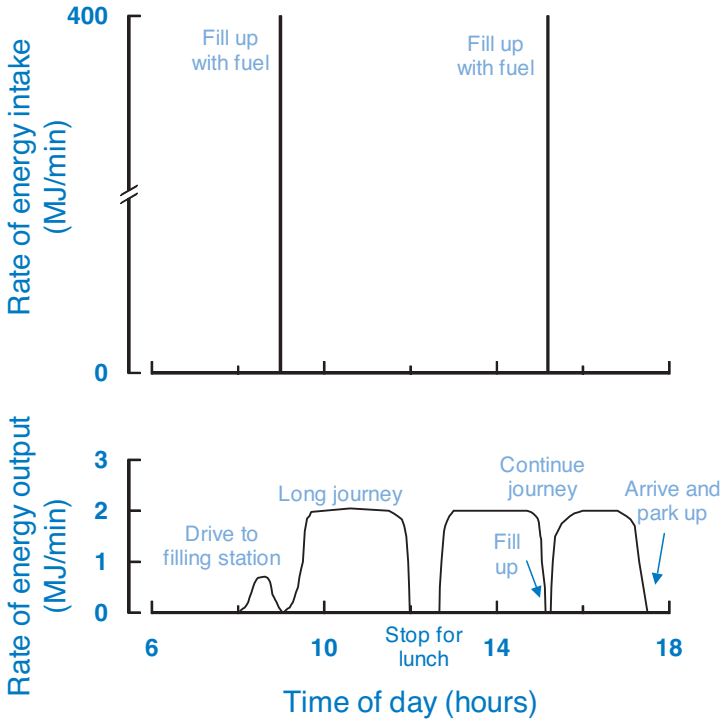

Figure 1.1 **Rates of energy intake and output for a motor vehicle**. The rate of intake (top panel) is zero except for periods in a filling station, when it is suddenly very high. (Notice that the scales are different for intake and output.) The rate of output is zero while the car is parked with the engine off; it increases as the car is driven to the filling station, and is relatively high during a journey. When totaled up over a long period, the areas under the two curves must be equal (energy intake = energy output) – except for any difference in the amounts of fuel in the tank before and after.

with occasional extra bursts of external work (Figure 1.2). It is clear that we, like the motor vehicle, must have some way of storing food energy and releasing it when required. As with the motor vehicle, the human brain may also be at the beginning of the regulatory mechanism, although it is not the conscious part of the brain: we do not have to think when we need to release some energy from our fat stores, for instance. Some of the important regulatory systems that will be covered in this book lie outside the brain, in organs which secrete hormones, particularly the pancreas. But whatever the internal means for achieving this regulation, we manage to store our excess food energy and to release it just as we need.

This applies to the normal 24-hour period in which we eat meals and go about our daily life. But the body also has to cope with less well-organized situations. In many parts of the world, there are times when food is not that easily available, and yet people are able to continue relatively normal lives. Clearly, the body's regulatory mechanisms must recognize that food is not coming in, and allow an appropriate rate of release of energy from the internal stores. In other situations, the need for energy may be suddenly increased. Strenuous physical exercise may increase the total rate of

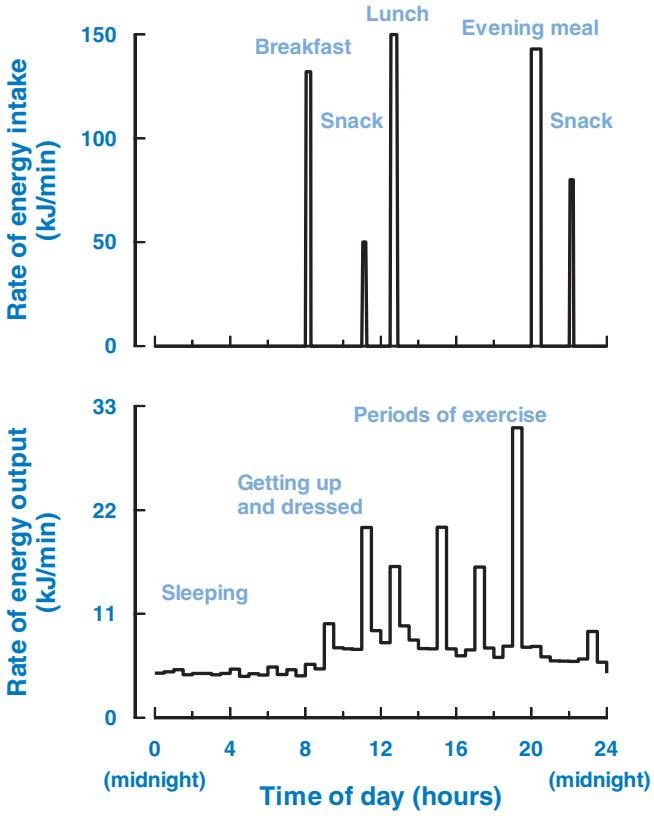

Figure 1.2 Rates of energy intake and output for a person during a typical day. The rate of energy intake (top panel) is zero except when eating or drinking, when it may be very high. The rate of energy output (heat + physical work) (lower panel) is at its lowest during sleep; it increases on waking and even more during physical activity. As with the car, the pattern of energy intake may not resemble that of energy expenditure, but over a long period the areas under the curves will balance – except for any difference in the amounts of energy stored (mainly as body fat) before and after. Data for energy expenditure are for a person measured in a calorimetry chamber and were kindly supplied by Dr Susan Jebb of MRC Human Nutrition Research, Cambridge.

metabolism in the body to twenty times its resting level. Something must recognize the fact that there is a sudden need to release energy at a high rate from the body's stores. During severe illness, such as infections, the rate of metabolism may also be increased; this is manifested in part by the rise in body temperature. Often the sufferer will not feel like eating normally. Once again, the body must have a way of recognizing the situation, and regulating the necessary release of stored energy.

What we are now discussing is, indeed, *metabolic regulation*. Metabolic regulation in human terms covers the means by which we take in nutrients in discrete meals, and deliver energy as required, varying from moment to moment and from tissue to tissue, in a pattern which may have no relationship at all to the pattern of intake. Metabolic regulation works ultimately at a molecular level, mainly by modulation of the activities

of enzymes. But one should not lose sight of the fact that these molecular mechanisms are there to enable us to lead normal lives despite fluctuations in our intake and our expenditure of energy. In this book, the emphasis will be on the systems within the human body which sense the balance of energy coming in and energy required, particularly the *endocrine* (hormonal) and the *nervous* systems, and which regulate the distribution and storage of nutrients after meals, and their release from stores and delivery to individual tissues as required.

The intention of this preamble is to illustrate that, underlying our everyday lives, there are precise and beautifully coordinated regulatory systems controlling the flow of energy within our bodies. Metabolic regulation is not a dry, academic subject thought up just to make biochemistry examinations difficult; it is at the center of human life and affects each one of us every moment of our daily lives.

1.2 The Chemistry of Food – and of Bodies

Energy is taken into the body in the form of food. The components of food may be classified as *macronutrients* and *micronutrients*. Macronutrients are those components present in a typical serving in amounts of grams rather than milligrams or less. They are the well-known carbohydrate, fat, and protein. Water is another important component of many foods, although it is not usually considered a nutrient. Micronutrients are vitamins, minerals, and nucleic acids. Although these micronutrients play vital roles in the metabolism of the macronutrients, they will not be discussed in any detail in this book, which is concerned with the broader aspects of what is often called *energy metabolism*.

The links between nutrition and energy metabolism are very close. We eat carbohydrates, fats, and proteins. Within the body these are broken down to smaller components, rearranged, stored, released from stores, and further metabolized, but essentially whether we are discussing food or metabolism the same categories of carbohydrate, fat, and protein can be distinguished. This is not surprising since our food itself is of organic origin, whether plant or animal.

In order to understand metabolism and metabolic regulation, it is useful to have a clear idea of some of the major chemical properties of these components. This is not intended as a treatise in physical or organic chemistry but as a starting point for understanding some of the underlying principles of metabolism. The discussion assumes a basic understanding of the meaning of atoms and molecules, of chemical reactions and catalysis, and some understanding of chemical bonds (particularly the distinction between ionic and covalent bonding).

1.2.1 Some Important Chemical Concepts

1.2.1.1 Polarity

Some aspects of metabolism are more easily understood through an appreciation of the nature of polarity of molecules. *Polarity* refers to the distribution of electrical charge over the molecule. A non-polar molecule has a very even distribution of electrical

charge over its surface and is electrically neutral overall (the negative charge on the electrons is balanced by the positive charge of the nucleus). A polar molecule has an overall charge, or at least an uneven distribution of charge. The most polar small particles are ions – that is, atoms or molecules which have entirely lost or gained one or more electrons. However, even completely covalently bonded organic molecules may have a sufficiently uneven distribution of electrical charge to affect their behavior. Polarity is not an all-or-none phenomenon; there are gradations, from the polar to the completely non-polar.

Polarity is not difficult to predict in the molecules which are important in biochemistry. We will contrast two simple molecules: water and methane. Their relative molecular masses are similar – 18 for water, 16 for methane – yet their physical properties are very different. Water is a liquid at room temperature, not boiling until 100 °C, whereas methane is a gas ('natural gas') which only liquifies when cooled to – 161 °C. We might imagine that similar molecules of similar size would have the same tendency to move from the liquid to the gas phase, and that they would have similar boiling points. The reason for their different behaviors lies in their relative polarity. The molecule of methane has the three-dimensional structure shown in Figure 1.3a. The outer electron 'cloud' has a very even distribution over the four hydrogen atoms, all of which have an equal tendency to pull electrons their way. The molecule has no distinct electrical poles – it is non-polar. Because of this very even distribution of electrons, molecules near each other have little tendency to interact. In contrast, in the water molecule (Figure 1.3b) the oxygen atom has a distinct tendency to pull electrons its way, shifting the distribution of the outer electron cloud so that it is more dense over the oxygen atom, and correspondingly less dense elsewhere. Therefore, the molecule has a rather negatively charged region around the central oxygen atom, and correspondingly positively charged regions around the hydrogen atoms. Thus, it has distinct electrical poles – it is a relatively polar molecule. It is easy to imagine that water molecules near to each other will interact. Like electrical charges repel each other, unlike charges attract. This gives water molecules a tendency to line up so that the positive regions of one attract the negative region of an adjacent molecule (Figure 1.3b). So water molecules, unlike those of methane, tend to 'stick together': the energy needed to break them apart and form a gas is much greater than for methane, and hence water is a liquid while methane is a gas. The latent heat of evaporation of water is 2.5 kJ/g, whereas that of methane is 0.6 kJ/g. Note that the polarity of the water molecule is not as extreme as that of an ion – it is merely a rather uneven distribution of electrons, but enough to affect its properties considerably.

The contrast between water and methane may be extended to larger molecules. Organic compounds composed solely of carbon and hydrogen – for instance, the alkanes or 'paraffins' – all have the property of extreme non-polarity: the chemical (covalent) bond between carbon and hydrogen atoms leads to a very even distribution of electrons, and the molecules have little interaction with each other. A result is that polar molecules, such as those of water, and non-polar molecules, such as those of alkanes, do not mix well: the water molecules tend to bond to each other and to exclude the non-polar molecules, which can themselves pack together very closely because of the lack of interaction between them. In fact, there is an additional form of direct attraction

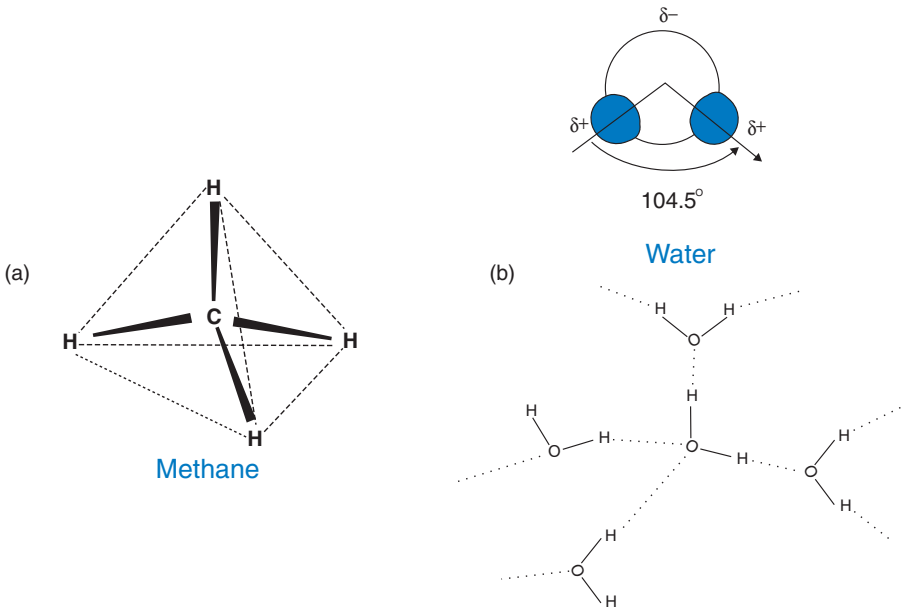

Figure 1.3 (a) Three-dimensional structure of the methane molecule and (b) the molecular structure of water. (a) The hydrogen atoms of methane (CH_4) are arranged symmetrically in space, at the corners of a tetrahedron. (b) The molecular structure of water. Top: view of the 'electron cloud' surrounding the molecule; bottom, interactions between water molecules. The molecule has a degree of *polarity*, and this leads to electrical interactions between neighboring molecules by the formation of *hydrogen bonds*. These bonds are not strong compared with covalent bonds, and are constantly being formed and broken. Nevertheless, they provide sufficient attraction between the molecules to account for the fact that water is a liquid at room temperature whereas the non-polar methane is a gas.

between non-polar molecules, the *van der Waals* forces. Random fluctuations in the density of the electron cloud surrounding a molecule lead to minor, transient degrees of polarity; these induce an opposite change in a neighboring molecule, with the result that there is a transient attraction between them. These are very weak attractions, however, and the effect of the exclusion by water is considerably stronger. The non-polar molecules are said to be *hydrophobic* (water fearing or water hating).

A strong contrast is provided by an inorganic ionic compound such as sodium chloride. The sodium and chlorine atoms in sodium chloride are completely ionized under almost all conditions. They pack very regularly in crystals in a cubic form. The strength of their attraction for each other means that considerable energy is needed to disrupt this regular packing – sodium chloride does not melt until heated above 800 °C. And yet it dissolves very readily in water – that is, the individual ions become separated from their close packing arrangement rather as they would on melting. Why? Because the water molecules, by virtue of their polarity, are able to come between the ions and reduce their attraction for each other. In fact, each of the charged sodium and chloride ions will become surrounded by a 'shell'" of water molecules, shielding it from the

attraction or repulsion of other ions. Sodium chloride is said to be *hydrophilic* – water loving. The terms *polar* and *hydrophilic* are for the most part interchangeable. Similarly, the terms *non-polar* and *hydrophobic* are virtually synonymous.

Ionic compounds, the extreme examples of polarity, are not confined to inorganic chemistry. Organic molecules may include ionized groups. These may be almost entirely ionized under normal conditions – for instance, the esters of orthophosphoric acid ('phosphate groups'), as in the compounds AMP, ADP, and ATP, in metabolites such as glucose 6-phosphate, and in phospholipids. Most of the organic acids involved in intermediary metabolism, such as lactic acid, pyruvic acid, and the long-chain carboxylic acids (fatty acids), are also largely ionized at physiological hydrogen ion concentrations (Box 1.1). Thus, generation of lactic acid during exercise raises the hydrogen ion concentration (the acidity) both within the cells where it is produced, and generally within the body, since it is released into the bloodstream.

As stated earlier, polarity is not difficult to predict in organic molecules. It relies upon the fact that certain atoms always have *electronegative* (electron withdrawing) properties in comparison with hydrogen. The most important of these atoms biochemically are those of oxygen, phosphorus, and nitrogen. Therefore, certain functional groups based around these atoms have polar properties. These include the hydroxyl group (—OH), the amino group ($—NH_2$), and the orthophosphate group ($—OPO_3^{2-}$). Compounds containing these groups will have polar properties, whereas those containing just carbon and hydrogen will have much less polarity. The presence of an electronegative atom does not always give polarity to a molecule – if it is part of a chain and balanced by a similar atom this property may be lost. For instance, the ester link in a triacylglycerol molecule (discussed below) contains two oxygen atoms but has no polar properties.

Examples of relatively polar (and thus water-soluble) compounds which will be frequent in this book are sugars (with many —OH groups), organic acids such as lactic acid (with a COO^- group), and most other small metabolites. Most amino acids also fall into this category (with their amino and carboxyl groups), although some fall into the *amphipathic* ('mixed') category discussed below.

Another important point about polarity in organic molecules is that within one molecule there may be both polar and non-polar regions. They are called amphipathic compounds. This category includes phospholipids and long-chain fatty acids (Figure 1.4). Cell membranes are made up of a double layer of phospholipids, interspersed with specific proteins such as transporter molecules, ion channels and hormone receptors, and molecules of the sterol, cholesterol (Figure 1.5). The phospholipid bilayer presents its polar faces – the polar 'heads' of the phospholipid molecules – to the aqueous external environment and to the aqueous internal environment; within the thickness of the membrane is a non-polar, hydrophobic region. The physicochemical nature of such a membrane means that, in general, molecules cannot diffuse freely across it: non-polar molecules would not cross the outer, polar face and polar molecules would not cross the inner, hydrophobic region. Means by which molecules move through membranes are discussed in Chapter 2 (Box 2.1, p. 31).

The long-chain fatty acids fall into the amphipathic category – they have a long, non-polar hydrocarbon tail but a more polar carboxylic group head ($—COO^-$).

Box 1.1 Ionization State of Some Acids at Normal Hydrogen Ion Concentrations

The normal pH in blood plasma is around 7.4. (It may be somewhat lower within cells, down to about 6.8.) This corresponds to a hydrogen ion concentration of 3.98×10^{-8} mol/l (since $-\log_{10}$ of 3.98×10^{-8} is 7.4).

The equation for ionization of an acid HA is:

$$HA \Leftrightarrow H^+ + A^-;$$

this equilibrium is described by the equation:

$$\frac{[H^+][A^-]}{[HA]} = K_i$$

where K_i is the dissociation or ionization constant, and is a measure of the strength of the acid: the higher the value of K_i the stronger (i.e., the more dissociated) the acid.

K_i in the equation above relates the concentrations expressed in molar terms (e.g., mol/l). (Strictly, it is not the concentrations but the 'effective ion concentrations' or ion *activities* which are related; these are not quite the same as concentrations because of inter-ion attractions. In most biological systems, however, in which the concentrations are relatively low, it is a close approximation to use concentrations. If activities are used, then the symbol K_a is used for the dissociation constant of an acid.)

Some biological acids and their K_a values are listed in Table 1.1.1, together with a calculation of the proportion ionized at typical pH (7.4).

Table 1.1.1

Acid	K_a	% ionized at pH 7.4
Acetic, CH_3COOH	1.75×10^{-5}	99.8
Lactic, $CH_3CHOHCOOH$	0.38×10^{-4}	99.9
Palmitic acid, $CH_3(CH_2)_{14}COOH$	1.58×10^{-5}	99.8
Glycine, CH_2NH_2COOH (carboxyl group)	3.98×10^{-3}	100

The calculation is done as follows (using acetic acid as an example):

$$K_a = 1.75 \times 10^{-5} = \frac{[H^+][Ac^-]}{[HAc]}$$

(where HAc represents undissociated acetic acid, Ac^- represents the acetate ion). At pH 7.4, $[H^+] = 3.98 \times 10^{-8}$ mol/l. Therefore,

$$\frac{[Ac^-]}{[HAc]} = \frac{1.75 \times 10^{-5}}{3.98 \times 10^{-8}} = 440$$

(i.e., the ratio of ionized to undissociated acid is 440:1; it is almost entirely ionized).

The percentage in the ionized form $= \frac{440}{441} \times 100\% = 99.8\%$.

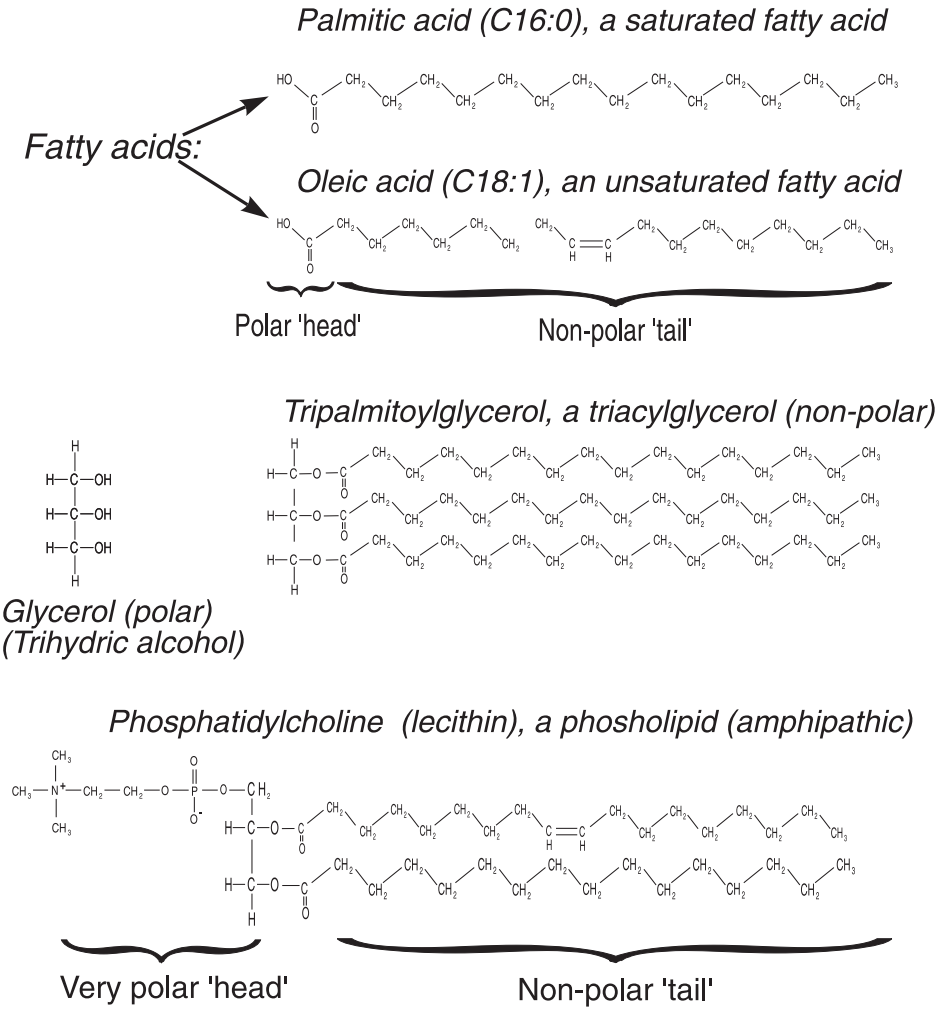

Figure 1.4 **Chemical structures of some lipids**. A typical saturated fatty acid (palmitic acid) is shown with its polar carboxylic group and non-polar hydrocarbon tail. *Glycerol* is a hydrophilic alcohol. However, it is a component of many lipids as its hydroxyl groups may form ester links with up to three fatty acids, as shown. The resultant *triacylglycerol* has almost no polar qualities. The *phospholipids* are derived from phosphatidic acid (diacylglycerol phosphate) with an additional polar group, usually a nitrogen-containing base such as choline (as shown) or a polyalcohol derivative such as phosphoinositol. Phospholipids commonly have long-chain unsaturated fatty acids on the 2-position; oleic acid (18:1 *n*-9) is shown.

Another compound with mixed properties is cholesterol (Figure 1.6); its ring system is very non-polar, but its hydroxyl group gives it some polar properties. However, the long-chain fatty acids and cholesterol may lose their polar aspects completely when they join in ester links. An ester is a compound formed by the condensation (elimination of a molecule of water) of an alcohol (—OH) and an acid (e.g., a carboxylic

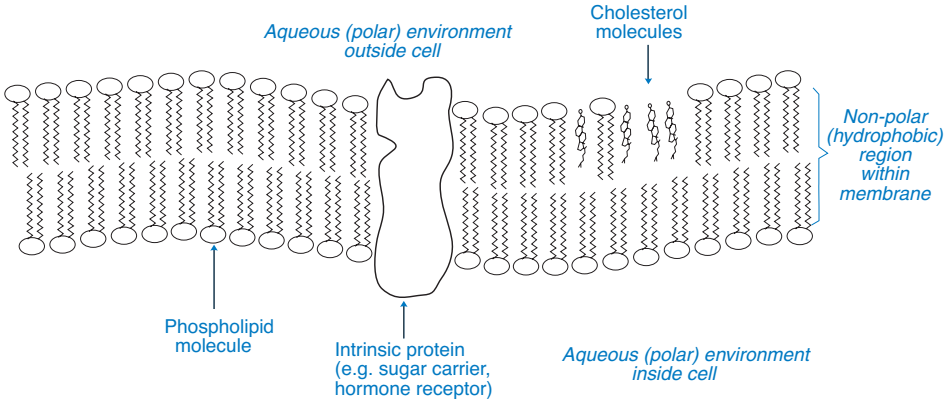

Figure 1.5 Structure of biological membranes in mammalian cells. Cell membranes and intracellular membranes such as the endoplasmic reticulum are composed of bilayers of phospholipid molecules with their polar head-groups facing the aqueous environment on either side and their non-polar 'tails' facing inwards, forming a hydrophobic center to the membrane. The membrane also contains *intrinsic proteins* such as hormone receptors, ion channels, and sugar transporters, and molecules of cholesterol which reduce the 'fluidity' of the membrane. Modern views of cell membrane structure emphasize that there are domains, known as 'rafts,' in which functional proteins co-locate, enabling interactions between them. These lipid rafts are characterized by high concentrations of cholesterol and of certain phospholipids (glycosphingolipids): see Further Reading for more information.

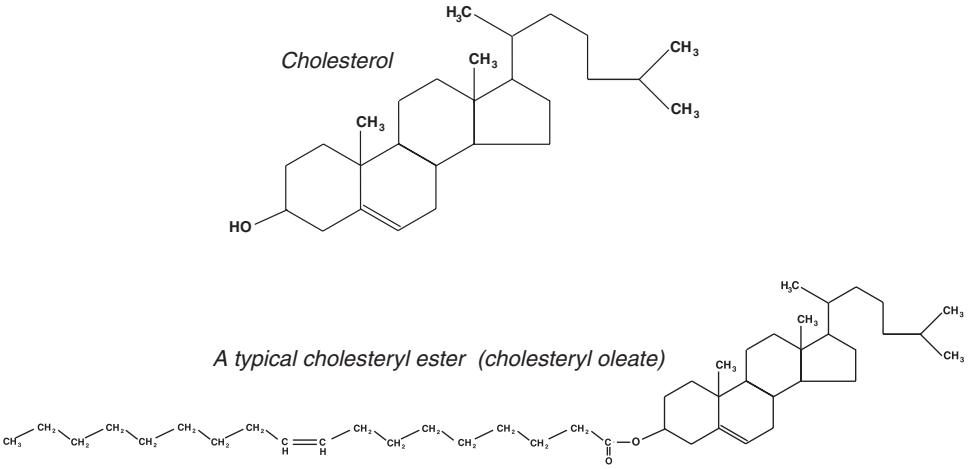

Figure 1.6 Cholesterol and a typical cholesteryl ester (cholesteryl oleate). In the structure of cholesterol, not all atoms are shown (for simplicity); each 'corner' represents a carbon atom, or else –CH or $-CH_2$. Cholesterol itself has amphipathic properties because of its hydroxyl group, but when esterified to a long-chain fatty acid the molecule is very non-polar.

acid, —COO^-). Cholesterol (through its —OH group) may become esterified to a long-chain fatty acid, forming a *cholesteryl ester* (e.g., cholesteryl oleate, Figure 1.6). The cholesteryl esters are extremely non-polar compounds. This fact will be important when we consider the metabolism of cholesterol in Chapter 10. The long-chain fatty acids may also become esterified with glycerol, forming triacylglycerols (Figure 1.4). Again, the polar properties of both partners are lost, and a very non-polar molecule is formed. This fact underlies one of the most fundamental aspects of mammalian metabolism – the use of triacylglycerol as the major form for storage of excess energy.

Among amino acids, the branched-chain amino acids, leucine, isoleucine, and valine, have non-polar side chains and are thus amphipathic. The aromatic amino acids phenylalanine and tyrosine are relatively hydrophobic, and the amino acid tryptophan is so non-polar that it is not carried free in solution in the plasma.

The concept of the polarity or non-polarity of molecules thus has a number of direct consequences for the aspects of metabolism to be considered in later chapters. Some of these consequences are the following:

(1) Lipid fuels – fatty acids and triacylglycerols – are largely hydrophobic and are not soluble in the blood plasma. There are specific routes for their absorption from the intestine and specific mechanisms by which they are transported in blood.
(2) Carbohydrates are hydrophilic. When carbohydrate is stored in cells it is stored in a hydrated form, in association with water. In contrast, fat is stored as a lipid droplet from which water is excluded. Mainly because of this lack of water, fat stores contain considerably more energy per unit weight of store than do carbohydrate stores.
(3) The entry of fats into the circulation must be coordinated with the availability of the specific carrier mechanisms. In the rare situations in which it arises, uncomplexed fat in the bloodstream may have very adverse consequences.

1.2.1.2 Osmosis

The phenomenon of *osmosis* underlies some aspects of metabolic strategy – it can be seen as one reason why certain aspects of metabolism and metabolic regulation have evolved in the way that they have. It is outlined only briefly here to highlight its relevance.

Osmosis is the way in which solutions of different concentrations tend to even out when they are in contact with one another via a *semipermeable membrane*. In solutions, the *solvent* is the substance in which things dissolve (e.g., water) and the *solute* the substance which dissolves. A semipermeable membrane allows molecules of solvent to pass through, but not those of solute. Thus, it may allow molecules of water but not those of sugar to pass through. Cell membranes have specific protein channels (*aquaporins*, discussed in Section 2.2.1.6) to allow water molecules to pass through; they are close approximations to semipermeable membranes.

If solutions of unequal concentration – for instance, a dilute and a concentrated solution of sugar – are separated by a semipermeable membrane, then molecules of

solvent (in this case, water) will tend to pass through the membrane until the concentrations of the solutions have become equal. In order to understand this intuitively, it is necessary to remember that the particles (molecules or ions) of solute are not just moving about freely in the solvent: each is surrounded by molecules of solvent, attracted by virtue of the polarity of the solute particles. (In the case of a non-polar solute in a non-polar solvent, we would have to say that the attraction is by virtue of the non-polarity; it occurs through weaker forces such as the van der Waals.) In the more concentrated solution, the proportion of solvent molecules engaged in such attachment to the solute particles is larger, and there is a net attraction for further solvent molecules to join them, in comparison with the more dilute solution. Solvent molecules will tend to move from one solution to the other until the proportion involved in such interactions with the solute particles is equal.

The consequence of this in real situations is not usually simply the dilution of a more concentrated solution, and the concentration of a more dilute one, until their concentrations are equal. Usually there are physical constraints. This is simply seen if we imagine a single cell, which has accumulated within it, for instance, amino acid molecules taken up from the outside fluid by a transport mechanism which has made them more concentrated inside than outside. Water will then tend to move into the cell to even out this concentration difference. If water moves into the cell, the cell will increase in volume. Cells can swell so much that they burst under some conditions (usually not encountered in the body, fortunately). For instance, red blood cells placed in water will burst (*lyse*) from just this effect: the relatively concentrated mixture of dissolved organic molecules within the cell will attract water from outside the cell, increasing the volume of the cell until its membrane can stretch no further and ruptures.

In the laboratory, we can avoid this by handling cells in solutions which contain solute – usually sodium chloride – at a total concentration of solute particles which matches that found within cells. Solutions which match this osmolality are referred to as *isotonic*; a common laboratory example is *isotonic saline* containing 9 g of NaCl per liter of water, with a molar concentration of 154 mmol/l. Since this will be fully ionized into Na^+ and Cl^- ions, its particle concentration is 308 'milliparticles' – sometimes called milliosmoles – per liter. We refer to this as an osmolarity of 308 mmol/l, but it is not 308 mmol NaCl per liter. (Sometimes you may see the term *osmolality*, which is similar to osmolarity, but measured in mmol per kg solvent.)

The phenomenon of osmosis has a number of repercussions in metabolism. Most cells have a number of different 'pumps' or active transporters in their cell membranes which can be used to regulate intracellular osmolarity, and hence cell size. This process requires energy and is one of the components of basal energy expenditure. It may also be important in metabolic regulation; there is increasing evidence that changes in cell volume are part of a signaling mechanism which brings about changes in the activity of intracellular metabolic pathways. The osmolarity of the plasma is maintained within narrow limits by specific mechanisms within the kidney, regulating the loss of water from the body via changes in the concentration of urine. Most importantly, potential problems posed by osmosis can be seen to underlie the metabolic strategy of fuel storage, as will become apparent in later sections.

1.2.2 The Chemical Characteristics of Macronutrients

1.2.2.1 Carbohydrates

Simple carbohydrates have the empirical formula $C_n(H_2O)_n$; complex carbohydrates have an empirical formula which is similar to this (e.g., $C_n(H_2O)_{0.8n}$). The name carbohydrate reflects the idea, based on this empirical formula, that these compounds are hydrates of carbon. It is not strictly correct, but illustrates an important point about this group of compounds – the relative abundance of hydrogen and oxygen, in proportions similar to those in water, in their molecules. From the discussion above, it will be apparent that carbohydrates are mostly relatively polar molecules, miscible with, or soluble in, water. Carbohydrates in nature include the plant products starch and cellulose and the mammalian storage carbohydrate glycogen, as well as various simple sugars, of which glucose is the most important from the point of view of human metabolism. The main source of carbohydrate we eat is the starch in vegetables such as potatoes, rice, and grains.

The chemical definition of a sugar is that its molecules consist of carbon atoms, each bearing one hydroxyl group (—OH), except that one carbon bears a carbonyl group (=O) rather than a hydroxyl. In solution, the molecule exists in equilibrium between a 'straight-chain' form and a ring structure, but as the ring structure predominates sugars are usually shown in this form (Figure 1.7). Nevertheless, some of the chemical properties of sugars can only be understood by remembering that the straight-chain form exists. The basic carbohydrate unit is known as a monosaccharide. Monosaccharides may have different numbers of carbon atoms, and the terminology reflects this: thus, a hexose has six carbon atoms in its molecule, a pentose five, and so on. Pentoses and hexoses are the most important in terms of mammalian metabolism. These sugars also have 'common names' which often reflect their natural occurrence. The most abundant in our diet and in our bodies are the hexoses *glucose* (grape sugar, named from the Greek *glykys* sweet), *fructose* (fruit sugar, from the Latin *fructus* for fruit), and *galactose* (derived from lactose, milk sugar; from the Greek *galaktos*, milk), and the pentose *ribose*, a constituent of nucleic acids (the name comes from the related sugar arabinose, named from *Gum arabic*).

Complex carbohydrates are built up from the monosaccharides by covalent links between sugar molecules. The term *disaccharide* is used for a molecule composed of two monosaccharides (which may or may not be the same), *oligosaccharide* for a short chain of sugar units, and *polysaccharide* for longer chains (> 10 units), as found in starch and glycogen. Disaccharides are abundant in the diet, and again their common names often denote their origin: *sucrose* (table sugar, named from the French, *sucre*), which contains glucose and fructose (Figure 1.7); *maltose* (two glucose molecules) from malt; *lactose* (galactose and glucose) from milk. The bonds between individual sugar units are relatively strong at normal hydrogen ion concentrations, and sucrose (for instance) does not break down when it is boiled, although it is steadily broken down in acidic solutions such as cola drinks; but there are specific enzymes in the intestine (described in Chapter 3) which hydrolyze these bonds to liberate the individual monosaccharides.

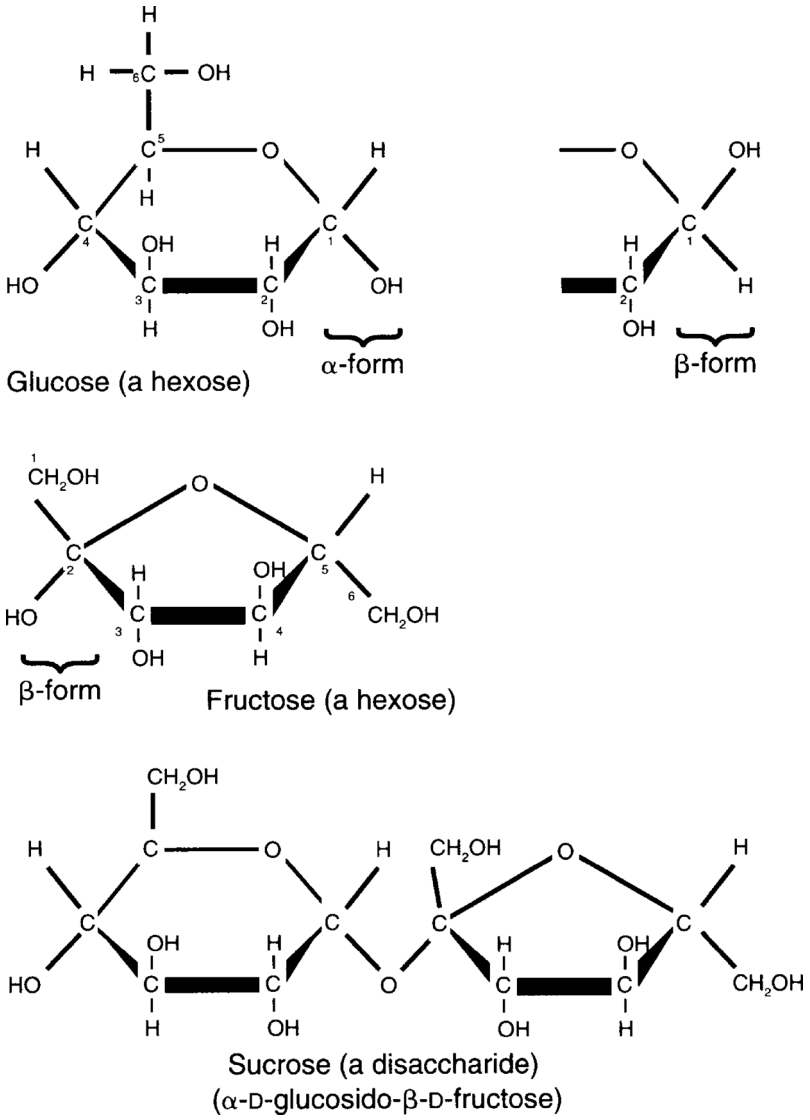

Figure 1.7 **Some simple sugars and disaccharides**. Glucose and fructose are shown in their 'ring' form. Even this representation ignores the true three-dimensional structure, which is 'chair' shaped: if the middle part of the glucose ring is imagined flat, the left-hand end slopes down and the right-hand end up. Glucose forms a six-membered ring and is described as a pyranose; fructose forms a five-membered ring and is described as a furanose. In solution the α- and β- forms are in equilibrium with each other and with a smaller amount of the straight-chain form. The orientation of the oxygen on carbon atom 1 becomes fixed when glucose forms links via this carbon to another sugar, as in sucrose; α- and β-links then have quite different properties (e.g., cellulose vs starch or glycogen).

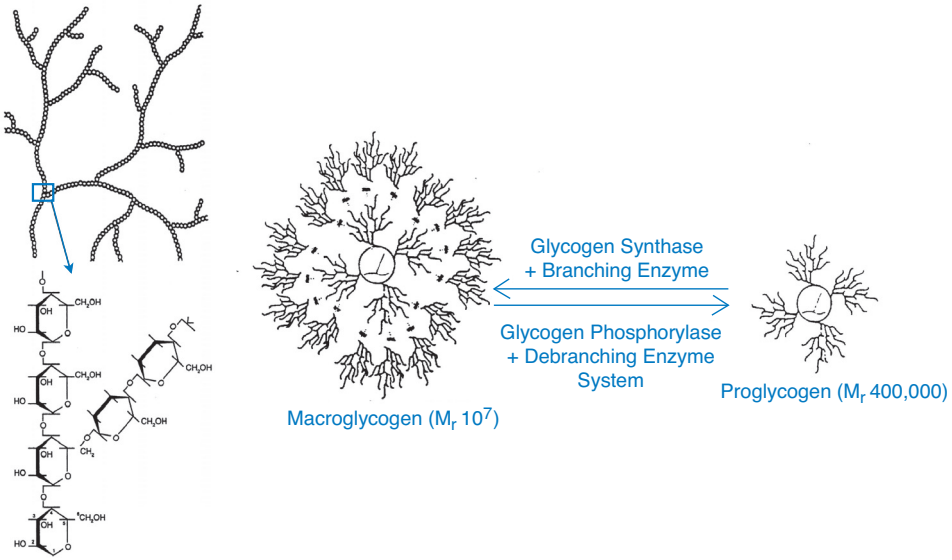

Figure 1.8 **Structure of glycogen**. Left-hand side: each circle in the upper diagram represents a glucosyl residue. Most of the links are of the α-1,4 variety. One of the branch points, an α-1,6 link, is enlarged below. Amylopectin, a component of starch, has a similar structure. Amylose, the other component of starch, has a linear α-1,4 structure. Right-hand side: glycogen is built upon a protein backbone, glycogenin. The first layer of glycogen chains forms proglycogen, which is enlarged by addition of further glucosyl residues (by glycogen synthase and a specific branching enzyme, that creates the α-1,6 branch-points), to form macroglycogen. When glycogen is referred to in this book, it is the macroglycogen form that is involved. Pictures of proglycogen and macroglycogen taken from Alonso *et al.* (1995), *FASEB Journal*, Copyright 1995 by Fedn of Am Societies for Experimental Bio (FASEB). Reproduced with permission of Fedn of Am Societies for Experimental Bio (FASEB).

Polysaccharides differ from one another in a number of respects: their chain length, and the nature (α- or β-) and position (e.g., ring carbons 1–4, 1–6) of the links between individual sugar units. Cellulose consists mostly of β-1,4 linked glucosyl units; these links give the compound a close-packed structure which is not attacked by mammalian enzymes. In humans, therefore, cellulose largely passes intact through the small intestine where other carbohydrates are digested and absorbed. It is broken down by some bacterial enzymes. Ruminants have complex alimentary tracts in which large quantities of bacteria reside, enabling the host to obtain energy from cellulose, the main constituent of its diet of grass. In humans there is some bacterial digestion in the large intestine (Chapter 3). Starch and the small amount of glycogen in the diet are readily digested (Chapter 3).

The structure of glycogen is illustrated in Figure 1.8. It is a branched polysaccharide. Most of the links between sugar units are of the α-1,4 variety but after every 9–10 residues there is an α-1,6 link, creating a branch. Branching makes the molecules more soluble, and also creates more 'ends' where the enzymes of glycogen synthesis and breakdown operate. Glycogen is stored within cells, not simply free in solution but