1

Introduction to metabolism

Overview of the chapter

In this chapter we will consider definitions of metabolism; the biochemistry–physiology continuum. The concept of metabolic pathways and their organization and control of metabolism are likened to a road map involving 'flow' of substrates but with mechanisms to accelerate or slow down pathways or to direct substrates through alternative routes.

Introductions to enzyme kinetics and bioenergetics are given with explanations of key terms such as K_m and V_{max}; coenzymes, cofactors and inhibitors; typical metabolic reactions; free energy; exergonic and endergonic reactions, catabolism and anabolism.

Guidance on how to study metabolic pathways is given using glycolysis as a model pathway.

1.1 Introduction

Movement; respiration; excretion; nutrition; sensitivity; reproduction. These are the six criteria often used by biologists to define 'life'. Whilst physiologists describe many processes of human biology at the tissue and organ level, a biochemist studies the same processes but at a 'higher magnification'. To a biochemist, the six features listed above can all be described in terms of chemical events, so a useful definition of biochemistry is 'the study of life at the molecular level'. The discipline of cell biology fits between physiology and biochemistry, but the three disciplines together form a continuum of knowledge and investigation.

Biochemical studies follow several themes. For example, investigations can be focussed on the chemical *structures* of molecules, (for example the structure of glycogen, DNA or protein conformation) or the structural inter-relationship between molecules (e.g. enzymes with their substrates, hormones with their receptors). The other branch of biochemical enquiry is into those numerous '*dynamic*' events known collectively as 'metabolism', defined here as 'all of the chemical reactions and their associated energy changes occurring within cells'. The purpose of metabolism is to provide the

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energy and building materials required to sustain and reproduce cells and thereby the organism.

It is estimated that there are between 2000 and 3000 different types of metabolic reaction occurring, at various times, within human cells. Some of these are common to all cell types whilst others are restricted to one or two particular tissues whose specialized physiological functions reflect the specialized metabolic changes occurring within them. Metabolism is a fascinating, yet at first sight, complicated process apparently representing a daunting challenge for the learner. For most students, it is unnecessary to learn *every* reaction in *every* pathway; what is important is that there an *understanding of concepts* of metabolism so that what appears to be a complicated set of reactions and pathways can be seen in terms of relatively few chemical and thermodynamic (energetic) principles. Metabolism may be likened to a journey; there is a starting place and a destination and there will be some important intermediate stops, perhaps where a change of mode of transport will be necessary, there will be points of interest and also places *en route* which deserve little or none of our attention. This analogy will be further developed later in the text. Furthermore, it is vital to realize that metabolism is adaptable; changes in physiological situations, for example fed or fasting, resting or exercising, health or ill- health will result in changes in particular aspects of metabolism.

The purpose of this book is to present metabolism in an organ-based fashion to make clear the links between biochemistry and physiology. By presenting metabolism in an appropriate tissue-context, the significance of pathways and their inter-relationships should be more meaningful.

1.2 Metabolic pathways

The term 'intermediary metabolism' is used to emphasize the fact that metabolic processes occur via a series of individual chemical reactions. Such chemical reactions are usually under the control of enzymes which act upon a substrate molecule (or molecules) and produce a product molecule (or molecules) as shown in Figure 1.1. The substrates and products are referred to collectively as 'intermediates' or 'metabolites'. The product of one reaction becomes the substrate for another reaction and so the *concept* of a metabolic pathway is created.

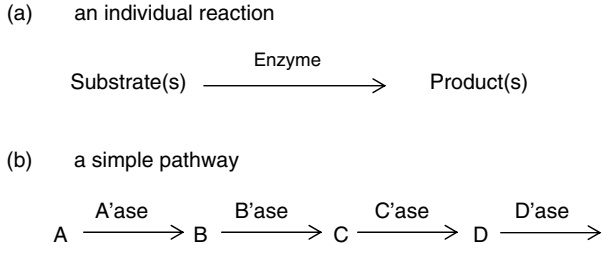


Figure 1.1 Simple representation of a metabolic pathway. 'Compound' B is the product of the first reaction and the substrate for the second reaction, and so on. Capital symbols represent metabolic intermediates and lower case letters with the suffix 'ase' represent enzymes

Metabolism and the individual reactions which comprise a pathway represent a dynamic process. Terms such as 'flow', 'substrate flux', 'rate' and 'turnover' are all used to communicate the idea of the dynamic nature of metabolism.

The student should be aware that a pathway is essentially a conceptual 'model' developed by biochemists in order to represent the flow of compounds and energy through metabolism. Such models are simply ways of trying to explain experimental data. A potential problem in representing metabolic pathways as in Figure 1.1 is that there is an implication that they are physically and/or topographically organized sequences. This is not necessarily true. With some exceptions (described in Section 1.3), most enzymes are likely to be found 'free' within the cytosol or a compartment of a cell where reactions occur when an enzyme and its substrate meet as a result of their own random motion. Clearly this would be very inefficient were it not for the fact that cells contain many copies of each enzyme and many molecules of each type of substrate.

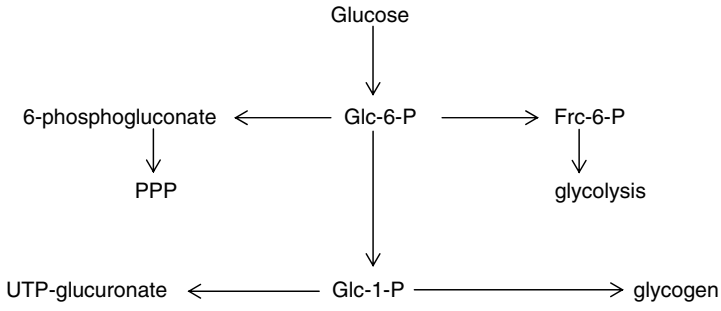
Think again about making a journey; a useful analogy is a road map of a city centre where there are main and subsidiary routes, one-way systems and interchanges, where traffic flow is controlled by signals and road-side signs. Very few people would attempt to learn, that is memorize, a complete route map, but learning the 'rules of the road' coupled with basic map reading skills and knowledge of main roads will enable most people to negotiate successfully a journey from one place to another. A complete diagram of intermediary metabolism appears to be as complicated as a road map of a city or region, that is a tangle of individual reactions with numerous substrates.

Understanding biochemical pathways is somewhat similar to map reading. The flow of traffic along roads and through the city is conceptually similar to the flow of substrates within the cell. Rather than visualizing cars, vans and trucks, think about the numerous carbon, hydrogen, nitrogen, phosphorus and oxygen atoms 'flowing' as component parts of substrate molecules, through pathways within the cell. Just as the traffic flow is regulated and directed with signals and restrictions, so too is the flow of substrates. Vehicles (metabolites) join or leave a particular traffic flow at intersections (converging or diverging pathways); the rate of flow is affected by traffic signals (enzymes), by road works or accidents (defective enzymes) and by the number of vehicles using the road (concentration of substrate molecules); they may need to take short-cuts or be diverted to avoid congested areas. Similarly, substrate molecules also may be routed via alternative pathways in a manner which best serves the physiological requirements of the cell at any particular moment. At times vehicles will need to take on fuel and some molecules need to be 'activated' by attachment to coenzyme A or uridine diphosphate (UDP), for example).

A note about terminology.

Glucose-1-phosphate (Glc-1-P) means that glucose has a phosphate attached at carbon 1 in place of a hydrogen atom.

Fructose-6-phosphate (Frc-6-P) means that fructose has a phosphate attached at carbon 6 in place of a hydrogen atom.



The relative activities of the enzymes which use glc-6-P as substrate determine the net flow.

Frc-6-P = fructose-6-phosphate
Glc-1-P = glucose-1-phosphate
PPP = pentose phosphate pathway
UTP = uridyl triphosphate

Figure 1.2 Glucose-6-phosphate is at a 'metabolic cross-roads'

1,3-bis phosphoglycerate (1,3-BPG) glyceric acid (glycerate) has 2 phosphate groups attached at carbons 1 and 3.

NB: 'bis' formerly designated as 'di'

Cells contain a large number of individual *types* of substrates; this is often referred to as the 'pool of intermediates'. One type of substrate may have a role to play in two or more pathways at different times according to the physiological demands being made on the cell. Metabolic regulation involves enzymes operating on substrates that occur at junctions of two or more pathways to act as flow-control points, rather like traffic signals. A good example of a substrate at a crossroads is glucose-6-phosphate (Glc-6-P), an intermediate that is common to glycolysis, glycogen turnover, the pentose phosphate pathway (PPP) and via UDP-glucose, the uronic acid pathway (Figure 1.2).

Clearly, substrates such as Glc-6-P do not 'belong' to a particular pathway but may occur within several routes. Channelling of the compound through a particular pathway will be determined by the relative activity of the enzymes using the substrate which in turn will be determined (regulated) by cellular requirements. Different pathways become more or less significant according to the physiological conditions (e.g. fed or fasting state, active or resting) in which the cell or organism finds itself.

1.3 Organization of pathways

Pathways can be illustrated in a metabolic map as linear, branched or cyclic processes (Figure 1.3) and are often compartmentalized within particular subcellular location: glycolysis in the cytosol and the Krebs tricarboxylic acid (TCA) cycle in

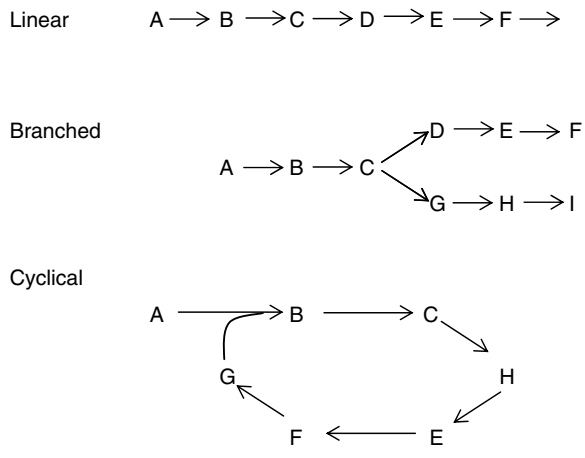


Figure 1.3 Conceptual arrangement of pathways

mitochondria are obvious examples. However, not all reactions of a particular pathway necessarily occur in the same organelle or location. Haem synthesis and urea synthesis (both described in Section 6.2) for example occur partly in the mitochondria and partly in the cytosol of liver cells.

Once an enzyme-catalysed reaction has occurred the product is released and its engagement with the next enzyme in the sequence is a somewhat random event. Only rarely is the product from one reaction passed directly onto the next enzyme in the sequence. In such cases, enzymes which catalyse consecutive reactions, *are* physically associated or aggregated with each other to form what is called a multi enzyme complex (MEC). An example of this arrangement is evident in the biosynthesis of saturated fatty acids (described in Section 6.30). Another example of an organized arrangement is one in which the individual enzyme proteins are bound to membrane, as for example with the ATP-generating mitochondrial electron transfer chain (ETC) mechanism. Intermediate substrates (or electrons in the case of the ETC) are passed directly from one immobilized protein to the next in sequence.

Biochemical reactions are interesting but they are not 'magic'. Individual chemical reactions that comprise a metabolic pathway obey, obviously, the rules of organic chemistry. All too often students make fundamental errors such as showing carbon with a valency of 3 or 5, or failing properly to balance an equation when writing reactions. Furthermore, overall chemical conversions occur in relatively small steps, that is there are usually only *small* structural changes or differences between *consecutive* compounds in a pathway.

To illustrate this point, consider the following analogy. The words we use in everyday language are composed from the same alphabet of letters. Changing even one letter within a word changes the meaning. Try converting the word WENT into COME by changing *only one* letter at a time. Each intermediate must be a meaningful English word.

This exercise is conceptually similar to biochemical conversions. One of the skills of the experimental biochemist is to identify metabolic intermediates and then to

arrange them in a chemically sensible sequence to represent the pathway, that is develop the model to explain the experimental results. A model answer to the word puzzle cited above is given at the end of the chapter.

1.4 Enzymes and enzyme-mediated reactions

This section deals with the nature of enzymes and their importance in metabolic control is discussed more fully in Chapter 3. Enzymes are biocatalysts whose key characteristics are as follows;

Enzymes are:

- Proteins;
- Chemically unchanged at the end of the reaction they catalyse and so are reusable;
- Required in small amounts because they are recycled;
- Able to act upon a specific substrate or structurally very similar substrates;
- Able to act on a particular part (functional group) within the substrate;
- Able to catalyse a specific type of chemical reaction;
- Able to operate under mild conditions of pH, temperature and pressure (if gases are involved).

1.4.1 Equilibrium or steady state?

The majority of biochemical reactions are *reversible* under physiological conditions of substrate concentration. In metabolism, we are therefore dealing with chemical *equilibria* (plural). The word equilibrium (singular) signifies a balance, which in chemical terms implies that the *rate* of a forward reaction is balanced (i.e. the same as) the *rate* of the corresponding reverse reaction.

$$r \rightleftarrows p \text{ which may also be written as } r \leftrightarrow p$$

r = reactant(s)

p = product(s)

$$r \rightarrow p \text{ is the forward reaction}$$

and

$$p \rightarrow r \text{ is the reverse reaction}$$

Many chemical reactions (especially those occurring within cells) are theoretically reversible under reasonable conditions of pressure (when gasses are involved, which is rare), temperature and concentration.

In a closed system, that is one in which there is no addition of 'r' nor any removal of 'p', the reaction will come to a perfect balance; 'the point of equilibrium'. A common misunderstanding of the concept of this point of equilibrium is that it implies an *equal concentration* of r and p. This is not true. The point of equilibrium defines the relative concentrations of r and p when the *rate* of formation of p is exactly equal to the *rate* of formation of r. The point of equilibrium value for a chemical reaction can be determined experimentally. If the starting concentration of the reactant is known, then it follows that the *relative* concentrations of r and p when equilibrium has been reached must reflect the *relative* rates of the forward and reverse reactions. For a given reaction, under defined conditions, the point of equilibrium is a constant and given the symbol K_{eq}.

Thus

$$K_{eq} = \frac{[p]}{[r]}$$

where [ ] indicates molar concentration

When the equilibrium concentration of p is *greater* than the equilibrium concentration of r, we can say that the forward reaction is favoured (faster) and $K_{eq} > 1$;

$$r \rightleftharpoons p \qquad \text{so at equilibrium, } [p] > [r]$$

NB: the weight and size of the arrows represents the relative rates of reaction

The higher the value of K_{eq}, the more difficult it is for that reaction to 'go backwards' so effectively it becomes unidirectional.

Conversely, if [r] is greater then [p], the reverse reaction is favoured and $K_{eq} < 1$ because $[p] < [r]$ signifies that the forward reaction becomes increasing less likely and the value becomes smaller. It could be argued that a 'true' equilibrium occurs only when $[r] \approx [p]$, but K_{eq} is a measure of the relative *rates* of the forward and reverse reactions. An important consequence of the magnitude of K_{eq} is that the further away a reaction is from a true equilibrium, the greater the energy change involved in that reaction. This is explained in more detail later in this chapter and also in Chapter 2.

Most individual biochemical reactions are reversible and are therefore quite correctly considered to be chemical equilibria, but cells are not closed systems; fuel (e.g. a source of carbon and, in aerobic cells, oxygen) and other resources (e.g. a source of nitrogen and phosphorus) are continually being added and waste products removed, but their relative concentrations within the cell are fairly constant being subject to only moderate fluctuation. Moreover, no biochemical reaction exists in isolation, but each is part of the overall flow of substrate through the pathway as a whole.

Stated simply, biochemical reactions never reach a true equilibrium because the product of one reaction is the substrate for the next and so the reaction is 'pulled' towards completion achieving net formation of product. Indeed, if reactions inside a cell were *true equilibria*, there would be no net flow of substrate, no formation of end

products and therefore no metabolic pathway. Biologically, this would not be very desirable! The situation which exists within cells is better described as a *steady state*. In this condition, there *is* net flow of matter but the instantaneous concentrations of intermediates fluctuate relatively little, unless a 'stress' for example the need to respond to a physiological challenge, is placed on the system.

Although there is a bewildering array of individual reactions occurring within cells they can be classified into a small number of groups. Learning the types of reactions and then identifying particular examples as and when they arise is easier than trying simply to memorize a sequence of chemical changes. Typical biochemical reactions include the following (Figures 1.4 to 1.17).

1. **Atomic and molecular rearrangements**
   Isomerization involving (a) a change in functional group or (b) the repositioning of atoms within the same molecule, for example

   a. ribose-5-P $\xrightarrow[\text{(aldehyde to ketone)}]{}$ ribulose-5-P

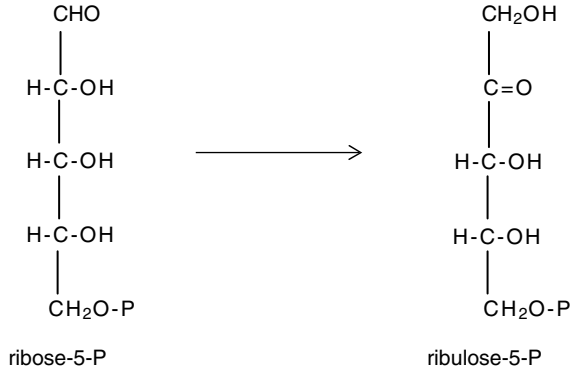


Figure 1.4 Enzyme: phosphoriboisomerase

   b. 2-phospho glycerate $\xrightarrow[\text{(repositioning of the P group from carbon 2 to carbon 3)}]{}$ 3-phospho glycerate

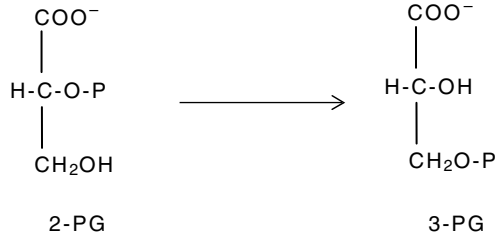


Figure 1.5 Enzyme: phosphoglyceromutase

2. **Substitution**
Replacement of one atom or group with another, for example, a hydrogen atom is replaced by a methyl group;

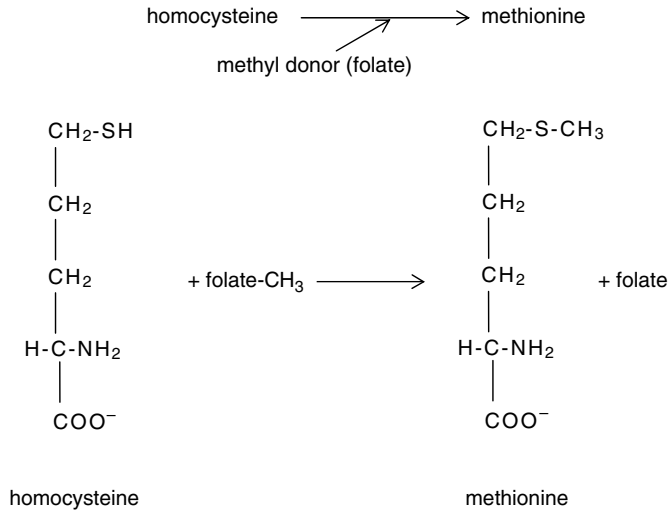


Figure 1.6 Enzyme: methionine synthase

3. **Redox reactions** ***These Are Very Important***
Oxidation and reduction reactions always occur together and are usually easily spotted because of the involvement of a coenzyme.

$$\text{A-H}_{(\text{red})} + \text{coenz(ox)} \leftrightarrow \text{B}_{(\text{ox})} + \text{coenz-H(red)}$$

Coenz = coenzyme;
(red) = reduced form;
(ox) = oxidized form (i.e. fewer hydrogen atoms/electrons than the reduced form);
for example, lactate dehydrogenase

$$\text{lactate} + \text{NAD}^+ \leftrightarrow \text{pyruvate} + \text{NADH} + \text{H}^+$$

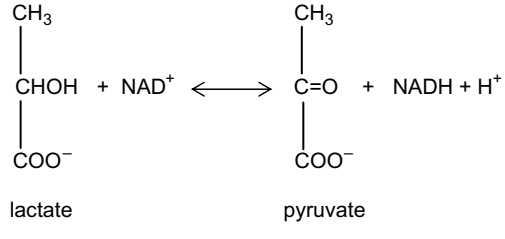


Figure 1.7 Enzyme: lactate dehydrogenase

The lactate is oxidized (two hydrogen atoms removed) and the NAD^+ is reduced to $NADH + H^+$

Oxidation sometimes occurs simultaneously with another chemical change. For example, oxidative decarboxylation or oxidative deamination.

a. Oxidative decarboxylation; CO_2 released

$$\text{pyruvate} + NAD^+ + \text{CoASH} \rightarrow \text{acetyl CoA} + CO_2 + NADH + H^+$$
$$(\text{CoASH} = \text{co enzyme A})$$

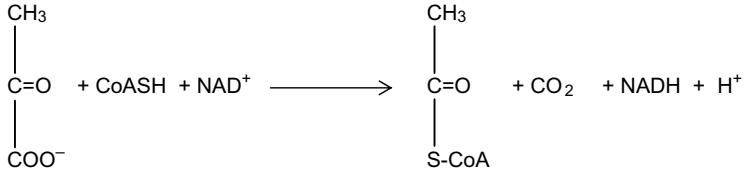


Figure 1.8 Enzyme: pyruvate decarboxylase

b. Oxidative deamination; NH_3 released

$$\text{glutamate} + NAD^+ + H_2O \rightarrow NH_3 + \text{2-oxoglutarate} + NADH + H^+$$

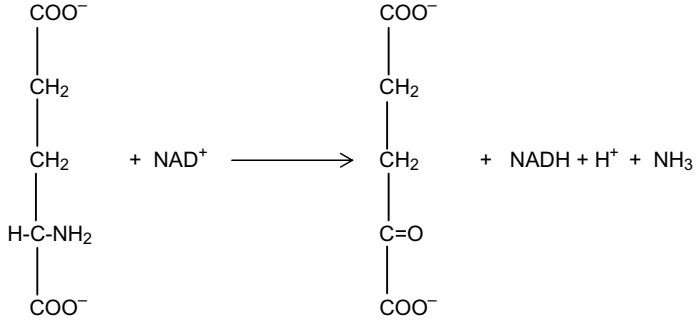


Figure 1.9 Enzyme: glutamate dehydrogenase

4. **Cleavage**

a. Hydrolysis, if water is used to break a bond

$$\text{Glucose-6-P} + H_2O \rightarrow \text{Glucose} + \text{Pi}$$ Pi symbolises free inorganic phosphate

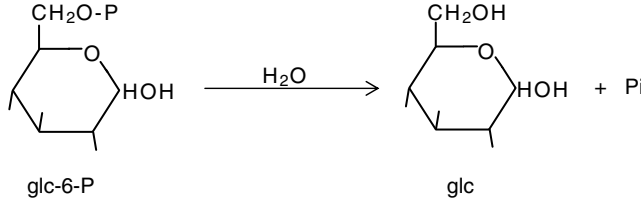
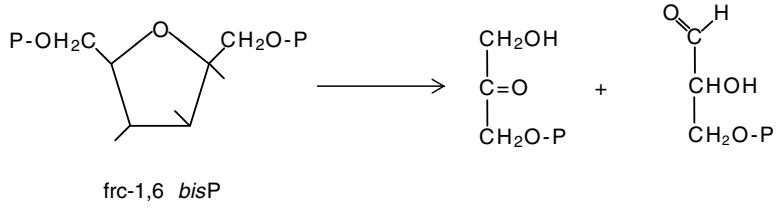


Figure 1.10 Enzyme: glucose-6-phosphatase

b. One molecule is split into two

$$\text{F-1, 6-}\textit{bis}\text{ phosphate} \rightarrow \text{glyceraldehyde-3-P} + \text{dihydroxyacetone-P}$$



frc-1,6 *bis*P

Figure 1.11 Enzyme: aldolase

5. **Condensation**

Two molecules join together with the elimination of a H_2O. Condensation reactions are used when macromolecules are being formed. Amino acids are joined via peptide bonds and monosaccharides via glycosidic bonds, both of which are condensation reactions.

$$2\ \text{glucose} \rightarrow \text{maltose} + H_2O$$

$$2\ \text{amino acids} \rightarrow \text{dipeptide} + H_2O$$

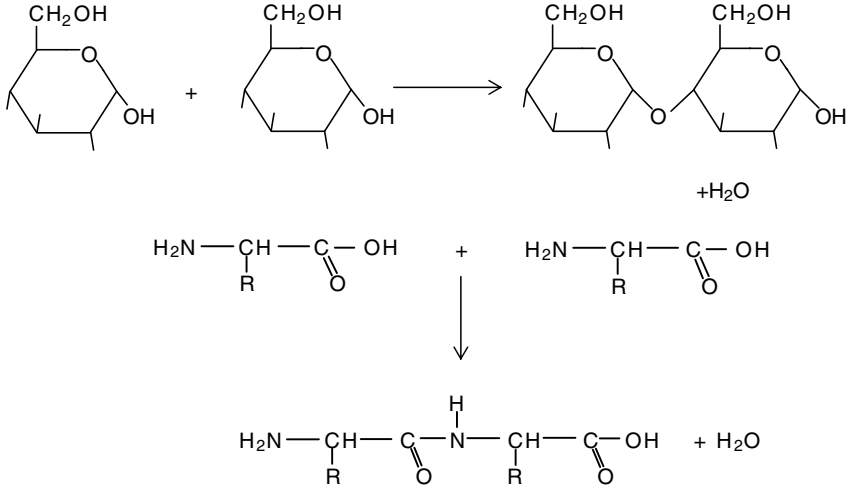


Figure 1.12 Enzymes: synthases

6. **Addition**

a. Two molecules are joined together but water is not eliminated. Often ATP is used to provide energy.

$$\text{glutamate} + NH_3 + \text{ATP} \rightarrow \text{glutamine} + \text{ADP}$$

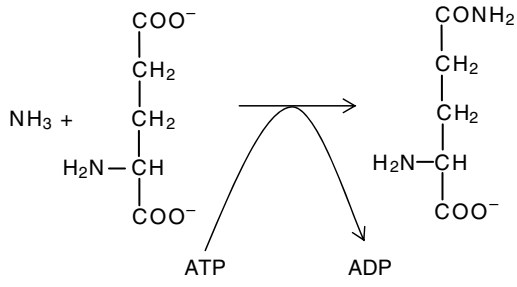


Figure 1.13 Enzyme: glutamine synthase

b. Alternatively, addition may across a double bond

$$\text{fumarate} + H_2O \rightarrow \text{malate}$$

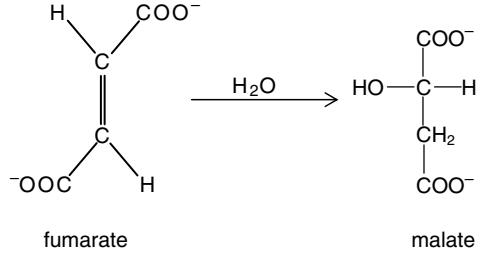


Figure 1.14 Enzyme; fumarase

7. **Transfer**

a. A phosphate group may be transferred from ATP to a substrate;

$$\text{glucose} + \text{ATP} \rightarrow \text{G-6-P} + \text{ADP}$$

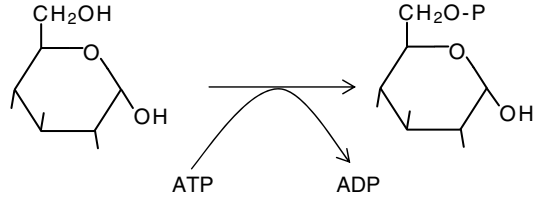


Figure 1.15 Enzyme: glucokinase or hexokinase

b. A functional group may be 'swapped' between two molecules

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

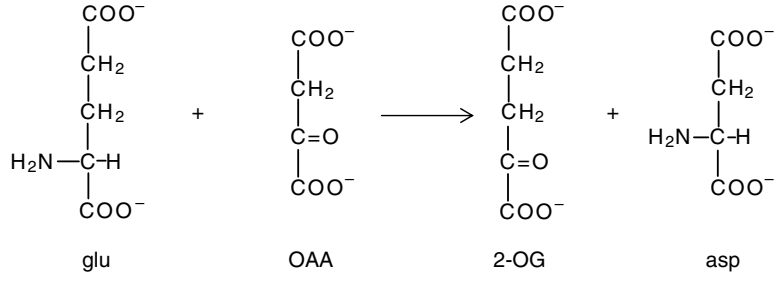


Figure 1.16 Enzyme: aspartate transaminase (= aspartate aminotransferase)

c. Quite complex chemical groupings may be transferred

sedoheptulose-7-P + glyceraldehyde-3-P → erythrose-4-P + fructose-6-P

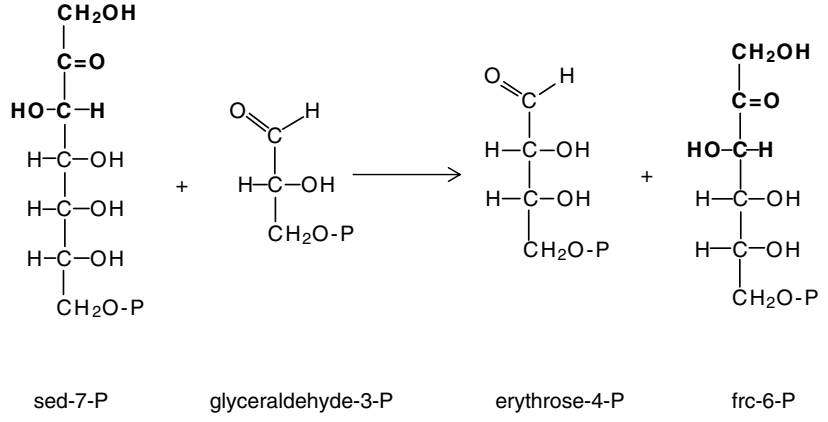


Figure 1.17 Enzyme: transaldolase

Here, a C3 unit (bold) has been transferred from sedoheptulose-7-P to glyceraldehyde-3-P.

The reactions given above illustrate the chemical changes that frequently occur in biochemistry. When you meet a reaction for the first time, it is a good idea to first of all identify the type of reaction occurring, and then look at the specific details.

Enzyme-mediated catalysis requires the breaking and making of chemical bonds between atoms; this involves changes in energy and is described by thermodynamics. Enzymes reduce the activation energy, that is make the reaction process easier to initiate but do not alter the overall energy change, which is determined by the free energy difference between the substrate(s) and the product(s). The change in free energy determines the *spontaneity* or likelihood of a reaction but the *speed* (kinetics) of an enzyme-catalysed reaction is governed by factors such substrate concentration,

enzyme concentration, pH temperature and the presence of activators or inhibitors. Principles of enzyme kinetics and thermodynamics as applied to biochemistry are dealt with in Sections 1.4.2 and 1.5 respectively, whilst a more detailed analysis and explanation of these topics can be found in Chapter 2.

1.4.2 Enzyme kinetics: an introduction

Kinetics is the study of the factors which influence reaction rates. Enzyme-catalysed reactions are subject to the same principles of rate regulation as any other type of chemical reaction. For example, the pH, temperature, pressure (if gases are involved) and concentration of reactants all impact on the velocity reactions. Unlike inorganic catalysts, like platinum for example, there is a requirement for the substrate (reactant) to engage a particular region of the enzyme known as the active site. This binding is reversible and is simply represented thus:

$$E + S \rightleftarrows [ES] \rightarrow P + E$$

Where,

E = enzyme

S = substrate

[ES] = enzyme-substrate complex

P = product

The relative rates of formation and dissociation of [ES] is denoted as K_m, the Michaelis constant. Each enzyme/substrate combination has a K_m value under defined conditions. Numerically, the K_m is the substrate concentration required to achieve 50% of the maximum velocity of the enzyme; the unit for K_m is therefore the same as the unit for substrate concentration, typically μmol/l or mmol/l. The maximum velocity the enzyme-catalysed reaction can achieve is expressed by the V_{max}; typical unit μmol/min. The significance of K_m and V_{max} will be discussed in greater detail in Chapter 2.

In a cell, enzymes do not always work at their V_{max}. The precise rate of reaction is influenced by a number of physiological (cellular) factors such as:

- [S]
- [coenzyme]
- presence of activators or inhibitors.

Because enzymes are proteins, they are subject to all of the factors (e.g. pH, temperature) which affect the three-dimensional integrity of proteins in general.

The ability of some organisms to control the pH and temperature of their cells and tissues represents a major biological development. Homeothermic animals (e.g. mammals) maintain a constant temperature of about 37 °C as this corresponds to the temperature of optimum activity of most enzymes. Poikilothermic or so-called cold-blooded animals (e.g. reptiles) have to sun themselves for sometime every morning in order to raise their body temperature in order to optimize enzyme activity within their cells.

Plants and single celled organisms have no means of autoregulating their operating temperature and thus their growth and replication are influenced by external conditions. Hence, we keep food at 4 °C in a refrigerator to prevent spoilage yet we incubate bacterial cultures at 37 °C and usually in a buffered medium when we wish to cultivate the cells for further study.

Homeostatic mechanisms also allow animals to control their intracellular pH very strictly. In humans for example, blood pH (usually taken as a reliable but indirect measure of cellular pH) is 7.4 ± 0.04. At 37 °C cytosolic pH is actually slightly lower at about 7.0 but different compartments within the eukaryotic cells may have quite different pH, for example, lysosomes have an internal pH of about 5; the inside of a mitochondrion is more alkaline than the outside whilst the inside of a phagosome in a white blood cell is more acidic than its surrounding cytosol, both situations arising due to proton pumping across a membrane.

Except in a few instances, the enzyme molecule is very much larger than the substrate(s) upon which it works. The reason for this great disparity in size is not entirely obvious, but the possibility of the enzyme binding with more than one small molecule (e.g. regulator molecules, see Section 1.4.3) arises when we are dealing with large structures.

1.4.3 Enzyme ligands: substrates, coenzymes and inhibitors

As we saw earlier in this chapter, substrates are the molecules which undergo chemical change as a result of enzyme activity. Many enzymes will only operate when in the presence of essential co-factors or coenzymes. The term 'coenzyme' is not entirely appropriate as it implies that, like enzymes themselves, these compounds do not undergo chemical change. This is not true and more accurate terminology would be co-substrate. Coenzymes are always much smaller than the enzymes with which they operate and are not heat sensitive as are the proteins.

Examples of coenzymes: vitamin-derived nucleotides; for example adenosine phosphates; ATP, ADP, AMP; nicotinamide derivatives; NAD^+, NADH, $NADP^+$, NADPH; flavin derivatives; FAD, $FADH_2$; coenzyme A (abbreviated to CoA, CoASH or CoA-SH).

Not all vitamin coenzymes need to be in the form of a nucleotide (base, sugar, phosphate). For example; thiamine; biotin; pyridoxine; vitamin B_{12}.

Some enzymes also require inorganic factors to achieve full activity. Such co-factors include metal ions, Mg^{2+}, Mn^{2+}, Zn^{2+} and non-metals, Cl^-.

Inhibitors are compounds which reduce the efficiency of an enzyme and are important in directing and regulating the flow (or flux) of substrates through a pathway. Inhibitors which bind strongly to the enzyme for example, poisons such as cyanide, cause irreversible effects, but inhibition is rarely 'all or nothing' in a cell. Most inhibitors bind reversibly (as does the substrate of course) to the enzyme. Inhibitors which are structurally very similar to the true substrate effectively 'block' the active site and are called competitive inhibitors, because they compete with the true substrate for binding to the enzyme. Here, the ratio of substrate [S] to inhibitor [I] is critical in determining the quantitative effect of the inhibitor. Non-competitive inhibitors are also act reversibly by preventing the release of the product or by distorting the shape of the enzyme so preventing the substrate accessing the active site.

1.5 Bioenergetics: an introduction to biological thermodynamics

Thus far, our discussion has considered the chemical changes which constitute metabolism. We must now introduce some fundamental ideas of bioenergetics. Further details can be found in Chapter 2.

All molecules have an amount of energy determined mainly by their chemical structure. Metabolism involves chemical change. Inevitably therefore, energy changes always accompany the chemical changes which occur in metabolism. Our understanding of bioenergetics arises from physics and the laws of thermodynamics.

The First Law of Thermodynamics states that energy can be neither created nor destroyed but different forms of energy can be interconverted. The three forms of energy which are important to us are enthalpy (heat or 'total energy', represented by the symbol H), free energy ('useful energy' symbol G, in recognition of Josiah Gibbs) and entropy ('wasted energy', symbol S). Free energy is termed 'useful' energy because it can bring about useful work such as biosynthesis, transmembrane secretion or muscle contraction. Entropy however is not available for work but is the energy associated with chaos, disorder, loss of organization or an increase in randomness. Imagine a building, a castle, a tenement, or an office block which has not been maintained and thus shows the ravages of time and neglect. The building has lost its initial organization and structure because insufficient energy has been expended on its upkeep. You are now imagining entropy.

These three energy terms we have met are related by the following equation:

$$\Delta H = \Delta G + \mathrm{T}\Delta S$$

where Δ indicates 'change in' and T is absolute temperature (Kelvin; °C + 273).

Rearranging this equation gives $\Delta G = \Delta H - \mathrm{T}\Delta S$, which shows that as entropy increases as a function of temperature, free energy decreases.

The Second Law of Thermodynamics states that the entropy of the universe is constantly increasing. Cells are of course highly organized, a state which like the building referred to above, can only be maintained if free energy is expended. In other words, metabolism provides via catabolic (energy liberating, degradative) reactions free energy to prevent cells falling into disrepair by ensuring that biosynthesis and other cellular work can occur via anabolic (energy consuming, synthetic) reactions. *Homo sapiens*, like all animals is a heterotroph, meaning that the energy and raw materials required to maintain cellular structure and integrity are derived from the diet.

Figure 1.18a illustrates a phenomenon known as 'coupling'. Energy liberated from one process is used to drive forward an energy-requiring process. Individual biochemical reactions may be viewed similarly. Reactions which occur with a net loss of free energy ($-\Delta G$, termed exergonic) are spontaneous and favourable whereas those in which the products have more free energy than the reactants ($+\Delta G$, termed endergonic) will *not* occur spontaneously. An endergonic reaction can be driven forward by utilizing some of the energy liberated by the previous reaction in the pathway. Alternatively, ATP, the 'universal energy currency' of the cell can be called upon to provide the energy needed to overcome an endergonic reaction. The hydrolysis of ATP to ADP and inorganic phosphate (Pi) lies very far from equilibrium, has a very large Keq, and so is associated with a large energy change. It is not strictly true to state that energy is liberated from the *bond* (often incorrectly referred to as a 'high energy' bond) between the second and third phosphate groups of ATP. Figure 1.18b illustrates the coupling of energy liberating (catabolic) and energy consuming (anabolic) processes.

1.6 Enzyme-mediated control of metabolic pathways

Previously, the analogy was drawn between substrate flow in metabolism and traffic flow in towns, with enzymes acting as the 'traffic signals'; let us return to that image and consider where and how enzymes fulfil their control function. In the absence of traffic control measures, it is not difficult to imagine a situation of complete 'grid-lock' arising with no vehicles moving anywhere. Enzymes fulfil a regulatory function and prevent metabolic grid-lock by directing substrates along one pathway or another, by accelerating or slowing a particular pathway. Further details of metabolic control are given in Chapter 3.

Theoretically, all enzyme reactions are reversible but the overall flux (flow) of substrate in a pathway is unidirectional. To extend our road map analogy, this type of reaction acts as a control point in a pathway, rather like a one-way street, allowing substrates to flow in only one direction.

Such a 'metabolic one-way street' comes about in large part due the fact that certain chemical reactions are associated with a large energy change, which in chemical terms mean that the reaction is operating far away from its true equilibrium. Reactions of this nature are difficult to reverse under the conditions of pH, temperature and substrate concentration which exist inside cells and so become 'physiologically irreversible'.

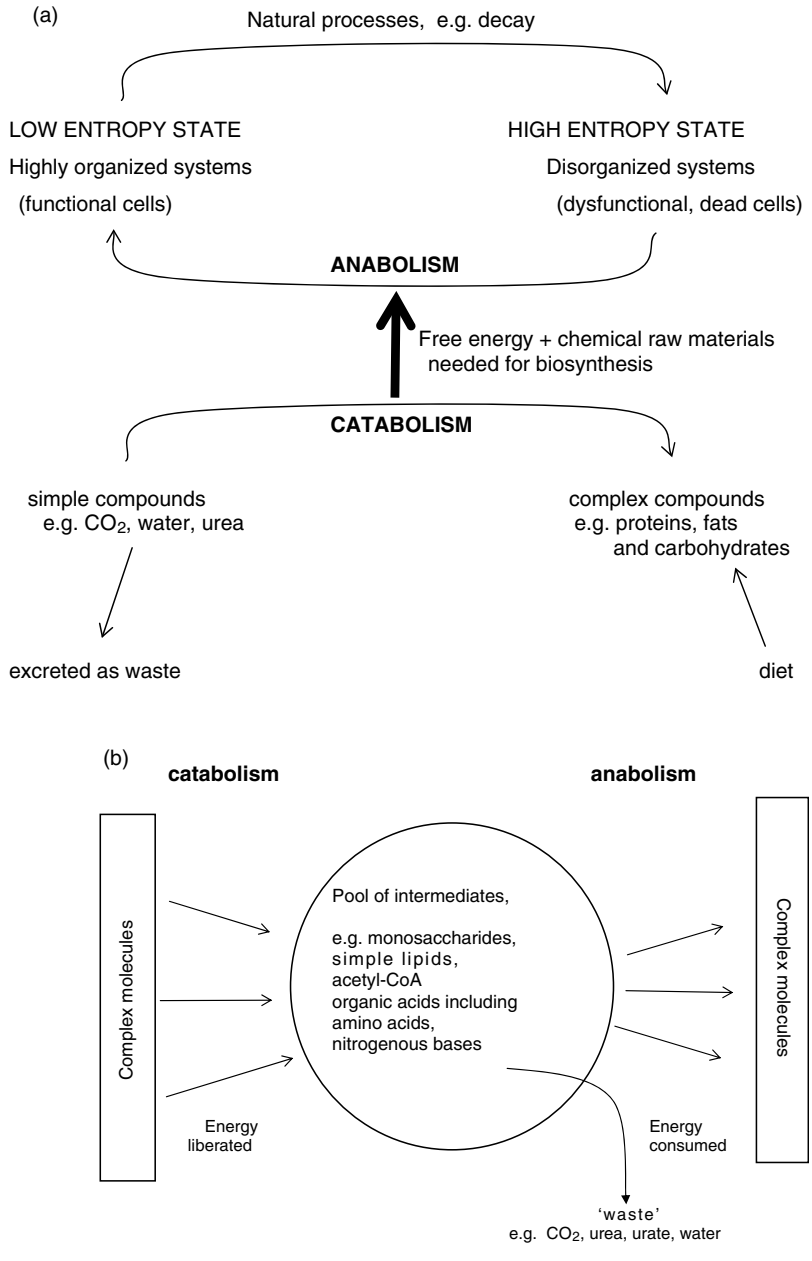


Figure 1.18 Energy flow in biological systems

Reactions operating far from their equilibrium position are not easy to identify merely by looking at the metabolic map, although the involvement of ATP is often a significant clue to a reaction being irreversible. Stated simply, hydrolysis of ATP 'energizes' a reaction which is normally irreversible. However, we can often predict the existence of

flow-control enzymes at or near to the beginning of a pathway or at branch points (junctions) in pathways.

Enzyme activity is affected by changes in pH, temperature, substrate concentration, enzyme concentration and the presence of activators or inhibitors. Inside cells, both pH and temperature are normally tightly regulated so neither is able to influence greatly the physiological action of enzymes (a notable exception being the marked pH change seen in vigorously exercising muscle, see Chapter 7). Substrate concentration certainly does vary considerably within cells due, for example, to recent food intake or physical activity. Finally, enzyme concentration and the presence of activators or inhibitors also affect the rate of a reaction.

Enzymes are proteins (gene products) synthesized by DNA transcription and messenger RNA (mRNA) translation. Many enzymes are described as being 'constitutive', meaning they are present at all times. Others are 'inducible', meaning that their synthesis can be increased on-demand when circumstances require. By increasing the concentration of certain enzymes, induction allows more substrate to undergo chemical reaction and the pathway accelerates.

Activators and inhibitors regulate not the amount of enzyme protein but the activity ('efficiency') of that which is present. Two principal mechanisms of control are (i) competitive and (ii) allosteric. Competitive control (inhibition) occurs when a compound which is structurally similar to the true substrate binds to the active site of the enzyme. This is how a number of drugs and poisons bring about their effect. For example, a group of therapeutic drugs called statins are used to treat heart disease because by inhibiting a key enzyme called HMGCoA reductase, they reduce the hepatic synthesis of cholesterol and therefore the plasma concentration of that lipid.

Allosterism (Greek 'other place') is the name given to the mechanism whereby endogenous regulators, compounds found within or associated with the pathway in which the target enzyme occurs or from a related pathway, control a particular reaction. These regulators, allosteric activators and allosteric inhibitors, bind to the enzyme at identifiable allosteric sites, not the active site. The activity of the target enzyme changes as the cellular concentration of the allosteric regulators rise or fall. Details of allosteric control are given in Chapter 3.

Fluctuation in regulator concentration reflects the metabolic status of the cell and so the regulators themselves are acting as intracellular 'messengers'. For example, ATP, ADP and AMP act as allosteric regulators in glycolysis. When the cytosolic concentration of ATP in the liver or muscle is high, the cell has enough 'energy currency' so to process more glucose through to pyruvate would be wasteful. It is more useful to divert the glucose in to glycogen synthesis, an effect which is achieved by the allosteric inhibition of phosphofructokinase (PFK). Conversely, if the cytosolic concentration of ADP is high, PFK activity is accelerated, allowing more pyruvate to be synthesized leading to increased production of acetyl-CoA to be used in the Krebs TCA cycle and ultimately the synthesis of ATP. Here we have a good example of biochemical feedback.

Allosteric regulators bind to the target enzyme in a non-covalent manner. An entirely different enzyme control mechanism is covalent modification. Here, the conformation of the enzyme protein, and thereby its activity, is changed by the

attachment of, usually, phosphate donated by ATP. Reversible phosphorylation is itself mediated via protein kinases (which transfer inorganic phosphate from ATP to a substrate) and protein phosphatases (which remove, by hydrolysis, inorganic phosphate from a substrate).

Not all allosteric proteins are enzymes. In fact, probably the best-known and characterized allosteric protein is haemoglobin, which like an enzyme binds ligands (small molecules) to itself, for example, oxygen rather than a substrate.

1.7 Strategy for learning the details of a pathway: 'active learning' is essential

When asked to learn a pathway, the temptation is to sit down and memorize each step in turn from top to bottom. A common failing in students who are new to metabolic biochemistry is in trying to memorize the whole of a pathway at the outset: *Rule 1 Resist the temptation to memorize*! This approach leads to 'knowing' but not really 'understanding'. Moreover, memorizing individual reactions/pathways is not always helpful. To use a microscopical analogy, begin with a low power view, try to see the pathway in relation to others and be clear about the physiological purpose of the pathway. Metabolism is a mosaic of component parts; pathways do not exist in isolation and taking the time see the broad picture at the start of the learning process will make the learning process more meaningful and therefore easier.

Rule 2 Be positive: *Don't* think about pathways simply as information gathered from experiments carried out in test tubes. *Don't* think about biochemistry as a body of knowledge that has to be mastered to pass an exam. *Do* think about what is going on inside your own cells and tissues at various times; having read a chapter in this book or after attending a lecture, think about how reactions and pathways in your own cells and tissues respond to your changing physiological circumstances, such as sleeping, sitting, walking, running, fasting, after food. Biochemistry is dynamic and its about you.

The following strategy should help put the pathway into its proper context.

The overview

1. **WHERE** does the pathway occur?
   that is in which cell types (prokaryotic or eukaryotic or both, in which tissue(s) of a multicellular organism, in which compartment of the eukaryotic cell, (cytosol, mitochondria, lysosomes etc.).

2. **WHAT** is the biochemical purpose of the pathway?
   for example, to release energy; to produce reducing power, to produce a key functional molecule, to synthesize a macromolecule.

3. **WHAT** links are there between the pathway and any others?

4. **WHERE** are the control points within the pathway?

5. **WHEN** does the pathway operate? Is it always active or is it an 'adaptive' pathway?

The answers to all of these questions may not be evident immediately, but are usually to be found by diligent study active learning.
Once the overview is clear, begin to look in more detail at the chemistry and mechanisms of process. Here are some more suggestions of points to look for when studying an unfamiliar pathway in more detail.

The details

Skeletal view	
	What are the first and last substrates? Is the pathway linear, branched or cyclical? How many intermediate substrates are present? Learn the names of the intermediates. Which coenzymes are involved and where?
Chemistry of the intermediates and the reactions:	
	Look at them as organic chemicals; How many carbon atoms are present and what types of functional groups are present? What structural similarities and differences are there between the intermediates? What sort of chemical reactions are occurring, for example, oxidation, condensation, hydrolysis. Don't worry about getting the right sequence at this stage
Learn the names of the enzymes in sequence.	
	Use of the EC naming system will help you deduce the name of the substrate and the chemical change occurring
Learn the structures of the intermediates.	
	Use cue cards with the structure of an intermediate on one side and its name on the other; Test yourself by selecting at random the name of an intermediate substrate and then draw from memory its structure.
Redraw the pathway in a different way	
	Include structures and all names in a different way; Design a different image thus avoiding merely reproducing diagram from a book or the one given during a lecture. Be creative; make the diagram as vivid and memorable as possible.

1.7.1 An Example: glycolysis as a model pathway

You will probably be familiar with glycolysis (the Embden–Meyerhof pathway, Figure 1.20) from previous studies at school perhaps, so let's use this important pathway to illustrate some points in the recommended strategy.

1. **The overview**

Where?	Universal, occurs in all cell types. Cytosolic
What purpose?	To begin the oxidative catabolism of glucose. The production of ATP is small so this is not a prime role in most tissues. The end products pyruvate (or lactate) are important compounds for other pathways.
What are the links to other pathways?	Pentose phosphate pathway and glycogen metabolism (both are linked via glucose-6-P); glycerol from lipids may enter at the level of triose phosphate
Where are the control points? Reactions catalysed by	Hexokinase/Glucokinase Phosphofructokinase Pyruvate kinase
When does the pathway operate?	All of the time (constitutive).

2. **The details**

Skeletal view	glucose $\rightarrow 2 \times$ pyruvate($C_3H_3O_3$)if operating aerobically (or $2 \times$ lactate, $C_3H_5O_3$, if anaerobic)
Carbon balance:	$C_6H_{12}O_6 \rightarrow 2 \times C_3H_3O_3$ compounds (or $2 \times C_3H_5O_3$ if anaerobic)
	number of intermediates = 11 including glucose and pyruvate 10 enzyme-catalysed reactions
Coenzymes	2 molecules of NADH + H^+ are generated per molecule of glucose oxidized; net gain of 2 molecules of ATP per molecule of glucose oxidized, that is, 2 molecules ATP consumed and 4 molecules produced per molecule of glucose.

Chemistry of the intermediates	4 hexoses 3 of which are phosphorylated, one of which is bis-P i.e. two phosphates on different carbons within the same molecule)
	one aldehyde/ketone combination, both phosphorylated
	5 organic acids (all have 3 carbon atoms) 4 of these are phosphorylated
Reactions:	2 phosphorylations directly from ATP + 1 oxidative phosphorylation when Pi is added
	3 isomerizations
	1 cleavage
	2 dephosphorylations
	1 rearrangement

Names of the enzymes

Hexokinase/glucokinase	(HK/GK)
Phosphohexoisomerase	(PHI)
Phosphofructokinase	(PFK)
Aldolase	(ALDO)
Triose phosphate isomerase	(TPI)
Glyceraldehyde-3-P dehydrogenase	(Gly'ald-3-P D'ase)
Phosphoglycerokinase	(PGK)
Phosphoglyceromutase	(PGM)
Enolase	(ENO)
Pyruvate kinase	(PK)

Let's try applying the active learning model approach. The chemical structure of each glycolytic intermediate substrate is shown in Figure 1.19. Remembering that each individual reaction in any pathway brings about a small chemical change, arrange the structures in a logical sequence. The names of the intermediates are given in Figure 1.20.

Hint: think back to the word puzzle in which you changed the word 'went' into 'come'. The same process of small discrete changes of chemical structure can be seen to apply here.

First, name the intermediates using knowledge of simple organic chemistry and chemical nomenclature.

Start with the easy ones! Glucose [compound (iv)] should be familiar to you *and* it is one of only two substrates in glycolysis which is not phosphorylated; the other one being pyruvate [compound (i)].

From glucose, we can easily identify glucose-6-P (Glc-6-P) [compound (v)].

Similarly, fructose-6-P, one of the five-sided furan ring sugars we meet in metabolism. [Compound (x)] and fructose,-1,6-bis P [compound (xi)] should be obvious from their structures.

There is only one compound which carries an aldehyde group, so glyceraldehyde-3-P must be compound (viii) and acetone you may already know as a ketone, so compound (ii) is dihydroxyacetone phosphate, DHAP.

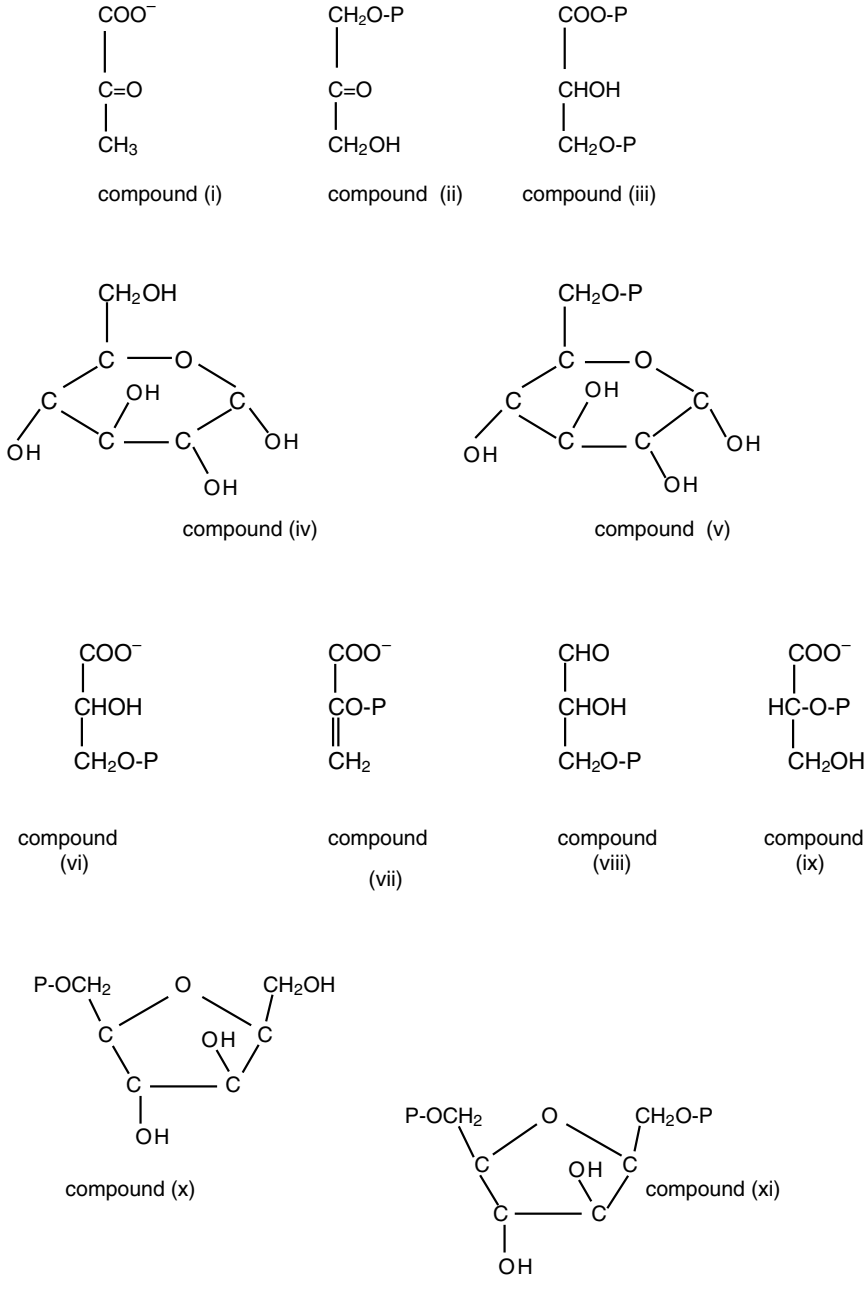


Figure 1.19 The chemical intermediates of glycolysis

Now for the glycerates. 1,3 bis-phosphoglycerate [compound (iii)] is the only molecule with two attached P groups. When we number the carbon atoms in an aliphatic organic compound we invariably start at the most oxidized carbon (drawn at the top of the chain), so carbon 2 of the glyceric acid derivatives must be the middle

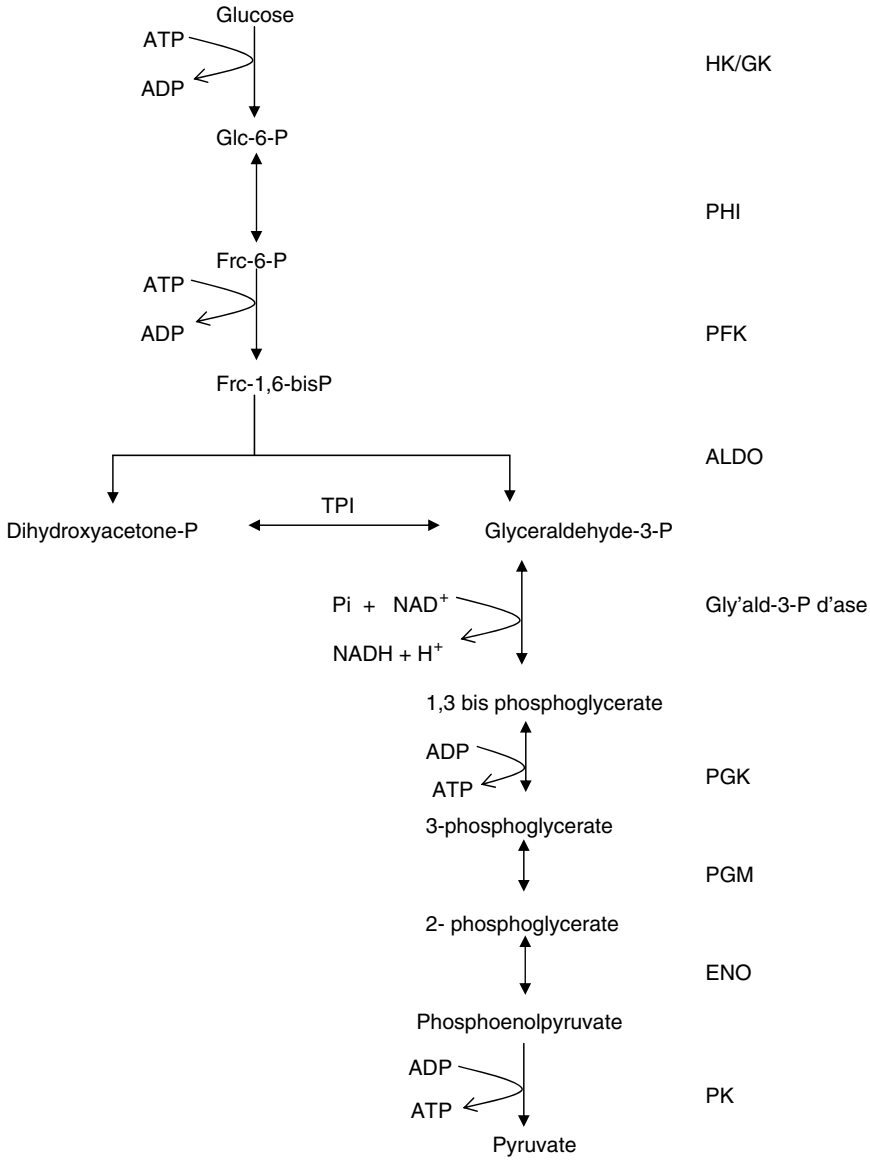


Figure 1.20 Glycolysis

one, so **2**-phosphoglycerate is compound (ix), and so **3**-phosphoglycerate must be compound (vi).

This leaves only one compound which must be phospho enol pyruvate (PEP) as compound (vii).

Metabolic pathways are better learnt as an exercise in logic than pure memory work!! Working from first principles with a firm underpinning knowledge will seldom

let you down, whereas rote learning is superficial. We all suffer from 'memory blank' at various times!

Chapter summary

Metabolism describes the processes which allow energy to be utilized to maintain the integrity of an organism. Catabolic reactions usually liberate energy which the cell uses to drive forward anabolic reactions. Energy changes are associated with chemical changes which would normally occur far too slowly to be of biological use to an organism, so enzymes are used to accelerate reactions. Enzymes are catalysts but share few characteristics with inorganic catalysts such as platinum. The relative specificity of each enzyme for its substrate(s) means that each cell of the body requires hundreds of different types of enzyme and each type must be present in multiple copies. Enzyme-catalysed reactions are arranged into pathways; sequences of individual reactions in which each enzyme brings about a small chemical change. Keep in mind the road traffic analogy. Pathways are controllable and adaptable.

Learning metabolism requires a step back to focus, initially at least, not on the minute details but on the biological purpose(s) of a pathway. Look for patterns and similarities between pathways and always ask the questions 'what does this pathway do for *me*?' and 'how does this pathway adapt to changing physiological situations?' Be an active learner and make it personal!

The word puzzle. There are probably several ways to do this, here is one way:

went → want → wane → cane → came → come

Notice that apart from the number of letters, the first and last words are structurally very different and indeed have opposite meanings yet there is a logical progression.

Problems and challenges

1. Distinguish between . . . free energy, entropy and enthalpy
2. Define the terms endergonic and exergonic
3. What information is given by the sign ($+$ or $-$) of the free energy value?
4. Why does metabolism *not* grind to a resounding halt when an endergonic reaction occurs within a pathway?
5. *Without* performing any calculation, state with reasons if the following reactions are likely to be strongly exergonic, weakly exergonic, strongly endergonic or weakly endergonic:
   i. R $\rightarrow$ P $K_{eq} = 0.005$
   ii. R $\rightarrow$ P $K_{eq} = 127$
   iii. R $\rightarrow$ P $K_{eq} = 2.5 \times 10^{-4}$
   iv. R $\rightarrow$ P $K_{eq} = 0.79$
   v. R $\rightarrow$ P $K_{eq} = 1.27$
6. Like glucose-6-P, pyruvate and acetyl-CoA are at metabolic cross-roads. Consult a metabolic map and identify these important compounds and note the ways in which they may be formed and metabolized.
7. Refer to Section 1.4. What type of enzyme-catalysed reaction is occurring in each of the following examples?
   a. Glucose-6-phosphate $\rightarrow$ Fructose-6-phosphate
   b. Fructose-6-P + ATP $\rightarrow$ Fructose-1,6 *bis*phosphate + ADP
   c. pyruvate + CO_2 $\rightarrow$ oxaloacetate
   d. Fructose-1,6-*bis*phosphate + H_2O $\rightarrow$ Fructose-6-phosphate + Pi
   (NB: Pi is an abbreviation for inorganic phosphate)