6

How Fast, How Far? Chemical Kinetics and Equilibrium

An aim of this book is to demonstrate how energy is accessed and utilized to sustain life on Earth. Chemical reactions play a major role in this, so the more we understand about chemistry, the easier the task should become. Let's reflect on what needs to be addressed. Ideally, scientists would like to be able to predict the likely result of any proposed reaction. That would provide confidence that chemical theory is soundly based. It would encourage further work to refine the ideas—no one would suggest that everything is known. Experience has shown that deeper understanding leads to better prediction, with the result that knowledge is continually expanding.

Much of what we need to know about chemical reactions can be summarized in a few words:

Which way?
How fast?
How far?
What?
How?

For any given reaction, these aspects are totally integrated and any order of treatment will be somewhat arbitrary. How far have we come so far in the search to understand chemical reactions?

Chapter 4 dealt rather briefly with *which way*, by introducing the Second Law of thermodynamics and a means to determine the direction of a spontaneous change—a negative ΔG is required for any spontaneous process, with contributions from enthalpy and entropy. Entropy might be conceptually challenging, but its effects are very real and in some processes a dominating influence.

Chapter 5 described the way in which atoms combine to form molecules: the importance of relative electron energies and the formation of molecular orbitals. The section on mechanisms provided some insight about the rearrangements of bonding electrons to form new compounds. It partially answers the *how* question.

The *what* question is mostly about identifying the products of a reaction. Predicting the products beforehand is a harder proposition, but ultimately the one that provides the richest rewards. A systematic knowledge of reaction mechanisms is important for full understanding and prediction.

The remaining questions—'*how fast, how far*'— are the topics to be addressed in this chapter.

How fast is the topic of chemical kinetics. I won't treat this topic formally, concentrating instead on the basic concepts in the context of biological reactions.

Consider the simple organic reaction between chloromethane and hydroxide ion in water:

$$HO^- + CH_3Cl \longrightarrow CH_3OH + Cl^- \qquad (6.1)$$

This reaction proceeds spontaneously and the products, methanol and chloride ion, can be isolated in high yield. This is far from the whole story, so let's look at the details.

1) We have seen in Chapter 4 that if ΔG is negative, a reaction will be spontaneous. That is the case here, as $\Delta G^0 = -105.6$ kJ mol^{-1}. This answers the question *which way* quantitatively. It is a large value, confirming the high yield of products. As a comparison, it can be shown that any reaction that goes to 99.9% completion at 25°C has a ΔG of -17.1 kJ mol^{-1} (Streitwieser and Heathcock 1981).
2) How fast? This is our interest here. What factors determine the rate of a reaction?

Though ΔG^0 in reaction (6.1) is large in favour of the products, the reaction is actually quite slow. A 0.05 M solution of chloromethane in 0.1 M aqueous NaOH solution reacts to only to the extent of about 10% in 2 days at room temperature (Streitwieser and Heathcock 1981). Obviously, favourable thermodynamics is not enough. There must be a suitable pathway available. Chemical reactants usually encounter an energy barrier before any reaction takes place. This is particularly the case in organic reactions when covalent bonds are broken and formed. This energy barrier is formally called the activation energy for the particular reaction. The values of activation energies can vary greatly from reaction to reaction. A useful way to visualize the energy changes during the course of a reaction is to plot a reaction profile. Potential energy is plotted vs the reaction coordinate to represent the progress of a reaction (Figure 6.1).

High activation energies lead to slow rates, and vice versa. The ΔG^* for reaction (6.1) is about 105 kJ mol^{-1}.

The average kinetic energy of molecules at room temperature is about 2.5 kJ mol^{-1}. However, this is an average value, and molecules are constantly colliding

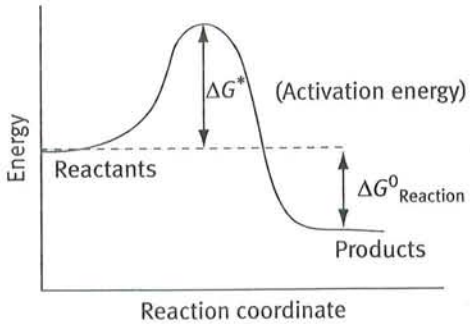


Figure 6.1. A reaction profile for a one step chemical reaction that has a negative $\Delta G_{reaction}$ and activation energy ΔG^*. Reactions with high values of ΔG^* tend to be slower and *vice versa*.

and exchanging energy. There will be a few with energies above 105 kJ mol^{-1}, hence the 10% reaction over 2 days at room temperature. At a higher temperature the average kinetic energy of the molecules will be greater, a larger number will achieve the activation energy in a given time, so the rate will increase (Figure 6.2). Thus, the rate of reaction (6.1) at 50°C is about 25 times that at 25°C.

Under any set of conditions, molecules have a distribution of kinetic energy depending on the absolute temperature. As the temperature is increased, the distribution of energy broadens, and shifts to higher-energy values (Figure 6.2) The curves are skewed slightly in the high-energy direction; a small percentage of molecules has very high kinetic energy. The result is that, even when the activation energy is high, some reactions will occur at room temperature.

Reaction (6.1) provides a useful example. It answers the which way, how fast, and how far questions quantitatively. It is important to note that the magnitude of $\Delta G_{reaction}$ tells us nothing about the rate. The sign of $\Delta G_{reaction}$ tells us the direction of change (which way) and its magnitude informs us of the position of equilibrium (how far). The position of equilibrium will be considered in some depth later in the chapter.

Consider another example. If I were to mix 2 L of hydrogen and 1 L of oxygen in a glass flask and seal it, what would happen? At room temperature, nothing much. One could observe the flask for days and still see no change. However, if I were to arrange to generate a small electric spark inside the flask, you would certainly notice a difference. There would be a flash, a few drops of water would form in the flask, and the temperature would rise slightly, showing that some energy was liberated overall. Temperature is a reflection of the average kinetic energy in a molecular system. If the glass of the flask were too thin it might collapse—3 moles of gas becoming 2 moles of liquid water would result in a partial vacuum inside. The equation for this reaction is:

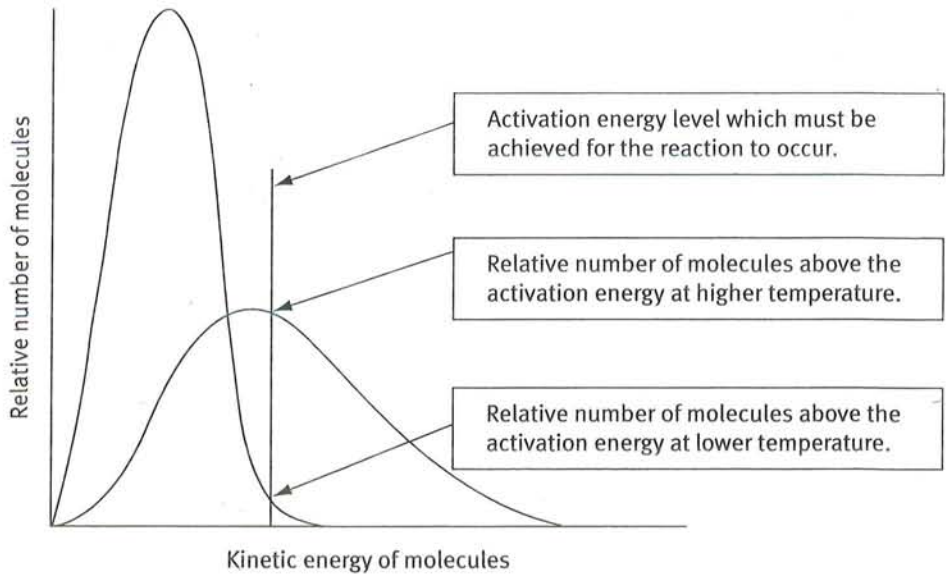


Figure 6.2. Distribution of molecular kinetic energy. At higher temperatures, a greater number of molecules will be above the activation energy level, resulting in a faster reaction rate.

$$2H_2(g) + O_2(g) \longrightarrow 2H_2O(l) \qquad (6.2)$$

The spark provides enough energy to initiate the reaction, but why was energy needed to set it off under the prevailing conditions, and once started, why did it continue?

The covalent bonds in hydrogen and oxygen molecules are quite stable at room temperature and pressure. To react, the molecules must be brought into intimate contact and any repulsion between them must be overcome. In the case of hydrogen and oxygen at room temperature, the energy distribution is such that an insignificant number of molecules possess the activation energy. Once the kinetic energy of a few more molecules of hydrogen and oxygen has been boosted above the activation energy level by the energy from the spark, there is enough heat energy liberated by the reaction itself to break a few more H–H and O=O bonds, which rearrange to form more water, liberating more energy to break more bonds, and so on. All this happens very fast so that the hydrogen and oxygen form water literally in a flash.

The involvement of the spark is another example of the statement that a chemical reaction will proceed only if there is a suitable pathway available for it to do so. Many chemical reactions need an energy boost to get them going at a reasonable rate at room temperature. Some, like the formation of water above, once given the initial boost will generate enough energy to keep going without the need for any more external energy. Such reactions liberate energy overall and are therefore exothermic. Other reactions might need to be heated continuously to provide sufficient activation energy for the molecules to be converted to products at a reasonable rate. Most chemical and biochemical reactions need some activation energy; some need much more than others. In the case of industrial processes, slow reaction rates may not be economical. One solution to this problem is to raise the temperature of the entire reaction, increasing the average kinetic energy of the molecules. More molecules will achieve the activation energy, so more will react in a given time. A useful rule of thumb is: for each 10°C increase in temperature, the rate of a reaction will approximately double.

Another approach is to use a catalyst, which alters the rate of a chemical reaction. Catalysts have proved to be extremely valuable. Intense efforts by the chemical and pharmaceutical industries have produced an array of catalysts, many of which are protected by patents. The reaction between hydrogen and oxygen mentioned above may be brought about at room temperature by a catalyst such as finely powdered platinum metal. To merely say that catalysts speed up a chemical reaction by lowering the activation energy is an oversimplification. Usually the detailed mechanism is changed, so that the new, catalysed pathway has lower activation energies (Figure 6.3).

For a reaction to proceed spontaneously under the given conditions, the Gibbs energy change, ΔG, must be negative. Note that the free energy change for the overall reaction is the same with or without the catalyst. Usually, only a small amount of catalyst is required, much less than the stoichiometric amounts of the reactants. This is because the catalyst, once it has done its job, can be released from the products to catalyse more reactant molecules. A true catalyst

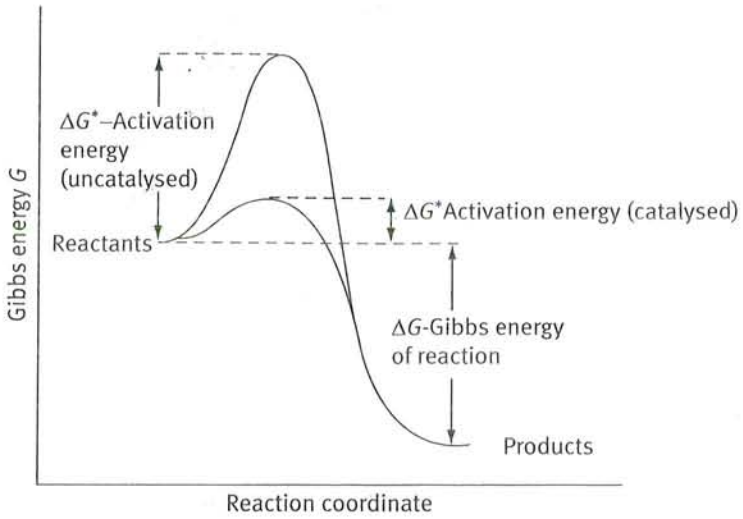


Figure 6.3. Reaction coordinate for uncatalysed and catalysed reactions. The activation energy, ΔG^{*}, is lower for the catalysed reaction, so the reaction rate is increased. The Gibbs energy change of the overall reaction, $\Delta G_{\text{reaction}}$, is not altered by the presence of a catalyst.

is not permanently changed by the reaction (6.1) catalysed. This is important industrially, as catalysts are usually expensive. Catalysis is vitally important in biology, as the enzymes that catalyse and modulate biochemical reactions are normally present in quite small amounts, and are a precious resource for the organism involved. Much more about enzymes, their very special properties, and the energetics of enzyme-catalysed reactions will be discussed in Chapter 8.

We haven't yet addressed the question 'why should activation energy be required at all?'

As we have seen, the rate of a reaction depends on the fraction of molecules above the activation energy. It also depends on the concentration of reacting molecules because concentration directly determines the probability that any two molecules will collide and initiate a reaction. Reaction rates are directly proportional to concentration, so if we let the rate of reaction equal v, then

$$v \propto [\text{reactants}]$$

or

$$v = k\,[\text{reactants}]$$

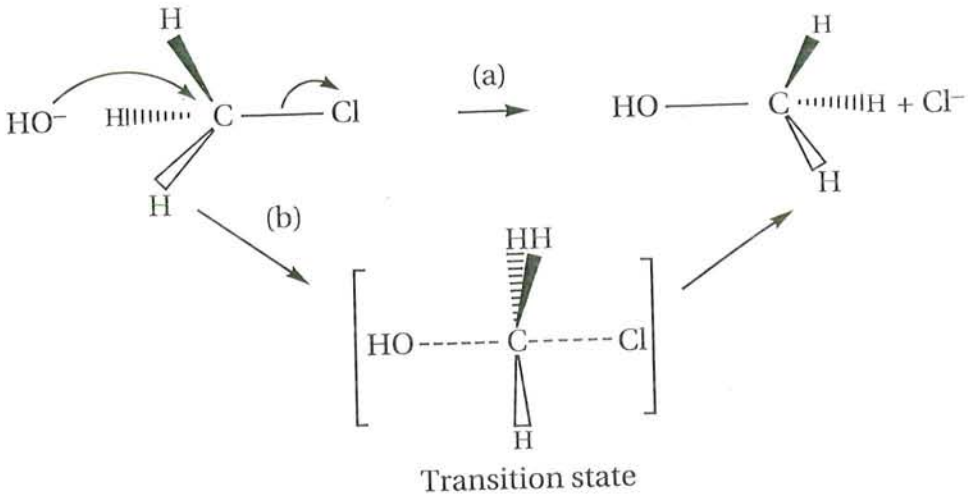
where k is the rate constant. Square brackets are used from here on to denote the concentration in moles per litre (mol L^{-1}).

The rate of reaction (6.3) depends on two reactant concentrations and is classified as a second-order reaction.

$$HO^{-} + CH_3Cl \xrightarrow{k} CH_3OH + Cl^{-} \qquad (6.1)$$

$$v = k\,[HO^{-}][CH_3Cl]$$

Reaction (6.1) probably involves a single-step mechanism. The short picture is the direct displacement of Cl^{-} by HO^{-} (a). The route via a transition state (b) is more realistic and more informative.



The transition state model explains much of the energetics and kinetics of reactions. In general, as the reaction proceeds along the reaction coordinate, the potential energy increases until it reaches a maximum, which determines the activation energy ΔG^*. In molecular terms, the energy maximum corresponds to the transition state. The transition state is the position of maximum energy partly because to achieve it there often is a crowding of different reacting species into a relatively small volume. In the case of reactions such as (6.1) the transition state involves a fleeting situation where all the groups attached to the central carbon atom are coplanar, as shown in route (b) above, where steric interference is at a maximum. The dotted bonds indicate that as the C–Cl bond is breaking, the HO–C bond is forming in a concerted manner. The molecule is on its way to being flipped inside out like an umbrella, or inverted. This inversion process can have a particularly large effect on ΔG^* when the attached groups are bigger than the hydrogens in reaction (6.1). For a reaction with a single transition state as proposed for reaction (6.1), the formation and breakdown of the transition state will be the rate-determining step of the reaction.

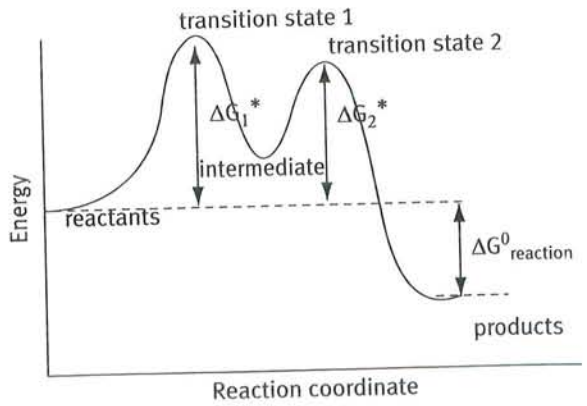


Figure 6.4. Profile of a reaction involving one relatively stable intermediate species. The rate-determining step in such a reaction will be formation of the transition state with the greater activation energy, ΔG^*. In this example formation of transition state 1 is rate determining.

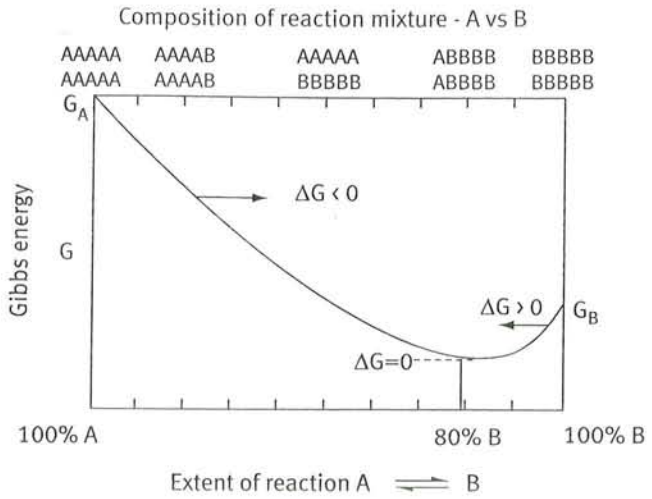


Figure 6.5. A plot of Gibbs energy vs extent of reaction for the conversion of compound A to compound B. The composition at equilibrium is 80% B. For any composition below 80% B, $\Delta G < 0$ and the reaction will be spontaneous in the A–B direction. For any composition above 80% B, it will be spontaneous in the B–A direction. At equilibrium, 80% B, $\Delta G = 0$, and the composition will remain constant.

Some reactions are more complex than (6.1) and may involve a relatively stable intermediate species. Such a reaction profile is shown in Figure 6.4. There would be two activated complexes, and the one with higher activation energy (ΔG_1^*) would be the rate-determining step. There are many examples of such stable intermediates in enzyme-catalysed reactions such as the protease chymotrypsin (see Figure 8.15).

To answer *how far*, we must deal with the state of equilibrium for chemical reactions. This will allow us to discuss the principles of chemical reactions as applied to metabolism, especially what needs to be considered when a number of reactions are involved in metabolic pathways. It is an interesting and useful fact that few chemical reactions proceed only in one direction. To some extent, most reactions are reversible. Most often we write reactions so that they proceed from left to right on the page. This is just a convenient convention. The reactants appear on the left of the equation and the products on the right, again by convention. To indicate a reaction is at least partially reversible, a double arrow is used:

$$A \rightleftharpoons B \tag{6.3}$$

These arrows alone don't tell us much. To understand the full implications of a reversible reaction, including the energetics involved, we need to express what is happening quantitatively, just as we have with ΔG, ΔH, etc.

As an introduction to this, let's look at a graphical way to represent Gibbs energy changes in a reaction.

Figure 6.5 shows a plot of Gibbs energy G vs extent of reaction. Several points are important to note:

- The reaction as written will be exothermic and will proceed spontaneously ($\Delta G < 0$) from a maximum value of G, when the composition is 100% of A molecules, towards the formation of B.
- When the composition reaches 80% B/20% A, the slope of the curve is zero ($\Delta G = 0$) and the reaction will be at equilibrium.

- If we were to start with 100% B, the reaction would take place in reverse (B $\longrightarrow$ A) until the composition reached 80% B and 20% A.
- The plot is a curve with a minimum point in G rather than a straight line going from 100% A to 100% B. This is in part because there is a contribution to G arising from the mixing of A and B molecules. The A/B ratio changes continuously during the course of the reaction A $\longrightarrow$ B, so its contribution to G will vary during the course of the reaction (see the composition diagram at the top of Figure 6.5).
- As soon as the reaction starts, there will be some B formed. The concentration of B will increase until the forward reaction A $\longrightarrow$ B occurs as the same rate as the reverse reaction (B $\longrightarrow$ A). This occurs at 80% B/20% A in our example.
- At 80% B, the situation is described as a dynamic equilibrium. Despite the constant overall composition, some individual molecules of B are reacting to form new molecules of A, at the same rate that some molecules of A are reacting to form new molecules of B. At the molecular level, plenty is happening, but overall the reaction is going nowhere.
- As the magnitude of G varies with the concentration of A relative to that of B, we can calculate the value of G at any [A]/[B] ratio. Importantly for our understanding of biological reactions, the curve in Figure 6.5 shows that ΔG for such a reaction will be greatest the farther the reaction is from equilibrium. This has two implications for regulation of metabolism: (i) a reaction with a large negative ΔG is often a site for regulation by allosteric control and (ii) reactions close to equilibrium can be reversed in some metabolic pathways. This is useful if the cell needs to change the pathway from a catabolic to an anabolic role (see Chapter 11).

How do we express all this information quantitatively? Firstly, a few more words about rates of reaction.

For the reaction:

$$\mathrm{A} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{B} \tag{6.4}$$

k_1 and k_2 are the rate constants for the forward and reverse reactions, respectively. Let the molar concentrations of reactant A and product B be indicated by square brackets [A] and [B].

The rate of the forward reaction is given by: $v_{\text{forward}} = k_1[\mathrm{A}]$.

The rate of the reverse reaction is given by: $v_{\text{reverse}} = k_2[\mathrm{B}]$.

At equilibrium, the forward and reverse rates are equal, so at equilibrium,

$$k_1[\mathrm{A}] = k_2[\mathrm{B}] \tag{6.5}$$

This rearranges to:

$$\frac{\text{rate constant for the forward reaction}}{\text{rate constant for the reverse reaction}} = \frac{k_1}{k_2} = \frac{[\mathrm{B}]}{[\mathrm{A}]} = K_{\text{eq}} \tag{6.6}$$

To make the treatment applicable to all chemical reactions, I will change from the simple reaction (6.4) used so far to a general expression for a reaction:

$$\mathrm{aA} + \mathrm{bB} \rightleftharpoons \mathrm{cC} + \mathrm{dD} \tag{6.7}$$

where the reactants A and B, plus the products C and D are present in their stoichiometric (balanced equation) ratios *a*, *b*, *c*, and *d*, respectively. Expressing the equilibrium constant for this reaction leads to:

$$K_{eq} = \frac{[C]^c[D]^d}{[A]^a[B]^b} \tag{6.8}$$

The overall Gibbs energy change can be shown (but not here) to be:

$$\Delta G = \Delta G^0 + RT \ln \frac{[C]^c[D]^d}{[A]^a[B]^b} = \Delta G^0 + RT \ln K_{eq} \tag{6.9}$$

where ΔG^0 is the standard free energy change for the reaction (that is, the ΔG under standard conditions) T is the absolute temperature, R the gas constant (Boltzmann's constant, k_b, per mole) and $[A]^a$, etc. means the molar concentration of reactant A raised to the power '*a*'.

Equation (6.10) consists of two parts: a constant term, ΔG^0, which depends only on the particular reaction taking place, and a variable term, $RT \ln = \frac{[C]^c[D]^d}{[A]^a[B]^b}$, which depends on the temperature, concentrations of the reactants and products, and the stoichiometric relationships *a*, *b*, *c*, and *d*.

At equilibrium, the forward reaction is exactly balanced by the reverse reaction, so $\Delta G = 0$.

It follows that at equilibrium:

$$\Delta G^0 = -RT \ln \frac{[C]^c[D]^d}{[A]^a[B]^b} = -RT \ln K_{eq} \tag{6.10}$$

where the subscript 'eq' signifies equilibrium, that is the concentrations refer to the concentrations at equilibrium. If the equilibrium constant can be determined, ΔG^0 can be calculated, and vice versa. The standard states agreed by convention and used in most thermodynamics tables are:

$$\text{pressure: } 10^5 \text{ Nm}^{-2} = 10^5 \text{ Pa (pascal)}$$

$$\text{temperature : } 273.15 \text{ K or } 25^\circ\text{C}$$

For pure solids and liquids the standard state is the pure solid or pure liquid. For solutes the standard state is 1.00 M concentration.

Different standard conditions have been adopted by biologists, who use 25°C and pH = 7.0, as these are close to physiological conditions for many organisms. When the biological standard states are used, this is indicated by the addition of a prime (′), after the symbol. Thus ΔG^0 becomes $\Delta G^{0\prime}$, ΔH^0 becomes $\Delta H^{0\prime}$, and ΔS^0 becomes $\Delta S^{0\prime}$. Thermodynamic values can vary substantially between the two standard states because the normal thermodynamic standard state concentration for H^+ is 1 M, or pH = 0. This is obviously unsuitable for most biochemical reactions, which take place in buffered solutions close to pH 7. Major differences will occur between ΔG^0 and $\Delta G^{0\prime}$ when H_2O and H^+ are involved in the reaction. This is the case in many biological reactions. Rather than incorporate specific terms for $[H^+]$

and $[H_2O]$ in equations such as (6.10), they are usually incorporated into the $\Delta G^{0\prime}$ and K_{eq} values. When water and protons are *not* involved, $\Delta G^0 = \Delta G^{0\prime}$.

We'll see in Chapter 11 that ΔG values under conditions in the cell (physiological conditions) can be markedly different from the ΔG^0 or $\Delta G^{0\prime}$ values under standard conditions. It is useful to bear in mind that the ΔG of a chemical reaction is just another way of expressing the equilibrium constant, and vice versa. Because the relationship between ΔG and K_{eq} is logarithmic, small changes in ΔG result in large changes in K_{eq}.

To gain some idea of the relative magnitudes of ΔG^0 and the corresponding K_{eq}, let us use some specific values.

Applying equation (6.10)

$$\Delta G^0 = -RT \ln K_{eq}$$

Using values of 2.26×10^{-4}, 1, and 2.26×10^4 as examples of K_{eq}, we can draw up a table to show how the values of ΔG^0 vary with changes in K_{eq}.

ΔG^0 (kJ mol^{-1})	K_{eq}*
+20.9	2.26×10^{-4}
0	1
−20.9	2.26×10^4

*K_{eq} has no units. It is the ratio of k_1/k_2 so is a simple number.

A value of $K_{eq} > 10^4$ ($\Delta G^0 < -20.9$ kJ) means that the rate constant for the forward reaction is $>10^4$ times that of the reverse reaction. As a rule of thumb, this means the reaction goes to completion as written.

Conversely, if $K_{eq} < 10^{-4}$ ($\Delta G^0 > +20.9$ kJ) the reverse reaction will be favoured to completion.

The above relationships between ΔG^0 and K_{eq} can be summarized to:

If $\Delta G^0 < 0$ $K_{eq} > 1$

If $\Delta G^0 = 0$ $K_{eq} = 1$

If $\Delta G^0 > 0$ $K_{eq} < 1$

Suppose we have a reaction which has come to equilibrium. Equation (6.10) shows that if some change moves the reaction away from equilibrium, this will stimulate a change in the direction of restoring the equilibrium. The system will change to restore the equilibrium concentrations of the reactants and products. This is known as Le Chatelier's principle (after French chemist H.L. Le Chatelier (1850–1936)).

What factors influence the position of equilibrium? Firstly, let us consider the effect of temperature on the equilibrium constant.

Using eqn (6.10), $\Delta G^0 = -RT \ln K_{eq}$, and $\Delta G^0 = \Delta H^0 - T\Delta S^0$, we can show that

$$\ln K_{eq} = -\Delta G^0/RT = -(\Delta H^0/R)(1/T) + \Delta S^0/R \qquad (6.11)$$

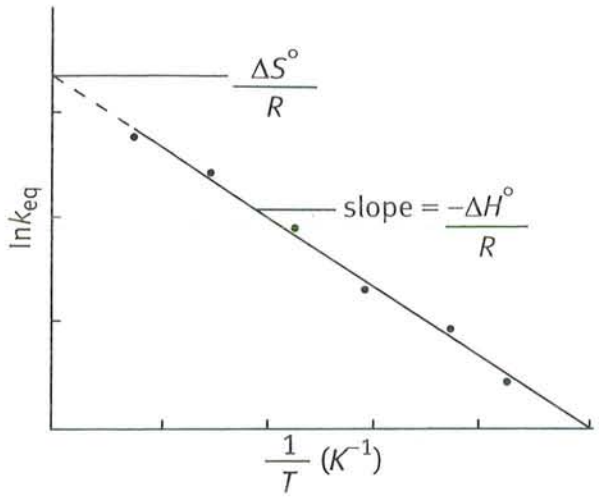


Figure 6.6. A typical van't Hoff plot of ln K_{eq} vs $1/T$.

Equation (6.11) implies that $\ln K_{eq}$ is inversely proportional to the absolute temperature. If H and S are not greatly influenced by a small change in temperature, often the case over the relatively small biological temperature range, a plot of $\ln K_{eq}$ vs $1/T$ should be linear, with a slope equal to $-\Delta H^0/R$ and an intercept equal to $\Delta S^0/R$.

This is called a van' Hoff plot, after J.H. van't Hoff' (1852–1911) a Dutch physical chemist, the first Nobel laureate in chemistry, awarded in 1901 (Figure 6.6).

The value of such a plot is that it can yield values for ΔH^0 and ΔS^0, so long as K_{eq} can be measured experimentally over a suitable temperature range (Haynie 2001).

Equation (6.11) also shows that the equilibrium constant depends on $\Delta H^0{}_{\text{reaction}}$. If the reaction is endothermic ($\Delta H^0{}_{\text{reaction}}$ is positive) then $(-\Delta H^0/RT)$ will be negative, so as T is increased, K will increase.

We can therefore conclude that:

a) for an endothermic reaction, increasing the temperature will increase K, that is will shift the equilibrium in favour of the products
b) if the reaction is exothermic, increasing the temperature will, for similar reasons, cause a decrease in K and shift the equilibrium to favour the reactants.

Both conclusions are probably familiar from elementary chemistry as examples of Le Chatelier's principle.

Secondly, let us consider the influence of concentration on the position of equilibrium. For the reaction:

$$A + B \rightleftharpoons C + D$$

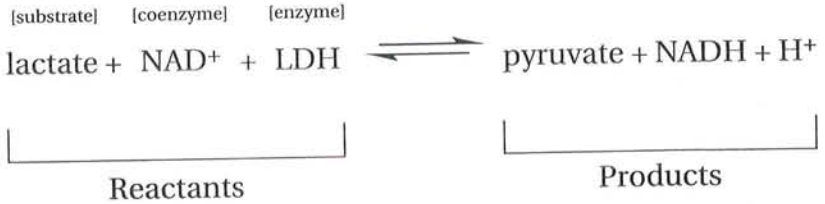
$$K_{eq} = \frac{[C][D]}{[A][B]}$$

Suppose fresh A and B atoms were to be added to those already present at equilibrium (without changing the volume). What will occur? This amounts to

an increase in concentration of A and B, which will increase the forward reaction rate to form new C plus D, until the new C plus new D molecules reach a concentration such that the reverse reaction rate once again is equal to the forward rate, and equilibrium will be re-established—another example of Le Chatelier's principle at work. During the many processes involved in metabolism in a biological system, we seldom have the simple system described above. Usually there are other reactions feeding in to form A and B, while C and D are usually removed by further reactions in the metabolic pathway. In living organisms, there obviously needs to be some control over the production of A and B, and over the removal of C and D.

The *how far* of the chapter title also concerns how far towards or how far from equilibrium living organisms exist, and how they achieve and maintain these positions. Supposing we have a situation inside a cell in which the equilibrium constant of a reaction is small. Its ΔG is small and positive, meaning that there is very little tendency for the reaction to proceed spontaneously in the direction written.

Now suppose the cell is in need of the products C and D, or perhaps needs to get rid of A and B. How can this be achieved? This is a real situation for cells, an example being the oxidation of lactate to pyruvate at pH 7. Lactate (lactic acid) builds up in muscle cells when they work vigorously, say during a sprint race, as aerobic respiration can't keep up with energy demand. This can only be tolerated for a short time. We experience pain as lactate builds up, and eventually are forced to stop, pant and use the gulped air to remove the lactate from our aching muscle cells. The cells use the gulped air to oxidize the lactate to pyruvate, which then enters aerobic metabolic pathways and is oxidized further. The oxidation reaction requires the involvement of an enzyme, lactate dehydrogenase (LDH), and a coenzyme, NAD. An enzyme doesn't alter the equilibrium constant of the reaction it catalyses; it increases the rate at which the reaction occurs. A coenzyme is usually a small molecule which, as its name suggests, helps some enzymes to achieve their purpose. Not all enzymes require a coenzyme.



(6.12)

At pH 7 and 37°C, ΔG^0 is positive, and the oxidation of lactate is not possible under physiological conditions. However, if the concentrations of lactate and NAD^+ are in great excess over pyruvate and NADH (which is the case after enough vigorous exercise) the overall ΔG actually becomes negative and the reaction will proceed spontaneously in the direction written. Actually it is the concentration ratio

$$\frac{[\text{pyruvate}][\text{NADH}][\text{H}^+]}{[\text{lactate}][\text{NAD}^+]}$$

rather than the absolute concentrations, that are the key factors determining the direction of the reaction. To keep the reaction running, it is necessary to keep the above concentration ratio small, that is much less than 1.

This can be achieved by removing the products, pyruvate and NADH, as rapidly as they are made. This has important consequences for an energetically unfavourable reaction embedded in a metabolic pathway. The statement can be made:

It is possible to keep energetically unfavourable reactions running by continuously removing the products.

In the above example, pyruvate is removed by immediate conversion to another compound, acetyl coenzyme A, in a manner that will be described later (Chapter 11). Everyone at some time has surely felt the discomfort of lactate build up in the muscles after strenuous exercise and knows that it takes a while for this pain to ease. Once again a knowledge of thermodynamics, this time as applied to chemical equilibria, has helped us to explain the workings of part of a biological system with which we are all familiar.

Let us consider at this point some more of the requirements for effective metabolism in a living organism.

All metabolic reactions are governed by the same laws of chemistry described above. There is nothing special or mystical about the basic chemistry of life, but there are many things very special about the way this chemistry is arranged, regulated, and coordinated. If we take humans as the example organism, all the enormous variety of reactions needs to occur at about body temperature, 37°C. This is a considerable constraint, or at least would be for traditional synthetic chemists. In addition, the reactions occur mainly in an aqueous environment, they must usually avoid strongly acidic or strongly alkaline conditions (which are damaging to our tissues), and be protected against too much oxygen. Such constraints would send most synthetic chemists crazy, for they usually need to spend a great deal of time and effort to prevent getting water, and often oxygen, into their reactions. The fact that so many biological reactions are able to take place under mild aqueous conditions is a tribute to the power of biological evolution, which has, over time, been able to hone the workings of life with such exquisite subtlety that even we, arguably its cleverest product, can't come close to matching them.

Why should there be so many reactions needed by a living organism, even a simple one? One reason is that to obey the laws of thermodynamics, under mild conditions and in water surroundings, some rather round-about pathways need to be followed. Suppose an organism needs endothermic reactions to take place, for example a plant needs to produce cellulose to strengthen it or an animal needs to produce collagen, a protein in tendons and connective tissues. Putting glucose molecules together to form cellulose requires an input of energy; so does putting amino acids together to form proteins and two-carbon units to synthesize fatty acids.

What are the sources of energy available to the plant or animal? The reactants can't be heated to boiling, as a chemist might be able to do, so where can the energy be accessed? The answer is deceptively simple.

The energy released by a spontaneous, exothermic chemical reaction ($\Delta G < 0$) may be used by the plant or animal or any living system to 'drive' an endothermic reaction ($\Delta G > 0$) of whatever type may be needed. To achieve this in a controlled manner requires the two reactions to be 'coupled' in a specific way. This coupling of reactions is used by living organisms for a number of purposes:

1) to allow the synthesis of molecules needed for various structures such as cellulose or proteins
2) to maintain the temperature of warm-blooded creatures
3) to synthesize a range of essential biological molecules which cannot be provided directly by the diet
4) for movement
5) for transport of molecules around the body and through biological membranes.

All of these processes are endothermic: they require an input of energy to proceed. The use of coupled chemical reactions is one of the ingenious means by which biology provides such specific energy requirements.

As an example of energy coupling, firstly of a non-chemical nature, let us look at a mechanical analogy. Suppose a builder wants to raise a block of stone, mass 50 kg, from the ground to a ledge 5 m above. This is an energy-requiring, endothermic process, and will certainly never proceed spontaneously. Suppose the builder arranges to have a pulley system attached to a beam about 7 m above ground, with one end of a rope tied around the block of stone, running over the pulley to the other end, which is held by a 80-kg man standing on the 5 m ledge. If the 80-kg man were to jump from the ledge, his gravitational potential energy would become available, through the rope, to lift the block. The tendency of the man to fall to ground would be his weight, 80-kg, and the tendency to stop him falling would be the weight of the block, 50-kg weight. The net tendency of the block to move upwards would be about 30 kg, that is $\Delta G = -30$ units for the upwards direction. The 30 kg should be plenty to overcome the small amount of friction in the pulley. The block would move up and the man would fall to the ground, but more slowly (and safely) than if he had jumped freely. Thus an exothermic process, an 80-kg man falling under gravity, will have been coupled to an endothermic process, the block being raised against gravity, in such a way that the block is raised successfully. The whole process will occur spontaneously once the man jumps free of the ledge. In our example of mechanical work, the intensity factor, gravitational potential, is the height the weight was lifted, and the capacity factor is the mass of the block.

So much for the mechanical analogy. Now let's consider an actual example of energy coupling for a biochemical reaction. The amide group of the common amino acid glutamine is a major donor of nitrogen in the biosynthesis of compounds such as purines, pyrimidines, and some other amino acids. Glutamine is formed from glutamate and ammonium ion, catalysed by the enzyme glutamine synthetase. The reaction is ATP-dependent, and requires Mg^{2+} ion, as many ATP reactions do. It is a central control point in nitrogen metabolism. Glutamine is the amino group donor in many biosyntheses and is a storage form of ammonia.

The two reactions involved are:

$$\text{glutamate} + NH_3 \longrightarrow \text{glutamine} + H_2O \quad \Delta G^{0\prime} = +14.2 \text{ kJ mol}^{-1} \tag{6.13}$$

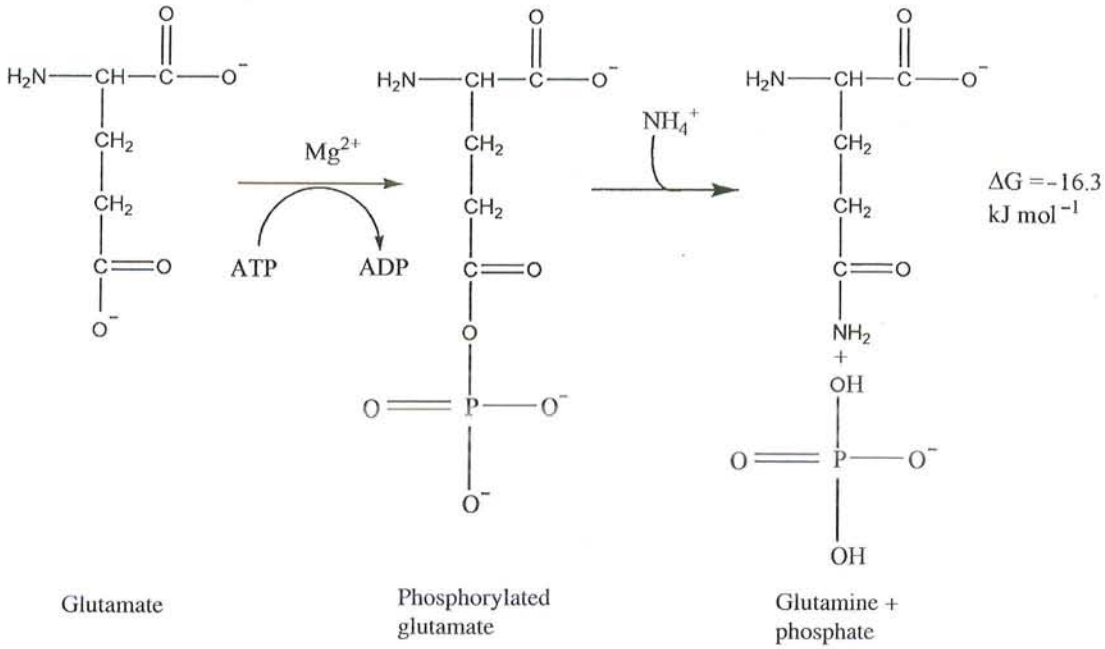
$$ATP + H_2O \longrightarrow ADP + \text{phosphate} \quad \Delta G^{0\prime} = -30.5 \text{ kJ mol}^{-1} \tag{6.14}$$

Reaction (6.13) is endothermic, and won't proceed spontaneously. Reaction (6.14) is exothermic and if coupled to (6.13) the overall reaction should be exothermic by $(14.2 - 30.5) = -16.3$ kJ mol^{-1} and spontaneous.

Adding eqns (6.13) and (6.14) leads to:

$$\text{glutamate} + NH_3 + ATP \longrightarrow \text{glutamine} + ADP + \text{phosphate}$$
$$\Delta G^{0\prime} = -16.3 \text{ kJ mol}^{-1} \tag{6.15}$$

The reaction mediated by glutamine synthetase, and coupled via ATP, becomes:



This example reinforces what we have shown above—it is possible to predict whether or not pairs of coupled reactions will occur spontaneously simply by summing the Gibbs energy changes for each reaction and looking at the sign of the resulting ΔG. Let us take as a second example adenosine triphosphate, ATP itself. ATP is used to drive many metabolic reactions, but then it must be regenerated from the 'lower-energy' compound such as adenosine diphosphate (ADP) formed as a result of the reaction.

The energy-producing reaction is:

$$ATP + H_2O \longrightarrow ADP + \text{phosphate} \quad \Delta G^{0\prime} = -30.5 \text{ kJ mol}^{-1} \tag{6.16}$$

The reverse, or regeneration, step is:

$$ADP + \text{phosphate} \longrightarrow ATP + H_2O \quad \Delta G^{0\prime} = +30.5 \text{ kJ mol}^{-1} \tag{6.17}$$

Reaction (6.17) is obviously an endothermic reaction that will not proceed without some energy input. One way in which the required energy is obtained is by coupling to an exothermic reaction, such as that involving the conversion of another 'high-energy' compound, phospho[enol]pyruvate (PEP) to pyruvate:

$$\text{PEP} + \text{H}_2\text{O} \longrightarrow \text{pyruvate} + \text{phosphate} \quad \Delta G^{0\prime} = -62.2 \text{ kJ mol}^{-1} \tag{6.18}$$

Adding eqns (6.17) and (6.18) we obtain:

$$\text{PEP} + \text{ADP} \longrightarrow \text{ATP} + \text{pyruvate} \quad \Delta G^{0\prime} = 30.5 - 62.2 = -31.7 \text{ kJ mol}^{-1} \tag{6.19}$$

As a shorthand way of expressing what is taking place, it is legitimate to eliminate phosphate and water from eqn (6.19), as each occurs on both sides when we add eqns (6.17) and (6.18), thus cancelling one another out. In this sense, we are treating eqn (6.19) as the algebraic sum of eqns (6.17) and (6.18). The resulting coupled reaction (6.19) is exothermic and will occur spontaneously from left to right as written. The reaction is catalysed by pyruvate kinase, an important reaction in glycolysis, that we will return to in Chapter 11.

This involvement of an enzyme is a vital aspect of coupled biochemical reactions. Reactions (6.17) and (6.18) would not be coupled if the reagents were merely mixed together in a test-tube, just as the block in the example above would not move upwards unless it was connected via the rope to the man when he jumped. Many ATP-coupled reactions involve the phosphorylation of an intermediate such as the phosphorylated glutamate above. The presence of the enzyme doesn't alter the overall thermodynamics of eqns (6.15) and (6.19), which on the basis of their negative ΔG values we would predict to occur spontaneously.

Just how great an effect can ATP coupling have on the equilibrium position of a reaction? Suppose we wish to know quantitatively by how much the equilibrium and the product/reactant ratio change for an ATP-coupled *in vivo* reaction. We saw in the LDH example above the importance of concentration ratios in keeping an energetically unfavourable reaction running. Here, we must include biologically realistic concentrations of ATP and other reactants in the calculation. In a cell, the concentrations of metabolites, ions, coenzymes, etc., vary with time and location. As pointed out above, the standard ΔG^0 or $\Delta G^{0\prime}$ values we have been using never occur in real cells. These values are used in many textbook examples because the real cellular concentrations of many species are difficult to determine experimentally. The standard values give a useful picture, but sometimes more realistic ones must be used.

Before answering the question at the beginning of the last paragraph, let's look at an example involving 'real' values of ΔG for physiological conditions.

For the hydrolysis of ATP:

$$\text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{ADP} + \text{P}_\text{i} \quad \Delta G^{0\prime} = -30.5 \text{ kJ mol}^{-1} \tag{6.20}$$

$$K_{eq} = \frac{[ADP][P_i]}{[ATP]}$$

$$\Delta G = \Delta G^{0\prime} + RT \ln \left[\frac{[ADP][P_i]}{[ATP]}\right]$$

Taking some 'typical' cell values:

$$[ATP] = 3.0\text{mM}, [ADP] = 0.8\text{mM}, \quad \text{and}$$

$$P_i = 4.0 \text{ mM at } 37^\circ\text{C (Voet } \textit{et al.}\text{ 2002)}$$

This reduces to $\Delta G = -30.5 - 17.6 = -48.1$ kJmol^{-1}

This value of -48.1 is much more exothermic than the standard state one of -30.5 kJ mol^{-1}.

Other values quoted are (i) -47.6 kJ mol^{-1} for a bacterial cell, using [ATP], [ADP], and [P_i] values of 8 mM, 8 mM, and 1 mM, respectively, at 25°C (Garrett and Grisham 2010), and (ii) -55 kJ mol^{-1} in human erythrocytes (Garrett and Grisham 1999). The results reinforce the effect of conditions on real ΔG values.

To our question. Using the values provided by Garrett and Grisham (2010) for a bacterial cell; ATP is typically present in 1000-fold excess over its hydrolysis products:

$$\frac{[ADP][P_i]}{[ATP]} = 10^{-3} \text{ approx.}$$

We can take any hypothetical equation having a $\Delta G^{0\prime}$ of our own choosing:

$$X \rightleftharpoons Y \qquad \Delta G^{0\prime} = +20.9 \text{ kJ mol}^{-1}$$

Then from the equation

$$\begin{aligned} \Delta G^{0\prime} &= -RT \ln K_{eq} \\ 20\,900 &= -(8.31)(298) \ln K_{eq} \\ \ln K_{eq} &= -3.6642 \\ K_{eq} &= 2.26 \times 10^{-4} = [Y_{eq}]/[X_{eq}] \end{aligned}$$

This is an energetically unfavourable reaction.

Coupling the same reaction to the hydrolysis of ATP, we obtain the overall equation:

$$X + ATP \rightleftharpoons Y + ADP + P_i$$

This is the same as the sum of the two uncoupled reactions:

$$X \rightleftharpoons Y \qquad \Delta G^{0\prime} = +20.9 \text{ kJ mol}^{-1}$$

$$ATP + H_2O \rightleftharpoons ADP + P_i \qquad \Delta G^{0\prime} = -30.5 \text{ kJ mol}^{-1}$$

$$\text{Overall } X + ATP + H_2O \rightleftharpoons Y + ADP + P_i \quad \Delta G^{0\prime} = -9.6 \text{ kJ mol}^{-1}$$

So
$$-9600 = -RT \ln K_{eq}$$

$$\ln K_{eq} = \frac{9600}{2476} = 3.87$$

$$K_{eq} = 48$$

By definition:

$$K_{eq} = \frac{[Y_{eq}][ADP][P_i]}{[X_{eq}][ATP]}$$

$$48 = \frac{[Y_{eq}][8 \times 10^{-3}][10^{-3}]}{[X_{eq}][8 \times 10^{-3}]}$$

$$\frac{[Y_{eq}]}{[X_{eq}]} = 48000$$

$$\text{For the uncoupled reaction } \frac{[Y_{eq}]}{[X_{eq}]} = 2.26 \times 10^{-4}$$

$$\text{So the ratio } \frac{K_{coupled}}{K_{uncoupled}} = \frac{4.8 \times 10^4}{2.26 \times 10^{-4}} = 2.12 \times 10^8$$

The equilibrium ratio of products:reactants in the coupled reaction is about 2×10^8 times that of the uncoupled one, turning an energetically unfavourable reaction into one that will proceed spontaneously to completion.

The above example illustrates a general principle. It reveals why coupling of reactions to ATP hydrolysis is utilized in so many different ways in living cells. The reaction coupled can be of any type, such as formation of a covalent bond in biosynthesis, ion transport across a membrane against a concentration gradient, or a change in conformation of a protein, as in muscle contraction or assisted protein folding.

A few more words on 'high-energy' biomolecules are appropriate here. Although most of the energy for life comes from the Sun, not all living organisms can process sunlight directly. Those which can are able to capture and store the light energy in various chemical compounds, such as carbohydrates. Examples are algae and green plants, which store mainly the carbohydrates starch or sucrose. Other organisms, such as mammals, including humans, are able to feed on these stored compounds, releasing the energy in reactions usually involving oxygen (recall the 'controlled burning' of sugars mentioned earlier) during the process of respiration. Despite some differences, both types of organism share mechanisms for generating useful forms of chemical energy. One of these is the use of high-energy phosphate compounds, such as ATP, GTP, ADP, PEP, acetyl phosphate, glucose-1-phosphate, and creatine phosphate. There are 'high-energy' compounds other than phosphates that are also used (see Box 11.1). Compounds are classified as being

high energy if they have a ΔG of hydrolysis more negative than about -25 kJ mol^{-1}. Equation (6.19) illustrates this: $\Delta G^{0\prime} = -31.7$ kJ mol^{-1}.

Hydrolysis is the name given to reactions involving compounds that are 'split' (lysed) with the involvement of water. In the case of phosphorylated compounds, during hydrolysis the phosphate group is transferred to water. It is important to distinguish between these high-energy phosphate compounds and the long-term energy storage compounds such as starch in plants and glycogen and fat in animals. The ATP type of high-energy compounds are transient forms of energy storage, molecules which carry energy from point to point for the moment-to-moment running of the cell. The high-energy compounds themselves must be regenerated for further use when the demand arises later. ATP in eukaryotes, for example, is largely synthesized from ADP during the process of respiration, in a key series of reactions called oxidative phosphorylation, details of which are discussed in Chapter 10.

This completes the discussion on the way in which coupled reactions may be used to utilize special high-energy compounds to form essential biomolecules. The reasons why some compounds have a large negative ΔG of hydrolysis are discussed in Box 6.1.

So far in this chapter we have seen two ways in which energetically unfavourable reactions can be made to proceed:

1) by removing the products of the reaction as fast as they are produced – see eqn (6.12)
2) by coupling of reactions involving, for example, ATP (eqns (6.15) and (6.19)). As explained in Box 6.1, this latter process is classified as a group transfer reaction. In these cases the essential group was phosphate.

Another mechanism of energy transfer, termed electron transfer, is important in biological energy processes and is discussed in Chapter 10. We have discussed endothermic and exothermic reactions and processes, and the fact that exothermic reactions and processes proceed spontaneously if there is a suitable pathway available. We have rationalized this by saying that spontaneous chemical reactions occur because the products are at a lower Gibbs energy than the initial reactants (ΔG is negative).

Reversible reactions are very useful in metabolism as they can be controlled to go one way under one set of conditions and in the opposite direction under a different set of conditions. This property is important in regulation of metabolic pathways (Chapter 11). We have stated that isolated systems, which don't exchange matter and energy with their surroundings, if left to their own devices will eventually reach a state of thermodynamic equilibrium.

However, in living systems, reversible reactions do not necessarily reach thermodynamic equilibrium as described above; in fact they mainly do not. A living organism that fits our definition of a system is an example of an open system, which may exchange matter and energy with its surroundings and is not at thermodynamic equilibrium. Such a system normally exists in a state of dynamic energy balance, sometimes called a steady state, but not in a state of thermodynamic equilibrium.

Box 6.1 ATP: the energy currency of cells

ATP is the canonical 'high-energy' compound of the biological world. Almost all cellular chemical energy use can be traced back to it. We have defined high-energy compounds as those having a standard Gibbs energy of hydrolysis, $\Delta G^{0\prime}$, more negative than about -25 kJ mol^{-1}. Thus:

$$\text{ATP} + \text{H}_2\text{O} \longrightarrow \text{ADP} + \text{phosphate} \quad \Delta G^{0\prime} = -30.5 \text{ kJ mol}^{-1} \qquad (6.20)$$

The structure and roles of ATP are worthy of further attention.

Coupled reactions using ATP and other high-energy compounds take advantage of their phosphoryl group transfer potential. Phosphoryl group transfer potential is a measure of the tendency of such compounds to transfer their phosphoryl groups to water, expressed in order of their $\Delta G^{0\prime}$ values (Table 6B1).

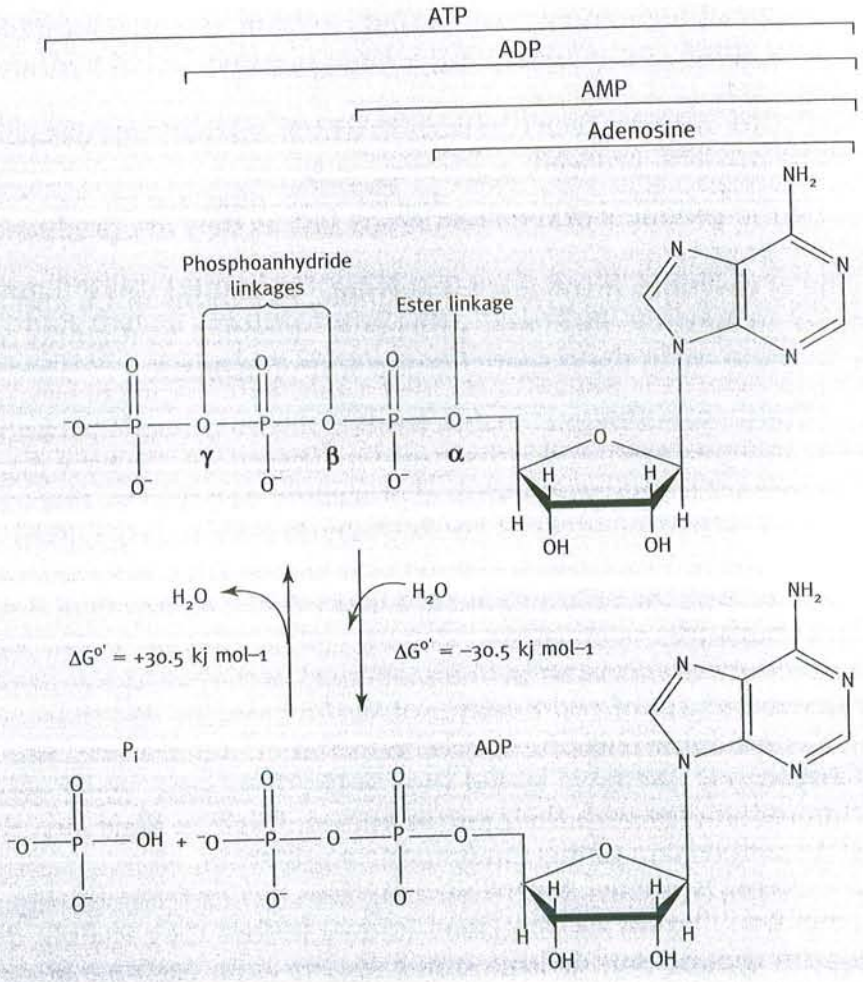


Figure 6.B1. ATP and related structures, showing the energetics of hydrolysis to ADP and inorganic phosphate (P_i) and the reverse reaction. The free energy of hydrolysis of ATP to AMP and inorganic pyrophosphate (PP_i) is also widely utilized in biological systems.

Box 6.1 (continued)

Table 6.B1. Some standard free energies of hydrolysis.

Compound	$\Delta G^{o\prime}$ (kJ mol^{-1})
Phosphoenolpyruvate	−61.9
1, 3-Bisphosphoglycerate	−49.4
Acetyl phosphate	−43.1
Phosphocreatine	−43.1
PP_i	−33.5
ATP ($\rightarrow$ AMP + PP_i)	−32.2
ATP ($\rightarrow$ ADP + P_i)	−30.5
Glucose-1-phosphate	−20.9
Fructose-6-phosphate	−13.8
Glucose-6-phosphate	−13.8
Glycerol-3-phosphate	−9.2

Source: Jencks, W.P., in Fasman, G.D. (ed.) (1976) *Handbook of Biochemistry and Molecular Biology*, (3rd edn.), Physical and Chemical Data, Vol. I, pp. 296–304, CRC. Press.

Table 6.B1 shows that ATP is in about the middle range. The compounds above ATP in the table can, with suitable enzyme coupling, form ATP from ADP and P_i by substrate level phosphorylation. This can be used as an alternative to oxidative phosphorylation that takes place in mitochondria.

Why should ATP and other compounds have such exothermic $\Delta G^{0\prime}$ of hydrolysis values?

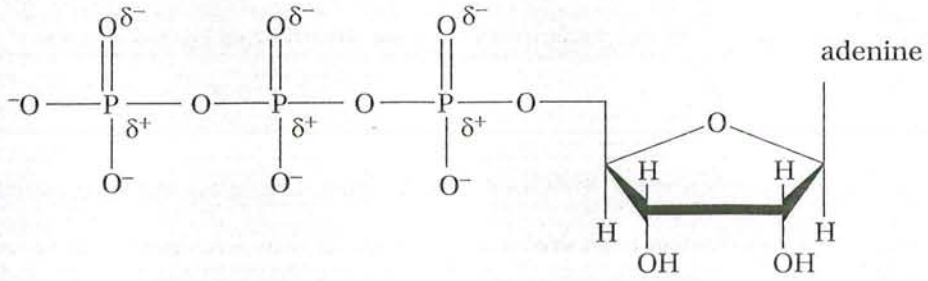
Bear in mind that, in general, $\Delta G^{0\prime}$ values depend on concentrations of reactants and products. In addition, in the case of ATP and its hydrolysis products, there is a dependence on pH and ionic strength, as ions are involved. Let us look specifically at ATP and other phosphorylated compounds.

The bonds/linkages involved are labelled β- and γ- in the ATP structure in Figure 6.B1. They are phosphoanhydride bonds, in contrast to the phosphate ester bond labelled α.

The 'high energy' involved here has nothing to do with bond energy, which is a measure of the energy needed to break a covalent bond. ATP-coupled reactions involve the energy liberated during the process of hydrolysis. The explanation is in what happens *after* hydrolysis, relative to what happens before. We are looking for reasons why, energywise, the components of ATP would 'prefer' the energy state after hydrolysis to that before hydrolysis. There are several proposed contributions:

1. ATP is destabilized by electrostatic repulsion between relatively close positive charges. The electronegative O atoms polarize the P–O bonds as shown below, increasing the electrostatic repulsion between them.

Box 6.1 (continued)

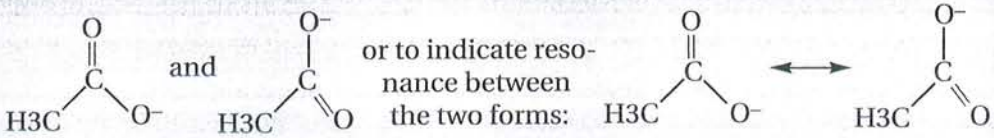


Hydrolysis relieves this source of instability by separation of the charges.

2. Entropic factors: There is an increase in the number of molecules after hydrolysis, allowing for an increase in entropy.
3. Smaller solvation energy of ATP relative to ADP + P_i. In the latter there are two smaller molecules with five negative charges to be solvated by water. ATP has four negative charges spread over a larger molecule. The magnitude of this effect is difficult to assess, but is probably quite significant.
4. To help with this explanation, we need to deal with an aspect of the stabilization of molecular species that was not mentioned in Chapter 5. This is the concept of resonance.

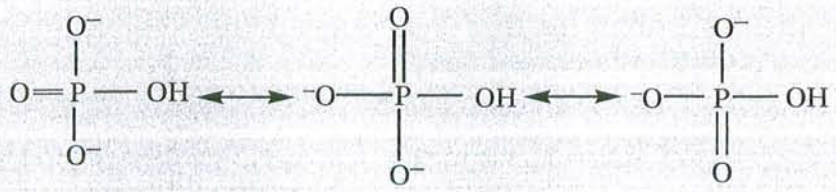
Some molecules can exist in two or more forms by rearrangement of their electrons. This can only happen to any significant extent if the electron orbitals involved are in 'allowed' orientations, with little or no energy barrier between them.

Thus, acetate ions can be written in two equi-energy forms:



As these forms are equal in energy, then entropy will ensure that both are equally likely to occur. Entropy favours the distribution of energy over as many states as possible.

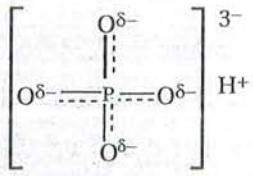
In the case of the inorganic phosphate ion from the hydrolysis of ATP, there are at least three resonance forms capable of existing simultaneously:



inorganic phosphate (P_i) resonance forms

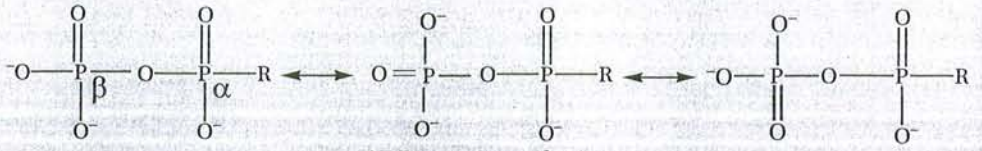
Box 6.1 (continued)

We could imagine more, formed by locating the proton on other oxygen atoms. This can be illustrated with a diagram of what is known as a resonance hybrid:



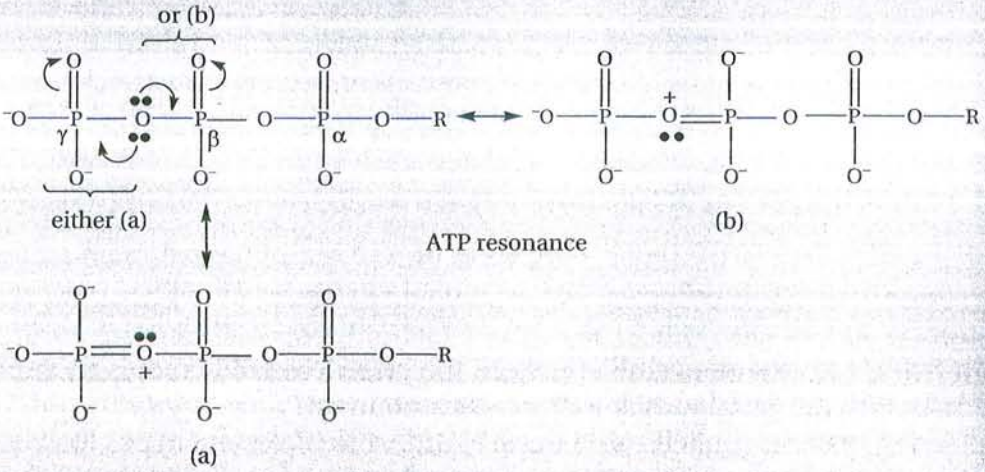
This shows that the H^+ and double bond are equally likely to involve any one of the four oxygen atoms.

After hydrolysis, there are at least three viable resonance forms for the remaining ADP:



ADP resonance forms

By contrast, in the case of ATP, there will be three forms for the end (γ) phosphate group, corresponding to the three for the β-phosphate in ADP above. There will be two competing forms, (a) and (b) below, that can exist alternately, but not simultaneously. This limits ATP to about four viable resonance-stabilized forms.



Overall, the resonance stabilization for ATP is much less than for its hydrolysis products ADP and P_i.

All these influences add up to produce a more favourable, that is lower overall, energy distribution for the hydrolysis products over the unhydrolyzed ATP. Similar arguments can be made of other 'high-energy' compounds.

Box 6.1 (continued)

The above discussion is for the thermodynamics of group-transfer reactions, which are vitally important in biology. Group transfer potential is discussed in terms of free energy of hydrolysis, but in most biological examples water doesn't even enter the equation. Consider the general case of the biosynthesis of a molecule that involves the condensation of two compounds (de Duve 2005):

$$XOH + HY \longrightarrow X\text{–}Y + H_2O \qquad (6.B1)$$

If this is carried out by a coupling reaction with ATP as the energy source, the steps involved are:

$$ATP + XOH \longrightarrow ADP + X\text{–}O\text{–}P \qquad (6.B2)$$

In the second step, X is transferred to Y–H:

$$X\text{–}O\text{–}P + HY \longrightarrow X\text{–}Y + P_i \qquad (6.B3)$$

Adding (6.B2) and (6.B3):

$$ATP + XOH + HY \longrightarrow X\text{–}Y + ADP + P_i \qquad (6.B4)$$

No water in its free form is involved. It is transferred via the XOP intermediate from XOH to P_i. Instead of the energy of ATP hydrolysis being dissipated as heat, this vitally important mechanism results in the synthesis of a covalent bond. The step in (6.B2) to form X–O–P is called the activation step, as XOH is activated chemically by the phosphoryl transfer to form XOP, which is capable of reacting with Y–H.

As pointed out by de Duve, many important biochemical processes involve the activation of X–O–H by transfer of inorganic pyrophosphate, PP_i, which involves splitting of the inner (β-) phosphoanhydride linkage rather than the outer (γ-) linkage (Figure 6.B1).

This summarizes to:

$$ATP + X\text{–}O\text{–}H \longrightarrow AMP + X\text{–}O\text{–}PP$$

$$X\text{–}O\text{–}PP + HY \longrightarrow X\text{–}Y + PP_i$$

$$\text{Overall} \quad ATP + X\text{–}O\text{–}H + H\text{–}Y \longrightarrow X\text{–}Y + AMP + PP_i$$

Aerobic cells maintain a high level of ATP via photosynthesis or respiration to ensure that a ready supply of energy is available.

For living systems an overall approach too close to equilibrium is an approach to death. Why do I make such a dramatic statement?

All living systems must be able to carry out work of various types to stay alive. Systems at equilibrium cannot carry out work, which is reflected in the fact that $\Delta G = 0$ in such systems. In one sense, life can be considered as a struggle against reaching equilibrium. When death occurs, the organism ceases its normal energy-driven processes and the inevitable 'downward' path of chemical reactions in its body towards equilibrium commences. Constantly during its lifetime, each beetle, each eagle, each whale, and each of the 6.8 billion, and rising, humans on Earth, every last bacterium of the billions seething about in

your gut is 'battling' against thermodynamics to survive, each an improbable island of order in a sea of chaos. Each can only succeed in this by processing the energy in its food to maintain an ordered state. All are non-equilibrium systems, engaged in dissipating some of the concentrated energy of the universe. They take in 'high-energy' chemical compounds and degrade them to 'low-energy' compounds, making use of the released potential energy for their own survival.

Suppose we look at a living organism in terms of the basic processes involved in its existence. In the broadest sense, a living organism is a transducer of energy. By transducer I mean that an organism has the ability to take energy in one form and to transform at least some of that energy to other forms, with the 'objective' of its own survival. The energy that is transformed goes, in part, towards maintaining the high degree of organization necessary to sustain all the life functions of the organism. We have discussed many of those functions, and in most cases it is easy to recognize why a supply of energy is necessary to carry them out. Maintenance of body temperature, growth, and muscular activity all need a source of energy. In addition, there is the less obvious yet crucial energy function of maintaining the degree of order we associate with a living organism. All its organs and systems are specifically constructed and integrated in such a way as to function as a whole. Failure of one or more vital organs, such as a kidney, the liver, the brain, or the heart, can lead to the death of a mammal. After death, the ability of an animal or plant to maintain its ordered structure is lost, as it can no longer act as a successful transducer of energy. Processes of decay commence as bacteria and other biological consumers take control and convert whatever energy is stored in the body of the deceased animal to their own advantage. Most of this residual energy is recycled in such a way because the living world is adept at survival and the death of one creature usually means life for others. The ultimate fate of the energy of life is low-intensity heat.

In order to maintain the dynamic energy balance mentioned in Chapter 1, we humans must achieve an overall daily balance of energy intake, anabolism, catabolism, and energy output. The maintenance of this balance is the role of metabolism, assisted by our own conscious contribution in the form of a suitable diet and lifestyle.

As I have stressed previously, the simple maintenance of our energy balance is not the whole story.

Most biological molecules are 'turned over'; they are replaced periodically. Most of us are familiar with the fact that most of the cells in our bodies are replaced a number of times during our lifetime; nerve cells seem to be a notable exception, although even the consensus on this is changing. The rates of molecular turnover may vary greatly. Enzymes and some other proteins have a half-life of 2–8 days. Structural proteins such as collagen in connective tissue and cartilage last much longer, often having a half-life of greater than 200 days. We shed hundreds of thousands of dead skin cells every day. Much of the 'dust' that gathers so annoyingly in our bedrooms and bathrooms consists of skin cells. Red blood cells work hard, being squeezed through narrow capillaries as well as being pushed through the major blood vessels at a punishing rate, and as a result they suffer much wear and tear. They are replaced after about 120 days.

Similarly, most of our carbohydrates, fats, and nucleic acids are being continuously replaced, although we don't notice this process at all.

As a result of all this replacement activity, each of us is literally not the person he or she used to be. Each of us is a temporary, reasonably organized arrangement of chemical compounds, but over our lifetime we do not even consist of the same molecules. Suppose it were to become possible to label each individual molecule in a newborn female baby. After 15 years, how many labels would we be likely to find in her body (assuming the ethics of the time allowed us to look)?

Apart from entirely new sets of molecules such as those arising at puberty, the same types of molecules—same proteins, same enzymes, same fats, same sugars—would be found. However, most—I can't hazard a guess at the percentage, but it would be low—would be unlabelled because of turnover. Turnover occurs from the molecular to the cellular level. Over time, most of the original labels will disappear, but our girl as an individual organism will continue to exist. The more widely she travelled, the broader the spread of her molecular labels would be. She would probably pick up a few of her own labels as they were recycled. Is this the origin of the saying 'getting your own back'?

Many of the individual atoms that once made up your body may well be spread widely through the neighbouring biosphere. Some might well be inspiring the leaps of your favourite sporting hero or fuelling the gyrations of a pop star. This turnover of molecules is one of the unique characteristics of living organisms. In contrast, inanimate objects such as motorcar engines simply wear out and need to be replaced. Your shoes will stretch and wear out, but the cells of your feet will replace themselves.

What are the origins of the order of life? Order and organization are consumers of energy, but how is it that out of the chaos and disorder of non-living matter, life has managed to arise? From a purely thermodynamic point of view, a living creature is a most improbable occurrence. So long as it remains alive, a plant or animal is able to convert some of the energy it consumes to resist the tendency to become disordered and fall apart. As soon as its normal energy flow ceases, the resistance disappears, the plant wilts, the animal collapses, and the inexorable degradations characteristic of death are set in motion. The highly structured leaves and flowers, the stems and roots, the legs, the paws, the heart and the brain all too soon lose their familiar form. Soon only the harder parts, the teeth and the skull, the skin, and the trunk of a tree, will be left to view. Of these, a tiny fraction may later be preserved as fossils, of which an even smaller fraction may become exposed by scouring wind or water, or by the probing tools of humans. Energy is 'running down' for these examples of ours and in the universe as a whole, as it expands, cools, and approaches maximum entropy. The stars are constantly generating and radiating vast amounts of energy, but will eventually 'burn out'. They pass through a series of evolutionary stages that are quite well understood. Our own Sun is burning away its mass at about 4,674,000 tonnes per second as it converts hydrogen to helium by nuclear fusion, with the generation of a vast amount of energy that radiates away into space in all directions. We alive today need not be concerned with this seemingly wasteful dissipation of our major source of energy. The Sun contains over 99.8% of all the mass in the

solar system and has been estimated to have more than half of its predicted lifetime of 10 billion years remaining. As far as we know, Earth is the only place in the solar system where life has evolved. Life as we are privileged to observe it may well be unique to our galaxy, if not to the universe itself.

Apart from a few glitches, such as the major extinctions, life on Earth has not only survived for 3.5 billion years, overall it has positively thrived. Its complexity, diversity, and sheer extravagance fascinate all who take the time to wonder at its workings. Life's underlying order is another of its intriguing assets. The maintenance of order brings us back to the inescapable laws of thermodynamics. Any real-world process involves energy changes. There are macroscopic forms of energy, involving the properties of the system as a whole, such as the kinetic and potential energies of rocks, cars, bullets, or people. Microscopic forms of energy are related to molecular structure and molecular activity, and are essentially independent of what is happening outside. This microscopic energy includes the motion of molecules as they vibrate, rotate, stretch, break, and reform their bonds. The sum of all these microscopic forms of energy is called the internal energy. The internal energy associated with chemical bonds is called chemical or bond energy. During a typical chemical reaction some bonds are destroyed and new ones are formed. The internal energy changes and some chemical energy may be stored in a 'concentrated' form for later access. In order to have access to reactions with a large negative ΔG, living organisms need to have access to such compounds with concentrated, high-intensity energy. We can argue that the quality/intensity of this chemical potential energy, largely stored in food, is the ultimate result of the 'intensification' of solar energy by the process of photosynthesis.

The Second Law says that in any spontaneous process the overall entropy of the universe will increase. At the same time it does not exclude the possibility that locally the entropy may actually decrease during a process. This aspect is one that has confused some who have tried to justify the existence of God on thermodynamic grounds. Such people assert that as living organisms exhibit order, that is the origins of their highly organized bodily systems are characterized by a decrease in entropy (as when a large protein is synthesized from a number of small amino acids), they defy the Second Law and only a God can do that. The misconception arises because the whole picture is not being considered. Only a local part (you) of the whole system (the universe) is being considered in the argument. Consider what has really happened to yourself during your lifetime so far. You as an organized system have probably existed essentially unchanged since reaching maturity. That is only part of the story. As we have seen, most of our body cells will be replaced a number of times during our lifetime. What has happened to all the food you have consumed and all the water you have drunk? The individual atoms in all the chemicals in the mountain of faeces and the small lake of urine you have generated could be scattered almost anywhere on Earth, especially if you are well travelled. The same applies to the water vapour you have breathed out or perspired into the air. The carbon dioxide you have exhaled, millions of litres of it probably, could have been through plants and recycled to animals which yourself or almost anyone else could have eaten. Some of your carbon dioxide molecules may have reached the tables of people as far away as

America or Africa. If that isn't an example of an increase in overall disorder and spreading out of the energy involved, I don't know what is.

Other statements of the Second Law include:

'It is impossible to turn all heat into work.' (Sadi Carnot)
'Heat can never pass spontaneously from a body at lower temperature to one at a higher temperature.' (Clausius)

'No process is possible whose only result is the abstraction of heat from a single heat reservoir and the performance of an exactly equivalent amount of work.' (Kelvin-Planck)

Systems tend to proceed from ordered (low entropy or low probability) states to disordered (high entropy or high probability) states.

All naturally occurring processes proceed towards equilibrium, that is towards a state of minimum potential energy.

The total amount of entropy in the Universe is increasing.

There are others. The simplest I know is 'things wear out'. The story of Humpty Dumpty is another. Machines begin to wear out spontaneously immediately they start to be used. They never spontaneously repair themselves.

Why are there so many statements of the Second Law? One reason is that the Second Law has wide applicability, so the wording can be expressed in many ways. Another is that the pioneers of thermodynamics were not always clear in their own minds about entropy. Let's look at a few more everyday examples.

We all have experience of the tendency of things in our daily lives to proceed, annoyingly in most cases, spontaneously towards disorder. My teenage son's room is an example. Any handyman is soon amazed at the amount of disorder he creates in a short time. Tools become scattered, bits of timber lie everywhere, wood shavings, nails, and sandpaper clutter the workplace. All this is a result of a redistribution of some of the handyman's concentrated, high-intensity energy into a more disordered form. In the process, the mess was made and the handyman's muscles produced a lot of heat. Another familiar example is the incredible mess created during the cooking of a three-course meal. All these need an input of our energy to reorganize. Unfortunately, the utensils will never spontaneously reorganize themselves into storage. We don't really expect this, for it would be against all experience. Nevertheless, it is the result of the Second Law. Similarly, improbable arrangements don't occur spontaneously. Tossing a handful of childrens' blocks into the air will not result in a model medieval castle.

Irreversible processes are sometimes described as examples of 'time's arrow' and are characterized by an increase in entropy.

Many of the things worth achieving in life seem to involve creating order—building a complex structure or piece of machinery, creating the ordered beauty of a work of art, writing a novel by ordering your thoughts into a sequence then typing them out, composing a symphony. I can't recall an example of a noteworthy human achievement that has occurred without considerable organizational effort occurring at some level.

There are examples of good fortune, such as a hole in one at golf or a spectacularly successful gambling win. Statistically, these little-effort events are possible, but rare, and don't really count as achievements in the way I mean.

They are highly improbable, which is reflected in the very low frequency with which they occur.

Although perhaps not everyone would express it this way, real achievements are admired because most people appreciate the difficulty and effort involved in overcoming the natural tendency towards disorder and in bringing about a decrease in entropy, even at a local level.

The fundamental role of thermodynamics in living organisms was lucidly described by the physicist Erwin Schrodinger in his masterful little book *What is life*? First published in 1944, 9 years before the structure of DNA was determined, it throws a little more light on the nature of entropy (Schrodinger 1967). From Chapter 6:

'It is by avoiding the rapid decay into the inert state of equilibrium that an organism appears so enigmatic; so much so, that from the earliest of times of human thought some special non-physical or supernatural force was claimed to be operative in the organism... How does a living organism avoid decay? The obvious answer is: by eating, drinking, breathing, and (in the case of plants) assimilating. The technical term is metabolism... Every process, event, happening—call it what you will; in a word, everything that is going on in Nature means an increase of the entropy of the part of the world where it is going on. Thus a living organism continually increases its entropy... and thus tends to approach the dangerous state of maximum entropy, which is death. It can only keep aloof from it, i.e. alive, by continually drawing from its environment negative entropy—which is something very positive as we shall immediately see. Or to put it less paradoxically, the essential thing in metabolism is that the organism succeeds in freeing itself from all the entropy it cannot help producing while alive.... The device by which an organism maintains itself stationary at a fairly high level of orderliness (fairly low level of entropy) really consists in continually sucking orderliness from its environment. This conclusion is less paradoxical than it appears at first sight... in the case of higher animals we know the kind of orderliness they feed upon well enough, *viz*, the extremely well-ordered state of matter in... complicated organic compounds, which serve them as foodstuffs. After utilizing it they return it in a very much degraded form—not entirely degraded however, for plants can still make use of it.'

In the last few sentences you will recognize echoes of what I mentioned in Chapter 1, where I discussed the utilization of available energy in the food we consume. We have now been able to deal not only with the relatively simple concept of energy release from our food, but also with the less obvious and more fundamental considerations of entropy compensation and its role in the maintenance of life.

Finally, it is interesting to contemplate two great evolutionary concepts of the nineteenth century: entropy and evolution by natural selection. Both are evolutionary in the sense that their results and predictions unfold with the passage of time. Darwin claimed that the living world was not created as it is now, but rather that living organisms are and always have been undergoing constant change and adaptation, evolving over time into many different forms. Life is characterized by complexity (high degrees of order and a multiplicity of chemical reactions), diversity (of species and of form), and randomness (of mutations). On the other hand, entropy predicts simplicity, maximum disorder, lack of motion, and the same ultimate thermodynamic fate for everything in the universe.

A burning question in the nineteenth century was, could these two concepts be reconciled? It seemed an impossible task in those times, and as a result the biological and physical sciences continued to follow their own 'laws' and to develop different ways of thinking about the world. Some scientists believed for a time that biology was based on a number of yet-undiscovered laws of nature. This was understandable but incorrect. As we have just seen, thermodynamics and biology are interrelated, but this was not widely recognized until about the 1960s.

We are getting closer to an explanation of our dynamic energy balance in terms of fundamental chemical and physical laws. Here I stress again that I am not implying that living organisms, either their workings or their behaviour, are fully explicable by the simple application of purely physical and chemical laws. There is more to life than this, but whatever processes are involved in the workings of biology, they do not and cannot violate those laws.

When developments in physics and chemistry led to the elucidation by Watson and Crick of the structure of DNA in 1953, the science of molecular biology became possible. The determination of the chemical structure of the genetic material allowed application of the laws and techniques of the physical scientist to the most fundamental biological questions and processes. When it became possible to determine the sequence of the components of DNA, genes were 'mapped' at the molecular level. Definite sequences of DNA eventually became identified with specific genes on specific chromosomes. Further developments in X-ray crystallography and nuclear magnetic resonance spectroscopy led to the determination of high-resolution, three-dimensional structures of proteins and many other biomolecules, and the era of genetic engineering was just around the corner.

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