

Cellular Thermodynamics

Joe Wolfe, *University of New South Wales, Sydney, Australia*

Energy of various types is shared among molecules according to Boltzmann's distribution. This simple law is used to explain aspects of the function and structure of cells, including reaction rates, electrochemical equilibria, osmosis, phase equilibria, molecular aggregation and the stability of membranes.

Introduction

Thermodynamics and thermal physics deal with many quantities and phenomena that are literally vital to the structure, function, operation and health of cells. These include temperature, energy, entropy, concentrations, molecular motions, electrochemistry, osmotic pressure, reaction rates, changes of phase molecular aggregation and much else. This article explains these ideas and serves as an introduction to the material of several other articles. The approach is to introduce the important and global ideas with biological examples and then to concentrate on explanations at the molecular level rather than to follow the traditional development using macroscopic variables and the ideal gas, although the two equivalent pictures are related via the chemical potential. The mathematical and formal rigour that is thus bypassed may be readily found in much longer accounts in classic texts in thermal physics (e.g. Landau and Lifschitz, 1969).

Equilibrium

By definition, equilibrium is achieved when the relevant parameters cease to vary with time. If a mammal dies in a cool, dry environment, its body, initially warmer than its surroundings, gradually comes to thermal equilibrium with them. Over a much longer time scale, its water content falls to several percent: it is approaching hydraulic equilibrium. Complete, global chemical equilibrium is rarely of direct interest to biologists: even seeds are not completely equilibrated with their environment. In many cases, however, local equilibria are achieved, to an excellent approximation. Two adjacent cells are usually extremely close to both thermal equilibrium and hydraulic equilibrium because of their proximity: if there were a temperature difference over the small distance between them, heat would usually flow rapidly from the hotter to the colder. Similarly, water can usually leave and enter cells relatively rapidly, because of the permeability of membranes to water. Therefore, if the cells are close, diffusion of water, in the liquid or vapour phase, can allow rapid approach to hydraulic equilibrium.

Secondary article

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Steady State

In a system in steady state, energy and/or matter enter the system and leave the system at the same rate. The energy may leave in a different form or at a different temperature. For example, a portion of human skin approximates steady state with respect to some variables: it is losing heat via radiation and evaporation but is receiving heat via conduction from subcutaneous tissue and the flow of blood; it is losing water and some volatiles by evaporation but receiving them via bulk flow, diffusion and permeation. In steady state, most of the parameters of the system do not change with time. Steady state is a more obvious approximation to the behaviours that interest biologists, although it does not include such important features as growth.

The maintenance of a steady state requires a continuous throughput of energy, which is schematized in **Figure 1**. Remove the source or the sink of energy, and the living system comes to equilibrium – it dies.

Heat, Light, Photons and 'Useful Energy'

The illustrations of steady state in **Figure 1** introduce some important ideas about the transmission of heat, and other forms of energy, in systems approximating steady state. The temperatures are given in the thermodynamic or

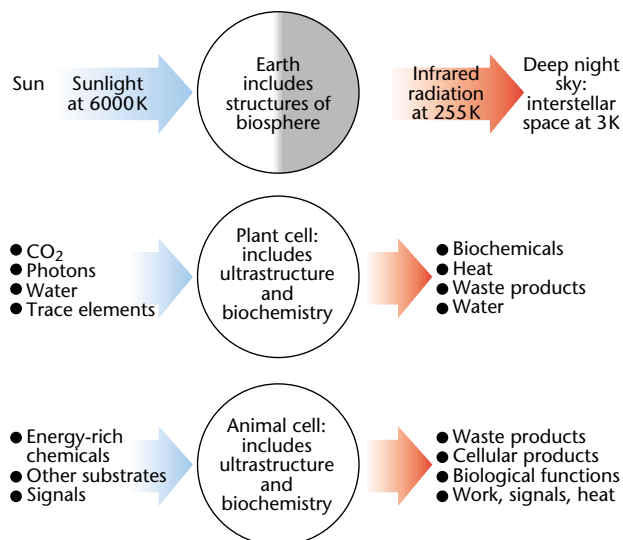


Figure 1 A thermal physics cartoon of the Earth, a plant cell and an animal cell. Their macroscopically ordered structure – and anything else they do or produce – is ultimately maintained or ‘paid for’ by a flux of energy from the sun (at 6000 K) to the night sky (at 3 K). The entropy produced by these processes is ultimately exported into interstellar space. (A few exceptional ecosystems on the ocean floor obtain their energy from cosmogenic chemistry rather than from sunlight, but most of us depend on photosynthesis.) The Earth absorbs solar energy at a rate of about 5×10^{16} W, so the rate of entropy input is about 5×10^{16} W/6000 K $\approx 8 \times 10^{12}$ WK⁻¹. The greenhouse effect notwithstanding, the Earth radiates heat at approximately the same rate, so its rate of entropy output is 5×10^{16} W/255 K $\approx 2 \times 10^{14}$ WK⁻¹. Thus the biosphere creates entropy at a rate of nearly 2×10^{14} WK⁻¹. Living things contribute only a fraction of this entropy, but that is still a huge amount. Despite the disingenuous claims of some anti-evolutionists, biological processes are in no danger of violating the Second Law of thermodynamics.

absolute scale (in kelvins, K); to convert to Celsius (°C), subtract 273.14 from the temperature in K.

In one sense, ultraviolet (UV) and visible light and infrared (IR) radiation (radiant heat) differ only quantitatively: they are all electromagnetic waves but have different wavelengths. In biochemistry, the difference is qualitative: visible and ultraviolet light are either useful energy for photosynthesis or photoreception, or potentially dangerous radiation for photolabile molecules, while the heat radiated by an organism at ~ 300 K to its surroundings (or from the surroundings to the organism) is not directly useful in biochemistry. The difference is due to the quantization of energy.

Like matter, light is not infinitely divisible. Light of a given colour (and thus, given frequency and wavelength) has a minimum quantity or quantum of energy. This is conveyed by a photon. A photon may be imagined as a minimum ‘packet of energy’, while in transit as light. The energy of a photon is hc/λ , where h is the Planck constant, c is the velocity of light and λ is the wavelength. For the sun, radiating at a temperature of 6000 K, most of the energy is carried by photons with wavelengths of typically 0.5 μ m

(visible light) and energies of about 4×10^{-19} J, which gives 250 kJ per ‘mole of photons’. For living things, radiating at ~ 300 K, the corresponding values are 10 μ m (far infrared), 2×10^{-20} J per photon or 10 kJ per ‘mole of photons’. The quantum of energy of visible (and especially ultraviolet) light is comparable with the separation of the quantized energy levels of electrons in atoms and molecules. Photons with this energy can activate many chemical reactions directly, as in photosynthesis and vision. The quantum energy of infrared radiation from a body at 300 K (~ 10 kJ per mole) is comparable with the typical kinetic energies of molecules due to their thermal motion. This energy can usually only activate a reaction indirectly: reactions that occur at temperatures of ~ 300 K are indeed activated by the thermal motion of molecules, but, as we shall see, it is usually only a small proportion of molecules, having rather more than the average energy, that produce the reaction.

Entropy

This difference between the chemically ‘useful’ energy (light or UV), and the ‘waste’ energy (infrared heat) can be quantified using the concept of entropy. When an amount of heat ΔQ is transferred at temperature T , the system gaining the heat has its entropy increased by $\Delta Q/T$. Thus sunlight (which has, roughly speaking, a radiation temperature of ~ 6000 K) is radiation with low entropy. A joule of thermal radiation from the surface of an organism at ~ 300 K (~ 20 times cooler) transmits 20 times as much entropy as a joule of solar radiation. This theme is a common one in biology: energy is acquired from low-entropy sources, and is used in a variety of different biochemical, physiological and ecological mechanisms. Ultimately, the energy is lost to a ‘sink’ for energy at low temperature and correspondingly high entropy. Traditional thermodynamics discusses heat engines (such as the steam turbines at thermal power stations) taking heat from the high-temperature source, converting part of it into work, rejecting the rest as heat into the low-temperature sink (the cooling tower) and generating entropy in so doing. The biology of (most of) the biosphere depends thus not only upon the energy from the sun (a source at $T \sim 6000$ K) but also upon the ultimate sink of energy, the night sky (at ~ 3 K). See Figure 1.

Macroscopic and Microscopic Views

A cell is, for our purposes, a macroscopic object. It contains perhaps 10^{14} molecules, and we can define and measure such parameters as its temperature, the pressure inside a vacuole, the tension in a membrane and so on. This is the domain of thermodynamics. The power of this

discipline derives from the fact that one can often apply thermodynamics to the overall behaviour of systems without knowing the molecular details.

For a single small molecule, on the other hand, temperature and pressure are neither well-defined nor measurable. The complementary discipline of statistical mechanics applies the laws of physics to individual molecules, atoms and photons and, by considering the statistical behaviour of large numbers, deduces the behaviour of macroscopic systems. It thus relates thermodynamics to Newtonian or quantum-mechanical laws at the molecular level.

From cells to ultrastructure to molecules, we move from the macroscopic to microscopic points of view. For instance, work, kinetic energy and heat are forms of energy and, in the macroscopic world, they are clearly distinguished. This difference almost disappears at the molecular level.

The laws of thermodynamics apply to macroscopic systems. The Zeroth Law states that if one system is in thermal equilibrium with a second, and the second is in thermal equilibrium with a third, the first and the third are in thermal equilibrium. This law makes temperature a useful concept: temperature is defined as that quantity which is equal in two systems at thermal equilibrium. The First Law is the statement of conservation of energy at the macroscopic, thermal level. It states that the internal energy U of a system is increased by the net amount of heat ΔQ added to it and decreased by the amount of work ΔW that the system does on its surroundings: $\Delta U = \Delta Q - \Delta W$, and that the internal energy of a system is a function of its physical parameters (i.e. it is a state function). The Second Law states that the total entropy generated by a process is greater than or equal to zero. It is possible for the entropy of a system to decrease (e.g. the contents of a refrigerator during cooling), but in doing so the entropy of the surroundings is increased (the refrigerator heats the air in the kitchen). The Third Law states that no finite series of processes can attain zero absolute temperature. (Different but equivalent statements of the second and third law are possible.)

These laws apply to macroscopic systems, where entropy and temperature have a clear meaning, and where heat is clearly distinguished from other forms of energy. At the microscopic level, they may be applied to a large number of molecules in a statistical way, but not to individual molecules. For example, the kinetic energy of a small molecule *is* heat. If it is accelerated by interaction with another molecule, has work been done on it, or has heat been given to it? For a large collection of molecules, however, collective organized motion (say a current of air) can do work, whereas random individual motion (in a hot, stationary gas) can supply heat. The second law can be stated thus in molecular terms: on average, systems go from improbable states (states having a small number of possible configurations) to probable states (having many

possible configurations). For very small numbers of molecules, however, the improbable is possible. If you toss 10 coins, you will occasionally (0.1% of the time) get 10 heads. If you could toss 6×10^{23} coins, you would never toss 6×10^{23} heads.

Distribution of Molecules and Energy: Low Energy versus High Entropy

How is energy distributed among molecules? How are molecules distributed in states or regions having different energies? How do systems compromise between maximizing entropy and minimizing energy? (The distinctions between internal energy, enthalpy and free energy are introduced later.) At equilibrium, the answer to these questions is usually given by the Boltzmann distribution. This distribution, shown in **Figure 2**, explains several important phenomena (chemical and ionic equilibration, reaction rates, osmotic pressure and more). It can be viewed as a compromise or balance involving energy and entropy: an important theme in cellular physics and biochemistry. In the state of highest entropy, all molecules or atoms would be distributed homogeneously in space. The state of lowest energy is equally uninteresting to biologists: the biosphere would consist of large crystals of nitrogen, oxygen, ice, and other substances. Equilibrium is a compromise between the two extremes. Because entropy is heat (energy) divided by temperature, entropy appears multiplied by T in equations concerning equilibrium (the product having units of energy). Consequently, entropic effects tend to dominate at high temperature, i.e. molecules spread out to achieve maximum molecular disorder. At low temperature, minimizing energy is more important, so molecules distribute more strongly into the lower-energy states.

Figure 2 shows that, at any temperature, the molecules are concentrated in states with low energy. A familiar example

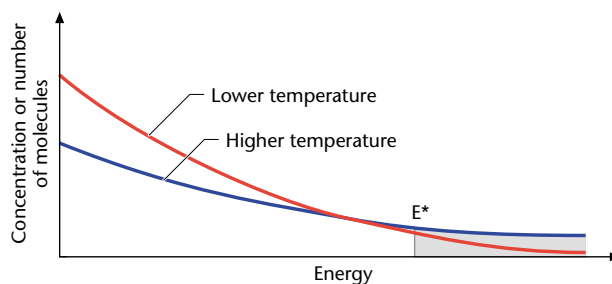


Figure 2 At equilibrium, molecules and their energies are distributed according to the Boltzmann distribution, sketched here. In states, phases or regions having different energies E , the concentrations or numbers of molecules are proportional to $\exp(-E/kT)$, where E is the energy per molecule, $k = 1.38 \times 10^{-23} \text{ J K}^{-1}$ is the Boltzmann constant and T is the absolute temperature.

is the concentration of gas in the atmosphere, where concentration is high at ground level (low gravitational potential energy), and falls as the altitude h increases. The gravitational potential energy is Mgh per mole, where g is the magnitude of the gravitational field and M is the molar mass. If we neglect the variation in temperature, we can derive the Boltzmann distribution easily in this case. If we consider the volume element with thickness dh and area A , the pressure difference dP acting on the area A supports the weight of the element, so $A dP = -\rho g A dh$, where ρ is the density of the gas. The equation of state $PV = nRT$ gives $\rho = nM/V = MP/RT$. Combining these equations an expression for dP/dh .

$$\frac{dP}{dh} = -\left(\frac{Mg}{RT}\right)P = -\frac{P}{\lambda}$$

where $\lambda = RT/Mg \approx 8$ km.

Solving the equation gives $P = P_0 \exp(-Mgh/RT)$, and substitution in the equation of state gives $C = C_0 \exp(-Mgh/RT)$. Thus at low altitude the potential energy is low, the concentration is high and the entropy per mole is low, and conversely for high altitudes.

Higher concentration, we shall see, implies lower entropy. The Boltzmann distribution gives the proportion of molecules in particular states as a function of the energy of the state. In many cases this corresponds to the concentration c in a region with energy E (eqns [1a,b]).

$$c = c_0 \exp(-E/kT) \quad [1a]$$

$$C = C_0 \exp(-E_m/RT) \quad [1b]$$

Here c_0 or C_0 is the reference concentration at which $E = 0$. The relation is given in two forms. Equation [1a] refers to molecules, so E is the energy per molecule and k is Boltzmann's constant. Equation [1b] refers to moles, so E_m here is the energy per mole and $R = N_A k$ is the gas constant, where N_A is the Avogadro number. The application of this equation is broader: for any region with a given potential energy, it also gives the distribution of molecules among states with different kinetic energies (Landau and Lifschitz, 1969). In a gas or a solution, the number of molecules in a state having a given energy is also given by eqn [1]: very few molecules have an energy more than several times kT per molecule or RT per mole, which have the values of about 4×10^{-21} J per molecule and 2 kJ mol^{-1} respectively for ordinary temperatures. People who deal with or think about molecules tend to use the first form, and those who think about macroscopic quantities tend to use the latter, so it is useful to be able to convert between them. kT is called the thermal energy because the various thermal motions of molecules have energies of this order.

Figure 2 also shows us that, at higher temperatures, the distribution varies less strongly with energy. At the molecular level of explanation, high temperature corresponds to high average levels of molecular energy. To

increase the average energy, fewer molecules have low energy and more have high. Alternatively, we could say that entropy becomes more important at higher temperature. The entropy is increased if the molecules are distributed more uniformly among the different energies. At the macroscopic level, we could say that the temperature is raised by putting in heat, and that by definition raises the entropy.

In some systems, the variation of concentration with energy may include other terms, although the Boltzmann factor, eqn [1], is always present. In some systems, the number of available states may vary as a function of energy; however, in such systems the Boltzmann term dominates at high energies. In concentrated systems there are other complications, discussed below under 'Osmotic effects'. In most texts on thermodynamics and physical chemistry, the Boltzmann factor appears via the chemical potential, which we shall introduce later in this molecular-level development.

Reaction Rates and Equilibria

The unidirectional rates of reactions are often determined by the Boltzmann term (provided that quantum tunnelling is negligible). If the activation energy of a reaction is E^* in one direction, then inspection of **Figure 2** shows that the proportion of molecules having more than that energy is proportional to the shaded portion of the area under the curve. Qualitatively, we see immediately why reaction rates increase at high temperature: more molecules (greater shaded area) have sufficient energy to react. Quantitatively, integration from E^* to ∞ gives this proportion as $\exp(-E^*/kT)$. The forward reaction rate r is proportional to this factor, as well as others, such as the rate of collisions, the fraction having appropriate orientation, etc. Taking logs gives eqn [2].

$$\ln r = -E^*/kT + \text{constant} \quad \text{(for molecules)} \quad [2a]$$

$$\ln r_m = -E_m^*/RT + \text{constant} \quad \text{(for moles)} \quad [2b]$$

If only one direction of a reaction is considered and if all other effects are temperature-independent, then the reaction rate is given by eqn [2], which is Arrhenius's law. Thus the negative slope of an Arrhenius plot – a plot of \ln (reaction rate) vs $1/T$ (in kelvin) gives the activation energy divided by k or R , subject to the caveats above, i.e. a unidirectional reaction and no other temperature-dependent effects. The caveats are important and are only rarely satisfied in biology. For instance, many enzymes become inactive at very low and very high temperatures, owing to such processes as denaturation, de-oligomerization and

encapsulation in ice or vitrified solutions. So the slope of a typical Arrhenius plot goes from plus infinity to minus infinity over several tens of kelvins. This does not imply that the activation energy is infinite and/or negative: rather it means that the local slope of such a plot, divided by k or R , should usually be called an 'apparent' activation energy.

Further, it is rare to have a reaction proceed in one direction only. If the concentration of reaction products is initially zero, then the initial reaction rate is that of the forward reaction. If no reagents or products are supplied or removed, the rate of the reverse reaction increases until chemical equilibrium is achieved, when the net reaction rate is zero. Applying the Boltzmann factor leads to the equations of chemical equilibrium, which are discussed in the article Thermodynamics in Biochemistry.

To deal with chemical and biochemical processes, we need an energy 'accounting system' that includes the possibility that a reaction involves doing work on the environment and/or receiving heat from the environment. For this purpose, enthalpy and Gibbs free energy are introduced.

Enthalpy

The enthalpy is defined as $H = U + PV$, where U is the internal energy, P is the pressure and V is the molecular or molar volume. This quantity appears because, under usual conditions such as constant (atmospheric or other) pressure, molecular volumes may change during biochemical processes. A change in volume ΔV at pressure P requires the performance of work $P\Delta V$, which is the work required to push the surroundings away to allow the expansion ΔV . In a reaction, the energy for this work must be provided by the reacting molecules, and so an increase in V of the activated state will make a reaction a little slower, and a positive ΔV of reaction will lead to a smaller equilibrium constant. For instance, in the process of evaporation of water at atmospheric pressure, a mole of liquid water (0.018 L) expands to become a mole of water vapour (~ 20 – 30 L, depending on the temperature), so the work done in 'pushing the atmosphere out of the way' is ~ 2 – 3 kJ mol⁻¹. At lower pressures, less work is required and so the boiling temperature is lower.

In biochemical processes at atmospheric pressure that do not involve the gas phase, however, the value of $P\Delta V$ is usually negligible. A molecular volume change of one cubic nanometre (the volume of thirty water molecules) would be very large in most biochemical contexts, yet here $P\Delta V$ is only 10^{-22} J per molecule, which is ~ 40 times smaller than kT , the characteristic thermal energy of molecules.

Gibbs Free Energy

Biochemical processes often occur at constant temperature: this means that any heat that is generated is transferred elsewhere (usually by conduction), and that heat absorbed in a process is likewise provided from neighbouring regions. For such cases, it is useful to use the Gibbs free energy, defined as $G = H - TS = U + PV - TS$, where S is the entropy. Now $T\Delta S$ is the heat gained at constant temperature (see above), so the Gibbs free energy adds to the internal energy the work done in volume changes and also the heat that the process loses to its environment. In the spirit of this article, however, we need to explain why G is the appropriate energy accounting at the molecular level, where the distinction between heat and other energy disappears.

For this, we need Boltzmann's microscopic definition of entropy, $S = k \ln w$, where w is the number of different possible configurations of a system. This expression gives rise to the idea that entropy quantifies disorder at the molecular level. For example, when a solid melts, it goes from an ordered state (each molecule in a particular place) to a state in which the molecules may move, so the number of possible configurations increases enormously. The entropy produced by equilibrium melting is just the latent heat divided by the temperature. For 1 g of ice at 273 K, this gives 1.2 J K⁻¹. Note that this is disorder at the molecular level: macroscopic disorder is unimportant. If one shuffled an initially ordered pack of cards, the entropy associated with the disordered sequence is about 3×10^{-21} J K⁻¹. The entropy produced by one's muscles during the shuffling would be roughly 10^{20} times larger. Another example to stress the irrelevance of macroscopic order is to consider the cellular order of a human. Biochemical procedures often begin by dividing a tissue into cells and then mixing or 'shuffling' them. If we took the $\sim 10^{14}$ cells in a human and 'shuffled' them, we would lose not only a person, but a great deal of macroscopic order. However, we would produce an entropy of only $k \ln(10^{14}) \cong 4 \times 10^{-8}$ J K⁻¹, 30 million times smaller than that produced by melting the 1 g ice cube. Again, the heat of the 'shuffling' process would be vastly larger.

So how does this molecular disorder affect equilibria and how does it relate to the Gibbs free energy? Consider this example: if a reaction has (for every initial state) w different activated states, all with approximately equal energies, then, all else equal, the reaction is w times more likely. The reaction rate would be proportional to $w \exp(-E^*/kT) = \exp(\ln w - E^*/kT) = \exp[-(E^* - TS)/kT]$. If we include the possibility of volume change, $E^* = \Delta U^* + P\Delta V$ term, and we have $\exp(-G^*/kT)$, where G^* is the free energy of activation.

The TS term in the Gibbs free energy allows for entropic forces. The forces discussed in mechanics usually involve the internal energy U of a system. The gravitational potential energy of a system comprising the Earth and a

mass m is mgh . Here the force is $-\partial U/\partial h = -mg$ (the weight of the mass). In biological systems, displacements (in a direction x , say) often change the Gibbs free energy, and so the force is $-\partial G/\partial x$. Thus the forces depend on changes both in energy (often electric potential) and entropy. One example is given in **Figure 3**.

Other Equilibria

From eqn [1] we can simply obtain expressions for electrochemical equilibria (the Nernst equation), for maximal reaction rates, for osmotic pressure, for phase equilibria and for the chemical potential. These are derived from the macroscopic view in classical texts, but here we sketch molecular explanations.

Electrochemical effects

Positively charged ions tend to accumulate in regions of low electrical potential Φ , where their electrical energy is low. Entropy, on the other hand, tends to distribute them with equal concentration everywhere. For an ion of charge q , the electrical potential energy is $q\Phi$. At equilibrium,

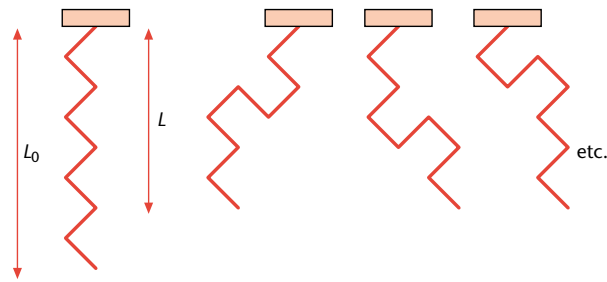


Figure 3 This cartoon of a polymer, fixed at one end, illustrates how the entropy term in the Gibbs free energy contributes to a force. Force times change in length gives the work done in stretching the polymer, so we examine states with different lengths. In the all-*trans* configuration, the polymer has a length L_0 , but there is only one configuration corresponding to that length, so the entropy associated with the internal bending is $k \ln 1 = 0$. At a shorter length L , there are N possible configurations, of which only a few are shown. The entropy associated with bending is here $k \ln N > 0$. Suppose that the extra potential energy associated with the *cis* bonds is ΔU , and neglect changes in the molecule's partial volume. The change in G is $\Delta G = \Delta U - TS_{\text{bend}}$. We consider the case where $TS_{\text{bend}} > \Delta U$, where the shorter state is stable (has a lower G) with respect to the longer. In molecular terms, we would say that the Boltzmann distribution indicates that the ratio of the number of molecules with length L to that with length L_0 is $N \exp(-\Delta U/kT)$, which for this case is > 1 . Note that, when we increase the temperature, the length decreases – which also happens when one heats a rubber band. We could also calculate the (average) force exerted by this molecule, $\partial G/\partial L$, and, from a collection of such molecules, we can find the elastic properties of this (idealized) polymer. Finally, note that the entropic force here becomes stronger as T increases, because T multiplies S . (In Boltzmann terms, more molecules are in the higher energy state.) This increasing effect with temperature is important for entropic forces, including for the hydrophobic interaction, discussed later in the text and in the article *The Hydrophobic Effect*.

positive ions accumulate preferentially in regions of low electric potential, and vice versa. From the Boltzmann distribution, $c = c_0 \exp(-q\Phi/kT)$. (In mole terms, $C = C_0 \exp(-ZF\Phi/RT)$, where Z is the valence and F is the charge on a mole of monovalent ions.) This is called the Nernst equation, and it is usually written taking logs of both sides: $\ln(c/c_0) = -q\Phi/kT = -ZF\Phi/RT$. Note that the characteristic voltage $kT/e = RT/F \cong 25$ mV at ordinary temperatures, so local biological potential differences are typically of this order.

Ionic distributions across membranes

Because the cytoplasm has a lower electric potential than the external solution, the cytoplasmic concentration of Cl^- ions is lower at equilibrium. Permeating cations have higher cytoplasmic concentration at equilibrium, as shown

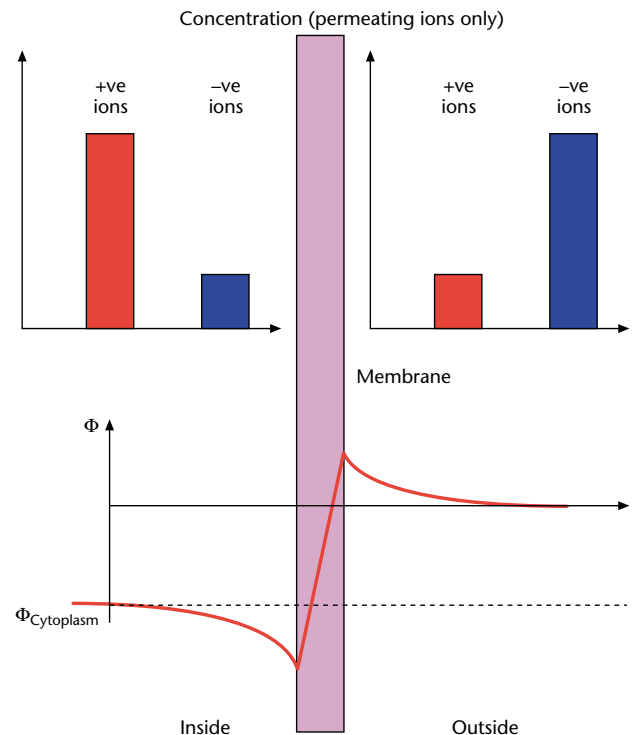


Figure 4 The Boltzmann distribution explains the equilibrium distribution of permeating positive (+ve) and negative ions. The electric potential energy per unit charge is called the electric potential or voltage (Φ). The state of lowest energy for the permeating ions would have all permeating cations on the left (low Φ) and all permeating anions on the right (assuming that we maintain the potential difference). The highest-entropy state would have equal concentrations of the permeating ions everywhere. Note that this distribution applies only to freely permeating molecules that are not 'actively pumped'. In practice, the total number of positive charges inside a cell differs only by a very tiny fraction from the total number of negative charges. In terms of this diagram, many of the extra intracellular anions required to approach electroneutrality are found on nonpermeating species, while the extracellular solution will also contain many cations that are expelled from the cell by active (energy-using) processes.

in **Figure 4**. Note, however, that many ions and other charged molecules do not equilibrate across membranes (over ordinary time scales). Some do not permeate (proteins and other large molecules), while others are continuously maintained in a nonequilibrium state. The negative potential in the cytoplasm is in part maintained by 'pumps', i.e. mechanisms that, by expending energy, produce a flux of specific ions in a particular direction.

Ionic distributions near charged membranes and macromolecules

Similarly, in the vicinity of the negatively charged surface of a membrane or a macromolecule, the concentration of cations in the solution is higher than in the bulk solution. The surfaces of many membranes are (on average) negatively charged, so there is usually an excess of cations and a depletion of anions near the membrane, as shown in **Figure 5**. Many macromolecules have a small excess of negative charge and so are surrounded by a region of similarly perturbed ion concentration.

Figure 5 shows the mean field approximation: the assumption that the distribution of an individual ion is determined by the average distribution of all other ions. Locally, this is a severe simplification: near an anion one is more likely to find a cation than another anion, so there are local correlations in the distribution. These are important in the electrical interaction between macromolecules or membranes and can even lead to counterion-mediated attractions between like-charged macromolecules (Grønbech-Jensen *et al.*, 1997).

The local correlations in ion distribution also lead to electrostatic screening: electrical interactions are weaker in solution than they would be in pure water because ions of the opposite sign (counterions) tend to accumulate near charges and screen their effects. Further, electrical effects in pure water are much weaker than they would be in vacuum because water molecules are dipolar, and they have a

tendency to rotate so that the positive charge at the hydrogen side of the molecule and the negative charge on the oxygen side act to reduce any electrical effects. Once again, the highest entropy would yield a random orientation of water molecules, but this is compromised because the energy is lower in the arrangement just described. (An empirical way of describing this effect for large volumes is to say that water has a dielectric constant of about 80: electrical effects in water are about 80 times weaker than those produced by the same charge distribution in vacuum.)

Osmotic effects

A difference in hydrostatic pressure produces a difference in energy: to move a molecule whose partial molecular volume is V from a reference pressure of zero to a higher pressure P requires an energy PV . Substituting this energy in the Boltzmann distribution (eqn [1]) gives the equilibrium osmotic distribution. Usually we are concerned with the equilibration of water, because water permeates most biological barriers more easily than most solutes. Pressures are usually measured with respect to atmospheric pressure.

Most membranes are permeable to water but impermeable to macromolecules, sugars and some ions. (Some small organic molecules such as ethanol and dimethyl sulfoxide pass relatively easily through membranes, so their osmotic effects are usually transient.) **Figure 6** shows a semipermeable membrane with pure water on the left and a solution of an impermeant solute on the right. Suppose that initially the pressure on both sides is zero (**Figure 6a**). We notice that the solutes are not in equilibrium because their concentration is higher on the right, so they would diffuse to the left if there were no membrane. The equilibrium of the solute does not interest us, however, because the semipermeable membrane prevents it from being achieved. Water can permeate the membrane,

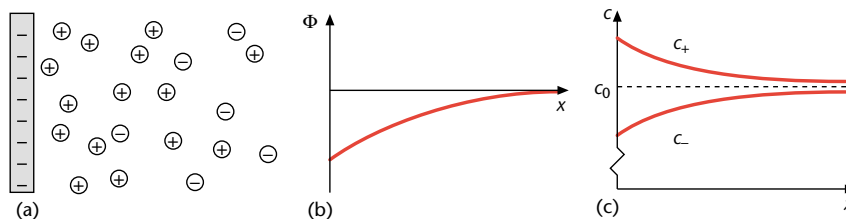


Figure 5 A charged surface in an ionic solution gives a nonuniform ion distribution over a scale of nanometres (a). Positive ions are attracted towards the interface, negative ions are repelled. The layer of excess positive ions (called a double layer) counteracts the electrical effect at increasing distance x . (b) and (c) show how the electrical potential Φ and the ionic concentrations c_+ and c_- vary near the surface, but approach their bulk concentration c_0 at large x .

The electric charge of ions has another effect that contributes to their distribution. The self-energy or Born energy of an ion – the energy associated with the field of the ion itself – depends upon the medium, and is lower in polar media such as water than in nonpolar media such as the hydrophobic regions of membranes or macromolecules. Thus ions are found in only negligible concentration in the hydrocarbon interior of membranes. This energy is also thought to affect the distribution of ions inside aqueous pores or near protein molecules: if the ion is sufficiently close (\sim nm) to a nonpolar region of membrane or macromolecule, it has a high Born energy. This energy and the Boltzmann distribution mean that the concentration of ions of either sign is lower than would be otherwise expected, not only in nonpolar regions, but also in the aqueous solution very near such regions. Again, the equilibrium distribution will be a compromise between minimizing the Born energy (ions keep away from nonpolar regions) and maximizing entropy (ions distribute uniformly everywhere).

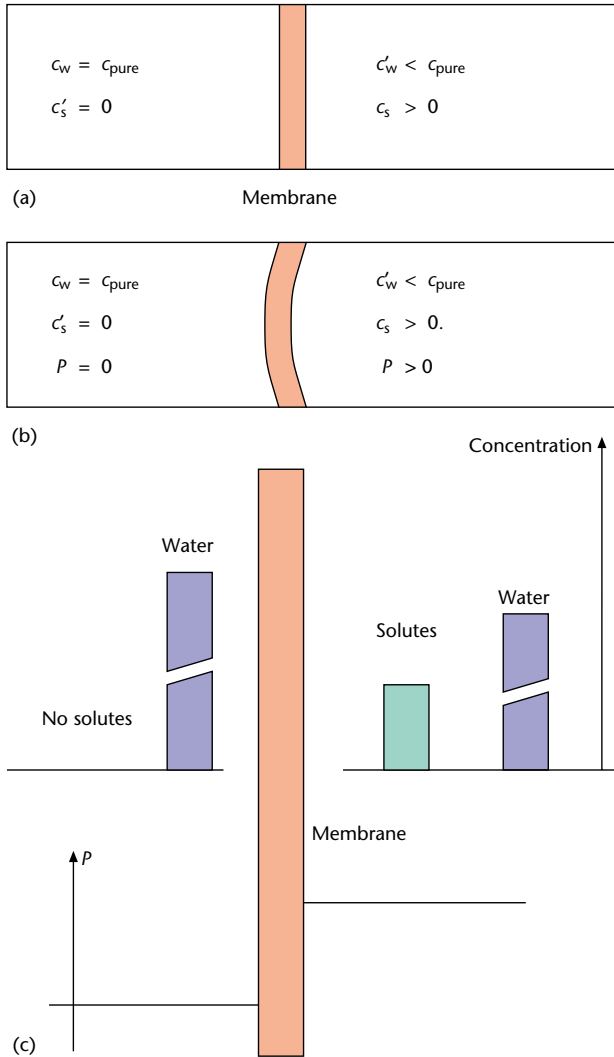


Figure 6 The membrane shown in (a) is permeable to water but not to solutes. Water flows towards the more concentrated solution (which has a lower water concentration). This flow stops and hydraulic equilibrium is achieved when the pressure P in the concentrated solution is sufficiently high. The system in (b) has a higher energy than (a), but it also has higher entropy. Entropy will be maximized if the membrane bursts and the solutions mix freely. Using the Boltzmann distribution with PV as the energy difference and making the approximations that solute volume and concentrations are small, one obtains the condition for osmotic equilibrium – the value of the hydrostatic pressure difference required to stop further flow of water into the solution side. In the case of a solution, the number fraction of water molecules, rather than their concentration, must be used in eqn [1]. Using the approximation that solute volume and concentration are small, one readily obtains an approximate expression for the equilibrium pressure difference, $P_{\text{osm}} \cong kTC_s = RTC_s$, where the solute concentration is c_s in molecules m^{-3} or C_s in kmol m^{-3} respectively. Note that water concentration is usually much higher ($\sim 50 \text{ kmol m}^{-3}$) than solute concentrations, and so the proportional difference in water concentration is small. However, rates of diffusion and permeation depend on the absolute difference. A solution of 1 kmol m^{-3} or 1 mol L^{-1} of nondissociating solute gives an osmotic pressure of approximately 2 MPa, or 20 atmospheres, the exact value depending on temperature and on some corrections due to solute–solvent interactions.

however. Further, water is not in equilibrium in this situation, because the concentration of water is higher on the left: if there is no pressure difference, water will move from the pure water on the left into the solution on the right where the water is, one might say, diluted by the solutes. So water moves through the membrane from pure water (low solute concentration) into the side with higher solute concentration. If we put, say, a blood cell of any sort into pure water, water would flow in and the cell would expand until the membrane burst. If the membrane is supported by something stronger, such as a plant cell wall, the expansion continues only until the mechanical tension in the wall is large enough to resist a substantial difference in hydrostatic pressure. Hydraulic equilibrium is achieved when the lower concentration of water in the high-pressure side is balanced by the higher pressure and therefore higher PV energy on that side. Large osmotic pressure differences (typically 1 MPa or 10 atmospheres) are commonly found in plants, where water is close to equilibrium between the extracellular solution (with low solute concentrations) and the much more concentrated intracellular solution. The plant cell wall resists the difference in hydrostatic pressure (Slatyer, 1967). In animal cells, there is no comparably strong structure, so the hydrostatic pressure differences are much smaller.

Further osmotic effects

The semipermeability of membranes is not the only thing that prevents solutes and water from mixing completely. Sufficiently large solutes are prevented from entering the narrow spaces between closely packed macromolecules or membranes. Further, the centre of a large solute cannot usually approach more closely than about one radius from an ultrastructural surface (called the excluded volume effect). These effects complicate the osmotic forces acting on macromolecules and membranes. When cells are dehydrated by extracellular freezing or air drying, the highly concentrated solutes produce extremely large osmotic effects. In these cases, studies of the phase equilibria show how the mechanical stresses and strains associated with freezing and dehydration damage in cells can be substantially reduced by osmotic effects (Wolfe and Bryant, 1999).

The Chemical Potential

This represents another way of writing the Boltzmann equation $C = C_0 \exp(-E/RT)$. Taking logs and rearranging gives eqn [3].

$$RT \ln C_0 = RT \ln C + E \quad [3]$$

E , the difference in energy between the state considered and the reference state, may include the work done against pressure, the electrical potential energy and other terms if

necessary. The utility of this formulation is that, at equilibrium at a given temperature T , one sees that the quantity $(RT \ln C + E)$ does not vary with position in a given phase. For the moment, let PV_m be the only energy term, as is the case for an electrically neutral molecule, where V_m is the partial molar volume. The chemical potential μ is defined as in eqn [4].

$$\mu = \mu^\circ + RT \ln C + PV_m = \mu^\circ + RT \ln C_0 \quad [4]$$

where μ° , called the standard chemical potential, is the value of μ in some reference state with concentration C_0 . (The reference state is often the pure substance). Thus the Boltzmann distribution translates as the statement that the chemical potential is uniform at equilibrium. The significance of μ° is seen when we consider the distribution of molecules among different phases.

Effects of different molecular environments

Energy is required to move a molecule from a solid, where it is usually strongly bound to its neighbours, into a liquid, where it is less strongly bound. Further energy is required to take it out of the liquid into a gas. (As an approximate guide for small molecules: in a solid, the intermolecular energy of attraction is $\gg kT$; in a gas it is $\ll kT$, in a liquid it is comparable with kT .) The entropy in a solid is lowest because the molecules have least motion and therefore relatively few different configurations, and it is greatest in a gas. The energy also increases from solid to liquid to gas, as we supply the latent heats of fusion and evaporation. Thus the coexistence of two phases in equilibrium, such as aqueous solution and water vapour, gives another example of the entropy–energy compromise.

Similarly, the energy of a solute molecule is different in different solvents such as water, alcohol and oil. The energy of a molecule (e.g. a nonpolar anaesthetic) is different when it is in an aqueous solution from that when it is partitioned into the interior of a membrane. These differences in what we might call the energy of the molecular environment may similarly be included in the standard chemical potential μ° .

If molecules in phase 1 and phase 2 equilibrate at temperature T and pressure P , then substituting the difference in environmental energy ($\mu^\circ_1 - \mu^\circ_2$) into equation (1), taking logs and rearranging gives:

$$\mu^\circ_1 + kT \ln c_1 + PV_1 = \mu^\circ_2 + kT \ln c_2 + PV_2 = \mu \quad [5a]$$

or, for moles:

$$\mu^\circ_{m1} + RT \ln C_1 + PV_{m1} = \mu^\circ_{m2} + RT \ln C_2 + PV_{m2} = \mu_m \quad [5b]$$

where the chemical potential is μ per mole and μ_m per molecule.

If we include in the energy terms the electrostatic potential energy discussed above ($Ze\Phi$ or $ZF\Phi$), we obtain the electrochemical potential

$\tilde{\mu} = \mu^\circ + kT \ln c + PV + Ze\Phi$, which should be used when discussing the equilibrium of ions. In principle, one could also include the gravitational potential energy $mg \Delta h$ in the chemical potential. This is rarely necessary. Because diffusion is slow, one usually only applies the chemical potential in cases for which $mg \Delta h$ is much smaller than the other terms. In footnote 2 the term is important because we consider heights in kilometres.

In eqn [5a], $(k \ln c)$ represents the entropy associated with a solute/gas molecule's freedom to translate, to sample the volume available. We return to this under 'Molecular Aggregation'. We can now rearrange eqn [5] to retrieve the Boltzmann distribution (eqn [1]).

Now, using the standard chemical potentials to quantify the energy and entropy changes between different phases or molecular environments, we relax the constraint concerning a single phase in the section above. The chemical potential is a quantity that is equal in any regions in which molecules are distributed according to the Boltzmann distribution, so the chemical potentials are equal throughout a region in that molecules are in equilibrium.

Further, in the absence of active processes, molecules diffuse so as to achieve equilibrium, i.e. they diffuse from high μ to low μ , at a rate proportional to the gradient in μ . Molecular diffusion (individual molecular motion) follows the gradient in μ , but this does not apply to bulk flow (collective molecular motion). If a jug of milk is held above a cup of water, the milk (water plus solutes) will *flow* from jug (high mgh) to cup (low mgh): macroscopic volumes of fluid behave according to the mechanics of the macroscopic world. However, if you sealed the cup and jug in a box, water would very slowly diffuse, via the vapour phase, from the cup (water at high μ) to the jug (low μ).

Surface Energies and Surface Tensions

A further important free energy difference in cellular biology is that between water molecules at the surface and water molecules in the interior of the water/solution volume. This is introduced in **Figure 7**. In surfaces between two fluids having a surface tension γ , the extra work done in introducing a molecule of area a to the interface is γa . Because one of the chief phenomena maintaining cellular ultrastructure is the surface tension of water, such a term must often be introduced into the Boltzmann distribution and thus to the chemical potential.

Molecular Aggregation

When molecules aggregate to form oligomers or macroscopic ultrastructure, the entropy of translation is shared, so the entropy per molecule is divided by the number N in the aggregate. Thus, if the energy of aggregation is

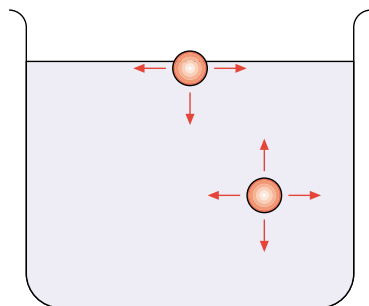


Figure 7 The surface free energy of water. In bulk solution, water molecules are transiently hydrogen-bonded to their neighbours. A water molecule at an air–water surface or a hydrocarbon–air interface has fewer neighbours with which to form hydrogen bonds, so its energy is higher. Because molecules will tend to rotate to form bonds with their neighbours, the surface is more ordered and has a lower entropy per molecule. Together, these effects give the water surface a relatively large free energy per unit area, which gives rise to the hydrophobic effect. The work done per unit area of new surface created is numerically equal to the surface tension γ , which is the force per unit length acting in the surface. This is simply shown: if one expands the surface by drawing a length L a perpendicular distance dx , the force acting along the length L is γL , so the work done is $\gamma L dx = \gamma da$, where da is the increased area.

independent of T , the proportion of monomers will increase with T as this entropy becomes more important. Molecular aggregation is important in various processes, including the aggregation of several proteins to produce an oligomeric enzyme.

We shall consider simple lipid aggregations and membranes as an example, because it involves another important energy term. Consider a single lipid molecule, in water, with a standard chemical potential μ_1° in equilibrium with a micelle or vesicle comprising N molecules (**Figure 8a** and **b**). The standard chemical potential μ_N° in the aggregate is much lower, because the aggregate has little hydrocarbon–water interface per molecule, while the monomer has much. (Note that, in this case, the surface energy between the hydrocarbon and water has been incorporated into μ° , rather than considered as a separate term. See Israelachvili *et al.* (1980) for details.)

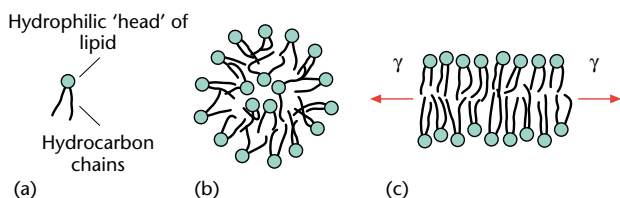


Figure 8 The equilibria among a lipid monomer (a), a vesicle containing N molecules (b) and a macroscopic lipid bilayer membrane (c) shows the effect of molecular aggregation and mechanical stress.

The membrane may be subject to a tensile force per unit length γ , and this may do work by changing in area. The equilibrium here is given by:

$$\mu_1^\circ + kT \ln X_1 = \mu_N^\circ + \frac{kT}{N} \ln \frac{X_N}{N} = \mu_m^\circ - \gamma \frac{a}{2}$$

where μ_m° is the standard chemical potential of lipids in the membrane and X_1 and X_N are the number fraction of lipids in the monomer and vesicle state, respectively. Because of the large number of molecules in the membrane, the translational entropy per molecule is negligible. The factor $\frac{1}{2}$ in the last term arises because each lipid molecule has an area a in only one face of the membrane. Notice the effect of the various terms: if the lipid has more or longer hydrocarbon tails, then the extra ‘environmental energy’ difference due to the hydrophobic effect will increase ($\mu_1^\circ - \mu_N^\circ$) giving rise to a lower monomer concentration. Increasing the tensile stress in the membrane (larger γ) makes the last term more negative, so monomers (and perhaps even vesicles, if a suitable mechanism exists) will move into the membrane, increasing the area and perhaps relaxing the stress. Alternatively, changing the area of a membrane causes a possibly transient mechanical change in γ , and thus a transfer of membrane material between membrane and a reservoir of material. Although the exchange mechanisms are not understood, it is possible that such processes occur in some cell membranes, where regulation or homeostasis of membrane tension and cell area have been reported (Wolfe and Steponkus, 1983; Morris and Homann, 2001).

Conclusion

The ubiquitous Boltzmann distribution explains, at a molecular level, a range of effects and processes important to biochemistry and cell biology. This chapter may also be considered as an introduction to further reading in other articles including those dealing with aspects of thermodynamics in biochemistry, cell biophysics, membranes, macromolecules, water, hydrophobic effect, plant stress, and in other works in these fields.

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Glossary:

Temperature (T)	That quantity that is equal in two objects in thermal equilibrium. (A measure of how hot a body is, or how much energy is associated with each type of random motion of and in molecules. Of itself, heat flows from higher to lower temperature.)
Heat (Q)	Energy that is transmitted only by a difference in temperature. Adding heat may increase the energy associated with molecular motion (and thus increase the temperature), or it may increase intermolecular and/or intramolecular potential energies (in a change of phase or in endothermic reactions).
Work (W)	Energy transmitted when the point of application of a force is displaced. (When the point of application of a force \mathbf{F} is displaced a distance ds , the work done dW is the scalar product $\mathbf{F} \cdot ds$, <i>ie</i> the magnitude of \mathbf{F} times the component of ds parallel to \mathbf{F} .)
Entropy (S)	The ratio of the heat energy transferred to the temperature at which it is transferred. (It is also a measure of disorder <i>at the molecular level only</i> .)
Equilibrium	In a system into which no matter or energy enters and from which none leaves (a closed system), equilibrium is the state achieved when parameters no longer change over time.
Steady state	A state of a system in which parameters are not changing with time, but in which energy and/or matter may be entering and leaving the system (an open system).
Gibbs free energy (G)	G is a measure of the work that may be extracted from a system at a constant pressure and temperature. $G \equiv U + PV - TS$
Osmotic pressure (Π)	If a solvent equilibrates between two bulk solutions with different composition, the solution with the lower mole fraction of solvent (<i>ie</i> the more concentrated solution) must have a higher (hydrostatic) pressure. This difference in pressure is called the difference in osmotic pressure.
Chemical potential μ	μ is a quantity of a particular component that has the same value at all places where that compound is in equilibrium. (μ for a component equals the variation in a thermodynamic potential with respect to change in the amount of that component.)
Surface tension (γ)	The force per unit length required to increase the area of a surface. (γ equals the change in surface energy per unit area.)
Photon	The carrier of the minimum quantity of electromagnetic radiation of a given wavelength. (Just as a molecule is the possible smallest quantity of water, a photon is the smallest possible quantity of light.)