carbon dioxide to dissolved carbonic acid, while iron is present in hemoglobin and myoglobin to reversibly bind dioxygen. Iron is also found in systems, which add oxygen to organic substrates or transfer energy between biological components in metabolic energy transfer chains. Cobalt, in the form of vitamin B_{12} coenzyme, is used to carry out chemical transformations associated with structural changes in carbon-compounds, and copper is present in some superoxide dismutase enzymes to convert superoxide to dioxygen and peroxide. In each case the metal brings its own distinctive properties to the task and these are exploited by the biochemical system in which the metal participates.

Living organisms require efficient systems for managing metals. The bulk metals tend to be quite freely mobile but selective means of transporting them across cell membranes are necessary to maintain a delicate electrolyte balance and for nerve cells to function efficiently. Trace metals may be transported and stored by proteins. In particular iron is transported by transferrin and stored in ferritin while copper can be transported and stored in ceruloplasmin. Metals are also stored in the proteins, *e.g.* cytochromes or enzymes, within which they express their biological function. Some clinical applications of the chemistry of metals are concerned with the management of metals in the body. Metal containing pharmaceutical formulations can be used to treat deficiencies of a particular metal, *e.g.* iron for anaemia or cobalt as vitamin B_{12} for pernicious anaemia. Conversely the chemistry of metals may be exploited in treating problems arising from an excess of a metal in tissues, *e.g.* copper in Wilson disease or iron overload in patients receiving repeated blood transfusions. One approach to the treatment of iron overload exploits a natural microbial iron-sequestering agent. Since iron in the environment is generally found in a rather insoluble and inaccessible form, some micro-organisms excrete agents known as siderophores which strongly bind iron and facilitate its absorption into the micro-organism. Agents of this type can be adapted to bind iron in the body and promote its excretion providing an example of the application of bioinorganic chemical knowledge in a clinical application.

Beyond clinical applications which relate to the natural utilisation of trace metals, others exploit quite unnatural and non-physiological features of metal chemistry. As examples the toxicity of platinum complexes is managed and targeted in treatments involving anticancer drugs such as cisplatin while, in diagnostic medicine, the differing biodistributions of various technetium complexes can be exploited in imaging applications.

1.3 Metallopharmaceuticals

1.3.1 General Requirements

Metals may not seem an obvious choice as components of pharmaceuticals and it is a common perception that metal compounds are toxic, unstable and generally not well suited for pharmaceutical applications. Certainly in some historic uses of metal compounds, the treatment may have been as dangerous as

the disease and early beneficial applications exploited the microbiocidal properties of metals such as mercury, arsenic and bismuth. Pharmaceuticals are more usually expected to be organic carbon based compounds, *e.g.* aspirin or penicillin, these being relatively unreactive chemically, often uncharged and amenable to structural variation to optimise their medicinal properties. However, over the past 30 years the particular chemical reactivities of metals, their magnetic and nuclear properties and the structural variety of their compounds, have become important in a variety of medical applications. Although not exactly Erlich's 'magic bullet', the organ specific uptake of technetium radiopharmaceuticals and the highly specific nature of the binding of cisplatin to DNA demonstrate the potential of this class of compound in specific medicinal applications.

In order to be useful in medicine, chemical compounds need to meet a variety of criteria. The most obvious requirement is that they must exhibit a medically beneficial effect with minimal toxic side effects. The relative benefit of a drug compared to its toxicity can be expressed in terms of its therapeutic index. This can be defined as the ratio of the dose required to kill 50% of test animals (LD_{50}) to the dose required to produce an effective therapeutic response in 50% of test animals (ED_{50}), *i.e.* Therapeutic Index = LD_{50}/ED_{50}. A high therapeutic index is clearly desirable, indicating that a relatively large dose is required to produce a toxic effect compared to that required to produce the therapeutic effect. However, because LD_{50} values cannot be obtained using human subjects, the therapeutic index can only have limited value for comparing different test compounds as it cannot be based on wholly human data.

As the dose of a compound with some medically beneficial effect is increased, and its *in vivo* concentration in the subject rises, it may be expected that the therapeutic benefit will increase. However, at some point this benefit will start to be negated by toxic effects until the dosage becomes so high as to be positively detrimental to the patient (Figure 2). Thus there will be a particular concentration range, associated with a particular dosage regime, which maintains the desired therapeutic effect. (A similar argument could be applied to foodstuffs. As an example carrots, a source of vitamin A, will produce a beneficial effect in normal dietary amounts. However, excessive consumption can lead to chronic or acute toxic effects, particularly in the liver where vitamin A accumulates. Those with liver damage, *e.g.* from over consumption of alcohol, are especially at risk). Typically the concentration of a drug will rise after administration then fall again as the compound is processed in the body and excreted. The timescale over which this process takes place, and any tendency for the drug to accumulate, will affect the dose regime necessary to maintain concentrations within the optimal therapeutic range.

A pharmaceutically useful compound must have an appropriate bioavailability and biodistribution, allowing it to pass through barriers such as cell membranes which lie between the outside world and the site of action (Figure 3). The rates of absorption, distribution, metabolism and elimination of a drug determine its pharmokinetics. Parenteral drugs enter the bloodstream directly, typically by intravenous injection, but oral drugs must be absorbed

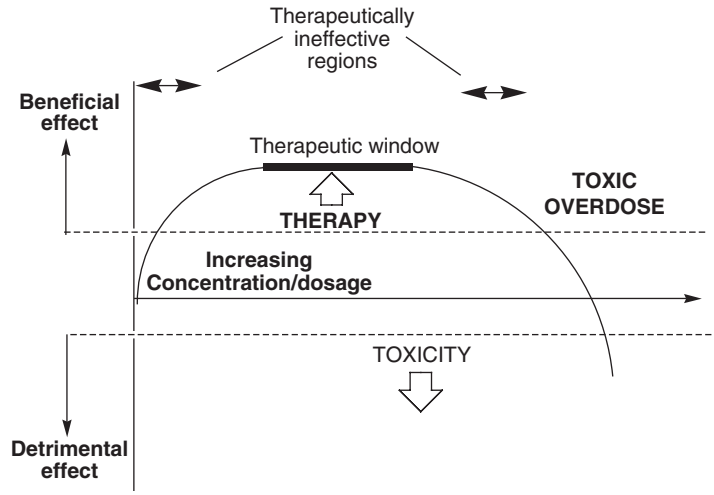

Figure 2 *The effect of increasing pharmaceutical dosage, or concentration in vivo, on benefit to the patient. Initially the beneficial effect increases with increasing concentration but at high doses, toxic effects predominate. Dosage regimes need to be adjusted to keep concentrations within the therapeutic window*

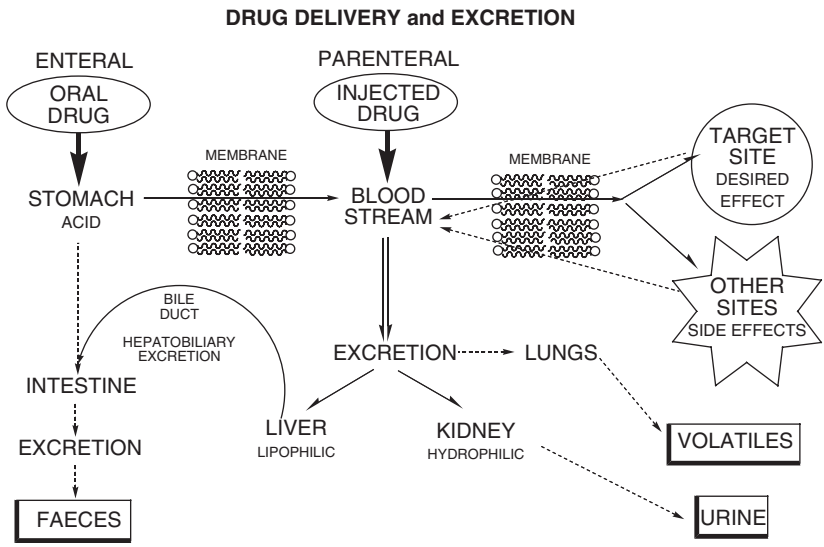

Figure 3 *A diagram summarising the intake, distribution and excretion routes of a pharmaceutical*

through the gut (Figure 3). Consequently, oral drugs must be able to withstand the acidic conditions in the stomach and pass through the gut wall to enter the bloodstream in a suitable form. This presents further challenges in the design of oral drugs compared to parenteral drugs. Since uncharged compounds are

generally absorbed through membranes more readily than charged compounds, neutral compounds, or those which become neutral under acidic conditions, tend to be more promising candidates for the formulation of oral drugs.

To be effective a drug will need to be sufficiently stable *in vivo* that it is not degraded by biological processes before having had chance to exert its effect. It will also need to reside in the body long enough to exert its effect but not so long as to irreversibly accumulate to the extent that unacceptable toxic effects start to arise. Sometimes drugs are administered in a form which is not the therapeutically active agent. Rather they are precursors which will be converted to the active form by metabolic processes *in vivo*. Such compounds are known as prodrugs and must show suitable pharmokinetics before and after their conversion to the active form.

The primary means of distributing a drug around the body will be *via* the bloodstream. Here pharmaceutical compounds can encounter a variety of challenges. Firstly, the compound will need to retain sufficient solubility in the aqueous saline environment of the blood. Secondly, interactions with the proteins and other species present in blood need to be considered. This is particularly important for metal containing drugs since proteins may compete to bind the metal and so influence its biodistribution and properties. In some cases this may be a desirable effect, in others it may not. As an example, radioactive gallium is easily converted to an insoluble form in water and so is injected as its citrate which is more stable. In the blood the iron transport protein transferrin competes with citrate for the gallium and transfers it to other sites where it is deposited. In other cases binding to serum proteins in the blood may lead to an unwanted distribution of the metal, so it may be necessary to inject it in a form resistant to competitive binding by serum proteins. A further challenge is provided by natural metabolic processes for affecting the breakdown of chemicals in the body. In the case of metal containing drugs, this process can sometimes release the metal and allow it to be converted to a form which may accumulate in the body. This issue needs to be considered in the design and formulation of a metallopharmaceutical agent. The elimination and excretion of a drug and its metabolic products can usually take place through the liver or kidneys. Water soluble, hydrophilic, compounds are generally excreted in urine *via* the kidneys (Figure 3). Here the process of glomerular filtration eliminates simple salts and small molecules while tubular secretion deals with larger molecules such as proteins, some of which may be readsorbed. Fatty, lipophilic, compounds tend to be processed through the liver and bile duct (hepatobiliary system) passing into the gut for readsorption or excretion in faeces.

1.3.2 Structure-Activity Relationships

Understanding the relationship between the molecular structure, and properties, of a chemical compound and its biological activity can provide an important tool to aid the design of new pharmaceuticals. Unfortunately, the

complicated nature of the interactions between chemical compounds and biological systems does not make it easy to devise accurate quantitative structure-activity relationships. However, by collecting data on the behaviour of a large number of closely related compounds it may sometimes be possible to develop a general understanding of the importance of different structural features in the molecule. In some cases more quantitative correlations can be developed between a parameter describing a particular property of the compound and a particular biological effect.

One example of a parameter which has been used in this way is provided by a partition coefficient describing the distribution of a compound between water and a selected oily liquid which does not mix with water. The partition coefficient provides a numerical value reflecting the relative preference of a compound for oily organic regions, such as membranes, compared to aqueous media, such as blood serum. The numerical value may be correlated with a chosen pharmacological parameter such as the minimum concentration required to induce a particular physiological response. Data from a large number of experiments can be used to define the relationship between the partition coefficient and the physiological response. Once the form of this relationship has been established, the chemical structure of new trial compounds can be designed so as to optimise the partition coefficient without modifying other structural features essential for biological activity. In this way the search for new active compounds can be focused on those most likely to prove effective.

Usually drugs will need to interact with a site in the body which has a specific chemical structure so that the size and shape of a molecule are important design features. The presence of specific chemical groups at particular locations in the molecule can also be important as can the polarisability of parts of the molecule. Modern computational methods offer a powerful tool for modelling the properties of compounds and their compatibility with potential binding sites. Together with structure-activity relationships, such computer modelling can facilitate the design of new active compounds offering significant economies in the costly process of drug development. This approach is now well established for organic compounds but the inclusion of metals creates some additional complications when it comes to the design of new metallopharmaceuticals.

Where a metal is fully contained within an organic host, modification of the host structure to optimise biological distribution and activity might follow precedents set with non-metal containing drugs. However, often the properties or reactivity of metal will be an important feature of a metallopharmaceutical. In such cases the host structure containing the metal must not be modified in a way which might impair the ability of the metal to perform its function. Thus, although modelling and structure-activity relationships can be applied to metallopharmaceuticals, it is necessary to introduce additional considerations relating to the rôle of the metal and the nature of its interaction with the host structure in which it is contained. Some examples of the importance of structure in determining the efficacy of specific metallopharmaceuticals can be found in Chapters 3 and 4.

1.3.3 Clinical Trials

The evaluation of new compounds for clinical use involves several stages. There will be a discovery or design phase in which a serendipitous discovery, or a new example of a class of compound known to, or thought likely to, have medicinal properties, is evaluated. If sufficiently promising results are obtained, and acceptable toxicity levels established, the drug may proceed into clinical trials. These can be divided into four phases as follows:

PHASE I — Small groups of healthy volunteers receive the drug so that assessments can be made of its absorption, biodistribution, pharmokinetics, accumulation, side effects and dosage. Initially the trial will start with smaller doses than needed for therapeutic effects to check for adverse reactions. If the drug is shown to be safe in humans the trial may proceed to Phase II.

PHASE II — Small groups of patients suffering from the ailment to be treated receive the drug to establish its effectiveness. The optimum dosage regime and any adverse reactions are assessed.

PHASE III — A large number of patients with the ailment are evaluated in double blind trials involving the new drug, comparison treatments and placebos to establish the efficacy of the new drug and obtain safety data to support applications for its licensing and approval for clinical use.

PHASE IV — Monitoring of patients treated with the drug continues following approval and throughout general clinical use in order to further optimise procedures and check for previously undetected side effects.

This whole process can take many years. As an example US FDA approval for the clinical use of cisplatin was granted in 1978, some 14 years after the serendipitous discovery that platinum compounds suppressed cell division and 7 years after the start of Phase I clinical trials.

1.4 The Special Properties of Metals

1.4.1 Comparison with Organic and Biological Compounds

In aqueous solutions metals differ from organic compounds in a number of important respects. In organic compounds carbon–carbon bonds are generally rather stable and provide the robust skeleton, which gives parts of biological molecules their shape and stability. Where there is a need for carbon containing units, such as amino acids in proteins, to be connected and disconnected, carbon–nitrogen or carbon–oxygen bonds are usually used to form the linkages. Although bonds of this type are stable, compared to carbon–carbon bonds they can be more easily broken or formed through the addition or

elimination of water. This can be done under biochemical control allowing proteins, for example, to be broken down and reassembled with comparative ease. Metals are often quite reactive towards changing the atoms they are bonded to in aqueous media. However, rates of reaction can vary substantially between different metals and between different compounds of the same metal. This offers a wide range of chemical behaviour. Sometimes it is useful to have metal compounds, which are inert and do not readily convert to other forms. Sometimes it is important for the metal compound to be sufficiently reactive to allow the metal to be exchanged and incorporated into specific biological systems. Controlling the reactivity of the metal compound is one way of controlling which biological system can have access to the metal.

The interactions between charged metal atoms and other species in solution are somewhat different from those involved in the interactions between organic biological compounds. Two biological compounds may enter into quite complicated interactions through mutual recognition and binding between several complementary sites in the two different species (Figure 4). The interactions between enzymes and their substrates or the complementary strands of DNA in a DNA duplex offer examples of this type of behaviour. The individual linkages may not be particularly strong compared to conventional chemical bonds, but can act in concert to give quite strong overall binding interactions. Unlike organic compounds, metals typically show what is known as 'Lewis acid' behaviour, that is they bind to atoms such as oxygen in water or nitrogen in ammonia. This allows them to spontaneously assemble groups of atoms around them to form a 'metal complex'. They can also bind to certain oxygen, nitrogen or sulfur atoms in proteins and other biochemical species. In this way they can influence the structures of organic substrates to which they become attached (Figure 5). They can also form reactive centres within biological species to which they have become bound. Metals bound within enzymes may act to

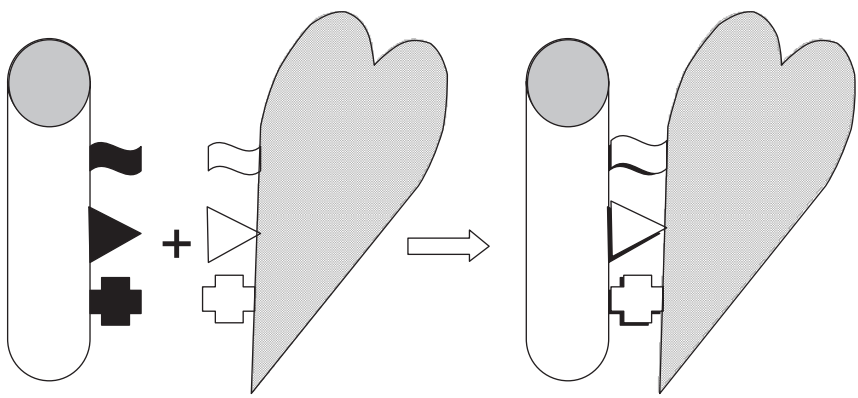

Figure 4 *A schematic representation of two biological molecules with complementary binding sites recognising each other and combining to form a ''complex''. Examples might be an enzyme and its substrate or the two strands of a DNA double helix*

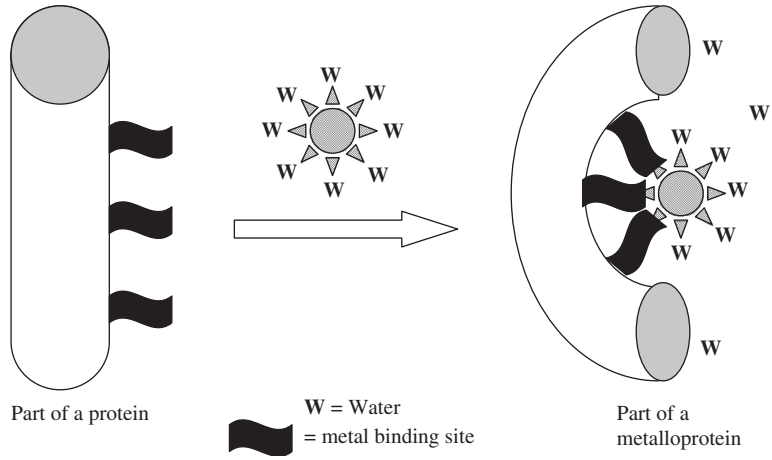

Figure 5 *A schematic representation of an aquated metal ion interacting with part of a biological substrate, such as a protein, to release some water and make the protein fold. This may just have a purely structural effect, or the metal may become a reactive centre within the biological molecule*

polarise a substrate to promote a particular reaction. An example is provided by zinc in carboxypeptidase acting as a centre catalysing protein degradation through the addition of water. Some metals can also change their charge and so act as electron transfer agents. This behaviour is found with iron in the cytochromes involved in mitochondrial electron transfer chains. Copper is also used as an electron carrier, for example in catalysing the conversion of superoxide to dioxygen and peroxide. Metals can also bind and activate small molecules such as dioxygen or nitric oxide in a much simpler and more reversible manner than would be possible using organic molecules. The use of iron centres to transport dioxygen in hemoglobin provides one obvious example.

Beyond the particular chemical features which metals bring to living systems and pharmaceuticals, they offer other attributes important for medical applications. Metals can form stable magnetic materials of a type becoming important in Magnetic Resonance Imaging applications. Organic compounds with magnetic properties tend to be very reactive and unstable *in vivo*. In addition the magnetism they can create is very limited compared to metals such as iron, manganese or gadolinium. Stabilising suitable forms of magnetic metals for use in magnetic resonance imaging applications presents a particular chemical challenge in (i) maintaining suitable magnetic behaviour (ii) controlling their biodistribution and (iii) preventing unwanted accumulation of the metal, for example in the liver. Radioactivity is another phenomenon with significant medical applications and a number of metallic elements are available in radioactive forms suitable for clinical use. In order to exploit this radioactivity it is again important to incorporate the metal in a compound with biodistribution and pharmokinetic behaviour suitable for the clinical application. This

requires careful selection of the form in which the metal is administered and this, in turn, requires a good understanding of the chemistry of the metals involved.

1.4.2 Coordination Chemistry

The metallic elements used in medicine have characteristic features crucial to their particular application and not shared with organic compounds. In order to understand why a particular metal should be chosen for a particular medical application it is necessary to appreciate the properties and chemistry of the metals concerned. The branch of chemistry most closely concerned with the behaviour of metals under conditions relevant to living systems is known as coordination chemistry. This encompasses the chemistry of metals in aqueous media and their interactions with materials such as those encountered within living organisms or used in the formulation of metallopharmaceuticals. The origins of coordination chemistry as a distinct branch of chemistry date back to the beginning of the 20th century and are marked by the award of a Nobel prize to Alfred Werner in 1913. Among other achievements, Werner established the structure of the compound now know as cisplatin and used in cancer therapy.

To understand why metal atoms form compounds with particular structures and reactivities, it is necessary to have some appreciation of atomic structure and of chemical bonding models which go beyond those applicable to lighter elements such as carbon. The Crystal Field Theory proposed by Bethe in 1929 offered a starting point, although this was not developed into a generally used bonding model for coordination compounds until the 1950s. More elaborate theoretical models of bonding in metal compounds have since been developed but the Crystal Field Theory model continues to offer a relatively simple and accessible insight into the chemistry and magnetic properties of many metals. Chapter 2 attempts to provide a concise introduction to coordination chemistry. Those with prior experience of the chemistry of metals may wish to skip part or all of this chapter. Those with a limited chemistry background will hopefully find it a useful introduction to coordination chemistry, which underpins the subsequent chapters on diagnosis and therapy, and a platform for further reading on the chemistry of metals.