Preface

Two roads diverged in a wood, and I –
I took the one less traveled by,
And that has made all the difference.

Robert Frost 'The road not taken' from Mountain Interval (1916).

I still feel that 'the road not taken' reflects many of the choices that I made early in my career – deciding to read biochemistry instead of continuing with the rest of my schoolmates to read chemistry; carrying out my final year project in a protein chemistry laboratory when the rest of J.N. Davidson's Department in Glasgow was working on nucleic acids; going off on a post-doctoral fellowship to Germany, without having any more competence in the language of Goethe and Schiller than that required to translate a passage of scientific German (still a requirement for science graduates in the 1960s) with the aid of the faithful Patterson's German/English scientific dictionary. Yet, on more considered reflection of the really significant roads that one has taken over the years, it is striking how many of them seem to involve an important element of predestination[1].

My 'Blood and Iron' connection began during my final year undergraduate project in Glasgow, when my supervisor Dr George Leaf suggested that I should work on the haemopeptide from horse heart cytochrome c. Although my subsequent doctoral thesis involved gas chromatography of amino acid derivatives[2] and mass spectrometry of peptides, I was working in a laboratory just next door to that of Hamish Munro, who, with his doctoral student Jim Drysdale, was studying the regulation of ferritin synthesis in rat liver. In May 1966 my head of Department, J. Norman Davidson, informed me that there would be a lectureship vacant in the autumn, so I had better write up my thesis[3], and – by the way – if I wanted to have a post-doctoral year abroad I should get going fast to organise it! An interview for the post of lecturer and, two days later, a doctoral viva (these were, after all, the golden sixties) in early September, led to me turning up on the doorstep of the Max-Planck Institut für Biochimie in Munich in January 1967 with a PhD and a lectureship in Biochemistry in my pocket. There I began isolating and characterising insect haemoglobins

[1]The notion that events somehow conspire to lead to decisions which are not really taken, but rather impose themselves. I already referred in previous editions to the impact of the writings of Soren Kierkegaard, in many ways the philosophical father of predestination, on my thinking, particularly when I was envisaging entering theology.

[2]The method failed to be of any practical use for the analysis of hydrolysates of peptides purified by 2-dimensional paper chromatography and electrophoresis, no doubt because of the lack of specificity of the detection system.

[3]My doctoral students will understand my reluctance to believe that it needs 6–8 months to write a thesis – I wrote mine between the end of May and the middle of August, while still carrying out final experiments and travelling to Edinburgh four nights a week for rehearsals with the Edinburgh Festival Chorus. We performed the Mahler symphony number 8 and the Britten War Requiem (with Peter Pears, Dietrich Fischer-Dieskau and Galina Vishneskya as soloists, the Melos Ensemble conducted by Benjamin Britten himself, with the Scottish National Orchestra and the Chorus conducted by Sir Alexander Gibson).

in Gerhard Braunitzer's laboratory, working together with Volkmar Braun, which resulted in my first publication (Braun *et al.*, 1968).

Back in Glasgow, no doubt influenced by Hamish Munro, I began my long involvement with ferritin. But, at the International Congress of Biochemistry in Switzerland, an invitation from Heinz-Günther Wittmann to give a seminar in Berlin led to my leaving the permanent position in Glasgow for a nonpermanent position. So, from 1970 till 1973 I was a Senior Fellow of the European Molecular Biology Organisation (EMBO) at the Max-Planck Institut für Genetik in Berlin. There, I continued my work on ferritin, but also worked on ribosomal protein–RNA interactions. During my stay in Berlin (still then within the confines of the Wall), I travelled extensively, attending symposia and workshops on protein structure and on protein synthesis. Among the many colleagues and friends whom I met were Goetz Domagk, then Professor of Biochemistry at the Catholic University of Louvain, and Hubert Chantrenne, of the Free University of Brussels, whom I had entertained during his visit to Glasgow to give a lecture on protein biosynthesis in my capacity as Secretary of the student Biochemical Society[4]. Without my realising this, they played key roles in my being invited to succeed Domagk in autumn 1973 as chair of biochemistry in the Chemistry Department of the Catholic University of Louvain in Louvain-la-Neuve. In none of these decisions was I remotely involved – things simply happened. So it was that I came in a preordained fashion to protein chemistry, to iron and to Belgium.

My interest in the broader field of biological inorganic chemistry developed during the organisation, with Cees Veeger[5], of seventeen Advanced Courses on 'Chemistry of Metals in Biological Systems' (with the financial support of the Federation of European Biochemical Societies (FEBS), the European Science Foundation and the European Union). With the enthusiastic help of a devoted faculty, we have trained more than 750 young scientists in the techniques required to investigate the role of metals in biological systems. It is with pride and a great deal of pleasure that I continue to see on the platforms of both European and International Congresses of Biological Inorganic Chemistry many of the students who passed through the Louvain-la-Neuve courses. In many ways, the frustration of not being able to secure the funding to continue these courses over the last few years has resulted in my first venture into writing a textbook for students (Crichton, 2008).

A relatively limited number of inorganic elements play important roles in biology, the environment and medicine. Their relative abundance in the earth's crust, in seawater, and examples of their specific functions, are presented in Table P.1 (as are those of a few selected nonmetals). The basic principles involved in the bioselection of elements conform to four fundamental rules: (i) abundance, (ii) efficacity, (iii) basic fitness for a given task, and (iv) evolutionary pressure (Frausto da Silva and Williams, 2001; Crichton, 2008). A rapid examination of Table P.1 shows that abundance, for example, is not an adequate requirement for biological fitness (aluminium is perhaps the best example, and owes its inclusion to the fact that it has more or less been brought into our present day biological environment by man himself). Individual elements are particularly fitted for specific functions, often as a direct consequence of their chemical properties. Na^+ and K^+, which form complexes of very low stability and are therefore very mobile in biological media, are ideally suited for use in ionic

[4]In the same capacity I also entertained Feodor Lynen, whom I had got to know during my stay in Munich – the cost being, as I recall, a bottle of Glenfiddich after a well lubricated dinner!

[5]Cees and I were also involved as members of the Steering Committee of the European Science Foundation 'Chemistry of Metals In Biological Systems' Programme, which was largely responsible for the creation of both the *Journal of Biological Inorganic Chemistry* and the Society of Biological Inorganic Chemistry.

Table P.1 *Relative abundance and examples of functions of inorganic elements (and a few selected nonmetals) which play an important role in biology*

Metal	Crustal Average (ppm)	Seawater mg/l	Examples of specific functions
Sodium	2.8×10^4	1.1×10^4	osmotic control, electrolytic equilibria, currents
Magnesium	2.1×10^4	1.4×10^3	phosphate metabolism, chlorophyll
Aluminium	8.1×10^4	1×10^{-3}	neurotoxic, solubilised by acid rain
Silicon	2.8×10^5	3	prevents aluminium toxicity
Potassium	2.6×10^4	3.9×10^{-2}	osmotic control, electrolytic equilibria, currents
Calcium	3.6×10^4	4.1×10^{-2}	second messenger, muscle activation, biominerals
Vanadium	135	2×10^{-3}	nitrogenase, peroxidases
Chromium	100	5×10^{-4}	glucose metabolism ?
Manganese	950	2×10^{-3}	oxygen production and metabolism, structure
Cobalt	25	4×10^{-4}	B_{12} coezymes, alkyl transfer
Nickel	75	7×10^{-3}	hydrogenases, urease
Copper	55	3×10^{-3}	electron transfer, oxidases, oxygen transport
Zinc	70	1×10^{-2}	Lewis acid catalysis, regulation (DNA binding)
Selenium	5×10^{-2}	9×10^{-9}	glutathione peroxidase
Molybdenum	1.5	1×10^{-2}	nitrogenase, oxidases, oxo-transfer
Tungsten	1.5	1×10^{-4}	dehydrogenases
Iron	$\mathbf{5 \times 10^4}$	$\mathbf{3 \times 10^{-3}}$	**Oxygen transport, storage, activation and detoxification, electron transfer, nitrogen fixation, ribose reduction, etc.**

Source: from Mason and Moore (1982).

equilibria and electrolytic circuits. Mg^{2+} is generally involved with phosphate compounds and phosphate metabolism, while Ca^{2+}, in addition to its structural role in biological minerals like bone, plays a key role as an intracellular signalling messenger. Zn^{2+}, redox inactive, is found in more than 300 enzymes, functions in many situations as a Lewis acid, but is also involved as a component of gene regulatory proteins. Copper, like iron is involved in many electron transfer reactions as well as in enabling cells to cope with dioxygen. Manganese, also redox active, constitutes the catalytic centre of the photosynthetic water splitting complex, oxidising two molecules of water to dioxygen (and in the process transforming our atmosphere from reducing to oxidising). And so one could continue; yet, if each of these elements have their own particular specificities with regard to biological function, we will consider one metal only (with the exception of a brief excursion into its interactions with other metals in Chapter 12) in what follows. This metal, which I consider to be of capital importance, is **iron**, which, as a glance at the table will show, has a multiplicity of functions. The reader will, I trust, forgive me this selectivity, for it is with iron that I have passed the last four decades, and it is the metal with which I am the most familiar.

As I set out on this next step along a road already well travelled, I would like to address myself first of all to you, my dear readers and colleagues, and to thank you for the enormous encouragement that you have given me over the last two decades to continue, and develop, the project which I began in 1990 and continued in the 2001 edition. It has been an important stimulus to undertake the preparation of this third edition, to seeing well-thumbed copies of both editions on your desks and bookshelves, and of hearing from so many how useful you,

and particularly your students, have found them to be in giving a critical as well as panoramic view of iron metabolism. I continue to adopt the position that when writing a review (or even more important, an overview), it is important not simply to provide a seed catalogue of the current literature, but to take a reasoned position concerning the probability that one particular viewpoint is correct. As Aneurin Bevan[6] pointed out, '*We know what happens to people who stay in the middle of the road. They get run down*'. But, enough of this; rather than the critique emitted by one of the characters in John Osborne's, '*Look back in Anger*', '*They spend their time mostly looking forward to the past*', let us address what has been happening since the last edition.

The importance of well-defined amounts of iron for the survival, replication and differentiation of the cells of animals, plants and almost all microorganisms is well established (Crichton, 2001). One notable exception is constituted by the so-called 'lactic acid bacteria', which include members of the *Lactobacillus* family together with some *Lactococcus* and *Streptococcus*. They are of particular importance in the manufacture of dairy products, including cheeses[7] and yoghurt, as well as in the spoiling of milk. Their adaptation to growth in the presence of the strongly iron binding protein of milk, lactoferrin (Chapter 5), has led to the conclusion that they can grow in the absence of iron, although unequivocal proof of this is not easy to obtain.

However, for most living organisms, iron in excess is toxic, and iron deficiency is also a general problem in biology, which means that iron homeostasis is extremely important, both at the cellular and the systemic level. And since humans have little capacity to excrete iron, it follows, as was originally suggested by McCance and Widdowson (1937), that iron balance in man is primarily determined by iron absorption. Some examples of the multiple roles of iron in biology have been selected here, to give the reader a clear impression of the importance of this element. This panorama is by no means comprehensive, nor is it intended to be. It should, like the 'ameuse geule' served before the meal in many French restaurants, simply whet the appetite of the reader for what is to follow.

While iron is the fourth most abundant element in the earth's crust, it is only present in trace concentrations in seawater (Table P.1). In particular, the surface waters of the Southern Ocean contain extremely low concentrations of iron (20–50 pM) thereby limiting primary production of phytoplankton (Martin and Fitzwater, 1988). Since the Southern Ocean exerts a major control on the partial pressure of carbon dioxide (pCO_2) in the atmosphere, low rates of photosynthesis and biological carbon export in Antarctic waters result in macronutrients being largely unused. The resulting up-welled CO_2 enters the atmosphere, sustaining the relatively high interglacial atmospheric pCO_2 of the present day. Martin subsequently proposed (Martin, 1990) that natural variations in the atmospheric iron flux could ultimately regulate primary production in the Southern Ocean and influence the pCO_2 of the atmosphere, potentially contributing to global warming of the planet.

Mesoscale iron addition experiments (FeAXs) have unequivocally shown that iron supply limits production in one third of the world's oceans, where surface macronutrient concentrations are perennially high (Boyd *et al.*, 2007). The findings of these 12 FeAXs show that iron supply exerts control over the dynamics of plankton blooms, which in turn affects the

[6]The left-wing miner and trades union official from South Wales became Minister of Health in the 1945 post-war Atlee Government, and was responsible for establishing the UK National Health Service. The NHS was created sixty years ago, on the 5 July 1948, enabling the government to take over responsibility for all medical services and supplying free diagnosis and treatment for all.

[7]One is reminded of the classic reply of Charles de Gaulle, in response to the question 'Why is France such a difficult country to govern?' – 'Do you know of any other country that produces more than 360 different sorts of cheese'.

biogeochemical cycles of carbon, nitrogen, silicon and sulfur, and ultimately influences the world's climate system. In the Southern Ocean Iron Experiment in 2002, in which 1.7 tonnes of iron sulfate was dropped in the sea (Coale *et al.*, 2004), the data accumulated imply that each atom of iron added to the sea could pull down between 10 000 and 100 000 atoms of carbon out of the atmosphere by encouraging plankton growth, which captures carbon and sinks it deep towards the ocean floor (Bishop *et al.*, 2004). Scaling up of such iron fertilization of the sea could have a real impact on the high level of carbon dioxide in the atmosphere, which is causing global warming, with some researchers estimating that, in the Southern Ocean alone, the technique could absorb 15% of the carbon dioxide build-up. However, ecologists are quick to warn that the technique could damage marine ecosystems in ways that remain to be established, and the future of massive fertilization of the oceans is still in a state of turmoil (Buesseler *et al.*, 2008).

Iron added to so-called high nitrate low chlorophyll (HNLC) regions of the world's oceans has clearly been shown to promote growth of autotrophic as well as heterotrophic microorganisms. What, then, are the molecular mechanisms by which marine microorganisms compete for the added iron (reviewed in Butler, 2005)? In common with other bacterial species many marine bacteria have been shown to produce siderophores (see next paragraph). The marinobactins, aquachelins, amphibactins, ochrobactins and synechobactins are siderophores isolated from distinct genera of marine bacteria. They all contain a unique head group that coordinates iron(III) and one of a series of fatty acid tails (C_8–C_{18}). The marinobactins, aquachelins and most likely the amphibactin lipopeptides are characterized by low critical micelle concentrations (CMCs). At concentrations exceeding their CMC, the apo marinobactins form spherical micelles which shrink in diameter upon coordination of Fe(III). Upon addition of excess Fe(III), the Fe(III)-marinobactin micelles undergo a transition to form vesicles (Martinez *et al.*, 2000; Owen *et al.*, 2005). These properties seem to have evolved as a common iron acquisition strategy for marine bacteria, the properties of which remain to be elucidated (Martinez *et al.*, 2003).

Ironically, the propensity of some pathogenic bacteria, and particularly highly virulent strains, to infect their hosts, whether other microbes, plants or mammals, depends on their capacity to produce highly effective iron uptake systems. The first to be clearly identified was the aerobactin system for iron uptake in virulent strains of *E. coli*, which is encoded by the ColV plasmid (Williams, 1979). To combat the effectiveness of the siderophores produced by highly virulent bacterial strains, animals may synthesize specific proteins, like lipocalin, to bind and nullify their action. However, these countermeasures can be thwarted by at least one bacterium, *Salmonella*, glycosylating its siderophore (enterobactin/enterochelin) so that binding of the modified siderophore (now termed salmochelin) with lipocalin no longer occurs (Ratledge, 2007). This early conceptual observation has subsequently been underlined by the identification, not only of pathogenic solutions to gaining access to host iron supplies, but also to the increasing realisation that horizontally transmissable mobile genetic elements, designated as 'pathogenic islands' constitute a source of enhanced virulence, frequently associated with uptake of essential nutrients, like iron (Hacker and Kaper, 2000).

In previous editions of this book I have evoked the way in which many plants in the *Season of mists and mellow fruitfulness* (John Keats *To Autumn*) shed their leaves, and with it much of their mineral content, including the iron, so essential for photosynthetic electron transfer pathways. Come the spring, they must recuperate this iron from the soil and send it, sometimes tens of metres high in the air, to regenerate their photosynthetic capacity in the newly formed leaves. While we continue to enlarge our understanding of the uptake of soil iron by the

roots, we are now beginning to have a much better idea of how iron is transported around plants. Since the publication of the last edition of this book, the second fully sequenced and annotated plant genome, that of rice (International Rice Genome Sequencing Project, 2005), has joined that of *Arabidopsis* (Arabidopsis Genome Initiative, 2000) providing scientists with the possibility to compare a monocot against a dicot plant in terms of their gene content and genome structure. However, it could also allow the identification of possible candidate genes which play a role in iron uptake, transport and homeostasis by analogy with fungal and mammalian systems. These two genomes are both relatively small in size (125 Mbp and 389 Mbp for *Arabidopsis* and rice, respectively), and an even more abundant harvest is awaited from the ongoing analysis of the much larger cereal genomes, such as barley, maize and wheat. The abundance of highly conserved repetitive elements has contributed to the considerable increase in physical size that is observed in these cereal genomes when compared with rice and *Arabidopsis*. Approximately 80% of the maize genome[8] is composed of repetitive DNA mainly long terminal repeat (LTR)-retrotransposons.

Proteins with iron–sulfur ([Fe–S]) cofactors play important roles in many cellular processes, such as redox reactions, metabolic catalysis and the regulation of gene expression (Chapter 2). Mitochondria play a central role in [Fe–S] protein biogenesis and our understanding of the way in which these clusters are assembled has evolved rapidly in the last few years. [Fe–S] clusters are constructed from cysteine as a source of sulfur, combining it with iron to synthesize the [Fe–S] cluster on scaffold proteins, and the cluster is finally incorporated into recipient apoproteins. In eukaryotes, such as yeast and human cells, more than 20 components are known that facilitate the maturation of [Fe–S] proteins in mitochondria, cytosol, and nucleus (Lill and Muhlenhoff, 2006). These components are also involved in other cellular pathways, such as the regulation of iron homeostasis or the modification of tRNA. The final step of haem biosynthesis, the insertion of iron into protoporphyrin IX by ferrochelatase, also takes place in the mitochondria.

In both [Fe–S] cluster formation and haem biosynthesis, the protein frataxin is thought to the play the role of an Fe^{2+} chaperone (Lill and Muhlenhoff, 2006; Zhang *et al.*, 2005, 2006). The expansion of a trinucleotide repeat in the first intron of the frataxin gene is the origin of Friedreich's ataxia[9], the most common hereditary ataxia and the most prevalent cerebrellar ataxia among children and adults in Europe. This neurodegenerative disease is characterized by the accumulation of large amounts of iron within the mitochondria, as is also observed in a number of haematological disorders associated with defects in Fe/S protein biosynthesis. One such example is cross-linked sideroblastic anaemia with associated ataxia (Bekri *et al.*, 2000), in which a mutation in a membrane transporter results in impaired transport of [Fe–S] clusters from the mitochondria to the cytoplasm.

I referred earlier to the studies of Hamish Munro on ferritin biosynthesis, which resulted in the identification in the 5'-Untranslated Region (UTR) of both H and L chain ferritin mRNAs of putative stem loops consisting of a highly conserved sequence of 28 nucleotides termed Iron Regulatory Elements (IREs). These IREs, are found in both the 3'- and 5'-UTRs of a number of other proteins involved in iron metabolism. With the discovery of iron regulated cytosolic RNA binding proteins, Iron Regulatory Proteins (IRPs), which bind to the IREs (Liebold and Munro, 1988; Rouault *et al.*, 1988), it became clear how in many mammalian cells there is a reciprocal regulation, at the level of mRNA translation, of iron uptake and intracellular iron

[8] The 2.4–2.7 gigabase pairs (Gbp) maize genome is almost as large as the human genome (2.8 Gbp) and the determination of its complete DNA sequence is imminent.

[9] Ataxia – total or partial inability to coordinate volantary bodily movements, particularly muscular movements.

utilisation. IRP1 exists in an iron free form, which binds to the IRE stem loop, and an iron bound form, with an [Fe–S] cluster, which has aconitase activity. Our understanding at the molecular level of the conformational changes involved in this transition have been greatly advanced by the determination of the structure of the protein in both its forms (Dupuy *et al.*, 2006; Walden *et al.*, 2006).

In the previous edition we highlighted the impressive developments in our understanding of iron metabolism through the application of the revolutionary techniques of molecular biology. This resulted in the identification of new genes and their gene products involved in iron uptake and cellular utilisation, with the prospect of progressively approaching an understanding of their function. The role of many of these proteins has become increasingly clear in the last few years. But we can also hail the appearance of an impressive number of new genes and gene products in the increasingly complex network of reactions that constitute iron metabolism. Not the least impressive of these is the antimicrobialpeptide hormone, hepcidin, which has swept over the mammalian iron field like some kind of virus[10] (which it is certainly not!).

The initial discovery in Rennes that hepcidin was significantly up-regulated (Pigeon *et al.*, 2001) in the liver of iron overloaded mice was obtained by performing a suppressive subtractive hybridisation between cDNAs from the liver of iron overloaded and control mice – the same strategy that had been employed to identify IREG1, the iron transporter up-regulated in conditions of increased iron absorption in the duodenum (McKie *et al.*, 2000). This was followed by the serendipitous discovery in Paris that inactivation of the mouse hepcidin gene resulted in iron overload (Nicolas *et al.*, 2001) – the authors were seeking to knock-out an adjacent gene, but the genetic 'scissors' used to excise it removed hepcidin expression as well. In summary (for more detail see Chapter 8), the way in which hepcidin exerts its key role in the regulation of systemic iron homeostasis is as follows (Nemeth *et al.*, 2004; de Dominico *et al.*, 2007). Hepatocytes secrete hepcidin, which binds to IREG1 (ferroportin), the iron efflux transporter expressed in all iron exporting cells. This results in the phosphorylation, internalization, ubiquitination and ultimate degradation of ferroportin. When hepcidin concentrations are high, ferroportin degradation exceeds its rate of synthesis, resulting in decreased iron uptake from the intestine, and retention of iron within macrophages and hepatocytes. In contrast, when hepcidin concentrations are low, ferroportin synthesis exceeds its rate of degradation, increased amounts of iron are released at the basolateral membrane of intestinal enterocytes, from macrophages (iron derived from senescent erythrocytes) and from hepatocytes into the extracellular fluid.

On account of our incapacity to excrete significant amounts of iron, pathological disorders of iron metabolism associated with excessive iron accumulation, principally in parenchymal tissues, are often observed in man. These diseases, designated as Hereditary Haemochromatosis (HH), are now known to be a family of conditions, characterised by excessive dietary iron absorption. The classical form of HH (type 1) is an autosomal recessive, HLA linked disease. It is the most widely prevalent form of HH, and indeed one of the most frequent genetic disorders in man, more common than cystic fibrosis, muscular dystrophy and phenylketonuria combined, with an estimated carrier frequency of 1 in 200 in Caucasian populations. The identification of the candidate gene, *Hfe*, the determination of its X-ray structure, the demonstration of its interaction with transferrin receptor 1 (TfR1), and the determination of the structure of its complex with the transferrin receptor, were all described in the previous edition. But it is only with the recognition that the site of action of HFE is not in the gastrointestinal

[10]Since the six publications in 2000 and eight in 2001, the total to date (August 2008) is in excess of 1100 (PubMed).

tract, but rather in the liver, that we have recognised that, as with all of forms of HH, the primary defect in type I HH is in the regulation of hepatic hepcidin synthesis. Mice with hepatocyte specific *Hfe* ablation develop systemic iron overload, whereas enterocyte specific or macrophage specific disruption of *Hfe* results in a normal iron phenotype (Vujic Spasic *et al.*, 2007, 2008). Taken together with the lack of progression of hereditary haemochromatosis in human liver transplant recipients receiving normal livers, it seems clear that hepatocytes are the principal cells controlling systemic iron homeostasis, through the secretion of hepcidin. Indeed, we might consider that hepcidin is to iron homeostasis what insulin is to the homeostasis of blood glucose (Pietrangelo, 2007).

Iron is particularly indicated for catalysing reactions which necessitate a free radical mechanism, like the transformation of ribose to deoxyribose. This reaction, which is of primordial importance for DNA replication and cell division, is catalysed by ribonucleotide reductases, the best class of which has a dinuclear iron centre and a stable tyrosyl free radical as cofactor. However, as we will see in Chapters 10 and 11 (which have been prepared in collaboration with my long-standing (and long suffering) collaborator Roberta Ward), iron can also catalyse reactions with molecular oxygen to produce highly reactive oxygen species (ROS). This is the so-called oxygen paradox – oxygen is an absolute necessity for our energy-economical anaerobic life style, yet it is a potential toxin. On the plus side, the arrival of oxygen enabled organisms which developed respiratory chains to extract almost 20 times more energy from metabolism than was available when using redox balanced fermentations. The down-side was that molecular oxygen proved to be toxic, generating the potentially dangerous hydroxyl ion, $OH^{\bullet}$, a short lived but highly reactive free radical, which causes enormous damage to all biological molecules. As we develop in Chapter 11, this has enormous consequences for age related disorders, particularly neurodegenerative disorders. As we have argued (Crichton and Ward, 2006; Crichton *et al.*, 2008), dysregulation of metal ion homeostasis, particularly of redox active metals like iron and copper in specific brain regions, leads to the generation of ROS, which can either directly damage proteins, or lead to the formation of highly reactive aldehydes. These, in turn, generate protein carbonyls, resulting in protein denaturation, aggregation and a subsequent failure of the ubiquitin/proteasome system to eliminate these defective proteins. The end result, as in Alzheimer's, Parkinson's, Huntington's disease and countless others, is neurodegeneration accompanied by the appearance of neuropathological lesions characterised by intranuclear, cytoplasmic or extracellular protein aggregates (Crichton and Ward, 2006; Crichton *et al.*, 2008).

Finally, we can cite the powerful influence on human civilization of the materials out of which weapons could be manufactured, reflected in the so-called three ages of Man (Stone, Bronze and Iron). While the Stone Age, which began around two million years ago, left many vestiges, it used stone, in the apparent absence of metals, as the sole means of making martial instruments. It was superseded by the Bronze Age, which was marked by important inventions, like the wheel and the ox-drawn plough, and was characterised by the use of metals, notably copper. True bronze, an alloy of copper and tin, in Europe, was heavily dependent on the tin deposits of Cornwall. But by around 1200 BC, the ability to heat and to forge iron saw the end of the Bronze Age, and ushered in the Iron Age, of which few artefacts remain on account of the poor stability of iron when confronted by oxygen and water (rust is no way to preserve archaeological artefacts!). As we discuss in Chapter 12, iron does interact with copper, as well as with a number of other metals. While little remains from the Iron Age, compared to the Stone, to the Bronze, or even to the Gold and Silver, iron always has the last word.

> *This policy cannot succeed through speeches and shooting matches and songs; it can only be carried out through blood and iron*

Otto von Bismarck, speech to the Prussian House of Deputies, 28 January 1886

These few examples give only the superficial mariner's view of the iceberg that remains unseen concerning the fundamental importance of iron in biological systems. There remains a great deal more that we have not seen in this titillation of the reader's palate, and we shall surely make amends in what follows. For the vast majority of living organisms iron is absolutely necessary for their maintenance, defence, differentiation and last, but by no means least, for their growth and cellular division.

That is why I have devoted this book to the inorganic biochemistry of iron. I accept that any errors or omissions are my responsibility, and I would be most grateful to my readers to let me know what needs to be amended in the next edition.

Robert Crichton

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