WOLFGANG KAIM
BRIGITTE SCHWEDERSKI
AXEL KLEIN

SECOND EDITION

BIOINORGANIC CHEMISTRY: INORGANIC ELEMENTS IN THE CHEMISTRY OF LIFE

AN INTRODUCTION AND GUIDE

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Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life

An Introduction and Guide

Second Edition

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Contents

Preface to the Second Edition

The predictably enormous growth of bioinorganic chemistry has made a second edition of this text both necessary and difficult. While there are several extensive and often specialized reviews, major texts and handbooks on this subject, our experience in teaching it has suggested the provision of an updated overview of the classical, novel and applied sections of the field, which has not only become one of the major subdisciplines of inorganic chemistry but, due to its highly interdisciplinary nature, has also pervaded other areas of the life sciences.

The second edition contains updates of many kinds. New structure information on some intricate metalloproteins, such as water oxidase and the molybdopterin-based enzymes, has been included, replacing the earlier speculative models. Emerging developments are referred to at various points, covering such topics as bioorganometallic chemistry, nucleic acid ligation, gasotransmitters, nanoparticles and global cycles of the elements C, P and N. The vastly increased focus on the medical applications of inorganic compounds has required that more space be devoted to this particular aspect. Nonetheless, we have tried to keep the amount of material at a constant, manageable level suitable for an introductory overview, rather than the typical condensed fragments presented in general textbooks of inorganic chemistry or biochemistry. To achieve this, we have tried to concentrate on the facts and on descriptions of function, rather than on model compounds or mechanistic hypotheses (which may vary with time); excellent treatments of the reaction mechanisms of bioinorganic systems are available in T. D. H. Bugg's *Introduction to Enzyme and Coenzyme Chemistry*, third edition (John Wiley & Sons, 2012) and D. Gamenara, G. Seoane, P. Saenz Mendez and P. Dominguez de Maria's *Redox Biocatalysis: Fundamentals and Applications* (John Wiley & Sons, 2012). A basic knowledge of inorganic, organic, physical and biological chemistry remains necessary to make optimal use of this text.

Throughout this book, we have made reference to the RCSB Protein Data Bank for biological macromolecules. Each structure deposited therein is given a unique PDB code (e.g. 1SOD), and all information pertaining to that structure can be found using its code. For easy reference, we have included this code with all the structures in this book, so that the reader can refer to the original data online.

For comments and encouragement during the planning and completion of this edition, we thank many of our colleagues. We thank the publishers for their support and patience and Martina Bubrin for help in retrieving crystal structure files and drawing the structures. Most special thanks are due to Angela Winkelmann for her continued contributions to the preparation of the manuscript.

Wolfgang Kaim
Brigitte Schwederski
Axel Klein
Stuttgart and Cologne, January 2013

Instructors can access PowerPoint files of the illustrations presented within this text, for teaching, at: http://booksupport.wiley.com.

Preface to the First Edition

This book originated from a two-semester course offered at the Universities of Frankfurt and Stuttgart (W.K). Its successful use requires a basic knowledge of the modern sciences, especially of chemistry and biochemistry, at a level that might be expected after one year of study at a university or its equivalent. Despite these requirements we have decided to explain some special terms in a glossary and, furthermore, several less conventional physical methods are briefly described and evaluated with regard to their practical relevance at appropriate positions in the text.

A particular problem in the introduction to this highly interdisciplinary and not yet fully mature or definitively circumscribed field lies in the choice of material and the depth of treatment. Although priority has been given to the presentation of metalloproteins and the electrolyte elements, we have extended the scope to therapeutically, toxicologically and environmentally relevant issues because of the emphasis on functionality and because several of these topics have become a matter of public discussion.

With regard to details, we can frequently only offer hypotheses. In view of the explosive growth of this field there is implicit in many of the statements regarding structure and mechanisms the qualification that they are "likely" or "probable". We have tried to incorporate relevant literature citations up to the year 1993.

Another difficult aspect when writing an introductory and, at the same time, fairly inclusive text is that of the organization of the material. For didactic reasons we follow partly an organizational principle focused on the elements of the periodic table. However, living organisms are opportunistic and could not care less about such systematics; to successfully cope with a problem is all that matters. Accordingly, we have had to be "nonsystematic" in various sections, for example, treating the hemerythrin protein in connection with the similarly O_2-transporting hemoglobin (Chapter 5) and not under 'diiron centers' (Section 7.6). Several sections are similarly devoted to biological-functional problems such as biomineralization or antioxidant activity and may thus include several different elements or even organic compounds. The simplified version of the P-450 monooxygenase catalytic cycle which we chose for the cover picture illustrates the priority given to function and reactivity as opposed to static-structural aspects.

We regret that the increasingly available color-coded structural representations of complex proteins and protein aggregates cannot be reproduced here. General references to the relevant literature are given in the bibliography at the end of the book while specific references are listed at the end of each chapter in the sequence of appearance.

For helpful comments and encouragement during the writing and correction of manuscripts we thank many of our colleagues. Recent results have become available to us through participation in the special program "Bioanorganische Chemie" of the Deutsche

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Wolfgang Kaim
Brigitte Schwederski
Stuttgart, December 1993

1 Historical Background, Current Relevance and Perspectives

The progress of an inorganic chemistry of biological systems has had a curious history.

R. J. P. WILLIAMS, *Coord. Chem. Rev.* **1990**, *100*, 573

The description of a rapidly developing field of chemistry as "bioinorganic" seems to involve a contradiction in terms, which, however, simply reflects a misconception going back to the beginning of modern science. In the early 19th century, chemistry was still divided into an "organic" chemistry which included only substances isolated from "organisms", and an "inorganic" chemistry of "dead matter".[1] This distinction became meaningless after Wöhler's synthesis of "organic" urea from "inorganic" ammonium cyanide in 1828. Nowadays, organic chemistry is defined as the chemistry of hydrocarbons and their derivatives, with the possible inclusion of certain nonmetallic heteroelements such as N, O and S, regardless of the origin of the material.

The increasing need for a collective, not necessarily substance-oriented designation of the chemistry of living organisms then led to the new term "biochemistry". For a long time, classical biochemistry was concerned mainly with organic compounds; however, the two areas are by no means identical.[2] Improved trace analytical methods have demonstrated the importance of quite a number of "inorganic" elements in biochemical processes and have thus revealed a multitude of partially inorganic natural products. A corresponding list would include:

- metalloenzymes (ca. 40% of the known enzymes, especially oxidoreductases (**Fe**, **Cu**, **Mn**, **Mo**, **Ni**, **V**) and hydrolases (e.g. peptidases, phosphatases: **Zn**, **Mg**; **Ca**, **Fe**);
- nonenzymatic metalloproteins (e.g. hemoglobin: **Fe**);
- low-molecular-weight natural products (e.g. chlorophyll: **Mg**);
- coenzymes, vitamins (e.g. vitamin B_{12}: **Co**);
- nucleic acids: (e.g. $DNA^{n-}(M^{+})_n$, M = **Na**, **K**);
- hormones (e.g. thyroxine, triiodothyronine: **I**);
- antibiotics (e.g. ionophores: valinomycin/**K**);
- biominerals (e.g. bones, teeth, shells, coral, pearls: **Ca**, **Si**, . . .).

[1] There is increasing evidence that much of the "inorganic" material on the surface of the earth has undergone transformations during long-term contact with organisms and their metabolic products, such as O_2 [1].

[2] The term "bioorganic chemistry" is increasingly being used for studies of organic compounds that are directly relevant for biochemistry.

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Some (by today's definition) "inorganic" elements were established quite early as essential components of living systems. Examples include the extractions of potassium carbonate (K_2CO_3, potash) from plants and of iron-containing complex salts $K_{3,4}[Fe(CN)_6]$ from animal blood in the 18th century, and the discoveries of elemental phosphorus (as P_4) by dry distillation of urine residues in 1669 and of elemental iodine from the ashes of marine algae in 1811.

In the middle of the 19th century, Liebig's studies on the metabolism of inorganic nutrients, especially of nitrogen, phosphorus and potassium salts, significantly improved agriculture, so that this particular field of science gained enormous practical importance. However, the theoretical background and the analytical methods of that time were not sufficient to obtain detailed information on the mechanism of action of essential elements, several of which occur only in trace amounts. Some very conspicuous compounds which include inorganic elements like iron-containing hemoglobin and magnesium-containing chlorophyll, the "pigments of life", were analyzed and characterized later within a special subfield of organic chemistry, the chemistry of natural products. It was only after 1960 that bioinorganic chemistry became an independent and highly interdisciplinary research area.

The following factors have been crucial for this development:

1. Biochemical isolation and purification procedures, such as chromatography, and the new physical methods of trace element analysis, such as atomic absorption or emission spectroscopy, require ever smaller amounts of material. These methodical advances have made it possible not only to detect but also to chemically and functionally characterize trace elements or otherwise inconspicuous metal ions in biological materials. An adult human being, for example, contains about 2 g of zinc in ionic form (Zn^{2+}). Although zinc cannot be regarded as a true trace element, the unambiguous proof of its existence in enzymes was established only in the 1930s. Genuine bioessential trace elements such as nickel (Figures 1.1 and 1.2), (Chapter 9) and selenium (Chapter 16.8) have been known to be present as constitutive components in several important enzymes only since about 1970.

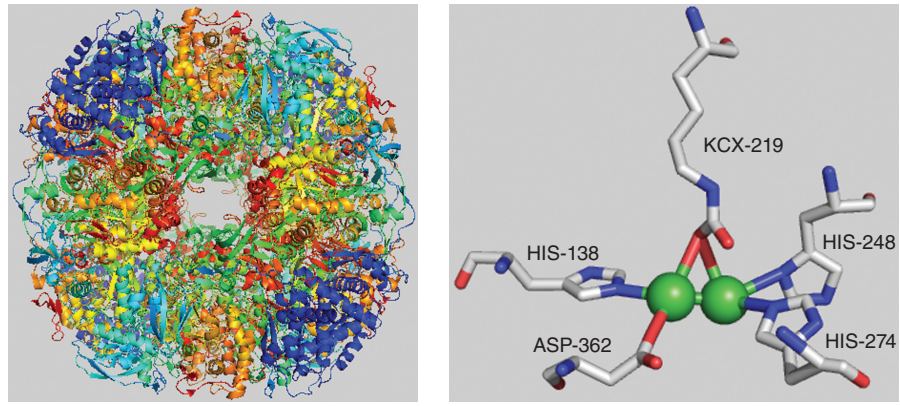

Figure 1.1
Nickel-containing urease, the first enzyme to be crystallized [2]. (a) Crystal structure of the full assembly of *Helicobacter pylori* urease, redrawn from [3] (PDB code 1E9Z). (b) Active site with two nickel centers (green spheres); histidine, aspartate, and a carbamylated lysine as ligands (Section 9.2).

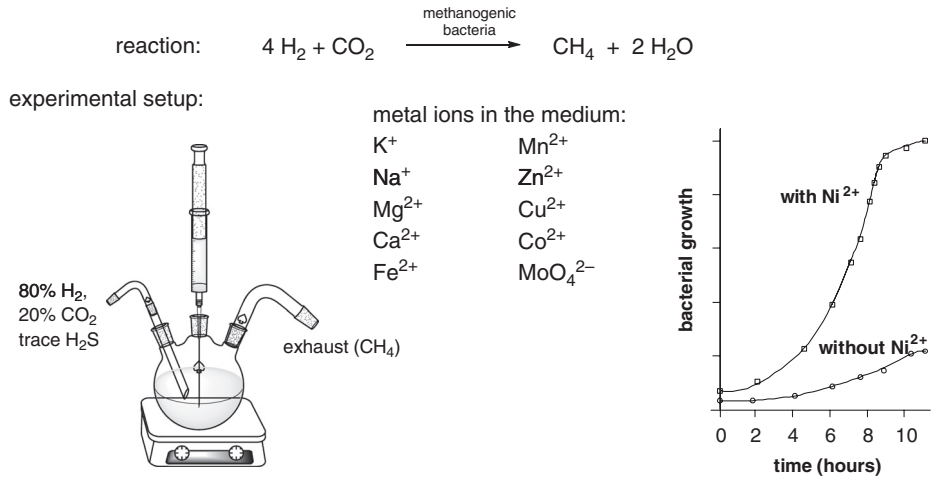

Figure 1.2
Discovery of nickel as an essential trace element in the production of methane by archaea.

In a desire "to accomplish something of real importance", the biochemist James B. Sumner managed to isolate and crystallize in 1926 a pure enzyme for the first time [2], much to the skepticism and disbelief of most experienced scientists. The chosen enzyme, urease (from jack beans), catalyzes the hydrolysis of urea, $O{=}C(NH_2)_2$, to CO_2 and $2\,NH_3$. It contains two closely associated nickel ions per subunit (Section 9.2). It was believed by many then that pure enzymes contained no protein, and only after other enzymes were crystallized was Sumner's discovery accepted. He was honored in 1946 with the Nobel Prize in Chemistry. However, Sumner's belief that urea contained *only* protein was corrected in 1975 when Dixon *et al.* proved that urease is a nickel metalloenzyme (Section 9.2).

In a very different research area, the biological reduction of carbon dioxide by hydrogen to produce methane has been investigated by studying the relevant archaebacteria, which are found, for example, in sewage plants. Even though the experiments were carried out under strictly anaerobic conditions and all "conventional" trace elements were supplied (Figure 1.2), the results were only partly reproducible. Eventually it was discovered that during sampling with a syringe containing a supposedly inert stainless steel (Fe/Ni) tip, minute quantities of nickel had dissolved. This inadvertent generation of Ni^{2+} ions led to a distinctive increase in methane production [4], and, in fact, several nickel containing proteins and coenzymes have since been isolated (see Chapter 9). Incidentally, a similar unexpected dissolution effect of an apparently "inert" metal led to the serendipitous discovery of the inorganic anti-tumor agent *cis*-$PtCl_2(NH_3)_2$ ("cisplatin", Section 19.2.1).

2. Efforts to elucidate the mechanisms of organic, inorganic and biochemical reactions have led to an early understanding of the specific biological functions of some inorganic elements. Nowadays, many attempts are being made to mimic biochemical reactivity

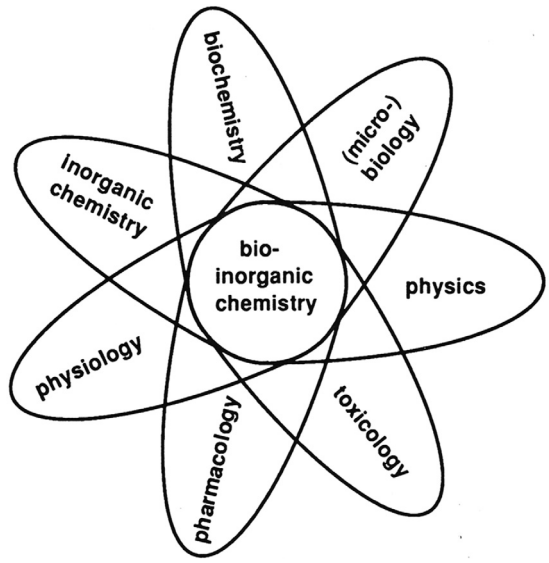

Figure 1.3
Bioinorganic chemistry as a highly interdisciplinary research field.

through studies of the reactivity of model systems, low-molecular-weight complexes or tailored metalloproteins (Section 2.4).

3. The rapid progress in bioinorganic chemistry, an interdisciplinary field of research (Figure 1.3), has been made possible through contributions from:
   - ○ physics (→ techniques for detection and characterization);
   - ○ various areas of biology (→ supply of material and specific modifications based on site-directed mutagenesis);
   - ○ agricultural and nutritional sciences (→ effects of inorganic elements and their mutual interdependence);
   - ○ pharmacology (→ interaction between drugs and endogeneous or exogeneous inorganic substances);
   - ○ medicine (→ imaging and other diagnostic aids, chemotherapy);
   - ○ toxicology and the environmental sciences (→ potential toxicity of inorganic compounds, depending on the concentration).

A list of examples illustrating the *application potential of bioinorganic chemistry* could include the following:

- Industrial sector:
  - ○ anaerobic bacterial degradation in sewage plants or in sediments: **Fe, Ni, Co**;
  - ○ biomining (bacterial leaching; ≈ 15% of the global copper production): **Cu, Au, Fe, U**.

- Environmental sector:
  - ○ agricultural trace element problems: nitrogen fixation (**Fe, Mo, V**); **Mo/Cu** antagonism; **Se** content of soil;
  - ○ pollution through metal species: **Pb, Cd, Hg, As, Al, Cr**;
  - ○ detoxification, for example via peroxidases: **Fe, Mn, V**.

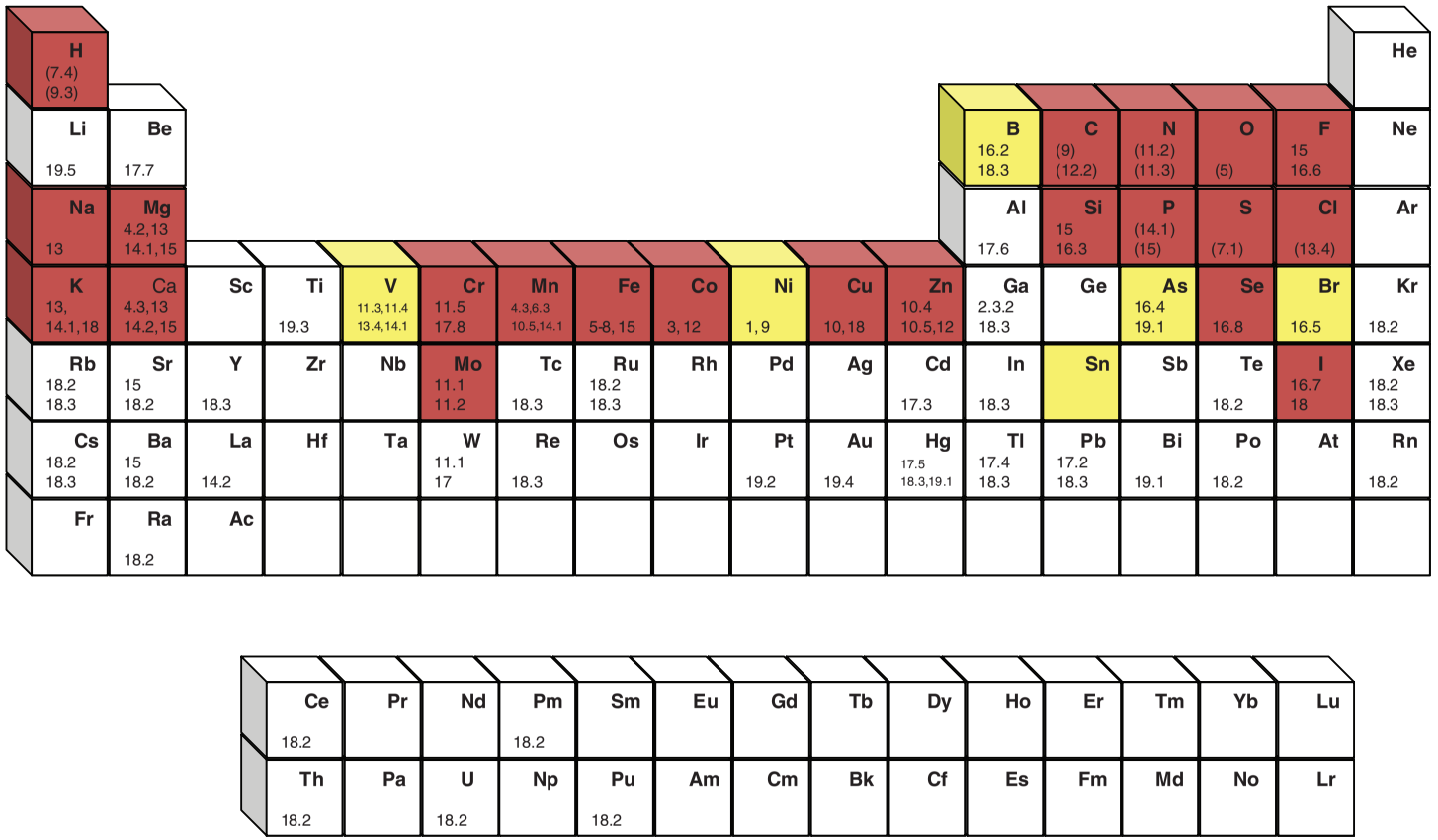

Figure 1.4

Periodic table of the elements. Indicated are the chapters and sections in which each element is discussed in this book. essential element; presumably essential element for human beings.

- Biomedical sector:
  - radiodiagnostics (single-photon emission computed tomography (SPECT), positron emission tomography (PET)), radiotherapy: **Tc, I, Ga, In, Re**;
  - other imaging techniques (magnetic resonance imaging (MRI), x-ray: **Gd, Ba, I**);
  - chemotherapy: **Pt, Au, Li, B, Bi, As**;
  - biominerals (biocompatible materials, coping with demineralization processes): **Ca, P, C, F**;
  - "inorganic" nutrients and noxious food components: deficiency, poisoning; physiological dynamics of resorption, transport, storage, excretion;
  - drug development (oxidative metabolism, metalloenzyme inhibitors): **Fe, Zn**;
  - biotechnological options: specific mutation, metalloprotein design.

A particularly spectacular example of applied bioinorganic chemistry is the successful use of the simple inorganic complex *cis*-diamminedichloroplatinum, *cis*-$Pt(NH_3)_2Cl_2$ ("cisplatin"), in the therapy of certain tumors (Section 19.2). This compound has been the subject of one of the most successful patent applications ever granted to a university.

Even those areas of chemistry that are not primarily biologically oriented can profit from the research in bioinorganic chemistry. Due to the relentless pressure of evolutionary selection, biological processes show a high efficiency under preset conditions. These continuously self-optimizing systems can therefore serve as useful models for problems in modern chemistry. Among the most current topics of this type are:

- the efficient collection, conversion and storage of energy;
- the catalytic activation of inert substances, especially of small molecules under mild conditions in stepwise fashion;
- the (stereo)selective synthesis of high-value substances with minimization of the yield of unwanted byproducts; and
- the environmentally benign degradation and recycling of substances, especially the detoxification or recycling of chemical elements from the periodic table (Figure 1.4).

Beyond a presentation and description of bioinorganic systems, the major purpose of this book is to reveal the correlation of function, structure and actual reactivity of inorganic elements in organisms. The more biological than chemical question of "Why?" should eventually stimulate a more purposeful use of chemical compounds in nonbiological areas as well.

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2 Some General Principles

2.1 Occurrence and Availability of Inorganic Elements in Organisms

"Life" is a process which, for an adult organism, can be characterized as a controlled stationary flow equilibrium maintained by energy-consuming chemical reactions ("dissipative system"). *Input* and *output* are essential requirements for such open systems. They differ very much from the more familiar and mathematically far more easily described "dead" thermodynamic equilibria (Figure 2.1).

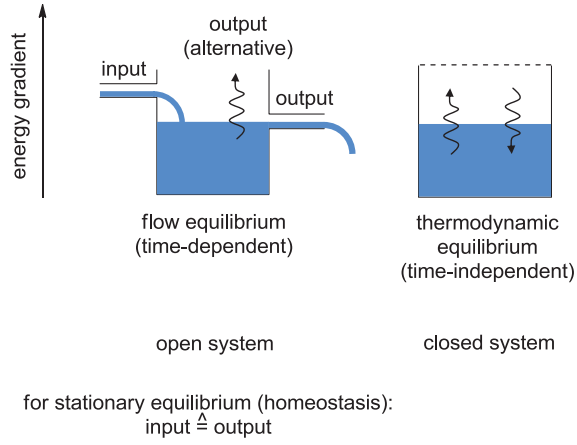

Figure 2.1
Two kinds of "equilibrium".

In addition to the energy flux, life requires a continuous material exchange which, in principle, includes *all* chemical elements (see Figure 1.4). The occurrence of these elements in organisms depends on external and endogeneous conditions; elements can be "bioavailable" to variable extents but can also be enriched ("bioaccumulated") by organisms using active, energy-consuming processes involving a local reduction of entropy. Some trends are obvious from the most familiar example, the elemental composition of the human body (Table 2.1).

The values for O and H in Table 2.1 reflect the high content of (inorganic) water; the "organic" element carbon only comes in third. Calcium as the first metallic element ranks fifth, its main quantitative use being the stabilization of the endoskeleton. Table 2.1 further shows relatively large quantities of potassium, chlorine, sodium and magnesium,

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Table 2.1 Average elemental composition of a human body (adult, 70 kg) [1].

Element	Symbol	Mass (g)
oxygen	O	43 000
carbon	C	16 000
hydrogen	H	7000
nitrogen	N	1800
calcium	Ca	1200
phosphorus	P	780
sulfur	S	140
potassium	K	125
sodium	Na	100
chlorine	Cl	95
magnesium	Mg	25
fluorine	F	5.0 (var.)
iron	Fe	4.0
zinc	Zn	2.3
silicon	Si	1.0 (var.)
titanium[a]	Ti	0.70
rubidium[a]	Rb	0.68
strontium[a]	Sr	0.32
bromine[a]	Br	0.26
lead[b]	Pb	0.12
copper	Cu	0.07
aluminum[a]	Al	0.06
cerium[a]	Ce	0.04
tin[b]	Sn	0.03
barium[a]	Ba	0.02
cadmium[b]	Cd	0.02 (var.)
boron[b]	B	0.018
nickel	Ni	0.015
iodine	I	0.015
selenium	Se	0.014
manganese	Mn	0.012
arsenic[b]	As	0.007 (var.)
lithium[a]	Li	0.007
molybdenum	Mo	0.005
chromium	Cr	0.002 (var.)
cobalt	Co	0.002

[a] Not rated essential.
[b] Essential character not unambiguous.

the "mass" or "quantity elements" or "macronutrients". Iron, zinc and fluorine are distinctly less abundant inorganic elements. According to one definition, "trace elements" with regard to the human body involve a daily requirement of less than 25 mg (see Table 2.3), and some of them, such as boron, arsenic and tin, have not yet been unambiguously defined with regard to amount, essential character and function [2]. Since humans coexist with a host of supporting "lower" organisms – the "microbiome" – their requirements for trace elements will also have to be added. Elements are *essential* if their total absence in the organism causes severe, irreversible damage. Sometimes essentiality is invoked if the optimal functioning of organisms is impaired; in such instances the corresponding elements would be better referred to as "beneficial". Table 2.1 illustrates the occurrence of non-negligible quantities of obviously nonessential elements such as Ti, Rb, Sr, Br, Al and Li in the human body.

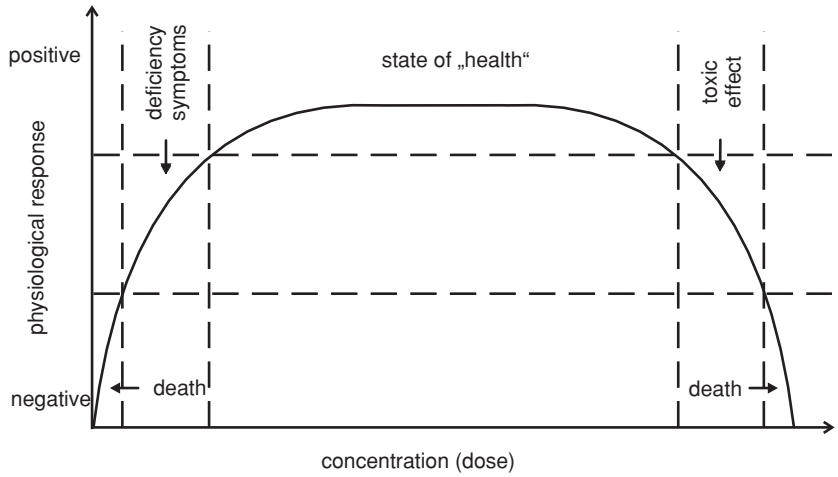


Figure 2.2
Schematic dose–response relationship (Bertrand diagram) for an essential element (compare Figure 17.1 for exclusively toxic elements).

These elements are probably incorporated due to a chemical similarity (indicated by ↔) with important essential elements (Li^+, Rb^+, $Cs^+ \leftrightarrow Na^+$, K^+; Sr^+, $Ba^{2+} \leftrightarrow Ca^{2+}$; $Br^- \leftrightarrow Cl^-$; Al^{3+}, $Ti^{4+} \leftrightarrow Fe^{3+}$). Elements that are known to be mainly toxic, such as As, Pb and Cd, deserve special attention; a positive effect of traces has been discussed for some of these elements, pointing to the ambivalence of many trace elements and to the problem of threshold values (see Figures 2.2 and 2.3). Possibly a physiological – if not always essential – function developed for all naturally occurring elements during the evolution of life [3].

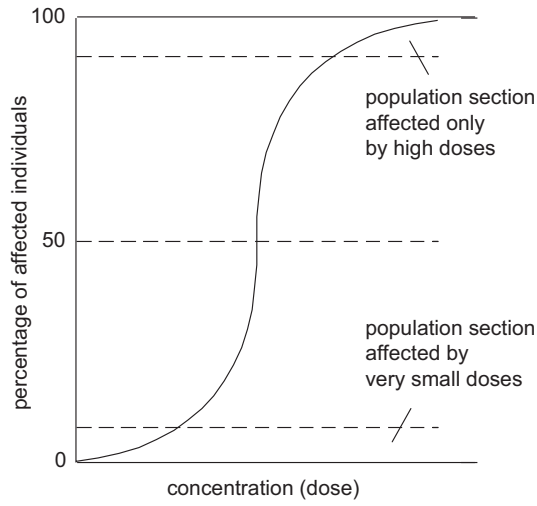


Figure 2.3
Typical variance of the (toxic) effect of a substance within a population.

The elements silicon, aluminum and titanium, which are prominent as components of minerals in the earth's crust, play only a marginal role in the biosphere. Under normal physiological conditions (pH ≈ 7), these elements in their usual high oxidation states exist as nearly insoluble oxides or hydroxides and are therefore not (bio)available. Molybdenum, on the other hand, is a rare element in the earth's crust but is quite soluble at pH 7 as MoO_4^{2-} and thus relatively abundant in sea water; it has therefore been found as an essential element in many organisms. As a rule, metallic elements are soluble in neutral aqueous media and thus bioavailable in either low (+I, +II, i.e. as hydrated cations) or very high oxidation states (+V, +VI, +VII), as hydrated oxoanions such as MO_4^{n-}. However, one should not underestimate the ability of organisms to actively transport and accumulate inorganic substances. As pointed out in Chapter 13, living systems have developed elaborate mechanisms and use much energy to create and maintain concentration gradients for inorganic ions between membrane-separated compartments inside of organisms. Similarly, there are efficient biological mechanisms to accumulate silicate or Fe^{3+} ions, both of which are practically insoluble at pH 7, and thus make them bioavailable for structural or other purposes (see Chapters 8 and 15). Not surprisingly, the elemental compositions are highly variable for different species and even different parts of higher organisms, depending *inter alia* on the kind of metabolism and on the biotope.

The flow equilibrium character of life processes (Figure 2.1) implies that the individual inorganic elements are continuously excreted and replenished even though their overall stationary concentration remains approximately constant ("homeostasis"). The rate of exchange is strongly dependent on the type of compound (chemical speciation) and on the site of action or storage in the individual organism. According to established principles of reaction kinetics, ions of low charge are exchanged relatively quickly (K^+, Mn^{2+}, MoO_4^{2-}), whereas more highly charged species such as Fe^{3+} have longer physiological half lives. It is not surprising that elements such as Ca^{2+}, which find their main quantitative use in the solid-state skeleton, are on the whole exchanged very slowly. The biological half life can then amount to several years; nevertheless, a continuous metabolism takes place even for "biominerals" (see Chapter 15).

Which elements are essential, which are beneficial and which are toxic for a certain organism? Such questions are continuously being discussed in popular science, particularly for humans. Quantitatively, this is a matter of the physiological state (i.e. of the ability to "function" properly) or even of the individual disposition of an organism, depending on the concentration or "dose" of an element, which is often related to its share in the food supply. A dose–response diagram of the type in Figure 2.2 can thus be discussed; this shows the ambivalent effects of many substances and illustrates the principle of Paracelsus: "The dose makes the poison". An important term here is the *therapeutic window*, which characterizes the concentration range causing advantageous physiological effects.

In a more detailed approach, the following aspects have to be considered:

- The type of chemical compound, of which the element is a part, is often crucial for the response of the organism (chemical speciation). The pathway, the extent and the rate of uptake, metabolism, storage and excretion can differ greatly; poor utilization of an otherwise bioavailable essential trace element may thus be responsible for deficiency symptoms. The absorption of inorganic compounds by the organism depends primarily on the solubility and therefore the charge of the system; humans resorb molybdate MoO_4^{2-} quite well, whereas slowly reacting Cr^{3+} is resorbed only to a small extent.

- Given the essentiality of biological compartmentalization within and between cells, the toxicity of an (inorganic) species may depend strongly on the site of action. Many bioessential elements are thus also potent carcinogens, depending on their location.
- It cannot be expected that higher organisms will react uniformly within a population or in the course of their individual development. Therefore, only average statements can be made with regard to a certain situation, such as for the adult state of a preferentially homogeneous population (Figure 2.3).
- The concentration variation of one particular element frequently affects the concentrations and physiological effects of other substances, including compounds of other inorganic elements. This multidimensional interdependence has been known qualitatively for a number of elemental nutrients since the experiments of Liebig. Two components can interact by mutually promoting corresponding effects (synergism) or by competing and suppressing each other's effects (antagonism).

An antagonistic relationship in a two-component system can be the result of displacement ($Zn^{2+} \leftrightarrow Cd^{2+}$, Pb^{2+}, Cu^{2+} or Ca^{2+}) or mutual deactivation ($Cu^{2+} + S^{2-} \rightarrow CuS$ (insoluble)). With three components, for example in the system Cu/Mo/S (Chapter 10 and Section 11.1.2), matters get more complicated, and in reality there is a multidimensional network of synergistic and antagonistic relationships, which is further complicated by the spatially unsymmetrical distribution of inorganic elements in organisms [4]. For instance, there are strongly contrasting distributions of soluble monocations (Na^+ vs K^+), dications (Ca^{2+} vs Mg^{2+}) and monoanions (Cl^- vs $H_2PO_4^-$) in the extra- and intracellular regions, respectively, as outlined in Chapter 13.

Despite this complexity, some deficiency symptoms of individual inorganic elements are quite familiar, particularly as they concern human beings [2,5] (see the incomplete list in Table 2.2). As far as causal connections are known for the single elements, these will be discussed in the relevant chapters within this book. A general syndrome of (trace) element deficiency is growth retardation: the number of truly essential elements seems to be smaller in fully developed organisms than during growth periods. This assumption was

Table 2.2 Some characteristic symptoms of chemical element deficiency in humans.

Deficient element	Typical deficiency symptoms
Ca	retarded skeletal growth
Mg	muscle cramps
Fe	anemia, disorders of the immune system
Zn	skin damage, stunted growth, retarded sexual maturation
Cu	artery weakness, liver disorders, secondary anemia
Mn	infertility, impaired skeletal growth
Mo	retardation of cellular growth, propensity for caries
Co	pernicious anemia
Ni	growth depression, dermatitis
Cr	diabetes symptoms
Si	disorders of skeletal growth
F	dental caries
I	thyroid disorders, retarded metabolism
Se	muscular weakness, esp. cardiomyopathy
As	impaired growth (in animals)

Table 2.3 Essential elements in food for adults and infants.

Inorganic constituent	RDA (adults),[a] in mg	AI (infants),[b] in mg
K	4700	400–700
Na	1200–1500	120–370
Ca	100–1300	200–260
Mg	310–420	30–75
Zn	8–11	2–3
Fe	8–18	0.27–11.0
Mn	1.6–2.3	0.003–0.6
Cu	0.9	0.2
Mo	0.045	0.002
Cr	0.02–0.035	0.0002–0.005
Co	~0.0024 (vitamin B_{12})	0.0004
Cl	1800–2300	180–570
PO_4^{3-}	700	100–275
F	3–4	0.01–0.5
I	0.15	0.11–0.13
Se	0.055	0.015–0.020

[a] Recommended Dietary Allowance (RDA) is derived from the Estimated Average Requirement (EAR [6]). 97.5% of the population meets or exceeds the EAR. Data taken for male and female age groups from 19 to 70 years.
[b] Adequate Intake (AI) is used when an EAR/RDA cannot be developed. The AI level is based on observed or experimental intakes. Data reflect mean values for infants 0–6 months and 6–12 months old (lower and higher values, respectively).
Sources: National Research Council. *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride,* 1997; *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline,* 1998; *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids,* 2000; *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*, 2001; *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate,* 2005; *Dietary Reference Intakes for Calcium and Vitamin D,* 2011. Washington, DC: The National Academies Press.

confirmed by pioneering experiments in the 1960s, which were designed to guarantee a nutritionally complete diet for astronauts during long space flights. The inorganic contents of such synthetic food are summarized in Table 2.3 in the form of the RDA (Recommended Dietary Allowance [6]) values of the US Food and Drug Administration (FDA). Whether such a composition is really sufficient or guaranteed in today's food supply and how far it can be exceeded via increased uptake or separate supplementation without detrimental consequences are still open questions in dietetics, particularly from the popular scientific point of view.

According to Figure 2.3, there are not only deficiency symptoms from the lack of essential elements but also toxic effects resulting from an excess of these, whether caused by insufficient excretion or by excessive uptake [7]. Such poisoning can be treated using "bioinorganic" measures, namely through the application of antagonists or a "chelate therapy" [8,9], which involves the complexation and excretion of acutely toxic metal ions using multidentate chelate ligands ((2.1), Table 2.4). Considering that there are a number of essential metal ions present in organisms, the problem of selectivity is obvious; selectively coordinating ligands have thus been developed for some specific heavy-metal ions. The most successful such ligands offer selectivity either (i) according to the preferred size of the coordinated ion or (ii) with respect to favored coordinating atoms (S for "soft" heavy metals, N especially for Cu^{2+}, O for "hard" metal centers). Furthermore, suitable

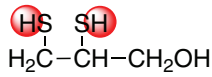
Table 2.4 Chelate ligands for detoxification after metal poisoning.

Ligand (2.1)	Trade or trivial name	Preferably coordinated metal ions	Detailed description in Chapter/Section
(a) 2,3-dimercapto-1-propanol	dimercaprol, BAL	Hg^{2+}, As^{3+}, Sb^{3+}, Ni^{3+}	17
(b) D-2-amino-3-mercapto-3-methylbutyric acid (D-β,β-dimethylcysteine)[a]	D-penicillamine	Cu^{2+}, Hg^{2+}	10, 17
(c) ethylenediaminetetraacetate	EDTA	Ca^{2+}, Pb^{2+}	
(d) deferrioxamine B	DFO, desferal	Fe^{2+}, AI^{3+}	8.2, 17.6
(e) 3,4,3-LICAMC		Pu^{4+}	18.1.3.3

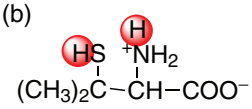
[a]The L-enantiomer is toxic.

chelate ligands must (iii) form kinetically and thermodynamically stable complexes and (iv) facilitate rapid renal excretion, for example by containing hydrophilic hydroxyl groups.

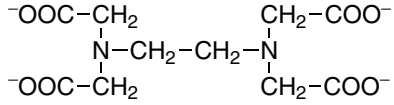
(a)



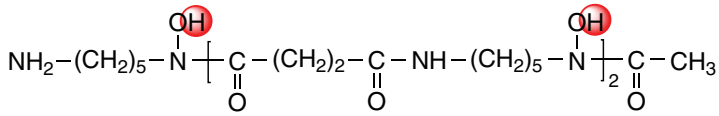
(b)



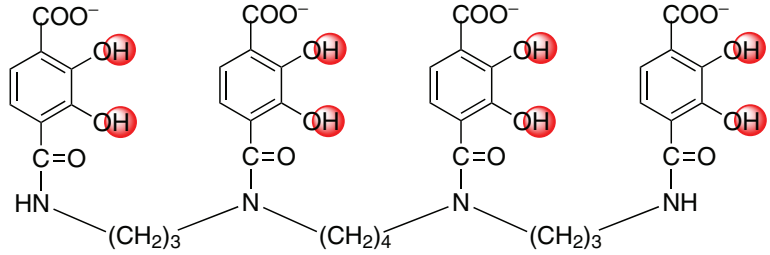
(c)



(d)



(e)



H: acidic protons which may be substituted by chelated metal ions

(2.1)

The Chelate Effect

Ligands that can use more than one nonadjacent donor atom for binding to a metal center are referred to as "chelate ligands". The corresponding chelate complexes contain at least one "metallacycle" ring structure, which restricts the torsional mobility of the system and contributes to enhanced selectivity. Due to the preference of sp^2- and sp^3-configured atoms for 120° and 109° bond angles, the optimum ring size of the metallacycles in chelate complexes is five, although four- and six-membered rings can also occur for very small and very large metal ions, respectively.

Chelate complexes can exhibit enhanced thermodynamic and kinetic stability (the "chelate effect").

- In addition to the enthalpy gain from optimum metal–donor interaction in suitable cyclic arrangements, an entropic factor favors the formation of chelate complexes because the number of free particles (including no-longer-coordinated individual solvent ligands) is increased.
- The kinetic stabilization of chelate complexes reflects the most unlikely complete dissociation of such species, which would require the simultaneous breaking of several metal–donor bonds.

Other things being equal, the chelate effect increases with the ligand "denticity"; that is, with the number of donor atoms and metallacycles being formed. An additional stabilization is achieved when the chelate rings are part of a larger preformed macrocyclic arrangement that can be constructed in two (planar tetrapyrrole complexes) or three dimensions (ionophor complexes, cryptates; Section 2.3.2).

"Hard" and "Soft" Coordination Centers

The susceptibility of atoms and ions to experience a charge shift in their electron shell through interaction with a coordination partner differs considerably. This has led to an often loosely used distinction between little-affected "hard" and easily polarizable "soft" coordination centers. Among the soft electron pair donors are thiolates (RS^-), sulfide (S^{2-}) and selenides; on the other hand, the fluoride anion (F^-) and ligands with negatively charged oxygen donor centers are classified as hard. In many cases, the observed affinities between metal ions and ligand atoms can be interpreted in such a way that interactions between centers of the same type – that is, hard–hard (highly ionic bond) and soft–soft (partly covalent bond) – are preferred. One possible quantitative approach to this rather intuitively used concept is based on a correlation between the ratio charge/ionic radius of a metal dication and the measurable second ionization energy.

2.2 Biological Functions of Inorganic Elements

The great efforts made by organisms to take up, accumulate, transport and store inorganic elements are justified only by their important and otherwise unguaranteed function. For