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Review

Arsenic–selenium and mercury–selenium bonds in biology

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Contents

Abstract

When rabbits are simultaneous injected with arsenite and selenite or mercuric chloride and selenite, compounds with As–Se and Hg–Se bonds are formed in the bloodstream. The combined application of liquid chromatography-inductively coupled plasma atomic emission spectrometry (ICP-AES) and X-ray absorption spectroscopy (XAS) has revealed the molecular structure of these toxicologically important compounds and provided insight into their mechanism of formation. The glutathione-driven formation of these compounds in the bloodstream fundamentally links the metabolism of the environmental pollutants mercuric mercury and arsenite with that of the essential ultratrace element selenium, which establishes a feasible mechanism by which the chronic low-level exposure of various human populations to these toxic metals and metalloid compounds is linked to human diseases, including cancer and neurodegenerative diseases.

Keywords: As^{III}; As^{V}; Se^{IV}; Se^{VI}; Hg^{2+}; Metabolism; Endogenous thiols; Antagonism; Mechanism of chronic toxicity; Environmental bioinorganic chemistry

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1. Aims and scope

Over the last 40 years bioinorganic chemistry has matured into an important branch of modern chemistry which links classical coordination chemistry with biochemistry [1,2]. Various spectroscopic techniques, such as UV–vis spectroscopy (e.g. absorption, circular dichroism) together with advanced spectroscopic techniques (e.g. X-ray absorption spectroscopy, electron paramagnetic resonance spectroscopy), have revealed the active site structure of numerous metalloproteins, which has contributed to a better understanding of the biochemical role of essential elements in mammalian organisms [2]. The assembly of biologically active metalloproteins in mammalian cells, however, requires a continuous supply of the corresponding essential metal or metalloid compound at the site of synthesis and is orchestrated by ingenious homeostatic regulation mechanisms [2]. These mechanisms intricately tune: (a) the gastrointestinal absorption of metals and metalloid compounds into the bloodstream [3], (b) their subsequent transport to organs (mediated by plasma transport proteins) [2], (c) their uptake into various organs (e.g. by specific receptors) [4] and (d) their biliary, urinary or pulmonary excretion, in order to maintain the organ concentrations of essential elements constant throughout life. In view of the many vital functions that metalloproteins play in mammalian cells, it is not surprising that the disruption of homeostatic regulation mechanisms is directly linked to human diseases [5]. The disruption can be brought about by a nutritional deficiency of a particular essential metal/metalloid compound [6], the ingestion of dietary constituents which inhibit the intestinal absorption of an essential metal/metalloid compound [7], and/or mutations in genes which code for proteins that are actively involved in the homeostatic regulation mechanism itself (a–c) [4,8,9]. Another, previously neglected, but nonetheless important mechanism for the disruption of homeostatic regulation mechanisms of essential elements – and this review will exclusively focus on this one – is their *in vivo* disruption by simultaneously ingested toxic metals and metalloid compounds based on the formation of compounds with As–Se and Hg–Se bonds in the bloodstream. In view of the concomitant reduction of the organ availability of the essential element Se, this molecular mechanism provides a conceptually novel functional connection between the increased dietary exposure of various human populations to toxic metals and metalloid compounds and human health [5], which remains one of the greatest challenges for biology in the postgenomic era [10]. By embracing the biological chemistry of toxic metals and metalloid compounds, the emerging new branch of bioinorganic chemistry – environmental bioinorganic chemistry – is destined to not only provide fundamentally new insights into the bioinorganic basis of chronic human diseases (e.g. cancer, neurodegenerative diseases, etc.), but also to uncover hitherto unknown mammalian detoxification mechanisms which can ultimately be exploited in a practical fashion to mitigate biogeochemistry related human diseases.

In this review I attempt to present a unique "environmental bioinorganic chemistry" perspective of environmentally relevant forms of the metal Hg and the metalloids As and Se. After summarizing the known health effects that are associated with the exposure of humans to these persistent inorganic pollutants, a brief overview of their individual mammalian toxicology and metabolism will be provided. Thereafter, a synopsis of the knowledge regarding the antagonistic interactions between As^{III} and Se^{IV} and between Hg^{2+} and Se^{IV} in mammals will be presented. Finally, the structural basis of these interactions will be revealed and some of the far-reaching consequences that the *in vivo* formation of compounds with As–Se and Hg–Se bonds has with regard to a better understanding of the intricate connection between the geochemistry and human health will be discussed.

2. Toxic metals and metalloids in the environment

The earth's crust is comprised of 90 elements which – owing to their different chemical properties – can be broadly classified into metals, metalloids and non-metals. The crustal abundance of these elements ranges between 474,000 ppm (oxygen) and 6×10^{-7} ppm (radium) and covers ~11 orders of magnitude [11]. Hydrological cycle-driven chemical weathering reactions together with deep sea vent activity continuously mobilize these elements from igneous and sedimentary rocks/minerals of the earth's crust to the hydrosphere [12,13]. Determined by their individual chemical properties, each element subsequently undergoes a series of abiotic (e.g. dissolution, hydrolysis, oxidation of primary minerals) and/or microbially mediated reactions (e.g. methylation, oxidation, reduction) to yield chemical "species", such as molecules, ions and complexes with other elements. These element "species" are subsequently exchanged between the major environmental compartments, namely the geosphere, the atmosphere, the hydrosphere and the biosphere (all living organisms). The circulation of all species of an element between all environmental compartments is described by its global biogeochemical element (GBCE) cycle [14], which intrinsically links the health of organisms ("bio") with the earth's crust ("geochemical element" cycle). The GBCE cycles of all elements are intertwined to a complex and dynamic system that has been steadily evolving ever since the surface of the earth had formed. Consequently, each element must be recognized as part of this steadily changing system and changes in the GBCE cycle of one element can either directly (e.g. burning sulfur-rich fossil fuel causes acid rain) or indirectly (e.g. acid rain can mobilize toxic metals and metalloids [15]) affect the health of living organisms. In order to better understand the complex functional connections between the geochemical environment and human health [16], however, we have to consider the evolution of life itself.

Life evolved in a predominantly inorganic environment. Following what can be described as a natural selection of the chemical elements by biological systems, some elements of the earth's crust were recruited to actively participate in the biochemistry of life and became absolutely necessary for the organisms maintenance, its well-being and propagation. The 11 elements that are absolutely essential for all biological systems are C, O, H, N, Na, K, Ca, Mg, P, S and Cl [2]. Since they are the most abundant elements in all organisms they are referred to as bulk elements. Another 15 essential elements are also required by

most biological systems and are classified according to their abundance in organisms into trace elements (dietary requirement in mg/day; Fe, Cu, Zn) and ultratrace elements (dietary requirement in μg/day; V, Cr, Mn, Co, Ni, Mo, W, B, Si, Se, F, I) [2,17]. The relevance of these trace and ultratrace elements for the biochemistry of life is reflected by the fact that ∼1/3 of all mammalian proteins are metalloproteins (with one or more of these elements in the active center) [18]. The necessity to provide the organism and, more specifically, its internal organs with a constant supply of adequate amounts of essential trace and ultratrace elements resulted in the evolution of efficient mechanisms to "extract" these elements from ingested food and drinking water. Figuratively speaking, organisms invented ways to extract essential elements from the earth's crust and in turn became in itself an important component in the global network of GBCE cycles, the biosphere. The earth's crust, however, not only contains elements that are beneficial for organisms, but also elements that are detrimental and toxic.

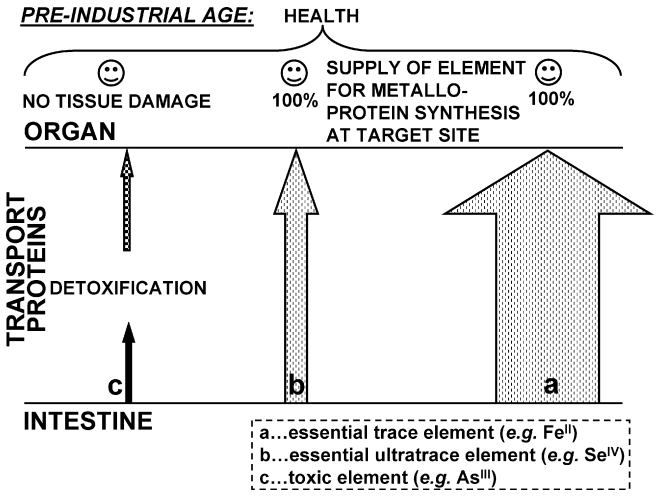

Fig. 1. Schematic transport of intestinally absorbed essential trace and ultratrace elements to organs and detoxification of ingested toxic elements in the mammalian bloodstream during the pre-industrial age. Note that the flux of toxic elements (represented by the thickness of arrow (c) is small compared to that of essential ultratrace elements (arrow b).

2.1. Exposure of organisms to toxic metals and metalloids during evolution

The regular ingestion of food and drinking water – the chemical composition of which closely reflects their geochemical origin – represents the major nutritional source of essential elements for all living organisms. Natural processes, such as chemical weathering reactions, volcanic activity, deep sea vent activity and the outgassing of the earth's mantle (in case of Hg^0), however, have always mobilized toxic metals and metalloids from the earth's crust. As a result, life on earth has evolved in the presence of and has been continually exposed to "background" concentrations of these elements (or compounds thereof). It is, therefore, not surprising that many organisms, including mammals, have evolved detoxification mechanisms against the detrimental effects of ingested toxic metals and metalloid compounds, which is schematically depicted in Fig. 1. Even though the ingested daily doses of any particular toxic metal or metalloid compound fluctuated throughout evolution (which essentially corresponds to a flux), detoxification mechanisms evolved to minimize tissue damage (Fig. 1c).

Bacteria, for example, detoxify absorbed CH_3Hg^+ by cleaving the C–Hg bond followed by the reduction of the generated Hg^{2+} to elemental Hg^0. Finally, the latter can diffuse through the cell membrane and is thereby removed from the organism [19]. Conversely, mammalian organisms have evolved metal-binding proteins, such as metallothionein, which – even though a primary role for this protein has not yet been established – protect mammalian organisms from Cd toxicity by sequestering toxic Cd ions [20,21]. Similarly, the intracellular sequestration of heavy metals by phytochelatins detoxifies inorganic pollutants, such as Cd in animals [22].

2.2. Industrial revolution and increased mobilization of toxic metals and metalloids to the environment

The industrial revolution represents the starting point for the ongoing anthropogenic large-scale mobilization of toxic metals and metalloid compounds from the earth's crust (geosphere) to the global environment (hydrosphere, atmosphere and biosphere) [23]. Over the past ∼200 years, gradually increasing anthropogenic activities, such as fossil fuel consumption [24], heavy metal mining [25], smelting [26], fertilizer application [27,28], municipal waste incineration [29], leaching of toxic metalloids from coal ash [30] and the use of growth promoters [31] have emerged important sources of toxic metals and metalloids in the global environment. The total Hg concentration in alpine snow (Mont Blanc, France), for instance, has increased five-fold between 1885 and 1965 [32] and a quantitative assessment of the worldwide anthropogenic contamination of air, water and soils with toxic metals has revealed that human activities significantly perturb the biogeochemical element cycles of at least 11 elements on a global scale [29]. The anthropogenic emissions of As and Hg, for example, now rival or exceed their natural emissions [33,34] and significantly increased concentrations of toxic metals and metalloids have been detected in lake sediments compared to pre-1900 background concentrations [35–37]. Additionally, increasing concentrations of toxic metals and metalloids, particularly As and Cd, have been detected in U.S. rivers in long-term water quality studies [38]. A likely source for Cd in river water is the application of Cd-containing fertilizers, such as superphosphate, which in one instance contained ∼7 mg Cd/kg [28]. In another case rock phosphate was identified as the source of Hg pollution in a marine ecosystem in Western Australia [27]. The magnitude of anthropogenic emissions of As, Hg and Cd as compared to their mobilization by natural processes is reflected by the fact that current depictions of GBCE cycles contain a previously non-existent compartment, the anthrosphere (the sum of all anthropogenic activities), which has also triggered legislative action to decrease the emission of certain toxic metals and metalloids in the U.S. and Europe. The current global anthropogenic emission of environmentally persistent

toxic metals and metalloid compounds, however, must be predicted to increase as populous countries like India and China emerge and aspire to first world standards of living which is inevitably going to increase their fossil fuel consumption and could compensate for the decreasing emissions in the U.S. and Europe.

The accumulation of As^{III}, Cd^{2+}, Hg^{2+} and CH_3Hg^+ in human tissues is well established. In an area where coal with a high As content was burned, for example, humans had hair As concentrations of 10.3 mg As/kg [39]. Conversely, Cd^{2+} predominantly accumulates in the kidneys in humans [40], where an average concentration of ~40 mg Cd/kg (wet weight) has been reported [40]. Hg^{2+} also accumulates in the kidneys [41–44] and chloralkaline plant workers had an average kidney concentration of 26 mg Hg/kg [43]. CH_3Hg^+ accumulates in the liver, the kidneys, the brain [45], and hair, where total Hg concentrations of up to 2400 mg Hg/kg have been reported [46]. In addition, a survey which was conducted by the Center of Disease Control revealed that almost 8% of women of childbearing age in the US have blood Hg levels exceeding 5.8 ppb, which represents the precautionary level that was set by the Environmental Protection Agency and is also recommended by a National Research Council (NRC) report [47].

The main reason for concern with regard to the increasing concentrations of toxic metals and metalloid compounds in the environment is that the chronic ingestion of relatively small daily doses of these pollutants is associated with dramatic overall health effects in humans. With regard to As, for instance, the chronic ingestion of 200–250 μg of inorganic As per day will eventually result in cancer in humans [48]. On the basis of the drinking water guidelines of the World Health Organisation, it is estimated that up to 75 million people are currently exposed to concentrations of inorganic As in drinking water that are associated with the aetiology of internal cancers [49]. Another established inorganic carcinogen, Cd^{2+}, was found to mimic the *in vivo* effects of estrogen in the uterus and the mammary gland after the administration of as little as 5 μg Cd^{2+}/kg body weight in rats [50]. The health effects that are caused by the exposure of humans to toxic metals, such as CH_3Hg^+, are also associated with significant economic losses because of the resulting diminished economic productivity [51]. Hence, the exposure of certain human populations to increasing dietary levels of inorganic and organic forms of toxic metals and metalloid compounds will significantly increase the risk of chronic diseases. Consequently, studies into the elucidation of the complex functional connections between the anthropogenic perturbation of GBCE cycles and human health are of practical relevance and urgently needed [16]. Conceptually, two major mechanisms can be identified by which the increased dietary ingestion of toxic metals and metalloids can adversely affect human health (Fig. 2, mechanisms 1 and 2). So far mechanism 1, the direct effect of a toxic metal or metalloid compound on a particular internal organ has received considerable attention. The main focus of the present review, however, is mechanism 2, a largely neglected mechanism which is fundamentally involved in the chronic toxicity of metal and metalloid compounds in humans.

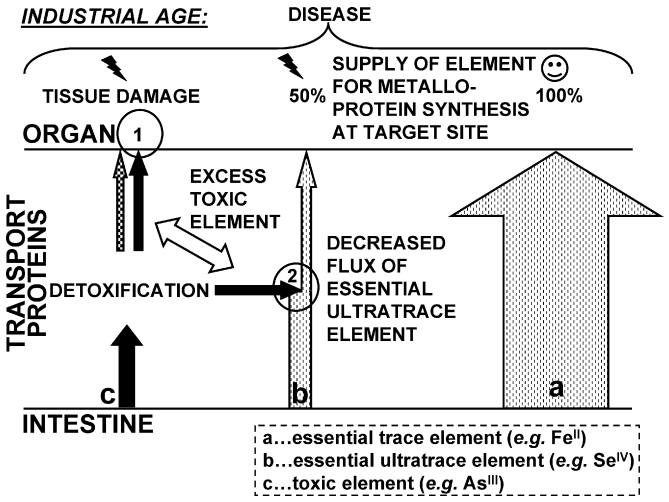

Fig. 2. Transport of intestinally absorbed essential trace elements to organs and tissue damage caused by excess of toxic elements (left over after detoxification, mechanism 1) and decreased organ-availability of essential ultratrace elements (due to interactions with toxic elements, mechanism 2) in the mammalian bloodstream during the industrial age. Note that the flux of toxic elements (represented by the thickness of arrow c) is now comparable to that of the essential ultratrace elements (arrow b).

3. Mammalian toxicology of metals and metalloid compounds

Several poisoning incidences after World War I, which involved the exposure of humans to CH_3Hg^+ [52,53], Cd^{2+} [25], As^{III} and As^V [49,54–57] and Se [54,58], have revealed that the health of mammalian organisms can be greatly affected by natural local geochemical conditions [54–56,59], the careless anthropogenic emission of toxic metals into the surrounding environment [52], mining activities [60] and accidental poisoning [53]. From a toxicological point of view it is important to distinguish between the exposure to a toxic metal or metalloid compound following a single incident (e.g. a chemical disaster) or the exposure over weeks, months or years (e.g. elevated concentrations of As in drinking water). In addition, one must differentiate between the ingestion of large doses (acute toxicity) and the ingestion of comparatively smaller doses (chronic toxicity). In the context of this review, chronic toxicity is defined as the long-term exposure of humans to low levels of a particular toxin. It is important to emphasize at this point that the underlying molecular mechanism(s) for the acute toxicity (Fig. 2, mechanism 1) of a metal or metalloid compound can, but not necessarily are the same as those involved in the mechanism of chronic toxicity (Fig. 1, mechanism 2).

3.1. Acute toxicity of individual metals/metalloid compounds and mechanisms of toxicity

The aforementioned human poisoning epidemics greatly increased the public awareness of the danger that is associated with the unwitting exposure of humans to toxic metals and metalloid compounds through the ingestion of food and/or drinking water. As a consequence, toxicologists began to systematically investigate the toxicity of numerous organic and

inorganic metal and metalloid compounds by exposing animals to increasing doses of the individual compounds. This eventually led to a measure for their acute toxicity (LD_{50} value) and revealed that the acute toxicity of organic and inorganic forms of metals and metalloid compounds strongly depends on their molecular form. In rats, for instance, the minimum lethal dose (MLD) for As^{III} was one third of that for As^{V} (4.0 mg As/kg for As^{III}; 12.0 mg As/kg for As^{V}), whereas the MLD for Se^{IV} was 2.5 mg Se/kg compared to 3.8 mg Se/kg for Se^{VI} [61]). The striking effect of methyl-groups on the toxicity of a metalloid compound is readily illustrated by comparing the LD_{50}'s of $NaAsO_2$ (As^{III}) and the sodium salts of the As^{V}-compounds methylarsonic acid [$CH_3As(O)(OH)_2$](MMA^{V}) and dimethylarsinic acid [$(CH_3)_2As(O)OH$](DMA^{V}) of 41, 700 and 2600 mg/kg, respectively [62].

Investigations into the molecular basis for the acute toxicity of As^{III}, Cd^{2+}, Hg^{2+} and CH_3Hg^+ in mammals revealed that these compounds selectively inhibit enzymes with vicinal dithiols in their active center [63–67] (with regard to CH_3Hg^+, its reaction with plasmalogens, a major constituent of the phospholipids backbone in cell membranes, has been proposed to explain its neurotoxicity [68]). This must be attributed to their unusually strong affinity for thiol-groups and is readily explained by the fact that the soft Lewis acids As^{III}, Cd^{2+}, Hg^{2+} and CH_3Hg^+ (the most simple soft Lewis acid) have a high chemical affinity for soft Lewis bases, such as thiols [69]. In case of As^{III}, for instance, the reaction with dithiols is essentially irreversible with formation constants in the order of $10^7\ M^{-1}$ [70]. Even though As^{III} has been demonstrated to inhibit more than 100 mammalian enzymes [64,71], the detailed molecular mechanism of inhibition has not been established [64,72–74]. Today the acute toxicity, however, is rather irrelevant from a public health point of view, since only a few cases involving the acute exposure of humans to toxic metals and metalloids occur on an annual basis. Conversely, the chronic exposure of the general population to increasing low-levels of toxic metals and metalloid compounds through their diet and drinking water is disproportionately more important and warrants studies into the elucidation of the molecular mechanisms underlying their chronic toxicity. Conceptually, the mechanisms of acute toxicity and chronic toxicity are likely to be fundamentally different in nature. This is because enzyme inhibition – an established mechanism of acute metal and metalloid toxicity – inherently requires stoichiometric amounts of the chemical agent at the site of toxicity (Fig. 2, mechanism 1). Conversely, chronic toxicity, which is observed after the ingestions of much lower doses compared to acute toxicity, is therefore likely to be based on an entirely different biomolecular mechanism than enzyme inhibition (a possible mechanism is illustrated in Fig. 2, mechanism 2).

3.2. Chronic toxicity of individual metals/metalloid compounds and unsolved questions

A plethora of molecular mechanisms have been proposed to be involved in the carcinogenicity and neurotoxicity of metals and metalloid compounds in humans, including the induction of cell apoptosis [75–78], NF-κB activation [79–82], the inhibition of calcium channels [83,84], the inhibition of DNA repair [85–88], the inhibition of protein synthesis [84], the alteration of signal transduction [89], oxidative stress [88] and – probably the most widely cited – the generation of free radicals (Fig. 2, mechanism 1) [73,90–96]. Despite this, the biomolecular mechanisms that form the mechanistic basis for their chronic toxicity in humans, including their carcinogenicity and neurotoxicity, remain elusive [97]. Even though empirical dose-effect relationships have been established for several toxic metals and metalloid compounds in humans [48,98], non-linearities at the lower-dose end make it notoriously difficult to extrapolate an observed response (e.g. cancer) to smaller dose levels. This makes it impossible to determine with certainty how much of an individual toxic metal or metalloid compound can safely be ingested by humans [98] and indicates that if less than a certain threshold dose of a particular toxic metal/metalloid compound is ingested, the dose no longer results in a measurable response. The fact that the metal/metalloid apparently no longer reaches the target organ can be explained by its "interaction" with other chemically reactive constituents (either endogenous or simultaneously ingested) in the bloodstream to toxicologically important novel metabolites with a dramatically different biological activity compared to the ingested metal/metalloid compound. This review will focus on "interactions" between toxic metals/metalloid compounds and the essential ultratrace element Se and identifies the *in vivo* formation of compounds with As–Se and Hg–Se bonds as an important aspect of the mammalian toxicology of As^{III} and Hg^{2+}. The fate of the identified compounds suggests that their formation represents a mammalian detoxification mechanism by which mammals protect themselves from ingested toxic metals and metalloid compounds (Fig. 2, mechanism 2) [99,100].

3.3. The mixture toxicity problem and synergistic/antagonistic toxic effects

Although the general population is simultaneously exposed to low levels of several toxic metals and metalloid compounds, the vast majority of toxicological studies that have been conducted so far did not address this "mixture toxicity" problem. The latter refers to the fact that it is impossible to predict the toxic effect of a mixture of two compounds (additive toxicity – where the overall toxicity of a mixture is the sum of the individual toxic effects – will not be considered here) and arguably poses the most difficult problem in modern environmental toxicology [101,102]. Conceptually, the "mixture toxicity" problem is ultimately based on synergistic and antagonistic toxic effects that are mediated by the organism itself. Synergistic toxicity refers to a situation in which the administration of two toxic compounds results in a greater overall toxicity than the sum of the individual responses. Conversely, antagonistic toxicity refers to a decreased overall toxicity of a mixture as compared to the toxicity of the individual compounds. These "interactions" between individually toxic metals and metalloid compounds are driven by the biochemical machinery of the organism itself and are – as discussed below – ultimately based on endogenous constituents which maintain

reducing conditions inside mammalian organisms. Since these "interactions" likely represent the root cause for non-linearities in the dose-response relationship [97] and could be involved in the etiology of chronic diseases in humans, they should be considered when the risk that is associated with the exposure of an organism to a mixture of toxic chemicals is to be assessed.

4. Mammalian metabolism of individual toxic metals and metalloid compounds

Over the past three decades, analytical chemists have greatly contributed to a better understanding of the mammalian metabolism of toxic metals and metalloid compounds by identifying toxicologically relevant metal or metalloid-containing metabolites using advanced instrumental analytical techniques. The direct analysis of urine, for instance, was greatly facilitated by hyphenated techniques, such as the coupling of high performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (ICP-MS) [103–105]. The molecular mechanism of toxicity itself, however, cannot be established by the analysis of urine alone since "this would be like trying to tell what happens inside a house by watching what goes in by the door and what comes out by the chimney" in the words of the father of physiology, Claude Bernard [106]. Accordingly, the molecular basis of the toxicity of metals and metalloid compounds can only be understood by addressing their mammalian biochemistry inside the organism, which necessitates appropriate instrumental analytical techniques. Following the scope of this review I will now briefly summarize the individual mammalian metabolism of As^{III}, As^{V}, Hg^{2+}, CH_3Hg^+, Se^{IV} and Se^{IV} since this will identify the metabolites, which are critically involved in the antagonistic "interactions" (between $As^{III} + Se^{IV}$ and $Hg^{2+} + Se^{IV}$) that will be discussed thereafter. The following concise summaries are not intended to be thorough reviews covering all aspects of their mammalian metabolism. Urinary metabolites, enzymatic biotransformations, free radical damage and apoptosis, for instance, will not be covered in any particular detail. The aim is rather to provide the molecular basis for an understanding of the organism-mediated "interactions" that will be discussed.

4.1. Arsenite metabolism

In aqueous solution and at physiological pH, As^{III} is present as neutral $As(OH)_3$ ($pK_1 = 9.2$). Since As^{III} has a particularly high chemical affinity for thiols, As^{III} reacts with endogenous L-glutathione (GSH) and L-cysteine (Cys) in aqueous solution to form complexes, such as $(GS)_3As$ [107–112] and $(Cys)_3As$ [108]. Studies which were reported in the 1960s provided the first strong link between As in drinking water (essentially As^{III} and As^{V}) and lung and urinary tract cancer in humans [113]. This together with the subsequent identification of MMA^{V} ($pK_1 = 4.1$, $pK_2 = 9.1$ [114]) and DMA^{V} ($pK_1 = 6.3$ [114]) in human urine in 1973 [115] greatly stimulated investigations into the mammalian metabolism of the apparent carcinogen As^{III}. The oral administration of As^{III} (50 and 500 μg of As) to human volunteers followed by the analysis of their urine for total As revealed that As^{III} is efficiently absorbed from the gastrointestinal tract into the bloodstream (>80%) [116–119] (Fig. 3). Even though it has been observed that As^{III} induces the synthesis of GSH in the gastrointestinal mucosa in rats [120], virtually nothing is known about the intestinal uptake mechanism itself. This, however, must be regarded as highly desirable, since knowledge about the specific uptake mechanism would represent an important starting point for the development of an inexpensive, palliative dietary additive to specifically disrupt the gastrointestinal absorption of As^{III} similar to the established disruption of the intestinal absorption of Zn^{2+} by dietary phytic acid [7].

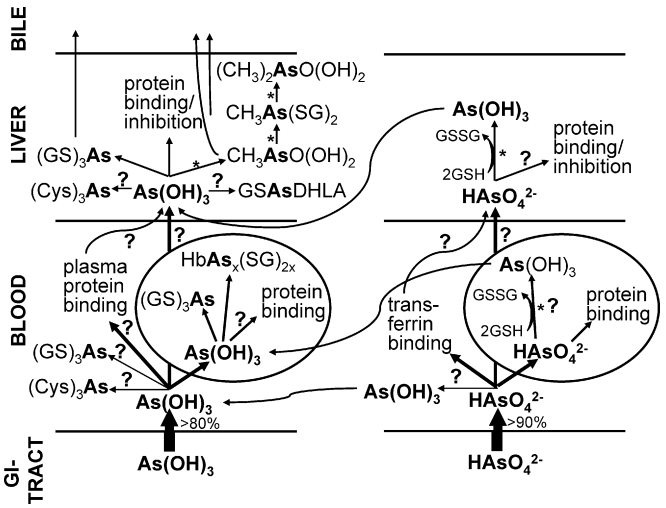

Fig. 3. Schematic illustration of the mammalian metabolism of As^{III} and As^{V}. Oval shape: erythrocyte; Cys: L-cysteine; GSH: L-glutathione; GSSG: oxidized L-glutathione; DHLA: dihydrolipoic acid. Asterisk indicates enzymatically mediated biotransformation.

In the bloodstream, As^{III} can bind to plasma GSH (2.8 μM in human plasma [121]), plasma Cys (10 μM in rat plasma [122]) or plasma proteins (Fig. 3). Even though these events critically determine the half-life of As^{III} in the bloodstream and therefore are of fundamental toxicological significance to better understand how As^{III} is delivered to its target organs, few studies have specifically investigated the binding of As^{III} to plasma constituents in plasma or serum (^{74}As-labeled As^{III}) [123–125]. Despite the fact that 10–50% of As^{III} were reported to be bound to plasma proteins (depending on the animal species) [123,124], the major As^{III}-binding plasma protein has not been identified. In view of the fact that human serum albumin (HSA) represents the largest fraction of free sulfhydryls (Cys-34) in plasma (5 g/100 ml blood [126]), it is somewhat surprising that no binding of As^{III} to HSA has been reported in the literature (Au^{3+}, Ag^+, Hg^{2+}, Cd^{2+} and Cu^{2+} all bind to Cys-34 [126,127]). This could be explained by the fact that $As(OH)_3$ does not react with deprotonated sulfhydryls (e.g. GS^- [111]) and that the Cys-34 of HSA is reported to have an unusually low pK_{SH} of ~5 [126]. Hence, Cys-34 of HSA is deprotonated at pH 7.4 and this would explain why HSA does not bind $As(OH)_3$. Despite the lack of evidence of binding of As^{III} to plasma proteins, the identification of putative As^{III} transport proteins in plasma or serum would contribute to a much better understanding with regard to how mammals "handle" As^{III}. The accumulation of As^{III} by

erythrocytes represents another potential fate in the bloodstream and several studies have conclusively demonstrated that As^{III} is accumulated by eryhthrocytes (Fig. 3) [128–130]. In view of intra-erythrocytic GSH concentrations of ~3.0 mM [131,132], it is not surprising that strong evidence in favor of the formation of $(GS)_xAs(OH)_{3-x}$ ($x = 1–3$) complexes in intact rabbit erythrocytes has been obtained using ^1H spin-echo NMR spectroscopy [133,134]. These complexes, however, were demonstrated to be labile in aqueous solution [70,111]. Apart from GSH, erythrocytes also contain another major sulfhydryl-group containing constituent, hemoglobin (Hb) and mixed complexes between As^{III}, GSH and Hb have been detected in erythrocytes (Fig. 3) [130,133]. More recent studies have revealed that rat Hb, which contains a total of 10 cysteines has a 3–16 times stronger affinity for As^{III} [and other As^{III} compounds, such as monomethylarsonous acid $CH_3As(OH)_2$ (MMA^{III}) and dimethylarsinous acid $(CH_3)_2AsOH$ (DMA^{III})] than human Hb, which contains a total of 6 cysteines [74,135].

Even though As^{III} is rapidly translocated from the bloodstream to the liver (<1 h after oral or iv administration) [136–138], the molecular form of As^{III} that is translocated and the underlying mechanism have yet to be elucidated. Inside hepatocytes, several potential molecular targets of As^{III} must be considered. With a hepatocyte cytosol concentrations of ~7.5 mM GSH [139] and 0.2–0.5 mM Cys [140], these endogenous thiols must be identified as putative molecular targets of As^{III}. In fact, the formation of $(GS)_3As$ complexes was observed by chromatographic means when intrahepatic conditions (7.5 mM GSH, PBS-buffer, 37 °C) were simulated using the "retention analysis method" [111]. The endogenous dithiol dihydrolipoic acid (DHLA) must also be considered as a potential molecular target of As^{III} since As^{III}-dithiol-complexes have been described in the literature [112,141–143] and since protein bound lipoic acid (LA) concentrations in mammalian liver of 11.6 nmol/g have been reported [144]. Studies aimed at the identification of As^{III}-binding proteins in rabbit liver cytosol have identified three major binding proteins (100, 450 and >2000 kDa) [145], whereas five As^{III}-binding proteins were identified in rat liver cytosol (only 3, however, were characterized by their MW of >1000, 135 and 38 kDa) [146]. The 450 kDa protein that was detected in rabbit liver must be tentatively identified as the iron-storage protein ferritin, since *in vitro* studies have demonstrated that As^{III} can release Fe from this Fe-storage protein [147]. The established enzymatic methylation of As^{III} in hepatocytes [148] suggests that at least one of the other As^{III}-binding proteins in liver cytosol is an As^{III}-methyltransferase, which has been recently isolated from rat liver (41 kDa) [149]. Pyruvate-dehydrogenase represents another likely As^{III}-binding protein in liver cytosol, since it contains DHLA-moieties which are known to have a strong affinity for As^{III} (thereby inhibiting this enzyme) [150–152]. Investigations directed toward the identification of As-containing metabolites in bile have so far identified $(GS)_3As$ [137,153], $CH_3As(GS)_2$ [137], MMA^{III} (based on the concentration of free GSH in bile, it cannot be excluded that actually $CH_3As(GS)_2$ was present) [154] and MMA^V [137] (Fig. 3). With regard to the translocation mechanism itself, multidrug resistance-associated protein 2 (MRP2/cMOAT) has been demonstrated to be involved in the transport of $(GS)_3As$ and $CH_3As(GS)_2$ in mammals [153]. This mechanism, however, is significantly different from the ATP-driven efflux of As^{III} from *E. coli* [155,156]. Taken together, several potential endogenous monothiol and dithiol-containing molecular targets of As^{III} have been identified in hepatocytes. Even though the exact sequence of As^{III} binding events in hepatocyte cytosol could involve the simple transfer of As^{III} from intracellularly abundant monothiols (Cys, GSH) to dithiol-containing molecules (e.g. DHLA) [111,157], the detailed sequence of events that occur *in vivo* remain to be elucidated.

4.2. Arsenate metabolism

As^V, which at physiological pH is present as a mixture of $H_2AsO_4^-/HAsO_4^{2-}$, has been demonstrated to be chemically reduced to As^{III} by GSH [107,158] and Cys [159]. Like As^{III}, As^V has been found to be efficiently absorbed (>90%) from the gastrointestinal tract in humans and most experimental animals [116,118,119,160] (Fig. 3). In rats, the gastrointestinal absorption of As^V was strongly inhibited by the simultaneous oral administration of DHLA [161], which is difficult to explain since there are no reports of a chemical reaction between these two compounds. Based on the structural similarity between As^V and P^V ($H_2PO_4^-/HPO_4^{2-}$ at pH 7.4), it is likely that As^V is absorbed from the gastrointestinal tract into the bloodstream via the known P^V uptake mechanisms (one Na^+-dependent, one Na^+-independent) [162].

In blood, the binding of As^V to plasma proteins has been investigated in mouse and rabbit plasma [124,163], and transferrin was identified to be a major As^V-binding protein in human serum [164]. A reduction of As^V to As^{III} has been reported in dog plasma [165] and could be mediated by plasma GSH and Cys (combined concentration ~13 μM). Several studies have demonstrated that intravenously administered As^V is reduced to As^{III} *in vivo* (As^{III} was detected in plasma) [118,125,163,165–168] and *in vitro* (in blood) [130,169]. The accumulation of As^V in rabbit and human erythrocytes [133,170] – apparently mediated by the Na^+-pump and the erythrocyte anion transport protein AE1 [170] – suggested that these cells could be an important site for the reduction of As^V and was subsequently confirmed [130]. Subsequent studies confirmed that the reduction of As^V to As^{III} in eryhtroctes is accomplished by GSH and not by purine nucleoside phosphorylase [169]. Further studies are required, however, before an enzymatic reduction in erythrocytes can be definitively excluded. Inside erythrocytes, As^V has also been shown to bind to unidentified high molecular weight proteins (MW > Hb)[163].

The mechanism of translocation of As^V from the bloodstream to the liver and the nature of the molecular species that is translocated are unknown (it is likely that As^V accesses the P^V translocation mechanism). In the liver, As^V has been show to be reduced to As^{III} [159] (Fig. 3). This reduction can be accomplished either chemically by GSH or Cys [159] or enzymatically by an As^V-reductase, which has been partially purified from human liver [171,172]. Hepatic As^V-reductase activity has also been reported for several primates [173]. In the liver, As^V has

been demonstrated to be uptaken by subcellular compartments, such as the nucleus and the mitochondria [163,174]. As^{V} uncouples oxidative phosphorylation [175–177] in the liver and thus displays a fundamentally different molecular mechanism of toxicity than As^{III} [64].

4.3. Mercuric mercury and methylmercury metabolism

$HgCl_2$ in solution with physiological levels of Cl^- exists as an equilibrium mixture of $HgCl_2$, $HgCl_3^-$ and $HgCl_4^{2-}$ in roughly equal concentrations [178]. Even though CH_3Hg^+ is most likely to be ingested in form of CH_3HgCys [179], it cannot be entirely excluded that – owing to the conditions in the stomach (low pH, high Cl^- concentrations) – a small amount of lipid-soluble CH_3HgCl is formed that will enter the gastrointestinal tract [180]. Studies which involved the oral administration of humans with tracer doses of either Hg^{2+} or CH_3Hg^+ have revealed that their absorption from the gastrointestinal tract into the bloodstream greatly depends on the molecular form. Almost 100% were absorbed by this route in case of CH_3Hg^+ (administered as $CH_3{}^{203}HgNO_3$) [181], whereas only ~7% of Hg^{2+} were absorbed when $^{203}HgCl_2$ was administered [41] (Fig. 4). Interestingly, $HgCl_2$ has been demonstrated to be microbially methylated to CH_3Hg^+ in humans by intestinal microbes [182] including most strains of *staphylococci* and *E. coli* [183]. Even though the gastrointestinal uptake mechanisms have not been investigated, the striking difference in their absorption rates can be rationalized in general terms based on their permeability through lipid bilayer membranes. Because of the hydrophobic nature of CH_3HgCl (uncharged at physiological pH), lipid bilayer membranes do not pose a significant permeability barrier for this Hg compound [184]. Similarly, $HgCl_2$ has been demonstrated to cross artificial lipid bilayer membranes in form of neutral $HgCl_2$ [178].

After their absorption into the bloodstream, both Hg^{2+} and CH_3Hg^+ – owing to their extremely high propensity to react with thiols [185] – are likely to bind to plasma constituents which contain sulfhydryl groups (Fig. 4). The addition of $HgCl_2$ to an aqueous solution of HSA, for instance, resulted in the formation of a $(HSA)_2Hg$ complex [186] and subsequent studies involving size-exclusion chromatography (SEC) have revealed that this also occurs in human serum (in the presence of the plasma proteome) and that >90% of the Hg was associated with this plasma protein [187]. ~5% of the Hg, however, eluted in the low molecular weight fraction and could be explained by the formation of $(Cys)_2Hg$ and/or $(GS)_2Hg$ complexes which exhibit a characteristic linear S-Hg-S coordination chemistry [188]. The formation constant for the binding of CH_3Hg^+ to mercaptalbumin [189,190] has been determined to be considerably larger than that for GSH and Cys [180]. Only one study, however, has reported the binding of CH_3Hg^+ to albumin in plasma after the oral administration of mice with CH_3HgCl [191]. In view of the permeability of artificial lipid bilayer membranes for CH_3HgCl and $HgCl_2$, it is not surprising that both mercurials have been demonstrated to permeate the erythrocyte membrane. ^1H spin-echo NMR-spectroscopy was employed to detect the binding of $HgCl_2$ to intracellular thiols in intact erythrocytes (suspended in an isotonic saline glucose solution) [192]. $HgCl_2$ rapidly diffused through the cell membrane (intracellular equilibrium distribution was established within 4 min) and reacted with intracellular GSH and Hb to form a mixed-ligand complex GS–Hg–Hb (Fig. 4), which had a lifetime of less than 30 s (25 °C). The addition of $HgCl_2$ to whole blood, however, demonstrated that it did not significantly penetrate the erythrocyte membrane [193–195], which must be most likely attributed to the fact that Hg^{2+} is complexed by plasma HSA. Conversely, the oral administration of humans with $CH_3{}^{203}HgNO_3$ (11 μg $MeHg^+$) revealed that after 15 min the ^{203}Hg concentration was ten times higher in erythrocytes than in plasma [181]. ^1H spin-echo NMR-spectroscopy revealed that CH_3Hg^+ rapidly diffuses through the cell membrane of human erythrocytes and binds to GSH [196] (Fig. 4). The average lifetime of the formed CH_3Hg–SG complex, however, was 0.01 s and much shorter than that for the GS–Hg–Hb complex. Interestingly, the treatment of human blood with CH_3Hg^+ (12 nmol/ml, 30 min, 37 °C) followed by the suspension of the isolated "CH_3Hg^+-loaded erythrocytes" in buffered solutions only released CH_3Hg^+ (from intact erythrocytes) when bovine serum albumin (0.2 mM) was present. This illustrates the mobility of this mercurial in biological systems and implies communication across the erythrocyte membrane [197]. The incubation of human blood with CH_3HgCl (12 nmol/ml, 30 min) followed by the SEC analysis of erythrocyte lysate showed that ~30% of the CH_3Hg^+ was associated with GSH (the remainder eluted in the high molecular weight fraction) [197]. CH_3Hg^+ – which should have a strong affinity for the sulfhydryl-groups of Hb [190] – has also been demonstrated to bind to Hb inside rabbit erythrocytes [197] (Fig. 4).

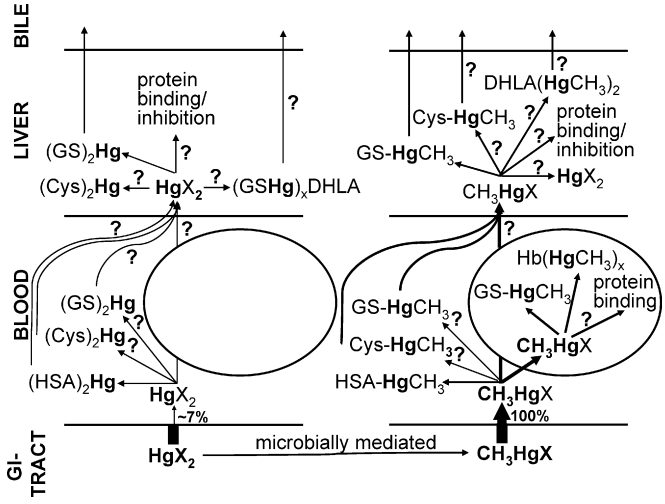

Fig. 4. Schematic illustration of the mammalian metabolism of Hg^{2+} and CH_3Hg^+. Oval shape: erythrocyte; Cys: L-cysteine; GSH: L-glutathione; HSA: human serum albumin; DHLA: dihydrolipoic acid; X = Cl^- or Cys.

The molecular mechanisms by which Hg^{2+} and CH_3Hg^+ are translocated from the blood to the liver are not completely understood. With regard to CH_3Hg^+, the intravenous co-administration of Cys or GSH increased the hepatic uptake of CH_3Hg^+, which indicates that these thiols may be involved in the translocation mechanism [190]. Subsequent studies which

involved the perfusion of rat livers with solutions containing thiol-complexed $CH_3{}^{203}Hg$ (10-fold molar excess of the thiol in Krebs–Henseleit buffer) revealed that the dose of Hg that was recovered in the liver decreased in the order GSH (17.7%), Cys (15.7) and HSA (6.9%) and therefore strongly depended on the chemical form of CH_3Hg^+ in the perfusing solution (and therefore likely also in plasma) [198]. Even though no comparable perfusion studies have been reported for $HgCl_2$, it could be demonstrated that the intravenous co-administration of Cys or GSH (along with $HgCl_2$) decreased the hepatic Hg^{2+}-uptake [190]. These findings, however, should not be interpreted as evidence that Cys or GSH are not involved in the translocation mechanism, since excess thiol could have competitively inhibited the hepatic uptake of a putative Hg^{2+}-thiol-complex or could have resulted in the formation of Hg-species that is not translocated. In fact, it has been recently reported that a Hg^{2+}-conjugate of Cys was uptaken by human kidney proximal tubular epithelial cells in cell culture experiments [199]. The incubation of equimolar HSA, $HgCl_2$ and Cys, for instance, resulted in the formation of a ternary HSA–Hg–Cys complex, which may play an important role in the translocation of Hg^{2+} from the blood to the liver [187]. The translocation of Hg^{2+} from the bloodstream to the liver appears to be mediated by a Na^+–K^+–ATPase [200]. Inside hepatocytes, Hg^{2+} and CH_3Hg^+ can bind to several endogenous ligands. In spite of the comparatively large cytosolic GSH concentration (7.5 mM) [139], this thiol must be identified as a likely intracellular target of both mercurials (Fig. 4). With regard to Hg^{2+}, circumstantial evidence for the intrahepatic formation of GS–Hg–SG comes form studies which exposed animals to $HgCl_2$ and tentatively identified GS–Hg–SG in bile using size exclusion chromatography [190,201,202]. In addition, the experimental inhibition of GSH-synthesis *in vivo* markedly enhanced the lethal toxicity of $HgCl_2$ [203]. Similarly, the administration to animals of CH_3Hg^+ resulted in the biliary excretion of a CH_3Hg–SG complex [190,204–207] which appears to be a substrate for canalicular carriers that transport GSH [207]. Endogenous dihydrolipoic acid (LA concentration in mammalian liver 11.6 nmol/g [144]) must also be considered as a potential intracellular target for Hg^{2+} since the administration of LA to rats dramatically enhanced the biliary excretion of Hg^{2+} and could be explained by the intrahepatic formation of a $(GSHg)_x$DHLA complex [208]. The fact that little hepatotoxicity has been observed after the administration of Hg^{2+} and CH_3Hg^+ to animals, suggests that both mercurials do not appreciably interact with intracellular proteins and are most likely efficiently delivered to the bile across the canalicular plasma membrane for the excretion in the intestine [202]. This could also explain the fact that no comprehensive studies into the identification of Hg^{2+} and CH_3Hg^+-binding proteins in liver cytosol have been reported. Interestingly, the chronic administration of mice with $HgCl_2$ (4–7 weeks, 1 mM $HgCl_2$ in drinking water) has resulted in the accumulation of Hg in hepatocyte nuclei [209], which was later demonstrated to occur within 15 min post-injection in rats [200]. Subsequent results demonstrated that Hg^{2+} can induce the transcription of the metallothionein-I gene in mouse liver [210]. Direct experimental evidence for the binding of Hg^{2+} to DNA has been recently obtained by identifying a Hg^{2+}-crosslinked base pair (thymine–Hg–thymine) which stabilized a DNA-duplex [211].

4.4. Selenite and selenate metabolism

In contrast to As^{III}, Hg^{2+} and CH_3Hg^+ (except As^V), endogenous reduction is the dominating feature of the mammalian metabolism of Se^{IV} and Se^{VI} [212]. In aqueous solution and at physiological pH, Se^{IV} is predominantly present as $HSeO_3^-$ ($pK_1 = 2.6$, $pK_2 = 7.3$) and Se^{VI} as SeO_4^{2-}. In aqueous solution, GSH and Cys chemically reduce Se^{IV} to GS–Se–SG and Cys–Se–Cys [213,214]. Conversely, Se^{VI} is not reduced by GSH [215]. In humans, orally ingested Se^{IV} and Se^{VI} are readily absorbed from the gastrointestinal tract into the bloodstream (Se^{IV} 62–81%, Se^{VI} ~95%) [216–219] (Fig. 5). In rats both Se^{IV} and Se^{VI} are most efficiently and rapidly absorbed (within 15 min) from the small intestine (ileum) [220]. Since Se^{VI}, however, was absorbed ~3–7 fold more efficiently than Se^{IV} [220,221], these oxo-anions appear to be absorbed by different uptake mechanisms. Se^{IV} is not actively transported and is believed to be absorbed by diffusion [221–224]. A more specific transport mechanism, however, cannot be entirely excluded [223] (HSe^- has also been demonstrated to be absorbed from the gastrointestinal tract in chicken [225]). Studies into the role of ingested dietary thiols on the mucosal uptake of Se^{IV} across the intestinal brush border membrane in rats have revealed that the absorption of Se^{IV} by the jejunum was stimulated by Cys [226] and that the preincubation of rat ileum with GSH resulted in a significantly increased uptake of Se^{IV} (uptake of Se^{VI} was unaffected by GSH) [227]. These results are in accord with the observation that an inhibition of endogenous GSH-synthesis in rat tissues (including the intestinal mucosa) decreased the transepithelial transport of Se^{IV} [220]. Thus, exogenous (dietary) and endogenous thiols (excreted from the liver via bile to the small intestine), appear to be involved in the gastrointestinal absorption and the bioavailability of Se^{IV} [228]. With regard to Se^{VI}, the

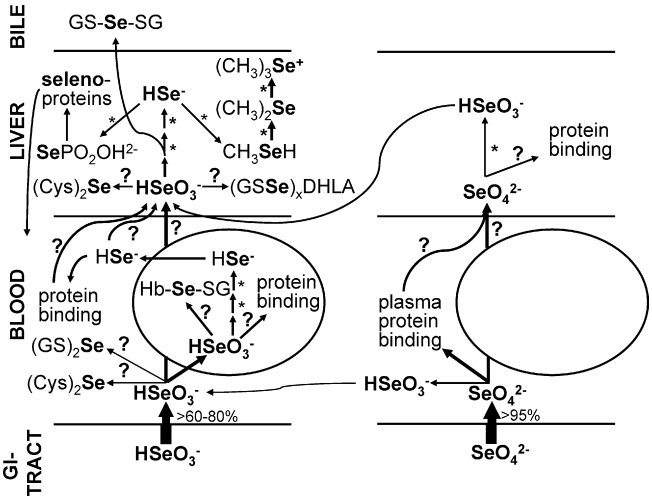

Fig. 5. Schematic illustration of the mammalian metabolism of Se^{IV} and Se^{VI}. Oval shape: erythrocyte; Cys: L-cysteine; GSH: L-glutathione; DHLA: dihydrolipoic acid; Hb: hemoglobin. Asterisk indicates enzymatically mediated biotransformation.

inhibition of its absorption from the small intestine (the ileum) by a 1000-fold excess of SO_4^{2-} in rats suggests a common, relatively unspecific, but active transport mechanism [221,222,227], which is energized in part by the Na^+-gradient across the intestinal brush border membrane [222,227].

After the absorption of Se^{IV} and Se^{VI} into the bloodstream, their individual binding to plasma proteins must be considered (Fig. 5). Even though the binding of Se^{VI} to plasma proteins has not been investigated, it has been reported that plasma, but not erythrocytes, will reduce Se^{VI} to Se^{IV} [229,230]. In rats, Se^{VI} was not appreciably uptaken by erythrocytes and disappeared from the bloodstream much faster than Se^{IV} [215]. Studies of the binding of Se^{IV} to proteins in plasma have revealed a negligible incorporation of Se into plasma proteins [231–234], which is also in accord with the detection of free Se^{IV} in human plasma [231]. When incubated with blood, however, Se^{IV} was rapidly taken up by erythrocytes (50–70% within 1–2 min) [233–238] (Fig. 5). This uptake is mediated by the erythrocyte anion transport protein (AE1) which transports a wide variety of oxy-anions and is presumably based on the structural similarity of the Se^{IV}-anion ($HSeO_3^-$) with the bicarbonate anion (HCO_3^-) [239]. A study into the transport of oxy-anions in human erythrocytes has revealed that Se^{VI} influx was two orders of magnitude slower than that of Se^{IV}, which substantiates the *in vivo* results that were observed in rats [215]. The inhibition of the extrusion of ^{75}Se from erythrocytes by high extracellular concentrations of Se^{IV} [230,233] suggests that the AE1 transport protein is involved in the regulation of the uptake of Se^{IV} by erythrocytes [240]. Evidence in support of this hypothesis comes from experiments which showed that the reaction of Se^{IV} with Cys-residues of the cytosolic domain of AE1 is associated with a conformational change, that could be transmitted to the extracellular domain of the erythrocyte membrane [240] and is in accord with the predicted large conformational change in the structure of the anion-loaded AE1 protein during the translocation process of oxy-anions [239]. Inside erythrocytes, Se^{IV} was found to be metabolized [230,236,237] involving the GSH-mediated stepwise reduction [241,242] to a reduced selenium compound – presumably HSe^- [231,232,242,243] (Fig. 5). The latter species is rapidly effluxed from the erythrocytes (within 15–20 min). This, however, only occurs in the presence of plasma or albumin in the supporting medium [234,241,244] and implies a feedback mechanism across the erythrocyte membrane which could involve the AE1 transmembrane protein [240]. The expelled HSe^- has been demonstrated to loosely bind to plasma proteins [230,233,237,245], including albumin [218,234,238,243]. The easy removal of protein-bound Se (within 60 min after treatment) by urea, Cys and mercaptoethanol has been rationalized in terms of the formation of –S–Se– bonds with surface accessible protein sulfhydryl groups, but was not experimentally verified [218,245]. With regard to albumin, the erythrocyte-effluxed Se compound was demonstrated to not bind to Cys-34 of bovine serum albumin [243], which is expected in view of the fact that sulfhydryl Cys-34 is deprotonated at pH 7.4 [126]. Another interesting observation that still needs to be explained is the fact that HSe^- is sequestered by only about 5% of the albumin existing in plasma [234]. In a study with human cancer patients, ^{75}Se (given as Se^{IV}) rapidly disappeared from the plasma within 1 h, but returned to the plasma (where it was bound to proteins) between 1 and 6 h [245]. This suggests that erythrocyte generated HSe^- is rapidly translocated to the liver and reappears in the plasma in form of selenoproteins (proteins containing endogenously synthesized Se–cysteine groups), since it was impossible to remove Se by dialysis in the presence of mercaptoethanol [245]. After the addition of $^{75}Se^{IV}$ to blood, four major Se-containing entities were identified in plasma of humans [231,245] and rhesus monkeys [246]. The Se-containing peak that was detected in the low-molecular weight region within 1 h rapidly disappeared over time (within 6 h) and likely corresponds to either GS–Se–SG, Cys–Se–SG or free Se^{IV} (the latter has been identified in rat and human serum) [247,248]). The other selenium-containing peaks were selenoprotein P, the major selenoprotein (accounts for ~40–60% of total Se in humans), glutathione peroxidase pGSH–Px (23%) and albumin (16%) [249–252]. Only one study has reported the binding of Se^{IV} to erythrocyte proteins. After the injection of Se-depleted rhesus monkeys with $^{75}Se^{IV}$, size-exclusion chromatography (Sephadex-150) of erythrocyte lysate (prepared after 3, 32 and 48 h) revealed four major Se-containing entities [246]. The ^{75}Se-peak that eluted in the void volume (peak 1 > 150 kDa) remained constant throughout the observed time-period, but the protein was not identified. Since no protein with a size > 150 kDa is known to exist in human erythrocyte lysate [253], this Se-containing peak may be the product of Se^{IV}-induced cross-linking between two sulfhydryl-containing proteins similar to the known reaction of Se^{IV} with biological thiols [254]. Another ^{75}Se-peak (peak 2) eluted at the position of cGSH–Px (cytosolic GSH–Px was the first mammalian selenoprotein to be isolated from rat erythrocytes in 1973 [255]). This ^{75}Se-peak was hardly recognizable after 3 h, but was greatly increased at the 32 and the 48 h time-point, indicating endogenous selenoprotein-biosynthesis. Another ^{75}Se-peak (peak 3) co-eluted with Hb which is in accord with studies that detected Se in Hb following the injection of dogs with Se^{VI} [256]. A putative selenohemoglobin (possibly Hb–Se–SG) has been postulated to be formed similar to sulfhemoglobin [257,258] (Fig. 5). Se-peak 3 was formed transiently and was most intense between 9 and 24 h post-injection. The ^{75}Se that eluted in the low-molecular weight region (referred to as peak 4, but actually comprised of several small peaks) was intense up to 3 h, but gradually decreased in intensity to level off at the 24 h time-point and likely corresponds to small molecular weight metabolites of Se^{IV}, such as GS–Se–SG.

With regard to the translocation from the bloodstream to the liver, Se^{VI} is believed to be translocated in its unmetabolized form [215,259]. Conversely, several Se^{IV} species and metabolites thereof must be considered. Se^{IV} could be translocated in itself, since free Se^{IV} has been identified in human plasma [247]. In plasma, the nonenzymatic reaction of Se^{IV} with GSH and Cys could result in the formation of species (GS–Se–SG and Cys–Se–Cys) that may be involved in the translocation to the liver [260]. In contrast to this, the translocation of erythrocyte-processed Se^{IV} (presumably HSe^-) is supported by experiments [245]. Virtually nothing, however, is known about the translocation mechanism(s) themselves (Fig. 5). In the liver,

both Se^{IV} and Se^{VI} were shown to be reduced to HSe^- [261] (other nutritional Se compounds are also catabolized to HSe^- in the liver [262,263]). The reduction of Se^{IV} involves endogenous GSH and glutathione reductase [264], whereas Se^{VI} is believed to be enzymatically reduced to Se^{IV} involving its enzymatic activation (with ATP) to adenosine-5′-selenophosphate, followed by the non-enzymatic GSH-mediated reduction to Se^{IV} [264,265]. In case of excess dietary intake of Se, HSe^- is utilized for the biomethylation to $(CH_3)_2Se$, which is pulmonary excreted (an thus detoxified) [266–270], or $(CH_3)_3Se^+$ which has been claimed to be a major urinary metabolite in humans and rats [271–273], but urgently needs to be confirmed [104,274] (Fig. 5). If nutritionally adequate amounts of the essential trace element Se [275] are ingested, HSe^- is used for selenoprotein synthesis [276–278]. This involves the conversion of HSe^- to selenophosphate ($SePO_2OH^{2-}$) and is mediated by an ATP-consuming reaction involving $SePO_2OH^{2-}$-synthetase [279,280] (Fig. 5). $SePO_2OH^{2-}$ is subsequently used for the synthesis of selenocysteyl-tRNA (the mechanism is not completely understood in mammals [280]) which finally attaches selenocysteine to the growing peptide chain that is assembled by the ribosome. More than 25 selenium-containing proteins have so far been detected in liver cytosol alone after *in vivo* labeling of rats with $Na_2{}^{75}SeO_3$ followed by gel-electrophoresis [281]. Reasonable speculations estimate that up to 100 mammalian selenoproteins may exist [276]. With regard to the biliary excretion of Se, approximately similar bile Se concentrations were observed after the intravenous administration of rats and rabbits with Se^{IV} and Se^{VI} [167,282]. Studies into the molecular form of Se in bile after the treatment of rats with Se^{IV} revealed the presence of GS–Se–SG [283]. In summary, HSe^- emerges as a key metabolite in the mammalian metabolism of Se [262,264,270,284,285].

5. Interactions between metals and metalloid compounds in mammals

A concise summary of the phenomenological observations regarding "interactions" until the year 2000 will now be presented.

5.1. Arsenite–selenite interaction

Elevated levels of Se in soils represent a serious problem in agriculture since certain plants can accumulate large quantities of Se and since feeding of these plants to livestock will result in "alkali disease" and "blind staggers", two different forms of Se poisoning [286]. Enormous losses of livestock caused by feeding toxic vegetation in South Dakota in the 1920's and the eventual identification of the culprit – elevated Se concentrations in grain (10–30 mg/kg) [59,287] – triggered an investigation into the effect of a variety of metals and metalloid compounds on the toxicity of seleniferous grains in rats. Rather unexpectedly, the addition of As^{III} in form of $NaAsO_2$ to drinking water (5 mg As/l) of rats offered full protection against liver damage caused by seleniferous wheat and Se^{IV} (∼15 mg Se/kg), whereas 2.5 mg As/l only partially protected against the toxicity of seleniferous wheat (∼11 mg Se/kg) [288–291]. Feeding studies with rats revealed that As^{III} added to the diet similarly offered protection against the toxicity of Se^{IV} or seleniferous grains [292]. Subsequent studies confirmed this striking mineral antagonism between two individually highly toxic metalloid compounds in rats [293–295], mice [296], hogs [297], steers [298], dogs [299], chicken [300–303], mallards [304] and some findings suggest that the same holds true for humans [305–310]. The prevention of the Se^{IV}-induced inhibition of the liver succinic dehydrogenase activity by including As^{III} in the diet of rats represents further proof for this antagonism in mammals using an enzymatic assay [311]. Taken together, these phenomenological findings hinted toward the existence of a common underlying biomolecular detoxification mechanism which operates in all higher animals. The first indication of a possible connection between this antagonism and cancer was provided by a feeding study with rats which demonstrated that As^{III} (2 ppm As in diet) abolishes the anticarcinogenic effect of Se^{IV} (2 ppm Se in diet) in inbred female C_3H/St mice (an animal model prone to the spontaneous formation of mammary adenocarcinoma following the exposure to arsenite in drinking water) [312] and in a dimethylbenz[a]anthracene-induced mammary tumor model in rats [313]. Even though these studies involved the exposure of animals to doses of both metalloids that are only rarely encountered in the environment, the results conclusively demonstrated that As^{III} can interfere with the mammalian metabolism of Se^{IV}. Direct experimental evidence for the occurence of this antagonism at the cellular level has been observed in cultured human lymphocytes [314–317], human leukemia HL-60 cells [318], porcine endothelial cells [319], rat hepatocytes [320], human hepatocytes [321] and – on a subcellular level – in Se-deficient rat mitochondria [322].

The first insight into the mechanism of this mineral antagonism was provided by a study which demonstrated that an oral, toxic dose of Se^{IV} that was administered to rats was detoxified by As^{III} regardless whether the latter was administered orally or subcutaneously [323]. This strongly suggested that the As^{III}–Se^{IV}-antagonism was not based on the inhibition of the gastrointestinal absorption of Se^{IV} by As^{III}. Other studies demonstrated that the simultaneous administration of As^{III} along with Se^{IV} markedly inhibited the pulmonary excretion of $(CH_3)_2Se$ in rats [269,324–326] and hamsters [327], which was difficult to rationalize at the time since the exhalation of $(CH_3)_2Se$ had been previously established as a major detoxification pathway for excess selenite in mammals. As^{III} was also shown to greatly affect the body distribution of Se among internal organs [282,326,328] and significantly retarded the passage of Se (given as Se^{IV}) from the blood to the liver [128,295]. In addition, As^{III} dramatically decreased the retention of Se in the liver of rats in short-term (3–24 h after dosing) [128,282,326,328–330] and long-term feeding experiments (∼2 to 18 months) in rats (seleniferous wheat [289], selenite [325]), hogs (seleniferous corn [297]), and mice (SeO_2 [312]). Other long-term feeding experiments which involved As^{III}-fortified diets, however, did not result in significantly decreased Se concentrations in the liver (Se was administered as selenifer-

ous wheat/corn or Se^{IV}) of rats [292,294]. The discovery that As^{III} markedly increased the biliary excretion of Se into the gastrointestinal tract (when the latter was administered Se^{IV}) [282,327,329] and vice versa [128,129,326] prompted Orville A. Levander to postulate the *in vivo* formation of an As and Se-containing detoxication conjugate in the liver [331], which is readily excreted in the bile [282,286,331]. The inhibition of the Se^{IV}-mediated reduction of methemoglobin by As^{III} in the presence of GSH indicated that erythrocytes were possibly involved in mediating this antagonism [332]. Subsequent studies demonstrated that As^{III} also inhibited the formation of H_2Se from Se^{IV} in a system which contained GSH, glutathione reductase and bovine serum albumin (the As^{III} concentrations that were used in this study did not inhibit the glutathione reductase itself) [333]. These results prompted the authors to postulate "a reaction between arsenite and a product of selenite reduction" and provided an important clue with regard to the nature of the putative As and Se-containing detoxication conjugate itself. The same authors later corroborated these findings [212,284] and proposed that "arsenite was reacting in a stoichiometric fashion with reduced intermediates formed from selenite" [284]. Seemingly unrelated studies into the enzymatic methylation of As^{III} in mammals revealed that Se^{IV} inhibited the methylation of As^{III} in rat liver cytosol [334,335]. This result was eventually verified in an enzyme essay using partially purified As^{III}-methyltransferase which had been isolated from rabbit liver (12.6 μM Se^{IV} resulted in 50% enzyme inhibition) [336]. The latter study also reported that Se^{VI} and HSe^- inhibited the methylation of As^{III} much less effectively than Se^{IV}. Collectively, these investigations strongly indicated that the biochemical interaction between As^{III} and Se^{IV} occurs in blood and the liver.

5.2. Arsenate–selenite interaction

Studies involving the simultaneous exposure to As^V and Se^{IV} established an antagonistic interaction between these metalloid compounds in mallards [304,337], rats [330,338–340], hamsters [341] and mice [342]. The simultaneous administration of rats with As^V and Se^{IV} revealed an increased fecal excretion of both metalloids (compared to the corresponding control groups) which is in accord with observations reported for the As^{III}–Se^{IV} interaction [343]. Subsequent studies showed that As^V increased the biliary excretion of Se (given as Se^{IV}) [128,326] and vice versa [128,129] and long term feeding studies (between 4 and 8 weeks) revealed that the chronic exposure to Se^{IV} decreased the *in vivo* methylation of simultaneously administered As^V [342]. Conversely, As^V significantly increased the fecal excretion of ^{75}Se [344]. Combined, these results indicated that the interaction between As^V and Se^{IV} is related to the As^{III}–Se^{IV} interaction. Interestingly, the consecutive injection of rats with As^V (3 mg As_2O_5/kg) and Se^{IV} (0.5 mg SeO_2/kg) twice a week for 4 weeks resulted in the formation of a precipitate in the kidney lysosomes, which was characterized as As_2Se [340].

5.3. Mercuric mercury–selenite interaction

Another perplexing antagonism between two individually highly toxic compounds was discovered in 1967, when the $HgCl_2$-induced renal and intestinal necrosis in rats (4.0 mg Hg/kg body weight) was demonstrated to be completely abolished by the simultaneous administration of Se^{IV} (2.4 mg Se/kg body weight; molar ratio Se:Hg = 1.5) [345]. Using the same doses, the injection of Se^{IV} 1 h after $HgCl_2$ increased the survival rate of rats by ∼94% (on day 7) compared to the $HgCl_2$-only group. This dramatic effect of Se^{IV} on the toxicity of $HgCl_2$ in rats (and vice versa) was subsequently confirmed by others [293,346]. In addition, the administration of Se^{IV} (0.8 mg Se/kg body weight) to rats treated with $HgCl_2$ (2.0 mg/kg body weight) fully blocked the depletion of the GSH concentration in the liver by Hg^{2+} [347]. Se^{IV} was also found to considerably prolong the half-life of ^{203}Hg (given as $^{203}HgCl_2$) in the bloodstream of mice over a 4 weeks period [348,349]. After the confirmation of this phenomenon in rats [350] and rabbits [351], Se^{IV} was demonstrated to decrease the Hg concentration of the kidneys and the intestine, but to increase those of the liver and blood (24 h and 2 weeks after treatment) [349,352,353]. These findings were in qualitative agreement with studies which showed that the simultaneous administration of Se^{IV} dramatically decreased the urinary excretion of Hg (given as $HgCl_2$) [354,355] and vice versa [356]. Hence, the simultaneous administration of Se^{IV} greatly affects the organ distribution of $HgCl_2$ [354,357–361] (and vice versa [328,361–364]) and the excretion of Hg^{2+}. Since the administration of 2,3-dimercaptopropanol, which is generally used to remove Hg from the body [365], did not markedly effect the excretion of Hg from $HgCl_2$ and Se^{IV}-treated animals, the authors concluded that "the compounds which are formed in the organism by the reaction of selenium with mercury must be relatively stable" [349], which was later confirmed by others [366]. A long-term feeding study in which rats were exposed to $HgCl_2$ in drinking water (10 ppm) and given Se^{IV} in the diet (10 ppm) for 7 weeks, resulted in marginally decreased Se-concentrations in the liver (87% of Se-only control) and a more pronounced decrease in the kidney (66% of Se-only control) [325]. The inhibition of the pulmonary excretion of volative Se compounds (following the administration of Se^{IV}) by simultaneously administered $HgCl_2$ [325,354,367], the mutual decrease in the translocation of Se (given as Se^{IV}) and Hg (given as $HgCl_2$) from the mother via the placenta to the foetus [348,354,368,369], and the prevention of the inhibitory effect of $HgCl_2$ on the GSH-peroxidase activity in the kidney by Se^{IV} [370] strongly indicated that $HgCl_2$ interfered with the metabolism of Se^{IV} [368,371], possibly in blood and involving reactions with proteins [348,368,372]. The Se^{IV}-induced diversion of $^{203}HgCl_2$ binding from the small to the high molecular weight region in liver and kidney cytosol (by size exclusion chromatography) suggested that the interaction between Se^{IV} and $HgCl_2$, however, also occurs in the liver and the kidney [352,353,373–383] and also in erythrocytes [195,351,384,385]. Direct experimental evidence in favor of an interaction between Se^{IV} and $HgCl_2$ in the liver comes from studies which showed that Hg^{2+} inhibited the swelling of Se-deficient rat liver mitochondria caused by GSH and Se^{IV} [322]. The spatial co-localization of Se and Hg in liver and kidney (but not in brain [386]) after the exposure of rats to Se^{IV} and $HgCl_2$ was another observation

which implied their interaction in organs [387–391]. A study into the effect of varying doses of Se^{IV} on the tissue distribution of Hg (administered as $HgCl_2$) and measured 24 h after administration revealed that an equimolar dose of Se^{IV} produced the maximum retention of Hg in blood and the maximum reduction in renal Hg [350]. This suggested a 1:1 stoichiometry between Se^{IV} and Hg^{2+} with regard to the underlying detoxification mechanism between Se^{IV} and $HgCl_2$ and was eventually confirmed in chicken [392], mice [393] and pigs [394].

In 1974 Raymond Burk's group reported that the co-injection of rats with $^{75}Se^{IV}$ (0.4 mg Se/kg body weight) and $^{203}HgCl_2$ (1.0 mg Hg/kg body weight) resulted in the formation of a single Se and Hg-containing compound in plasma (detected by size exclusion chromatography followed by radiodetection) [395], which was confirmed by others [374,396–399]). A compound with a 1:1 molar Se:Hg ratio was detected in rats plasma even when different doses of Se^{IV} and $HgCl_2$ were injected [395]. Furthermore, the isolated ^{75}Se and ^{203}Hg-containing peak maintained its 1:1 stoichiometry even after it was subjected to ion-exchange chromatography [382,395]. Even though these results strongly suggested that Se and Hg were attached to a single plasma protein, other researchers concluded from their studies that the Hg–Se complex in plasma was an inorganic mercuric selenide colloid which did not contain protein [400]. The observed time lag after the injection of Se^{IV} before the 1:1 Se:Hg containing protein could be detected, suggested that Na_2SeO_3 must be metabolized in the bloodstream before binding with $HgCl_2$ to the plasma protein can occur [395]. Interestingly, a water soluble "black complex" which apparently contained GS-moieties and equimolar amounts of Hg and Se was synthesized by reacting 4 mmol GSH with 0.5 mmol $HgCl_2$ and 0.45 mmol of Na_2SeO_3 (followed by the removal of a precipitate) and was revealed to be non-toxic when fed to mice [401]. Even though this "black complex", very likely represented the detoxification product that is formed *in vivo* between Se^{IV} and $HgCl_2$ in mammals, the authors did not structurally characterize it at the time.

In 1983 Akira Naganuma's group revealed that GSH is fundamentally involved in the formation of the Hg–Se-containing plasma protein in rabbit blood [385]. This finding further implied that erythrocytes were possibly involved in driving the antagonism between Se^{IV} and $HgCl_2$ in the bloodstream since the concentrations of GSH in plasma are 100-fold lower than those in erythrocytes [122]. Interestingly, the administration of Se^{IV} 1 h before $HgCl_2$ markedly enhanced the lethality of the latter [372,402], which implies a synergistic toxicity between a Se^{IV}-metabolite and Hg^{2+} and emphasizes that the order of injection critically determines the nature of the "interaction" (antagonistic versus synergistic) *in vivo*.

The next big step forward with regard to a better understanding of the molecular mechanism regarding the Se^{IV}–$HgCl_2$ antagonism in mammals was reported in 1997 by Kazuo Suzuki's group. They identified the plasma protein to which a structurally unknown Hg–Se entity bound as selenoprotein P [403]. Based on a subsequent study the same group concluded that ~35 Hg–Se-containing entities, which are formed in blood and contain ~100 atoms of Hg and Se bind to selenoprotein P (Sel P) [404,405]. The structure of the Hg–Se entity itself, however, remained elusive.

6. Elucidation of the structural basis of the arsenite–selenite antagonism

Despite the fact that all studies which involved the simultaneous administration of animals with As^{III} and Se^{IV} collectively identified the mutual biliary excretion of As^{III} and Se^{IV} as the most prominent feature of this antagonism, the underlying molecular mechanism remained elusive. One possible explanation for the As^{III}-induced increased biliary excretion of Se (given as Se^{IV} as outlined in Section 5.1) was a "postulated reaction between these two elements to form some sort of detoxication conjugate readily excreted into the bile" [282]. The same authors , however, also suggested that "another possibility could be a change in the permeability of the blood/bile barrier caused by a reaction of arsenic with essential structural units in membranes or with certain energy-producing enzyme systems necessary for the maintenance of membrane integrity". In order to elucidate which one of these explanations was the correct one, evidence in favour of the biliary excretion of a putative As–Se-compound had to be gathered and its structure had to be elucidated. Since As^{III} and Se^{IV} are well known to individually react with GSH [107,213] and since GSH represents the most prevalent low molecular weight thiol in the liver of mammals [139], the *in vivo* formation and biliary excretion of a ternary $As_xSe_y(GS)_z$ compound was envisioned.

A systematic investigation into the reaction of equimolar As^{III} and Se^{IV} with increasing amounts of GSH (in aqueous solution) showed that at least 8 mol-equivalents of GSH were required to prevent the formation of a red precipitate (α-Se) [406]. The obtained reaction mixture presumably contained a water-soluble Se compound, which was oxygen-sensitive and decomposed below pH 7.0. Electrospray ionization mass spectroscopy (ESI-MS) of this reaction mixture did not reveal any useful results, but Raman spectroscopy identified a unique peak at 290 cm^{-1} and – more importantly – two peaks at 510 and 660 cm^{-1}, which implied the presence of oxidized GSH (GSSG). This implied that a redox reaction had occurred in which GSH was consumed [406]. ^{77}Se NMR spectroscopy of the reaction mixture resulted in a single ^{77}Se signal with an unusual ^{77}Se chemical shift of −5.7 ppm. This finding provided the first experimental evidence in favor of the formation of a novel Se compound (the ^{77}Se chemical shift of the starting material, Na_2SeO_3, has been reported at 1260 ppm [407]). Studies which involved size exclusion chromatography (SEC) and radio-detection (γ-counter) of the injected $H^{75}SeO_3^-$ and/or $^{73}As(OH)_3$ in the collected fractions provided further evidence for the formation of an As–Se-compound from As^{III}, Se^{IV} and excess GSH (Fig. 6).

The subsequent analysis of the obtained reaction mixture by As and Se K-edge extended X-ray absorption fine structure (EXAFS) analysis (in collaboration with Graham N. George and Ingrid J. Pickering at the Stanford Synchrotron Radiation Laboratory, SSRL) revealed an As–Se bond length of 2.32 Å and

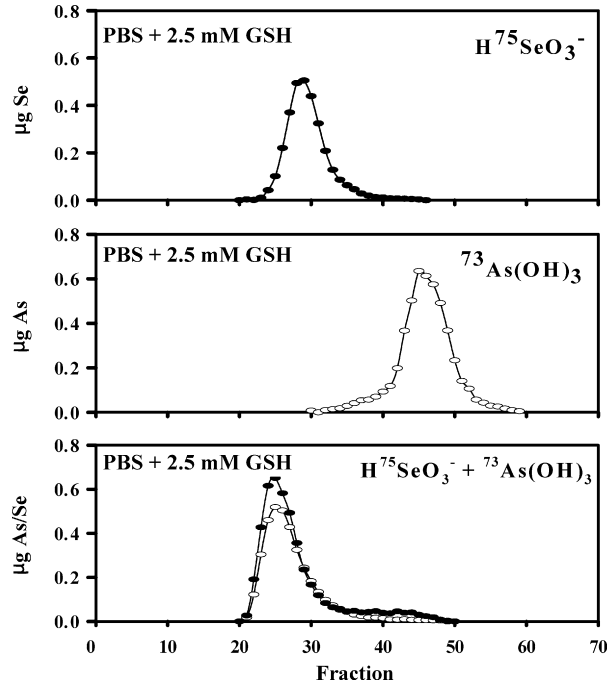

Fig. 6. Size exclusion chromatography (Sephadex G-10) of $^{73}As(OH)_3$ (5 μg As) and/or $H^{75}SeO_3^-$ (5 μg Se) and radiodetection in the collected fractions (γ-counter). PBS: phosphate buffered saline; GSH: L-glutathione. All mobile phases pH 7.4.

two As-S interactions at 2.25 Å [406]. According to reported bond distances for As–Se single and double bonds in the Cambridge Structural Database, the observed bond length suggested an As=Se double bond with significant single bond character. Hence, the EXAFS data revealed the solution species as the seleno-bis (*S*-glutathionyl) arsinium ion, $[(GS)_2AsSe]^-$. This EXAFS-derived structure allowed the assignment of the 290 cm^{-1} Raman peak as the ν(As–Se) mode of $[(GS)_2AsSe]^-$. The fact that this frequency was lower compared to what had been previously reported for As=Se bonds, was consistent with a bond order of slightly less than two and in accord with the EXAFS data [406]. In order to ascertain the EXAFS-derived structure with a different analytical technique, the retention behavior of the obtained reaction mixture (with nine GSH to prevent oxidation) was investigated by size exclusion chromatography (SEC, Sephadex-25) and hexadecyl-trimethylammonium bromide-containing mobile phases (30–50 mM in 0.1 M Tris-buffer, pH 8.0) followed by the simultaneous As, Se and S-specific detection of the column effluent by inductively coupled plasma atomic emission spectrometry (ICP-AES) [408]. Based on the interaction of the negatively charged species of the reaction mixture [GSSG net charge: −2; $(GS)_2AsSe^-$ net charge: −3 if the EXAFS-derived structure was correct] with the positively charged micelles of the mobile phase, the comparative migration behavior of GSSG and $[(GS)_2AsSe]^-$ provided direct experimental evidence for additional negative charge on $(GS)_2AsSe^-$ compared to GSSG. Taken together, the ^{77}Se NMR, Raman and SEC-ICP-AES data unequivocally confirmed the EXAFS-derived solution structure and established the overall reaction according to Eq. (1):

$$As(OH)_3 + HSeO_3^- + 8\,GSH \rightarrow (GS)_2AsSe^- + 3GSSG + 6H_2O \quad (1)$$

With a structurally characterized synthetic model compound in hand, the K-edge As and Se X-ray absorption near edge spectra of $[(GS)_2AsSe]^-$ were measured (SSRL). These spectra essentially provide a fingerprint of this compound and can be used to qualitatively identify this compound in complex biological matrices. In order to probe if the model compound was identical to the putative As–Se compound that was excreted in the bile, rabbits were injected with As^{III} (0.60 mg As/kg body weight) followed 3 min later by Se^{IV} (0.63 mg Se/kg body weight) and bile was collected for 25 min [406]. Atomic fluorescence spectroscopy analysis (SSRL) revealed that the metalloid concentrations in these bile samples were 20.9 ppm for As and 21.6 ppm for Se (rabbits injected with either As^{III} or Se^{IV} had bile metalloid concentrations of 1.7 ppm As and 0.1 ppm Se). The As/Se molar ratio in these bile samples was 0.97 and strongly indicated the presence of an As–Se compound with equimolar As and Se. The obtained K-edge Se X-ray absorption near edge spectra of bile (the Se spectrum provides a more characteristic "fingerprint" of this compound than the As spectrum) was almost identical to that of synthetic $[(GS)_2AsSe]^-$ and indicated the presence of $[(RS)_2AsSe]^-$ (where R is an organic donor) since X-ray absorption near edge spectroscopy cannot explicitly identify the nature of the sulfur donor itself. In order to identify the sulfur donor in the bile species, bile form similarly treated rabbits was analyzed by SEC-ICP-AES. Simultaneous multielement-specific detection of As, Se and S revealed a single peak which contained these elements (the As/Se molar ratio was 1:1), which greatly increased in intensity upon spiking the bile with synthetic $[(GS)_2AsSe]^-$. Hence, SEC-ICP-AES unequivocally identified GS as the sulfur donor in the bile species.

Investigations aimed at an elucidation of the chemical reactions underlying Eq. (1) revealed, that each metalloid oxo-anion individually reacts with GSH first and that the reaction products finally react to $[(GS)_2AsSe]^-$ in virtually quantitative yield [409]. In particular, $HSeO_3^-$ is reduced by 6 mole-equivalents GSH to HSe^- (this involves three consecutive $2e^-$ reduction steps) [213]. Conversely, $As(OH)_3$ reacts with 2 mol-equivalents GSH to $(GS)_2As$–OH [111]. HSe^- will then nucleophilically attack the As-atom of $(GS)_2As$–OH to form a four-coordinate intermediate species $[(GS)_2As(OH)(SeH)]^-$, which loses H_2O to form $[(GS)_2AsSe]^-$. In the presence of excess GSH – the prevalent situation in the liver [139] and erythrocytes [131] – it is possible that HSe^- rather reacts with $(GS)_3As$ to $[(GS)_2AsSe]^-$ and GSH (Fig. 7).

A subsequent investigation of a potential *in vivo* interaction between other environmentally common oxy-anions of As and Se in rabbits (As^{III}–Se^{VI}, As^{V}–Se^{IV}, As^{V}–Se^{VI}) revealed that $[(GS)_2AsSe]^-$ was only excreted in bile (within 25 min) when As^{III} (2.40 mg As/kg body weight) and Se^{VI} (2.52 mg Se/kg body weight) were injected (Se compound followed 3 min later by As compound) [167]. Since whole blood (collected at the

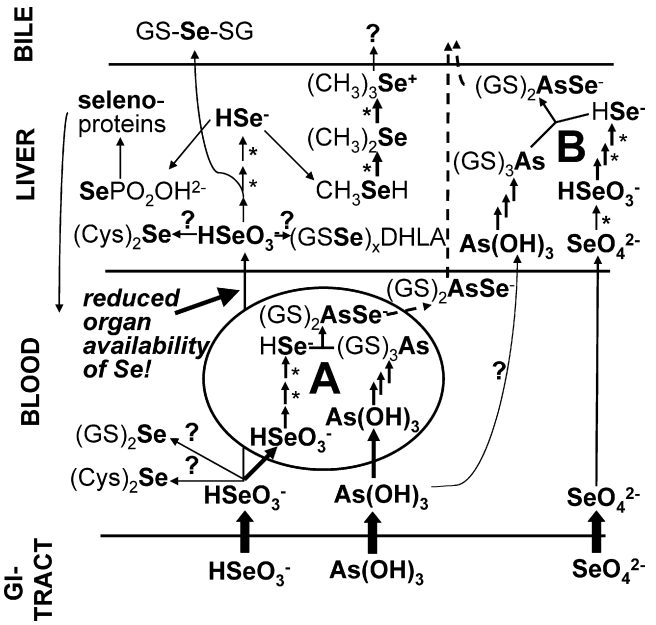

Fig. 7. Interaction between As^{III} and Se^{IV} in erythrocytes (A) and between As^{III} and Se^{VI} in the liver (B). Oval shape: erythrocyte; Cys: L-cysteine; GSH: L-glutathione; DHLA: dihydrolipoic acid. Asterisk indicates enzymatically mediated biotransformation.

25 min postinjection) contained predominantly unchanged Se^{VI}, these results (the presence of $[(GS)_2AsSe]^-$ in bile) implied that Se^{VI} must have been reduced to Se^{IV} in the liver (Fig. 7). Consequently, these results clearly indicated that $[(GS)_2AsSe]^-$ was assembled in the liver. More recent results – and arguably the most important with regard to the relevance of this As^{III} and Se^{IV} antagonism for environmental toxicology – have provided experimental evidence in favor of the *in vivo* formation and biliary excretion of $[(GS)_2AsSe]^-$ after the intravenous administration of rabbits with environmentally relevant doses of As^{III} and Se^{IV} [410]. In fact, an increased mutual biliary excretion of As and Se was observed after the intravenous administration of doses as small as 5 μg of As^{III} and Se^{IV}/kg body weight (100-times lower dose compared to the previous study) [406]. This study also revealed that the intravenous injection of rabbits with 50 μg As^{III}/kg body weight mobilized endogenous Se to the bile (evidenced by the increased Se concentration in bile compared to the control). Combined, these results established a causal relationship between the exposure of rabbits to As^{III} and Se-deficiency [410]. The most recent results established that $[(GS)_2AsSe]^-$ is rapidly formed in blood (after the addition of As^{III} and Se^{IV}) and that erythrocytes are an important site for the *in vivo* assembly of this toxicologically important metabolite [411]. These findings suggest that the erythrocyte-mediated assembly of $[(GS)_2AsSe]^-$ represents an evolved mammalian detoxification mechanism, which – if overwhelmed – represents the molecular basis for the systemic toxicity of As^{III} [136]. An overview of the antagonism between As^{III} and Se^{IV} and between As^{III} and Se^{VI} is given in Fig. 7.

7. Elucidation of the structural basis of the mercuric mercury–selenite antagonism

In order to elucidate the structure of the Hg–Se entity that binds to selenoprotein P in the bloodstream, a systematic investigation into the reaction of equimolar Hg^{2+} ($HgCl_2$) and Se^{IV} with increasing amounts of GSH was conducted [412]. Whereas colorless solutions were obtained with <3 mol equivalents of GSH, progressively darker solutions were obtained with up to 6 mol equivalents. When ≥7 mol equivalents of GSH were used, a black precipitate was formed. Since the 6 mol-equivalent mixture resulted in the formation of a precipitate within 10 min, 5 mol-equivalents of GSH were used to synthesize a putative Hg–Se compound. Since the characterization of this black solution by ESI-MS, MALDI-TOF, FAB-MS, ^{77}Se NMR and ^{199}Hg NMR did not yield any useful results, the black solution species was analyzed by size exclusion chromatography (Sephadex G25) followed by simultaneous Hg, Se and S-specific detection using ICP-AES. The two most prominent features of the obtained chromatogram were (a) the elution of a sulfur compound which was be identified as GSSG and (b) the elution of a single Hg/Se/S-containing species in the void volume. In analogy to the aqueous solution chemistry between As^{III}, Se^{IV} and GSH (Eq. (1)), the detection of GSSG in the reaction mixture implied that GSH had been consumed in a redox reaction. More importantly, the Hg/Se molar ratio of the peak that eluted in the void volume was 1.14 and contained a small, but significant amount of S (which corresponded to ∼5 mol equivalents per 100 atoms of Hg or Se). The fact that the black Hg/S/Se-species eluted in the void volume implied a size of >5 kDa, which corresponds to a Stokes radius of 65 Å. Subsequent Hg (L-edge) and Se K-edge EXAFS spectroscopy of the purified black Hg/S/Se-species (SSRL) revealed a four-fold coordination of Hg with Se (2.61 Å) and a small amount of a Se-S interaction (∼2.0 Å), which was consistent with the chromatography results and implied the presence of (GS–Se–) entities. Taken together, the SEC-ICP-AES and the EXAFS results suggested a core of mercuric selenide (Hg–Se) with the ligation of ∼5 GS-moieties on the surface (Fig. 8). The GS-moieties on the Hg–Se core explained the overall water solubility of this complex since HgSe in itself is virtually insoluble in water ($K_L = 10^{-59}$). The structure of this $(Hg–Se)_{core}(GS)_5$-species is reminiscent of water soluble CdS particles which are formed with plant phytochelatins [413].

In order to elucidate if this $(Hg–Se)_{core}(GS)_5$-species is formed *in vivo*, the Hg and Se X-ray absorption near edge spectra were measured (SSRL) and compared to those of plasma which was collected from rabbits 20 min after they had been injected with Na_2SeO_3 (0.63 mg Se/kg body weight) followed 3 min later by $HgCl_2$ (1.57 mg Hg/kg body weight). The Hg and Se X-ray absorption near edge spectra of plasma were essentially identical to those of the synthetic $(Hg–Se)_{core}(GS)_5$-species, which suggested that this species is readily formed *in vivo*. Additional evidence for the *in vivo* formation of the synthetic $(Hg–Se)_{core}(GS)_5$-species was the fact that the Hg/Se ratio in plasma (1.12) was in agreement with that of the synthetic model compound (1.14) and the results reported by others [395].

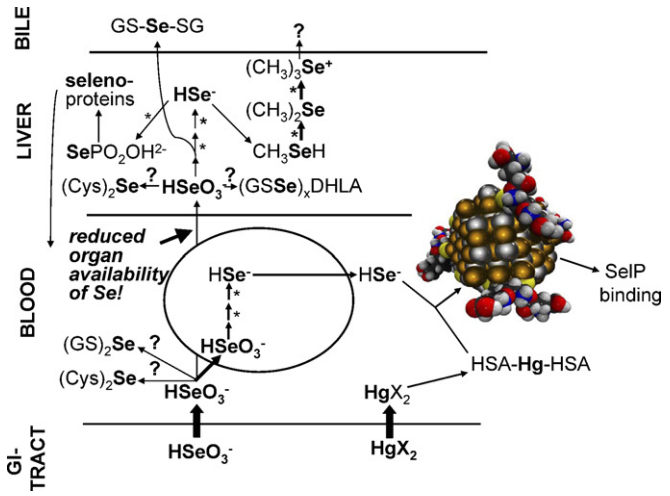

Fig. 8. Interaction between Hg^{2+} and Se^{IV} in the bloodstream. Oval shape: erythrocyte; Cys: L-cysteine; GSH: L-glutathione; DHLA: dihydrolipoic acid; SelP: selenoprotein, coloured species represents $(Hg–Se)_{100}(GS)_5$ species. Asterisk indicates enzymatically mediated biotransformation.

According to the molecular mechanism which was proposed for the mutual detoxification of Se^{IV} and Hg^{2+} by Sasakura and Suzuki [405], the species that is formed *in vivo* can be characterized as $(Hg–Se)_{100}(GS)_5$ which subsequently binds to selenoprotein P [403]. Since 35 of these $(Hg–Se)_{100}(GS)_5$ entities can bind to 1 selenoprotein P molecule [404], the latter plasmaprotein is fundamentally involved in the detoxification of Hg^{2+} and the chemically related Cd^{2+} in mammals [405]. An overview of the overall detoxification mechanism is depicted in Fig. 8.

8. Concluding remarks

Because of the ongoing anthropogenic emission of toxic metals and metalloid compounds into the global environment, certain populations are simultaneously exposed to increasing levels of toxic metals and metalloid compounds through their diet and drinking water. The toxicity of a mixture, however, cannot be accurately predicted since numerous antagonistic and synergistic effects are known to exist between individual compounds. The simultaneous administration of rabbits with two individually highly toxic compounds followed by the analysis of various biological fluids (e.g. bile, plasma) by XAS and SEC-ICP-AES has revealed the structural basis of the antagonistic interactions between the inorganic pollutants As^{III} and Hg^{2+} and the essential ultratrace element Se (administered as Se^{IV} or Se^{VI}). The fact that both interactions are driven by endogenous GSH (which reduces Se^{IV} to highly reactive HSe^- in erythrocytes) and occur in the bloodstream reinforces the previously expressed notion that erythrocytes represent the first line of defence against ingested toxic metals and metalloid compounds [5,202]. The As^{III}–Se^{IV} antagonism is based on the assembly of $[(GS)_2AsSe]^-$ in erythrocytes followed by its extrusion to plasma, the liver and finally bile. The Hg^{2+}–Se^{IV} antagonism involves the erythrocyte mediated formation of $(Hg–Se)_{100}(GS)_5$ in blood plasma and its subsequent binding to selenoprotein P. Since the As=Se bond in $[(GS)_2AsSe]^-$ and the four-coordinate Hg–Se bonds in $(Hg–Se)_{100}(GS)_5$ are chemically more stable that the corresponding As–S and Hg–S single bonds (e.g. between As^{III}/Hg^{2+} and endogenous GSH), the biochemistry and toxicology of As^{III} and Hg^{2+} are not only driven by their interactions with endogenous thiols as previously thought, but also – and much more importantly so – by their reaction with endogenously generated highly reactive selenium metabolites, such as HSe^-. The *in vivo* formation of metabolites with As–Se and Hg–Se bonds therefore implies that ingested As^{III} and Hg^{2+} both target the metabolism of Se. Several details regarding the *in vivo* assembly and the fate of $[(GS)_2AsSe]^-$ and $(Hg–Se)_{100}(GS)_5$, however, still need to be elucidated.

Since Se is an essential ultratrace element for all higher animals including humans, the chronic low-level exposure of humans to As^{III} and Hg^{2+} will render part of the ingested dietary Se^{IV} (and Se^{VI}) organ-unavailable. In view of the fact that Se-deficiency is associated with cancer in humans, the As^{III} and Hg^{2+} induced Se-deficiency of internal organs (i.e. the organavailability of Se) will inevitably increase the human cancer risk and therefore represents a conceptually novel mechanism for the chronic toxicity of As^{III} and Hg^{2+} [5]. In view of the fact that numerous synergistic and antagonistic interactions involving the essential ultratrace element Se are known [5] and given that many other interactions between toxic and essential ultratrace elements are likely to exist, the elucidation of the underlying molecular mechanisms will provide exciting new insights into mammalian detoxification mechanisms, which – if overwhelmed – will result in chronic toxicity. The basic tenet of this review is that the molecular basis for the chronic toxicity of metals and metalloid compounds cannot be elucidated based on the reductionistic approach alone (e.g. to study the effect of a toxic metal in cell culture; Fig. 2, mechanism 1). Instead, the constant flux of essential *and* toxic elements through whole mammalian organisms must be taken into account and can provide important new insights into mammalian detoxification mechanisms (Fig. 2, mechanism 2). Following R.J.P. Williams advice that "Living organisms cannot be understood by studying extracted (dead) molecules. We have to study flow systems" [5] the emerging new science of environmental bioinorganic chemistry is destined to uncover the inorganic basis of chronic human diseases, such as cancer and neurodegenerative diseases, since ultimately every toxicological phenomenon rests on a chemical foundation.

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