1. Introduction

1.1 Trace elements

Living organisms are exposed every day to tremendous amounts of substances in our environment, which are widely distributed in the air, water and soil. The regulation of the mineral balance in the body is essential to survival. These substances include major and trace elements that may or may not be essential for sustaining life. Major elements reported to be essential are carbon, hydrogen, oxygen, nitrogen, sulfur, calcium, phosphor, potassium, sodium, chlorine and magnesium. Trace elements reported to be essential are iron, zinc, copper, manganese, iodine, selenium, cobalt, chromium. Other elements are not known to be essential but are constantly found in living tissue (these include aluminum, antimony, cadmium, arsenic, mercury, rubidium, fluorine, vanadium, silver and lead) . Their levels in the body appear to parallel environmental exposure. Of these elements that have no known nutritional value, some have been found to be toxic at concentrations well below those of other nonessential elements. Lead, cadmium and mercury are examples of elements that are toxic when present at relatively low levels [1]. However, even the essential metals can have harmful effects depending on their concentration and their chemical forms. The specific chemical form of a given element influences strongly its intake, accumulation, transport, storage and activity in the living organisms. To ensure that essential metals are delivered to the right tissues, cells or cellular compartments at the right concentration at the right time, living organisms have developed sophisticated mechanisms for transporting, storing and discarding metals. These mechanisms are collectively known as trafficking, the process of moving metals about to meet the biochemical need of an organism. A number of proteins are involved in transporting and storing metals and in transforming them into forms that are useful to the body or forms that can be eliminated from the body.

Trace elements have an important biochemical and physiological roles in the body, which are based on their concentrations and chemical speciation in the tissues. Some proteins contain metals and metalloids and it is well established that their biological activity depend on the presence of these elements. Trace elements play a part in the synthesis and structural stabilization of both proteins and nucleic acids. They are also constituents of hormones. In addition, they are involved in the function of subcellular systems such as mitochondria, as well as in membrane transport, nerve conduction, and muscle contraction. Some of them (Cu, Zn, Mn, and Se) act as antioxidants.

1.2 Selenium

Selenium was discovered by Jöns Jacob Berzelius in 1817, who found it in a Swedish factory as an impurity in the waste from sulfuric acid. He named it after Selene, the Greek goddess of the moon. Selenium is a naturally occurring element that is distributed widely in nature in most rocks and soils and is usually combined with sulfur in silver, copper, lead, and nickel minerals. Selenium is found in the VI group of the periodic table. It is an element with an atomic number of 34 and an atomic weight of 78.96 g/mol. It is a non-metal (metalloid) that is chemically related to sulfur and tellurium. In compounds its most common valence states are 6, 4, 2, and -2. The element is a member of the sulfur family and resembles sulfur both in its various forms and in its compounds [2]. Selenium exists in several allotropic forms. It can be prepared either as an amorphous or a crystalline form. The color of amorphous selenium is either red, in powder form, or black, in vitreous form. Crystalline monoclinic selenium is deep red; crystalline hexagonal selenium, the most stable variety, is metallic grey. Natural selenium has six stable isotopes: ^{74} Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se and ^{82}Se. Fifteen other isotopes have been characterized.

There are several selenium ores, but most of the selenium is produced as a byproduct of copper refining. It also accumulates in the residues from sulfuric acid production.

Selenium is used in the electronics industry (photo cells and solar cells), in the glass industry, and as a component of pigments in plastics, paints, enamels, inks, and rubber. It is used to remove color from glass, as it will counteract the green color of ferrous impurities and in the preparation of pharmaceuticals. It also finds application in photocopying and as a nutritional supplement. Radioactive selenium is used in diagnostic medicine.

1.2.1 Biological role of selenium

Selenium is essential to mammals and higher plants, but only in very small amounts. The essentiality of selenium for animals was first reported by Schwarz and Foltz in 1957. It was found that selenium administered to vitamin E-deficient rats prevented liver necrosis [3]. Subsequently, it was recognized that certain livestock diseases, like: white muscle disease in lambs and cattle, mulberry heart in pigs, muscle dystrophy in horses, infertility in ewes and reproductive disorders in cattle were associated with low selenium intake. In 1973 it was discovered that selenium is an essential component of glutathione peroxidase, an enzyme which prevents free radical formation by breaking peroxide into alcohol and water [4, 5]. The comparison of the complete amino acid sequence of glutathione peroxidase with the cDNA of the mouse glutathione peroxidase gene helped to reveal that a TGA codon is responsible for

the insertion of the selenocysteine into amino acid chain of this enzyme [6]. At the same time it was shown that the TGA codon is also responsible for encoding selenocysteine in *E.coli* [7]. A series of studies was carried out to elucidate the mechanisms of the selenoprotein formation. In 1991 Böck and co-workers clarified the biosynthesis of bacterial selenoproteins and termed selenocysteine the 21st amino acid [8]. Further investigations have revealed the biosynthesis of the eukaryotic selenoproteins [9, 10]. Simultaneously research interest has been directed towards the identification of novel selenoproteins and their biological functions. Here the important findings were: the identification of selenium as a catalytically active component of the type I iodothyronine deiodinase [11, 12], the importance of selenocysteine in the biosynthesis of thioredoxin reductase [13], the indispensability of the phospholipid hydroperoxide glutathione peroxidase [14] and sperm nuclei glutathione peroxidase [15] for male fertility. Scanning of the nucleotide sequence databases for the elements necessary for the decoding of UGA as selenocysteine gave information on the existence of further mammalian selenoproteins [16, 17].

Selenium plays important or essential roles in many fields, including antioxidation, maintenance of the integrity of muscle cells and red blood cells, DNA-RNA synthesis, detoxification of poisonous metals, cellular respiration and energy transfer, production of sperm cells, fetal development, integrity of keratinous tissue (skin, hair, nails), pancreatic function, antibody synthesis, and the production of ubiquinones (substances believed to help to protect against infectious diseases and malignancies, inflammatory diseases, heart disease, and high blood pressure).

Human selenium deficiency occurs in the countries or regions, where the soil concentration of selenium is low (north and East Asia) [18]. There is evidence that selenium deficiency may contribute to the development of a form of heart disease, hypothyroidism, and a weakened immune system [19, 20]. Three specific human diseases have been associated with selenium deficiency:

- Keshan Disease, which results in an enlarged heart and poor heart function, occurs mainly in selenium - deficient children [21, 22]
- Kashin-Beck Disease, which results in osteoarthropathy
- Myxedematous Endemic Cretinism, which results in mental retardation.

There is also evidence that selenium deficiency does not usually cause illness by itself. Rather, it can make the body more susceptible to illnesses caused by other nutritional, biochemical or infectious stress (in the case of Keshan disease by the Coxsackie virus) [21].

High intake of selenium can result in a condition called selenosis [23]. Symptoms of selenosis include gastrointestinal upsets, hair loss, white blotchy nails, garlic breath odor, fatigue, irritability, and mild nerve damage [24].

1.2.2 Formation of selenocysteine-containing proteins

The mechanisms for encoding selenocysteine are very interesting. The genes for two Sec-containing proteins, glutathione peroxidase 1 (GPx1) in mammals [6, 25, 26] and formate dehydrogenase in *Escherichia coli* [7], were the first genes found to contain TGA in the open reading frame. The selenocysteine (Sec) is encoded by the UGA codon, which serves as both a termination and a Sec codon. Sec insertion requires a specific mRNA stem-loop structure which in bacteria is situated immediately downstream of the UGA codon and four unique gene product: SelA, SelB, SelC and SelD. SelB is an elongation factor, like the better known EF-Tu. A protein SelB forms a complex with tRNA carrying Sec. This complex binds to the stem loop of the selenoprotein mRNA and mediates the incorporation of Sec into the protein. The Sec-bound tRNA (SelC) is delivered by SelB directly to the UGA codon, which is recognized by the anticodon of this specific tRNA. Sec tRNA is the only known tRNA that governs the expression of the selenoproteins. Sec is the only amino acid that is synthesized on its tRNA [27, 28]. Sec $tRNA^{[Ser]Sec}$ is initially aminoacylated with serine in both prokaryotes [27] and eukaryotes [28], and serine provides the carbon skeleton for the Sec synthesis [27, 28, 29]. The biosynthesis of Sec from serine on Sec $tRNA^{[Ser]Sec}$ has been completely characterized for *E. coli* [27,8]. In *E. coli*, a pyridoxal phosphate-dependent Sec synthase (SelA) catalyses the replacement of the side-chain oxygen of serine by selenium and thus the conversion of Seryl- $tRNA^{[Ser]Sec}$ into Sec-$tRNA^{[Ser]Sec}$[27, 8]. In prokaryotes selenophosphate, which is synthesized from selenide and ATP by selenophosphate synthetase (SelD), was identified as selenium donor [30]. Two selenophosphate synthetase genes in mammals (designated *Sps1* and *Sps2*) have been identified [31, 32, 33]. It is likely that in eukaryotes the selenium donor has the same form [31, 32, 33].

In mammals the signal redefining UGA, called SECIS (selenocysteine insertion sequence) [9], has a more complicated stem loop structure and the functions of SelB are divided into two proteins called: SBP2 (SECIS-binding protein 2) [34, 35, 36], and eEFsec (the Sec-specific elongation factor also called mSelB) [37, 38]. SECIS is located in the 3’ untranslated region of all eukaryotic selenoproteins genes and not adjacent to the UGA codon like in the prokaryotes [39]. Eukaryotic SECIS elements are composed of:

- two helixes separated by an internal loop

- SECIS core structure - Quartet, located at the base of helix 2;
- apical loop.

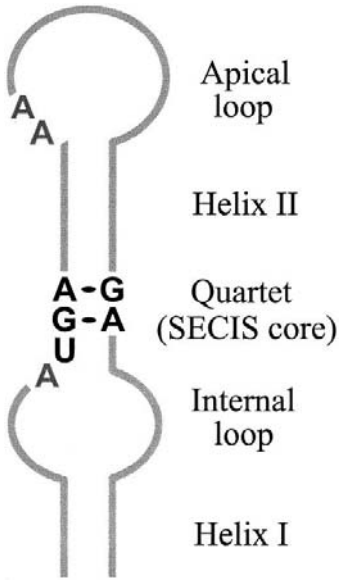


Figure 1-1 SECIS element consensus structure.

The Quartet is formed by four non-Watson-Crick interacting base pairs and is the main functional site of the stem-loop structure (see Figure 1-1) [40]. SBP2 forms with the SECIS element a tight SECIS-SBP2 complex [34, 35, 36] but it does not have the task of binding to the Sec $tRNA^{[Ser]Sec}$. SBP2 binds EFsec, which in turn recruits Sec $tRNA^{[Ser]Sec}$ and inserts Sec into nascent polypeptides in response to UGA codons [37, 38]. EFsec is specific for Sec and is different from EF1A, which is involved in the insertion of the other 20 amino acids. The SECIS element in the form of a complex with two proteins, SBP2 and EFsec, can recruit Sec-carrying tRNAs, as is schematically shown in Figure 1-2.

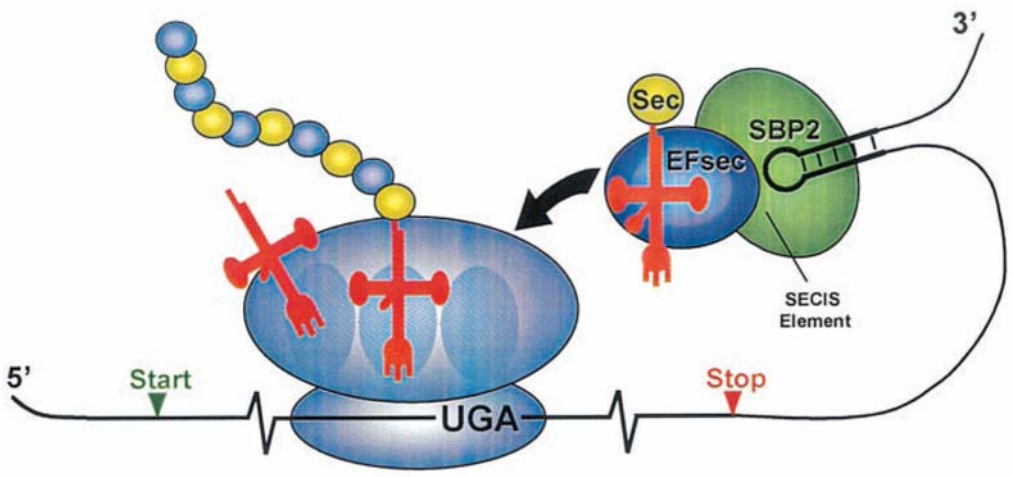


Figure 1-2 The suggested mechanism of Sec insertion in eukaryotes [41]. Selenocysteyl-tRNA (in orange with Sec in yellow) is shown in a complex with EFsec (in blue) and SBP2 (in green) and the SECIS element (shown as a hairpin loop in black) that is ready for donation to the ribosomal A site to be decoded by UGA (shown in the selenoprotein mRNA in black). Once the Sec $tRNA^{[Ser]Sec}$ complex is donated to the A site, Sec $tRNA^{[Ser]Sec}$ is transferred to the peptidyl site and Sec is incorporated into the nascent selenopeptide. The growing selenopeptide is shown as alternating gold and blue balls attached to the tRNA in the peptidyl site. The mRNA (shown in black with its start and stop codons indicated) is attached to the smaller of the two ribosomal subunits, and the unacylated tRNA is shown leaving the ribosomal exit site.

1.2.3 Selenium-containing proteins in mammals

The number of selenium-containing proteins has increased during the recent years. There are more than 20 known selenium-containing proteins in mammals [41, 42, 43, 44], and it would seem very likely that several of these are mediators of health benefits of dietary selenium. In naturally occurring selenium-containing proteins selenium can be inserted cotranslationally as the amino acid selenocysteine (Sec). The posttranslationally (as a dissociable cofactor) [45] insertion of this element take place in the bacteria and plants.

The occurrence of this element in proteins is widespread throughout all major domains of life and is responsible for the majority of biological effects of selenium. It should be noted that selenium can also be incorporated nonspecifically into proteins [46]. This occurs when selenium replaces sulfur in the biosynthesis of cysteine or methionine and the resulting selenoamino acid (Sec or selenomethionine) is inserted in place of the natural amino acid. The known selenoproteins are shown in the Table 1-1.

Table 1-1. Survey of all known mammalian selenoproteins

Selenoproteins	Tissue distribution	Subcellular distribution	Function	References
Cytosolic Glutathione peroxidase (Gpx1)	ubiquitous	cytosolic	antioxidant	[5, 6]
Gastrointestinal Glutathione peroxidase (Gpx2)	gastrointestine	cytosolic	antioxidant?	[47]
Plasma Glutathione hydroperoxidase (Gpx3)	kidney, plasma	extracellular	plasma antioxidant	[48]
Phospholipid hydroperoxide glutathione (Gpx4)	various tissues	cytosol and membrane associated	intracellular antioxidant	[49]
Sperm nuclei glutathione peroxidase (snGpx)	testis, spermatozoa	nucleus	protamine condensation, antioxidant	[15]
Thioredoxin reductase 1 (TRx1)	various tissues	cytosolic, mitochondria	multiple roles, redox control	[13]
Thioredoxin reductase 2 (TRx2)	various tissues	mitochondria	multiple roles	[50, 51]
Thioredoxin reductase 3 (TRx3)	various tissues	cytosolic	multiple roles	[52]
Thyroid hormone deiodinase 1	thyroid, liver, kidney, pituitary	membrane associated	catalyses the deiodination of T4 to T3; bioactivation	[11, 12]
Thyroid hormone deiodinase 2	brain, brown adipose tissue, pituitary, placenta	membrane associated	catalyses the deiodination of T4 to T3; bioactivation	[53, 54, 55]
Thyroid hormone deiodinase 3	CNS, placenta, skin	membrane associated	catalyses the deiodination of T4 to reverse T3 and of T3 to T2, inactivation	[53, 55]
Selenophosphate synthetase 2 (SPS2)	highest in the liver and testis	cytosolic	catalyses the production of selenophosphate	[56]
15 kDa Selenoprotein (Sel15)	Various tissues	cytosolic	unknown	[57, 58]
18 kDa Selenoprotein (Sel18)	various tissues	membrane associated	unknown	[42]
Selenoprotein M (SelM)	various tissues	perinuclear	unknown	[59]
Selenoprotein N (SelN)	skeletal muscle, liver, brain, heart, stomach	membrane associated	cell proliferation and regeneration?	[17, 60, 61]
Selenoprotein P (SelP)	liver, ubiquitous	secreted protein, binds to heparin	Se transport, antioxidant?	[62, 63]
Selenoprotein R (SelR, SelX)	various tissues	cytosolic	R-methionine sulfoxide reductase	[64]
Selenoprotein W (SelW)	skeletal muscle, heart, brain, testis, spleen	cytosolic	antioxidant	[65]
Selenoprotein H	various tissues	cytosolic	unknown	[66]
Selenoprotein O	various tissues	cytosolic	unknown	[66]
Selenoprotein I	various tissues	cytosolic	hypothetical CDPalcohol transferase	[66]
Selenoprotein K	various tissues	plasma membrane	unknown	[66]
Selenoprotein S	various tissues	plasma membrane	unknown	[66]
Selenoprotein V	only testis	cytosolic	unknown	[66]

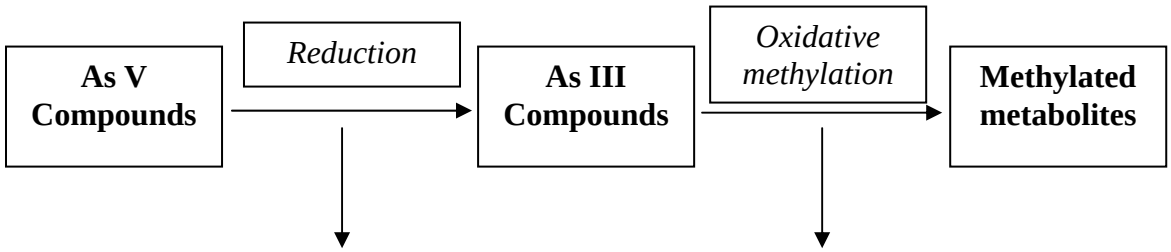
1.3 Arsenic

Arsenic (Greek Arsenikon) is a metalloid with an atomic number of 33 and an atomic weight of 74.92 g/mol. It belongs to the nitrogen group of elements (group V in the periodic table). It is believed that Albertus Magnus detected the element in 1250 A.D. In 1649 Schroeder published two methods of preparing the element. Arsenic is a naturally occurring element widely distributed in the earth's crust. It may exist in two solid modifications: yellow, and grey or metallic It is found in nature in a number of minerals including realgar (As_4S_4), orpiment (As_2S_3), arsenolite (As_2O_3), and iron minerals such as arsenopyrite (FeAsS) and loellingite ($FeAs_2$). In soils arsenic is present in two valence states: +3 as arsenite and +5 as arsenate [67]. Industrial and agricultural sources of arsenic may enhance, sometimes in a dramatic way, the natural levels of arsenic (mining activities, smelters, coal and coal combustion by-products, withdrawal sludges, pesticides) [67].

Arsenic salts are used as pesticides, wood preservatives, in glass manufacturing, in alloys, electronics, paint pigment and in the manufacture of dyestuffs. Arsenic preparations are no longer recommended and are rarely used for medical purposes. Some homeopathic preparations (arsenicum album: As_2O_3) [68] or "natural" remedies or preparations (Asian herbal remedies: e.g. herbal tea for example) [69] may contain different arsenic compounds. Arsenic-containing drugs may be applied to treat leukaemia. They were used more widely before antibiotics were discovered and produced, and before the toxic effects of arsenic at lower than lethal doses were known. For a while, Fowler’s solution (which contains arsenic) was used to treat everything from skin problems to arthritis to bacterial infections.

1.3.1 Metabolism of arsenate and arsenite

Studies of the arsenic metabolism in humans primarily relied on the characterization of arsenic metabolites in the faeces and urine following intake of various forms of arsenic. Those studies suggested that two processes are involved: reduction/oxidation reactions that interconvert arsenate and arsenite, and methylation reactions that convert arsenite to monomethyl arsonic acid (MMA) and dimethyl arsenic acid (DMA). Reduction of arsenate to arsenite is necessary before methylation can occur and it may happen in two ways: (1) enzymatically (purine nucleoside phosphorylase converts inorganic AsV to inorganic AsIII, glutathione-S-transferase converts MMAsV to MMAsIII), and (2) non-enzymatically by glutathione (GSH) [76, 77]. The methylation of trivalent arsenicals is an enzymatic transfer of the methyl group from S-adenosylmethionine (SAM) catalyzed by As(III)-methyltransferases.



1. Reduction-oxidation **AsO_4^{3-} + 2e → AsO_3^{3-}** *iAsV* *iAsIII*	*2. Oxidative methylation* **AsO_3^{3-} + SAM → $CH_3AsO(OH)_2$** *iAsIII* *MMAsV*
3. Reduction-oxidation **$CH_3AsO(OH)_2$ + 2e → $CH_3As(OH)_2$** *MMAsV* *MMAsIII*	*4. Oxidative methylation* **$CH_3As(OH)_2$ + SAM → $(CH_3)_2AsO(OH)$** *MMAsIII* *DMAsV*
5. Reduction-oxidation **$(CH_3)_2AsO(OH)$ + 2e → $(CH3)_2As(OH)$** *DMAsV* *DMAsIII*	

Figure 1-3 Arsenic metabolism by mammalian methylators.

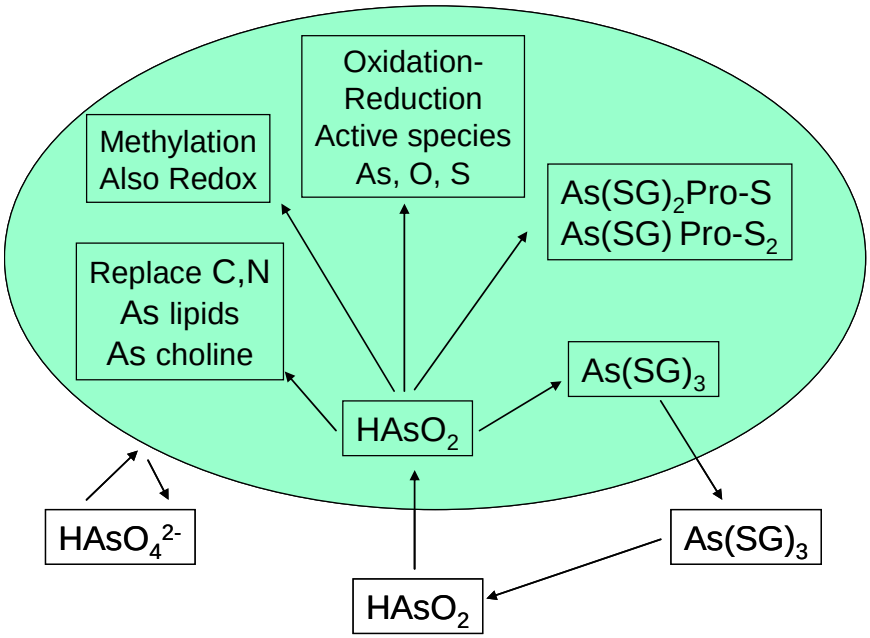


Figure 1-4 The disposition of As III in the cell: Arsenic (III) reacts using three mechanisms: binding to RSH, transferring electron oxidation-reduction reactions and substitution for carbon.

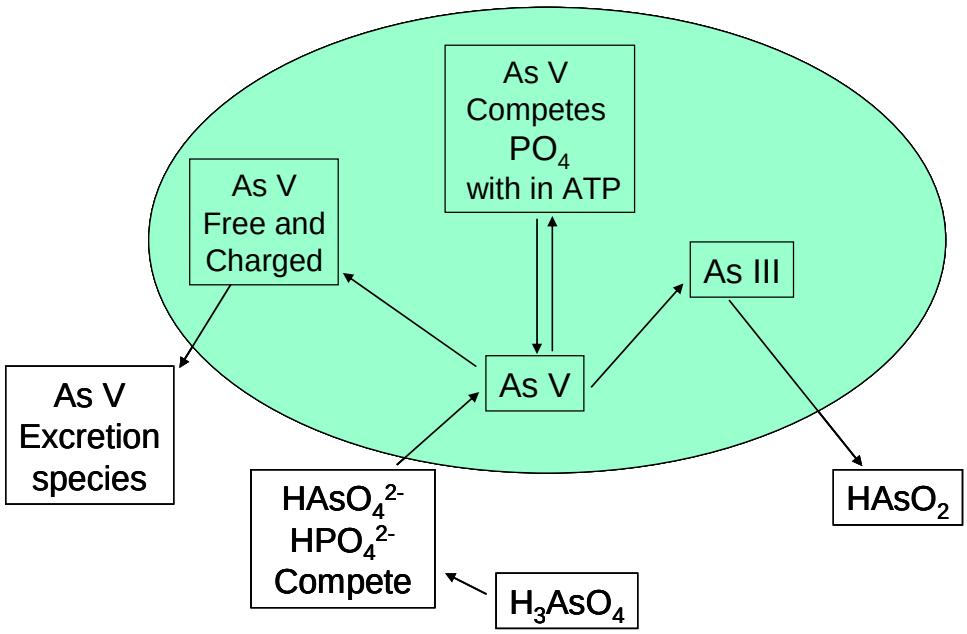


Figure 1-5 The disposition of As V in the cell: the As (V) is the charged salt of arsenic acid and it has to be transported across the cell membrane. It has a very limited range of actions because it binds like phosphate are not stable. As V can be reduced to As III and it can undergo the intracellular As III reactions.

In the previous reviews those methylation reactions were generally considered to be a protective mechanism against the acute toxicity of inorganic arsenic- a critical detoxification step. Styblo et al. characterized the role of methylation in the protection against toxicity of arsenicals. Among the arsenicals examined, trivalent monomethylated species were the most cytotoxic in all cell types. Pentavalent arsenicals were significantly less cytotoxic as their trivalent analogs [83, 84]. The cytotoxicities of trivalent arsenicals were similar in the cells with high, low or no methylation capacity, suggesting that methylation plays a minor role in protection of cells against acute toxic effects of those species [83]. Those results interpreted the methylation of arsenic as pathway for its activation, not as a mode of detoxification. Other mechanisms than methylation are likely to play a critical role in detoxification of inorganic arsenicals like e.g. binding of arsenicals to proteins [84, 85].

Once absorbed, arsenic is bound to hemoglobin, leucocytes, and plasma proteins. It is cleared from the intravascular space within 24 hours, and distributed in most tissues [70, 71].The ratio between red cell and plasma arsenic concentrations increases with the level of exposure: from 1/1 for low exposures to 3/1 for high environmental exposures [72]. Soluble inorganic

compounds, well absorbed by the gastro-intestinal tract or by the lungs, are rapidly distributed to organs or tissues rich in proteins containing sulfhydryl groups, and accumulate mainly in liver, kidney, spleen and adrenal gland [73]. In humans, not exposed occupationally or environmentally to arsenic compounds, arsenic binds to the sulfhydryl groups in keratin and can be detected in hair, nails, and skin 2 to 4 weeks after exposure. After 4 weeks, arsenic accumulates in the bone, coinciding with decreasing levels in the liver and kidneys [70, 71, 74]. Arsenic compounds can cross the placental barrier [75].

1.3.2 Arsenic toxicity

Among the chemical species of arsenic that are present in the environment, inorganic arsenicals are considered more hazardous for human health. Drinking water contaminated with inorganic arsenicals, along with industrial emissions, are the major sources of exposure for populations worldwide.

The toxicity of arsenic compounds is generally linked to the soluble inorganic trivalent forms. The toxicity of pentavalent inorganic compounds seems to be related to the in vivo reduction of As(V) to As(III) [78]. Many of the toxicological effects of inorganic arsenic, especially of the trivalent form, are believed to be associated with its reaction with cellular sulfhydryl (-SH) groups of cellular proteins, thereby inhibiting cellular oxidative processes (pyruvate and succinate oxidative pathways), and enzyme systems essential to cellular metabolism [71, 78, 79]. Pentavalent arsenic is capable of uncoupling mitochondrial oxidative phosphorylation. This effect may be due to a competitive substitution of arsenate for inorganic phosphate and the formation of an arsenate ester which is quickly hydrolyzed [78].

The diffuse toxic process of arsenic poisoning causes widespread endothelial cellular toxicity, resulting in capillary damage and tissue hypoxia precipitating generalized vasodilatation and transudation of plasma. Gastrointestinal, cardiac, renal, bone marrow, central nervous system, and hepatic damage may be noted at different stages of arsenic poisoning [70, 71, 74, 80, 81]. Exposure to arsenicals has been associated with cancer of the skin, lung, liver, kidney, urinary bladder and disorders of the circulatory system [82]. Because inorganic arsenicals have not been conclusively demonstrated to be carcinogens in other species, it has been speculated that their carcinogenic potency in humans is modulated by concurrent exposure to other agents that modify the risk of cancer [86]. For example, an interaction between occupational exposure to inorganic arsenicals and smoking in the risk of lung cancer induction has been described [87]. Thus inorganic arsenic does not act through classic genotoxic and mutagenic mechanisms, but might be a tumor promoter that modifies signal transduction pathways

involved in cell growth and proliferation [88]. It has been shown that trivalent inorganic arsenicals modulate expression and DNA-binding activities of several key transcription factors like nuclear factor kappa B [89], tumor suppressor 53 (p53) [90], and activating protein-1 (AP-1) [91].

1.4 Arsenic - selenium interactions

Arsenic and selenium are metalloids with very similar chemical properties but with distinctly different biological effects. Selenium is an essential element [92], known for its antitumorogenic properties [93, 94]. In contrast, arsenic is an environmental toxicant and a carcinogen in humans. Interestingly in humans and most animal species, inorganic selenium and arsenic undergo similar metabolic pathways. Inorganic forms of selenium (selenite and selenate) are reduced in the first step to selenodiglutathione with a help of glutathione and then converted to hydrogen selenide in the reaction catalyzed by glutathione reductase. Hydrogen selenide is an important metabolite which can be either a precursor for the synthesis of selenocysteine or for the methylated forms such as methylselenol, dimethylselenide and the trimethylselenonium cation [95]. Like selenium, inorganic arsenic compounds undergo reactions with oxidative methylation to yield the mono-, di-, trimethylated metabolites [96]. In this case glutathione is a donor of electrons for the reduction of pentavalent arsenicals [76]. This reduction can also be catalyzed by enzymes. There is one more common feature in the metabolisms of those metalloids. The methylation of both selenium and arsenic is catalyzed by specific methyltransferases that use S-adenosylmethionine as the methyl group donor [97, 98, 99].

Previous studies showed that inorganic arsenic protects experimental animals against the toxicity of selenium compounds [99]. Inorganic arsenic compounds, mostly trivalent interfere with the metabolism of selenium and inhibit the formation of hydrogen selenide [100] and the activity of selenium-methyltransferase [101]. Most of those arsenic selenium interaction studies were focused on the metabolism of selenium. Thus, there is not so much information about the effect of selenium on the metabolism and biological activities of arsenic. There is already some indication that selenium can interfere with processes involved in the detoxification of inorganic arsenicals. Styblo et al. examined the effects of prior or concurrent exposure to selenite on metabolic conversion and toxicity of arsenic in primary rat hepatocytes [102]. This work showed that a micromolar selenite concentration inhibited methylation of inorganic arsenicals and increased its cellular retention and cytotoxicity.

Regrettably, the significance of this observation for the metabolism and toxicity of inorganic arsenicals *in vivo* has not been investigated.

1.5 The respiratory system

The respiratory system includes two lungs and the passages by which their inertial cavities are connected to the exterior. These respiratory passages are the nasal cavities, the pharynx, the larynx, the trachea and the bronchi [103].

1.5.1 Trachea and bronchi

The trachea forms the main part of the windpipe. It lies ventral to the oesophagus and is supported by C-shaped bands of cartilage which keep it open against the pressure from the surrounding organs but allow the stretching as the bolus passes down the oesophagus during swallowing. The trachea extends into the thorax dorsal to the heart and divides into two bronchi, one to each lung. These are supported by complete rings of cartilage and branch to form the bronchial tubes within the lungs. The fines branches are called bronchioles. The trachea, the bronchi, the bronchial tubes and the bronchioles are all lined through with ciliated mucous membrane in which there are numerous goblet cells. The mucus produced by these cells is distributed and moves slowly away from the lung by cilia. This helps to remove any dust particles which may enter the respiratory passages and to prevent them from accumulating in the lungs [104].

The respiratory mucosa contains sparse glands and subepithelial lymph follicles. Within the pseudo stratified epithelium ciliated and noncilliated cells, intermediate, brush and basal cells can be found. Two types of migratory cells (globule leucocytes, lymphocytes) are regularly observed, especially in the epithelium of the trachea. The epithelium is significantly thicker in the upper than in the lower trachea [105].

1.5.2 The lung

The lungs lie in the thorax on either side of the heart. Each lung lies in a pleural cavity which is lined with serous membrane. This membrane forms the parietal pleura and the visceral pleura (covering the lung surface) [103].

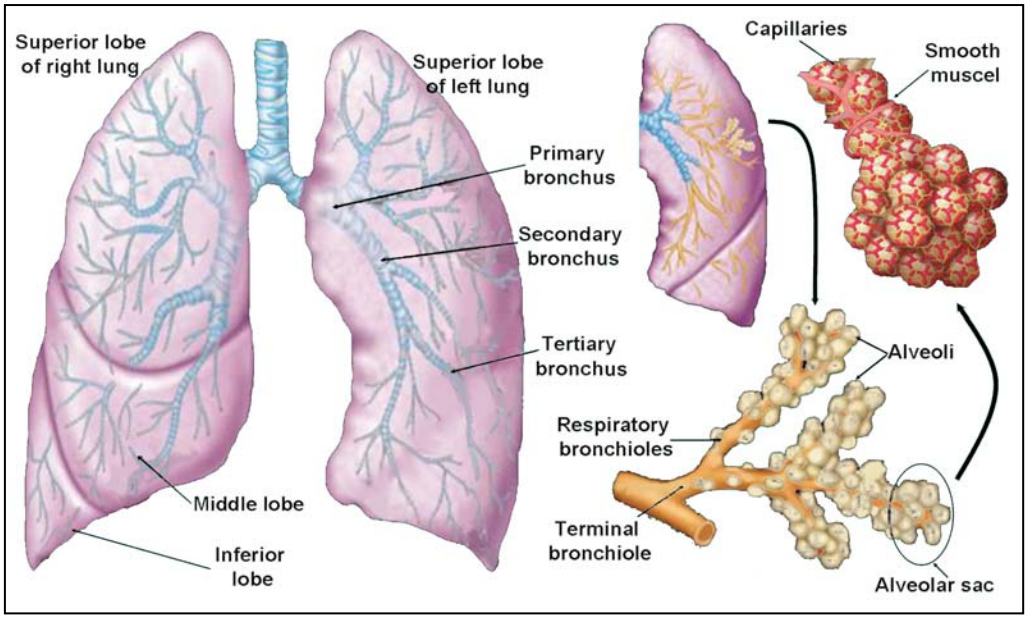


Figure 1-6 Anatomy of the lung and trachea

In the rat the right lung is divided into four lobes, but the left lung is undivided. The lungs of mammals are bright pink, spongy bags with internal cavities greatly subdivided to produce numerous air sacs, on the walls of which are microscopic pockets called alveoli. The air sac communicates with the bronchioles, via the main respiratory passages, with the outside air. The air sacs are bound together by loose connective tissue in which there are numerous blood vessels. The capillary blood vessels lie very close to the walls of the alveoli to facilitate gaseous exchange between the air and the blood.

Down to the level of the bronchioles the air conducting passages are lined with simple cuboidal ciliated epithelium. In the bronchioles the cilia are often absent. Goblet cells are found mainly in the larger bronchi. Pale columnar cells within the intrapulmonary airway epithelium were identified as neuroepithelial units. Subjacent to the epithelium lies a thin layer of connective tissue followed by a muscular layer [106].