REVIEW

Vanadium Detoxification: Chemical and Biochemical Aspects

by **Enrique J. Baran**

Centro de Química Inorgánica (CEQUINOR/CONICET, UNLP), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, C. Correo 962, 1900-La Plata, Argentina (e-mail: baran@quimica.unlp.edu.ar)

1. Introduction. – The biological effects, biodistribution, pharmacological activity, and toxicology of vanadium are areas of increasing research interest. Although numerous biochemical and physiological functions have been suggested for this element, and despite the amount of the knowledge so far accumulated, vanadium still does not have a clearly defined role in the higher forms of life [1–5].

So far, the best evidence for a biological role of vanadium comes from bacteria (the so-called alternative nitrogenases in which vanadium replaces molybdenum in the FeMo-cofactor of some *Azotobacter* species) [3][4][6–9] and from plants (vanadium-dependent haloperoxidases found in some algae, lichens and fungi) [3][4][8–10]. On the other hand, experiments with laboratory animals have shown that vanadium deprivation enhances abortion rates, reduces milk levels during lactation, and produces thyroidal disorders. It has also been suggested that vanadium participates in the regulation of ATP-ases, phosphoryl transferases, adenylate cyclase, and protein kinases and potentiate different growth factors [5][9][11][12].

Environmental contamination by vanadium has dramatically increased during the last decades, especially in the most developed countries, due to the widespread use of fossil fuels, many of which liberate finely particulate V_2O_5 to the atmosphere during combustion [13–15]. Therefore, and also owing to the emerging interest in the pharmacological effects of some of its compounds [16–20], the toxicology and detoxification of vanadium constitute areas of increasing research interest. The older literature about vanadium toxicology has been reviewed in the classical work of *Faulkner-Hudson* [21], and we have analyzed the most relevant aspects of its detoxification some years ago [22]. The pertinent information is extended and updated in the present review.

Vanadium toxicity has been reported in experimental animals and in humans. The degree of toxicity depends on the route of incorporation, valence, and chemical form of the element, and is also, to some extent, species-dependent [21][22]. In general, it increases as valence increases, pentavalent vanadium being the most toxic [22][23]. Although, under normal natural conditions, toxic effects do not occur frequently, at high doses or as a consequence of chronic exposure, it is a relatively toxic element for humans [24].

The upper respiratory tract is the main target in occupational exposure. Vanadium compounds, especially V_2O_5, are strong irritants of the airways and eyes. Acute and

chronic exposure gives rise to conjunctivitis, rhinitis, and to bronchitis, bronchospasms, and asthma-like diseases in more severe cases [21][22]. It can also produce fatigue, cardiac palpitation, gastrointestinal distress, kidney damage, and even neurological disorders. In human, acute toxicity has been observed in vanadium miners and industrial workers exposed to high doses of vanadium. The classic symptoms of this malady, referred to as 'green tongue' syndrome, are a green coloration of the tongue, accompanied by some of the above-mentioned disorders [5][21][22].

2. Detoxification Mechanisms. – All living organisms have developed defense mechanisms to deal with the reactive and potentially harmful by-products generated during cellular metabolism and to control the effects of exogenous substances that eventually invade the organism. This is called biological detoxification.

On the other hand, a series of drugs that are capable of chelating metal ions *in vivo* have been developed not only to eliminate excess of essential metals but also to prevent possible damage caused by nonessential, toxic elements. This is the basis of the so-called chelation therapies and constitute the chemical detoxification ways [25].

To understand how these mechanisms work in the case of vanadium, it is useful to give first a brief insight into the metabolism of this element in the higher forms of life.

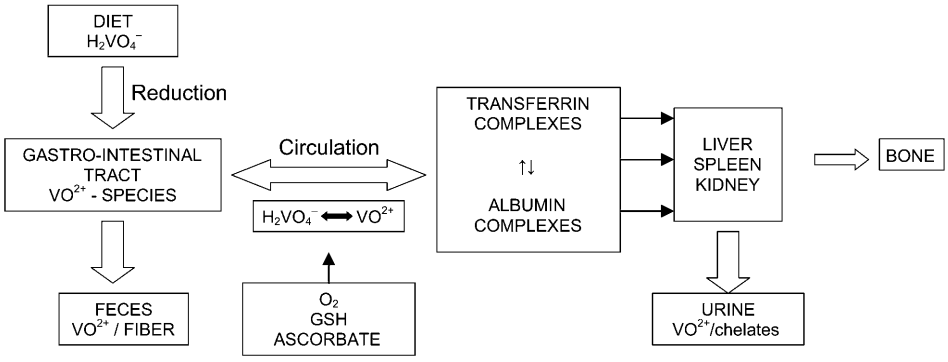
3. Vanadium Metabolism. – Although information about the metabolism of physiological amounts of vanadium in the higher forms of life remains scarce, an increasing amount of data has been accumulated during recent years, mainly from animal studies. Therefore, general aspects related to the absorption, transport, biological transformations, toxicity, and excretion of vanadium become understandable [5][24][26][27].

A summary of this knowledge is presented schematically in *Scheme 1* and briefly commented, as follows:

- Dietary vanadium occurs mainly in the anionic $H_2VO_4^-$ form and enters cells probably through the phosphate-transport mechanism. Most of the ingested vanadium(V) undergoes a rapid one-electron reduction in the gastrointestinal tract, generating oxovanadium(IV), VO^{2+}.
- Most of the ingested and reduced vanadium remains unabsorbed and is rapidly excreted by the fecal route. Strong association between VO^{2+} and dietary fiber is postulated.
- *In vivo*, all the vanadium is converted to a common form. The organ distribution is essentially independent of the oxidation state and the chemical nature of the originally administered form of the element. Endogenous reducing agents and dissolved oxygen ensure that both vanadium(V) and vanadium(IV) species are present in serum.
- Certain experimental evidences point to a relation between vanadium and iron metabolism. It has been suggested that the iron-transport protein transferrin may be also involved in vanadium transport.
- Interactions between vanadium species and serum albumin are probable, but very little is known about these processes as well as on the nature of the generated complexes.

- The fact that bone seems to be the major sink for retained vanadium has been unambiguously demonstrated by numerous studies.
- Final excretion of the small fraction of ingested and not retained vanadium occurs mainly through urine, preferably in the form of low-molecular-weight VO^{2+} complexes. Biliar excretion seems to be a secondary route.

Scheme 1. *Schematic Representation of Vanadium Metabolism in the Higher Forms of Life*



During the last years, we performed a series of model studies related to different aspects of this metabolic pattern in order to clarify some of its most important aspects [5][27].

4. Biological Detoxification of Vanadium. – Some aspects of the biological detoxification processes can be clearly interpreted on the basis of the described metabolic pattern. For example, the accumulation of vanadium in bone is surely one useful and very efficient biological detoxification mechanism. The high skeletal retention of vanadate can probably be related to its rapid exchange with bone phosphate, surely facilitated by the strong structural analogies between vanadate and phosphate. This exchange has been investigated using calcium hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ as a model for the inorganic phase of bone (*Eqn. 1*).

$$Ca_{10}(PO_4)_6(OH)_2 + n\ VO_4^{3-} \rightarrow Ca_{10}(PO_4)_{6-n}(VO_4)_n(OH)_2 + n\ PO_4^{3-} \quad (1)$$

It was found that the incorporation of moderate or low concentrations of vanadate in this lattice produces only very weak distortions at the macroscopic (crystallographic parameters, crystal ordering) and microscopic (local distortions, weakening of chemical bonds) levels of the apatite lattice [28]. Precipitation of calcium hydroxyapatite in the presence of the VO^{2+} cation [29] as well as interaction of apatite suspensions with this cation [30] demonstrated that VO^{2+} is not incorporated into the apatite lattice, but is strongly adsorbed onto its surface, suggesting that this process may be considered a second possible detoxification mechanism along with the participation of bone.

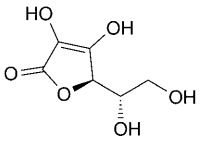
We have also investigated different reductive and complexation reactions, which may be involved in the biological detoxification of vanadium. For example, reduced glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH) is an important reducing agent of

vanadium(V) to vanadium(IV) (*cf. Eqn. 2*), and we could demonstrate that the generated oxovanadium(IV) cation interacts strongly with an excess of this reducing agent, generating different type of complexes, of which stoichiometry and structure is strongly dependent of the initial metal-to-ligand ratio, and the pH value of the reaction media [31–33]. This cation also interacts with the oxidized form of glutathione (GSSG) [34][35] which, in the pH range of 6–7, is even a more efficient VO^{2+} binder than GSH [33]. These results confirm that both GSH and GSSG may participate in the stabilization and transport of oxovanadium(IV) immediately after the GSH-mediated reduction of vanadate(V) in cellular systems [5][26][33].

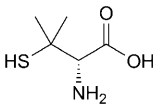
$$2\ VO_3^- + 2\ GSH + 6\ H^+ \rightarrow 2\ VO^{2+} + GSSG + 4\ H_2O \qquad (2)$$

Besides, the sulfur-containing amino acid L-cysteine is another potential reducing agent for vanadate in biological systems. Model studies in the VO_3^-/cysteine system show that vanadate is rapidly reduced, irrespective of the pH of the solution, followed by the formation of a purple 2 : 1 ligand-to-metal species. In this complex, the generated VO^{2+} cation interacts with an excess of L-cysteine through the amino N-atom and the deprotonated SH group of two amino acid molecules [36]. Also in this case, VO^{2+} can interact with cystine, the oxidation product of the amino acid, and coordination apparently occurs through the carboxylate and amino groups [37].

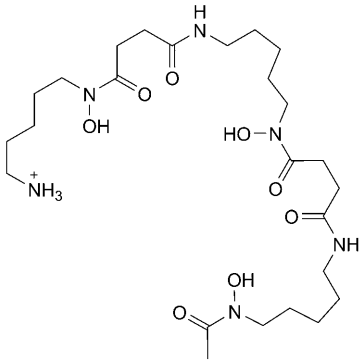
Ascorbic acid (vitamin C; **1**) is also a possible natural reducing and complexing agent for vanadium, which shall be discussed in greater detail in the next section. On the other hand, sugars and polysaccharides also play in general an important role in these biological processes, since they can interact with metal cations acting either as reductants and/or chelators [27][38]. This field of vanadium biochemistry has been reviewed recently [39] and, thus, shall not be discussed here more deeply. Finally, interaction of VO^{2+} with phosphate groups, which are also common ligands in biological systems, can also be considered as a possible detoxification route for reduced forms of vanadium. Some phosphate complexes of oxovanadium(IV) are very stable and have been well characterized [40–42]. Also phytic acid (*myo*-inositol hexaphos-



Ascorbic acid (**1**)



D-Penicillamine (**2**)



Desferrioxamine B (**3**)

phate), which forms both soluble and insoluble complexes with the VO^{2+} cation, should be considered in this context [43].

5. Chemical Detoxification of Vanadium. – Chelation therapy occupies a central place in modern medicine and pharmacology as extensive clinical experience, and continuous studies with laboratory animals demonstrate that acute or chronic human intoxications with a variety of metals can be considerably improved by administration of a suitable chelating agent [25][44–47]. Chelating agents usually diminishes metal toxicity by complexation of the toxic species, and subsequent excretion of the generated complex or preventing the absorption of the toxic species. On the other hand, important chelator characteristics are [25][46]:

- the chemical and biochemical stability, and the toxicity of the chelating agent and of the formed complex,
- side-effects generated by the chelator,
- lipophilicity/hydrophilicity of the chelating agent and of the resulting metal complex,
- stability of the generated complex (mainly determined by the hardness-softness character and the chelate effect),
- the possible route of excretion of the formed complexes.

In the case of vanadium, the biologically most relevant species, *i.e.*, V^{3+}, VO^{2+}, VO^{3+}, and VO_2^+, can be classified as hard acids [25][48]. Therefore, one may expect that the best chelating agents for these species are ligands that offer oxygen or nitrogen donors (hard bases in the HSAB classification).

Most of the so far known and well-established chelating agents employed in the clinical praxis have been tested, with varying success, for vanadium detoxification, generally with laboratory animal experiments [22]. Well-known chelating agents, such as ethylenediaminetetraacetic acid (EDTA), and related polyaminopolycarboxylic acids, have been profusely investigated. EDTA, in particular, shows a good chelating behavior for both vanadium(V) and vanadium(IV) species [22]. In the case of sulfur-containing chelating agents, such as 2,3-disulfanylpropanol (BAL), L-cysteine, or D-penicillamine (**2**), conflicting reports are found in the literature, concerning its efficacy in the case of acute intoxications [22]. Notwithstanding, for compound **2** , a good activity against both vanadium oxidation states has been reported [49], a fact that suggests that probably both its reducing power and its complexing capability play a role in its action.

In spite of the fact that phosphonic acids and related systems appear as suitable chelating agents for vanadium species [50] as predicted by the HSAB approach, these compounds have not been explored in detail up to now. The only system of this type so far investigated seems to be the calcium salt of (ethylenediamine)(tetramethylene)-phosphonic acid, which shows a high activity towards both vanadate(V) and oxovanadium(IV) [49].

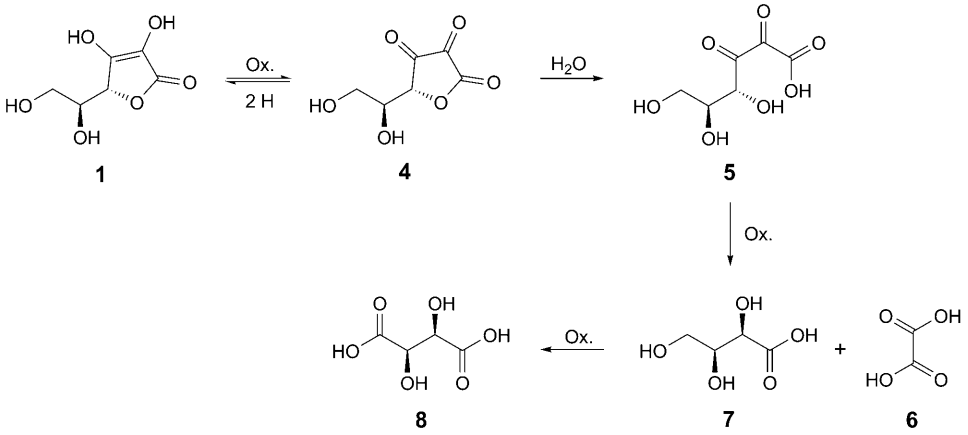
Desferrioxamine B (**3**), one of the best known siderophores and a widely used chelating agent for the treatment of iron overload conditions [25][44], merits a special comment, as it has also been shown to be a very effective antidote for vanadium poisoning, as demonstrated by animal studies [51]. It raises urinary and fecal vanadium

excretion and is effective in the removal of both vanadate and oxovanadium(IV) species [22].

From all the vanadium detoxification agents investigated so far, ascorbic acid (**1**) appears to be the most effective for human use, as shown by a systematic and comparative study of a great number of antidotes of very different chemical characteristics [49][52][53]. It is probably the least toxic of all the examined drugs and can be administered orally in relatively large doses. Its strong detoxification activity can surely by related to the facility with which it reduces vanadium(V) to VO^{2+} [54][55]. This cation could be then complexed by an excess of the vitamin but, at as it is known from the general behavior of metallic ascorbate complexes [54] and confirmed by detailed studies on the oxovanadium(IV)/ascorbate system [56], the stability constants of these complexes are relatively low, which, in the case of VO^{2+} species, is also in agreement with the absence of chelate binding [56]. This fact suggests that this type of complexes would not be useful for the stabilization and excretion of reduced vanadium. Consequently, we have suggested that a better way for the elimination of the generated oxovanadium(IV) may be its complexation with some of the oxidation products of the vitamin.

As it is known [39][56][57], dehydroascorbic acid (**4**), generated as the primary oxidation product, is also very unstable and undergoes a rapid series of transformations, as shown schematically in *Scheme 2*. It is degraded first to 2,3-dioxogulonic acid (**5**), which can further be degraded to a mixture of oxalic acid (**6**) and L-threonic acid (**7**). At higher pH values, the latter acid is oxidized to tartaric acid (**8**). Although all these species could interact with the VO^{2+} cation, a thorough investigation of these ligand systems showed that the primary complex generated by interaction of the oxocation with dehydroascorbic acid (**4**) is very unstable towards oxidation. It is hydrolyzed irreversibly with opening of the lactone ring generating 2,3-dioxogulonic acid (**5**), producing a 2:1 ligand-to-metal complex **9** [56], in which the enolized form of the mentioned acid acts a bidentate chelator of the cation (*Fig. 1*). This species seems to be very stable, as we could obtain different complexes of this type not only starting with

Scheme 2. *Schematic Representation of the Stepwise Oxidation of* L-*Ascorbic Acid* (**1**)



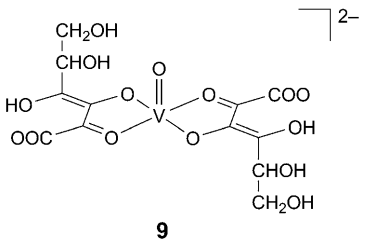


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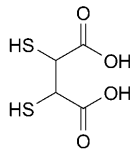
Fig. 1. *Proposed structural model for the complex anion* **9** *generated by interaction of* VO^{2+} *with 2,3-dioxogulonic acid* (**5**) (from [56])

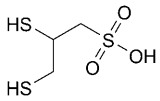
the system oxovanadium(IV)/dehydroascorbic acid (**4**) but also by direct reduction of vanadate with ascorbic acid (**1**) [58].

The results of these studies are also interesting in relation to different aspects related to vanadium excretion. In some recent studies, the low-molecular-weight vanadium species present in urine was identified as a vanadium/ascorbate complex [59][60]. But on the basis of the above mentioned results, it is most likely that the ligand may be any of the oxidation products of ascorbic acid (**1**), perhaps 2,3-dioxogulonic acid (**5**).

In recent years, two new and very promising chelating agents have been introduced into the medical praxis, they are *meso*-2,3-disulfanylsuccinic acid (DMSA; **10**) and 2,3-disulfanylpropane-1-sulfonic acid (DMPS; **11**) [45][46][61][62]. Both drugs, which are very stable, and show low toxicity and side effects, are available as tablets for oral administration [45][61–66].



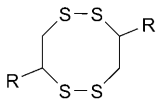
meso-2,3-Disulfanylsuccinic acid (DMSA, **10**)



2,3-Disulfanylpropane-1-sulfonic acid (DMPS, **11**)

In a search for new detoxification agents for vanadium, we have recently initiated some studies with these two chelating agents. We have found that both compounds are able to reduce rapidly vanadium(V) to oxovanadium(IV), which might be chelated by an excess of the acid [67][68].

In the case of DMPS (**11**), it is known that it is relatively rapidly oxidized to cyclic (*e.g.*, **12**) and acyclic polymeric sulfides (*e.g.*, **13**) [69–71] such as those shown in *Fig. 2*,



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$R = CH_2–SO_3^-$

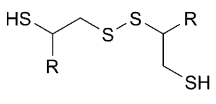

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Fig. 2. *Cyclic* (**12**) *and acyclic dimeric sulfides* (**13**), *some oxidation products of the interaction of vanadium(V) species with DMPS* (**11**)

and this are surely the reaction products of the interaction of vanadium(V) species with DMPS (**11**).

The interaction of VO^{2+} with DMSA (**10**) was thoroughly investigated by electron-absorption spectroscopy, in aqueous solution at different pH values. The spectral behavior, complemented with a spectrophotometric titration, shows the generation of a $[VO(DMSA)_2]^{2-}$ complex, in which the oxocation interacts with two pairs of deprotonated SH groups of the ligand [67]. In the case of DMPS (**11**), a similar complex of stoichiometry $[VO(DMPS)_2]^{4-}$ is generated [68]. Both agents are also able to produce the partial reduction of V_2O_5 suspensions at pH values between 5 and 7 [67][68]. The results of these studies clearly show that DMSA (**10**) and DMPS (**11**) appear as very interesting and promising agents for the detoxification of vanadium(V), whose merits are to be further explored, for example, with laboratory animals as next step.

It is a great pleasure to acknowledge the contributions of the colleagues and collaborators whose names appear in the references. Work from our laboratory reported here was supported by the *Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina* (*CONICET*) and by the *National University of La Plata*. The author is a member of the Research Career from CONICET.

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Received October 1, 2007