



Vanadium Biochemistry

Chapter 9

**DECAVANADATE TOXICITY EFFECTS FOLLOWING
in vivo ADMINISTRATION**

Sandra S. Soares¹, Carlos Gutiérrez-Merino² and Manuel Aureliano¹

1) Center for Marine Sciences (CCMAR) and Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; 2) Grupo de Bioenergética en Neuronas y Miocitos, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Extremadura, Av. Elvas s/n, 06071 Badajoz, Spain

Abstract

Very few in vivo animal studies involving vanadium consider the contribution of decavanadate (V₁₀) to vanadium biological effects. Recently, it is been suggested that decameric vanadate may not completely fall apart into other vanadate oligomers before induces changes in cell homeostasis, namely in several stress markers. An acute exposure of different fish species (Halobatrachus didactylus, Lusitanian toadfish, and Sparus aurata, gilthead seabream) to decavanadate, but not to other vanadate oligomers, induced different effects than vanadate in catalase activity, glutathione content, lipid peroxidation, mitochondrial superoxide anion production and vanadium accumulation, whereas both solutions seem to equally depress reactive oxygen species (ROS) production as well as total intracellular reducing power. Vanadium is accumulated in Sparus aurata mitochondria in particular when decavanadate is administrated. Moreover, exposure to different vanadate oligomers induced morphological changes in fish cardiac, hepatic and renal tissues causing tissues lesions in the liver and kidney, but not cardiac tissue. Nevertheless, the results highlight that different vanadate oligomers seem to follow, not only in vitro but also in vivo, different pathways, with different targets and effects. These recent findings, that are now summarized, point out the decameric vanadate species contributions to in vivo effects induced by vanadium in biological systems.

*Correspondence/Reprint request: Sandra S. Soares, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; e.mail: ssoares@ualg.pt

1. INTRODUCTION

Vanadium is a heavy metal with increased environmental circulation, resulting from various anthropogenic activities [1-3] and it is of great concern due to its toxicity and accumulative behaviour at specific target organs, such as the liver and kidney, inducing oxidative damage, lipid peroxidation and changes in haematological, reproductive and respiratory systems [4-9]. The formation of ROS induced by vanadium in biological systems may involve Fenton-like reactions [7], vanadate bioreduction mediated by reduced glutathione (GSH), flavoenzymes or nicotinamide adenine dinucleotide, reduced form (NADH), and nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), oxidases [10-12] or interaction with mitochondria [13].

Vanadate oligomers are often not taken in account in most biological studies, in spite that at the concentrations normally used the vanadate solutions also contains several vanadate oligomers besides the monomeric vanadate (V_1), such as dimeric (V_2) and tetrameric (V_4) vanadate species. Furthermore, it is recognized that the individual species may differently influence enzyme activities [14-19]. Decameric vanadate species (V_{10}), for instance, are well known to affect the activity of several enzymes [18-20]. The probably reason why *in vivo* toxicological studies of vanadate oligomers effects are scarce is that, it is considered that almost 98% of vanadium in cells is present as vanadyl (+4 oxidation state), being the intracellular concentration of vanadium (+5, vanadate) very low to vanadate oligomers be formed. However, it has been proposed that once formed the rate of decavanadate decomposition is slow (half-life time of hours) enough to allow to study its effects not only *in vitro*, but also *in vivo* [15-16, 21-28]. More recently, it was described that decameric vanadate can be stabilized upon interaction with cytoskeleton and membrane proteins [29]. It is believed that, due to its stability at physiologic conditions, decavanadate is not completely disintegrated into other vanadate oligomers before induces changes in several stress markers [24].

The pioneer studies on the interaction between decavanadate and proteins were performed with ribonucleases, in 1973 [30]. These and other studies demonstrated the decavanadate species as potentially responsible for the effects promoted in enzyme activities [14]. Since that, one thing is becoming clear, decavanadate seems to follow, not only *in vitro* but also *in vivo*, different pathways, with different targets and effects than other vanadate oligomers. The intracellular form of vanadium depends on the ratio of the whole pool of reductants to cell oxidants [31]. It is well known that, in the cell, vanadate is reduced to vanadyl, preventing the toxic effects of vanadium. However, vanadate reduction may not occur if the vanadate oligomers are present. Decameric species may eventually occur upon muscle cells acidification, becoming accessible to specific protein binding sites and inaccessible to reduction, thus inducing different cellular responses that the other vanadate species (Fig. 1). Therefore, a role of decameric vanadate species in the toxicological effects of vanadate in biological systems may be suggested.

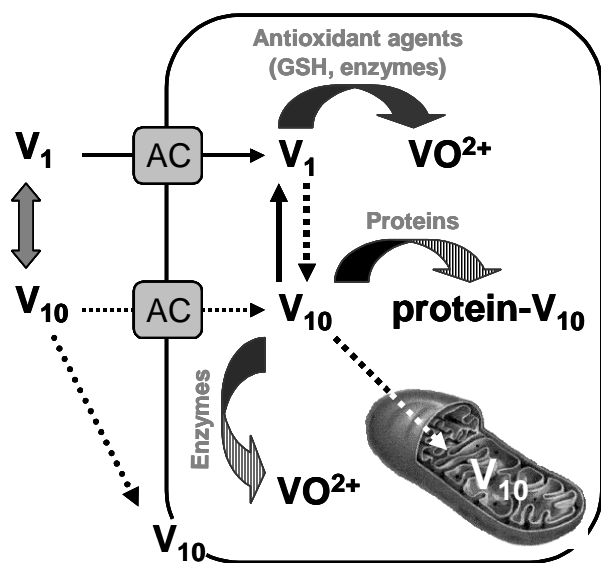


Figure 1. Scheme of the proposed cellular targets of decavanadate (V_{10}): it is suggested V_{10} uptake through anionic channels (AC) and/or V_{10} binding to membrane proteins, as well as, V_{10} formation upon intracellular acidification; it is also indicated the reduction of monomeric vanadate (V_1) by antioxidant agents and the reduction of V_{10} by enzymes, as well as, the binding of V_{10} to target proteins preventing its reduction; and finally it is proposed the accumulation of V_{10} into subcellular organelles, such as mitochondria (adapted from Aureliano and Gândara [24]).

It is becoming apparent the interest and usefulness of piscine models to oxidative stress studies [32], which are more exposed to environmental pollutants. In fact, several works have reported that some fish species are far more sensitive to heavy metals toxic effects than mammals. Fish species, which has been used as preferential experimental models in toxicology studies, are very easy to obtain, maintain and to handle. Therefore, and besides the interest of piscine models to oxidative stress studies, our knowledge show us to be very useful to study decavanadate toxicity in fish species, since at the fish physiological temperature decameric vanadate species is stable enough to induce different effects than vanadate itself.

Since 1999, our research group has performed *in vivo* administration of decavanadate in order to understand the contribution of decameric vanadate species to the toxic effects of vanadate [21-27]. Among the different experimental conditions, it was included different: (i) mode of decavanadate administration (intraperitoneal, i.p. versus intravenous, i.v.); (ii) fish species (*Halobatrachus didactylus* – Lusitanian toadfish – and *Sparus aurata* – gilthead seabream); (iii) vanadate concentration (1 and 5 mM); (iv) tissues (cardiac, hepatic, renal, blood); (v) subcellular fractions (cytosol, mitochondria, red blood cells, blood plasma); and (vi) exposure time (1, 6, 12, 24 hours, 2 and 7 days). A metavanadate solution, not containing decameric vanadate species, was always administered as a comparison group of study and, until now, following *in vivo* administration of decavanadate, several parameters were analysed such us: (i) vanadium subcellular distribution [21, 25-27]; (ii) histological changes on cardiac, hepatic and renal tissues [22]; (iii) effects on sarcoplasmic reticulum Ca^{2+} -pump; (iv) lipid peroxidation; and (v) antioxidants enzymes activities besides several oxidative stress markers in heart [21, 24, 27] and liver [24-25]. These previous reports indicated a different *in vivo* metabolic pattern for decameric vanadate species, pointing out the importance of vanadate speciation on the evaluation of vanadium toxicity in biology. Recently reported studies demonstrated that *in vivo* administration of different vanadate species induce different effects on vanadium subcellular distribution, tissue damage in liver, kidney and heart, lipid peroxidation and antioxidant enzymes [21-27].

Therefore, it cannot be excluded the hypothesis that decavanadate once formed at physiological conditions, it may live for long enough to induce different biological effects than the ones observed for vanadate alone. The role of decameric vanadate in biological systems is still to be clarified and to our knowledge, *in vivo* toxicological studies of these species are scarce. In this sense, in the present report it is presented a summarised review of the effects following decavanadate *in vivo* administration on: vanadium accumulation; methaemoglobin reductase and Ca^{2+} -pump activities; cardiac, hepatic and renal histology; and on overall oxidative stress markers such as: (i) reduced GSH content; (ii) overall rate of ROS production; (iii) mitochondrial superoxide anion radical ($\text{O}_2^{\cdot-}$) production and (iv) lipid peroxidation propagation.

2. STABILITY OF DECAMERIC VANADATE SPECIES

Only a few studies consider the hypothesis of a possible effect of decameric vanadate (V_{10}) at physiological conditions since, in these conditions, vanadate (+5 oxidation state) is considered to be almost totally reduced to vanadyl (+4) and decavanadate concentration is very low or is not present for long enough to induce any effects [33-36]. In order to correlate the effects observed *in vivo* with the decameric vanadate species present

in solution, it has been determined its stability in the injected solution, estimating the half-life time of decameric vanadate species on decavanadate solution.

2.1 Decameric species in vanadate solutions: NMR signals

Since most of the vanadate species can be detected by ^{51}V Nuclear Magnetic Resonance (RMN) spectroscopy, this technique has been often used to evaluate the compositions of vanadate solutions. As appraised by ^{51}V NMR spectroscopy, decavanadate stock solution pH 4.0, 50 mM in total vanadium, contains only decameric species since only signals from decameric vanadate species are observed: $\text{V}_{10\text{A}}$ at -515 ppm, $\text{V}_{10\text{B}}$ at -500 ppm and $\text{V}_{10\text{C}}$ at -424 ppm [27]. Conversely to decavanadate stock solution, that contains only decameric vanadate species, metavanadate stock solution contains ortho- and metavanadate species. However, after dilution in the injection medium containing 0.9% NaCl the NMR spectrum of the denominated decavanadate solution (containing presumably only decameric species) are also often contaminated with monomeric vanadate species, showing besides the decameric NMR signals, a signal at -562 ppm with a half-line broadening of 55 Hz (Fig. 2A). In fact, the spectra of decavanadate solution used in injections, 5 mM total vanadium in 0.9% NaCl at pH 7.0, contained mainly decameric species once the three signals from decameric vanadate species, ascribed to the three vanadium atoms of the decavanadate structure are identified [37]. On the other hand, in the metavanadate injected solution, 5 mM total vanadium concentration in 0.9% NaCl at pH 6.98, were detected monomeric (V_1), dimeric (V_2), tetrameric (V_4) and also pentameric (V_5) species, respectively, at -561 ppm, -575 ppm, -579 ppm and at -587 ppm, as described elsewhere [15] (Fig. 2).

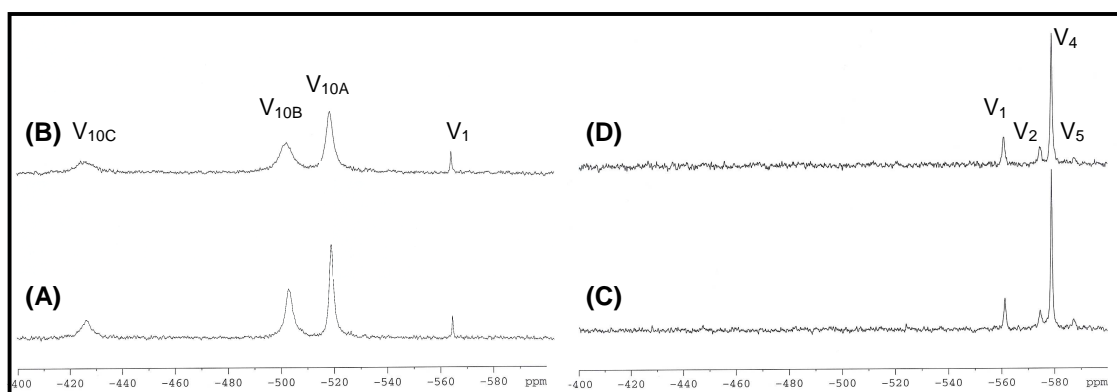


Figure 2. 105.2 MHz ^{51}V NMR spectra, at room temperature, of decavanadate, (pH 7.0) (A and B) and metavanadate (pH 7.0) (C and D) (5 mM total vanadium) in 0.9% NaCl, in the absence (A and C) or in the presence (B and D) of 5% blood plasma from *S. aurata*. All spectra were acquired in the presence of 10% D_2O . $\text{V}_{10\text{A}}$, $\text{V}_{10\text{B}}$ and $\text{V}_{10\text{C}}$ are signals of vanadium atoms from the decameric ($\text{V}_{10}\text{O}_{28}^{6-}$) species. V_1 and V_2 NMR signals correspond, respectively, to monomeric (VO_4^{3-} , HVO_4^{2-} and H_2VO_4^-) and dimeric ($\text{HV}_2\text{O}_7^{3-}$ and $\text{H}_2\text{V}_2\text{O}_7^{2-}$) vanadate regardless of the protonation state, whereas V_4 and V_5 correspond to cyclic tetrameric ($\text{V}_4\text{O}_{12}^{4-}$) and pentameric ($\text{V}_5\text{O}_{15}^{5-}$) vanadate species, respectively.

Vanadate studies in biological systems can be comparable to the iceberg phenomena: there is an invisible part, probably, not most interesting, but certainly the major

[24]. For this reason, it is of extreme importance to well characterize the vanadate species presence and the interactions with the system in study before attempting to understand the promoted effects. Therefore, the NMR studies must be performed in order to certify the presence of the vanadate species responsible for a specific effect. For instance, it was verified that in the presence of 5% blood plasma in the dilution medium, neither the concentration of the different vanadate species nor the ratio between vanadate oligomers in both solutions are drastically changed pointing out to vanadate/protein interactions (Table 1). Moreover, it is possible to deduce specific vanadate/protein interactions by analyzing the half-width broadening in the presence of the protein, as it is observed for the decameric signals that increase up to 1.5 (V_{10A}), at Table I, in the presence of blood plasma (B).

Furthermore, after dilution of the decavanadate solution into the injection medium (NaCl 0.9%), in the concentrations normally used in the present studies (1 and 5 mM in vanadium), the concentration of decameric vanadate increases linearly with total vanadate concentration whereas monomeric vanadate does not, as described recently [27, 29].

2.2. Decavanadate characterization and stability

UV/visible spectroscopic characterization of the injected vanadate solutions showed that the decavanadate solution present a characteristic spectrum (Fig. 3A) with visible absorbance up to 500 nm due to decameric vanadate species that absorbs at 360 and 400 nm, while metavanadate solutions spectra almost does not absorb at 400 nm.

Conversely to metavanadate, the decavanadate solution is unstable in the injection medium (NaCl 0.9%) as analysed by UV/visible spectroscopy [26]. In contrast to the labile oxovanadates present in the metavanadate solutions, which interconvert on the time scale of milliseconds to seconds, decameric vanadate has very slow decomposition kinetics upon dilution. Decameric vanadate present in decavanadate solutions decomposes into monomeric vanadate species (V_1) which solutions are colourless. The kinetics of decomposition of decameric species followed by UV/visible spectroscopy, at 25 °C, in the saline solution used for *in vivo* administration, assessed by measurements of the absorption at 400 nm has confirmed a first-order kinetic process, with an half-time of 16 hours (measured with 1 mM total vanadate concentration, i.e. 100 μ M decameric vanadate species) (Fig. 3B).

After this time, the NMR spectra contain not only decameric vanadate species but also the others species present in metavanadate solutions, in agreement to described elsewhere [15, 19, 24]. Therefore, this complex oxoanion remains relatively stable at room temperature, allowing studying its effects not only *in vitro* but also *in vivo*. Moreover, decameric vanadate is stabilized upon interaction with cytoskeletal and membrane-bound proteins [29].

At pH 7, decameric vanadate might not be present in solution depending on several parameters such as vanadium concentration, ionic strength or temperature [14]. However, once formed at pH 4 and upon the pH adjusted to a physiological value, it decomposes very slowly with a half-time that can reach up to 16 hours [21, 23, 27-28, 38]. This is an important feature, since decavanadate maintains its structure in an aqueous solution for a significant amount of time, allowing it to induce toxic effects in biological systems different from the ones promoted by vanadate itself.

Table 1. ^{51}V NMR spectral parameters, at room temperature, of the vanadate species present in decavanadate (pH 7.0) (A and B) and metavanadate (pH 7.0) (C and D) (5 mM total vanadium) in 0.9% NaCl, in the absence (A and C) or in the presence (B and D) of 5% fish blood plasma, namely chemical shift (δ), half line width ($\Delta\nu_{1/2}$) and concentration of the respective vanadate oligomeric species (C). The broadening factor of half line width (F) is defined as the coefficient between $\Delta\nu_{1/2}$ value in the absence and presence of 5% plasma. The concentration (C) of the vanadate species present in decavanadate and metavanadate solutions was determined from the fractions of the integrated areas observed in the corresponding spectrum (see Soares *et al.* [27]).

		V_1	V_2	V_4	V_5	V_{10A}	V_{10B}	V_{10C}
(A)	δ (ppm)	-561.7	-	-	-	-516.6	-500.8	-425.6
	$\Delta\nu_{1/2}$ (Hz)	55	-	-	-	206	318	393
	C (μM)	126	-	-	-	214	195	79
(B)	δ (ppm)	-561.1	-	-	-	-516.1	-500.0	-423.5
	$\Delta\nu_{1/2}$ (Hz)	55	-	-	-	318	581	807
	C (μM)	181	-	-	-	213	189	81
	F (relative to A)	(1.0)	-	-	-	(1.5)	(1.8)	(2.1)
(C)	δ (ppm)	-561.2	-574.5	-578.8	-587.2	-	-	-
	$\Delta\nu_{1/2}$ (Hz)	93	130	55	130	-	-	-
	C (μM)	443	389	903	33	-	-	-
(D)	δ (ppm)	-560.8	-574.4	-578.8	-587.4	-	-	-
	$\Delta\nu_{1/2}$ (Hz)	93	130	55	93	-	-	-
	C (μM)	878	332	865	nd	-	-	-
	F (relative to C)	(1.0)	(1.0)	(1.0)	(0.7)	-	-	-

$\Delta\nu_{1/2}$ values have a STD of ± 20 Hz; nd: not determined.

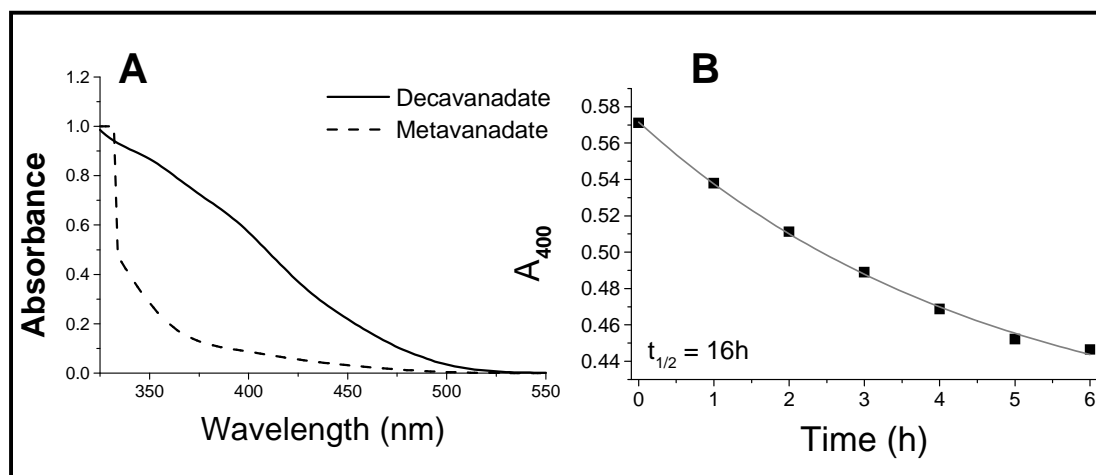


Figure 3. UV/visible spectra (A), at 25 °C, of decavanadate (solid) and metavanadate (dash) (1 mM total vanadium) solutions, in the injection dilution medium containing 0.9% NaCl, pH 8.0 and variation of the absorbance (B) of decavanadate solution (1 mM total vanadium) in 0.9% NaCl, at 400 nm, in function of time (adapted from Soares *et al.* [28]).

3. VANADIUM SUBCELLULAR DISTRIBUTION FOLLOWING DECAVANADATE ADMINISTRATION

The uptake of vanadium depends on the animal species and age, with the larger quantities of vanadium being found in bones, kidney, liver, spleen, plasma, muscle and fat, whereas the smallest amounts can be found in brain and other organs [39-41]. Toxicity studies with vanadium often disregard the contribution of vanadate oligomers, although recent studies demonstrated that the amount of vanadium found in blood plasma, red blood cells (RBC) and cardiac muscle subcellular fractions, after an *in vivo* acute exposure to sublethal vanadate concentrations, is affected upon decameric vanadate administration [21, 24-27]. Nevertheless in acute exposure experiments (from 1 hour to 7 days) following exposures to sublethal concentrations of both decavanadate or metavanadate (1 and 5 mM total vanadium), it is found that independently of the form of vanadium administered it can penetrate into internal organs of the fish *H. didactylus* and *S. aurata* with the following order of concentration: blood > heart > liver > kidney > muscle [21, 25-27, 42-43].

3.1. Blood and Heart

Following an acute exposure of two vanadate solutions – decavanadate and metavanadate – containing different vanadate oligomers, different patterns of vanadium subcellular distribution in fish blood plasma, red blood cells (RBC) and cardiac muscle subcellular fractions was described.

Indeed, upon an intravenous (i.v.) injection of 1 or 5 mM vanadate (total vanadium) in *S. aurata*, the highest amount of vanadium is found in blood plasma 1 hour after administration of both decavanadate and metavanadate solutions, being 80- to 1,000-fold higher than in RBC, respectively, if 5 or 1 mM total vanadium is administered [26-27]. Regarding cardiac mitochondria and cytosol, lower amounts of vanadium are found upon 1 mM vanadate (total vanadium) *in vivo* administration, not changing with exposure time in cytosol (about 50 ng/g dry tissue) for both solutions, while in mitochondria the amount of vanadium after the first hour (about 45 ng/g dry tissue) is about half of the vanadium content at 6 and 12 hours, for both solutions [27]. Apparently, 1 mM decavanadate does not affect vanadium distribution at the earlier times since similar values were observed for metavanadate. Previous works done with higher vanadate concentration (5 mM) with the same fish species and exposure time show a tendency to vanadium accumulation in mitochondria upon decavanadate i.v. administration in comparison to cytosol, being accumulated 2-fold higher in cardiac mitochondria [26] (Fig. 4).

The amount of vanadium in cardiac mitochondria was higher than in cytosol, earlier for metavanadate (6 hours) than for decavanadate (12 hours). After 12 hours of administration, the amount of vanadium in plasma, as well as in cardiac cytosol, decrease about 50% for both vanadate solutions, whereas it remains almost unchanged in mitochondria [26] (Fig. 4). In fact, it has been described that the binding of vanadium to plasma proteins is of special interest, as the vanadium binding proteins can play a role in the transport of vanadium in blood. Transferrin and albumin have been reported to be binding proteins for vanadium in human plasma [14]. Besides mitochondria, it has been suggested that, at a subcellular level, the majority of vanadium is found in the nucleus [41, 44-45]. When decavanadate is administered a different fate of intracellular vanadium

accumulation was observed in blood plasma and RBC, being vanadium mainly distributed in plasma, before accumulated into the mitochondrial fraction [26].

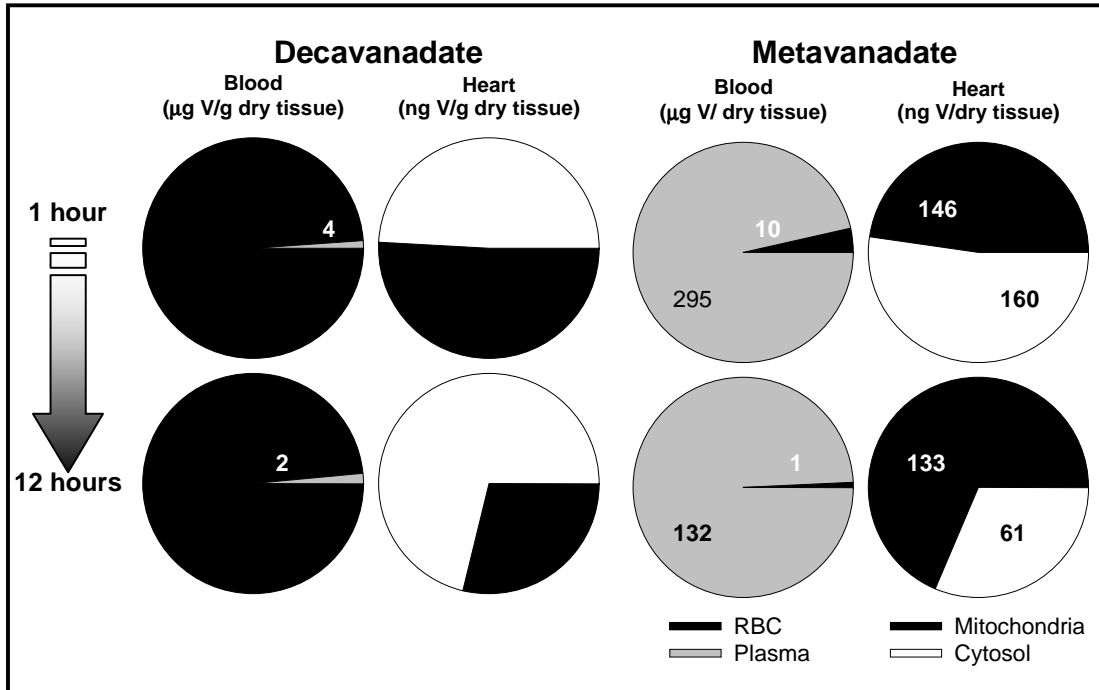


Figure 4. Vanadium concentration on red blood cells (RBC), blood plasma and cardiac mitochondria and cytosol of *Sparus aurata* individuals (n = 4) intravenously injected with 5 mM (total vanadium) decavanadate or metavanadate, 1 and 12 hours after exposure (adapted from Soares *et al.* [26]).

On contrary, for the same concentration (5 mM) and different mode of administration (intraperitoneal, i.p.), it was not observed the tendency of vanadium to accumulate in cardiac mitochondria upon decavanadate i.p. administration in *H. didactylus* [21].

In conclusion, in fish cardiac muscle, the vanadium distribution is dependent on the administration of decameric vanadate, with vanadium being mainly distributed in plasma, before being accumulated into the mitochondria.

3.2. Liver

In hepatic tissue, it has also been described that vanadium accumulation is favoured in mitochondria (2-fold higher than in cytosol), 24 hours upon 5 mM decavanadate (total vanadium) i.v. injection in *H. didactylus* [25] (Fig. 5). Moreover, decavanadate solution promotes a 5-fold increase (0.135 µg V/g dry tissue, $p < 0.05$) in the vanadium content of the mitochondrial fraction 7 days after exposure, whereas no effects were observed after metavanadate solution administration [25] (Fig. 5). The general tendency is that both liver and cardiac mitochondria may be a vanadium intracellular target. Apparently, for both tissues, the vanadium accumulation in mitochondria is favoured upon decavanadate administration.

As decavanadate is eventually less permeant through the anionic channel than the other vanadate oligomers (due to its molecular size) and likely to be bound with high affinity to proteins, therefore preventing its disintegration to vanadate oligomers and reduction to vanadyl, it has been suggested that the accumulation of vanadium in liver after decavanadate administration is a consequence of a higher level of exposure of liver to total vanadium. It is known that decavanadate binds with high affinity with proteins [18-20].

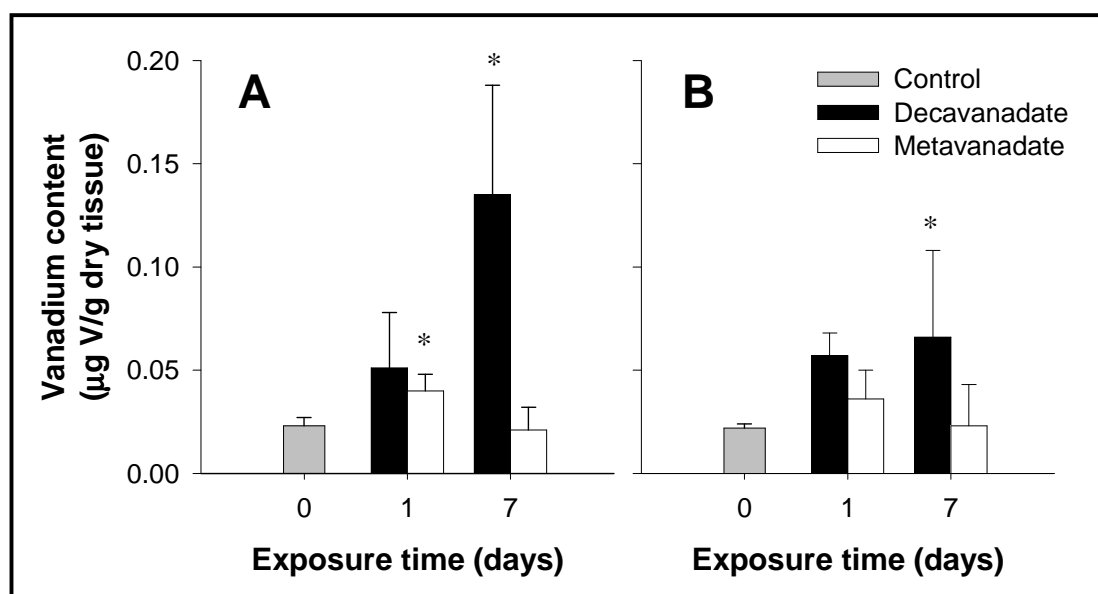


Figure 5. Vanadium amounts ($\mu\text{g V/g}$ dry tissue) in mitochondrial (A) and cytosolic (B) subcellular fractions of hepatic tissue from *Halobatrachus didactylus* individuals ($n = 5$) intravenously injected with 5 mM (total vanadium) decavanadate or metavanadate, 1 and 7 days after exposure (mean \pm STD); *significantly different from control, $p < 0.05$ (adapted from Gândara *et al.* [25]).

Furthermore, resembling heart tissue, vanadium accumulation in liver is dependent on the way of administration. The subcellular distribution of vanadium in liver appears to be different than in heart, since mitochondrial and cytosolic fractions of hepatic tissue accumulate 10% of total vanadium, while in cardiac tissue the same fractions accumulate 20% [21, 26-27].

3.3. Kidney

Also in renal tissue, mitochondria present more vanadium than cytosol, indicating that mitochondria were the main subcellular target. In a short-term exposure study [43], in renal mitochondria from *H. didactylus* intraperitoneally injected with 5 mM (total vanadium) decavanadate, vanadium concentration increased ($p < 0.05$) by 424% 1 day post-exposure, whereas a 98% increase was reported in metavanadate-treated fish. This experiment reveals that, even 7 days upon vanadate administration high amounts of vanadium were detected in the kidney of both decavanadate- and metavanadate-treated fish (+153% and +231% following decavanadate or metavanadate administration, respectively). Conversely, in kidney cytosol, metal concentration increased only upon 1 day of exposure,

being vanadate more abundant in cytosolic fraction after decavanadate exposure (+336%) than after treatment with metavanadate (+251%), while it were not observed a significant increase ($p > 0.05$) 7 days post-treatment. In conclusion, after decavanadate exposure vanadium tends to accumulate in subcellular compartments, such as mitochondria [43].

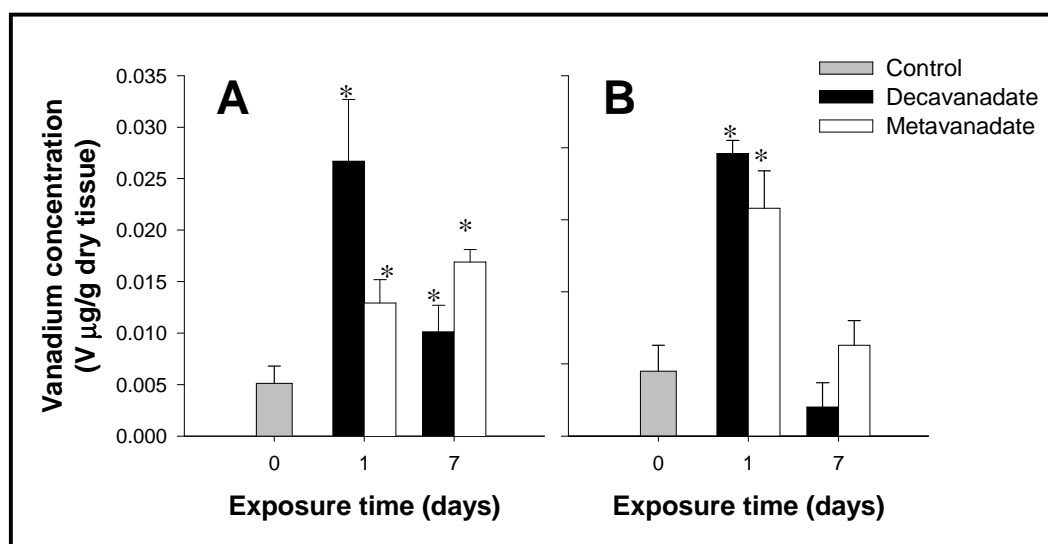


Figure 6. Vanadium concentration ($\mu\text{g V/g}$ dry tissue) in renal mitochondria (A) and cytosol (B) from *Halobatrachus didactylus* ($n = 5$) intraperitoneally injected with 5 mM (total vanadium) decavanadate or metavanadate, 1 and 7 days after exposure (adapted from Soares *et al.* [43]).

3.4. Muscle

Skeletal muscle is one of the tissues with the lowest vanadium accumulation described [45]. However, Sousa [42] reported that in *S. aurata* specimens, intravenously injected with 5 mM (total vanadium) decavanadate, the highest percentage of vanadium was found in muscle 1 hour after exposition (+96% relatively to control fish; 126 ± 12 ng/g dry tissue; $p < 0.05$), whereas the maximum amount of vanadium detected in skeletal muscle following metavanadate administration was acquired 12 hours after treatment (+94% relatively to non-treated fish; 135 ± 27 ng/g dry tissue; $p < 0.05$) (Fig. 7), suggesting that in this tissue decameric vanadate may be accumulated prior than other vanadates. A study with *H. didactylus* intraperitoneally injected with the same amount of vanadate, also revealed that the highest amount of vanadium in muscle is detected 24 hours upon exposure to decavanadate (+186% relatively to control).

Besides vanadium is more rapidly accumulated in muscle when administered in the form of decavanadate, nevertheless, conversely to observed in vanadium-target organs, in fish skeletal muscle it is not observed a preferential accumulation of vanadium upon the administration of a specific vanadate oligomeric species, being the accumulation of vanadium, administered as decavanadate or metavanadate, very similar ($p > 0.05$).

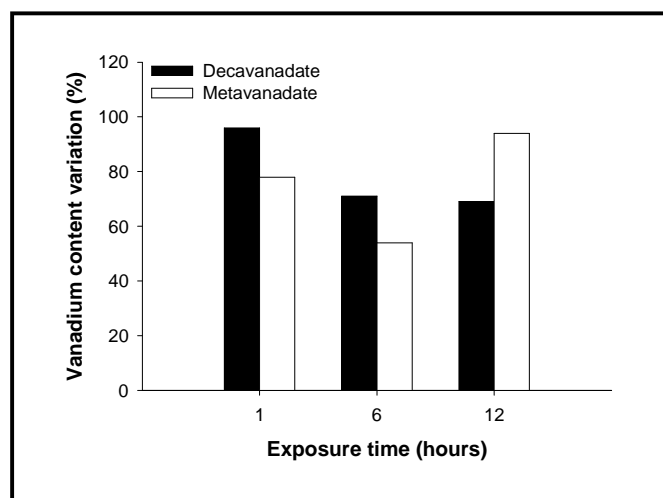


Figure 7. Variation of vanadium amount (%) relatively to control (non-treated fish) in *Sparus aurata* skeletal muscle (n = 4) intravenously injected with 5 mM (total vanadium) decavanadate or metavanadate, 1, 6 and 12 hours after exposure (adapted from Sousa [42]).

4. DECAVANADATE TOXICITY

Several haematological changes, compromising oxygen transport efficacy, namely cell destruction, haemolytic anemia and methaemoglobinemia – oxyhaemoglobin oxidation to methaemoglobin – have been described upon vanadium intoxication [5-7, 31, 46-53]. Moreover, in vanadium intoxication, besides the increase of red blood cells (RBC) breakdown, an increase on methaemoglobin levels has been reported [48, 54-56]. In the human erythrocyte, under normal conditions, a small proportion (about 1%) of haemoglobin exists as methaemoglobin [57-58]. On contrary, in fish species, haemoglobin in the form of methaemoglobin is ten fold greater than in humans [59]. Therefore, studies of toxic metals-induced methaemoglobinemia in piscine models are of extreme relevance.

Cardiovascular effects of heavy metals such as vanadium are also known, namely alterations of heart rate, cardiac inotropism and peripheral vascular resistance [60-61], although histopathological effects in cardiac tissue are not completely understood. There is a strict relationship between cardiac tissue morphology integrity and the maintenance of heart functional efficiency [62]. Therefore, characterization of the myocardial tissue may be important in evaluating the degree of cellular damage caused by exposure to toxic agents. To analysed V_{10} contribution to vanadate toxicity in haematological and cardiovascular systems, following an acute exposure to decavanadate solution, V_{10} -induced changes in methaemoglobin reductase and Ca^{2+} -pump activities were determined in intraperitoneally injected fish.

4.1. Methaemoglobin reductase activity

Following a short-term exposure (7 days), methaemoglobin reductase activity is affected differently after the *in vivo* administration of decavanadate or metavanadate solutions, metavanadate induced an increased of enzymatic activity by 67% ($p < 0.05$), while in the presence of decavanadate no significant changes were observed (Fig. 8) [23]. Soares and colleagues [23] injected intraperitoneally two vanadate 5 mM (total vanadium)

solutions, as decavanadate and metavanadate, in *H. didactylus*, in order to evaluate the effects of oligomeric vanadate species on methaemoglobin reductase activity from fish red blood cells (RBC). Although *in vivo* decameric vanadate did not induce changes in the methaemoglobin reductase activity, *in vitro* studies indicated that decavanadate besides strongly decreases methaemoglobin reductase activity (IC_{50} 50 μ M), also interact with haemoglobin, promoting haemoglobin oxidation to methaemoglobin, in concentrations as low as 50 μ M [23], suggesting that decameric vanadate is important to evaluate *in vivo* and *in vitro* vanadate effects on methaemoglobin reductase activity.

4.2. Decavanadate effects on the activity of sarcoplasmic reticulum Ca^{2+} -pump

Interaction of species present in vanadate solutions with ionic pumps has been described [15-16, 63]. In these studies, it is shown that, in non-damaged native vesicles decameric vanadate clearly differs from other oligomeric species in inhibiting Ca^{2+} uptake by sarcoplasmic reticulum (SR) coupled to ATP hydrolysis, Ca^{2+} efflux coupled with ATPase reversed activity (ATP synthesis) and H^+ ejection promoted by the SR ATPase [15-16, 19].

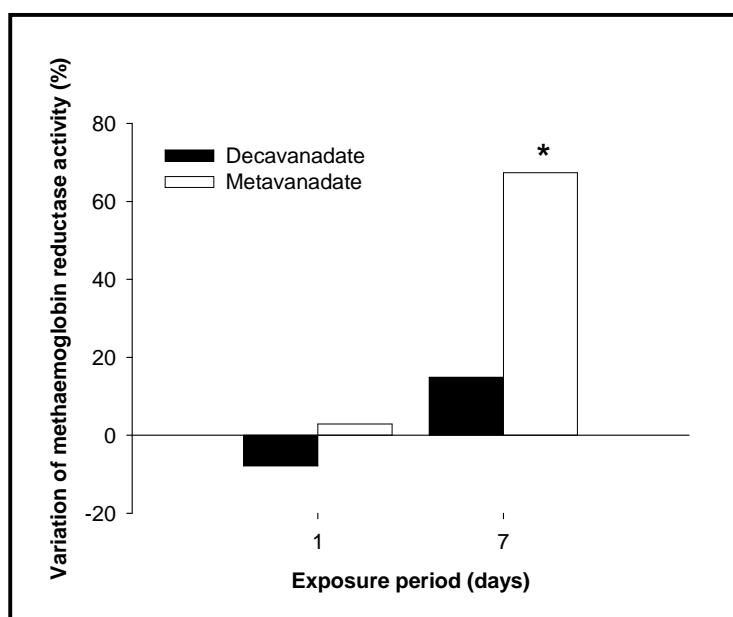


Figure 8. *Halobatrachus didactylus* methaemoglobin reductase activity (μ mol NAD^+ /min/g Hb) 1 and 7 days after 5 mM (total vanadium) decavanadate or metavanadate *in vivo* administration (n = 4); *significantly different from control, $p < 0.05$ (adapted from Soares *et al.* [23]).

In vivo measurements of SR calcium pump activity in *H. didactylus* skeletal muscle (70.4 ± 6.65 nmol Pi/min/mg SRV) reflect that ATP hydrolysis by Ca^{2+} -ATPase is significantly increased (+52%; $p < 0.05$) 48 hours upon *in vivo* administration of 5 mM (total vanadium) decavanadate, whereas only metavanadate solution decreased it by -15% ($p < 0.05$) 24 hours post-injection (Fig. 9); conversely, to the observed *in vitro* [64], where only decavanadate inhibits the calcium pump (coupled uptake). Therefore, decavanadate exerts noticeable effects on physiological calcium homeostasis, in particular when coupled

with ATP synthesis [64], having reactivity clearly different from the one observed for vanadate.

Different responses obtained on *in vivo* and *in vitro* studies prove that *in vivo* metabolism is very complex and great care must be taken on extrapolation from *in vitro* conditions. Biochemical aspects related with decavanadate effects on SR calcium pump structure and function are being reviewed in Chapter 7 of this book.

4.3. Histological changes on heart, liver and kidney

Borges and colleagues [22] analysed the contribution of vanadate oligomers to *in vivo* acute histological effects of vanadium in the heart, liver and kidney of *H. didactylus* intraperitoneally injected with a sublethal vanadium dose (5 mM total vanadium, 1 mg/kg) in the form of decavanadate or metavanadate. One and seven days post injection, stained sections of heart ventricle, renal and hepatic tissue reveal vanadium-induced tissue injuries. Both vanadate solutions produced similar effects in the renal tissue. Morphological alterations included damaged renal tubules showing disorganized epithelial cells in different states of necrosis. Reabsorbed renal tubules and hyperchromatic interstitial tissue were also observed. The hepatic tissue presented hyperchromatic and hypertrophied nuclei, along with necrosis and hypertrophied hepatocytes, with more severe changes observed upon exposure to decavanadate. Vanadate oligomers promoted evident tissue lesions in the kidney and liver, but not in the heart. However, cardiac tissue structural changes were produced. For instance, decavanadate induces a hypertrophy of the ventricle due to a decrease in the percentage of myocardium occupied by collagen fibres [22].

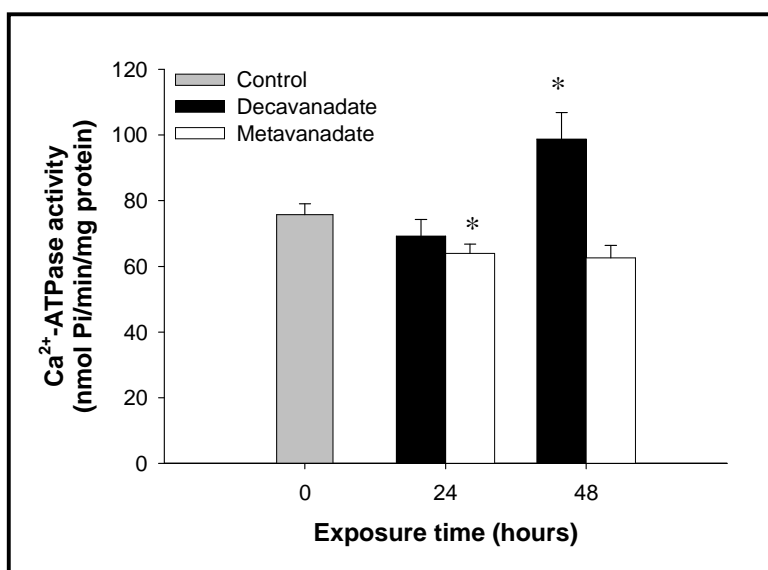


Figure 9. Sarcoplasmic reticulum vesicles (SRV) Ca^{2+} -ATPase activity (nmol Pi/min/mg SRV) from *Halobatrachus didactylus* skeletal muscle ($n = 3$), at 25 °C, in a reaction medium containing 0.1 M KCl, 25 mM HEPES (pH 7.0), 5 mM MgCl_2 , 50 μM CaCl_2 and 0.285 mg protein/ml (mean \pm STD); *significantly different from control, $p < 0.05$.

Vanadate treatment (either decavanadate or metavanadate) has no effect on the fractional area of the subepicardial and subendocardial cardiac tissues that was occupied by

various tissues components and no evidence of tissue lesions was found in any of the tissue layers. However, vanadate treatment induces changes on the fractional area of myocardium that consisted of the different cardiac tissue components (collagen type I, collagen type III, muscle tissue and lacunae). Exposure to decavanadate significantly decreased ($p < 0.05$) area fractions of collagen I (-69%, one day after vanadium exposure) and collagen III (-37% and -42%, 1 and 7 days after exposure, respectively) (Fig. 10). In contrast, the area occupied by muscle cells increased significantly ($p < 0.05$) by +11% and +8%, 1 and 7 days upon exposure, respectively. Furthermore, the exposure to metavanadate also decrease ($p < 0.05$) the percentage of myocardial area occupied by collagen I (-21%, one day after exposure to metavanadate) and collagen III (-18% and -44%, 1 and 7 days following vanadate treatment), whereas there was a significant increase ($p < 0.05$) in the area of muscle fibres (+13%, one day upon exposure to metavanadate) (Fig. 10).

Moreover, decavanadate-treated toadfish shows an increased in relative cardiac and ventricular masses, in simultaneous – but opposite – with the changes in the fractional myocardial area composed of collagen (decrease) and muscle fibres (increase) [22]. Further, because these effects are more pronounced upon decavanadate intoxication, versus metavanadate-treated fish, it is suggested that the exposure to decameric vanadate species might interfere with collagen metabolism and induce ventricular remodelling.

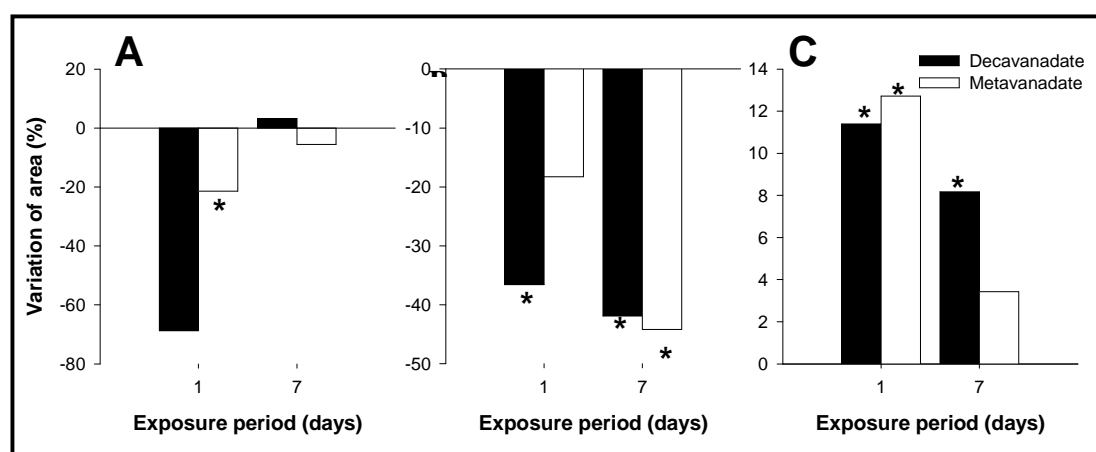


Figure 10. Variation of the fractional area (%) of myocardial images which is composed by the various tissue components: collagen type I (A), collagen type III (B) and muscle tissue (C) ($n = 5$); *significantly different from control, $p < 0.05$ (adapted from Borges *et al.* [22]).

5. LIPID PEROXIDATION AND OXIDATIVE STRESS MARKERS UPON DECAVANADATE *in vivo* ADMINISTRATION

Intracellularly vanadate (vanadium 5+) is reduced to vanadyl (vanadium 4+) by several antioxidant compounds, preventing vanadate toxic effects [9]. Several *in vivo* animal studies associate vanadate with oxidative stress and lipid peroxidation [7]. It was recently demonstrated that the acute exposure to decavanadate induce stronger effects than metavanadate increasing oxidative stress, by depressing the activity of antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) and increasing the lipid peroxidation in cardiac, hepatic and renal tissues [21, 24-25, 27, 42-43].

5.1. Glutathione (GSH) content

Reduced glutathione (GSH) is known to be one of the most important intracellular anti-oxidants, being considered the largest component of an endogenous cellular “redox buffer” [34, 65] and, since GSH was found to be involved in vanadate bioreduction [10-12, 66-68], it is expected that the levels of GSH should be affected when vanadate is administered. Several works has reported that the GSH pool is enhanced in the presence of vanadate [69-70]. More recently, Soares and colleagues observed a 135% ($p < 0.001$) increased in cardiac GSH levels 24 hours upon 5 mM (total vanadium) decavanadate intravenously (i.v.) administration and an enhancement of 171% and 120% 12 and 24 hours after metavanadate injection, respectively, in *H. didactylus* species (Fig. 11). The simplest explanation for this is that the decomposition of decavanadate will produce a delayed exposure of heart to metavanadate, although no similar observation can be made regarding other oxidative stress parameters.

On the other and, in hepatic tissue the GSH status is differently affected upon administration of both decavanadate and metavanadate solutions (5 mM total vanadium) in *H. didactylus* [25]. Whereas basal GSH content (7.8 ± 3.0 nmol/mg protein) did not shows significant changes after decavanadate solution i.v. administration, metavanadate solution administration increased GSH status up to 20 and 35%, after 12 and 24 hours, respectively. Therefore, cardiac muscle is considered to be more sensitive to oligovanadates oxidative stress than hepatic tissue. Once more, different organs show different sensitivities to decavanadate in comparison to other vanadate oligomers.

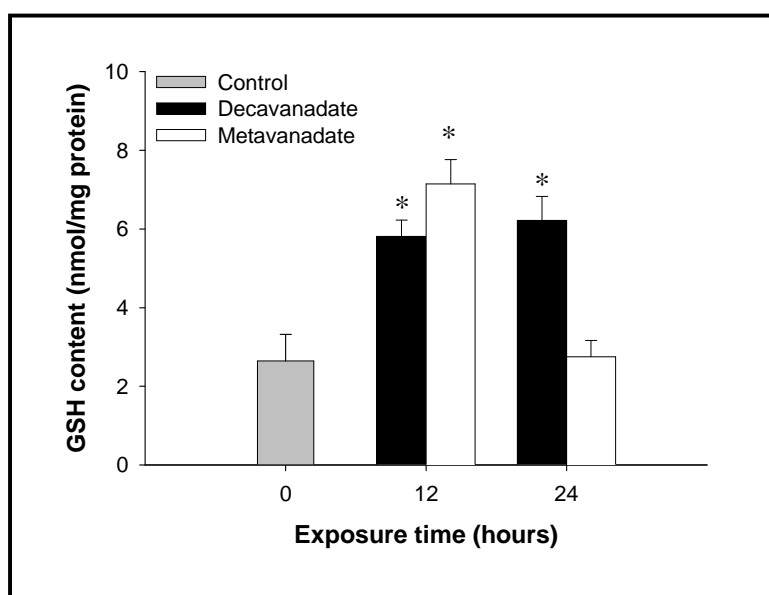


Figure 11. Reduced glutathione content (nmol GSH/mg protein) in the cardiac tissue of *Halobatrachus didactylus* ($n = 5$) intravenously injected with decavanadate or metavanadate (5 mM total vanadium; 1 mg/kg), 12 and 24 hours post-exposure (mean \pm STD); *significantly different from control, $p < 0.05$.

5.2. Reactive Oxygen Species (ROS) production

As well as other toxic metals, vanadium is known to exhibit the ability to produce ROS, resulting in lipid peroxidation and antioxidant enzyme alterations [9]. Vanadium can induce the formation of ROS in biological systems through: (i) Fenton-like reactions [7]; (ii) vanadate bioreduction mediated by GSH, flavoenzymes or NAD(P)H oxidases with ROS as a by-product [10-12, 66-68]; and (iii) recent evidences point out to an indirect promotion of ROS production, probably by interacting with mitochondria redox centres [13]. Moreover, vanadium toxicological effects in several cell types have been related with ROS production [13, 71-73]. Several suggestions on vanadate relation with ROS were made so far; nonetheless more work is needed to clarify whether vanadate speciation *in vivo*, its reduction, its action mechanisms and pathways could have different physiologic roles.

The overall pro-oxidant activity upon *in vivo* vanadate exposure has been determined by quantitative analysis of ROS production and shows that, in fish heart mitochondria, only metavanadate induces a significant and delayed increase ($p < 0.05$) in ROS production. The basal value for pro-oxidant activity in cardiac tissue 384 ± 140 a.u./min/mg protein increased above 198% ($p < 0.05$), 12 hours after metavanadate administration, whereas no significant effects were observed for decavanadate [27] (Fig. 12A). In liver tissue, both vanadate oligomers affect differently pro-oxidant activity: decavanadate enhances by about 80% the rate of ROS production for the duration of the exposure (12 hours), whereas metavanadate induces a 150% increase upon the first hour of exposure, being its pro-oxidant ability blocked throughout the 12 hours of exposure (40% and almost zero, 6 and 12 hours after vanadium exposure, respectively) (Fig. 12B).

Therefore, differential effects of decavanadate and metavanadate on ROS production should be expected on the light of the previous data. However, another similar *in vivo* study [25] reports an antioxidant action promoted by both vanadates in liver. Decavanadate-administered *H. didactylus* have a weak enhancement of ROS production 12 hours after exposition (about 15% increase), but 24 hours after ROS production decreased nearly 30%. On contrary, metavanadate-administered individuals showed a decrease of up to 40% in the overall rate of ROS production (12 and 24 hours), which is consistent with the observed increase in GSH content. Once more, the simplest explanation for the fact that decameric vanadate mimics the behaviour observed with metavanadate solution is that decomposition of decavanadate will produce a delayed exposure of the liver to metavanadate. In fact, this effect is also observed regarding the GSH content, described in the same conditions for the same organ.

Even though vanadium may participate in Fenton-like reactions [7], together with the proposed mechanisms of vanadate action that involves its bioreduction and ROS production [12, 72-74], results point out to a depression in the overall rate of ROS production. This observation is in good agreement with previous reports showing that vanadate supplementation diminished oxidative stress in certain experimental conditions, such as in rat-induced hepatocarcinogenesis [69] and in rat diabetic tissues [75]. Regarding superoxide anion radical ($O_2^{\cdot-}$) production in hepatic mitochondrial fraction, once again it was observed that metavanadate and decavanadate promoted opposite effects: 12 hours after exposition, the rate of $O_2^{\cdot-}$ production decreased 35% in decavanadate-administered individuals, while metavanadate administration induced an up to 45% increase in the rate. On the other hand, 24 hours after the results are reverted, decavanadate promoted a 30%

increase, and metavanadate decreased $O_2^{\cdot-}$ production in 40%. Thus, decameric vanadate species induced changes in $O_2^{\cdot-}$ mitochondrial production opposite to those produced by the oligomeric vanadate species that are present in metavanadate solution, from which it can be suggested again different pathways of biological action for the different oligomers. Several proposed vanadate action pathways within cells involves $O_2^{\cdot-}$ production mediated by NADPH oxido-reductases from the respiratory chain [12, 72-74]. If it is consider the action and detoxification mechanism proposed for vanadate, where vanadate is reduced to vanadyl with production of $O_2^{\cdot-}$, it is possible that decavanadate participates in such reactions in a different manner. Note also that after 24 hours, the increase in $O_2^{\cdot-}$ in decavanadate-administered individuals correlated with the increase of vanadium concentration in the mitochondrial fraction.

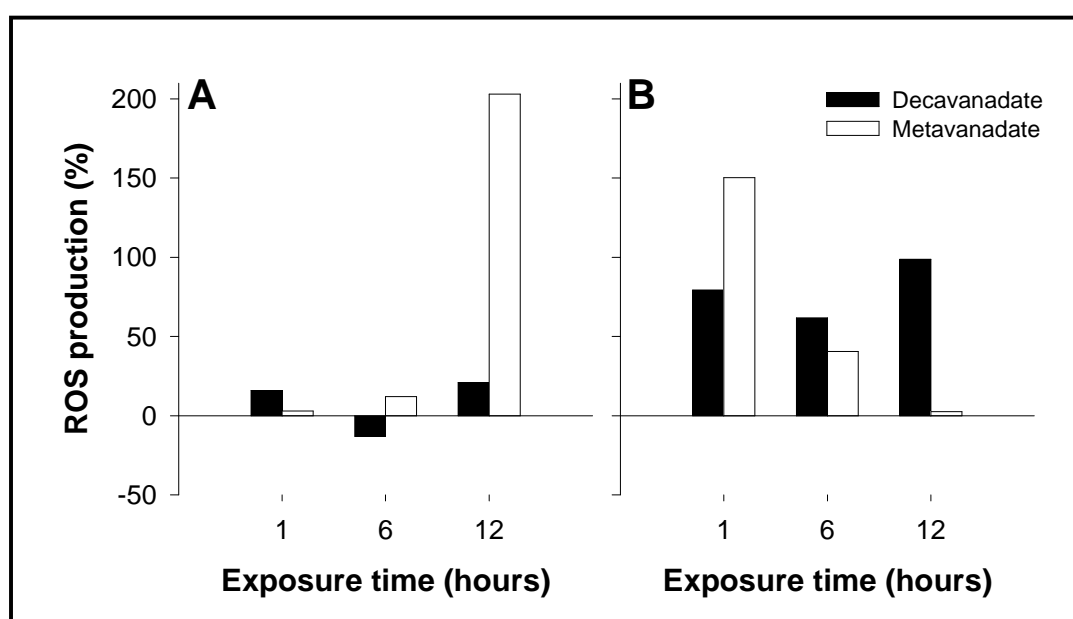


Figure 12. Overall rate of reactive oxygen species (ROS) production variation in cardiac (A) and hepatic (B) mitochondria of *Sparus aurata* (n = 6) intravenously injected with decavanadate or metavanadate (1 mM total vanadium; 1 mg/kg); *significantly different from control, $p < 0.05$ (adapted from Soares *et al.* [27]).

5.3. Antioxidant enzymes activities

Several biological studies associate vanadium with the ability to produce ROS, resulting in lipid peroxidation and antioxidant enzymes alterations, leading to oxidative stress [7, 9-10, 12, 66, 67-68, 74].

In agreement with the increase in ROS production previously reported (see section 6.2.), 1 mM metavanadate induces an increase (+115%) in mitochondrial superoxide dismutase (SOD) activity ($p < 0.05$), 12 hours after exposure, while decavanadate increases about 30% ($p < 0.05$) (at 1 and 12 hours after intravenous, i.v., administration) [27] (Table 2). It is known that SOD activity may increase with the need of protection against oxidant agents, such as vanadate. Apparently, only metavanadate act as a pro-oxidant since decavanadate does not induce the same overall response probably due to different

reactivities that can result from different mechanisms. Concerning mitochondrial catalase (CAT) activity, the basal CAT activity in mitochondria (1.63 ± 0.51 nmol/min/mg protein) decrease by -55% ($p < 0.05$) 12 hours after decavanadate exposure, whereas metavanadate induced no significant effects [27] (Table 2). Putting it all together, 1 mM decavanadate *in vivo* administration differs from metavanadate in not inducing cardiac mitochondrial ROS production and SOD activity besides decreasing CAT activity. Therefore, more pronounced pro-oxidant effects occur in cardiac mitochondria following i.v. metavanadate exposure whereas decavanadate administration seems to prevent this effect, probably by binding to target proteins that prevent its decomposition to vanadate that induces the producing of ROS or due to different reactivities. In conclusion, once formed decameric vanadate species has a different reactivity than vanadate, thus, pointing out that the differential contribution of vanadium oligomers should be taken into account to rationalize *in vivo* vanadate toxicity.

The decrease on the activity of CAT by decameric vanadate, is in agreement to previous results from the same laboratory, which reported that decavanadate induce a decrease in cardiac mitochondrial CAT activity (-60%), 7 days following 5 mM decavanadate intraperitoneal (i.p.) administration in *H. didactylus* [21] (Table 2). Cytosolic CAT activity decreased (15-20%) for both contaminated groups, upon vanadium exposure. However, in the metavanadate-contaminated group, the CAT activity decreased after 1 day of exposure (-19%) while the decavanadate group showed the strongest decrease ($p < 0.05$) of CAT cytosolic activity, observed after 7 days of decavanadate intoxication (-56%). Mitochondrial CAT activity showed less evident variations, with a slight increase after 1 and 7 days of metavanadate exposition and a minor decrease during the same period upon decavanadate exposure. The same group reported a SOD activity decrease, in cytosol, after vanadium intoxication, for both groups, with a strongest inhibition observed in decavanadate-treated group, 7 days after of exposure. For the mitochondrial SOD activity, also no significant responses were observed, although the decavanadate-treated group showed an increase of 23% after 1 days of exposure. GPx activity was not significant affected by vanadate. However, metavanadate induced a decreased in total-GPx, after 7 days of exposure (-35%), whereas the decrease observed in the decavanadate-treated group, observed upon 1 day (-20%), fades out 7 days after exposition. Conversely, both vanadate solutions intoxication decreased the Se-GPx activity, being the effects depressed or stimulated upon 1 or 7 days of metavanadate or decavanadate exposition, respectively. The activities of cytosolic SOD and CAT, exposed to 1 mg/kg of vanadium, registered a decrease immediately after 24 hours of exposure, suggesting an early cellular response to vanadium intoxication for both vanadate solutions.

Reduction of CAT activity was also described in fish exposure to cadmium [76] and silver [77]. On the other hand, a previous study [21] concluded that there are not any significant changes in mitochondrial SOD activity in *H. didactylus*, 1 and 7 days following i.p. exposure to 5 mM decavanadate or metavanadate (Table 2). In heart, more pronounced pro-oxidant effects occur in cardiac mitochondria following i.v. metavanadate exposure whereas decavanadate administration seems to prevent this effect. In hepatic tissue, it was described that decavanadate and metavanadate administration clearly induce different changes in oxidative stress markers [25]. Therefore, the antioxidants responses induced by vanadate may depend on the total vanadium concentration administered, on the way of exposure and/or vary between fish species, besides the vanadate species composition of vanadate solutions.

Table 2. Decavanadate and metavanadate effects (↗, enhancement; ↘, decrease) on cardiac mitochondria antioxidant enzymes activities (SOD, superoxide dismutase; CAT, catalase; total GPx, total glutathione peroxidases; Se-GPx, selenium-dependent glutathione peroxidases) from *Halobatrachus didactylus* and *Sparus aurata* injected with two different vanadate doses (1 and 5 mM total vanadium).

Organ	Vanadate	Enzyme	Effect	Author
Halobatrachus didactylus (5 mM total vanadium intraperitoneally injected)				
Heart	Decavanadate	SOD	↗ +23%	Aureliano <i>et al.</i> [21]
		CAT	↘ -60%	
		Total GPx	↘ -20%	
		Se-GPx	↘ -14%	
Heart	Metavanadate	SOD	=	Aureliano <i>et al.</i> [21]
		CAT	=	
		Total GPx	↘ -35%	
		Se-GPx	↘ -18%	
Sparus aurata (1 mM total vanadium intravenously injected)				
Heart	Decavanadate	SOD	↗ +30%	Soares <i>et al.</i> [27]
		CAT	↘ -55%	
Heart	Metavanadate	SOD	↗ +115%	Soares <i>et al.</i> [27]
		CAT	=	

5.4. Lipid peroxidation

Lipid peroxidation is commonly observed following enhanced ROS production in the cellular medium. Without specifying which vanadate species were present, it has been shown that vanadate can attenuate hepatic lipid peroxidation in induced hepatocarcinogenesis [69] and in 7,12-dimethylbenz(a)anthracene (DMBA) induced mammary carcinogenesis [78] and that vanadate does not change lipid peroxidation of control subjects [78].

Earlier cellular responses induced by decameric vanadate are currently being carried out looking for shorten times of exposition such as 1, 2 and 6 hours. However, it was observed that 1 hour upon intravenous (i.v.) administration both decavanadate and metavanadate (1 mM total vanadium) increase cardiac tissue lipid peroxidation in the same extent (about 20%) [27]. Obviously, for higher vanadate concentration (5 mM total vanadium), but different mode of administration (intraperitoneal, i.p.) – longer exposure times, different fish species (*H. didactylus*), but for the same tissue – it was described a significant increase ($p < 0.05$) in cardiac tissue lipid peroxidation propagation only 7 days after i.p. administration in *H. didactylus* (about 80% and 60% upon decavanadate and metavanadate, respectively) [21]. Recently, it was demonstrated that for higher vanadate concentration (5 mM), different longer exposure times, but for the same tissue (heart) and fish specie of *H. didactylus*, a significant increase ($p < 0.05$) in cardiac tissue lipid peroxidation propagation was observed after 1 and 7 days after decavanadate exposure whereas no effects were observed for metavanadate (Fig. 13) [64].

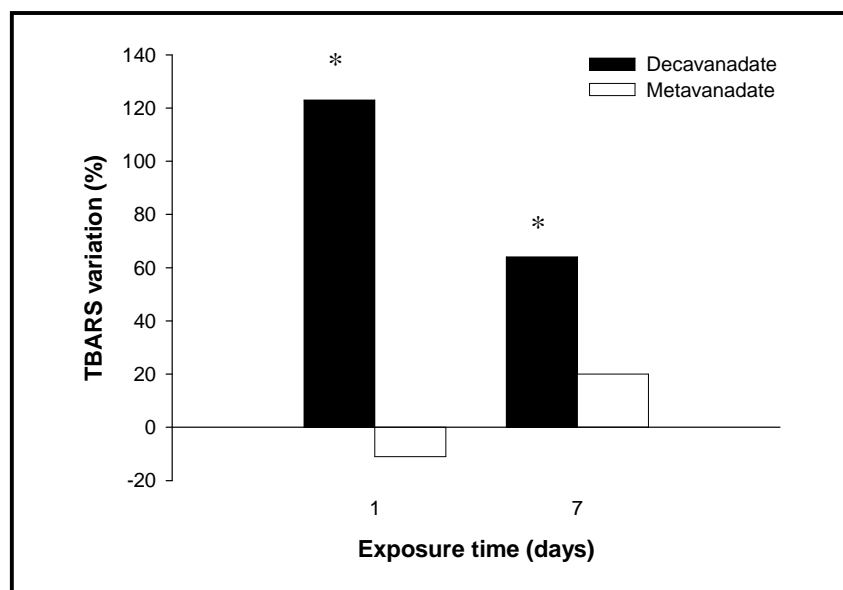


Figure 13. Lipid peroxidation propagation variation in *Halobatrachus didactylus* heart, 1 and 7 days following decavanadate and metavanadate (5 mM total vanadate) *in vivo* administration. Variation is calculated based on basal values. Values are present as means \pm SD (n = 6); *significantly different from control, $p < 0.05$ (adapted from Aureliano *et al.* [64]).

In fish liver tissue (*H. didactylus*) a 80% increase ($p < 0.05$) in lipid peroxidation was observed 24 hours after intravenous (i.v.) administration of both decavanadate or metavanadate solutions (5 mM total vanadium) [25]. In that study, decameric vanadate does not promote significant changes 12 hours after administration, in agreement to recently obtained in *S. aurata* individuals, i.v. injected with 1 mM total vanadium, where metavanadate increased lipid peroxidation products by about 55% ($p < 0.05$). As previously referred elsewhere [21, 79], decavanadate intoxication did not induce changes in the rate of lipid peroxidation till 12 hours, but later increased 80%, which is similar to the increase observed in metavanadate-administered individuals after 24 hours. Therefore, for higher (5 mM) decavanadate concentrations a delayed time effect in lipid peroxidation is observed for decavanadate in comparison to metavanadate, probably due to its stability at physiological pH.

For lower concentrations of vanadate (1 mM) both solutions induced lipid peroxidation, but once again the effects reveal to be different and dependent on the time after administration. It is suggested that decameric vanadate species induce peroxidation due to a different reactivity or even prevent the effects promoted by metavanadate since it does not maintain the levels exhibited by metavanadate, 6 and 12 hours after exposure. Eventually, after administration, the total decomposition of decameric species into metavanadate would not induce the same effects observed for the latter solution. Probably, the interactions promoted by the labile oxovanadates are different from the ones induced by decameric vanadate. Moreover, by the time that decavanadate would totally decompose into other vanadate oligomers the targets may not be the same in order to induce the same effects as before.

Clearly, the decameric species are the promoters of lipid peroxidation since metavanadate solution, that also contains monomeric species, has a lower effect. This peroxidative injury seems to be related to the reduction of the antioxidant enzyme activity.

6. MITOCHONDRIA AS A TARGET FOR DECAVANADATE TOXICITY

Mitochondria tend to be concentrated in tissues in which the energy demand is high. Thus, a compound that inhibits mitochondrial oxidative phosphorylation can therefore have a profound effect on the metabolism of important organs like the heart, kidney, liver and brain. Several reports have pointed out that mitochondria are one of the target organelles for vanadium [33, 80]. In fact, vanadium subcellular distribution results (see section 4) showed that the mitochondrial fraction tends to accumulate more vanadium after decavanadate solution administration [26-27] than upon vanadate exposure. Moreover, decavanadate *in vivo* administration points out to specific effects in mitochondrial activity [28] besides affecting mitochondrial antioxidants enzyme activities [21, 25, 27]. These results suggested that decavanadate is not behaving in the same way as other vanadate oligomers and indicated that, apparently, the mitochondria seem to be a target for decavanadate. All the above data support the hypothesis that mitochondria is might be a target for decameric vanadate species. This hypothesis has been further explored and recent evidences [28, 81] report that decameric vanadate inhibits mitochondrial respiration and induces mitochondrial membrane depolarization *in vitro* in a larger extent than metavanadate, in both rat liver and fish heart mitochondria [28, 64, 81]. For instance, decavanadate concentration as low as 0.5 μM , inhibits by about 50% oxygen consumption in intact cardiac mitochondria, while a 50-fold higher concentration of metavanadate (25 μM) is needed to induce the same effect (Fig. 14A) pointing out mitochondria as a potential cellular target for decavanadate toxicity [81]. Moreover, decameric vanadate also induced cardiac mitochondrial depolarization (IC_{50} 0.5 μM) strongly than metavanadate (IC_{50} 50 μM) (Fig. 14B) [81]. In intact mitochondria both oxygen consumption and membrane potential decreases with increasing concentrations of both vanadate solutions.

Decavanadate inhibits both mitochondrial oxygen consumption and membrane potential strongly than vanadate, acting as a potent respiratory chain inhibitor probably by affecting complex III [28].

7. CONCLUDING REMARKS

Although decameric vanadate is not frequently taken into consideration to account for the effect of vanadium in most biological studies, it is to be noted that due to its unusual long stability at physiological pH, it may not completely decompose into monomeric vanadate before inducing changes in several cellular stress markers, thus allowing to study its effects not only *in vitro* but also *in vivo*. Recent studies about the contribution of decameric vanadate to vanadate toxicity are useful to gain a deeper knowledge in vanadium biochemical effects. Moreover, piscine models seem to be adequate to study the effects of the administration of decavanadate in antioxidant stress markers, lipid peroxidation and vanadium subcellular distribution, not only due to the physiological temperature but also due to their sensitivity the metal toxicity.

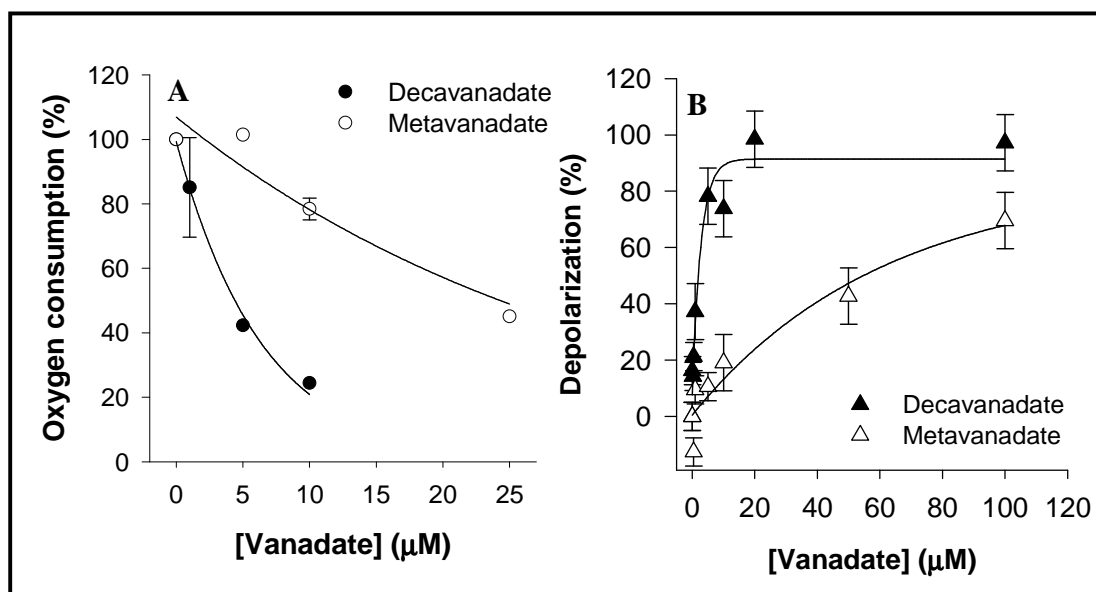


Figure 14. Decavanadate (solid circles) and metavanadate (open circles) *in vitro* inhibitory effect (up to 25 μM total vanadium) on fish heart mitochondrial (2 mg protein/ml) oxygen consumption (%), measured with an oxygen electrode (A); and, mitochondrial membrane depolarization (%) by decavanadate (solid triangles) and metavanadate (open triangles) on fish cardiac mitochondria (15 μg protein/ml), at 25 °C, in the presence of 5 mM GSH (B); mean ± STD (adapted from Soares *et al.* [81]).

Decameric vanadate promotes tissue lesions in liver and kidney, but not in the heart. However, cardiac muscle structural changes were produced, like a ventricular hypertrophy due to a decrease in the percentage of myocardial collagen fibres occupancy. However, the *in vivo* mechanisms involved in the described morphological alterations need to be further explored.

Vanadium subcellular distribution in cardiac, hepatic and renal mitochondria is dependent on total vanadium concentration and mode of administration. Moreover, mitochondria seem to be an intracellular target for vanadium accumulation following decameric vanadate *in vivo* administration. In fact, major alterations in mitochondrial antioxidant enzymes have been described upon exposure to decameric vanadate. Recent evidences reveal mitochondria as a target for decameric vanadate, reporting that decameric vanadate species inhibits mitochondrial respiration and induces mitochondrial membrane depolarization in a larger extent than vanadate, in both hepatic and cardiac mitochondria.

These results point out to the importance of taking into account decameric vanadate in the evaluation of vanadium biological effects. It is believed that the progress in understanding the role and the contribution of decavanadate to vanadium biological effects may be useful to gain a deeper knowledge in vanadium biochemistry. Therefore, based on the results of our experiments, in the present study it is proposed that decameric vanadate exhibits pro-oxidant activity through the promotion of superoxide anion formation ($O_2^{\cdot-}$); this burst is followed by a concomitant increase in superoxide dismutase (SOD) activity; the hydrogen peroxide (H_2O_2) thus produced will enhance the activity of glutathione peroxidases; leading to a raise in reduced glutathione (GSH) content; whereas $O_2^{\cdot-}$, by its turn, will induce lipid peroxidation through Haber-Weiss reaction (Fig. 15). It seems that the oxidant decavanadate activity does not follow through the direct induction of H_2O_2 production, as well as catalase antioxidant activity may not be related to the oxidative stress

responses against decameric vanadate toxic effects; thus, the observed peroxidation of lipid membranes upon *in vivo* administration of decavanadate is likely to be not linked to stimulation of Fenton reactions (Fig. 15). However, several questions remain to be address, such as: (i) Which are the subcellular and molecular targets of decavanadate with physiological relevance? (ii) Is decavanadate-induced oxidative stress due to its decomposition?

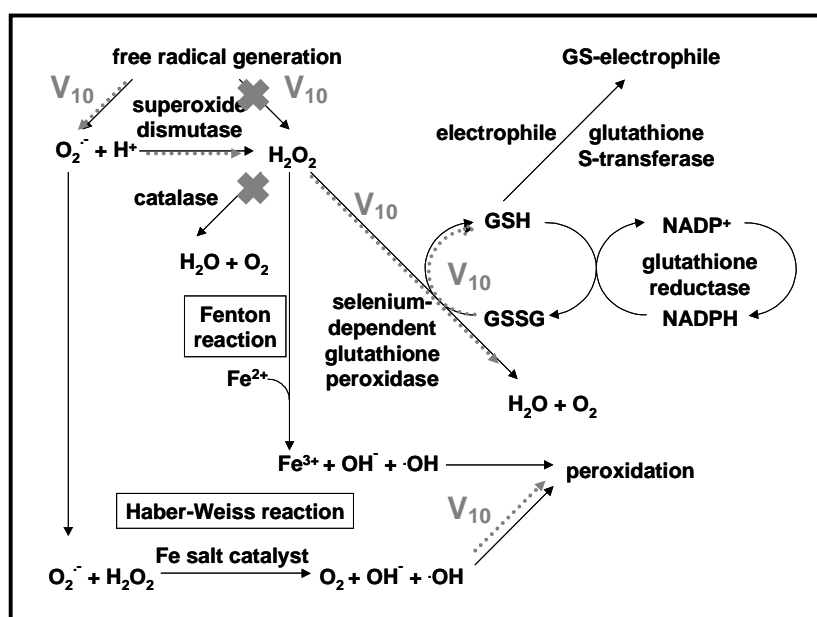


Figure 15. Summary of the proposed decavanadate pathways for the generation of reactive oxygen species and of the actions in some of the enzymes involved in antioxidant defences in cells (adapted from Storey [65]).

Putting it all together, it is proposed that once formed decameric vanadate species clearly induce differential biological responses than labile oxovanadates, probably due to different reactivities that can result from different mechanisms, thus pointing out the importance of taking in account the decavanadate contribution to the evaluation and rationalization of *in vivo* vanadate toxicity effects.

ACKNOWLEDGEMENTS

This work was supported by the POCTI program financed through FEDER, research project 38191/QUI/2001. S.S. Soares was supported by a PhD grant SFRH/BD/8615/2002 from Portuguese Foundation for Science and Technology (FCT).

REFERENCES

- [1] Nriagu, J.O. 1998, Vanadium in the environment, J.O. Nriagu (Ed.), John Wiley & Sons, New York, 1.

- [2] Beg, M.U., Al-Muzaini, S., Saeed, T., Jacob, P.G., Beg, K.R., Al-Bahloul, M., Al-Matrouk, K., Al-Obaid, T., and Kurian, A. 2001, *Arch Environ. Contam. Toxicol.*, 41, 289.
- [3] Grahame, T., and Hidy, G. 2004, *Inhal. Toxicol.*, 1, 143.
- [4] Zychlinski, L., Byczkowski, J.Z., and Kulkarni, A.P. 1991, *Arch. Environ. Contam. Toxicol.*, 20, 295.
- [5] Zaporowska, H., and Wasilewski, W. 1992, *Comp. Biochem. Physiol.*, 101C, 57.
- [6] Zaporowska, H., and Wasilewski, W. 1992, *Comp. Biochem. Physiol.*, 102C, 223.
- [7] Stohs, S., and Bagchi, D. 1995, *Free Radic. Biol. Med.*, 18, 321.
- [8] Domingo, J.L. 1996, *Reprod. Toxicol.*, 10, 175.
- [9] Byczkowski, J.Z., and Kulkarni, A.P. 1998, *Vanadium in the environment*, J.O. Nriagu (Ed.), John Wiley & Sons, New York, 235.
- [10] Ferrer, E.G., Williams, P.A.M., and Baran, E.J. 1993, *J. Inorg. Biochem.*, 50, 253.
- [11] Zhang, Z., Huang, C., Li, J., Leonard, S.S., Lanciotti, R., Butterworth, L., and Shi, X. 2001, *Arch. Biochem. Biophys.*, 392, 311.
- [12] Capella, L., Gefé, M., Silva, E., Affonso-Mitidieri, E., Lopes, A., Rumjanek, V., and Capella, M. 2002, *Arch. Biochem. Biophys.*, 406, 65.
- [13] Yang, X.G., Yang, X.D., Yuan, L., Wang, K., and Crans, D.C. 2004, *Pharm. Res.*, 21, 1026.
- [14] Chasteen, N.D. 1983, *Struct. Bond.*, 53, 105.
- [15] Aureliano, M., and Madeira, V.M.C. 1994, *Biochim. Biophys. Acta*, 1221, 259.
- [16] Aureliano, M., and Madeira, V.M.C. 1994, *Biochem. Biophys. Res. Commun.*, 205, 161.
- [17] Crans, D.C. 1994, *Comments Inorg. Chem.*, 16, 35.
- [18] Stankiewicz, P.J., Tracey, A.S., and Crans, D.C. 1995, *Metal ions in biological systems: vanadium and its role in life*, H. Sigel and A. Sigel (Eds.), Marcel Dekker, New York, 287.
- [19] Aureliano, M., and Madeira, V.M.C. 1998, *Vanadium in the environment*, J.O. Nriagu (Ed.), John Wiley & Sons, New York, 333.
- [20] Tiago, T., Aureliano, M., and Gutiérrez-Merino, C. 2004, *Biochemistry*, 43, 5551.
- [21] Aureliano, M., Joaquim, N., Sousa, A., Martins, H., and Coucelo, J.M. 2002, *J. Inorg. Biochem.*, 90, 159.
- [22] Borges, G., Mendonça, P., Joaquim, N., Coucelo, J., and Aureliano, M. 2003, *Arch. Environ. Contam. Toxicol.*, 45, 415.
- [23] Soares, S.S., Aureliano, M., Joaquim, N., and Coucelo, J. 2003, *J. Inorg. Biochem.*, 94, 285.
- [24] Aureliano, M., and Gândara, R.M.C. 2005, *J. Inorg. Biochem.*, 99, 979.
- [25] Gândara, R.M.C., Soares, S.S., Martins, H., Gutiérrez-Merino, C., and Aureliano, M. 2005, *J. Inorg. Biochem.*, 99, 1238.
- [26] Soares, S.S., Martins, H., and Aureliano, M. 2006, *Arch. Environ. Contam. Toxicol.*, 50, 60.
- [27] Soares, S.S., Martins, H., Duarte, R.O., Moura, J.J.G., Coucelo, J., Gutiérrez-Merino, C., and Aureliano, M. 2006, *J. Inorg. Biochem.*, doi:10.1016/j.jinorgbio.2006.08.002
- [28] Soares, S.S., Gutiérrez-Merino, C., and Aureliano, M. 2007, *J. Inorg. Biochemistry*, in press.
- [29] Ramos, S., Manuel, M., Tiago, T., Gândara, R.M.C., Duarte, R.O., Moura, J.J.G., Gutiérrez-Merino, C., and Aureliano, M. 2006, *J. Inorg. Biochem.*, 100, 1734.
- [30] DeMaster, E.G., and Mitchell, R.A. 1973, *Biochemistry*, 12, 2616.

- [31] Zaporowska, H., and Scibior, A. 1998, Vanadium in the environment, J.O. Nriagu (Ed.), John Wiley & Sons, New York, 135.
- [32] Kelly, K.A., Havrilla, C.M., Brady, T.C., Abramo, K.H., and Levin, E.D. 1998, *Environ. Health Perspect.*, 106, 375.
- [33] Edel, J., and Sabbioni, E. 1993, *Sci. Total Environ.*, 133, 139.
- [34] Hasspieler, B., Behar, J., and Di-Giulio, R. 1994, *Ecotox. Environ. Saf.*, 28, 82.
- [35] Baran, E. 1998, Vanadium in the environment, J.O. Nriagu (Ed.), John Wiley & Sons, New York, 318.
- [36] Yang, X., Wang, K., Lu, J., and Crans, D.C. 2003, *Coord. Chem. Rev.*, 237, 103.
- [37] Howarth, O.W., and Jarrold, M. 1978, *J. Chem. Soc. Dalton*, 22, 453.
- [38] Soares, S.S., Henao, F., Aureliano, M., and Gutiérrez-Merino, C. 2006, *J. Biol. Chem.*, submitted.
- [39] Hamel, F.G., Solomon, S.S., Jespersen, A.S., Blotcky, A., Rack, E., and Duckworth, W.C. 1993, *Metabolism*, 42, 1503.
- [40] Yuen, V.G., Orvig, C., Thompson, K.H., and McNeill, J.H. 1993, *Can. J. Physiol. Pharmacol.*, 71, 270.
- [41] Saeki, K., Nakajima, M., Noda, K., Loughlin, T.R., Baba, N., Kiyota, M., Tatsukawa, R., and Calkins, D.G. 1999, *Arch. Environ. Contam. Toxicol.*, 36, 81.
- [42] Sousa, A. 2004, Interaction of vanadium-citrate complexes with sarcoplasmic reticulum calcium pump [Portuguese], Biotechnology Master Thesis, Universidade do Algarve, Faro, 1.
- [43] Soares, S.S., Figueiredo, I., Coucelo, J., and Aureliano, M. 2007, submitted.
- [44] Zychlinski, L., and Byczkowski, J.Z. 1990, *Arch. Environ. Contam. Toxicol.*, 19, 138.
- [45] Carmignani, M., Volpe, A., Sabbioni, E., Felaco, M., Boscolo, P. 1998, Vanadium in the environment, J.O. Nriagu (Ed.), John Wiley & Sons, New York, 181.
- [46] Winterbourn, C.C. 1985, *Environ. Health Perspect.*, 64, 321.
- [47] Hogan, G.R. 1990, *Experientia*, 46, 444.
- [48] Nikinmaa, M. 1992, *Aquat. Toxicol.*, 22, 227.
- [49] Zaporowska, H., Wasilewski, W., and Slotwinska, M. 1993, *BioMetals*, 6, 3.
- [50] Russanov, E., Zaporowska, H., Ivancheva, E., Kirkova, M., and Konstantinova, S. 1994, *Comp. Biochem. Physiol.*, 107C, 415.
- [51] Zaporowska, H., and Slotwinska, M. 1996, *Folia Histochem. Cytobiol.*, 34, 99.
- [52] Zaporowska, H., Scibior, A., and Slotwinska, M. 1997, *Pol. J. Environ. Stud.*, 6, 206.
- [53] Chakraborty, A., Oinam, S., Karmakar, R., and Chatterjee, M. 1998, *Biometals*, 11, 95.
- [54] Garlej, T. 1974, *Pol. Tyg. Lek.*, 29, 2143.
- [55] Shakman, R.A. 1974, *Arch. Environ. Health*, 28, 105.
- [56] Garlej, T., Lis, E., and Mejran, S. 1975, *Bromat. Chem. Toksykol.*, 8, 213.
- [57] Schwartz, J.M., Reiss, A.L., and Jaffe, E.R. 1979, The metabolic basis of inherited diseases, J.B. Strambury, J.B. Wyngaarden, D.S. Fredencksun, J.L. Cooldstain and M.J. Brown (Eds.), McGraw-Hill, NewYork, 1654.
- [58] Di Giulio, R., Benson, W., Sanders, B., and Van Veld, P. 1995, Fundamentals of aquatic toxicology: Effects, environmental fate, and risk assessment, G. Rand (Ed.), Taylor & Francis, Washington, 523.
- [59] Lewis Jr., W.M., and Morris, D.P. 1986, *Trans. Am. Fish. Soc.* 115, 183.
- [60] Boscolo, P., Carmignani, M., Volpe, A.R., Felaco, M., del Rosso, G., Porcelli, G., and Giuliano, G. 1994, *Occup. Environ. Med.*, 51, 500.

- [61] Carmignani, M., Volpe, A.R., Masci, O., Boscolo, P., Giacomo, F., Grilli, A., del Rosso, G., and Felaco, M. 1996, *Biol. Trace Elem. Res.*, 51, 1.
- [62] Coucelo, J.M., Joaquim, N., Correia, V., Azevedo, J., and Coucelo, J.A. 1998, *Rev. Port. Cardiol.*, 17, 135.
- [63] Aureliano, M. 1996, PhD thesis, University of Coimbra, Coimbra.
- [64] Aureliano, M., Soares, S.S., Tiago, T., Ramos, S., and Gutiérrez-Merino, C. 2006, ACS Symposium Series, in press.
- [65] Storey, K.B. 1996, *Braz. J. Med. Biol. Res.*, 29, 1715.
- [66] Ghe, A.M., Stefanelli, C., Tsintiki, P., and Veschi, G. 1985, *Talanta*, 32, 359.
- [67] Kalyani, P., Vijaya, S., and Ramasarma, T. 1992, *Mol. Cell. Biochem.*, 111, 33.
- [68] Shi, S.J., Preuss, H.G., Abernethy, D.R., Li, X., Jarrell, S.T., and Andrawis, N.S. 1997, *J. Hypertens.*, 15, 857.
- [69] Chakraborty, A., Selvaraj, S., Sudarshan, M., Dutta, R.K., Ghugre, S.S., and Chintalapudi, S.N. 2000, *Nucl. Instrum. Meth. Phys. Res. Sect. B*, 170, 156.
- [70] Rinaldi, R., Eliasson, E., Swedmark, S., and Morgenstern, R. 2002, *Drug Metab. Dispos.*, 30, 1053.
- [71] Cortizo, A., Bruzzone, L., Molinuevo, S., and Etcheverry, S. 2000, *Toxicology*, 147, 89.
- [72] Huang, C., Zhang, Z., Ding, M., Li, J., Ye, J., Leonard, S., Shen, H., Butterworth, L., Lu, Y., Costa, M., Rojanasakul, Y., Castranova, V., Vallyathan, V., and Shi, X. 2000, *J. Biol. Chem.*, 275, 32516.
- [73] Gao, N., Ding, M., Zheng, J., Zhang, Z., Leonard, S., Liu, K., Shi, X., and Jiang, B. 2002, *J. Biol. Chem.*, 277, 31963.
- [74] Zhang, Z., Leonard, S., Huang, C., Vallyathan, V., Castranova, V., and Shi, X. 2003, *Free Radic. Biol. Med.*, 34, 1333.
- [75] Genet, S., Kale, R.K., and Baquer, N.Z. 2002, *Mol. Cell Biochem.*, 236, 7.
- [76] Pruell, R.J., and Engelhardt, F.R. 1980, *Mar. Environ. Res.*, 3, 101.
- [77] Atli, G., Alptekin, Ö, Tükel, S., and Canli, M. 2006, *Comp. Biochem. Physiol. C*, 143, 218.
- [78] Bishayee, A., Oinam, S., Basu, M., and Chatterjee, M. 2000, *Breast Cancer Res. Treat.*, 63, 133.
- [79] Al-Saleh, I., and Shinwari, N. 2002, *Chemosphere*, 48, 749.
- [80] Bracken, W.M., Sharma, R.P., and Elsner, Y.Y. 1985, *Cell Biol. Toxicol.*, 1, 259.
- [81] Soares, S.S., Gutiérrez-Merino, C., and Aureliano, M. 2006, *Aquat. Toxicol.*, accepted.