Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **27** (3): 468-76 (2008)

Review

Received: October 15, 2007
Accepted: March 11, 2008

Vanadium and its Complexes: the Renewed Interest in its Biochemistry

Maria T. PEPATO*, Najeh M. KHALIL, Maísa P. GIOCONDO & Iguatemy L. BRUNETTI

Clinical Analysis Department, School of Pharmaceutical Sciences
State University of São Paulo (UNESP), Rua Expedicionários do Brasil N° 1621,
Araraquara, CEP 14801- 902, São Paulo, Brazil.

SUMMARY. The field of bioinorganic chemistry, which explores the interactions between inorganic compounds and biological systems, has been growing rapidly in the last few years. Various elements and their compounds have attracted great interest, particularly vanadium. In this article we review the effect of vanadium and its complexes on diabetes mellitus.

RESUMEN. "Vanadio y sus Complejos: el Interés Renovado en su Bioquímica". Los estudios en el campo de la química bioinorgánica que exploran las interacciones entre los compuestos inorgánicos y los sistemas biológicos han crecido muy rápido en los últimos años. Algunos elementos y sus compuestos han despertado gran interés, particularmente el vanadio. Este trabajo es una revisión sobre el efecto del vanadio y sus complejos en la diabetes mellitus.

CHEMICAL PROPERTIES OF VANADIUM

Vanadium and its compounds have attracted great scientific interest [1]. It was discovered in 1801 by the Mexican chemist, del Rio, and named vanadin by the Swedish chemist Sefström in 1830. It is the nineteenth most abundant element in the earth's crust and belongs to the transition metals, of which it is the fifth commonest. Its content in the crust is 0.014%, making it as common as zinc, though it is more evenly dispersed. There are few concentrated deposits of vanadium, such as patronite [VS_4] in Peru, and most is extracted from mining byproducts, such as vanadinite [$PbCl_2$. $3Pb_3(VO_4)_2$], obtained during lead mining, and carnotite [$K_2(UO_2)_2.(VO_4)_2.3H_2O$], from uranium mining, and from some petroleum oils in Venezuela and Canada. When these oils are burnt, vanadium pentoxide (V_2O_5) is recovered as a product of total combustion [2-4].

In the oxidation states III, lV and V, vanadium easily forms V-O bonds and also bonds readily to N and S, forming a diverse range of coordination complexes [2,3]. The species vanadium (III) is unstable at physiological pH and in the presence of O_2., while vanadium (IV) is only stable in acid medium, existing as a blue cation that can be detected by electron paramagnetic spin resonance, since it possesses an unpaired electron. The most complicated of all the oxidation states is vanadium (V), which occurs as the HVO_4^{2-} anion at physiological pH. This anion tends to aggregate even at concentrations as low as 1mM, to form trimer or tetramer polyvanadates. At low pH, the decavanadate ($V_{10}O_{29}H_5^-$) is orange-colored and acts as a potent oxidant of aldehydes, catechols, olefins and sulfhydryl groups [5]. In aqueous solution exposed to the air, at room temperature, the oxidation states that predominate are V and IV, the former as orthovanadate (a mixture of HVO_4^{2-} and $H_2VO_4^-$ at pH7) and the latter usually as the

KEY WORDS: Complex [*bis*(morpholine-4-carbodithiolate)oxovanadium(IV)], Complex [(ethylenediaminetetraacetato) *bis*(oxovanadium(IV)], Diabetes mellitus, Toxicity, Vanadium.
PALABRAS CLAVE: Complejo [*bis*(morfolina-4- carboditiolato)oxovanadio(IV)], Complejo [(etilenodiaminatetraacetato) *bis*(oxovanadio(IV)], Diabetes mellitus, Toxicidad, Vanadio.

* Author to whom correspondence should be addresed. *E-mail:* pepatomt@fcfar.unesp.br

ISSN 0326-2383

vanadyl ion (VO^{2+}), which can suffer oxidation by dissolved O_2 in solutions of pH > 3 [6]. The chemistry of vanadium (V) shows parallels with that of pentavalent phosphorus and the V-O bond is about 1 Å longer than the P-O bond. This can lead the vanadates to interfere in biochemical reactions, notably those catalyzed by phosphohydrolases [5].

Vanadium (V) coordination compounds have a very flexible stereochemistry. Coordination geometries ranging from tetrahedral or octahedral to trigonal (pyramidal or bipyramidal) and pentagonal are thermodynamically plausible. The redox potentials, both for the V(V) / V(IV) and V(IV) / V(III) processes, increase even more the versatility of this transition metal in biological interactions [3].

BIOLOGICAL PROCESSES AND VANADIUM

The German chemist Henze discovered high concentrations of vanadium in blood cells of sea-squirts (marine invertebrates) in 1911. However, only in recent years has it been generally realized that vanadium is an essential nutrient for plants, animals and microorganisms. It is normally present in the mitochondria of all eukaryote cells at a concentration between 0.1 and 1.0 µM, yet its precise biological role is not at all clear [7].

In the human body, the total pool of vanadium is about 100 µg [6]. Approximately 90% of the vanadium circulating in the blood is bound to proteins and less than 10% is in a free state [8,9]. In plasma, the partial pressure of oxygen is such that vanadium complexes exist as both V(IV) and V(V) and, in the presence of endogenous reducing agents such as ascorbate and catecholamines, it forms complexes with proteins, in particular with transferrin [10] . A limited amount of free vanadate anion enters the cells via the anion transport system and is then quickly reduced to the vanadyl cation by glutathione [11,12]. Less than 1% of intracellular vanadium remains unbound [10].

Low vanadium content in the diet can lead to symptoms such as: decreased body growth and reproduction [6]; low survival of offspring; changes in the numbers of red-blood cells, iron metabolism and lipid levels, and in the metabolism of hard tissues such as teeth and bones [5].

Both in cationic and anionic forms, vanadium has an exceptional capacity to interact with biomolecules. Many potentially important therapeutic effects have been described, including hormonal, cardiovascular, anticarcinogenic and insulin-like activity [6].

Vanadium has a number of characteristics that would probably be required in a biologically essential element, including: low molecular weight and high catalytic activity of its compounds, suitable bonding structure, capacity to be chelated by biomolecules, ubiquity in the geosphere and probably in the biosphere, homeostatic regulation (controlled uptake and fast excretion) [13].

Regarding the toxicity of vanadium, acute and chronic symptoms due to occupational exposure are seen during combustion of vanadium-carrying fossil fuels and in the extractive processes employed to satisfy the growing demand for this element in the metal industry. In particular, irritation of the respiratory tract that can result in bronchitis or pneumonia is a common symptom [14].

OXIDATIVE STRESS AND VANADIUM

Oxidation of cell constituents by free radicals and/or reactive oxygen species (ROS) and reactive oxygen and nitrogen species is widely held to be a pathogenic factor in several human illnesses such as diabetes, atherosclerosis, cardiovascular illnesses, cancer, neurodegenerative disorders, and in the aging process [15]. The cellular production and removal of these species need to be critically balanced and to this end the cell strategically employs several antioxidant systems that scavenge or neutralize these molecules [16].

Among the ROS, the superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and hypochlorous acid (HOCl) are produced, for example, during the oxidative burst of neutrophils in response to infection (Fig. 1), when they play a crucial role in killing bacteria, but are also capable of doing considerable tissue damage, especially in inflamed areas, because of their biological reactivity [17].

For these reasons, the potential use of some organic metal compounds as oxidants or antioxidants in biological systems has aroused a lot of research interest. In the case of vanadium compounds, conflicting results have been published, concerning both their noxious and their beneficial effects.

Diverse studies have demonstrated that vanadium compounds can cause oxidative stress [18-20]. Vanadate-induced cell toxicity, ROS formation and production of thiobarbituric acid reactive substances (TBARS) increased as the vana-

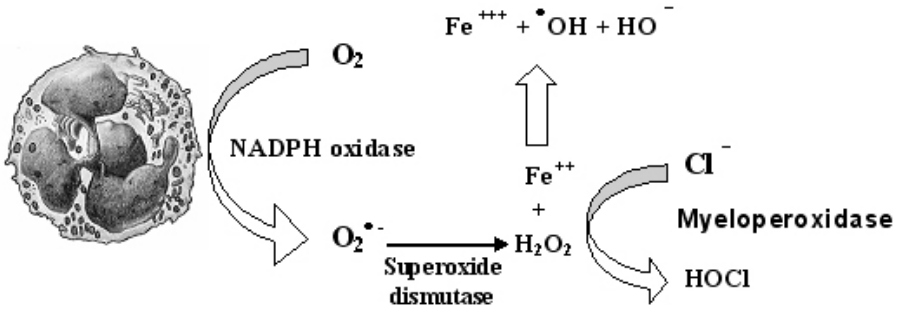

Figure 1.
Main reactive oxygen species (ROS) produced during the respiratory burst of neutrophils.

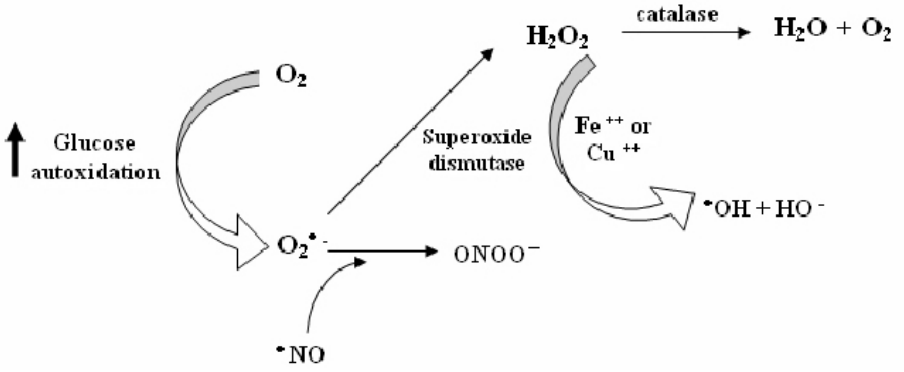

Figure 2.
Main reactive oxygen and nitrogen species and enzymes involved in glucose autoxidation.

date concentration was raised, in experiments with osteoblast and osteosarcoma cell lines [21]. *In vitro* incubation of vanadium (IV) with 2'-deoxyguanosine or DNA, in the presence of H_2O_2, resulted in enhanced 8-hydroxy-2'dG formation and substantial DNA strand breaks [22]. Recent evidence points to indirect promotion of ROS production, probably through mitochondrial interactions [19].

In diabetes, chronic hyperglycemia is a major causative factor of free-radical generation, owing to a rise in glucose autoxidation (Fig. 2), and this leads to many secondary diabetic complications, *via* damage to cellular proteins, membrane lipids and nucleic acids, and eventually to cell death [23].

A macrocyclic binuclear oxovanadium complex (6,6´-piperazine-1,4-diylmethylene-bis(4-methyl)-ethylenediimino-2-phenyl)dioxovanadyl sulfate) partially restored the level of lipid peroxides and the activity of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase, almost to control levels, in the pancreas of streptozotocin (STZ)-induced diabetic rats suffering oxidative stress [24].

Vanadate produced a synergistic effect with lithium, partially restoring the altered catalase, glutathione-peroxidase and CuZn-superoxide dismutase levels in diabetic kidneys and the depressed superoxide dismutase activity in diabetic liver [25].

Treatment with sodium orthovanadate restored the activity of antioxidant enzymes, levels of plasma lipid peroxide, glycoproteins and erythrocyte membrane phospholipids, in experimental diabetic rats [26].

The above evidence indicates that these vanadium compounds have a potential to counteract oxidative stress by the quenching or catalytic reduction of ROS and free radicals. In this laboratory, we are currently investigating interactions of some vanadium complexes with oxidative biological systems and preliminary results have demonstrated their efficacy as antioxidants.

Bifunctional vanadium compounds have also become available, consisting of antioxidants linked to vanadyl [27], an example being the complex bis[curcumino]oxovanadium(IV) whose *in vivo* hypoglycemic activity proved more effective than that of vanadyl sulfate [28]. The complex bis(allixinato)oxovanadium(IV) also had a strong hypoglycemic effect in diabetic animals [29].

PHARMACOLOGICAL INTEREST OF VANADIUM COMPOUNDS

The role of vanadium as a biometal became generally accepted when it was discovered, in 1977, that it inhibits the (Na, K) ATPase present in muscle [30]. Later, it attracted interest in the field of bioinorganic chemistry, on being found in the active site of certain enzymes, such as haloperoxidases (in marine algae and lichens) and one of the nitrogenases in the nitrogen-fix-

ing bacteria, *Azotobacter* [31]. There are several pharmacological applications of coordination compounds of vanadium, including: treatment of diabetes [3,32], cancer therapy [33,34], anti-inflammatory activity [32], normalization of hypertension and obesity [35] and spermicidal contraception [32,36,37]. In view of these recent applications, vanadium is the subject of a growing amount of research both on its therapeutic value in appropriate doses and its toxic effects in excess. The mechanisms of both kinds of effect must involve the biochemical events of cell regulation [5].

DIABETES AND VANADIUM

Studies on vanadium compounds and diabetes go back as far as 1899, when two diabetic patients were treated with sodium vanadate and experienced falls in their levels of glycosuria [38]. Research on vanadium and glucose metabolism was only taken up again 30 years ago [39] and intensified from 1985 by Heylinger *et al.* [40]. Many *in vitro* experiments performed on mammal cells, to assess the insulin-like effects of vanadium salts, revealed that vanadate mimics most of the actions of insulin in these cell cultures, *via* a post-receptor mechanism [5,41-45]. The main insulin-like actions manifested by vanadium are its capacity to: inhibit tyrosine phosphatases (PTPase) [46,47], lower the levels of blood sugar in diabetic rats [48-51] and in diabetic dogs [52] and lower the levels of serum lipids [39,53] in diabetic rats, boost glucose uptake by adipocytes [39,54] and muscle [55], stimulate both glycogen synthesis (in liver [39] and muscle [55]) and glycolysis [56], and inhibit glycogenolysis [54], liver gluconeogenesis [39] and lipolysis [53].

Research in this laboratory, involving continuous treatment of young STZ-induced diabetic rats (group DVO) with a solution of 1 mg/mL vanadyl sulfate ($VOSO_4$), which replaced the drinking water for periods of 19 and 29 days, revealed reductions in the hyperglycemia, liquid and food intakes and body weight of the rats, compared with untreated STZ-diabetic controls (group D) [57]. With regard to lipid metabolism, we found that in normal animals treated with VOSO4 (group NVO), there was no alteration in the serum levels of cholesterol and triglycerides, while in the diabetic animals treated with $VOSO_4$ (DVO), these serum levels tended to decrease after 19 days treatment, with a significant decrease in both levels after 29 days of treatment, relative to the control animals (D). These results suggest that greater effects may be obtained after longer periods of treatment. These experiments were also designed to test the possible toxic action of $VOSO_4$ and we found that at 1 mg/mL it had no detectable toxic effect on the liver or muscles of the young diabetic rats, according to assays of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LD) and creatine kinase (CK) in the serum [58].

The effects of vanadium treatment have also been tested in human beings [59]. It was found that patients with non-insulin-dependent diabetes (NIDDM), who suffered from obesity and insulin resistance, exhibited a moderate fall in fasting plasma glucose levels and glycosylated hemoglobin [60-62], when treated with $VOSO_4$ for 3-4 weeks. In light of these results, vanadium was thought to promote improved sensitivity to insulin in the liver and peripheral tissues [61,62]. Alongside these appreciable positive effects, there were also signs of gastrointestinal intolerance at the start of treatment, including nausea and diarrhea [60].

As already pointed out, vanadium has several oxidized forms, vanadyl and vanadate being the only ones that have shown insulin-like activity [40,48]. Given that vanadyl is known to be less toxic than vanadate and also more active in cells [63,64], researchers have focused on synthesizing new vanadyl complexes and investigating their insulin-like activity in order to develop more powerful and less toxic substitutes [59,64].

Three main classes of vanadium compounds have attracted interest in this field [65]: 1) inorganic salts of both vanadate anions $[VO_4]^{3-}$ and vanadyl cations VO^{2+}; 2) complexes resulting from the combination of pentavalent vanadium and hydrogen peroxide, mono- and diperoxovanadates: $[VO(O\text{-}O)(L\text{-}L')(H_2O)_2]^{n-}$ (n = 0,1) and $[VO(O\text{-}O)_2(L\text{-}L^1)]^{n-}$ (n = 1,2,3), where L, L' are coordinating ligands bonded into a bidentate pair; 3) chelated oxovanadium (IV) complexes. In this class, several types of coordination are possible. Some known complexes that have been tested successfully for insulin-like properties are displayed in Figure 3.

Studies of the complex VO^{2+}/Aspirin, for example, have shown that it works by inhibiting the enzyme tyrosine phosphatase (PTPase) [66,67] and thus regulating the level of phosphorylation of tyrosine residues in the tyrosine-specific kinase of the β-subunit of the insulin receptor, with consequent autophosphorylation and triggering of insulin-like actions. It has also been found that different vanadium compounds induce different patterns of phosphorylation of ty-

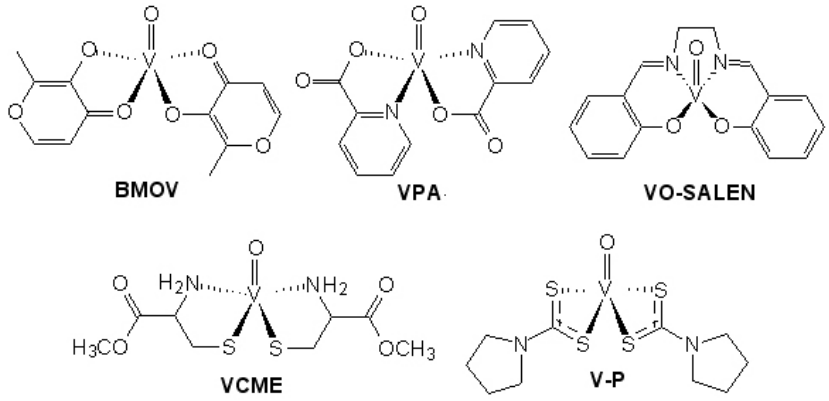

Figure 3. Chelation complexes of vanadium (IV) with insulin-like properties [3]. BMOV: bis(maltolato)oxovanadium (IV); VPA: oxobis(picolinato) vanadium (IV); VO(salen): [*N,N'*-disalicylideneethylenediamine]oxovanadium (IV); VCME: bis(methylcysteinato) oxovanadium (IV); V-P: oxobis(pyrrolidine-*N*-carbodithioato)vanadium (IV).

rosines [68]. Hence, we should expect different degrees of insulin-like action from different compounds.

In a continuation of the work on their new aspirin complex, the authors evaluated the effects of the complex $[VO(aspirin)ClH_2O]_2$ on two bone cell lines in culture and showed that its cytotoxic effects were stronger than those of VO^{2+}, as assessed by morphological changes and lipid peroxidation. In the same study, it is reported that the mechanism for these may involve induction of the expression of extracellular signal-regulated kinases (Erks) and inhibition of phosphotyrosine phosphatases (PTPaes) present in the cell extracts [69] .

Several other published investigations demonstrate the interest taken by scientists in synthesizing complexes of vanadium (IV) and vanadium (V) and testing them and the inorganic vanadyl and vanadate ions as antidiabetic agents [29,66,68-74]. Yuen *et al*., in 1993 and 1996 [75,76], found that both single-dose and continuous treatment of rats with the complex bis-(maltolato)oxovanadium(IV) (BMOV) led to a reduction in hyperglycemia at a dose 2 to 3 times lower than that needed for vanadyl or vanadate. Furthermore, compounds of vanadium chelated with a ligand containing thiazolidinedione produced positive results when the release of insulin was assessed in diabetic rats [77].

Another approach employed to test the insulin-like activity of vanadium complex has been to treat cell cultures with the complexes and assess their effects on the proliferation and differentiation of the cells and estimate the stimulation of glucose consumption, as well as to investigate their mechanism of action. This approach has been applied to a number of compounds, among then $Na_2[VO)(gluconate)_2].H_2O$, $K_2[VO(saccharate)_2].4H_2O$, $Na_4[VO(gluconate)_2].2H_2O$ and $K_5[VO(saccharate)_2].4H_2O$. In relation to cell differentiation, these compounds have been found to behave as inhibitory agents, as demonstrated by their inhibition of specific alkaline phosphatase (ALP) activity. As for as their metabolic effects are concerned, the four vanadyl complexes proved to be weaker stimulators of glucose consumption than the free VO^{2+} cation [78]. Oxovanadium (IV) complexes of general formula $Na_2[VO(L)_2].H_2O$, where the ligand L is one of the polyalcohols, sorbitol, galactitol or mannitol, exhibited a biphasic effect on cell proliferation: slight stimulation at low concentrations and inhibition in the range 25-100 µM; they also inhibited cell differentiation in tumor osteoblasts. Only Na_2 $[VO(galactitol)_2].H_2O$ stimulated glucose consumption to a similar extent to VO^{2+} [79]. Both the above studies [78,79] indicate that the bioactivity of each different complex depends on the type of complex, the ligand, the cell type and the dose.

One potential candidate for therapeutic is a complex of the vanadyl (IV) cation with the disaccharide trehalose (TreVO): $Na_6[VO(Tre)_2].H_2O$. When normal (MC3T3E1) and tumor (UMR 106) osteoblast cells were treated with TreVO, it showed insulin-like properties, inhibiting cell proliferation and differentiation. In the normal osteoblasts, TreVO behaved as a mitogen. It was found to enhance glucose consumption by a mechanism independent of phosphoinositol-3-kinase. All these effects depended on the concentration of the VO complex [80,81].

This laboratory has also investigated the effects of vanadyl ion complexes on physiological and biochemical variables, using a validated experimental model of diabetes mellitus [82]. The compounds we have tested were synthesized and characterized in collaboration with the laboratories of Dr. Alzir Azevedo Batista (Federal University of São Carlos, SP, Brazil) and Dr. Victor Deflon (University of Brasilia). Normal and diabetic rats (groups NVO, DVO) were treated by gavage for 34 days with 0.2 mmol/kg initial

body weight (b.w.) of the complex (ethylenediaminetetraacetato) bis(oxovanadium(IV)) [$(VO)_2$EDTA] (experiment approved by the Research Ethics Committee of Araraquara School of Pharmaceutical Sciences, UNESP: Article 29/2004). No change was observed in the rates of food and liquid intake, urine volume, urinary urea, serum cholesterol, high density lipoprotein (HDL) cholesterol or triglycerides, but the treatment significantly reduced the glycemia of the diabetic group (DVO) alone, though not sufficiently to reach a normal level of glucose. The mechanism responsible for this fall in the glycemia did not involve a reduction in glycogenolysis or a stimulation of glycogenesis [83]. Future studies with higher doses of this compound are planned.

Subsequently, we employed the same model for experimental diabetes to investigate the novel complex [bis(morpholine-4-carbodithioate)oxovanadium(IV)] [$VO(mor_2)$] (0.4 mmoles/kg b.w. given twice a day for the first 10 days and 0.6 mmoles/kg b.w. on the remaining 15 days - experiment approved by the Research Ethics Committee of Araraquara School of Pharmaceutical Sciences, UNESP: Article 29/2004). In the literature, models have been used to investigate synthetic or plant-based compounds with potential hypoglycemic action, in which the affected parameters are measured in both the fed [84,85] and overnight fasting [86,87] states. Our results for treatment with [$VO(mor)_2$] in both these states, fasting and fed (data not shown but be requested from: pepatomt@fcfar.unesp.br), showed both models to be equally sensitive. Treatment of the diabetic rats (group DVO) with this vanadyl complex reduced the food intake (fasting state) and the urine volume (fasting and fed states) to levels similar to those in an insulin-treated control group of diabetic rats (DI), which might be interpreted as a beneficial effect on diabetes, although the nutrients were probably not assimilated sufficiently well to promote an increase in the body weight (fed and fasting states). Furthermore, we found that treatment of the diabetic group with [$VO(mor)_2$] reduced their blood sugar to normal levels only in the fasting state, whereas the glycosuria was reduced in both the fasting and fed states to levels similar to group DI. Since glycosuria reflects a 24-hour period, it is a representative parameter of carbohydrate metabolism, implying that the metabolism was improved by this treatment of the diabetic animal. However, glycemia rather than glycosuria is routinely used as the analytic parameter for the diagnosis and classification of diabetes. The urinary urea level was reduced in the fed, but not in the fasting, treated group. Possibly, in this case, the fed state would be preferable for this test, due to the effect of amino acids, derived from proteins in the diet, on the production of urea in the liver. In fasting rats, urea would arise only as a result of proteolysis, which possibly was not reduced much more than in diabetic controls, since we found no difference between the weights of the muscles of groups DVO and diabetic (D).

It is well established that diabetes promotes an increase in proteinuria [88] and this condition was indeed reproduced in our experimental model in fasting animals. However, neither treatment with insulin nor with [$VO(mor)_2$] succeeded in correcting it.

The model studied here, in both fasting and fed rats, did not reveal any variation in lipid metabolism, raising doubts about the significance of the lack of effect of the treatment on group DVO, given that we also detected no changes in groups D and DI. The only exception was the reduction of triglycerides in the [$VO(mor)_2$]-treated normal group (NVO) relative to healthy controls, in the fed state. This reduction needs to be confirmed by further tests.

In experiments with fasting animals, there was a marked increase in the death rate (70%) during treatment of the diabetic group with the vanadyl complex. In order to find out whether the deaths were provoked by the diabetes per se or by the toxicity of the vanadyl complex, the treatment with [VO(mor)2] was also performed on normal rats, the variables again being measured in the fasting state. The results indicated that [$VO(mor)_2$] had no effect on the measured variables. The death rate in this case was 53%.

In experiments with normal and diabetic animals, in which the variables were determined in the fed state, the number of deaths was 40% in both groups NVO and DVO. Hence, it is probable that these deaths in the diabetic group were due neither to the diabetes *per se* nor to the overnight fasting. The results also showed that, in the fed state, there was a reduction of epididymal and retroperitoneal fat only in the group NVO, consistently with the lower rate of body growth observed in this group. However, this reduction in fat cannot be explained by a lower intake of food or by its being mobilized as triglycerides released into the blood, since neither of these events was observed.

Next, we measured the activities of the

marker enzymes for hepatotoxicity, AST, ALT and ALP, in diabetic and normal fasting rats, in order to investigate further the reason for the deaths. The decision to investigate hepatotoxicity followed a report from Dai *et al.* [89] that vanadyl sulfate had been found to accumulate in the liver and other organs; the complex was distributed as follows, in decreasing order of content: bone, kidney, testicle, liver, pancreas, plasma and brain. However, our results indicated that the rat deaths were not provoked by a hepatotoxic effect of vanadyl, since the hepatic marker enzyme activities in the blood were unchanged. Dai *et al.* [89] dissolved 0.5-1.5 mg/mL VOSO4 in the drinking water of diabetic and normal rats for a whole year, to assess its toxicity, and observed that no persistent change occurred in the plasma activity of ALT or AST. Of course, it is still theoretically possible that the vanadyl complex is an inhibitor of these enzymes, masking an increase in their synthesis in the liver.

The hypothesis that this toxic effect of [VO $(mor)_2$] is a result of an exceptionally high dose does not appear tenable, as the literature contains examples of similar doses of complexes such as bis(maltolato)oxovanadium (BMOV) being injected intravenously without such problems [74,90]. Human patients with type 2 diabetes have taken 50 mg vanadyl sulfate by mouth, twice a day, and tolerated it well. However, this treatment led only to a modest fall in blood sugar (20%) and no significant effects on the rate of glucose uptake by the liver, glycolysis, glycogen synthesis, oxidation of carbohydrates or lipolysis [60].

Published results on the toxicity of vanadium compounds show a degree of controversy [91-94]. The level of toxicity appears to vary with the valency: the higher the valency, the stronger the toxic effects, such as irritation of the eyes (conjunctivitis) and respiratory tract (nose-bleeds, rhinitis, asthma) and, less frequently, systemic toxicity [91]. Szakmary *et al.* [95] also noted a dependence on the specific compound; vanadium (V) pentoxide, for instance, was moderately embryotoxic, causing retarded skeletal development in rat fetuses, and significant maternal toxicity in rabbits. Lastly, the peroxovanadium complex bpV(phen) provoked cell death in RINm5F cells, predominantly by apoptosis, via a mechanism involving stress kinases and MKP-1 (mitogen-activated kinase phosphatase) [96].

In light of the observed rat deaths, the antidiabetic effects of the vanadyl complex [VO $(mor)_2$] reported here should be viewed with caution.

CONCLUSIONS

With regard to the antidiabetic properties of vanadium complexes, we conclude that it will be necessary to investigate further their effects on carbohydrate, lipid and protein metabolism, on the degradation pathways of proteins and on oxidative stress and cell toxicity so as to discover compounds with stronger beneficial effects on diabetes, associated with lower toxicity. Another aspect that requires more experimental study is the molecular properties of these complexes, specifically their interactions with biological macromolecules and enzyme systems connected with their observed effects.

Acknowledgments. We wish to thank the Foundation for the Development of the State University of São Paulo (FUNDUNESP), the Scientific Research Aid Program of the Araraquara School of Pharmaceutical Sciences, UNESP (PADC-FCF-Araraquara-UNESP) and the São Paulo State Research Aid Foundation (FAPESP) for financial support. We are also grateful for the expert help of the inorganic chemist Dr. Alzir Azevedo Batista, who provided valuable clarifications of chemical concepts during our preparation for writing this review. M P Giocondo is a Postgraduate student. We are also grateful to Prof. Dr. A A Batista and Prof. Dr. V Deflon for the synthesis and characterization of the vanadyl complex and to Ms TN Nunes and Mr MA Dangona for their technical assistance.

REFERENCES

1. Klotz, L.O., K.D. Kroncke, D.P. Buchczyk & H. Sies (2003) *J. Nutr.* **133**: 1448S-51S.
2. Sakurai, H., K. Fujii, H. Watanabe & H. Tamura (1995) *Biochem. Biophys. Res. Commun.* **214**: 1095-101.
3. Thompson, K.H., J.H. Mcneill & C. Orvig (1999) *Chem. Rev.* **99**: 2561-72.
4. Greenwood, N.N. & A. Earnshaw (1997) *"Chemistry of elements"*. 2nd ed. Oxford, Butterworth Heinemann.
5. Ramasarma, T. & F.L. Crane (1981) *Curr. Top. Cell. Regul.* **20**: 247-99.
6. Sigel, H. & A. Sigel (1995) *"Vanadium and its role in life"*. In: Orvig HK, Thomson M, Battell M, Mcneill Jr. Eds. *"Metal ions in biological systems"*. Marcel Dekker, New York, págs. 576-94.
7. Macara, I.G., K. Kustin & L.C. Cantley Jr. (1980) *Acta* **629**: 95-106.
8. Nechay, B.R. (1984) *Ann. Rev. Pharmacol. Toxicol.* **24**: 501-24.
9. Nechay, B.R., L.B. Nanninga, P.S.E. Nechay, R.L. Post, J.J. Grantham, I.G. Macara, L.F.

Kubena, T.D. Phillips & F.H. Nielsen (1986) *Fed. Proc.* **45**: 123-32.
10. Chasteen, N.D., E.M. Lord, H.J. Thompson & J.K. Grady (1986) *Inorg. Chem.* **884**: 84-92.
11. Sakurai, H., S. Shimomura, K. Fukuzawa & K. Ishizu (1980) *Biochem. Biophys. Res. Commum.* **90**: 293-8.
12. Rubinson, K.A. (1981) *Proc. Roy. Soc. London Ser B* **212**: 65-84.
13. Hopkins, Jr. l.l. & H.E. Mohr (1974) *Fed. Proc.* **33**: 1773-5.
14. Vouk, V. (1979) *"Handbook on the toxicology of metals"*. Elsevier, Amsterdam, págs. 659-74.
15. Podrez, E.A., H.M. Abu-Soud & S.L. Hazen (2000) *Free Radic. Biol. Med.* **28**: 1717-25.
16. Halliwell, B., R. Aeschbach & J. Löliger (1995) *Toxicol.* **33**: 601-17.
17. Hampton, M.B., A.J. Kettle & C.C. Winterbourn (1998) *Blood* **92**: 3007-17.
18. Stohs, S.J. & D. Bagchi (1995) *Free Radic. Biol. Med.* **18**: 321-36.
19. Yang, X.G., X.D. Yang, L. Yuan, K. Wanq & D.C. Crans (2004) *Pharm. Res.* **2**: 1026-33.
20. Halliwell, B. & J.M.C. Gutteridge (1999) *"Free radicals in biology and medicine"*. 3rd ed. Oxford University Press, Oxford.
21. Cortizo, A.M., L. Bruzzone, S. Molinuevo & S.B. Etcheverry (2000) *Toxicology* **147**: 89-99.
22. Shi, X.L., H.G. Jiang, Y. Mao, J. Ye & U. Saffiotti (1996) *Toxicology* **106**: 27-38.
23. Niedowicz, D.M. & D.L. Daleke (2005) *Cell. Biochem. Biophys.* **43**: 289-330.
24. Ramachandran, B., K. Ravi, V. Narayanan, M. Kandaswamy & S. Subramanian (2004) *Chem. Biol. Interact.* **149**(1): 9-21.
25. Scrivastava, P., A.K. Saxena, R. K. Kale & N.Z. Baquer (1993) *Res. Commun. Chem. Pathol. Pharmacol.* **80**(3): 283-93.
26. Sekar, N., A. Kanthasamy, S. Willian, N. Balasubramaniyan & S. Govindasamy (1990) *Acta Diabetol. Lat.* **27**(4): 285-93.
27. Joe, B., M. Vijaykumar & B.R. Lokesh (2004) *Crit. Rev. Food. Sci. Nutr.* **44**: 97-111.
28. Majithiya, J.B., R. Balaraman, R. Giridhar & M.R. Yaday (2005) *Trace Elem. Med. Biol.* **18**(3): 211-7.
29. Yasui, H., Y. Adachi, A. Katoh & H. Sakurai (2007) *Biol. Inorg. Chem.* **12**(6): 843-53.
30. Cantley, Jr. l.C., I. Josephson, R. Warner, M. Yanagisawa, C. Lechene & G. Guidotti (1977) *J. Biol. Chem.* **252**: 1421-3.
31. Robson, R.I., R.R. Eady, T.H. Richardson, R.W. Miller, M. Hawkins & J.R. Postgate (1986) *Nature, London* **322**: 388-90.
32. Thompson, K.H. & C. Orvig (2001) *Coord. Chem. Rev.* **219**: 1033-53.
33. Bakhtiar, R. & E. Ochiai (1999) *Gen. Pharmacol.* **32**: 525-40.
34. Chakraborty, T, A. Chatteriee, A. Rana, D. Dhachinamoorthi, P.A. Kumar & M. Chatterjee (2007) *Acta* **1772**: 48-59.
35. Hopfner, R.l., J.R. McNeill & V. Gopalakrishnan (1998) *Eur. J. Pharmacol.* **357**: 61-5.
36. D'Cruz, O.J., Y. Dong & F.M. Uckun (1999) *Biol. Reprod.* **60**: 435-44.
37. Ghosh, P., O.J. D'Cruz, D.D. DuMez, J. Peitersen & F.M, Uckun (1999) *J. Inorg. Biochem.* **75**: 135-43.
38. Hulin, B. (1994) *"New hypoglycaemic agents"*. In: Ellis G.P., Luscombe D.K. Eds. "Progress in medicinal chemistry". Elsevier, Amsterdam, págs. 1-31.
39. Tolman, E.l., E. Barris, M. Burns, A. Pansini & R. Partridqe (1979) *Life Sci.* **25**: 1159-64.
40. Heylinger, C.E., A.G. Tahiliani & J.H. McNeill (1985) *Science* **227**: 1474-7.
41. Shechter, Y. (1990) *Diabetes* **39**: 1-5.
42. Hori, C. & T. Oka (1980) *Biochim. Biophys. Acta* **610**: 235-40.
43. Dubyak, G.R. & A. Kleinzeller (1980) *J. Biol. Chem.* **55**: 5306-12.
44. Tamura, S., T.A. Brown, K.H. Whipple, Y. Fujita-Yamaguchi, R.E. Dubler, K. Cheng & J. Larner (1984) *J. Biol. Chem.* **259**: 6650-8.
45. Lyonnet, B., X. Martz & E. Martin (1899) *Presse Med.* **1**: 191-2.
46. Posner, B.I., R. Faure, J.W. Burgess, A.P. Bevan, D. Lachanced, G. Zhang-Sun, I.G. Fantus, J.B. Ng, D.A. Hall & B.S. Lum (1994) *J. Biol. Chem.* **269**: 4596-604.
47. Swarup, G., S. Cohen & D.L. Garber (1982) *Biophys. Res. Commun.* **107**: 1104-9.
48. Meyerovitch, J., Z. Farfel, J. Sack & Y. Shechter (1987) *J. Biol. Chem.* **262**: 6658-62.
49. Brichard, S.M., A.M. Pottier & J.C. Henquin (1988) *Endocrinology* **125**: 2510-6.
50. Ramanadham, S., J.J. Mongold, R.W. Brownsey, G.H. Cros & J.H. McNeill (1989) *Am. J. Physiol.* **257**: 904-11.
51. Dabros, W., A. Goc, B. Turyna & A.M. Kordowiak (2006) *Pol. J. Pathol.* **57**(2): 91-7.
52. Kim, J.M, J.Y. Chung, S.Y. Lee, E.W. Choi, M.K. Kim, C.Y. Hwang & H.Y. Youn (2006) *Vet. Sci.* **7**(4): 391-5.
53. Cros, G., J.J. Mongold, J.J. Serrano, S. Ramanadham & J.H. McNeill (1992) *Mol. Cell. Biochem.* **109**: 163-6.
54. Paquet, M.R., R.J. Romanek & R.J. Sargeant (1992) *Mol. Cell. Biochem.* **109**: 149-55.
55. Foot, E., T. Bliss, L.C. Fernandes, C. Da Costa & B. Leighton (1992) *Mol. Cell. Biochem.* **109**: 157- 62.
56. Clark, A.S., J.M. Fagan & W.E. Mitch (1985) *Biochem. J.* **232**: 273-6.
57. Pepato, M.T., M.R.T. Magnani, I.C. Kettelhut & I.L. Brunetti (1998) *Rer. Ciênc. Farmac., São Paulo* **19**(1): 93-108.
58. Pepato, M.T., M.R.T. Magnani, I.C. Kettelhut &

I.L. Brunetti (1999) *Mol. Cell. Biochem.* **198**: 157-61.
59. Tawa, R., K. Uchida, J. Taniyama, Y. Fujisawa, S. Fujimoto, T. Nagaoka, K. Kanamori & H. Sakuarai (1999) *J. Pharm. Pharmacol.* **51**: 119-24.
60. Boden, G., X. Chen, J. Ruiz, G.D. van Rossum & S. Turco (1996) *Metabolism* **45**(9): 1130-35.
61. Cohen, N., M. Halbertam, P. Shlimovich, C.J. Chang, H. Shamoon & L. Rossetti (1995) *J. Clin. Invest.* **95**: 2501-9.
62. Halbertam, M., N. Cohen, P. Shlimovich, L. Rosset & H. Shamoon (1996) *Diabetes* **45**: 659-66.
63. Nakai, M., H. Watanabe, C. Fujiwara, H. Kakegawa, T. Satoh, J. Takada, R. Matsushita & H. Sakurai (1995) *Biol. Pharm. Bull.* **18**: 719-25.
64. Sakurai, H., K. Tsuchiya, M. Nukatsuka, M. Nukatsukam, M. Sofue & J. Kawada (1990) *J. Endocrinol.* **126**: 451-9.
65. Orvig, C., K.H. Thompson, M. Battell & J.H. McNeill (1995) *"Vanadium compounds as insulin MIMICS". In: Sigel, H., Sigel, A. Eds. "Metals ions in biological Systems",* vol. 31: "Vanadium and its role in life". Marcel Dekker, New York, págs. 575-94.
66. Etcheverry, S.B., P.A. Williams, D.A. Barrio, V.C. Sálice, E.G. Ferrer & A.M. Cortizo (2000) *J. Inorg. Biochem.* **80**: 169-71.
67. Sekar, N., J. LI & Y. Shechter (1996) *Crit. Rev. Biochem. Mol. Biol.* **31**: 339-59.
68. Sálice, V.C., A.M. Cortizo, C.L.D. Gómez & S.B. Etcheverry (1999) *Mol. Cell. Biochem.* **198**: 119-28.
69. Etcheverry, S.B., P.A. Williams, V.C. Sálice, D.A. Barrio, E.G. Ferrer & A. M. Cortizo (2002) *Biometals* **15**: 37-49.
70. Kordowiak, A.M, B. Dudek & R. Grybós (2000) *Comp. Biochem. Physiol.* **125**: 11-6.
71. Melchior, M., S.J. Rettig, B.D. Liboiron, K.H. Thompson, V.G. Yuen, J.H. McNeill & C. Orvig (2001) *Inorg. Chem.* **40**: 4686-90.
72. Niu, Y., W. Liu, C. Tian, M. Xie, L. Gao, Z. Chen, X. Chen & L. Li (2007) *Eur. J. Pharmacol.* jun 19; [Epub ahead of print].
73. Sakurai, H., H. Sano, T. Takino & H. Yasui (2000) *J. Inorg. Biochem.* **80**: 99-105.
74. Takeshita, S., I. Kawamura, T. Yasuno, C. Kimura, T. Yamamoto, J. Seki, A. Tamura, H. Sakurai & T. Goto (2001) *J. Inorg. Biochem.* **85**: 179-86.
75. Yuen, V.G., C. Orvig & J.H. McNeill (1993) *Can. J. Physiol. Pharmacol.* **7**: 270-6.
76. Yuen, V.G., R.A. Pederson, S. Daí, C. Orvig & J.H. Mc Neill (1996) *Can. J. Physiol. Pharmacol.* **74**: 1001-9.
77. Storr, T., D. Mitchell, P. Buglyó, K.H. Thompson, V.G. Yuen, J.H. McNeill & C. Orvig (2003) *Bioconjug. Chem.* **14**: 212-21.
78. Williams, P.A., D. A. Barrio, S.B. Etcheverry & E.J. Baran (2004) *J. Inorg. Biochem.* **98**: 333-42.
79. Williams, P.A., S.B. Etcheverry, D. A. Barrio & E.J. Baran (2006) *Carbohydr. Res.* **341**: 717-24.
80. Barrio, D. A., P.A. Williams, A. M. Cortizo & S.B. Etcheverry (2003) *J. Biol. Inorg. Chem.* **8**: 459-68.
81. Barrio, D. A., E.R. Cuttáneo, M.C. Apeztequía & S.B. Etcheverry (2006) *Can. J. Physiol. Pharmacol.* **84**: 765-75.
82. Mori, D.M., A.M. Baviera, L.T.O. Ramalho, R.C. Vendramini, I.L. Brunetti & M.T. Pepato (2003) *Biotechnol. Appl. Biochem.* **38**: 183-91.
83. Paula, C.N., M.P. Giocondo, H.M. Baldan, A.T. Figueiredo, A.A. Batista, I.L. Brunetti, R.C. Vendramini & M.T. Pepato (2004) 56ª Reunião Anual da SBPC; Jul. 18-23; Cuiabá, MT, Brasil.
84. Aybar, M.J., A.N. Sánchez-Riera, A. Grau & S.S. Sánchez (2001) *J. Ethnopharmacol.* **74**: 125-32.
85. Gomes, A., M. Vedasiromoni, M. Das, R.M. Sharma & D.K. Ganquly (1995) *J. Ethnopharmacol.* **45**: 223-6.
86. Can, A., N. Akev, N. Ozsoy, S. Bolkent, B.P. Arda, R. Yanardag & A. Okyar (2004) *Biol. Pharm. Bull.* **27**: 694-8.
87. Venkateswaran, S., L. Pari & G. Saravanan (2002) *J. Med. Food.* **5**: 97-103.
88. Drewel University College of Medicine, Philadelphia: Drexel University Physicians (2005) Available from: http://www.topdocsonline.com/practices/PracticeInfo.asp?ID=511&PracID=18. Cited jul.8.
89. Daí, S., K.H. Thompson, E. Vera & J.H. McNeill (1994) *Pharmacol. Toxicol.* **75**: 265-73.
90. Yuen, V.G., C. Orvig & J.H. McNeill (1995) *Can. J. Physiol. Pharmacol.* **73**: 55-64.
91. Barceloux, D.G. (1999) *J. Toxicol. Clin.Toxicol.* **37**: 265-78.
92. Dabros, W., B. Kajda & A.M. Kordowiak (2002) *Pol. J. Pathol.* **53**: 123-8.
93. Sakurai, H. (1994) *Environ. Health Perspect.* **102** (suppl. 3): 35-6.
94. Thompson, K. H. & C. Orvig (2006) *J. Inorg. Chem.* **100**: 1925-35.
95. Szakmáry, E., M. Náray, E. Tátrai, A. Hudák & G. Ungváry (2002) *CEJOEM* **8**: 310-21.
96. Rumora, L., K. Barisic, D. Maysinger & T. Zanic-Grubisic (2003) *Biochem. Biophys. Res. Commun.* **300**: 77-83.