

Design of vanadium compounds as insulin enhancing agents

Katherine H. Thompson and Chris Orvig

Medicinal Inorganic Chemistry Group, Department of Chemistry,
The University of British Columbia, Vancouver, BC, V6T 1Z1, Canada

Received 6th April 2000, Accepted 16th June 2000

Published on the Web 2nd August 2000

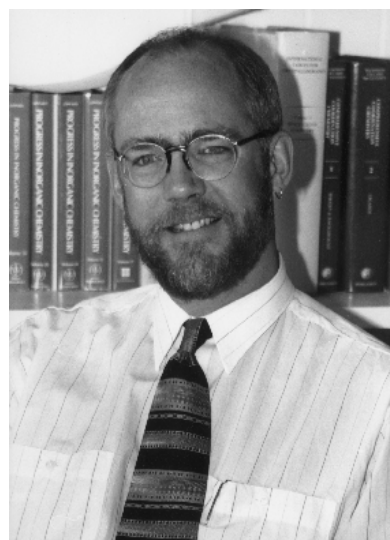
Vanadium compounds, *in vitro*, stimulate glucose uptake and inhibit lipid breakdown, in a manner remarkably reminiscent of insulin's effects. *In vivo*, vanadium enhances insulin's plasma glucose and lipid-lowering properties, leading to normalization of diabetic symptoms in the presence of only minimal endogenous insulin. The coordination chemistry of vanadium has great versatility for adjustment of pharmacological characteristics. Vanadium compounds are generally also very redox active, which is both advantageous and detrimental for optimizing biochemical function. In this perspective, we review and comment on how these properties have been revealed, what questions have arisen along the way, and where future investigations may be headed. While this is not a comprehensive overview of all available compounds touted as 'insulin enhancing agents', it will focus on those which are distinctive in some way, or which are representative

of the larger library of vanadium compounds, including all currently known relevant oxidation states of vanadium.

Introduction

The first demonstration that millimolar addition of sodium metavanadate to fat cells could stimulate glucose uptake and inhibit lipid breakdown was reported over two decades ago.¹ This serendipitous discovery was followed later by *in vivo* evidence that orally administered vanadate could reverse the overt symptoms of diabetes in experimentally diabetic animals.² Included in these symptoms were high blood sugar and plasma lipids, as well as the less obvious, but seriously pre-morbid, thyroid hormone abnormalities, and the cardiomyopathy associated with diabetes. Organic ligands, complexed to vanadium in coordination compounds, present ways to fine-

Chris Orvig was born and raised in Montréal. He received his B.Sc. in chemistry from McGill University in 1976 and, after a year as an itinerant skier, subsequently pursued graduate studies (as a Natural Sciences and Engineering Research Council (NSERC) of Canada scholar) in technetium chemistry at M.I.T. with Professor Alan Davison, receiving the Ph.D. in 1981. He was then an NSERC postdoctoral fellow with Professor Kenneth N. Raymond at the University of California, Berkeley in 1981–1983. After one year with the late Professor Colin J. L. Lock at McMaster University, he joined the University of British Columbia (U.B.C.) in 1984, where he is now Professor of Chemistry and Pharmaceutical Sciences, and Director of Medicinal Inorganic Chemistry Group. His scientific interests are firmly planted in the areas of medicinal inorganic chemistry and coordination chemistry; he has been involved over the years with radiopharmaceutical chemistry, metal ion decorporation, metal ion neurotoxicology, and chemotherapeutic metal complexes. Orvig is the inorganic chemistry editor of *Can. J. Chem.* and sits on numerous editorial boards. He has received various research and teaching awards, published more than 100 research papers, is a co-inventor on many issued patents (including eight from the USA), and is a certified ski instructor. He is, until late summer 2000, on sabbatical leave as a Humboldt Research Award winner in Münster, Germany with Professor Dr. F. E. Hahn, and travelling the world delivering lectures.



Chris Orvig



Katherine Thompson

Katherine Thompson was born and raised in Los Angeles. She received the B.A. in chemistry–zoology at Pomona College, and her M.Sc. (1977) at U.B.C. In the next 11 years she raised four children, and lived for extended periods in Durham, North Carolina and later, Canberra, Australia. She returned to U.B.C. in 1988, completing her Ph.D. in human nutrition, under Dr. Melvin Lee, 3 years later. Her interests in vanadium as a therapeutic adjunct in diabetes began with a postdoctoral fellowship with John McNeill from 1991 until 1994. She then took up a research fellowship in the Western Human Nutrition Research Centre, ARS, USDA, San Francisco, working on mathematical modeling of trace mineral metabolism. Since 1996, she has been a senior research associate in the Department of Chemistry at U.B.C., in the Medicinal Inorganic Chemistry Group.

Table 1 Selected milestones in the development of vanadium compounds as insulin mimetics

Corresponding author	Vanadium compound	Significance	Reference
Tolman, 1979	Na ₃ VO ₄	↑ Glucose uptake, ↓ lipolysis in fat cells	1
McNeill, 1985	Na ₃ VO ₄	↓ Plasma glucose, ↓ plasma insulin <i>in vivo</i>	2
McNeill, 1989	VOSO ₄	Better tolerated <i>in vivo</i> , lower toxicity	44
Sakurai, 1990	VCys, VTar	Comparison of insulin mimesis <i>in vivo</i>	45
Orvig, 1992	BMOV	First long-term study of novel orally available vanadium(IV) coordination complex	91
Kahn, 1995	Na ₃ VO ₄	First human clinical trial of vanadium(V)	79

tune the effects of vanadium, thereby minimizing any adverse effects without sacrificing important benefits.³ This area of research has recently grown enormously in an effort to find more effective, yet still orally available, complexes of vanadium which have insulin enhancing properties.^{3–8}

Three general classes of vanadium-containing compounds are of interest for their utility as insulin-mimetic agents: (1) inorganic vanadium, both anionic (vanadate, [VO₄]^{3–}) and cationic (vanadyl, VO²⁺), (2) coordination complexes, mostly of the general type VOL₂, and (3) peroxovanadium complexes (mono- and di-peroxovanadates, [VO(O₂)(H₂O)₂(L–L')]^{n–}, *n* = 0 or 1, and [VO(O₂)₂(L–L')]^{n–}, *n* = 1, 2 or 3). Coordinating ligands have specifically been chosen or designed to improve substantially the absorption, tissue uptake, and intracellular mobility of vanadium, thereby reducing the dose required for optimal insulin enhancement.⁹ In this perspective, the primary interest will be on coordination complexes, with some coverage of the currently most promising peroxovanadates. Inorganic vanadium salts as insulin enhancing agents will only be featured to illustrate the milestones of development in our understanding of insulin mimetic behaviour.

The term ‘insulin mimetic’ is something of a misnomer, since no vanadium compound can completely substitute for insulin (complete lack of insulin precludes effectiveness of any vanadium complex), but the actions of vanadium compounds are insulin-like, or at least insulin enhancing, for the most part. Poor absorption from the gastrointestinal (GI) tract into the bloodstream¹⁰ and narrowness of the window of optimal effectiveness *in vivo*¹¹ are the current limitations on administering vanadium to diabetic patients.

Design of vanadium-containing insulin enhancing agents

For vanadium to be useful as an orally available insulin mimetic agent (IMA) it must be able to cross biological membranes, both for the initial absorption process and for intracellular uptake. Very few metal ions have inherent active or facilitative transport mechanisms, exceptions being essential metal ions such as copper, zinc and iron.¹² Most others are assumed to cross cell membranes by passive diffusion, which requires that (for vanadium in a complex) the metal complex must have low molecular weight, no positive or negative charge, and a fair degree of resistance to hydrolysis. A high synthetic yield and known non-toxic metabolic products are also advantageous. The lipophilicity of the complex must be balanced with its hydrophilicity. Moreover, the metal–ligand complex should possess adequate thermodynamic stability.

Vanadium speciation and coordination bonding *in vivo*

Physiologically relevant oxidation states of vanadium include V^{III}, V^{IV} and V^V.¹³ In the early studies of vanadium speciation *in vivo* vanadate was considered to be the most relevant species in plasma.¹⁴ Experimental evidence, especially EPR studies, sup-

ported the idea that vanadate was reduced to vanadyl by intracellular binding to glutathione (γ-glutamylcysteinylglycine).¹⁵ Radiolabelled ⁴⁸VOCl₂ or NH₄⁴⁸VO₃ injected intravenously in rats,¹⁶ and ⁴⁸VOCl₂ in dogs,¹⁷ clearly showed vanadium(IV) binding to transferrin and, to a lesser extent, to other blood proteins, most probably albumin.^{17,18} Subsequent studies, incorporating ⁵¹V NMR techniques as well as EPR, revealed that V^{IV} and V^V tended to exist in equilibrium in plasma¹⁹ and that binding to plasma proteins such as transferrin and albumin kept a substantial portion of *in vivo* vanadium in a reduced form.^{20,21}

Nonetheless, vanadate's structural similarity to phosphate^{22,23} led researchers to expect that it might be the primary species in tissues such as bone,²⁴ the primary accumulator of vanadium *in vivo*,²⁵ where it could be expected to replace partially phosphate.²⁶ Recent electron spin echo envelope modulation spectroscopy (ESEEM) studies of kidney and liver samples from VOSO₄-treated rats found VO²⁺ nitrogen-ligated complexes in both tissues;²⁷ vanadium(IV) bound to phosphates in bone, most likely near the surface, has recently been demonstrated,²⁸ also by ESEEM. Thus, vanadium's role and distribution *in vivo* is still an ongoing area of investigation. Whether as vanadate, vanadyl, or an equilibrium mixture, vanadium has been shown clearly to possess insulin-like actions and, unlike insulin, to be orally active.⁵

Mechanisms of the insulin enhancing effects of vanadium

Vanadium is a required cofactor for several haloperoxidases,²⁹ and may play a role in regulation of thyroxine deiodinase;³⁰ however, a well defined biochemical role in mammals has remained elusive.^{10,31,32} Effects on various components of the intracellular signaling cascade are manifold;^{33–35} insulin-like stimulatory or inhibitory effects on specific glucose- and lipid-related enzyme systems have also amply been demonstrated.^{36,37} Insulin mimetic effects *in vitro* include stimulation of a plethora of enzyme systems in both the glucose and lipid metabolic pathways;^{1,38} it is worth noting, however, that most of these effects have been observed when millimolar concentrations of aqueous vanadium salts were added to cell cultures. These can at best, therefore, be considered to indicate insulin enhancing potential *in vivo*.

The mechanism of vanadium's *in vivo* effects has been the subject of much debate^{38–43} ever since the initial demonstration of these effects in chemically induced diabetic rats in the 1980s² (Table 1). This and subsequent studies demonstrated that vanadium's *in vitro* insulin-like effects could, in fact, translate into *in vivo* glucose- and lipid-lowering effects.⁵ In these early trials sodium chloride was added to the drinking water to improve palatability and reduce risk of dehydration; however, with the advent of vanadyl sulfate (VOSO₄, 0.5–1.25 mg mL^{–1}) as the form of vanadium added,⁴⁴ this addition was no longer necessary. Vanadium could never completely replace insulin,^{45,46} and the mechanism(s) were differentiable from those of insulin, both in enzyme systems and in cell culture studies.^{47–49} Some speculation as to the role of food restriction,^{44,50} or pancreatic

β -cell regeneration,⁵¹ in vanadium's insulin mimesis arose as a result of these seminal works; however, these occasional findings are clearly not causative factors in vanadium's insulin mimesis.⁵²

Early attempts to explain the mechanism of action of vanadium *in vivo* tended to assume that the membrane-spanning insulin receptor must be involved in some fashion.^{43,53} The obvious structural similarity between phosphate and vanadate ions tended to reinforce this notion.²³ Yet, approximately 10 years ago, conclusive evidence was presented that insulin receptor tyrosine phosphorylation was not required for vanadium's insulin enhancing effect.^{49,54} Vanadate can substitute for phosphate in transition state analogues of phosphotyrosine phosphates (PTPases) as shown by crystal structure studies,^{55,56} and sodium vanadate is routinely added to enzyme assays to preserve tyrosine phosphorylation.⁵⁷ The insulin enhancing mechanism of vanadium compounds appears, however, to be far more complex.^{41,58} A requirement for some degree of reversibility of this PTPase inhibition, and consequent stimulation of protein tyrosine kinases by intracellular vanadium, has more recently been hypothesized.³⁵ Some of vanadium's insulin mimesis appears to be due to stimulation of cytosolic tyrosine kinases which bypass at least some of the usual insulin-requiring pathways *in vivo*.⁵⁹ Most current evidence points to a site (or sites) of action downstream from the insulin receptor,⁶⁰ and considerable circumstantial evidence is accumulating which suggests that vanadium's effects on intracellular calcium metabolism may also be critical to a vanadium-containing compound's success as an IMA.⁶¹⁻⁶⁴

A key feature of vanadium's insulin mimesis is the multiplicity of its effects.^{65,66} In experimental animals (both spontaneously and chemically induced diabetic), vanadium alleviated not only the primary symptoms of diabetes, high blood glucose and lipid levels (triglycerides and cholesterol), but also the secondary complications, *e.g.* cardiomyopathy,² sorbitol accumulation,^{67,68} cataract development,^{69,70} thyroid hormone imbalance,^{2,44} alterations in kidney morphology,^{71,72} and adrenal hypertrophy.⁷² In partially pancreatectomized rats,⁷³ vanadium therapy reversed insulin resistance by restoration of muscle glycogen synthesis; in STZ-diabetic rats, it stimulated basal hexose uptake in muscle and liver,⁴³ and even in non-diabetic rat muscle, vanadium administration increased sensitivity to insulin stimulation of glycolysis and glycogen synthesis.⁷⁴ Since secondary complications of diabetes are known to have increased oxidative stress⁷⁵ as etiologic components, the possibility that vanadium's insulin mimesis is partially due to alterations in pro-oxidant/oxidant balance was also considered.^{76,77}

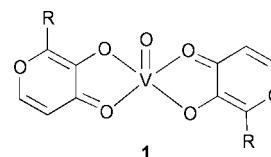
Within the last five years both inorganic and organic forms of vanadium have been tested in human subjects and shown considerable promise.⁷⁸⁻⁸² Doses required for human therapy are far lower than those in experimental animals, and modest improvements in insulin and glucose metabolism are seen within a few weeks of the start of the trials. The major drawback, as previously noted in experimental animals, is gastrointestinal (GI) distress. Since vanadium is absorbed at very low levels (generally < 2% of an oral dose),⁸³ a major avenue of continued research is development of appropriate ligands both to improve absorption and thus lessen the dose required, and to ameliorate the likelihood of gastric irritation.

3-Hydroxy-pyronate and -pyridinonate compounds

The pyrones, maltol (3-hydroxy-2-methyl-4-pyrone, Hma) and ethylmaltol (2-ethyl-3-hydroxy-4-pyrone, Hema), are particularly well suited for increasing absorption and bio-availability of metal complexes, since these compounds deprotonate easily (pK_a for Hma = 8.46; for Hema = 8.53) and form stable coordination complexes with a number of metal ions.^{84,85} When deprotonated, the maltolato ion is a known, non-toxic, anionic,

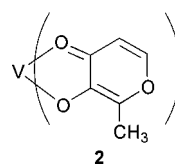
chelating, bidentate *O,O'* ligand for many metal ions, including iron,⁸⁶ aluminum,⁸⁷ gallium,⁸⁸ indium,⁸⁹ and molybdenum.⁹⁰ Both maltol (Hma) and ethylmaltol (Hema) are approved food additives in the US, the UK, and Canada.

Bis(maltolato)oxovanadium(IV) (BMOV) **1**^{91,92} and its close



analogue bis(ethylmaltolato)oxovanadium(IV) (BEOV)²⁸ can be synthesized by simple metathesis of an inorganic vanadium(IV) salt and the appropriately substituted 3-hydroxy-4-pyrone. Both are soluble (mM scale) in a number of organic solvents, as well as in water.^{92,93} Stability constants for the binding of **1** and **2** maltolato ligands to vanadyl in BMOV are $\log K_1 = 8.80$ and $\log K_2 = 7.51$; for the bis(ligand) complex, $\log \beta_2 = 16.31$. The geometry around the vanadium in $[\text{VO}(\text{ma})_2]$ is square pyramidal.⁹³

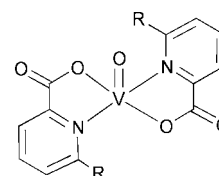
Six-coordinated vanadium(III)-pyrone and -pyridinone complexes are stable, lipophilic complexes, which are nonetheless water soluble.⁹⁴ $\text{V}(\text{ma})_3$, **2**, $\text{V}(\text{ema})_3$, $\text{V}(\text{koj})_3 \cdot \text{H}_2\text{O}$ and



$\text{V}(\text{dpp})_3 \cdot 12\text{H}_2\text{O}$ have been synthesized on a large scale by dithionite reduction of an aqueous vanadyl complex.⁹⁴

Vanadium(III) pyronate and pyridinonate compounds may serve as easily synthesizable pro-drugs that would readily convert into compounds of V^{IV} or V^{V} *in vivo*, with speciation dependent on pH and vanadium concentration.

Several other vanadium(IV) complexes have a $\text{VO}(\text{N}_2\text{O}_2)$ ligand coordination close to that found with the pyronates; examples are oxobis(picolinato)vanadium(IV) (VOPA), **3a**, and



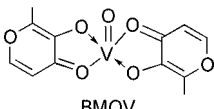
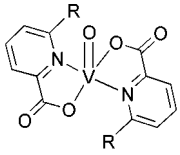
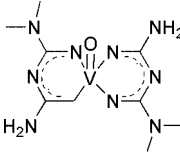
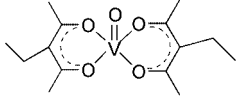
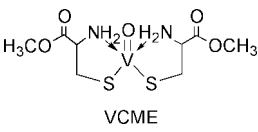
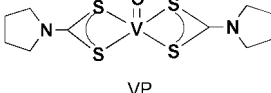
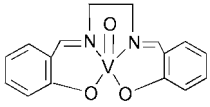
3a, VOPA ($\text{R} = \text{CH}_3$) **3b**, VOMPA ($\text{R} = \text{C}_2\text{H}_5$)

bis(methylpicolinato)oxovanadium(IV) (VOMPA), **3b**, the ligands being derivatives of 2-methylpyridine.^{95,96} VOMPA is more lipophilic than VOPA, and both compounds are insulin mimics.⁹⁵⁻⁹⁷

The redox chemistry of BMOV demonstrates an impressive lability in oxidation and reduction.⁹² In ambient methanol, or in any ambient alcoholic solvent, BMOV oxidizes to form an alkoxobis(maltolato)oxovanadium(V) complex, *cis*- $[\text{VO}(\text{OR})(\text{ma})_2]$, the oxidation kinetics being second order, a function of the concentrations of both the complex and molecular oxygen.^{93,98,99} The reaction between BMOV and molecular oxygen, in a 4:1 ratio, gives the vanadium(V) species, consistent with the fact that BMOV undergoes a 1-electron oxidation and O_2 is a 4-electron oxidant. The observed rate constant is directly proportional to the molecular oxygen concentration, consistent with this stoichiometry and the overall rate at 25 °C. Two pathways, aqua and hydroxo, give the analogue dioxoanion, *cis*- $[\text{VO}_2(\text{ma})_2]^-$, for oxidation of BMOV with O_2 in water.⁹⁹

With BMOV, it has been possible to achieve effective blood glucose lowering in STZ-diabetic rats at an initial dose of 0.4

Table 2 Comparison of bioactivity of vanadium-containing insulin mimetics tested *in vivo*^a

Vanadium compound	Dose; ^b method ^c	Result	Advantages	Disadvantages	Comments	Reference
 BMOV	0.1; i.p., 0.6; p.o. 0.2; p.o. 0.03; i.p.	Glucose lowering (GL) 2–3 × more effective than VS Maintenance dose for GL No glucose lowering	No toxicity in chronic use; ligand an approved food additive	Some GI distress above 0.8 mmol kg ⁻¹ i.p.	'Benchmark' compound; biodistribution more effective than VOSO ₄	100 25 91 7
 VOPA	0.06; i.p. 0.6; p.o. 0.1; i.p. 0.2; p.o.	33% Glucose lowering 64% Glucose lowering Glucose lowering in all test animals	Naturally occurring ligand Prolonged effect after cessation of treatment	Normoglycemia not sustained Slow onset	Calculated oral dose higher than with BMOV Maintenance dose lower than initial dose	95 96
 VO(metf) ₂	0.1; i.p. 0.6; p.o.	Glucose lowering in all test animals	Metformin in use as oral hypoglycemic with well studied pharmacokinetics	Not water soluble Some GI distress	No observed synergy	123
 VO(Etacac) ₂	0.3; i.p. 0.4; p.o.	No glucose lowering 35% Glucose lowering	Improved insulin sensitivity	Very unpalatable No correlation between plasma V and glucose lowering	Not an improvement over BMOV at the same dose	108
 VCME	0.1; p.o. 2.0; p.o.	Glucose lowering to 62% of initial level Mortality due to diarrhea	Readily available amino acid ligand	Not water soluble	Best of a series tested <i>in vitro</i> for FFA release	101
 VP	0.2; i.p. 0.2; p.o., ↓ 0.1; i.p., maintenance	Glucose lowering in all test animals; suppressed release of FFA	Prolonged normoglycemia following withdrawal	Not water soluble	Weight loss and ↑ bilirubin with i.p. dose	103
 VOSALEN	0.2; p.o.	Plasma glucose lowered to 'near normal' in diabetic rats	Water soluble and stable	Hypoglycemia; glucose-lowering not sustained	Tested in Alloxan diabetic rats; kidney and liver V higher in diabetic rats given same dose of complex as controls	107

^a In STZ-diabetic rats, except where noted. ^b mmol kg⁻¹. ^c p.o. = Oral.

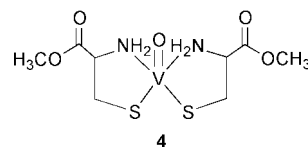
mmol kg⁻¹ d⁻¹, which can be decreased to 0.2 mmol kg⁻¹ d⁻¹ for maintenance, with no evidence of toxicity over a six month period of administration.⁹¹ The dioxoanion was, by contrast, not at all effective as an oral IMA.¹⁰⁰ Intraperitoneal (i.p.) and oral gavage demonstrated the glucose-lowering capability of several V^{III}-containing complexes, with V(ma)₃ proving to be the most potent by these standards, though still not as potent as BMOV, nor evincing a great redox stability.⁹⁴ Although no attempt has yet been made to establish the speciation *in vivo*, at physiological pH, vanadium(III) complexes should oxidize rapidly to analogues of V^{IV} or V^V.

Both VOMPA and VOPA have been proven as insulin-mimetic (Table 2), with the former more efficient⁹⁷ as an inhibitor of free fatty acid (FFA) release, a commonly used *in vitro* measure of insulin mimesis. The observed insulin mimesis is dependent on dose, but not in the most obvious (linear) dose-dependent fashion. Glucose lowering appears to have a slower onset and longer duration after cessation when fed at a lower

dose initially; the observed anti-diabetic response was, however, no less than when given initially at a higher dose.^{95,96}

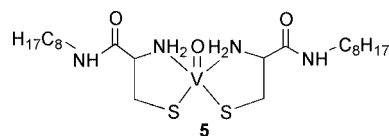
Thioligand derivatives

A variety of coordination spheres of vanadium(IV) and thioligands have been synthesized by simple methods. For example, vanadyl bis(cysteinate methyl ester), VCME, **4**¹⁰¹ was found to



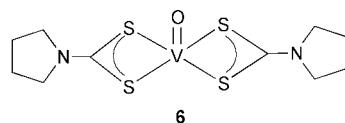
be a five coordinate vanadium(IV) complex, with a square pyramidal geometry, and was relatively stable. It was insoluble in water, and highly lipophilic.

Another N_2S_2 coordinated oxovanadium(IV) complex, bis-(*N*-octylcysteineamido)oxovanadium(IV), naglivan, **5**¹⁰² shared



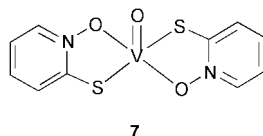
with VCME the same lipophilicity and poor aqueous solubility.

Oxobis(pyrrolidine-*N*-carbodithioato)vanadium(IV), V-P, **6**, has a VOS_4 coordination sphere, and is insoluble in water,



methanol, ethanol, diethyl ether and only slightly soluble in a variety of organic solvents, including pyridine, dimethyl sulfoxide, and dimethylformamide.¹⁰³ It is also unstable in air.

Bis(1-oxido-2-pyridinethiolato)oxovanadium(IV), VO(OPT)₂, **7**, with VO(S₂O₂) coordination has also been synthesized and

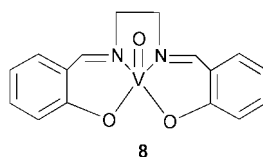


characterized.¹⁰⁴ Biological effectiveness of VO(OPT)₂ was evaluated *in vitro* only, by free fatty acid release from fat cells (adipocytes). In this system, VO(OPT)₂ was 4.7 times as effective as VOSO₄ (IC₅₀ = 0.19 and 0.90 mM, respectively).¹⁰⁴

Suspension of V-P, VCME or naglivan in 3–5% acacia gum made *in vivo* testing (by oral gavage) possible^{101–104} (Table 2). Naglivan, at doses of 5–15 mg V kg^{−1} d^{−1} (0.1–0.3 mmol kg^{−1} d^{−1}) effectively lowered blood glucose levels to near normal in STZ-diabetic rats; however, the onset of action was significantly slower than that seen with inorganic vanadium compounds.¹⁰² Despite its poor solubility characteristics, V-P was the most effective in a whole series of vanadium(IV) complexes of varying coordination modes, including V–O, V–N, and V–S, when tested for insulin mimetic activity by inhibition of free fatty acid release in rat fat cell preparations.¹⁰⁵ Significant mortality at doses 10 times the IMA dose was noted with the VCME series *in vivo*.¹⁰¹ By comparison with BMOV, none of these compounds was as orally available or as potent as IMAs.

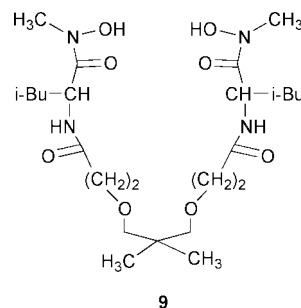
Acetylacetonate, β-hydroxamate, and phenolate compounds

[*N,N'*-Bis(salicylidene)ethane-1,2-diaminato]oxovanadium(IV), VOSALEN, **8**, was prepared by first synthesizing the ligand,



and then complexing the ligand to vanadium, by usual methods.¹⁰⁶ It was found to be air-stable and sparingly water-soluble. The coordination geometry was suggested to be octahedral in DMSO, but square pyramidal in the solid state. VOSALEN was orally effective (7.5 mmol kg^{−1} d^{−1} for 30 days) for glucose lowering in alloxan-induced diabetic rats; however, rats tended to become hypoglycemic, and withdrawal of treatment brought an immediate return to hyperglycemia.¹⁰⁷

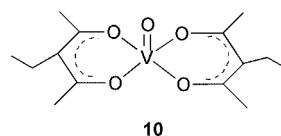
In an alternative strategy, design and preparation of ligands which might serve as *in situ* vanadium binding agents were undertaken, yielding a series of dihydroxamic acid chelators as hydrophobic carriers of vanadyl.¹⁰⁹ In an *in vitro* assay of lipogenic stimulation in rat adipocytes, RL-252, **9**, was maximally



effective at molar ratios of 10:1 vanadyl sulfate: chelator, suggesting a shuttle mechanism of action. Model compounds that were synthesized for chemical characterization were electrically neutral, lipid-soluble, and optically chiral; they released the bound metal ion when treated with aqueous glutathione solutions.

Concurrent *i.p.* administration of sodium metavanadate and L-glutamic acid γ-monohydroxamate in STZ-diabetic rats led to potentiation of the vanadate alone effect by 5–7 fold,¹¹⁰ with apparent *in situ* formation of an oxovanadium(V)-L-glutamic acid γ-monohydroxamate in a 1:1 or 1:2 ratio, as shown by ⁵¹V NMR studies.

Bis(3-methyl-2,4-pentanedionato)oxovanadium(IV), VO(acac)₂, and the close analogue bis(3-ethyl-2,4-pentanedionato)-



oxovanadium(IV), VO(Etacac)₂, **10**, have also recently been evaluated as possible insulin mimetic agents. The synthesis procedure for these compounds was simple metathesis of vanadyl sulfate trihydrate with the appropriate pentanedione and pH adjustment to pH 4 with sodium bicarbonate. The compounds were characterized by EPR and X-ray crystallographic studies.¹⁰⁸

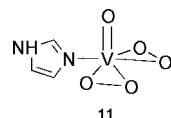
VO(Etacac)₂ (0.125 mg mL^{−1}, 0.4 mM) lowered plasma glucose in STZ-diabetic rats from 25 mM to approximately 16 mM within 8 weeks of treatment and closely paralleled the response to BMOV given in the same concentration in the drinking water.¹⁰⁸ VO(acac)₂ was proposed to effect somewhat more efficient glucose lowering;⁷ however, on close inspection, no physiologically significant difference among the three organically ligated vanadyl compounds can be discerned. No dose response was seen when calculated vanadium intake was compared with plasma glucose lowering for any of the three (nor for VOSO₄, corroborating earlier observations).⁴⁶ The three organo vanadium compounds were also not significantly different from each other in integrated plasma glucose or insulin response, according to the oral glucose tolerance test administered after 6 weeks of treatment. Intraperitoneal testing (25 μmol kg^{−1}) resulted in a slight (but statistically significant) glucose lowering (to 20 mM glucose, still severely diabetic) with VO(acac)₂ alone between 1 and 5 days after injection.⁷ This isolated finding hardly warrants a 'superior glucose-lowering' rating.⁷

Peroxo vanadates

Synthesis and characterization of a large number of peroxo-vanadate complexes of heteroligands, both mono- and poly-

dentate, revealed a range of stabilities toward decomposition in aqueous solution, depending on the nature of the hetero-ligand.^{111,112} None of the peroxovanadate complexes can be considered to be hydrolytically stable, and all are subject to redox processes, which ultimately result in radical formation with the concomitant potential for increased intracellular oxidative stress.^{113,114}

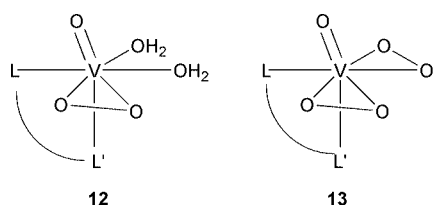
The 6-coordinate imidazole(oxo)bis(peroxo)vanadate(v), **11**, has been shown to enhance insulin receptor autophos-



phorylation in human liver cell culture, as well as to increase glucose transport in rat adipocytes, at concentrations ranging from 1 μM to 1 mM.¹¹⁵ The coordination of vanadium(v) to imidazole, an unusual pentagonal pyramid, presents structural analogies to the coordination of vanadium to histidine residues in vanadium-containing haloperoxidases and some phosphorylases.

Oxoperoxopicolinatovanadate, mpV(pic), **12**, with L and L' = picolinate, was effective in achieving a 20% decrease in plasma glucose in STZ-diabetic Sprague Dawley and BB rats at a lowest effective dose (LED) of 0.4 $\mu\text{mol kg}^{-1}$ while the lowest dose producing mortality was more than 15 times higher.¹¹⁶ By contrast, oxoperoxopyridine-2,6-dicarboxylatovanadate, mpV(2,6-pdc), had an LED = 24 $\mu\text{mol kg}^{-1}$ which was merely half the lowest dose producing mortality.¹¹⁶ *In vitro*, mpV(pic) was also most effective as an IMA, stimulating [^{14}C]glucose incorporation into diaphragm glycogen 9 times greater than in the absence of added peroxovanadate. The exact formulation appeared to be crucial, as another monoperoxovanadate, bis(oxalato)oxo(peroxo)vanadate, mpV(ox)₂, was only minimally stimulatory in the same assay.¹¹⁷ The apparent greater discrepancy between peroxovanadates in terms of their biological effectiveness, compared to oxovanadium(IV) complexes, which vary within a much smaller range in their relative biological effectiveness, may be due to a more specific nature of insulin mimesis, generally attributed to inhibition of PTPase activity.^{113,114} As PTPase inhibitors, steric and charge effects may be expected to play more prominent roles.

Two of the earliest discrete diperoxovanadate(v) compounds, **13**, potassium oxodiperoxo(pyridine-2-carboxylato)-



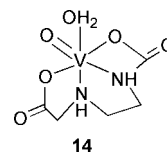
Mono- and bis-(peroxo)oxovanadate(v) complexes

vanadate(v) and potassium (3-hydroxypyridine-2-carboxylato)oxodiperoxovanadate(v), are prone to decomposition in aqueous solutions,¹¹¹ a limiting factor for potential pharmaceutical use. The most stable bisperoxovanadate compound of the $[\text{VO}(\text{O}_2)_2(\text{L}-\text{L}')]\text{V}^{+}$ type, potassium oxodiperoxo(1,10-phenanthroline)vanadate(v) trihydrate [bpV(phen)], has been tested *in vivo*, both by intrajugular injection of 6 $\mu\text{mol kg}^{-1}$,¹¹⁶ and by oral gavage, at 20 to 200 $\mu\text{mol kg}^{-1}$,¹¹⁸ and proved to be effective as a glucose lowering agent in very short-term trials. At doses of 0.75–6 $\mu\text{mol V kg}^{-1}$, bpV(pic), bpV(phen) and bpV(Me₂phen) were effective in lowering plasma glucose in BB rats (a spontaneously diabetic rat which is a good model of type I diabetes) whether given intravenously (i.v.), i.p., or subcutaneously, but not by oral gavage.¹¹⁸ The mechanism

of action of peroxovanadates unfortunately appears to involve increased intracellular oxidative stress,^{114,115} and PTPase inhibition is irreversible, unlike with oxovanadium(IV) compounds.^{119,120}

Peptide-bound oxovanadium(IV) complexes

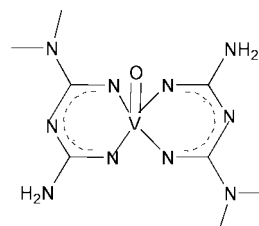
A very recent development in design of insulin enhancing agents is the preparation of vanadium-containing complexes of the $[\text{VO}(\text{XeX})(\text{H}_2\text{O})]$ type, **14**.^{121,122} Like VO(pic)₂ complexes, these tetradentate oxovanadium(IV) complexes, where XeX is N,N'-ethylenebis(amino acid), have N₂O₂ coordination.



Complexes containing glycine, alanine, valine, methionine and proline were prepared.¹²² Using FFA release from adipocytes as a measure of insulin mimesis, investigators found that complexes with Λ -type configuration, containing achiral or D-amino acids, at 0.01–1.0 mM V in the tissue homogenates, had a higher *in vitro* insulin-like activity than complexes with Δ -type configuration.¹²² Relative stimulation of FFA release was also correlated with increasing lipophilicity in this particular fat cell assay.

Vanadium-containing complexes with other oral Hypoglycemic agents

Another design strategy for insulin enhancing agents involves combining known hypoglycemic agents (or their close analogues) with oxovanadium(IV) in one, potentially synergistically effective, compound.¹²³ Biguanides, most notably metformin, are in pharmaceutical use as oral hypoglycemic agents, for treatment of type 2 diabetes. A variety of biguanide complexes were prepared and tested. Suspensions in 3% gum arabic were administered by oral gavage for *in vivo* testing, which showed both i.p. (at 0.12 mmol kg⁻¹) and oral (at 0.6 mmol kg⁻¹) insulin-mimetic activity, though without any sustained response, and with no evidence of additivity between the vanadyl and oral hypoglycemic components.¹²³



Conclusion

Design of vanadium-containing compounds as insulin enhancing agents can take advantage of vanadium's diverse characteristics, which render it an ideal, and perhaps unique, choice for stepping in as a regulator of cell metabolism, where insulin is insufficient or underutilized. In combination with appropriate ligands, with the potential to increase gastrointestinal absorption, target insulin-responsive tissues, and minimize toxicities, vanadium's potential as an insulin-enhancing agent can greatly be improved. Oxovanadium(IV) compounds, especially BMOV and analogues, are clearly effective in improving glucose uptake, decreasing plasma lipids, regulating thyroid hormone production, and ameliorating diabetic cardiomyopathy, retinopathy, and renal hypertrophy. Some oxovanadium(V) compounds have also shown promise as insulin

enhancing agents, especially in acute and *in vitro* studies. Determining the relative efficacy of new vanadium-containing insulin enhancing agents is an ongoing concern. Biological testing in whole animals is time-consuming, expensive, and has limited sensitivity to differentiate between structurally similar compounds. Cell culture and *in vitro* testing require sufficiently higher concentrations of compounds that the rankings may have limited transferability to the *in vivo* situation. As our understanding increases of the mechanisms of vanadium's insulin enhancing effects, our ability to devise a consistent, simple and fast assay will, no doubt, also increase. At this time, bis(maltolato)oxovanadium(IV) remains the 'benchmark' compound, against which all others are measured and, so far, generally found wanting in one respect or another.⁷

Acknowledgements

The authors gratefully acknowledge the Medical Research Council of Canada for grant support, the Natural Sciences and Engineering Research Council of Canada for personnel support and scholarships, and our many talented co-workers, who are listed in the references. We would also like to thank Nycomed Salutar, Kinetek Pharmaceuticals, Inc. and Angiotech Pharmaceuticals, Inc., for research support, and the Royal Society of Chemistry for a Journals Grant for International Authors.

References

- 1 E. L. Tolman, E. Barris, M. Burns, A. Pansisni and R. Partridge, *Life Sci.*, 1979, **25**, 1159.
- 2 C. E. Heyliger, A. G. Tahiliani and J. H. McNeill, *Science*, 1985, **227**, 1474.
- 3 K. H. Thompson, V. G. Yuen, J. McNeill and C. Orvig in *Vanadium Compounds: Chemistry, Biochemistry, and Therapeutic Applications*, eds. A. S. Tracey and D. C. Crans, Oxford University Press, New York, NY, 1998, pp. 329–343.
- 4 K. H. Thompson, J. H. McNeill and C. Orvig, *Chem. Rev.*, 1999, **99**, 2561.
- 5 C. Orvig, K. H. Thompson, M. Battell and J. H. McNeill, in *Metal Ions in Biological Systems*, eds. H. Sigel and A. Sigel, Springer-Verlag, Heidelberg, 1999, vol. 31, pp. 575–594.
- 6 C. Orvig, K. H. Thompson, M. C. Cam and J. H. McNeill, in *Uses of Inorganic Chemistry in Medicine*, ed. N. D. Farrell, The Royal Society of Chemistry, Cambridge, 1999, pp. 93–108.
- 7 B. A. Reul, S. S. Amin, J.-P. Buchet, L. N. Ongemba, D. C. Crans and S. M. Brichard, *Br. J. Pharmacol.*, 1999, **126**, 467.
- 8 K. H. Thompson, J. H. McNeill and C. Orvig, in *Topics in Biological Inorganic Chemistry*, eds. M. J. Clarke and P. J. Sadler, Springer-Verlag, Heidelberg, 1999, vol. 2, pp. 139–158.
- 9 K. H. Thompson, J. H. McNeill and C. Orvig, *Rev. Port. Quim.*, 1997, **4**, 3.
- 10 F. H. Nielsen, in *Vanadium Compounds: Chemistry, Biochemistry, and Therapeutic Applications*, eds. A. S. Tracey and D. C. Crans, The American Chemical Society, Oxford University Press, New York, NY, 1998, pp. 297–307.
- 11 G. R. Willsky, A. B. Goldfine and P. J. Kostyniak, in *Vanadium Compounds: Chemistry, Biochemistry, and Therapeutic Applications*, eds. A. S. Tracey and D. C. Crans, The American Chemical Society, Oxford University Press, New York, NY, 1998, pp. 278–296.
- 12 R. B. Rucker, B. Lonnerdal and C. L. Keen, in *Physiology of the Gastrointestinal Tract*, ed. L. R. Johnson, Raven Press, New York, 1994, pp. 2183–2202.
- 13 D. C. Crans, M. Mahroof-Tahir and A. D. Keramidas, *Mol. Cell. Biochem.*, 1995, **153**, 17.
- 14 K. A. Robinson, *Proc. R. Soc. London, Ser. B*, 1981, **212**, 65.
- 15 H. Degani, M. Gochin, S. J. D. Karlsh and Y. Shechter, *Biochemistry* 1981, **20**, 5795.
- 16 E. Sabbioni, E. Marafante, L. Amantini, L. Ubertalli and C. Birattari, *Bioinorg. Chem.*, 1978, **8**, 503.
- 17 W. R. Harris, S. R. Friedman and D. Silberman, *J. Inorg. Biochem.*, 1984, **20**, 157.
- 18 E. Sabbioni and E. Marafante, *Bioinorg. Chem.*, 1978, **9**, 389.
- 19 G. R. Willsky, D. A. White and B. C. McCabe, *J. Biol. Chem.*, 1984, **259**, 13281.
- 20 N. D. Chasteen, J. K. Grady and C. E. Holloway, *Inorg. Chem.*, 1986, **25**, 2754.
- 21 M. J. Gresser and A. S. Tracey, in *Vanadium in Biological Systems*, ed. N. D. Chasteen, Kluwer Academic, Dordrecht, 1990, pp. 63–80.
- 22 R. L. Van Etten, P. P. Waymack and D. M. Rehkop, *J. Am. Chem. Soc.*, 1974, **96**, 6782.
- 23 W. Plass, *Angew. Chem.*, 1999, **38**, 909.
- 24 E. Barrand, *J. Biol. Soc. Chil. Quim.*, 1987, **42**, 247.
- 25 I. A. Setyawati, K. H. Thompson, V. G. Yuen, Y. Sun, M. Battell, D. M. Lyster, C. Vo, T. J. Ruth, S. Zeisler, J. H. McNeill and C. Orvig, *J. Appl. Physiol.*, 1998, **84**, 569.
- 26 N. D. Chasteen, *Struct. Bonding (Berlin)*, 1983, **53**, 105.
- 27 K. Fukui, H. Ohya-Nishiguchi, M. Nakai, H. Sakurai and H. Kamada, *FEBS Lett* 1995, **368**, 31.
- 28 S. A. Dikanov, B. D. Liboiron, K. H. Thompson, E. Vera, V. G. Yuen, J. H. McNeill and C. Orvig, *J. Am. Chem. Soc.*, 1999, **121**, 11004.
- 29 A. Butler and J. V. Walker, *Chem. Rev.*, 1993, **93**, 1937.
- 30 E. O. Uthus and F. H. Nielsen, *Magnesium Trace Elem.*, 1990, **9**, 219.
- 31 I. G. Macara, *Trends Biochem. Sci.*, 1980, **5**, 92.
- 32 D. W. Boyd and K. Kustin, *Adv. Inorg. Biochem.*, 1984, **6**, 311.
- 33 B. Cheatham and C. R. Kahn, *Endocr. Rev.*, 1995, **16**, 117.
- 34 A. Morinville, D. Maysinger and A. Shaver, *Trends Pharmacol. Sci.*, 1998, **19**, 452.
- 35 G. Elberg, J. Li and Y. Shechter, in *Vanadium in the Environment. Part 2. Health Effects*, ed. J. O. Nriagu, John Wiley & Sons, Inc., New York, 1998, pp. 277–296.
- 36 Y. Shechter and A. Shisheva, *Endeavour, New Ser.*, 1993, **17**, 27.
- 37 P. J. Stankiewicz and A. S. Tracey, in *Metal Ions in Biological Systems*, eds. H. Sigel and A. Sigel, Marcel Dekker, Inc., New York, 1995, vol. 31, pp. 249–286.
- 38 Y. Shechter, J. Meyerovitch, Z. Farfel, J. Sack, R. Bruck, S. Bar-Meir, S. Amir, H. Degani and S. J. D. Karlsh, in *Vanadium in Biological Systems*, ed. N. D. Chasteen, Kluwer, Dordrecht, 1990, pp. 129–142.
- 39 Y. Shechter, J. Li, J. Meyerovitch, D. Gefel, R. Bruck, G. Elberg, D. S. Miller and A. Shisheva, *Mol. Cell. Biochem.*, 1995, **153**, 39.
- 40 I. G. Fantus, F. Ahmad and G. Deragon, *Diabetes*, 1994, **43**, 375.
- 41 J. Li, G. Elberg, D. C. Crans and Y. Shechter, *Biochemistry*, 1996, **35**, 8314.
- 42 A. Shisheva and Y. Shechter, *Endocrinology*, 1993, **133**, 1562.
- 43 J. Meyerovitch, Z. Farfel, J. Sack and Y. Shechter, *J. Biol. Chem.*, 1987, **262**, 6658.
- 44 S. Ramanadham, J. J. Mongold, R. W. Brownsey, G. H. Cros and J. H. McNeill, *Am. J. Physiol.*, 1989, **257**, H904.
- 45 H. Sakurai, K. Tsuchiya, M. Nukatsuka, M. Sofue and J. Kawada, *J. Clin. Biochem. Nutr.* 1990, **8**, 193.
- 46 K. H. Thompson, J. Leichter and J. H. McNeill, *Biochem. Biophys. Res. Commun.* 1993, **197**, 1549.
- 47 O. Blondel, J. Simon, B. Chevalier and B. Portha, *Am. J. Physiol.*, 1990, **258**, E459.
- 48 A. S. Clark, J. M. Fagan and W. E. Mitch, *Biochem. J.*, 1985, **232**, 273.
- 49 A. Green, *Biochem. J.*, 1986, **238**, 663.
- 50 A. K. Saxena, P. Srivastava and N. Z. Baquer, *Eur. J. Pharmacol.*, 1992, **216**, 123.
- 51 R. A. Pederson, S. Ramanadham, A. M. J. Buchan and J. H. McNeill, *Diabetes*, 1989, **38**, 1390.
- 52 M. C. Cam, B. Rodrigues and J. H. McNeill, *Eur. J. Endocrinol.*, 1999, **141**, 546.
- 53 A. S. Tracey and M. J. Gresser, *Proc. Natl. Acad. Sci. U.S.A.*, 1986, **83**, 609.
- 54 R. A. Mooney, K. L. Bordwell, S. Luhowskyj and J. E. Casnelle, *Endocrinology*, 1989, **124**, 422.
- 55 J. M. Denu, D. L. Lohse, J. Vijayalakshmi, M. A. Saper and J. E. Dixon, *Proc. Natl. Acad. Sci. U.S.A.*, 1996, **93**, 2493.
- 56 M. Zhang, M. Zhou, R. L. Van Etten and C. V. Stauffacher, *Biochemistry*, 1997, **36**, 15.
- 57 J. A. Gordon, *Meth. Enzymol.*, 1991, **201**, 477.
- 58 I. G. Fantus and E. Tsiani, *Mol. Cell. Biochem.*, 1998, **182**, 109.
- 59 A. Shisheva and Y. Shechter, *Biochemistry*, 1992, **31**, 8059.
- 60 E. Tsiani and I. G. Fantus, *Trends Endocrinol. Metab.*, 1997, **8**, 51.
- 61 M. David-Dufilho, M.-G. Pernollet, M. Morris, C. Astarie-Dekequer and M.-A. Devynck, *Life Sci.*, 1993, **54**, 267.
- 62 L. Sandrasegarane and V. Gopalakrishnan, *Life Sci.*, 1995, **56**, 169.
- 63 K. Terziyski, R. Tzenova, E. Milieva and S. Vladeva, *Folia Medica*, 1999, **41**, 34.
- 64 G. R. Ehring, H. H. Kerschbaum, C. M. Fanger, C. Eder, H. Rauer and M. D. Cahalan, *J. Immunol.*, 2000, **164**, 679.

- 65 B. R. Nechay, *Annu. Rev. Pharmacol. Toxicol.*, 1984, **24**, 501.
- 66 Y. Shechter, *Diabetes*, 1990, **39**, 1.
- 67 J. W. Lohr, M. I. Bennett, M. A. Pochal, J. McReynolds, M. Acara and G. R. Willsky, *Res. Commun. Chem. Pathol. Pharmacol.*, 1991, **72**, 191.
- 68 A. K. Saxena, P. Srivastava, R. K. Kale and N. Z. Baquer, *Biochem. Int.*, 1992, **26**, 59.
- 69 K. H. Thompson and J. H. McNeill, *Res. Commun. Chem. Pathol. Pharmacol.*, 1993, **80**, 187.
- 70 G. Ugazio, S. Bosia, E. Burdino and F. Grignolo, *Res. Commun. Mol. Pathol. Pharmacol.*, 1994, **85**, 313.
- 71 J. J. Mongold, G. H. Cros, L. Vian, A. Tep, S. Ramanadham, G. Siou, J. Diaz, J. H. McNeill and J. J. Serrano, *Pharmacol. Toxicol.*, 1990, **67**, 192.
- 72 S. Dai, K. H. Thompson, E. Vera and J. H. McNeill, *Pharmacol. Toxicol.*, 1994, **75**, 265.
- 73 L. Rossetti and M. R. Laughlin, *J. Clin. Invest.*, 1989, **84**, 892.
- 74 R. A. J. Challiss, B. Leighton, F. J. Lozeman, L. Budohoski and E. A. Newsholme, *Biochem. Pharmacol.*, 1987, **36**, 357.
- 75 S. P. Wolff, Z. Y. Jiang and J. V. Hunt, *Free Rad. Biol. Med.*, 1991, **10**, 339.
- 76 P. Srivastava, A. K. Saxena, R. K. Kale and N. Z. Baquer, *Res. Commun. Chem. Path. Pharmacol.*, 1993, **80**, 283.
- 77 K. H. Thompson and J. H. McNeill, in *Trace Elements in Man and Animals -9: Proc. 9th Int. Symp. Trace Elem. Man Animals*, eds. P. W. F. Fischer, M. R. L'Abbe, K. A. Cockell and R. S. Gibson, NRC Research Press, Ottawa, 1997, pp. 349–350.
- 78 G. Boden, X. Chen, J. Ruiz, G. D. V. van Rossum and S. Turco, *Metabolism*, 1996, **45**, 1130.
- 79 A. B. Goldfine, D. C. Simonson, F. Folli, M.-E. Patti and C. R. Kahn, *J. Clin. Endocrinol. Metab.*, 1995, **80**, 3311.
- 80 N. Cohen, M. Halberstam, P. Shlimovich, C. J. Chang, H. Shamoon and L. Rossetti, *J. Clin. Invest.*, 1995, **95**, 2501.
- 81 A. B. Goldfine, G. R. Willsky and C. R. Kahn, in *Vanadium Compounds: Chemistry, Biochemistry and Therapeutic Applications*, eds. A. S. Tracey and D. C. Crans, Oxford University Press, New York, 1998, pp. 353–367.
- 82 M. Halberstam, N. Cohen, P. Shlimovich, C. J. Chang, H. Shamoon and L. Rossetti, *J. Clin. Invest.*, 1996, **45**, 659.
- 83 F. H. Nielsen, in *Metal Ions in Biological Systems*, eds. H. Sigel and A. Sigel, Marcel Dekker, Inc., New York, 1995, vol. 31, pp. 543–574.
- 84 W. O. Nelson, T. B. Karpishin, S. J. Rettig and C. Orvig, *Can. J. Chem.*, 1988, **66**, 123.
- 85 D. J. Clevette and C. Orvig, *Polyhedron*, 1990, **9**, 151.
- 86 M. A. Barrand, B. A. Callingham and R. C. Hider, *Br. J. Pharmacol.*, 1987, **39**, 203.
- 87 D. J. Clevette, W. O. Nelson, A. Nordin, C. Orvig and S. Sjoberg, *Inorg. Chem.*, 1989, **28**, 2079.
- 88 W. O. Nelson, T. B. Karpishin, S. J. Rettig and C. Orvig, *Inorg. Chem.*, 1988, **27**, 1045.
- 89 B. L. Ellis, A. K. Duhme, R. C. Hider, M. B. Hossain, S. Rizvi and D. van der Helm, *J. Med. Chem.*, 1996, **39**, 3659.
- 90 S. J. Lord, N. A. Epstein, R. L. Paddock, C. M. Vogels, T. L. Hennigar, N. J. Zaworotko, W. R. Driedzic, T. L. Broderick and S. A. Westcott, *Can. J. Chem.*, 1999, **77**, 1249.
- 91 J. H. McNeill, V. G. Yuen, H. R. Hoveyda and C. Orvig, *J. Med. Chem.*, 1992, **35**, 1489.
- 92 P. Caravan, L. Gelmini, N. Glover, F. G. Herring, H. Li, J. H. McNeill, S. J. Rettig, I. A. Setyawati, E. Shuter, Y. Sun, A. S. Tracey, V. G. Yuen and C. Orvig, *J. Am. Chem. Soc.*, 1995, **117**, 12759.
- 93 G. R. Hanson, Y. Sun and C. Orvig, *Inorg. Chem.*, 1996, **35**, 6509.
- 94 M. Melchior, S. J. Rettig, K. H. Thompson, V. G. Yuen, J. H. McNeill and C. Orvig, manuscript in preparation.
- 95 M. Melchior, K. H. Thompson, J. M. Jong, S. J. Rettig, E. Shuter, V. G. Yuen, Y. Zhou, J. H. McNeill and C. Orvig, *Inorg. Chem.*, 1999, **38**, 2288.
- 96 H. Sakurai, K. Fujii, H. Watanabe and H. Tamura, *Biochem. Biophys. Res. Commun.*, 1995, **214**, 1095.
- 97 S. Fujimoto, K. Fujii, H. Yasui, R. Matsushita, J. Takada and H. Sakurai, *J. Clin. Biochem. Nutr.*, 1997, **23**, 113.
- 98 K. Elvingsson, A. González Baró and L. Pettersson, *Inorg. Chem.*, 1996, **35**, 3388.
- 99 Y. Sun, B. R. James, S. J. Rettig and C. Orvig, *Inorg. Chem.*, 1996, **35**, 1667.
- 100 V. G. Yuen, P. Caravan, L. Gelmini, N. Glover, J. H. McNeill, I. A. Setyawati, E. Shuter, Y. Zhou and C. Orvig, *J. Inorg. Biochem.*, 1997, **68**, 109.
- 101 H. Sakurai, Y. Hamada, S. Shimomura, S. Yamashita and K. Ishizu, *Inorg. Chim. Acta*, 1980, **46**, L119.
- 102 M. C. Cam, G. H. Cros, J.-J. Serrano, R. Lazaro and J. H. McNeill, *Diab. Res. Clin. Practice*, 1993, **20**, 111.
- 103 B. McCormick, *J. Inorg. Chem.*, 1968, **7**, 1965.
- 104 H. Sakurai, H. Sano, T. Takino and H. Yasui, *Chem. Lett.*, 1999, 913.
- 105 H. Watanabe, M. Nakai, K. Komazawa and H. Sakurai, *J. Med. Chem.*, 1994, **37**, 876.
- 106 J. A. Bonadies and C. J. Carrano, *J. Am. Chem. Soc.*, 1986, **108**, 4088.
- 107 N. Durai and G. Saminathan, *J. Clin. Biochem. Nutr.*, 1997, **22**, 31.
- 108 S. S. Amin, K. Cryer, B. Zhang, S. K. Durra, S. S. Eaton, O. P. Anderson, S. M. Miller, B. A. Reul, S. M. Brichard and D. C. Crans, *Inorg. Chem.*, 2000, **39**, 406.
- 109 Y. Shechter, A. Shisheva, R. Lazar, J. Libman and A. Shanzer, *Biochemistry*, 1992, **31**, 2063.
- 110 I. Goldwaser, J. Li, E. Gershonov, M. Armoni, E. Karnieli, M. Fridkin and Y. Shechter, *J. Biol. Chem.*, 1999, **274**, 26617.
- 111 A. Shaver, J. B. Ng, D. A. Hall, B. Soo Lum and B. I. Posner, *Inorg. Chem.*, 1993, **32**, 3109.
- 112 S. Kadota, G. Fantus, G. Deragon, H. J. Guyda, B. Hersh and B. I. Posner, *Biochem. Biophys. Res. Commun.*, 1987, **147**, 259.
- 113 B. I. Posner, R. Faure, J. W. Burgess, A. P. Bevan, D. Lachance, G. Zhang-Sun, I. G. Fantus, J. B. Ng, D. A. Hall, B. Soo Lum and A. Shaver, *J. Biol. Chem.*, 1994, **269**, 4460.
- 114 C. M. Krejsa, S. G. Nadler, J. M. Esselstyn, T. J. Kavanagh, J. A. Ledbetter and G. L. Schieven, *J. Biol. Chem.*, 1997, **272**, 11541.
- 115 D. C. Crans, A. D. Keramida, H. Hoover-Litty, O. P. Anderson, M. M. Miller, L. M. Lemoine, S. Pleasic-Williams, M. Vandenberg, A. J. Rossomoando and L. J. Sweet, *J. Am. Chem. Soc.*, 1997, **119**, 5447.
- 116 J.-F. Yale, D. Lachance, A. P. Bevan, C. Vigeant, A. Shaver and B. I. Posner, *Diabetes*, 1995, **44**, 1274.
- 117 A. P. Bevan, J. W. Burgess, J.-F. Yale, P. G. Drake, D. LaChance, G. Baquiran, A. Shaver and B. I. Posner, *Am. J. Physiol.*, 1995, **268**, E60.
- 118 J.-F. Yale, C. Vigeant, C. Nardolillo, Q. Chu, J.-Z. Yu, A. Shaver and B. I. Posner, *Mol. Cell. Biochem.*, 1995, **153**, 181.
- 119 C. Hiort, J. Goodisman and J. C. Dabrowiak, *Biochemistry*, 1996, **35**, 12354.
- 120 I. Castan, J. Wijkander, V. Manganiello and E. Degerman, *Biochem. J.*, 1999, **339**, 281.
- 121 K. Kawabe, M. Tadokoro, Y. Kojima, Y. Fujisawa and H. Sakurai, *Chem. Lett.*, 1998, 9.
- 122 K. Kawabe, M. Tadokoro, A. Ichimura, Y. Kojima, T. Takino and H. Sakurai, *J. Am. Chem. Soc.*, 1999, **121**, 7937.
- 123 L. C. Y. Woo, V. G. Yuen, K. H. Thompson, J. H. McNeill and C. Orvig, *J. Inorg. Biochem.*, 1999, **76**, 251.