# 4.5 Diabetes

## 4.5.1 Vanadium and Diabetes

The signalling hormone insulin is essential for the metabolism of carbohydrate and fat. It is secreted by the pancreas in response to elevated blood glucose levels and promotes glucose uptake by the liver, gut or muscle tissue leading to either its storage or use in energy production as required. A deficiency of insulin, or cellular resistance to its function, results in diabetes in humans. In Type I diabetes, known as insulin dependent diabetes, the pancreas secretes insufficient insulin and regular injections of insulin that are necessary to compensate. In Type II diabetes, which accounts for some 90% of cases, sufficient insulin is secreted but the cellular response is impaired leading to hyperglycemia. Type II diabetes is therefore described as insulin independent and patients show 'insulin resistance'. Administering doses of exogenous insulin to the patient offers a means of treating both types of diabetes and is the primary treatment for almost all Type I and many Type II cases. Insulin is a protein and, as a consequence, orally administered insulin does not deliver a biologically active hormone so that unpleasant and less convenient subcutaneous injections are necessary. Many other types of compound have been investigated for the treatment of diabetes and an oral drug which can mimic the effect of insulin is particularly desirable. Unfortunately, the required combination of good absorption, low toxicity, stability *in vivo* and insulin mimetic behaviour has proven elusive. In many examples promising *in vitro* results have not been translated into effective treatments *in vivo*.

The discovery by Lyonnet and Martin in 1899 that diabetic patients excreted less glucose in their urine after treatment with vanadate, containing V(+5) in $VO_4^{3-}$, indicated that transition metal compounds may have an important rôle to play in the treatment of diabetes. However the subsequent discovery of insulin directed the focus of research away from inorganic compounds and only more recently has interest in metal compounds revived following the finding that micromolar vanadate could inhibit phosphohydrolases. In fact several transition metal compounds have been found to promote glucose uptake and these are sometimes referred to as insulin mimics. However, such compounds do not exhibit the full functionality of insulin and, for example, they do not counteract catabolic hormones such as glucagon or suppress glucose production in the liver. Nonetheless there is continued interest in metal complexes which might have therapeutic applications for Type II diabetes where insulin is secreted but ineffective. In particular metal compounds offer the possibility of an oral drug which could overcome insulin resistance and, in this respect, vanadium complexes have become a focus of research.

## 4.5.2 Vanadium Chemistry

As an early 1st row d-block metal vanadium can exhibit a wide range of oxidation states among which +3, +4 and +5 are accessible under

physiological conditions. The oxidation state (+3) species typically contain simple $V^{3+}$ ions in an approximately octahedral coordination geometry, although 7-coordinate complexes are also known. The oxidation state +4 species typically contain the vanadyl ion, $VO^{2+}$, in a square pyramidal coordination environment. Vanadium in oxidation state +5 is present in the tetrahedral vanadate ion, $VO_4^{3-}$ which is usually present as a mixture of the protonated species $HVO_4^{2-}$ and $H_2VO_4^{-}$ rather like the hydrogen phosphates $HPO_4^{2-}$ and $H_2PO_4^{-}$. Complexes containing the vanadium(+5) dioxo ion, $VO_2^{3+}$, are also known and may exhibit coordination numbers ranging from 5–7 depending on the nature of the ligands present. Vanadium exhibits a rich redox chemistry but in the medical context the oxidation states V(+4) and V(+5) appear to be of primary importance, both being found to participate in extra- and intra-cellular equilibria. Vanadium has been shown to be important for development and growth in animal studies and the human body pool is typically in the range 100–200 micrograms. The toxic effects of vanadium depend on the route of administration and the chemical form in which the metal is initially present. The principle adverse effect is gastrointestinal distress. Vanadium accumulation in tissue, particularly in bone, does not appear to have significant harmful effects but could be a potential problem with its use in therapy if acceptable clearance rates cannot be demonstrated.

### 4.5.3 Vanadium Salts as Insulin Mimics

A variety of vanadium compounds have shown promising insulin mimetic activity *in vitro* but most have not proven to have suitable *in vivo* properties for therapeutic applications. Tests for *in vivo* insulin mimetic activity often involve the use of diabetic rodents as models. The diabetes may be spontaneously present in rats bred for the purpose or may be chemically induced. One widely accepted test for *in vivo* insulin mimetic activity involves rodents in which diabetes has been induced by treatment with streptozotocin (STZ). STZ is an antibiotic, which attacks the insulin secreting cells in the pancreas leading to reduced insulin secretion and the development of diabetic characteristics, including high glucose levels in blood and urine. Although not a complete model for Type I diabetes in humans, the STZ-diabetic rat does offer a reproducible and reliable test for *in vivo* insulin mimetic behaviour. Data from human studies is rather limited but studies of some vanadium compounds have been undertaken. Several inorganic vanadium compounds have been evaluated in limited clinical trials with otherwise healthy Type I and Type II diabetic subjects. Doses of 125 mg $day^{-1}$ of sodium orthovanadate ($Na_3VO_4$) over 2 weeks produced increased mean glucose metabolism rates in two out of five Type I subjects and all five Type II subjects. Treatment of Type II subjects with 100 mg $day^{-1}$ of vanadyl sulfate for 3 weeks produced improved insulin sensitivity, increased glucose disposal and reduced hepatic glucose production. These effects persisted for 2 weeks following the cessation of treatment but in both studies there was mild gastrointestinal intolerance. In another trial using

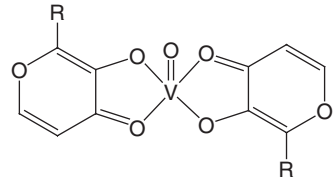
100 mg $day^{-1}$ of $VO(SO_4).3H_2O$ over 6 weeks, improved insulin sensitivity was observed in three out of five Type II subjects who were receiving oral hypoglycemics. Lower doses of vanadyl sulfate have been tried and at 25 mg V $day^{-1}$ there was no change in glucose and lipid metabolic parameters. However at 50 mg V $day^{-1}$ increased insulin sensitivity was found but with no change in plasma glucose levels.

Compared to the limited range of inorganic vanadium compounds which might be used in therapy, incorporating vanadium into a complex with organic ligands offers a far more versatile means of delivering the metal. The absorption, biodistribution, tissue uptake, retention and insulin mimetic activity of vanadium might be improved by the correct choice of ligand and useful therapeutic effects might be achieved at lower doses. In recent years a variety of complexes have been synthesised and tested for insulin mimetic properties. Related molybdate and tungstate compounds generally show lower insulin mimetic activity than vanadium compounds but are less toxic and may warrant further investigation.

### 4.5.4 Vanadium Coordination Compounds as Insulin Mimics

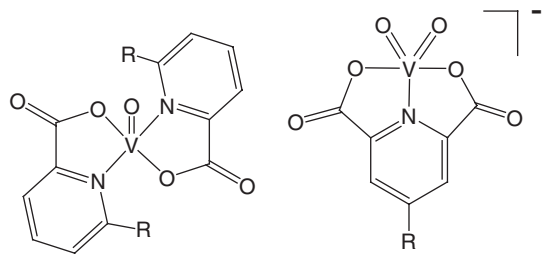
One of the most studied insulin mimetic vanadium complexes is the neutral water soluble vanadyl complex of maltol (maH) BMOV, **127a** $[VO(ma)_2]$. This complex has a square pyramidal coordination geometry and the stability constants for its formation are given by $\log K_1 = 8.80$, $\log K_2 = 7.51$, $\log \beta_2 = 16.31$. The complex is oxidised by dioxygen to the V(+5) dioxo complex $[VO_2(ma)_2]^-$ which does not show insulin mimetic properties. BMOV was found to be two to three times more effective as an insulin mimic than vanadyl sulfate. In STZ-diabetic rats 50% blood glucose lowering was achieved at an initial oral dose in drinking water of 0.4 mmol $kg^{-1}$ $day^{-1}$ decreased to 0.2 mmol $kg^{-1}$ $day^{-1}$ for maintenance. No evidence of toxicity was found over a 6-month period. BMOV has also been found to prevent some pathological consequences of diabetes, such as cardiomyopathy, and to attenuate hyperinsulinemia and hyperlipidemia in genetically diabetic rats. Vanadium residence times of 31 days, 7 h and 4 h were found respectively in bone, liver and kidney from studies with radiolabelled $[^{48}VO(ma)_2]$.

Compared to BMOV its ethyl substituted counterpart BEOV, **127b** $[VO(ema)_2]$, shows longer turnover times, is a little less soluble, has slightly higher lipophilicity and is more stable towards hydrolysis. This illustrates the way in which small structural changes in the organic ligands can be used to modify the properties of a metal complex and improve its suitability for therapeutic applications. BEOV successfully completed Phase 1 clinical trials in 2000 and doses to healthy human subjects approached therapeutic levels at 90 mg $day^{-1}$. The compound was well tolerated by the gastrointestinal tract, liver and kidneys and no serious side effects were found at three times the expected therapeutic levels in blood. Administration prior to meals improved oral availability of the drug.



**127 a** BMOV, R = $CH_3$, $[VO(ma)_2]$
**127 b** BEOV, R = $C_2H_5$, $[VO(ema)_2]$

A variety of other vanadium complexes have been investigated as potential insulin mimics. These include picolinic acid derivatives which afford square planar V(+4) complexes such as VOPA, **128a**, and its more lipophilic analogue VOMPA, **128b**, as well as the 5-coordinate dipicolinate V(+5) complexes $[VO_2(dipic)]^-$, **129a**, and $[VO_2(dipic\text{-}OH)]^-$, **129b**. In STZ-diabetic rats VOPA, given at an oral dose of 10 mg V $kg^{-1}$ day $^1$ for 2 days followed by 5 mg V $kg^{-1}$ $day^{-1}$ for 11 days produced normal plasma glucose levels after 7 days. The rats remained almost normoglycemic for a further 30 days without further treatment. A higher oral dose of 25 mg V $kg^{-1}$ $day^{-1}$ gave faster glucose lowering but with diarrhoea as one side effect and other tests at 60 mg V $kg^{-1}$ $day^{-1}$ produced evidence of gastrointestinal irritation. In comparison VOMPA administered orally at a dose of 10 mg V $kg^{-1}$ $day^{-1}$ produced a more sustained response persisting for 80 days after treatment ceased. Compared to BMOV, VOPA has lower aqueous solubility and, at an equivalent dose, appears to produce more gastrointestinal irritation.

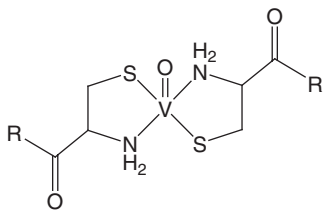


**128 a** VOPA, R = H
**128 b** VOMPA, R = $CH_3$,

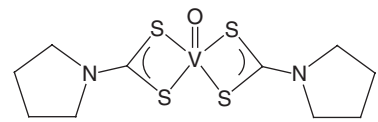
**129 a** R = H, $[VO_2(dipic]^-$
**129 b** R = OH, $[VO_2(dipic\text{-}OH)]^-$

Other V(+4) complexes which have been investigated include the cysteine methyl ester complex VCME, **130a** and the related cysteine octylamide complex Naglivan, **130b**, a dithiocarbamate complex V-P, **131**, the tetradentate ligand derivative [VO(salen)], **132**, and the $\beta$-diketonate derivatives **133a** and **b**. Some dipeptide derivatives and [VO(edda)], **134**, have also been studied. At a dose of 10 mg V $kg^{-1}$ $day^{-1}$ VCME was more effective in normalising glucose levels in STZ-diabetic rats than related complexes with malonate, oxalate or tartarate ligands but, although toxic effects were not apparent at the effective dose, the compound was lethal at 10 times this dose. At this dose V–P produced

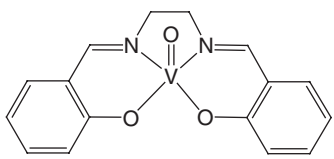
normoglycemia in 2 days and this was maintained using a dose of 5 mg V $kg^{-1}$ $day^{-1}$. However, oral administration was less effective than intraperitoneal injection which had the side effects of weight loss and increased bilirubin levels. [VO(salen)] was tested in alloxan-diabetic rats for 30 days at an oral dose of 7.6 mg V $kg^{-1}$ $day^{-1}$ and reduced blood glucose levels from hyper- to hypoglycemic levels. The complex [VO(edda)], **134**, has been tested *in vitro* for the inhibition of lipolysis and showed significant dose dependent activity. Similar chiral Δ-configuration complexes inhibited free fatty acid release at concentrations which decreased with increasing complex lipophilicity, an effect not found with similar achiral complexes. Several peroxo-complexes of vanadium have also been tested and shown promise in *in vitro* trials but have limited hydrolytic stability. They can also participate in reactions which may lead to radical formation making them unsuited for clinical use.



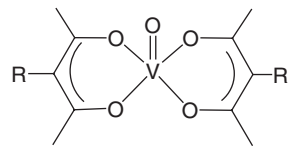
**130 a** VCME, R = $OCH_3$,
**130 b** Naglivan, R = $NHC_8H_{17}$,



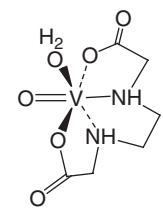
**131**
V-P



**132**
[VO(salen)]

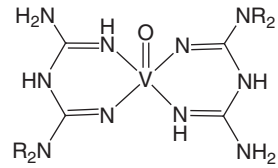


**133 a** R = H, $[VO(acac)_2]$
**133 b** R = $C_2H_5$, $[VO(Etacac)_2]$



**134**
[VO(edda)]

An attempt has been made to demonstrate a synergistic effect by coordinating vanadium to Metformin (metfH), a ligand with known insulin enhancing properties in its own right. At an oral dose of 30 mg V $kg^{-1}$ day $^{1}$ the complex $[VO(metf)_2]$, **135a**, normalised plasma glucose levels in STZ-diabetic rats within 24 h but this effect was not maintained. At this dose level metfH had no effect. The limited data available showed the glucose lowering capacity of $[VO(metf)_2]$ to be similar to that of BMOV.



**135 a** R = $CH_3$, $[VO(metf)_2]$
**135 b** R = H, $[VO(big)_2]$

Compared to V(+4), few V(+5) compounds have been shown to have insulin mimetic properties. The V(+5) complex $[VO_2(dipic)]^-$ (**129a**) as been shown to produce glucose-lowering in cats and STZ-diabetic rats but is labile at pH 7 and is less effective than $VOSO_4$. Its hydroxyl substituted analogue $[VO_2(dipic\text{-}OH)]^-$ (**129b**) is more stable at neutral pH and this is associated with blood glucose lowering effects comparable with those of $VOSO_4$ but at a lower dose. A dose of 1.6 mmol V $kg^{-1}$ $day^{-1}$ of $VOSO_4$ resulted a reduction in blood glucose from *ca.* 4.7 mg $ml^{-1}$ to *ca.* 3.0 mg $ml^{-1}$ after 6 days. Similar results were obtained using $[VO_2(dipic\text{-}OH)]^-$ but at a dose of 1.0 mmol V $kg^{-1}$ $day^{-1}$.

## 4.5.5 Mechanism of Action of Vanadium Compounds

Some vanadium compounds can mimic most of the metabolic effects of insulin *in vitro* and the exact mechanism by which vanadium produces this behaviour has been the subject of intensive study in recent years. However, the exact mechanism by which vanadium produces insulin mimetic effects *in vivo* is not yet fully understood, although some important biological effects of vanadium have been explained. A common feature of the organic complexes of vanadium, compared to inorganic salts, appears to be improved tissue permeation by passive diffusion without causing significant changes in toxicity indices.

At a superficial level, since both vanadium and phosphorus have five valence electrons, vanadate, $VO_4^{3-}$, exhibits an obvious similarity to phosphate, $PO_4^{3-}$. To some extent vanadate, which is mostly in the form $H_2VO_4^-$ under physiological conditions, can mimic $H_2PO_4^-$. However, the elements V and P are otherwise chemically rather dissimilar. In particular the redox properties of vanadium are quite different from those of phosphorus and, while the V(+4) $\{V{=}O\}^{2+}$ moiety can exist under physiological conditions, there is no counterpart for this in phosphorus chemistry. The vanadyl ion, $\{V{=}O\}^{2+}$ has also been said to resemble to $Mg^{2+}$ and vanadium may have important effects on intracellular $Ca^{2+}$ metabolism.

As with many other d-block metals, *in vivo* vanadium readily associates with proteins such as transferrin, albumin and hemoglobin in addition to GSH. Two proteins in the blood may be important in vanadium transport. One is serum albumin which has many potential metal binding sites, at least 5 of which can enter into weak non-specific binding (log $K = 4.38$) of $VO^{2+}$. There is also a strong $Cu^{2+}$ binding site which binds $VO^{2+}$ more strongly (log $K = 6.41$) than

the non-specific sites. BMOV also appears to bind to serum albumin but only at the strong binding $Cu^{2+}$ site. The other blood protein to consider is the iron transport protein transferrin. Apo-transferrin has two Fe binding sites and is known to transport two $VO^{2+}$ units.

Both V(+5) vanadate and V(+4) vanadyl mimic the effects of insulin on hexose uptake and glucose metabolism in rat adipocytes. Initially inhibition of $Na^+$, $K^+$-ATPase by vanadyl(+4) was thought to be important but this has been shown not to be the case. More recent *in vitro* studies have shown that vanadate inhibits several enzymes in the liver, muscle and adipose tissue. These act collectively in the storage or utilisation of glucose and their function may be modified by the presence of vanadium.

Vanadate also blocks the actions of hormones which oppose the action of insulin. It has been shown that insulin binding to the cell surface site of the insulin activated protein tyrosine kinase leads to the autophosphorylation of three tyrosine moieties located in the $\beta$ subunit of the insulin IGF-1 receptor. This triggers tyrosine kinase activity leading to the phosphorylation of tyrosine in several cytosolic docking proteins which are then recognised by effector molecules including P13-kinase. Woortmannin, a strong inhibitor of P13-kinase, blocks the activation of glucose uptake, glucose oxidation, lipogenesis and glycogenesis by vanadate but does not block its antilipolytic action. A cytosolic tyrosine kinase (cyt-PTK) that is activated by vanadate has been obtained from rat adipocytes and partly purified. Cyt-PTK is activated by V(+5) vanadate but not by V(+4). It has been suggested that cyt-PTK is involved in the activation of glucose metabolism by vanadate but not the activation of hexose transport or its antilipolytic action. It also appears that vanadium complexes can inhibit phosphotyrosine phosphatases so that enzymes remain in their phosphorylated active state.

An important feature of insulin mimetic vanadium compounds is the multiplicity of their effects in treating diabetes. In experimental animals not only were the primary symptoms alleviated but secondary effects such as cardiomyopathy, cataract formation, changes in kidney morphology and thyroid imbalance were also moderated. One important application of coordination chemistry in this context will be to use ligand design to improve vanadium bioavailability and *in vivo* distribution.

## 4.6 Cardiovascular System

### 4.6.1 *In vivo* Management of Gaseous Compounds

Two important applications of metal containing pharmaceuticals relating to the cardiovascular system are the management of nitric oxide, NO and dioxygen, $O_2$. Both exist as gases under ambient atmospheric conditions but must be stored or transported in an aqueous liquid medium. The other gaseous compound transported by the cardiovascular system is carbon dioxide, $CO_2$. This compound dissolves in aqueous physiological fluids in the form of