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The relationship between insulin and vanadium metabolism in insulin target tissues

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Abstract

Vanadium (V) is an orally effective treatment for diabetes, but relatively little is known about the mechanisms controlling its normal metabolism nor the long term pharmacokinetics of oral administration. We have examined the accumulation of V in various organs from rats fed liquid diet for up to 18 days, containing no additional V, 1.6, 80, or 160 μmole/kg/day as either sodium orthovanadate (SOV) or vanadyl sulfate (VS). V content was assayed using a sensitive neutron activation analysis method. The organs of the nonsupplemented animals contained widely varying concentrations (ng of V/g dry tissue weight) with brain < fat < blood < heart < muscle < lung < liver < testes < spleen < kidney. All organs accumulated V in a dose dependent manner. Not all organs showed steady state amount of V at 18 days, so additional rats were fed SOV or VS, switched to control diet, and assayed at 0, 4 and 8 days. From this data we calculated organ half lives of V. Insulin sensitive tissue tissues, such as liver and fat, had shorter half-lives than tissues that are relatively less insulin sensitive, such as spleen, brain and testes. SOV and VS fed rats showed similar patterns, but VS had somewhat shorter t½'s. Additional studies of old and young rats fed control diet for 45 days show accumulation of V in spleen and testes. These results indicate that vanadium metabolism varies widely among different organs, and that insulin, either directly or indirectly has effects on the retention of vanadium. This may have impact on the therapeutic use of vanadium in Type I diabetics with no insulin, or Type II patients who may be relatively hyperinsulinemic. (Mol Cell Biochem **153:** 95–102, 1995)

Key words: vanadium, insulin, radiochemical neutron activation analysis

Introduction

A consensus is forming that vanadium is likely an essential element [1–4], but definitive proof and the assignment of an exact function are still lacking. The majority of studies have found that its function is related to proper growth and development of the organism. Of related interest is the fact that vanadium has been shown to have growth factor-like effects. McKeehan *et al* [5] first showed that vanadium was required for optimal growth of fibroblasts in culture. Vanadium has been shown to have mitogenic responses in human fibroblasts and 3T3 and 3T6 cell lines [6–8]. Vanadium can have effects similar to fibroblast growth factor, epidermal growth factor and insulin [9–12]. Thus it appears that whatever vanadium's mechanism of action, it is related to similar actions of growth factors.

Vanadium's similarity to growth factors is probably best documented by its ability, at pharmacological doses, to mimic the actions of insulin. These effects and its important potential clinical use [13–17] have been demonstrated in studies with diabetic animals. Orally administered vanadate can normalize blood glucose levels in streptozotocin diabetic rats as well as restore cardiac function and increase lipogenesis [18, 19]. Glucose metabolism in general is normalized in diabetes [20, 21]. The vanadyl form of vanadium has also been shown to be effective in 'treating' diabetes [22–28].

However, oral administration of vanadium is not without its drawbacks. Toxic side effects have been noted, including some deaths, decreased weight gain, increased serum urea and creatine concentrations [29, 30]. Development and reproductive problems have also been noted with vanadium administration [31–33]. Vanadium has toxic effects on the immune system as well [34, 35] possibly mediated through alteration in microfilaments [36], cellular energy metabolism [37] or cytokine production [38]. Thus, as with any potential drug, the metabolism of this normally trace metal will be important if any significant clinical use is to be made of vanadium in humans.

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We have demonstrated (unpublished data) that a liquid diet is an effective and convenient means of administering vanadium compounds to experimental animals. We have used this method to examine the accumulation of vanadium in various tissues when administered at different doses, including one that would be effective at lowering blood glucose levels in diabetic rats. We have also demonstrated that the various organs retain vanadium at different rates. This work demonstrates the complex nature of vanadium metabolism, and identifies some of the organs which are most likely to be affected by long term administration of vanadium.

Materials and methods

Liquid Rat Diet, Original Lieber-DeCarli Control (product #F0005) was purchased from Bio-Serv (Frenchtown, NJ). Vanadyl sulfate was purchased from Fisher (Plano, TX). Cupferron and sodium orthovanadate were obtained from Sigma (St. Louis, MO). Male Sprague-Dawley rats were purchased from Sasco (Omaha, NE) and maintained and used in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The rats used in these studies were 140–160 g at the time of purchase, except for those used in comparing vanadium content of young versus old animals. The young animals were 39–41 days old (120–140 g) and the old animals were retired breeders 5–6 months old (approximately 500 g).

Rats were fed Purina Rat Chow and water *ad lib* for 4 days after their arrival at our facility. The rats were then switched to 90 ml per day Liquid Diet for 3 days prior to starting the vanadium treatment protocol. Sodium orthovanadate (SOV) or vanadyl sulfate (VS) was then added to 90 ml Liquid Diet at 1.6, 80, or 160 μmoles of vanadium/kg body weight/day. Control animals were given liquid diet alone without added vanadium. Animals were fed for 4, 11, or 18 days, with sacrifice at that time to determine organ vanadium content. The amount of liquid diet was recorded daily, and weights taken at the start of feeding and at 4, 11, and 18 days. Determination of vanadium content of the tissues was done by radiochemical neutron activation analysis as described [39] and as detailed below.

The rats used in the determination of the tissue half-lives were fed vanadium (either SOV or VS at 160 μmol V/kg*day) for one week, and then fed liquid diet alone without added vanadium until organ harvest. The animals were killed and organs taken at 0, 4, and 8 days and tissue levels determined as described below. The half lives were determined by curve fitting the data using the InPlot computer program (GraphPad Software Inc., San Diego, CA). The data were fitted to the equation

$$Y = A \times e^{-BX} + E$$

where Y is the vanadium concentration at time X (in days), A+E is the vanadium concentration at day 0, and E is the ending vanadium concentration. For our purposes, E was assumed to be the organ concentration of untreated control animals.

At the time of sacrifice the rats were anesthetized using 60 mg/kg sodium pentobarbital, and killed by pneumothorax and the subsequent removal of the organs. The following organs were routinely analyzed, though data is not presented for all the organs at all time points: brain; blood; epidymal fat pads; heart; kidney; liver; lungs; skeletal muscle (hind limb); spleen; and testes. The organs were removed using a Teflon-coated scalpel to assure no contamination of the sample from the stainless steel, which contains vanadium. The organs were placed in 50 ml Nalgene beakers and lyophilized to dryness. The organs were weighed, and digested with 10 ml of concentrated nitric acid at 56°C until dry. The samples had 10 ml of nitric acid added to them, were sonicated for 15 min and redigested as before. The sample was then dissolved in 5 ml 1 N nitric acid, sonicated for 15 min, and transferred to an irradiation vial. Samples were irradiated for 5 min at thermal neutron flux of 3.2×10^{11} n·cm^{-2} and a gamma-ray flux of 3×10^{17} eV/g·min in the Omaha Department of Veterans Affairs Medical Center TRIGA Mark I nuclear reactor by means of a pneumatic transfer tube. The sample was transferred to a separatory funnel 3 ml of fresh 6% cupferron solution were added and the funnel shaken for 30 sec. Five ml of chloroform were added, shaken for an additional 30 sec and the layers allowed to separate. The organic layer was withdrawn and 2 ml counted 5 min after removal of the sample from the reactor. The samples were counted for a live time of 200 sec in a 53 cm^3 closed end coaxial well Ge detector (Canberra, IN) with a relative efficiency of 10.4% for the 1332 keV gamma photon emitted by ^{60}Co. The gamma photons from the $^{51}V(n,\gamma)^{52}V$ reaction with an energy of 1.434 MeV were counted with Nuclear Data (Schaumberg, IL) ND 680 4096-channel analyzer employing ND 'Peak' software. The counts were compared to a known standard run on the same day to determine the vanadium content of the sample, with appropriate dilution factors taken into account for preparation and organic extraction of the sample. The vanadium value was divided by the dry weight of the sample to determine ng V/g dry tissue.

It should be noted here that fat tissue presented a difficult challenge in that it would not acid digest the way other tissues did. The reason for this was apparently the high lipid content which was insoluble in the aqueous nitric acid. To solve this problem we homogenized the wet tissue with chloroform:methanol (2:1) and separated the aqueous layer. The aqueous layer was dried down, and digested with nitric acid similar to the other tissues. Samples spiked with vanadium

standard prior to homogenization reveal a loss of about 50% using this procedure. Thus the fat data underestimate the vanadium content by half, and the results are not as consistent. However, using this method meant we could at least obtain some measure of the vanadium content of fat, which we were unable to do using the standard procedure.

For the study of vanadium effects on insulin binding and degradation, rats were fed Liquid Diet with or without 160 μmole V/kg body weight/day added in SOV form for 14 days. Hepatocytes were then prepared as described previously [40] and used to examine cell association and degradation of ^{125}I iodoinsulin labeled at the A- 14 position.

Results

Table 1 shows the vanadium content in control animals for the organs tested. The levels vary widely with kidney being the highest and brain the lowest. While the control groups varied somewhat over time and among the treatments, no significant pattern of change in vanadium content was seen for any organ. Thus these values represent the normal tissue levels against which the SOV and VS treated groups should be compared.

Figures 1 and 2 show the time course (Panel A) and the dose response (Panel B) curves for liver and testes, respectively. Note that the accumulation of vanadium is essentially linearly dependent on dose over the range tested. All organs tested showed similar dose response curves. Note that for liver their was no significant further accumulation from day 11 to day 18 (Fig. 1, Panel A). Kidney showed a similar pattern (data not shown). However testes (Fig. 2, Panel A) showed no tendency to plateau at later time points. Similar data was obtained for brain and spleen (not shown).

Figure 3 shows the binding and degradation of ^{125}I iodoinsulin to hepatocytes isolated from control rats and from rats fed SOV for 14 days. Essentially no difference is found in the affinity or number of insulin receptors, nor in degradation, as measured by trichloroacetic acid solubility. The time course of insulin association to hepatocytes was the same in

Table 1. Tissue vanadium levels

Organ	ng V/g Dry Tissue Weight	
Blood	11 ± 2	n = 26
Brain	2 ± 1	n = 29
Fat	5 ± 1	n = 23
Heart	16 ± 3	n = 25
Kidney	133 ± 14	n = 27
Leg Muscle	18 ± 5	n = 27
Liver	30 ± 2	n = 26
Lung	27 ± 3	n = 27
Spleen	64 ± 8	n = 26
Testes	38 ± 5	n = 24

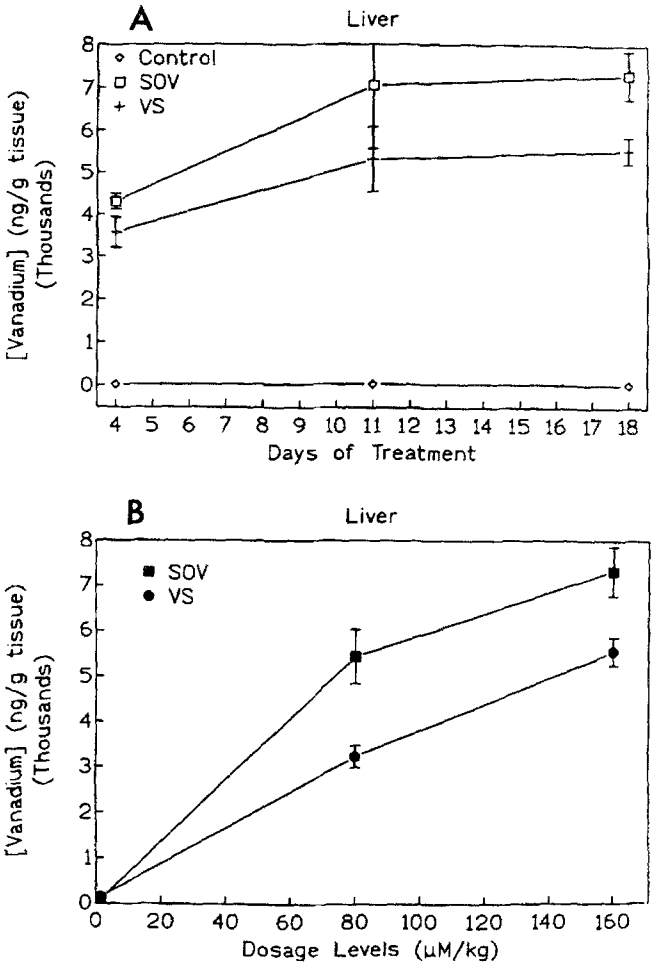

Fig. 1. Time course (Panel A) and dose-accumulation (Panel B) curves of vanadium for liver. Panel A shows the amount of vanadium (ng V/gram dry tissue weight) in the organs of animals fed liquid diet alone (Cont) or with 160 μmole Vlkg/day as SOV or VS at days 4, 11 and 18. Panel B shows the amount of vanadium (ng V/gram dry tissue weight) in the organs after 18 days of feeding vanadium as SOV or VS at the dose indicated on the x-axis.

control and treated animals (data not shown).

Table 2 shows the half-lives in various organs from animals treated with SOV or VS. Note that the values vary greatly among the organs, with liver and kidney having the shortest and testes the longest. Almost all of the tissues show a slightly longer half-life of vanadium for SOV fed rats compared to the VS fed rats.

Table 3 shows the vanadium content of the organs listed of young and old rats shortly after arrival at our facility and after 45 days of feeding control Liquid Diet. Brain and leg muscle showed no difference between animal groups before or after feeding. All other organs showed an increase over time in their vanadium content, suggesting the Liquid Diet may contribute more vanadium to the animal's diet than the chow fed by the supplier. Preliminary tests of the supplier's chow found them to be relatively low in vanadium content

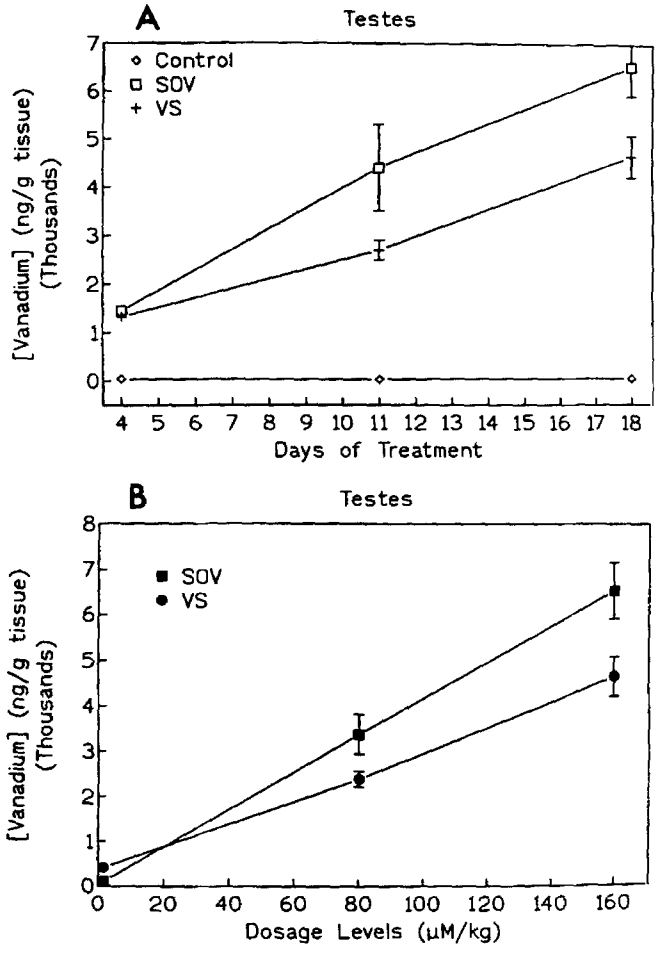

Fig. 2. Time course (Panel A) and dose accumulation (Panel B) curves of vanadium for testes. Panels are the same as described in the legend for Fig. 1.

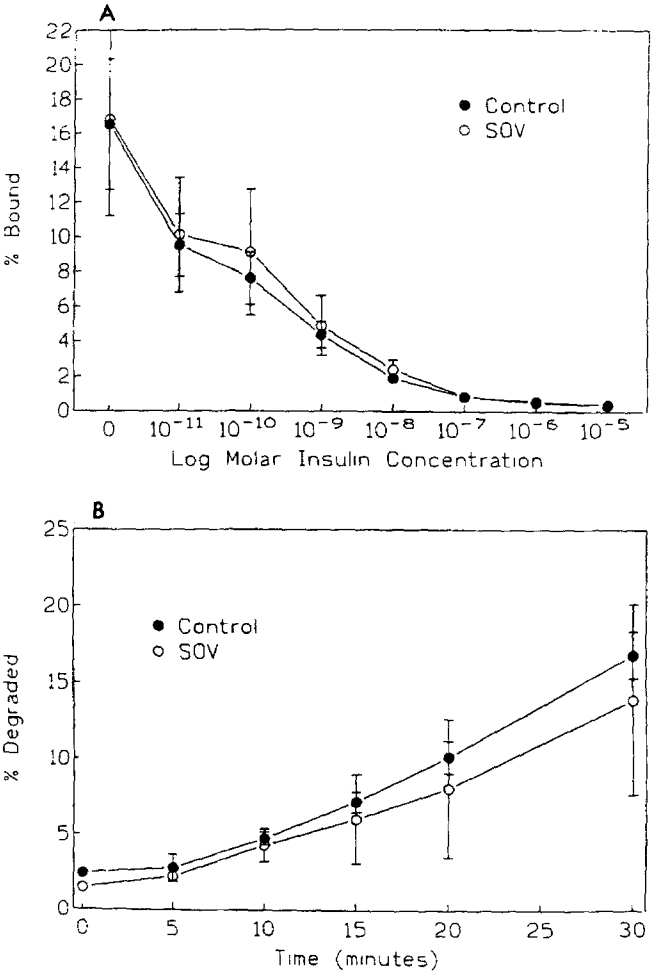

Fig. 3. Insulin binding and degradation in hepatocytes of control and SOV fed rats. Panel A shows displacement of tracer ^{125}I iodoinsulin by unlabeled insulin normalized to 10^6 cells/ml. Panel B shows tracer ^{125}I-iodoinsulin degradation in the media of cells incubated for the times shown.

(data not shown). This probably also explains why the values in Table 3 are lower than those in Table 1. Note that at the end of the age study the vanadium content of most of the organs is similar to that reported in Table 1. The slightly lower values probably reflect the lot to lot variability in the vanadium content of the diet that we have noted (unpublished observation). However spleen and testes showed a difference between age groups at the start and end of the feeding study. These results suggest these organs accumulate vanadium over time to a greater extent than the other organs tested.

Discussion

Vanadium is becoming increasingly important for its potential use in diabetes [13–17], its possible role as an essential element [1–4], and as an environmental toxicant [41]. To help understand its metabolic function and toxicology, it is important to know where the element resides in the body and where, under increased absorption, it may be concentrated. Because of its ability to decrease blood glucose concentrations we have examined vanadium levels in a variety of organs with varying sensitivities to insulin. Our hypothesis was that tissues in which glucose transport is sensitive to insulin may show greater levels of, or ability to concentrate, vanadium relative to those that are not insulin sensitive.

The data shown in Table 1 indicate that with respect to vanadium levels this hypothesis is false. Tissues such as fat and muscle, which have insulin sensitive glucose transport systems have relatively low levels of vanadium, while tissues that relatively insensitive to insulin, such as brain and kidney have widely disparate levels. The tissues with the lowest levels: brain; fat; heart; and muscle; have a large portion of their mass made up of components with which vanadium may not be expected to associate, i.e. lipid for fat and brain, and contractile proteins for heart and muscle. Thus vanadium

Table 2. Organ half lives (days)

Organ	SOV	r^2	VS	r^2
Liver	3.57	.997	3.18	.966
Kidney	3.93	.996	3.27	.999
Fat	4.06	.998	5.04	.983
Lung	5.52	.999	4.45	.998
Muscle	6.11	.999	4.49	.997
Heart	7.03	.994	5.05	.995
Spleen	9.13	.999	5.15	.993
Brain	11.17	.985	9.17	1.00
Testes	15.95	.987	13.50	.997

r^2 indicates the correlation coefficient of the data to the equation.

Table 3. Vanadium concentration in old and young rats

Organ /Rat# (Vng/gTis)	Young Day 0 (Vng/gTis)	Old Day 0 (Vng/gTis)	Young Day 45 (Vng/gTis)	Old Day 45 (Vng/gTis)
Brain	0	0	2 ± 2	0
Heart	3 ± 2	8 ± 3	12 ± 3	16 ± 1
Kidney	23 ± 2	18 ± 8	89 ± 13**	100 ± 8***
Leg muscle	6 ± 1	2 ± 2	6 ± 1	7 ± 1
Liver	6 ± 3	11 ± 1	18 ± 1*	24 ± 3*
Lung	5 ± 3	10 ± 1	17 ± 2**	21 ± 1**
Spleen	5 ± 4	40 ± 3^{++}	46 ± 6**	88 ± 6**
Testes	5 ± 5	23 ± 2^{+}	24 ± 3*	50 ± 3^{***++}

Values are mean ± S.E.M. Day 45 Different from Day 0: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Old different from Young: $^{+}$ = $p < 0.05$; $^{++}$ = $p < 0.01$. As determined by a Tukey-Kramer Multiple Comparisons test post ANOVA.

levels appear to correlate with the available 'space' it occupies in the tissue. A possible exception is the kidney, which had the highest levels of vanadium. However, this is not unexpected given the role the kidney plays in electrolyte metabolism. Thus the high levels probably represent vanadium which is being processed for excretion.

We have examined the accumulation of vanadium in animals orally treated with sodium orthovanadate (SOV) and vanadyl sulfate (VS) at three different doses. Liver and testes were typical (Panel B of Figs 1 and 2) of all organs tested, showing a linear dose-dependent increase in vanadium content. The organs did not accumulate vanadium uniformly, with kidney and liver increasing 150–200 fold at the highest dose by day 18 while fat only increased 10 fold at the same dose.

Panel A of Figs 1 and 2 show the time course of vanadium accumulation differs among the organs. Liver showed a plateau between day 11 and day 18, whereas testes appear to continue to accumulate vanadium at a relatively steady rate. Kidney behaved similarly to liver, while brain and spleen were more like testes. The other organs showed intermediate behavior, with a decrease in accumulation rate between day 11 and day 18 compared to between day 4 and day 11. These differences in accumulation rate could result from varying assimilation rates, or from different elimination rates. We therefore determined the half-life of vanadium for the various organs.

While vanadium levels do not correlate with insulin sensitive glucose transport, our studies of the organ half-lives reveals another general relationship. The organs with the shorter half-lives tend to be more insulin sensitive in general, while those with the longest are relatively insensitive. For example, testes, brain and spleen are not considered to be exquisitely sensitive target tissues of insulin, and they have the longest half-lives, indicating a relatively slow metabolic turnover of vanadium. Fat and liver however, are two model tissues for insulin sensitivity, and they have short half-lives. This suggests that vanadium metabolism may be controlled by or otherwise related to insulin or the metabolic effects under the influence of insulin.

We have recently shown [42] in a Type I diabetes model, (BB/Wor-UTM Wistar rats from the colony at University of Tennessee), that the vanadium content of livers from diabetic rats is decreased from their non-diabetic littermates. If vanadium is involved in insulin sensitive metabolic events in the cell, it is logical that the tissue levels would be influenced by insulin. However, we have also recently discovered (unpublished data) that streptozotocin diabetic rats have increased vanadium levels in liver, muscle and spleen. Heart and brain also showed increases, although they were not statistically significant. Kidney showed a decrease in vanadium content. Insulin treatment of the diabetic animals returned the vanadium values to normal. We believe the discrepancy in these findings is the result of insulin treatment of the BB/Wor-UTM increasing 150–200 fold at the highest dose by day 18 while fat only increased 10 fold at the same dose.

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It has been proposed that once vanadium, in either the SOV or VS form, enters the blood, it is converted to the vanadyl form within red blood cells, and that this is the form that resides in the tissues where it accumulates. If this were so, the organ half-life should be independent of the administered form. However our results suggest an alternative explanation. Whether administered as the orthovanadate or vanadyl species, equal vanadium concentrations are achieved in the blood. Even if there is some interconversion, some proportion of each remains in the blood, and the anionic vanadate as well as the cationic vanadyl are transported into the tissue. Once inside the cell there may be additional interconversion of the vanadium forms, but the vanadate species is either less metabolically active, preferentially converted to a more stable form, sequestered into a compartment which limits its turnover, or some combination of the above. Regardless of the exact mechanism, the vanadium involved is no longer able to be lost or transported out of the cell in the same manner as vanadium originally accumulated in the vanadyl form. Thus the administered form of vanadium can have an effect on its subsequent metabolism, which could have important consequences in increasing it effectiveness and decreasing toxicity if used therapeutically for diabetes mellitus. Furthermore, alternative forms of vanadium that have been proposed for therapetltic use, such as pervanadate [43, 44] or coordinated oxovanadium compounds [45–47], may exhibit different pharmacokinetic behaviors and should be examined with regard to organ ll accumulation.

These results may have important consequences with regard to the mechanism of vanadium's actions. Vanadate's activation of MPAP kinases [48] and glucose transport [49] are insulin receptor tyrosine kinase independent events. Pervanadate, however, appears to work by increasing insulin receptor tyrosine kinase activity by relatively specifically inhibiting the receptor's dephosphorylation by phosphotyrosine phosphatase(s) [44]. If the various vanadium compounds are handled differently by cells, and if the metabolism of the various forms of vanadium is affected differently by insulin, therapeutic use of specific compounds may be able to target and/or be limited by specific conditions or tissues. For example, differential sequestration of vanadate may mean it is more useful in some tissues than others, and its metabolic dependence on insulin may alter its usefulness between Type I and Type II diabetics. Furthermore, if vanadium compounds that work via the insulin receptor correct vanadium metabolism in diabetes, it may give insight into the mechanisms controlling vanadium levels.

Our results indicate a relationship between insulin and vanadium metabolism in some tissues. We also examined whether the converse were true, that vanadium could alter insulin metabolism. Figure 3 demonstrates that insulin binding and degradation were not altered in hepatocytes by oral treatment of rats with sodium orthovanadate. This indicates that insulin receptors are not affected by vanadium, and that subsequent processing of bound insulin by hepatocytes is also unchanged. Thus, the insulin-like effects of vanadium are unrelated to changes in insulin metabolism, at least in hepatocytes.

Vanadium content of the old and young animals organs before and after feeding liquid diet indicates two things. First, diet is very important in determining the levels of vanadium in some of the tissues. Forty-five days of consuming the liquid diet resulted in an increase in most, but not all organs. Thus, studies of vanadium levels depend on a consistent source of diet. Second, before and after liquid diet feeding, the older animals showed significantly higher levels of vanadium in spleen and testes. These data, combined with the long half-life of vanadium in these organs suggest they may be particular targets of vanadium toxicity. The continued accumulation of vanadium in tissues such as spleen, testes, or brain, may be a limiting factor in the use of vanadium for the treatment of diabetes mellitus. This deserves further study.

In conclusion, we have shown that organ concentrations

of vanadium vary widely, with tissues whose mass is made of largely a functional entity (e.g. contractile protein in muscle) tend to have less vanadium on a dry weight basis. The metabolism of vanadium, that is its turnover in organs, is roughly approximated by the tissue's sensitivity to insulin, with sensitive tissues having shorter half-lives. Two organs with longer half-lives, spleen and testes, tend to accumulate vanadium over time. These findings may relate to the immunotoxicity [34–38] and reproduction effects [31–33] found in animals. Finally, the nature of the vanadium compound exposed or administered to the animal can affect the metabolism of the element. Since the chemistry of vanadium is so complex, it will require more study to determine exactly which species are in the various organs. However, this study suggests there is a relationship between vanadium and insulin-stimulated metabolism, and the specific vanadium oxidation state and/or form is important in its metabolism.

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