A new experimental system of using fertile chick eggs to evaluate vanadium absorption and antidotal effectiveness to prevent vanadium uptake

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To establish the absorption of vanadium compounds such as vanadium chloride, vanadyl sulfate, and sodium metavanadate and to find effective chelating and reducing agents to cope with the absorption and uptake of vanadium compounds by embryonic chicks, aqueous vanadium solutions with or without chelating and reducing agent solutions were introduced into the air sacs of 14-day-old fertile chick eggs. After incubation of a further 5 days, mortality, embryonic weight, and vanadium content in the legs and toes were measured. Vanadium concentration in the legs and toes increased linearly as the administration of vanadium compounds increased and can be used as an index for vanadium absorption. Vanadium accumulation in the legs and toes, embryonic growth, and mortality showed no essential differences among VCl_3, $VOSO_4$, and $NaVO_3$. Among 19 antidotal substances tested, deferoxamine mesylate was the most effective, and Xylenol Orange, EDTA, and basophenathroline were secondarily effective to prevent vanadium uptake. Tiron could not prevent absorption from $VOSO_4$ as effectively as $NaVO_3$. Deferoxamine was the most effective in decreasing the death rate and increasing embryonic growth. Tiron was secondarily effective when excessive Tiron was administered. Antidotal effectiveness of deferoxamine was observed when it was simultaneously administered with vanadium compounds. Deferoxamine and $VOSO_4$ formed an equimolar complex that is unabsorbable. These results are comparable with the results shown in previous reports using rats and mice. The present experimental system using fertile chick eggs had advantages in the examination of mineral absorption and in the screening for effective antidotes from the viewpoints of ease of experimental procedure and animal welfare in comparison with conventional methods using laboratory animals.
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Keywords: vanadium; chelate; deferoxamine; chick embryo

Introduction

Vanadium has three oxidation states of +3, +4, and +5 in natural environments. Vanadate, or metavanadate, has a +5 oxidation state, and vanadyl has a +4 oxidation state. In animals, vanadate, or metavanadate, is reduced to vanadyl by reducing agents such as glutathione,[1,2] and intracellularly vanadyl complexed with protein and phosphate compounds becomes the main existing species.[3,4] Vanadium accumulation occurs in the kidneys, bones, and liver.[5–7] Vanadate is an inhibitor of (Na, K)-ATPase.[8] High vanadium uptake causes renal disorders.[9,10] Vanadium also has a pharmacological effect; reducing the blood glucose level in experimentally induced diabetic animals.[11,12] However, the therapeutic usage of vanadium to treat diabetes mellitus meets with an unsolved problem of vanadium accumulation in the kidneys. The uptake of high amounts of vanadium from anthropogenic sources by humans often causes acute or chronic toxicity problems.[13] These persons need treatment with chelating agents to reduce tissue vanadium accumulation. Several papers concerning the effects of chelating agents to cope with the vanadium accumulation and toxicity have been published using rats and mice.[14–18] Chelating agents can reverse vana-

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dium toxicity by chelation and subsequent excretion or by prevention of absorption.

The objectives of this work were to examine the absorption of different vanadium compounds of +3, +4, and +5 oxidation states and to screen for the effective antidotal agents to prevent vanadium absorption in a new experimental system using fertile eggs. Various vanadium compounds were administered into the air sacs of fertile eggs, and the vanadium concentration in the legs and toes of the chick embryos was used as an index for vanadium absorption. Antidotal effects of various chelating or reducing substances were also evaluated by simultaneously administering those substances with different vanadium compounds and by measuring death rate, embryonic weight, and vanadium contents in the legs and toes. The results obtained here were compared with previously published results using rats and mice.

Methods and materials

The following chemicals used were obtained from the companies indicated: VCl_3, $VOSO_4 \cdot 2H_2O$, $NaVO_3$ and L-ascorbic acid were from Wako Pure Chem. Ind. (Osaka, Japan); catechol from Tokyo Kasei Co.; Deferoxamine mesylate (Desferrioxamine mesylate) from Sigma Chem. Co.; D-penicillamine and 2,3-dimercapto-1-propane-sulfonic acid (sodium salt) from Aldrich Chem. Co.; dithiothreitol and glutathione (SH) from Boehringer Mannheim Co.; and acetylacetone, bathophenanthroline sulfonic acid (disodium salt), DTPA (Diethylenetriamine pentaacetic acid), EDTA-2Na (Ethylenediaminetetraacetic acid, disodium salt), EDTA-Ca (disodium calcium salt), EDTA-Zn (disodium zinc salt), PDTS (3-(2-Pyridyl)-5,6-bis(4-sulfophenyl)-1,2,4-triazine, disodium salt), Thorin (2-(2-Hydroxy-3,6-disulfo-1-naphthylazo)benzenearsonic acid, disodium salt), Tiron (1,2-Dihydroxybenzene-3,5-disulfonic acid, disodium salt), Variamine Blue B (N-(p-Methoxyphenyl)-p-phenylenediamine, hydrochloride), Xylenol Orange (3,3′-Bis[N,N-di(carboxymethyl)aminomethyl]-o-cresolsulfonphthalein) and Zephiramine (Tetradecyldimethylbenzylammonium chloride) from Dojindo Lab (Kumamoto, Japan).

Fertile chick (White Leghorn) eggs were incubated at 37.6°C and about 75% relative humidity in an incubator (Showa Furanki Lab., Saitama, Japan) with a constant rotating device (once in 1 hour) as shown previously.[19] Two small holes (about 1.5 mm ID) were made in the eggshells just above the air sacs of 14-day-old fertile eggs, and 100 μL of a freshly prepared, aqueous VCl_3, $VOSO_4$, or $NaVO_3$ solution was introduced into the air sacs. Immediately after the administration of vanadium compounds, 100 μL of an aqueous solution containing 1 μmol of chelating or reducing agents was separately introduced into the air sac through a different hole. After the holes were coated with vaseline, the eggs were incubated for a further 5 days. The mean weight of the 19-day-old fertile eggs (n = 701) was 55.5 ± 6.6 SD g. Wet embryonic weight was measured, and after the joints between the tibia and the metatarsus were cut off, the weight of all legs and toes was measured. All leg and toe parts were dried at 60°C for 22 hr, weighed, and subjected to nitric acid digestion with small amounts of H_2SO_4 and H_2O_2. The vanadium contents of the digested sample (10 mL) were analyzed by flameless atomic absorption spectrophotometry using the Hitachi spectrophotometer Z-8000. Vanadium standard solution (Wako Pure Chem. Ind.) for atomic absorption analysis containing 1 mg ml^{-1} of vanadium was diluted to 0 to 15 ng ml^{-1} of vanadium and used as analytical standard.

Data were analyzed by one-way analysis of variance using a statistical computer program (StatView ANOVA, Abacus Concepts, Berkeley, CA USA, 1987) and Fisher's Protected Least Significant Difference was used to assess the significant differences between groups. Death rate data were analyzed by the chi-square test. The levels of significant differences were determined at $P < 0.05$ throughout the experiments.

Results

The effects of VCl_3, $VOSO_4$, or $NaVO_3$ administration on the death rate, embryonic weight, legs and toes dry matter weight, and V concentration in the legs and toes are shown in *Figure 1 to 4*. The death rate (Y) increased linearly as the

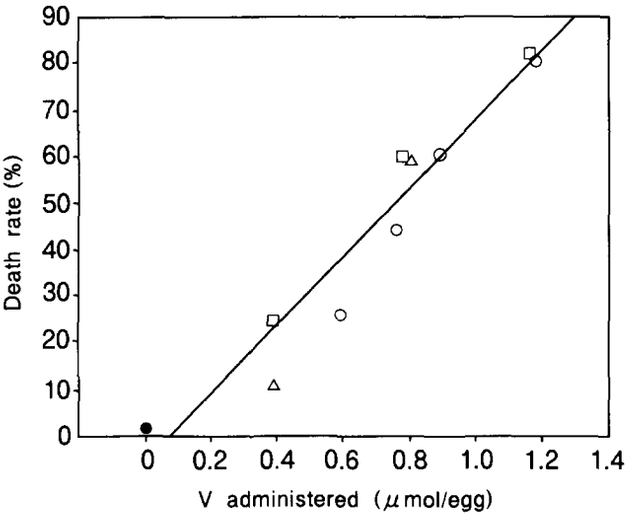

Figure 1 Relationship between vanadium administered per egg (μmole) and embryonic death rate (%). Black circle corresponds to control (no vanadium administration); triangles, VCl_3 administration; squares, $VOSO_4$ administration; and open circle, $NaVO_3$ administration. The numbers of eggs used were 39, 18, 25, 8, 94, 74, 82, 49, 33, and 10 in control, VCl_3 (0.39, 0.78 μmol), $VOSO_4$ (0.39, 0.78, 1.17 μmol), and $NaVO_3$ (0.59, 0.76, 0.89, 1.18 μmol) administration, respectively.

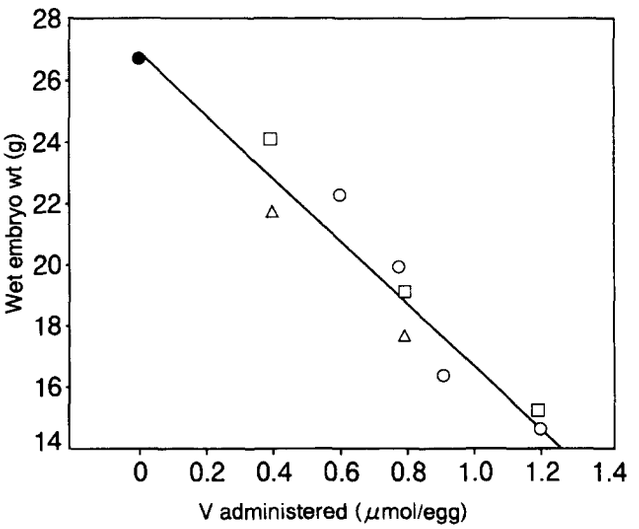

Figure 2 Relationship between vanadium administered per egg (μmol) and wet embryo weight (g). The symbols and the numbers of embryos used are the same as *Figure 1*.

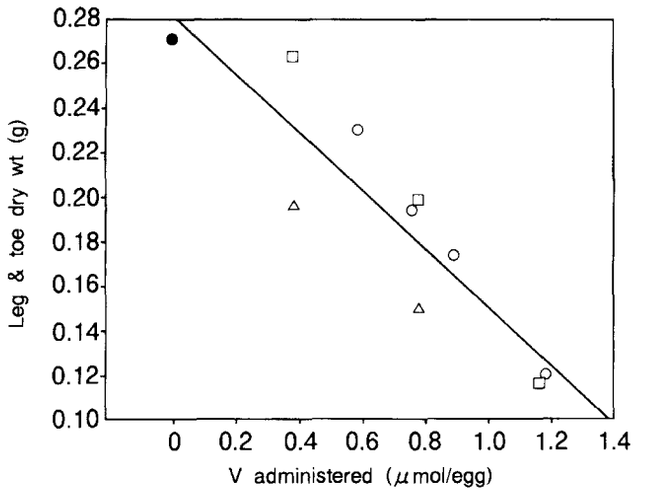

Figure 3 Relationship between vanadium administered per egg (μmol) and dry matter weight of legs and toes (g). The symbols are the same as *Figure 1*. The numbers of embryos used were 22, 15, 18, 5, 40, 36, 51, 23, 16, and 6 in control, VCl_3 (0.39, 0.78 μmol), $VOSO_4$ (0.39, 0.78, 1.17 μmol), and $NaVO_3$ (0.59, 0.76, 0.89, 1.18 μmol) administration, respectively.

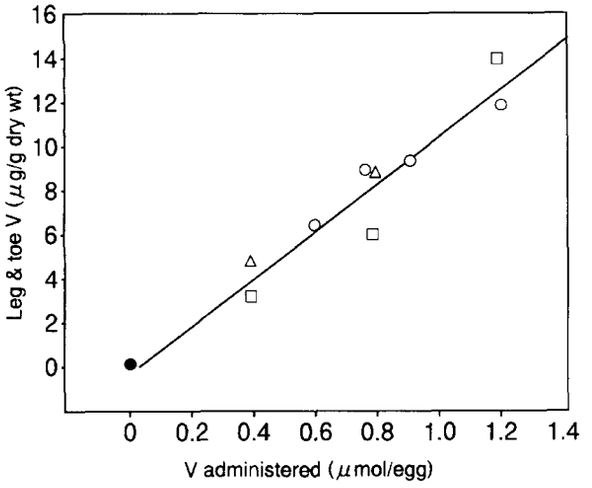

Figure 4 Relationship between vanadium administered per egg (μmol) and vanadium concentration in dry matter weights of legs and toes (μg g^{-1}). The symbols are the same as *Figure 1*. The numbers of embryos used are the same as *Figure 3*.

administration rate (X) of vanadium compounds increased *(Figure 1)*, and the linear relationship was: $Y = 73.13\ X - 5.4$ ($r^2 = 0.926$). Wet embryonic weight decreased linearly as the administration rate (X) of vanadium compounds increased *(Figure 2)*, and this relationship was also linear: $Y = -10.46\ X + 27$ ($r^2 = 0.935$). Dry matter content (Y) of whole legs and toes decreased as the administration rate (X) of vanadium compounds increased *(Figure 3)*, and the linear equation was: $Y = -0.132\ X + 0.283$ ($r^2 = 0.816$). Vanadium concentrations (Y) in the dry matter weights of legs and toes increased as the administration rate (X) of vanadium compounds increased *(Figure 4)*, and there was a linear equation: $Y = 10.956\ X - 0.165$ ($r^2 = 0.938$). Vanadium concentration in legs and toes can be used as a measure for vanadium absorption.

Effects of adding chelating or reducing agents on the increased death rate, the decreased embryonic weight, the decreased dry matter weights of legs and toes, and the increased vanadium concentration in dry matter weights of legs and toes caused by $VOSO_4$, $NaVO_3$, and VCl_3 administration are shown in *Tables 1 to 3*. In the total of 57 tests in *Tables 1 to 3*, correlation coefficients between death rate and either embryo weight, legs and toes dry matter, V concentration of legs and toes, or total amount of vanadium in legs and toes were −0.718, −0.719, 0.756, and 0.509, respectively; those between V concentration of legs and toes and either embryo weight, legs and toes dry matter, or total amount of vanadium in legs and toes were −0.537, −0.637, and 0.848, respectively; those between embryo weight and either legs and toes dry matter or total amount of vanadium in legs and toes were 0.935 and −0.119; and that between legs and toes dry matter and total amount of vanadium in legs and toes was −0.216. Although correlation between death rate and vanadium concentration in legs and toes was higher than that between death rate and total amount of vanadium in legs and toes, the death rate did not always correlate with vanadium concentration in legs and toes. Death rate and embryo weight may be used as indexes for vanadium toxicity. However, these indexes are more complex in nature than vanadium concentration in legs and toes, and as the basis for determining the efficacy of antidotal agents vanadium concentration in legs and toes was regarded as the first priority index.

In the administration of 0.78 and 1.17 μmol of $VOSO_4$ *(Table 1)*, 0.59 and 0.89 μmol of $NaVO_3$ *(Table 2)*, and 0.78 μmol of VCl_3 *(Table 3)*, bathophenanthroline, deferoxamine, EDTA, and Xylenol Orange significantly reduced vanadium concentrations of legs and toes. Tiron significantly reduced the vanadium concentration from $NaVO_3$ only. In the above five chelators under $VOSO_4$ and $NaVO_3$ administration, deferoxamine was the most effective antidote, not only in reducing vanadium absorption but also in decreasing death rate and increasing embryonic and legs and toes weights. In the other four chelators, Tiron was secondarily effective against $VOSO_4$ and $NaVO_3$ in decreasing death rate and increasing embryonic and legs and toes weights if the administration of $VOSO_4$ (1.17 μmol) was not considered. Tiron was effective when an excessive amount was administered. Although bathophenanthroline, EDTA, and Xylenol Orange were effective to prevent vanadium absorption, these were not as effective as deferoxamine in decreasing death rate and increasing embryonic growth.

In other substances that did not prevent vanadium absorption, acetylacetone was effective against $VOSO_4$ in decreasing death rate and increasing embryonic growth, while under $NaVO_3$ administration acetylacetone increased death rate and decreased embryonic growth; PDTS decreased death rate under $VOSO_4$ and $NaVO_3$ administration, and DTPA under $VOSO_4$ administration. Zephiramine (1 μmol) itself was toxic and Zephiramine aggravated vanadium toxicity. Under $NaVO_3$ administration, reducing agents such as dithiothreitol and ascorbic acid increased death rate. Under $VOSO_4$ administration, catechol and Variamine Blue B decreased embryonic growth.

Table 1 Effects of adding 1 μmol of chelating and reducing agents to $VOSO_4$ administration (0.78 and 1.17 μmol/egg) on death rate, wet embryo weight, dry weight of legs and toes, and vanadium concentration in legs and toes

	Death rate (%)	Wet embryo (g)	Leg & toe DM (g)	V concentration of leg & toe (μg/g DM)
$VOSO_4$ (0.78 μmol/egg)	60.6 (94)	19.1 ± 0.57	0.198 (40)	6.15 ± 0.66
+ Acetylacetone	22.2 (9)*	22.9 ± 2.0*	0.256 (6)*	6.37 ± 1.43
+ Bathophenanthroline	22.2 (9)*	20.1 ± 1.9	0.216 (5)	1.28 ± 0.86*
+ Catechol	50.0 (10)	16.9 ± 1.8	0.142 (6)**	8.68 ± 2.27
+ D-penicillamine	22.2 (9)*	21.7 ± 1.6	0.252 (5)	4.88 ± 1.21
+ Deferoxamine	5.3 (19)*	25.5 ± 1.2*	0.273 (8)*	2.74 ± 0.50*
+ Di-SH-propane-sulfate	66.7 (9)	17.0 ± 1.3	0.148 (5)	5.00 ± 0.25
+ Dithiothreitol	88.9 (9)	20.8 ± 1.5	0.195 (4)	8.18 ± 0.39
+ DTPA	20.0 (10)*	19.9 ± 2.0	0.214 (7)	5.00 ± 0.97
+ EDTA-2Na	22.9 (35)*	22.3 ± 1.3*	0.243 (18)*	2.00 ± 0.38*
+ EDTA-Ca	11.1 (8)*	20.4 ± 1.4	0.221 (5)	1.51 ± 0.30*
+ EDTA-Zn	33.3 (9)	21.7 ± 2.1	0.251 (5)	1.66 ± 0.84*
+ Glutathione (SH)	66.7 (9)	21.1 ± 1.5	0.214 (6)	6.99 ± 1.82
+ L-Ascorbic acid	42.3 (26)	20.2 ± 1.2	0.219 (11)	6.92 ± 1.13
+ PDTS	33.3 (18)*	20.2 ± 1.2	0.172 (9)	8.43 ± 0.89
+ Thorin	33.3 (9)	17.0 ± 1.9	0.169 (4)	5.90 ± 3.02
+ Tiron	19.2 (26)*	26.3 ± 1.1*	0.281 (13)*	5.61 ± 0.66
+ Variamine blue B	77.8 (9)	15.8 ± 1.2**	0.142 (4)**	7.90 ± 1.22
+ Xylenol orange	5.3 (19)*	20.8 ± 0.6	0.216 (8)	0.53 ± 0.10*
+ Zephiramine	66.7 (9)	16.5 ± 3.0	0.130 (6)**	13.37 ± 4.53**
$VOSO_4$ (1.17 μmol/egg)	81.1 (74)	15.2 ± 0.6	0.117 (36)	14.10 ± 1.30
+ Bathophenanthroline	28.6 (7)*	17.6 ± 1.1	0.168 (5)	5.26 ± 2.74*
+ Deferoxamine	7.1 (14)*	21.7 ± 0.9*	0.195 (8)*	2.15 ± 0.61*
+ EDTA-2Na	33.3 (6)*	17.3 ± 1.9	0.190 (2)	1.47 ± 0.94*
+ Tiron	72.2 (18)	13.8 ± 1.3	0.121 (10)	11.80 ± 2.81
+ Xylenol orange	20.0 (5)*	16.6 ± 2.3	0.176 (2)	1.80 ± 0.70*

Numbers in parentheses show the numbers of eggs or embryos used. In wet embryo weight and vanadium concentration, SEMs are also shown. Single asterisks (*) show significant differences ($P < 0.05$) from $VOSO_4$ administration toward the direction of reducing vanadium toxicity. Double asterisks (**) show significant differences ($P < 0.05$) from $VOSO_4$ administration toward the direction of increasing vanadium toxicity.

$VOSO_4$ (1.17 μmol) and variable amounts of deferoxamine (0 to 1.5 μmol) were simultaneously administered and vanadium accumulation in the legs and toes was determined (*Figure 5*). As the amount of deferoxamine increased, the vanadium concentration in the legs and toes decreased linearly, and when approximately the same amount of deferoxamine as the amount of $VOSO_4$ was administered, the vanadium content in the legs and toes reached the minimum plateau. This fact suggested that in the air sac compartment vanadyl and deferoxamine formed an equimolar complex that cannot be absorbed into the embryonic tissue. When deferoxamine (1.5 μmol) was administered 6.5 hr after $VOSO_4$ (1.17 μmol) administration, the protective effect of deferoxamine disappeared.

Discussion

Most treatments for acute and chronic metal poisoning use chelating agents forming soluble, stable, and excretable metal complexes.[20–23] Some previous works[3,24] show that vanadium toxicity increases as the valency increases, pentavalent vanadium being the most toxic.[4,24] For the mouse, the LD_{50} for vanadate is far less than for vanadyl.[15,17,18] Pentavalent vanadium penetrates the erythrocyte cell membrane faster than the quadrivalent vanadium.[14] However, for the chick, pentavalent vanadium and quadrivalent vanadium are equally toxic.[5,25] In the present experimental system, sodium metavanadate and vanadyl sulfate were equally toxic. According to Sabbioni et al.,[26] there are common pathways of different chemical forms of vanadium in animals. No significant difference is shown either in the rate or amount of hepatic subcellular uptake of the three oxidation states of vanadium.[27]

In the present work, deferoxamine mesylate was the most effective antidote. Xylenol Orange, EDTA, bathophenanthroline sulfonate, and Tiron were also effective antidotes. However, the capacity of Tiron to sequester quadrivalent vanadium appeared to be weaker than that of the other agents listed above. Deferoxamine is a ferric iron chelator and has been shown to be an antidotal agent for vanadium.[14,16,17,28–30] The effectiveness of Tiron[16–18,31] and EDTA[15,16,33] have also been shown. Although DTPA was not so effective in the present experiments, DTPA administration (i.p.) tends to raise fecal excretion of retained vanadium in rats.[14] In *Table 4* antidotal effects of several chelators used to reduce vanadium accumulation in kidneys are shown. Although EDTA appears to be the strongest antidote to reduce kidney vanadium in *Table 4*, EDTA has also shown some contradictory effects that increase brain, lung, and testes vanadium.[16] Most experiments of *Table 4* were to examine the effects of chelators in reversing vanadium toxicity by chelation and excretion. However, the present experiment using chick embryos was designed to examine the effects of chelators in preventing vanadium absorption. In this system, simultaneous administration of vanadium compounds and chelators are essential.

Table 2 Effects of adding 1 μmol of chelating and reducing agents to $NaVO_3$ administration (0.59 and 0.89 μmol/egg) on death rate, wet embryo weight, dry weight of legs and toes, and vanadium concentration in legs and toes

	Death rate (%)	Wet embryo (g)	Leg & toe DM (g)	V concentration of leg & toe (μg/g DM)
$NaVO_3$ (0.59 μmol/egg)	26.8 (82)	22.3 ± 0.4	0.230 (51)	6.59 ± 0.46
+ Acetylacetone	44.4 (9)	16.4 ± 1.4**	0.144 (5)**	7.81 ± 2.98
+ Bathophenanthroline	0.0 (9)	24.4 ± 0.9	0.258 (5)	2.78 ± 0.64*
+ Catechol	20.0 (10)	20.6 ± 1.8	0.237 (6)	6.45 ± 0.95
+ D-penicillamine	11.1 (9)	23.7 ± 0.9	0.248 (5)	5.24 ± 0.81
+ Deferoxamine	14.3 (14)	25.3 ± 0.6*	0.281 (6)*	1.26 ± 0.25*
+ Di-SH-propane-sulfate	33.3 (9)	23.0 ± 0.8	0.238 (5)	4.14 ± 0.62
+ Dithiothreitol	55.6 (9)**	17.5 ± 1.3**	0.176 (4)	5.45 ± 2.73
+ DTPA	20.0 (10)	21.7 ± 1.7	0.234 (6)	5.20 ± 1.49
+ EDTA-2Na	20.0 (30)	21.4 ± 0.8	0.229 (16)	3.78 ± 0.58*
+ EDTA-Ca	0.0 (7)	22.6 ± 0.8	0.231 (5)	3.22 ± 0.78*
+ EDTA-Zn	11.1 (9)	23.7 ± 0.9	0.243 (5)	4.40 ± 0.66*
+ Glutathione (SH)	44.4 (9)	22.1 ± 1.9	0.232 (7)	4.59 ± 1.42
+ L-Ascorbic acid	55.6 (9)**	20.1 ± 1.3	0.184 (4)	7.48 ± 1.13
+ PDTS	5.3 (19)*	21.5 ± 0.7	0.213 (10)	5.49 ± 0.85
+ Thorin	22.2 (9)	22.3 ± 2.3	0.266 (3)	4.34 ± 1.44
+ Tiron	0.0 (18)*	24.5 ± 0.5	0.257 (9)	2.87 ± 0.47*
+ Variamine blue B	22.2 (9)	24.0 ± 1.2	0.261 (4)	4.04 ± 1.25
+ Xylenol orange	14.3 (14)	23.9 ± 1.6	0.290 (6)*	1.06 ± 0.27*
+ Zephiramine	88.9 (9)**	8.7 ± 2.0**	0.084 (5)**	8.98 ± 2.27
$NaVO_3$ (0.89 μmol/egg)	60.6 (33)	16.4 ± 1.0	0.174 (16)	9.45 ± 2.28
+ Bathophenanthroline	25.0 (8)*	21.0 ± 1.1*	0.203 (5)	5.24 ± 2.18*
+ Deferoxamine	0.0 (10)*	22.9 ± 0.8*	0.254 (4)*	0.99 ± 0.34*
+ EDTA-2Na	0.0 (5)*	19.8 ± 1.4	0.203 (2)	0.30 ± 0.24*
+ Tiron	22.2 (9)*	19.8 ± 1.8	0.200 (6)	4.39 ± 0.56*
+ Xylenol orange	22.2 (9)*	17.6 ± 1.6	0.197 (4)	0.65 ± 0.33*

Numbers in parentheses show the numbers of eggs or embryos used. In wet embryo weight and vanadium concentration, SEMs are also shown. Single asterisks (*) show significant differences ($P < 0.05$) from $NaVO_3$ administration toward the direction of reducing vanadium toxicity. Double asterisks (**) show significant differences ($P < 0.05$) from $NaVO_3$ administration toward the direction of increasing vanadium toxicity.

Table 3 Effects of adding 1 μmol of chelating and reducing agents to VCl_3 administration (0.78 μmol/egg) on death rate, wet embryo weight, dry weight of legs and toes, and vanadium concentration in legs and toes

	Death rate (%)	Wet embryo (g)	Leg & toe DM (g)	V concentration of leg & toe (μg/g DM)
VCl_3 (0.78 μmol/egg)	60.0 (25)	17.6 ± 0.9	0.149 (18)	8.83 ± 0.97
+ Bathophenanthroline	0.0 (7)*	21.7 ± 0.4*	0.179 (4)	3.92 ± 1.19*
+ Deferoxamine	0.0 (8)*	26.7 ± 0.6*	0.301 (4)*	2.35 ± 0.23*
+ EDTA-2Na	28.6 (7)	20.8 ± 1.7*	0.213 (4)*	2.23 ± 0.25*
+ Xylenol orange	0.0 (8)*	26.6 ± 1.0*	0.297 (4)*	3.03 ± 0.70*

Numbers in parentheses show the numbers of eggs or embryos used. In wet embryo weight and vanadium concentration SEMs are also shown. Single asterisks show significant differences ($P < 0.05$) from VCl_3 administration toward the direction of reducing vanadium toxicity.

Vanadium (especially vanadyl) causes membrane lipid peroxidation through the generation of hydroxyl radical.[32–34] Vanadium stays as complexes with iron-binding proteins in vivo.[35,36] Bathophenanthroline sulfonate, a ferrous iron chelator, was an effective antidote in reducing vanadium absorption. It is interesting that iron and vanadium have the same binding substances; ferritin, transferrin, and lactoferrin become binding substances for iron and vanadium. Xylenol Orange, a fluorescent calcium-binding dye, was efficient as an antidotal agent for vanadium. Xylenol Orange is used to determine the vascularization of the bone[37] and the viability of skin flaps.[38] Although ascorbate was reported to reduce vanadium intoxication,[15,16,25,39] the present work did not support this theory. Ascorbate is effective in alleviating the toxic effects, but not effective in reducing vanadium accumulation.[16] Intraperitoneal administration of ascorbic acid does not affect serum mineral content in guinea pigs.[39]

Compared with conventional methods of using laboratory animals such as rats and mice, the advantages of the present experimental system were the usage of very small amounts of chemicals for many numbers of animals and the avoidance

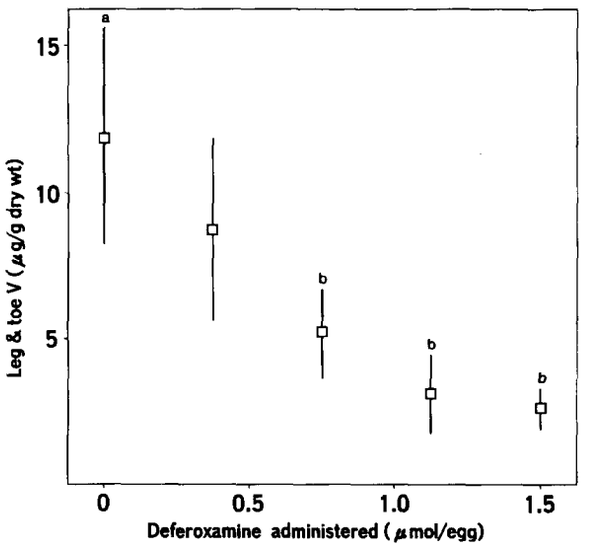

Figure 5 Effects of administration of $VOSO_4$ (1.17 μmol) with different amounts of deferoxamine mesylate (0 to 1.5 μmol) on vanadium concentration in the legs and toes. The bar shows SEM (n = 6). There are significant differences between the means denoted by a and b (P < 0.05).

of sacrificing sensitive animals. Animals in an embryonic stage are more able to tolerate pain and suffering in comparison to newborn, young, and adult animals. This is important given the recent trend of animal welfare. In the present experimental system, the influence of dietary composition can be avoided, and only the food from the yolk sac and the eggshell serves embryonic growth. The absorption of dietary vanadium appears to increase when a casein-based diet is fed to rats.[7]

The avian embryo has three circulation systems; the intraembryonic, the vitelline, and the allantoic, the latter two being extraembryonic systems. The vitelline circulation of the yolk sac and the allantoic circulation of the allantois are the major channels through which the embryo breathes, excretes, and is fed. The minerals administered into the air sacs can enter into the intraembryonic blood system through the allantoic circulation. The uptake of eggshell calcium occurs with allantoic membrane development from day 10 of incubation and during day 14 the membrane transports calcium at a maximum rate.[40]

Chelating agents such as deferoxamine and EDTA form complexes with vanadium compounds in the air sacs. When a VO-EDTA complex was administered directly into the air sac in the same experimental system, it neither remains in the air sacs nor accumulates into the legs and toes.[19] This is in contrast to the cases of vanadyl-cysteine methyl ester and Cu-EDTA complex administered into the air sacs. Vanadium and copper in these complexes can be retained well in the legs and toes and in the liver, respectively.[19,41] In contrast, the VO-EDTA complex is stable, and its vanadium is likely to be excreted as the original complex into the allantoic fluid. In the present work, vanadium complexed with strong chelating agents such as deferoxamine, EDTA, Xylenol Orange, and bathphenanthroline is assumed to have been excreted into the allantois compartment without breaking down. The chelating agents can contribute not only to prevent vanadium uptake but also to protect cell membrane from free vanadyl-induced radicals attack.[42]

Table 4 Effects of chelating agents to reduce vanadium concentrations of kidneys in vanadium-enriched animals

Animals used	V compounds administered*	Time and method of administration of chelators**	Chelators and extents of kidney V reduction***	Original literature
Chick, rat	V4, V5 included in diets (oral)	Included in diets (oral)	EDTA-92% (Chick) EDTA-76% (Rat)	5
Diabetic rat	V5 included in diets (oral)	After 3 weeks i.p. for 2 weeks	Tiron-41%	31
Rat	V4, V5 (i.p.)	After 24 hr i.p. once	Def-17% DTPA-7%	14
Mouse	V5 (i.p.)	Simultaneously i.p. once	Asc-4% Def-54% DTPA-41% EDTA-63% Tiron-65%	16
Mouse	V5 (i.p.)	Simultaneously; or after 8 hr i.p. once	Asc-27%; 11% Def-50%; 48% Tiron-65%; 4%	17
Mouse	V4 (i.m.)	After 10 min i.p. once	Asc-8% Def-22% DTPA-24% EDTA-32% Tiron-22%	18

*V4 and V5: vanadyl and vanadate compound, respectively.
**Chelators were administered immediately or some time after vanadium treatment.
***Asc and Def: ascorbic acid and deferoxamine, respectively.

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