# Peripheral erythrocyte levels, hemolysis and three vanadium compounds

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*Summary.* Three vanadium compounds of different valence states were administered to adult mice. Two, four, and eight days following treatment of vanadium, cardiac blood was collected. The blood sample was used to ascertain the peripheral erythrocyte count (cell/mm $^3$) and to determine the in vitro hemolytic index of erythrocytes obtained from mice treated in vivo with either the tri-, tetra-, or pentavalent vanadium compound. Data indicate that the tetravalent form was the most effective test substance in 1) promoting rupture of isolated erythrocytes compared to red cells retrieved from control mice and 2) depressing the erythrocyte count obtained from heart blood; maximum effects were manifest four days post-treatment. For all treatments there appeared to be a good correlation between the degree of vanadium-induced hemolysis and the peripheral erythrocyte count reduction following exposure to the vanadium.
*Key words.* Vanadium toxicity; hemolysis; erythropoiesis.

Vanadium has a wide natural occurrence in the environment and has been employed extensively in industrial processes [1, 2]. This trace metal has received considerable experimental attention because, above its essential micronutrient concentration, vanadium has been shown to induce a broad spectrum of pathophysiological conditions [3–5]. The demonstrated variable effects of vanadium include such factors as the mode of exposure [6], species of test animals [7], and chemical species of the test compound [8, 9]. In relation to the latter, vanadium has been reported to induce different effectivenesses in causing erythrocyte rupture, i.e., hemolysis, in vitro. The primary directives of the investigations reported here were to determine in vitro hemolytic indices of erythrocytes obtained from mice treated in vivo with three separate vanadium compounds, and to correlate such indices to changes in the number of peripheral erythrocytes at different times following vanadium treatment.

## *Materials and methods*

Vanadium compounds were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI 53233, USA). Because of the high instability of the divalent form of vanadium, only three of the oxidation states were tested. The vanadium salts were administered by a single intraperitoneal injection. Dosage of vanadium chloride [V(III)] was 28.3 mg/kg b. wt. Based upon comparable vanadium concentrations, vanadyl sulfate [V(IV)] and sodium orthovanadate [V(V)] injectates were 29.3 and 33.1 mg/kg, respectively. These represent a level of administration of vanadium at 9.2 mg/kg for each test compound.

Young adult female mice of the ICR strain were used for these investigations. Ten rodents comprised each experimental and control group. Water and standard rodent food pellets were available at all times. Mice weighed within a 31–37-g range, with an average weight of approximately 33 g. The vanadium compounds were prepared using triple-distilled water, and the pH of the injectate adjusted to pH 7.3. Animals were injected with their respective vanadium substance on day 0. Control mice were injected with 0.2 ml isotonic saline, which represented the average administered volume of aqueous vanadium salts. Two, four, and eight days following treatment, mice were sacrificed by cervical dislocation, and cardiac blood was withdrawn using sodium oxalate as the anticoagulant. A portion of the blood sample was retained to determine the erythrocyte count (No./mm$^3$) using the Coulter T-540 Hematology System. The packed red blood cell volume (hematocrit) was determined for each sample using a micromethod. The remaining part of the blood sample was used to establish the hemolytic index. All samples were corrected for hematocrit and were subjected to a chemical challenge to test for the in vitro susceptibility to hemolysis. The methods used to test for hemolysis were slightly modified from those described by Hansen and co-investigators [8]. Following the addition of reagents, blood samples were incubated at 37 °C for a 2-h period after which the samples were centrifuged and the supernatants collected. Absorbances of the supernatants containing hemoglobin released from the lysed erythrocytes were read at 540 nm using a Beckman spectrophotometer (Model DB-GT). The percentages of hemolyses for all groups were calculated by comparing the absorbance values of samples collected from vanadium-treated mice and control mice compared to those standard absorbance values of the 100% induced hemolysis by the standard treatment. The determined percentage hemolysis for each sample was expressed as a hemolytic index; this was calculated by dividing the percentage hemolysis obtained from the experimental animals' blood by the mean control's percentage. The control's hemolytic index was set at unity and the indices from vanadium-treated animals were compared to the normalized value of that of the control. Analysis of variances was used as the statistical test for the following results.

*Results and discussion*

Data in figure 1 reveal that V(IV) was the most effective treatment promoting an in vitro hemolytic effect. The days 2 and 4 V(IV)-induced effects (solid and striped bars, respectively) were approximately five and eight times those of the control's unity value. The most dramatic hemolysis was observed four days after treatment. This temporal response was mimicked by the blood obtained from the V(III) and V(IV) groups. It appears that the hemolytic effects of V(III) and V(IV), relative to the peak response of day 4, persisted for a longer period compared to the more abrupt decline in the day 8 ratio for the V(IV) group (stippled bars). The difference between the days 4 and 8 indices was approximately 4.5 for the V(IV) group's blood and only about 0.9 and 1.1 for indices determined from blood of V(III)- and V(V)-treated mice, respectively.

Blood withdrawn eight days after vanadium injection seemed to be equally susceptible to hemolysis, whereby, there were no significant differences among treatment groups. Thus, it appears that when considering the total sampling interval, the V(III) treatment was the least effective of the three. The samples obtained from V(V)-injected mice appeared to be secondary to the V(IV) group in their vulnerability to vanadium-induced erythrocyte rupture in vitro. Such conclusions were qualitatively substantiated by observations made during the collection of cardiac blood prior to manipulations for determination of hemolytic indices and erythrocyte counts. The fluids of supernatants obtained from centrifuged blood were least transparent for the V(IV) samples, followed by the V(V) and V(III) groups' samples, i.e., indicating greater erythrocyte sensitivity of the V(IV) samples to rupture during sample preparation for spectrophotometric analysis.

Data shown in figure 2 correlate with the hemolytic indices shown in the previous figure. Control mean erythrocyte counts were approximately $7.3 \times 10^6/mm^3$ for each of the sampling days. Such control counts are standard for this strain and sex of mouse[10]. The in vivo erythrocyte responses to V(III), V(IV), and V(V) treatments reflect an apparent reduction compared to control erythrocyte counts. The reduction was most pronounced four days post-injection for all three vanadium compounds tested. This time course is comparable to the maximum hemolysis effect. The most abrupt decrease in the erythrocyte titer during the experimental period was seen in the V(IV)-treated females on day 4. In regard to the V(III) and V(V) erythrocyte counts, there appeared to be little difference between days 2 and 8 counts from those non-vanadium-treated mice.

Data indicate that the V(IV) form was the most effective in depressing erythrocyte counts and in changing the erythrocyte membrane integrity, promoting greater hemolysis in vitro after in vivo vanadium-exposure of the erythrocytes. The four-day sampling period appeared to be the optimal time for the maximal responses of V(IV) as well as those of V(III) and V(V). Hansen et al.[8] have reported the hemolytic effects of vanadyl sulfate and sodium vanadate; the former compound was found to be the most potent in promoting hemolysis of isolated human erythrocytes. Results reported in these studies are in good agreement with theirs. However, the data presented here show that vanadyl sulfate affects erythrocytes in vivo as expressed by their increased hemolytic indices determined in vitro. Since all samples subjected to the hemolysis were corrected for hematocrit, one cannot argue that the differences in the observed absorbances were due to a discrepancy in the numbers of erythrocytes subjected to the hemolytic challenge among samples either within a group or among groups. It is tempting to postulate that erythrocytes exposed to vanadium in vivo became more subject to rupture due to an alteration of the erythrocyte's membrane. It is well established that vanadium upset the $Na^+K^+$-ATPase membrane system[11]. In addition, it has been published that vanadium increased the human erythrocyte membrane's permeability to sodium and potassium[12]. In either case, the erythrocyte may become more fragile due to changes in osmotic pressure. Thus, erythrocytes from vanadium-treated mice, although intact upon removal from the animal, may be more sensitive or vulnerable to rupture due to an osmotic effect or perhaps to an accelerated membrane-aging phenomenon. Therefore, when such cells are exposed to hemolytic agents, they would show greater hemolysis

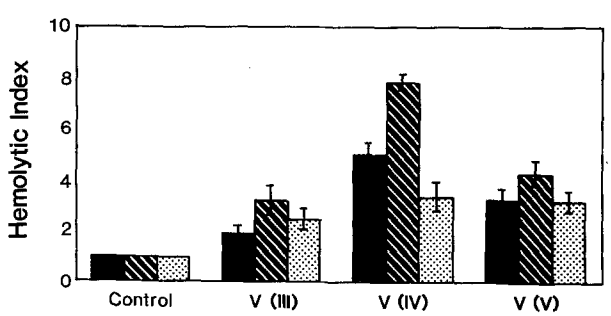


Figure 1. Hemolytic indices of erythrocytes in vitro obtained from vanadium-injected mice. The solid bars represent values obtained two days following treatment and the striped and stippled bars represent days 4 and 8 values, respectively.

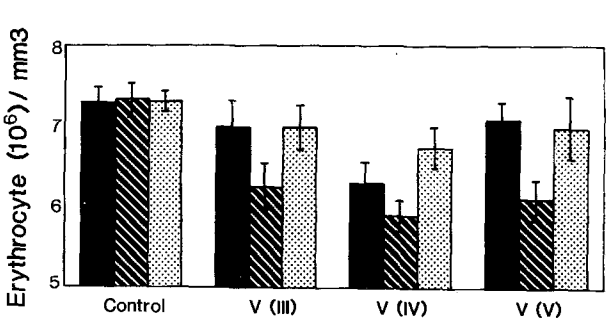


Figure 2. Number of peripheral erythrocytes obtained from vanadium-injected mice. The solid bars represent values obtained two days following treatment and the striped and stippled bars represent days 4 and 8 values, respectively.

compared to non-vanadium-exposed erythrocytes and those erythrocytes in which some vanadium compound was less effective in creating the membrane effect.

The reduction of the number of erythrocytes in V(IV)-treated mice was statistically significant on days 2, 4, and 8 compared to controls. It has been reported that vanadyl sulfate induced an increase in erythrocyte production as reflected by percentage of radioiron incorporation that occurred eight days after vanadium treatment [10]. Those data suggested that the production effect followed in the wake of an earlier hemolytic effect. This would, of course, lower the oxygen-carrying capacity of the blood and, in response to such a reduction, the homeostatic response of accelerated erythrocyte production would be expected. The results reported here agree quite well with the earlier reports. The depression of the peripheral erythrocyte count temporarily coincides with the maximum hemolytic effect for all three test vanadium compounds. Even though there seems to be variable potency concerning alternation of erythrocytic membranes via hemolysis and reduction in the number of circulating erythrocytes, it appears that all test vanadium compounds were most effective four days following exposure. In addition, there is a clear temporal correlation between the magnitude of the hemolytic index and the observed reduction in the number of peripheral erythrocytes obtained from vanadium-treated mice.

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## Critical period for induction of congenital hydrocephalus and dysplasia of subcommissural organ by prenatal X-irradiation in rats

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*Summary.* A single whole-body X-irradiation of pregnant Wistar rats at a dose of 1.05 Gy at 10.30, 12.30 and 14.30 h respectively, of gestational day 10 resulted in significantly high incidences of hydrocephalic offspring. No hydrocephalic offspring resulted from X-irradiation of pregnant rats with 1.05 Gy at 16.30 h, whereas a dose of 1.22 Gy at 16.30 h resulted in a low but statistically significant incidence of hydrocephalus. Neither 1.05 Gy nor 1.22 Gy X-irradiation of pregnant rats at 18.30 h resulted in any hydrocephalic offspring. Dysplasia of the subcommissural organ was noticed in all the hydrocephalic brains histologically examined.
*Key words.* Congenital hydrocephalus; subcommissural organ; prenatal X-irradiation; critical period; rat.

Congenital hydrocephalus has been shown to occur spontaneously or in inherited form in many mammalian species, including human beings. A number of investigators have already expressed several different ideas on the pathogenetic mechanism of congenital hydrocephalus [1]. Recently, we investigated the histological abnormalities in the congenital hydrocephalus spontaneously occurring in the MT/HokIdr mouse and the CWS/Idr rat, and suggested that the dysmorphogenesis of the subcommissural organ (SCO) may be involved in the causation of congenital hydrocephalus [2, 3]. Dysplasia of the SCO has also been reported by other authors in the inherited hydrocephalus occurring in rats [4, 5].

Congenital hydrocephalus has experimentally been induced in viable offspring in laboratory animals by treatment of the mothers with several teratogens [6, 7]. We have reported that the treatment of the mothers with 100 R (0.87 Gy) X-irradiation, or the administration of 5 mg/kg methylnitrosourea to pregnant rats on gestational day 9.5, results in high incidences of viable hydrocephalic offspring, and that the SCO of these hydrocephalic brains is reduced in size and developed only at the caudal roof of the third ventricle [7, 8].

The purposes of this study were to delineate the precise period of susceptibility of the embryo to X-irradiation which produces congenital hydrocephalus, and to deter-