

## Subacute Vanadium Toxicity in Rats

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Subacute vanadium toxicity was investigated in rats. Female Wistar strain rats, weighing 180 g (approximately 6 weeks old), were divided into three groups of seven each. They were fed a base diet with addition of various concentrations of vanadium (0, 50 or 100 ppm) for 10 weeks. The amounts of vanadium found in kidney and liver increased in response to vanadium exposure. A significant increase in metallothionein (MT) was observed in kidney of both the 100 ppm and 50 ppm vanadium-fed groups. Significant decreases in hemoglobin, hematocrit and leukocyte counts were also observed in the former, as well as decreased lymphocyte counts, B cells and immunoglobulin G and M levels.

**Key words** — vanadium, rat, leukocyte, lymphocyte, metallothionein

### INTRODUCTION

Vanadium in the air originates mainly from the combustion of fossil fuels, especially residual oils, which are known to be rich in this element. It has been shown that the burning of fossil fuels results in the release of 12000–24000 tons of vanadium per year, of which roughly 10–15% is deposited in the ocean as atmospheric fallout.<sup>1)</sup> In relation to this, epidemiological investigations have demonstrated a correlation between vanadium exposure and the incidence of lung cancer.<sup>2)</sup> The toxicity of vanadium is largely confined to the respiratory tract. Irritant activity with respect to the skin and eyes has also been described from industrial exposure.<sup>3)</sup> On the other hand, in recent years a number of experimen-

tal studies have shown an insulin-like action of different vanadium compounds.<sup>4,5)</sup> However, the major problem with vanadium treatment of diabetic animals has been its potential toxicity.<sup>6)</sup> Vanadium has been shown to be a potent inhibitor of many enzymes,<sup>7–9)</sup> including various ATPases such as those that are Na, K and Ca-dependent, dynein and myosin ATPases, many phosphatases and kinases, as well as ribonuclease. Vanadium inhibits the hepatic biosynthesis of cholesterol and phospholipids in experimental animals.<sup>10–12)</sup> We have already reported that relatively high concentrations of vanadium were found in the bones of rats fed a diet containing vanadium.<sup>13)</sup> This finding, therefore, suggests that vanadium may affect the hemopoietic system. From these aspects, we studied the toxicity of this element.

### MATERIAL AND METHODS

**Animals and Diets** — Female Wistar strain rats, approximately 6 weeks old and weighing 180 g (average), were purchased from Japan SLC Inc. (Shizuoka, Japan). The rats were divided into three groups of seven each. They were fed a base diet containing various concentrations of vanadium as sodium meta-vanadate (0, 50 and 100 ppm) for 10 weeks. Sodium metavanadate was purchased from Wako Pure Chem. Ind., Ltd. Japan. We have already reported the composition of the base diet.<sup>13)</sup> After 10 weeks, rats were killed by cardiac puncture under light ether anesthesia, and blood and tissues (kidney, liver and spleen) were removed immediately.

**Determination of Vanadium in Kidney and Liver** — The amounts of vanadium in tissues were determined by our previous method.<sup>14)</sup> Tissue samples (1–0.1 g) were dried at 120°C for 2 h on a hot plate and reduced to ash at 250°C for 2 h, then at 550°C for 6 h in a muffle furnace. The residues were dissolved in 5% HCl and adjusted to pH 4–6. The solution was transferred to a separatory funnel and 2 ml each of 10% ascorbic acid and 1% ammonium pyrrolidine dithiocarbamate (PDCA) solution were added. After allowing the mixture to stand for 5 min, 5.0 ml of xylene was added and the sample was vigorously shaken for 2 min. The xylene layer was subjected to atomic absorption spectrophotometry with a graphic furnace atomizer.

**Measurement of metallothionein (MT)** — The contents of MT in the kidney and liver were determined by the Cd-hem method.<sup>15)</sup>

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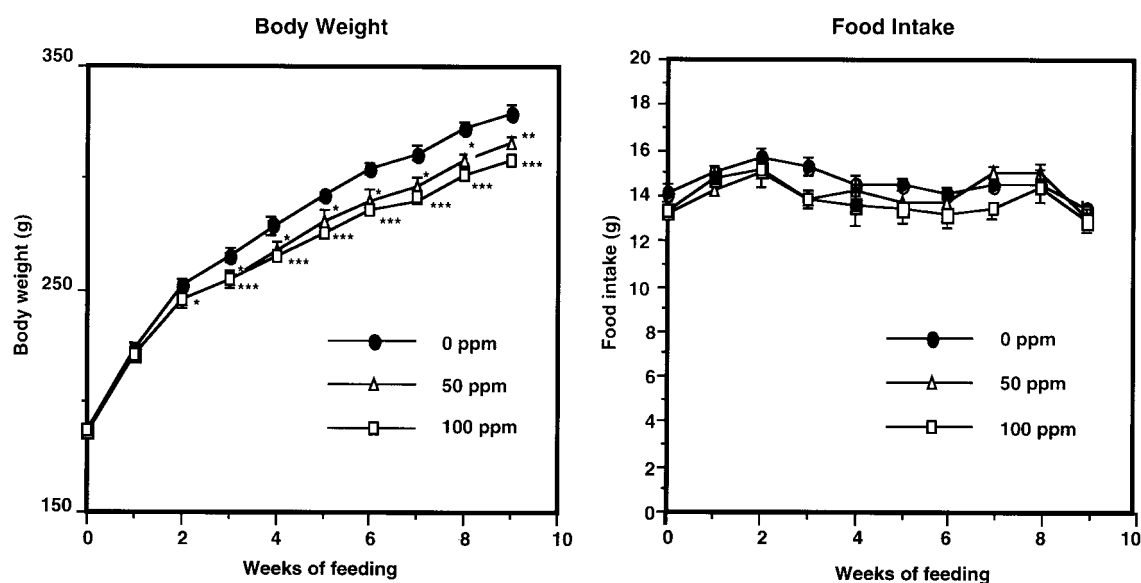


Fig. 1. Body Weights and Food Intake in Vanadium-Fed and Control Rats  
Significantly different from V 0ppm group : \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Determination of Glutamic Pyruvic Transaminase (GPT), Glutami Oxaloacetic Transaminase (GOT), Cholinesterase (ChE) and Alkaline Phosphatase (Alp) Activities** — Plasma GPT, GOT, ChE and Alp activities were measured by a colorimetric method (GPT-UV test, GOT-UV test, ChE B test and Al -K test, Wako Pure Products K. K., Japan).

**Determination of Thiobarbituric Acid Levels (TBA) in Tissues** — TBA levels were assayed by TBA reaction.<sup>16)</sup>

**Determination of Erythrocyte, Platelet, Reticulocyte and Leukocyte Counts, Hemoglobin Levels, Cell Number and Immunoglobulin Levels** — The erythrocyte, platelet and leukocyte counts were taken by an automatic blood cell counter, the reticulocyte count by staining with brilliant cresyl blue and the hemoglobin levels by cyanmet hemoglobin method. The lymphocytes, monocytes, neutrophils and eosinophils were counted by microscopy. Cell numbers were determined by flow cytometry (Becton Dickinson Immunocytometry Systems), and plasma immunoglobulins by Single Radial Immunodiffusion (Rat RID kit, Birmingham, U.K.).

**Statistical Analysis** — Values are shown as means and S.E.M., except where otherwise indicated. Data were analyzed using one-way ANOVA and, when appropriate, by a Student-Newman-Keul test. Results were considered significant at  $p < 0.05$ .

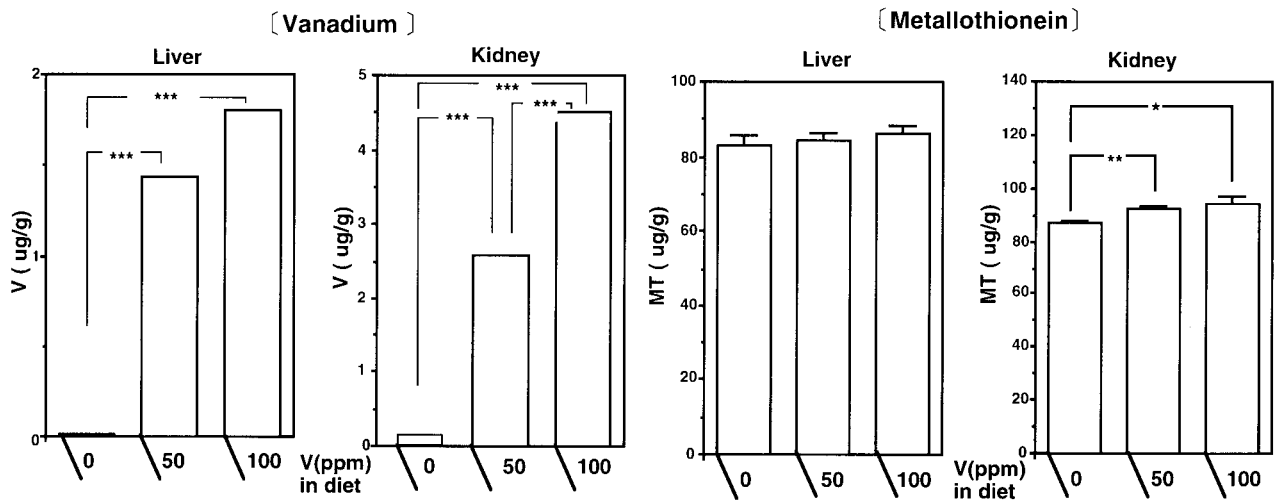
## RESULTS

As shown in Fig. 1, the vanadium-100 ppm-fed group showed significant decreases in body weight after 3 weeks of feeding. However, there was no significant difference in their food intake throughout the feeding period.

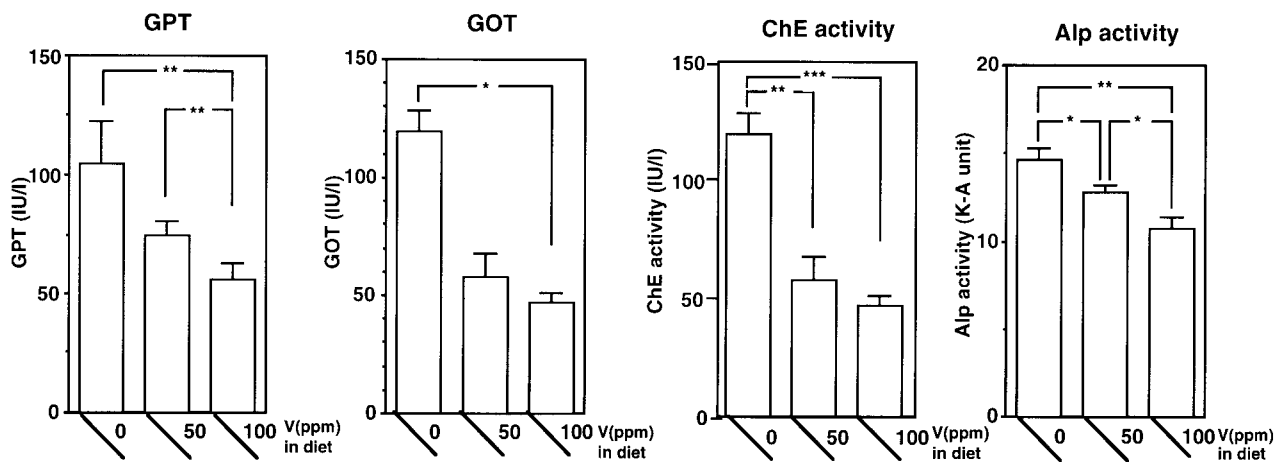
Figure 2 shows the contents of vanadium and MT in liver and kidney of the three groups. The contents of vanadium in both organs of all groups increased in response to vanadium exposure. A significant increase in MT was observed in kidney of both the vanadium-100 and 50 ppm-fed groups.

Plasma GPT, GOT, ChE and Alp activities in the vanadium-100 ppm-fed group were significantly lower than those in the control group (Fig.3). We have already found that vanadium directly inhibits these activities (Table 1).

Figure 4 shows TBA levels in liver and kidney of the three groups. The levels of TBA in kidney in the vanadium-100 ppm-fed group were significantly higher than those in the control group. Figure 5 shows hematological indices of the three groups. A significant increase in platelet and reticulocyte counts in the vanadium-100 ppm-fed group, and significant decreases in hemoglobin, hematocrit and leukocyte counts were also observed in the vanadium-100 ppm-fed group. Moreover, a significant decrease in lymphocyte counts was observed in the vanadium-100 ppm-fed group (Fig. 6).



**Fig. 2.** Vanadium and Metallothionein Contents in Liver and Kidney of Vanadium-Fed and Control Rats  
Significantly different from V 0ppm group : \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .



**Fig. 3.** Plasma GPT, GOT, ChE and Alp Activities in Vanadium-Fed and Control Rats  
Significantly different between the two groups: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Table 1.** Inhibitory Effect of Vanadium against GPT, GOT, ChE and Alp

Vanadium	Inhibition (%)			
	GPT	GOT	ChE	Alp
NaVO <sub>3</sub>				
3 $\mu$ M	75	86	88	78
0.3 $\mu$ M	27	34	35	30

Values are average of triplicate determinations. GPT, GOT, ChE and Alp were assayed as described in method section.

Figure 7 shows T cell and B cell counts for the three groups. A significant decrease in B cells was observed in the vanadium-100 ppm-fed group. Moreover, significant decreases in immunoglobulin

G and M levels were observed in the vanadium-100 ppm-fed group (Fig. 8).

## DISCUSSION

The vanadium-100 ppm-fed group showed significant decreases in body weight after 3 weeks of feeding. A.de la.Torre *et al.*<sup>17)</sup> reported that i.p. injections of sodium orthovanadate at 10 mg/kg/d for 8 consecutive days led to decreased food intake, diarrhea and weight loss in rats. In our experiment, diarrhea and small intake food decreases were seen during the breeding period. The contents of MT in kidney of the vanadium-100 and 50 ppm-fed groups

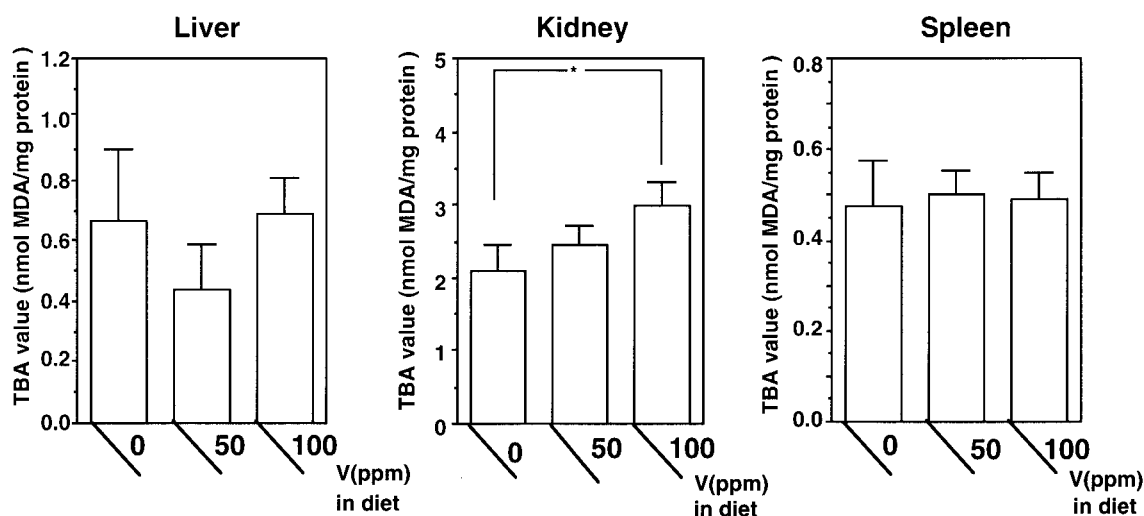


Fig. 4. TBA Values in Liver, Kidney and Spleen of Vanadium-Fed and Control Rats  
Significantly different between the two groups: \* $p < 0.05$ .

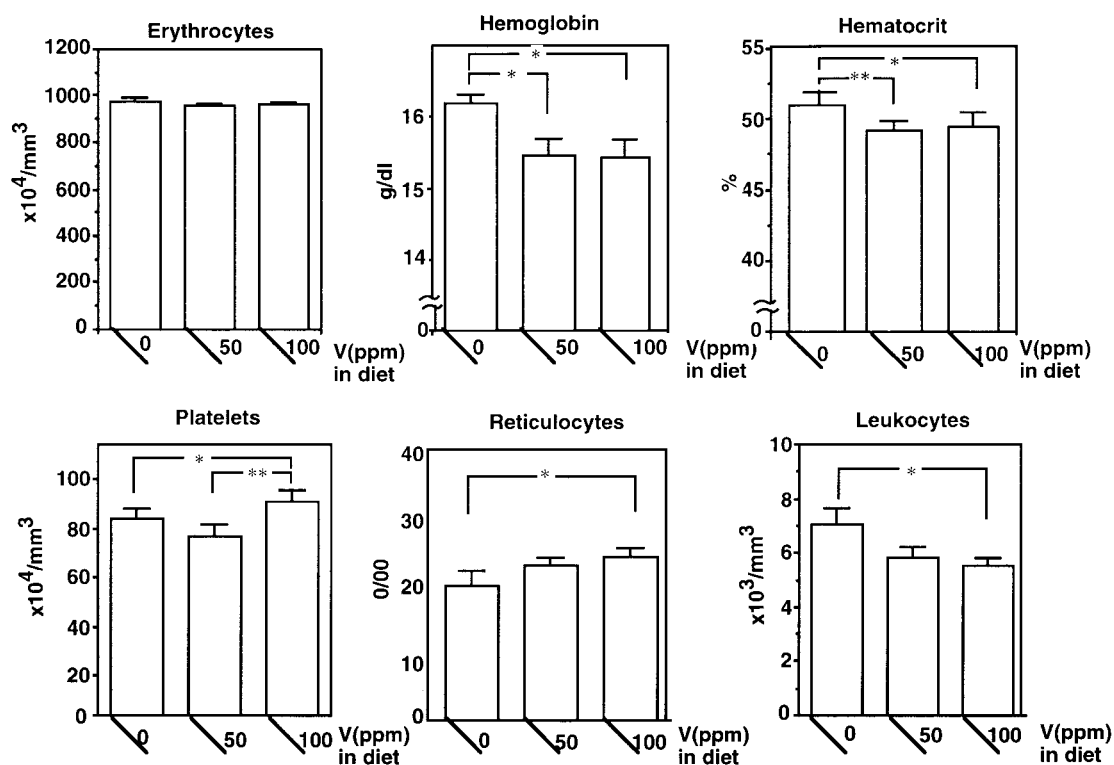


Fig. 5. Hematological Indices of Vanadium-Fed and Control Rats  
Significantly different between the two groups: \* $p < 0.05$ , \*\* $p < 0.01$ .

were significantly higher than those in the control group.

It is well known that the synthesis of MT is induced by heavy metals such as cadmium, zinc, copper and mercury. However, there is little information known on vanadium. We found that vanadium

is a weak inducer of MT (Fig. 2). Although MT was induced by vanadium, the renal TBA levels in the vanadium-100 and 50 ppm-fed groups increased. This increase in TBA value is probably attributed to the quite small induced amount of MT by vanadium in comparison with that by cadmium.

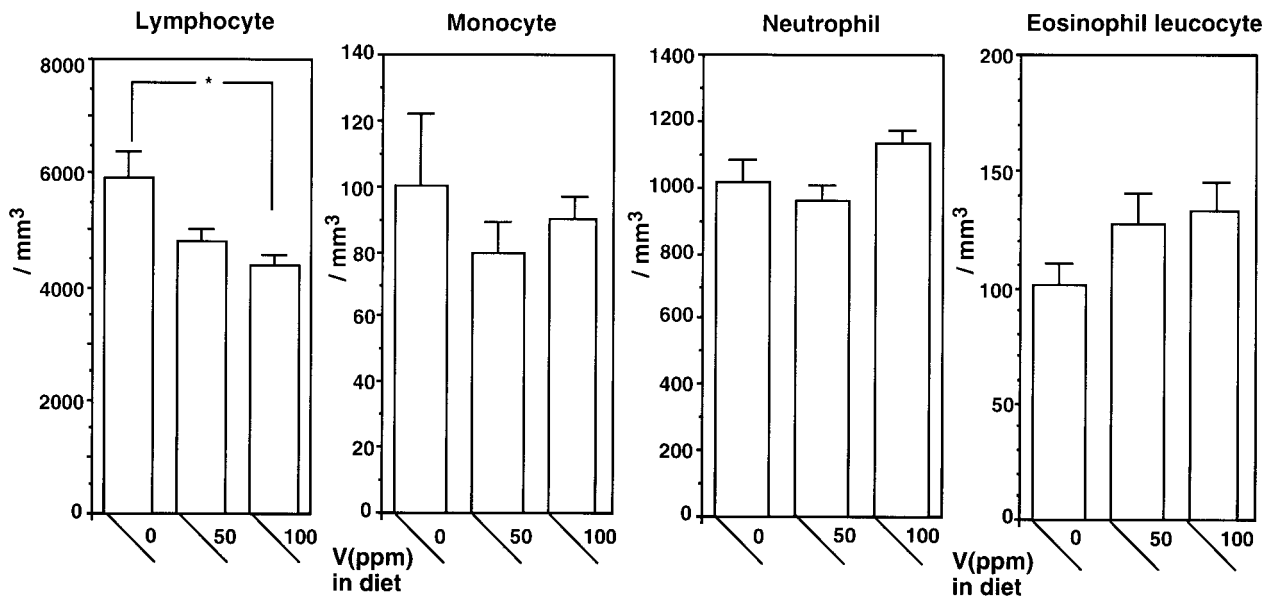


Fig. 6. Leukocyte Counts in Vanadium-Fed and Control Rats  
Significantly different from the two groups: \* $p < 0.05$ .

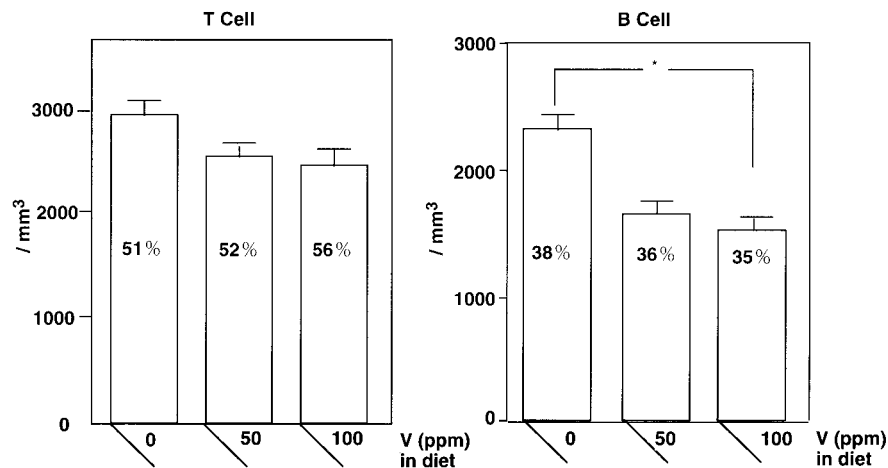


Fig. 7. T Cell and B Cell Counts in Vanadium-Fed and Control Rats  
Significantly different from the two groups: \* $p < 0.05$ , \*\* $p < 0.01$ .

Plasma GPT, GOT, ChE and Alp activities in the vanadium-100 ppm-fed group were significantly lower than those in the control group. We confirmed that vanadium directly inhibits these activities *in vitro* Lopez *et al.*<sup>18)</sup> described that both oxovanadium and vanadium ions are potent competitive inhibitors of the *Escherichia coli* alkaline phosphatase-catalyzed hydrolysis of *p*-nitrophenyl phosphate. Vanadium binds about as well as inorganic phosphate and has a pH-Ki profile similar to that of phosphate. They observed that vanadium ions can bind with high specificity to the phosphate-binding site on the enzyme. Kumar and Corder<sup>19)</sup> reported that

vanadate is a potent diuretic, natriuretic and vasoconstrictor in the isolated, perfused rat kidney and is nephrotoxic at high dose levels. Moreover, the renal effects of vanadate were reported to be similar to those of ouabain, and inhibitory effects on Na-K-ATPase and/or Ca-ATPase activity of renal vascular and tubular cells were suggested as possible mechanisms.<sup>20,21)</sup> Gibbons *et al.*<sup>22)</sup> reported that vanadium is a potent inhibitor of dynein 1 ATPase and thus the motility of cilia and sperm flagella.

More recently, Dafnis *et al.*<sup>23)</sup> showed that an i.p. injection of 5 mg vanadate/kg caused hypokalemic distal renal tubular acidosis in association with

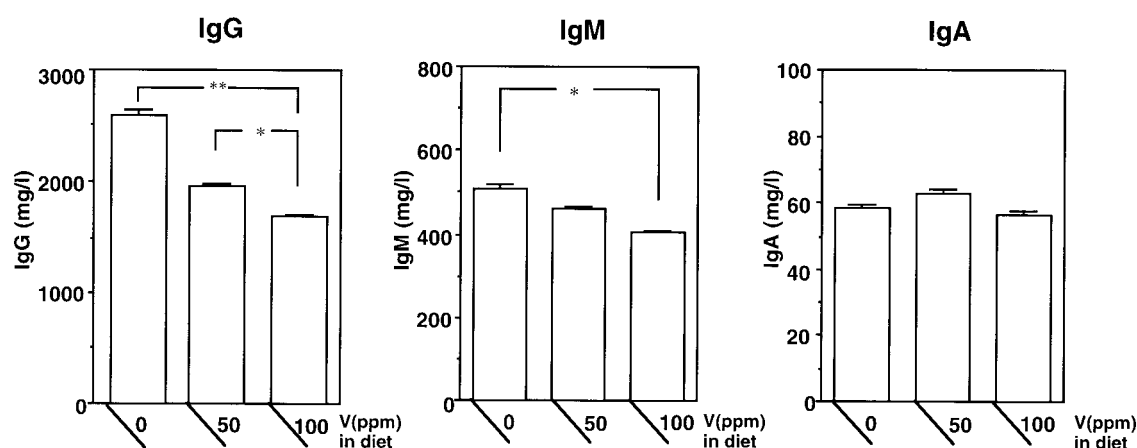


Fig. 8. Plasma Immunoglobulin Levels of Vanadium-Fed and Control Rats  
Significantly different from the two groups: \* $p < 0.05$ , \*\* $p < 0.01$ .

inhibition of collecting tubule H-K-ATP ase activity. It should be noted that vanadium anions tend to form a complex with phosphate.

In our experiment, significant decreases in lymphocyte counts, B cells and immunoglobulin G and M levels were observed in the vanadium-100 ppm-fed group. We previously showed that considerable retention (16%) of this element by the body, and relatively high concentrations of vanadium were found in the bones of rats fed a diet containing vanadium.<sup>13</sup> The findings of this study show that vanadium also affects the hemopoietic system. These findings can be of use in the risk assessment of environmental and occupational vanadium exposure. If in the future, vanadium compounds are used to treat human diabetes, great care must be taken because of its toxicity. In the future, experiments on chronic exposure are necessary at low doses to further understand chronic vanadium toxicity.

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