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Effect of chronic vanadium administration in drinking water to rats

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Two-month old Wistar rats of both sexes received, as sole drinking liquid, an aqueous solution of ammonium metavanadate (AMV) at a concentration of 0.01 or 0.05 mg V cm^{-3} (0.2 or 1.0 mM) for a period of 4 weeks. It was calculated that the animals took up doses of 1.5 and 5–6 mg V kg body weight $^{-1}$ 24 h^{-1}, respectively. Food and AMV solution consumption in the experimental groups was similar to food and water consumption in the control group. A statistically significant decrease of consumption of AMV solution at a concentration of 0.05 mg V cm^{-3} was noted only in males. Hematological examination demonstrated a decrease in the erythrocyte count, hemoglobin level and hematocrit index. This decrease in the erythrocyte count was associated with an increased percentage of reticulocytes in the peripheral blood of the animals drinking the solution with a higher vanadium content. Biochemical analyses demonstrated a decrease of L-ascorbic acid levels in the plasma and erythrocytes of animals drinking the AMV solutions. A distinct tendency for the malonyldialdehyde level to increase in the blood was also observed. Among the enzymes examined in the erythrocytes (catalase, glucose-6-phosphate dehydrogenase, lactate dehydrogenase and δ-aminolevulinic acid dehydratase [ALA-D]) only ALA-D activity was depressed.

Keywords: L-ascorbic acid, erythrocyte enzymes, hematological parameters, vanadium

Introduction

Vanadium is considered an essential trace element for chickens and rats, whereas its biological function for other animals and man has not yet been fully investigated (Anke *et al.* 1991, Nielsen 1991a, b, Rehder 1991, 1992).

Attention has recently been called to the role of vanadium in the metabolism of the thyroid (Nielsen 1990). An excess of vanadium is, however, toxic because of the relatively ready combination of this element with amino acids, peptides, proteins, enzyme substrates, nucleotides and carbohydrates (Urretavizcaya & Baran 1987, Tracey & Gresser 1988, Tracey *et al.* 1988, Crans *et al.* 1989, Geraldes & Castro 1989, Lord & Reed 1990).

A number of papers have appeared in recent years on the influence of vanadium on cell metabolism (Levine 1991, Sabbioni *et al.* 1991, Zaporowska *et al.* 1991), although only a few of them deal with the metabolism of blood cells (Vives-Corrons *et al.* 1981, Heller *et al.* 1987, Yoshino *et al.* 1989, Mendz 1991, Xu *et al.* 1991). Most of these studies, however, have been performed *in vitro*.

In our earlier papers we demonstrated the toxic influence of vanadium administered in drinking water on the red blood cell system of rats (Zaporowska & Wasilewski 1989, 1992a). The hematological consequences of a vanadium excess in animals and man were also presented in our mini-review (Zaporowska & Wasilewski 1992b). The present paper is a continuation of our previous investigations. Its aim is to investigate the influence of low vanadium concentrations administered in drinking water on the hematological indices, certain biochemical parameters and the activities of some enzymes in the erythrocytes of Wistar rats.

Materials and methods

Chemicals

Ammonium metavanadate (NH_4VO_3; AMV) was purchased from Reachim (Russia). 5-Aminolevulinic acid–HCl

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and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) were obtained from Serva Feinbiochemica (Heidelberg, Germany). Brilliant cresyl blue, NADP, sodium glucose-6-phosphate, sodium arsenate, sodium tungstate, triethanolamine, 2-thiobarbituric acid (TBA) and trichloroacetic acid were obtained from Merck (Darmstadt, Germany). Heparin was obtained from Polfa (Warsaw, Poland). Phosphate buffered saline (PBS) was obtained from WSiS (Lublin, Poland). All other reagents came from POCH (Gliwice, Poland).

Animals and treatments

Two-month old Wistar rats of both sexes (46 males and 47 females) were used in this study. Animals of each sex were randomly divided into three groups. Group I (control) received deionized water to drink, whereas the rats in groups II and III received, as sole drinking liquid, an aqueous solution of AMV at concentrations of 0.01 and 0.05 mg V cm^{-3} for 4 weeks, respectively. The rats were fed a standard granulated rodent laboratory chow (LSM; CLPP; Motycz, Lublin, Poland). Rats were housed singly in stainless steel cages in a vivarium (temperature, 19–20 °C; relative air humidity 60 ± 10%; natural day–night light cycles). The amounts of water, AMV solution and food consumed for the tested rats were checked daily during of the experimental period. The vanadium intake was calculated on the basis of the amount of AMV solution consumed for rats. Body weight was checked weekly.

Hematological parameters

The number of erythrocytes and leukocytes was counted in a Bürker chamber. Hemoglobin level was determined by the cyanmethemoglobin method and hematocrit by the micro-method. The percentage composition of leukocytes and polychromatophilic erythrocytes in the peripheral blood was counted in preparations stained by the Pappenheim method. The percentage of reticulocytes was determined in peripheral blood preparations stained with brilliant cresyl blue.

Enzyme measurements

Freshly drawn blood, collected with heparin as an anticoagulant, was centrifuged for 10 min at 800 × *g* at 4 °C. Plasma and buffy coats were removed by aspiration. The erythrocytes were washed 3 times with a double volume of cold isotonic PBS. Red blood cells were then hemolyzed by adding 2 volumes of distilled water. The hemolyzed blood samples were centrifuged and the enzyme activities were determined in the supernatant obtained.

The activities of the erythrocyte enzymes were measured by spectrophotometric methods: catalase (CAT: EC 1.11.1.6) according to Aebi (1970), glucose-6-phosphate dehydrogenase (G6P-DH; EC 1.1.1.49) after Beutler (1975), lactate dehydrogenase (LDH; EC 1.1.1.27) by using ready Cormay sets and δ-aminolevulinic acid dehydratase (ALA-D; EC 4.2.1.24) by the method of Berlin & Schaller (1974).

Other biochemical methods

The level of lipid peroxides in erythrocytes [expressed as malonyldialdehyde (MDA) equivalent] was determined by the method of Stocks & Dormandy (1971) modified by Gilbert *et al.* (1984). A molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} was used for calculations. The L-ascorbic acid concentration in plasma and red blood cells was determined according to the method of Kyaw (1978). The reduced glutathione level (GSH) in blood was estimated according to Beutler (1975).

Statistics

Student's *t*-test was used for statistical analysis. The value of $P < 0.05$ was assumed as the level of significance. All results are presented as mean values ± SEM.

Results

Rats receiving an aqueous solution of 0.01 or 0.05 mg V cm^{-3} as sole drinking liquid for 4 weeks did not show any differences in their external appearance or locomotor behavior as compared with control ones.

Food and AMV solution uptake in both experimental groups was similar to food and water uptake in the control group. Only males used a significantly lower quantity of 0.05 mg V cm^{-3} AMV solution (Table 1).

Body weight increases of the animals receiving aqueous AMV solutions were lower than those of controls (Table 1; Figure 1).

Hematological analyses showed a statistically significant decrease in the erythrocyte count in the animals receiving the 0.01 and 0.05 mg V cm^{-3} AMV solutions. The hemoglobin level and hematocrit value also decreased. The difference was significant for hemoglobin in animals receiving the higher vanadium concentration solution; however, hematocrit decreased significantly only in males (Table 2). The decrease in the erythrocyte count was accompanied by an increase in the percentage of reticulocytes and polychromatophilic erythrocytes in the peripheral blood of animals intoxicated with AMV. The difference was statistically significant only for reticulocytes in the animals receiving the 0.05 mg V cm^{-3} concentration AMV solution (Figure 2).

The leukocyte count was not changed under the influence of vanadium dosed in drinking water (Table 2). Neither was the leukocyte picture changed in these animals (Table 3).

Biochemical investigations showed a rise in the MDA blood level and a fall in the L-ascorbic acid level both in the plasma and erythrocytes of the rats intoxicated with AMV. The GSH level was slightly

Table 1. Food, fluid, vanadium intake and body weight gain in rats studied

Group of animals		Food intake (g rat^{-1} 24 h^{-1})	Fluid intake (cm^3 rat^{-1} 24 h^{-1})	Vanadium (mg kg body wt^{-1} 24 h^{-1})	Body weight gain (g)
Males					
control	(15)	24.38 ± 0.77	25.65 ± 0.65	–	74.77 ± 5.97
AMV 0.01	(15)	22.78 ± 0.36	26.11 ± 0.84	1.18 ± 0.01	63.83 ± 2.86
AMV 0.05	(16)	23.78 ± 0.88	22.04 ± 0.68***	4.93 ± 0.15	67.85 ± 3.10
Females					
control	(16)	20.22 ± 0.81	24.34 ± 0.94	–	35.03 ± 2.61
AMV 0.01	(15)	18.48 ± 0.58	24.48 ± 1.16	1.50 ± 0.23	27.88 ± 2.80
AMV 0.05	(16)	20.74 ± 0.90	23.51 ± 1.14	6.65 ± 0.27	31.92 ± 2.22

Number of animals in parentheses.
Significantly different from control group: *** $P < 0.001$.

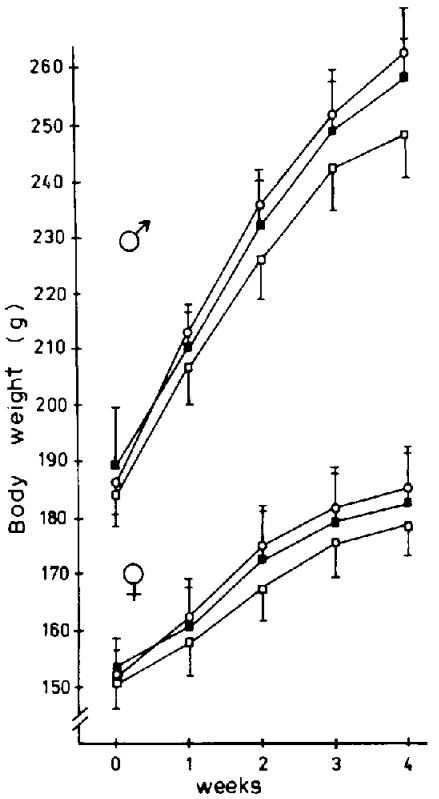

Figure 1. Changes in body weight of Wistar rats during the experimental period: ○, control group; □, rats drinking aqueous AMV solution at 0.01 mg V cm^{-3}; ■, rats drinking aqueous AMV solution at 0.05 mg V cm^{-3}.

depressed. The difference proved to be statistically significant only for L-ascorbic acid in the plasma of males (Table 4).

CAT, G6P-DH and LDH activities remained within the limits of the values recorded in the controls. Only a marked, but not significant, decrease of ALA-D activity was noted (Figure 3).

Discussion

Our earlier studies demonstrated that an aqueous solution of AMV at 0.15 mg V cm^{-3} (Zaporowska & Wasilewski 1992a) or 0.30 mg V cm^{-3} (Zaporowska & Wasilewski 1989, 1991) concentration, administered to rats as sole drinking liquid, frequently produced diarrhea ending, in some cases, in death. In the remaining animals a statistically significant decrease of food and AMV solution uptake and a diminished body weight increase were observed. The occurrence of diarrhea and a decrease in body weight appearing as a consequence of intoxication with vanadium compounds was also described by other authors (Roshchin 1967, Chakraborty *et al.* 1977, Meyerovitch *et al.* 1989, Domingo *et al.* 1991).

In the present experiment the rats received aqueous solutions of AMV at 0.01 and 0.05 mg V cm^{-3} concentration, corresponding to doses of 1.5 and 5–6 mg V kg body weight^{-1} 24 h^{-1}. The animals did not suffer from diarrhea, and their food and AMV solution consumption hardly differed from food and water consumption in the control group. Only males receiving a higher AMV concentration showed a significant lowering of food uptake. The decrease in body weight was not significant.

Despite the slight differences in the uptake of food and AMV solutions, as well as body weight increase, as compared with food and water consumption and body weight increment of controls, distinct changes appeared in the erythrocyte system of the animals tested. They were similar to those observed earlier after administration of vanadium concentrations many times higher. Thus, the application to rats of aqueous AMV solutions at 0.01 or 0.05 mg V cm^{-3} concentration in these investigations, and that at 0.15 or 0.30 mg V cm^{-3} in our earlier experiments

Table 2. Some hematological indices of peripheral blood in the tested rats

Group of animals	Erythrocytes ($\times 10^{12}$ dm^{-3})	Hemoglobin (mmol l^{-1})	Hematocrit (1)[a]	Leukocytes ($\times 10^{9}$ dm^{-3})
Males				
control	8.32 ± 0.17	9.37 ± 0.19	0.48 ± 0.001	11.59 ± 0.66
AMV 0.01	7.38 ± 0.20**	8.94 ± 0.28	0.47 ± 0.004*	11.26 ± 0.77
AMV 0.05	7.47 ± 0.27*	8.65 ± 0.26*	0.47 ± 0.003**	11.91 ± 0.68
Females				
control	8.24 ± 0.10	9.41 ± 0.12	0.48 ± 0.005	10.09 ± 0.63
AMV 0.01	7.38 ± 0.14***	8.76 ± 0.30	0.47 ± 0.003	9.26 ± 0.69
AMV 0.05	7.12 ± 0.17***	8.72 ± 0.20*	0.47 ± 0.002	10.13 ± 0.66

[a]Unit 'one'.
Significantly different from control group: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

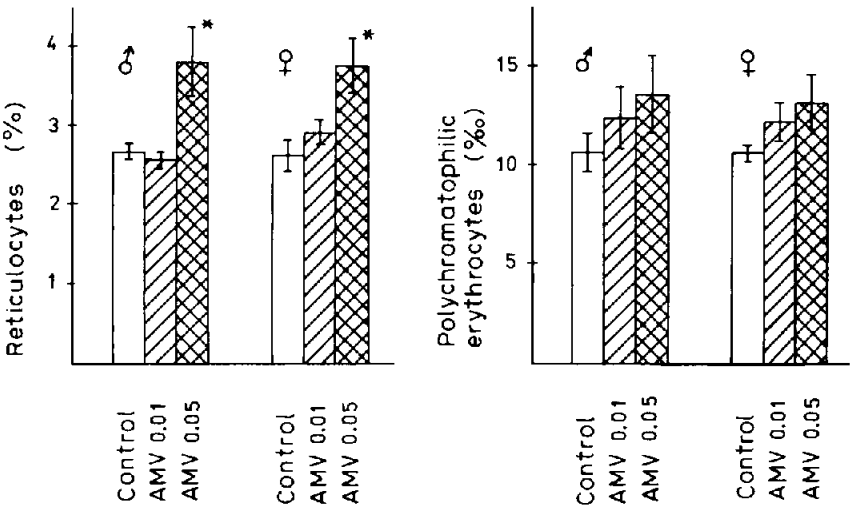

Figure 2. Reticulocyte and polychromatophilic erythrocyte percentage in peripheral blood of the tested rats. Significantly different from control group: *$P < 0.05$.

Table 3. Leukocyte composition of peripheral blood in the tested rats

Group of animals	Leukocytes ($\times 10^{9}$ dm^{-3})			
	neutrophils	eosinophils	monocytes	lymphocytes
Males				
control	2.55 ± 0.32	0.20 ± 0.032	0.54 ± 0.044	8.30 ± 0.42
AMV 0.01	2.48 ± 0.27	0.25 ± 0.024	0.48 ± 0.039	8.05 ± 0.64
AMV 0.05	2.16 ± 0.19	0.22 ± 0.022	0.58 ± 0.051	8.95 ± 0.52
Females				
control	1.92 ± 0.20	0.22 ± 0.021	0.52 ± 0.046	7.43 ± 0.50
AMV 0.01	2.05 ± 0.17	0.19 ± 0.025	0.46 ± 0.049	6.56 ± 0.52
AMV 0.05	1.82 ± 0.24	0.18 ± 0.031	0.45 ± 0.036	7.68 ± 0.47

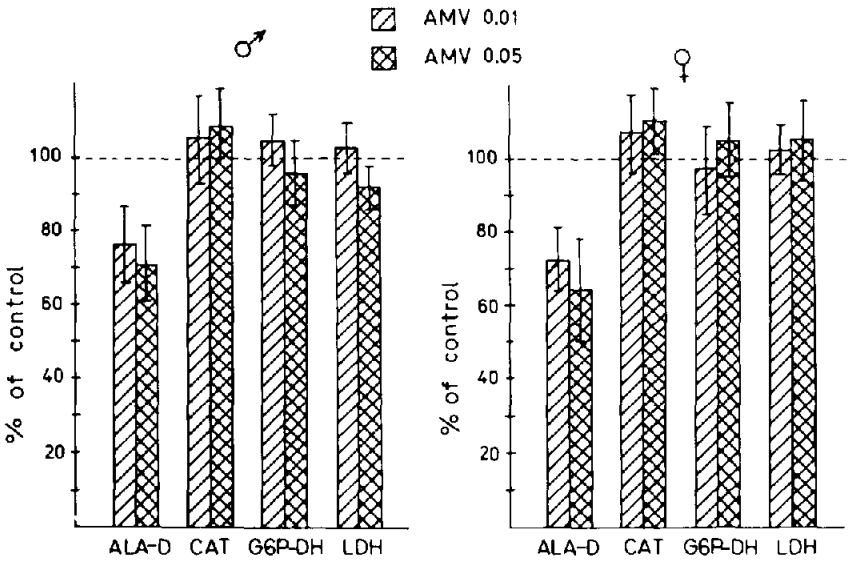

Figure 3. The activity of the erythrocyte enzymes: ALA-D, CAT, G6P-DH and LDH in the tested rat groups. The activities in the control group were: ALA-D, 2.94 ± 0.15 (males) and 2.86 ± 0.25 (females) U IRBC^{-1} min^{-1}; CAT, 2.90 ± 0.20 (males) and 3.10 ± 0.19 (females) E_{240} min^{-1} mg Hb^{-1}; G6P-DH, 8.50 ± 0.95 (males) and 8.10 ± 1.10 (females) IU g Hb^{-1}; LDH, 180.22 ± 4.50 (males) and 169.70 ± 5.85 (females) IU g Hb^{-1}.

Table 4. Levels of MDA, GSH and L-ascorbic acid in blood of rats tested

Group of animals	MDA (nM mg Hb^{-1})	GSH (mmol l^{-1})	L-ascorbic acid	
			plasma (μg cm^{-3})	erythrocytes (μg cm^{-3} packed cells)
Males				
control	0.439 ± 0.059	1.84 ± 0.19	14.09 ± 1.17	57.02 ± 6.30
AMV 0.01	0.457 ± 0.060	1.74 ± 0.15	10.67 ± 1.16*	46.56 ± 4.30
AMV 0.05	0.495 ± 0.084	1.67 ± 0.12	8.90 ± 1.69*	48.87 ± 6.04
Females				
control	0.350 ± 0.045	1.54 ± 0.08	7.99 ± 1.21	79.15 ± 8.30
AMV 0.01	0.403 ± 0.030	1.50 ± 0.08	7.29 ± 1.38	57.23 ± 5.94
AMV 0.05	0.405 ± 0.015	1.46 ± 0.05	6.81 ± 1.70	61.08 ± 9.51

Significantly different from control group: * $P < 0.05$.

(Zaporowska & Wasilewski 1989, 1991), gave similar depressions of the erythrocyte count, hemoglobin level and hematocrit index. It is difficult to explain the lack of any relation between the dose of vanadium applied and the values of the red blood cell parameters. This may be partially ascribed to the fact that diarrhea was observed in some of the animals drinking AMV solutions of 0.15 and 0.30 mg V cm^{-3} and that some of the rats did not survive the 4 week experimental period. Therefore, individuals more resistant to vanadium remained and provided the material subjected to examinations. It is also difficult to ascertain the direct cause of the decreased erythrocyte count. It may be a consequence of disturbances in their maturation, as might be indicated by the increased percentage of reticulocytes and polychromatophilic erythrocytes in the peripheral blood, with the simultaneous absence of changes in the percentage of cells making up the erythropoietic line in the bone marrow (Zaporowska & Wasilewski 1989). Such an interpretation seems to be supported by the investigations of English *et al.* (1983), who demonstrated that addition of 10–20 μM of AMV to an *in vitro* culture of Friend murine erythroleukemia cells inhibited the process of differentiation of these cells. It cannot be excluded that

the decrease in the erythrocyte count may be, at least partially, the consequence of shortened time of survival of these cells resulting from accelerated hemolysis, as suggested by Hogan (1990). The possibility of the occurence of hemolysis seems to be supported by the rise in the MDA level in the blood of the animals receiving AMV solutions to drink, as observed by us. As the product of lipid peroxidation in the erythrocyte membranes, this compound reduces their resistance and may lead to accelerated hemolysis (Inouye *et al.* 1980, Halliwell & Gutteridge 1985, Heller *et al.* 1987, Waniek *et al.* 1990).

Vanadium occurs mainly in its pentavalent form both in the systemic fluids and extracellularly. It undergoes reduction to vanadyl (VO^{2+}; +4 oxidation state) inside the cell. The process of vanadium reduction occurs, among other things, with the participation of glutathione, cysteine, NADH, NADPH and L-ascorbic acid (Macara *et al.* 1980, Heinz *et al.* 1982, Legrum 1986). In the investigations presented here a significant decrease in the L-ascorbic acid level was noted in the plasma of males. This agrees with our earlier findings (Zaporowska & Górski 1986) and the results of others (Roshchin 1967). A decrease in the L-ascorbic acid level was also noted in blood serum of subjects exposed to inhalation of vanadium (Gniot-Szulżycka *et al.* 1988). The decrease in the L-ascorbic acid level in the plasma and internal organs (Roshchin 1967, Chakraborty *et al.* 1977, Zaporowska & Górski 1986, Zaporowska 1991) of rats intoxicated with vanadium may be the result of enhanced catabolism of this compound under experimental conditions and also of diminished activity of the liver oxidase L-gulonolactone, an enzyme of the line of synthesis of this compound in the rat liver (Chakraborty *et al.* 1977).

The doses applied did not affect the activities of CAT, G6P-DH and LDH. Changes in G6P-DH and LDH activity were also not noted in our earlier studies (Zaporowska & Wasilewski 1992a), when rats were given, as only liquid, aqueous solution of AMV at a concentration of 0.15 mg V cm^{-3}. No changes in the activity of these enzymes were observed by Vives-Corrons *et al.* (1981) either in their studies on the influence of vanadium on erythrocyte enzymes *in vitro*. In the present study only the activity of ALA-D, an enzyme of the heme synthesis line, was reduced. This would confirm the earlier suggestions of Garlej *et al.* (1975) and Missenard *et al.* (1989) on the influence of this element on the process of heme synthesis.

Summing up, the doses of vanadium applied by us to rats in drinking water caused, above all, changes in the erythrocyte system. Knowledge of the range of these modifications is important, because the elements discussed have insulin-like effects on glucose metabolism. Therefore, in recent years studies on the possibility of utilization of vanadium in the treatment of diabetes have been conducted in many centres (Brichard *et al.* 1991, Domingo *et al.* 1991, 1992, Kimura *et al.* 1992). In these studies, vanadium was administered to animals most frequently in drinking water and the doses were similar to those in our experiments. Therefore, the description of possible side effects (such as changes in the erythrocyte system described here, and the decrease in the L-ascorbic acid level in the plasma and erythrocytes) may be helpful in establishing the optimal vanadium dose for pharmacological use.

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