STUDIES OF VANADIUM TOXICITY IN THE RAT

LACK OF CORRELATION WITH MOLYBDENUM UTILIZATION

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Received December 13,1973

SUMMARY: The toxic effects of vanadium in the rat are related to the extent of accumulation of the metal in the liver. The toxicity is not correlated with molybdenum utilization, since xanthine oxidase and sulfite oxidase activities as well as total liver molybdenum are unaffected by vanadium accumulation. The vanadium in liver and kidney displays an EPR signal which indicates that the metal has been reduced to vanadium (IV) from the pentavalent state and that it may exist in liver and kidney in a protein-bound form.

Molybdenum is known to be essential for nitrate reductase activity in plants and bacteria (1-4) and to be a component of the nitrogenase system in nitrogen-fixing organisms (5-7). In animals, the metal functions as a prosthetic group of the enzymes xanthine oxidase, aldehyde oxidase and sulfite oxidase (8,9). Tungsten has been well characterized as an antagonist of molybdenum in many of these systems (10-13). In addition, the relationship of vanadium to molybdenum utilization for the nitrogenase system in Azotobacter vinelandii (14) and for nitrate reductase in spinach (15) has been investigated. While vanadium does not appear to be incorporated into the nitrate reductase protein (15), isolation of a vanadium analogue of nitrogenase has been reported by Benemann, et al. (14). Their studies indicate that the vanadium-containing enzyme is inactive although the presence of the metal in some way promotes more effective utilization of molybdenum by the organism.

The toxic effects of vanadium in animals are well known (16); the molecular basis of the toxicity, however, has not been established. In view of the reported relationship between vanadium and molybdenum in nitrogen-fixing bacteria, the possibility of interaction between the two metals in animal systems was investigated as a potential explanation of the toxic effects of vanadium.

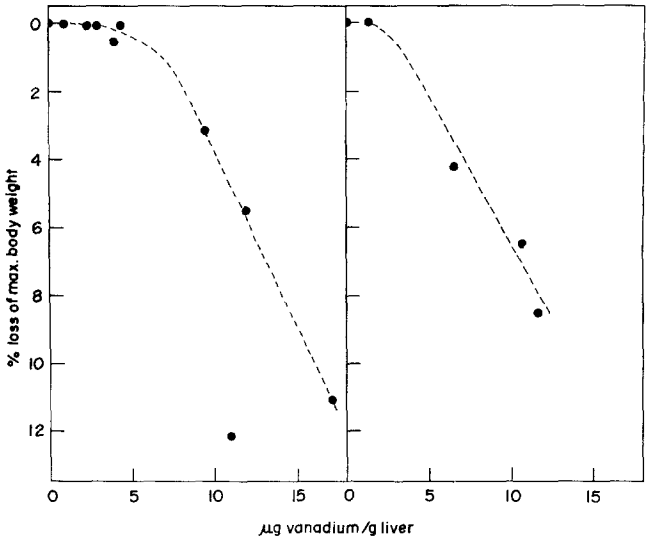

Fig. 1. Correlation between vanadium toxicity and accumulation of the metal in the liver. The results of two separate experiments are presented. Each point represents the results from an individual animal. The criterion of toxicity is defined in the text.

As the studies described below indicate, such a relationship does not in fact exist. Vanadium toxicity in the rat, therefore, is unrelated to molybdenum antagonism, and the molecular basis of the toxicity must be sought elsewhere.

MATERIALS AND METHODS: Male CD outbred rats weighing about 200 g were obtained from Charles River. They were maintained on deionized drinking water and a normal protein diet (Nutritional Biochemicals) which contained 20-30 ug of molybdenum per kg. Vanadium was administered by intraperitoneal injection as sodium metavanadate at doses of 1.25 to 2.5 mg vanadium per kg body weight. Molybdenum and vanadium in the tissues were assayed by colorimetric methods (17,18). In addition, vanadium was quantitated by EPR (electron paramagnetic resonance) spectroscopy on a Varian E-9 spectrometer, as described below. Xanthine oxidase and sulfite oxidase were assayed in various tissues as previously described (13).

RESULTS AND DISCUSSION: Rats receiving daily injections of sodium metavanadate at doses ranging from 1.25 to 2.5 mg per kg displayed symptoms of toxicity which included diarrhea and loss of weight. Higher doses of vanadium led to more severe symptoms and death. Very early in the experiments, however, it became apparent

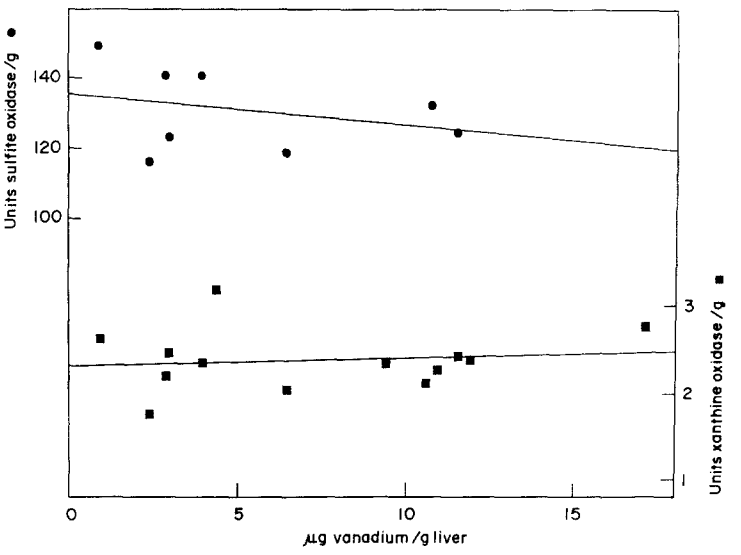

Fig. 2. Sulfite oxidase and xanthine oxidase activities in livers with varying amounts of accumulated vanadium. The lines representing least squares fits of the data have slopes of -0.9 ± 1.2 and 0.009 ± 0.21 for sulfite oxidase and xanthine oxidase, respectively. In both cases, the slopes do not vary significantly from zero, and thus neither activity is influenced by vanadium accumulation.

that there was a great deal of individual variation in the tolerance to a given dose of vanadium. It became necessary, therefore, to look at the animals individually in terms of the amount of vanadium actually accumulated in the tissues, rather than in terms of the administered dose of the metal.

As shown in Fig. 1, there is a clear correlation between the degree of sickness of an individual rat and the amount of vanadium present in the liver. The criterion of toxicity which is plotted in the figure is the weight loss of the animal (the difference between the maximum weight attained during the course of the experiment and the weight of the animal at the time of sacrifice), expressed as a percent of the maximum weight. The selection of such a measure to quantitate toxicity was arbitrary, but the method does appear to provide an adequate representation of the degree of sickness of the animals. As seen in Fig. 1, symptoms of vanadium toxicity were not evident in animals with low levels of hepatic vanadium; accumulation of the metal in excess of a specific threshold was well correlated with weight loss.

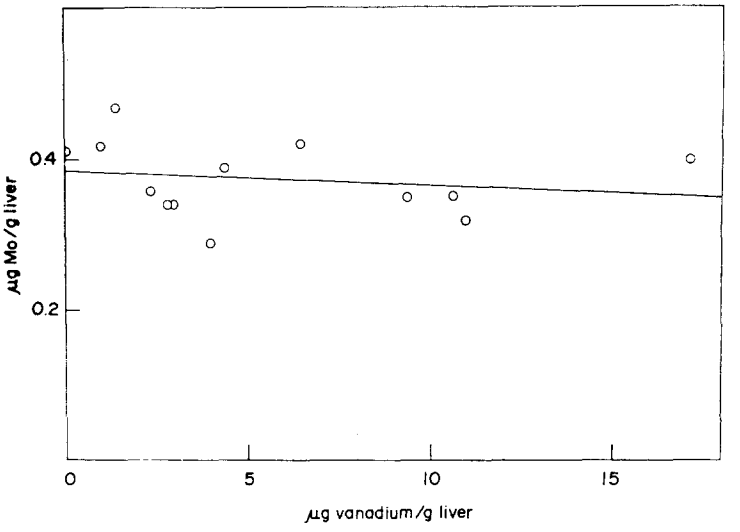

Fig. 3. Molybdenum contents of livers with varying amounts of accumulated vanadium. The line representing the least squares fit of the data has a slope of -0.002 ± 0.003. The slope does not vary significantly from zero, and thus hepatic molybdenum content is not influenced by vanadium accumulation.

The results of assays of hepatic xanthine oxidase and sulfite oxidase activitie are shown in Fig. 2. As can be seen, the activities of both of these enzymes were independent of the vanadium content of the liver. Aldehyde oxidase, the third molybdenum-containing enzyme identified in animal tissues, is present in very low amounts in rat liver (19) and was not examined in these investigations. As indicated in Fig. 3, the total liver molybdenum content was also not significantly altered by vanadium accumulation. This is in contrast to results of studies in this laboratory showing that tungsten is a molybdenum antagonist in rats, and lowers the activities of xanthine oxidase and sulfite oxidase to below 2% of control values (13). In the absence of an applied stress, animals deficient in the two molybdenum enzymes showed no signs of sickness whatsoever. Thus, it is clear that vanadium toxicity in rats is unrelated to antagonism of molybdenum utilization.

In the course of these studies, however, several additional observations were made which may be of assistance in future studies of the basis of vanadium toxicity. First of all, it was noted that livers of rats treated with vanadium

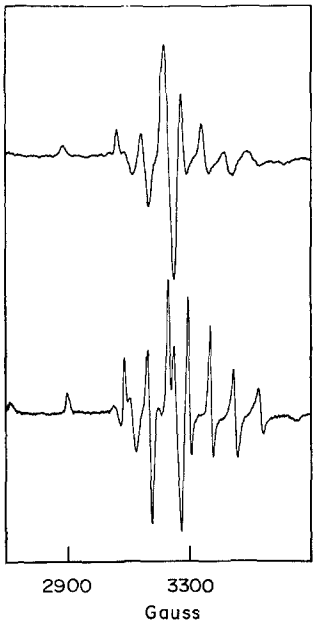

Fig. 4. EPR signals of vanadium (IV). The upper spectrum was obtained from a standard solution of sodium metavanadate reduced with dithionite. The lower spectrum was obtained from an isotonic homogenate of liver from a vanadium-treated rat, with no exogenous reductant. EPR conditions were as follows: Modulation frequency, 100 kHz; modulation amplitude, 8 gauss; microwave frequency, 9.12 GHz; microwave power, 5 milliwatts; time constant, 0.3 sec; gain, 1600; and temperature, -100°.

exhibited a multi-lined EPR signal characteristic of vanadium (IV). This finding was at first somewhat surprising since the metal was administered as metavanadate (VO_3) in the diamagnetic pentavalent state. Furthermore, as shown in Fig. 4, the vanadium EPR spectrum observed in the liver is somewhat broadened compared to the spectrum of dithionite-reduced metavanadate and shows clearly observable differences from the latter in the relative intensities of the various peaks. These differences are interpreted to indicate that the vanadium in the tissues exists in a protein-bound form.

Quantitation of vanadium by means of the EPR signal seen in homogenates of liver and kidney proved to be a simple and reliable procedure. Fig. 5 shows that colorimetric and EPR quantitations give directly comparable results over a wide range of tissue vanadium contents. Accumulation of large amounts of administered vanadium in the heart and lung of mice has been reported by earlier

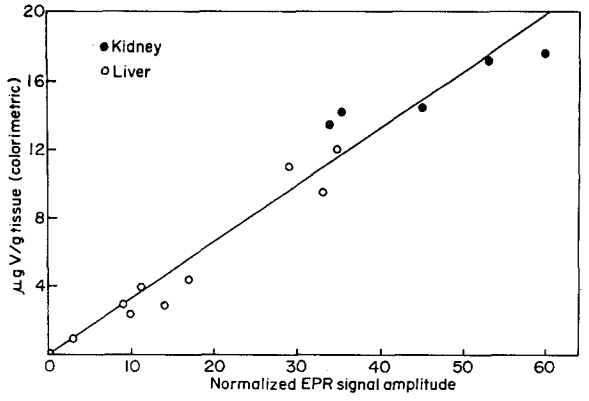

Fig. 5. Correlation between colorimetric quantitation and EPR signal of vanadium in isotonic liver and kidney homogenates. EPR quantitation was based on the amplitude of the low field line adjacent to the main line.

workers (20). In the present study, however, examination of these tissues by EPR spectroscopy revealed only small amounts of paramagnetic vanadium in the lungs and none at all in the heart. Thus, it is conceivable that the ability of the liver and kidney to reduce the vanadate by one electron is related to a specific detoxification mechanism which is present in these tissues. Alternatively, the production of the paramagnetic form of the metal may be directly related to the toxicity itself.

In summary, it has been established by several criteria that vanadium toxicity in the rat is unrelated to molybdenum antagonism. It is clear, however, that toxicity is related to accumulation of the metal in the liver and kidney and possibly to the ability of these tissues to reduce metavanadate to vanadium (IV). In addition, it appears that at least some of the metal is protein-bound. Further investigations into the altered oxidation state of vanadium and identification and characterization of the vanadium binding protein or proteins may provide clues as to the mode of action of the metal in producing symptoms of toxicity.

ACKNOWLEDGEMENTS: This work was supported in part by Grant GM 00091 from the National Institutes of Health. J.L.J. was supported by a Predoctoral Traineeship, Grant GM 00233 from the National Institutes of Health. H.J.C. is grateful to Dr. J.R. Fouts for his interest, encouragement and support.

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