Ascorbic Acid and Ethylene Diamine Tetraacetate as Antidotes in Experimental Vanadium Poisoning. (20831)

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It has been recently shown that the calcium disodium salt of ethylenediamine tetra-acetic acid ($CaNa_2EDTA$) affords protection against lethal doses of vanadium in mice(1). In a study of vanadium toxicity by Proescher *et al.*(2), it was suggested that the mode of detoxication of vanadium might be reduction in the body, from the evidence that the lower valence states of vanadium appeared to be less toxic than the higher valence states. It was therefore thought possible that ascorbic acid, a reducing substance, might also decrease the toxicity of vanadium. Accordingly, comparative tests of antidotal effectiveness of ascorbic acid and $CaNa_2EDTA$ were made in 3 animal species and the probable detoxication mechanism of the former antidote has been elucidated by polarography.

Methods. Mice of 20-29 g body weight of the Webster† strain and rats, 100-150 g body weight of a Wistar-Carworth† derived strain were used. Dogs were male mongrels, 6-12 kg in weight. The form of vanadium for administration was $NaVO_3 \cdot H_2O$‡ in isotonic saline solution. As used for mice, the concentration was 4 mg/ml (pH 7); for rats, 1% solutions (pH 7.85), and 3% solutions (pH 7)§ for the dogs. Calcium disodium ethylenediamine tetraacetate was chosen as the EDTA salt because of the reduced tendency of this salt to combine with the blood calcium(3). Ascorbic acid solutions (ca. pH 2.5) prepared without neutralization seemed to be well tolerated. Mice were injected intraperitoneally with ascorbic acid, 20 minutes prior to vanadium administration by the same route. Rats were injected with vanadium subcutaneously and were given the antidotes intraperitoneally 5 minutes later when signs of toxicity had become manifest. An observation period of 7 days was used in all mouse and rat tests. Dogs were injected intramuscularly with vanadium and the antidotes administered intraperitoneally after the first vomiting episode. Polarographic studies were made with a visible recording Sargent Model XXI instrument. Phosphate buffer, pH 7.4, gave no evidence of significant complex formation with vanadium. Each determination was made at 30°C on a 20-ml aliquot containing 0.01% gelatin as a maxima suppressor.

TABLE I. Protection of Mice by Ascorbic Acid against the Toxicity of Vanadium. Mortality ratios expressed as number of animals that died (numerator) to those used (denominator).

mg/kg $NaVO_3 \cdot H_2O$	mg/kg as V	Controls, no ascorbic acid	Ascorbic acid (1 g/kg), 20 min. before vanadium inj.
30	10.9	14/20	1/20
40	14.6	19/20	0/20

Results. Mice. Table I shows the protection afforded mice by ascorbic acid, intraperitoneally administered in a dose of 1 g/kg, 20 minutes prior to vanadium administration. Protection was essentially complete at doses corresponding to the LD_{70} and LD_{95} levels of $NaVO_3 \cdot H_2O$ (30 and 40 mg/kg, respectively). Ascorbic acid injected alone produced no signs of toxicity in 20 mice other than slight transitory depression. In subsequent tests, doses as low as 125 mg ascorbic acid/kg body weight afforded essen-

TABLE II. Antidotal Action of $CaNa_2EDTA$ and Ascorbic Acid in Vanadium-Poisoned Rats. Antidotes administered 5 min. after vanadium. Mortality ratios expressed as number of animals that died (numerator) to those used (denominator).

mg/kg $NaVO_3 \cdot H_2O$	mg/kg as V	Controls	$CaNa_2$-EDTA, 1 g/kg	Ascorbic acid, 250 mg/kg
60	21.9	20/20	7/20	6/20

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† Obtained from Hamilton Laboratory Animals, Inc., Cincinnati, O.

‡ Obtained from Fisher, Eimer and Amend.

§ The more concentrated 3% metavanadate solutions required neutralization with HCl to bring to a more physiologic pH value.

tially complete protection against an LD_{70} of $NaVO_3 \cdot H_2O$.

Rats. Table II shows the dosage-mortality data for rats when either $CaNa_2EDTA$ or ascorbic acid was administered after vanadium had given rise to manifest signs of toxicity. The 60 mg/kg dose of $NaVO_3 \cdot H_2O$ was MLD_{100} by subcutaneous administration. When $CaNa_2EDTA$ (1 g/kg) was administered 5 minutes after the vanadium, 65% of the rats survived. Ascorbic acid (250 mg/kg) administered in the same manner resulted in 70% survival.

Dogs. The intramuscular MLD of 10 mg $NaVO_3 \cdot H_2O$/kg was found to produce death within 18-36 hours with survival in only an occasional animal after a 7-day semicomatose state. Using this dose, 3 dogs were given $CaNa_2EDTA$ (100 mg/kg) intraperitoneally, after the first vomiting episode had occurred. Two more doses of EDTA at the same level and by the same route were administered at 2 and 4 hours after the first injection. Twenty-four hours later the dogs appeared to be in fair condition, and within 48 hours, the dogs were eating and showed few or no signs of vanadium toxicity. Similar responses were obtained after 3 more dogs were given 10 mg $NaVO_3 \cdot H_2O$/kg, followed by 50 mg ascorbic acid/kg‖ after the first toxic signs. Two subsequent injections of ascorbic acid were given, of the same dose and by the same route, at 2 and 4 hours after the initial injection. The ascorbic acid-treated dogs appeared to recover more quickly and showed fewer signs of toxicity than the EDTA-treated dogs. The dogs that had been treated with the antidotes appeared to be in good health and remained so for an observation period of 8 weeks after the V injection.

Polarographic studies. Ascorbic acid produced an anodic wave at an observed half-wave potential ($E_{1/2}$) of –0.01 volt. Current-voltage at different V^{+5} concentrations produced ill-defined, double waves measurable quantitatively within ±20%. A potential of –0.17 volt was taken as a reproducible $E_{1/2}$ for V^{+5} at pH 7.4 and was used as a reference for subsequent work. Similar findings have been previously described for V^{+5} in oxalate solutions(4). To determine the effects of ascorbic acid on V^{+5}, a polarogram was made of a solution containing 77 μg $NaVO_3 \cdot H_2O$/ml of phosphate buffer at pH 7.4; subsequently ascorbic acid, sufficient to give a concentration of 385 μg/ml, was added to the oxygen-free solution. Polarograms of this solution made at intervals of 15 minutes indicated that after the first interval approximately 50% of the V^{+5} had disappeared, and after one hour, 100%. This solution, buffered with acetate to pH 5.6 gave a double anodic wave characteristic of V^{+2}, thus suggesting that V^{+5} is reduced by ascorbic acid to V^{+2}. Hydrogen peroxide, an associated oxidation product of ascorbic acid, was believed not to be the agent involved in this reduction because the polarographic waves did not simulate those developed from the addition of ascorbic acid to V^{+5} solution. After reduction of V^{+5} with ascorbic acid bubbling air through the solution for several hours oxidized all the ascorbic acid, and the V returned to the pentavalent state as evidenced by the reappearance of the characteristic V^{+5} curve.

To observe the effect of an acid without strong reducing powers, gluconic acid was added to a fresh solution of $NaVO_3 \cdot H_2O$. The resulting curve resembled the V^{+5} curve, but was greatly expanded, and the observed $E_{1/2}$ was shifted slightly, thus suggesting the formation of a vanadium complex(5). The failure of V^{+5} to disappear in this experiment showed that gluconic acid does not reduce vanadium as does ascorbic acid.

$CaNa_2EDTA$ added to a solution of $NaVO_3 \cdot H_2O$ did not change the wave pattern, but shifted the $E_{1/2}$ slightly. ($CaNa_2EDTA$ concentration, 1540 μg/ml; $NaVO_3 \cdot H_2O$ concentration, 77 μg/ml.) The observed response suggests a very loosely-bound complex of vanadium with the chelating agent. The $E_{1/2}$ of a vanadyl sulfate (V^{+4}) solution was shifted slightly when treated with $CaNa_2EDTA$ indicating the probable formation of a weak complex similar to that found with V^{+5}.

Discussion. In mice and rats, signs of

‖ This dose of ascorbic acid for dogs was approximately 5 times that of the metavanadate on a mg basis; that for rats (250 mg/kg) and mice (125 mg/kg) approximately 4 times.

vanadium toxicity were usually present for 2 hours or more after $CaNa_2EDTA$ was administered to vanadium-poisoned animals; however, recovery gradually occurred in the majority of the animals. Dogs behaved similarly, with signs of vanadium toxicity being present for some time after $CaNa_2EDTA$ administration, with gradual, but eventual, recovery.

When ascorbic acid was administered to vanadium-poisoned animals, the signs of vanadium toxicity appeared to be arrested within one-half hour and recovery appeared to be more rapid than with $CaNa_2EDTA$. It has been reported(6) as well as indicated from the present studies, that EDTA forms a loose complex with vanadium which might explain why a short period of time is required before the signs of toxicity begin to subside in vanadium-poisoned animals, after EDTA administration. On the other hand, ascorbic acid was found to react rapidly with $NaVO_3 \cdot H_2O$ *in vitro* from polarographic studies, in which approximately 50% of the pentavalent vanadium disappeared from solution within 15 minutes after addition of ascorbic acid. Unfortunately it was not possible to decide from these determinations alone whether ascorbic acid formed a complex with the vanadium it reduced. Only 50% ascorbic acid was recoverable, however, by actual analysis from an equimolar mixture of vanadium and ascorbic acid shortly after mixing. A reasonable assumption is that the remainder was oxidized to noncomplexable moieties.

It has been shown(7) that acidity decreases the toxicity of injected vanadium; however, this is not believed to be the chief mode of action of ascorbic acid in these antidotal tests. Relatively large doses of ammonium chloride were required to give only a moderate prophylactic protection, whereas far smaller doses of ascorbic acid afforded high protection against vanadium. Moreover, in the smaller effective doses employed, 125 mg/kg, ascorbic acid would not be expected to alter appreciably the tissue milieu.

Summary. 1. Ascorbic acid at 1 g/kg when administered prior to vanadium antagonizes the toxicity of vanadium as $NaVO_3 \cdot H_2O$ in mice at the LD_{70} and LD_{95} levels. Dosage levels as low as 125 mg/kg ascorbic acid showed a protective action against the LD_{70} of $NaVO_3 \cdot H_2O$ in mice. 2. Antidotal effects of calcium disodium ethylenediamine tetraacetate and ascorbic acid have been demonstrated in vanadium-poisoned rats and dogs, after signs of toxicity had become manifest. 3. Ascorbic acid is probably the more rapidly-acting of the 2 antidotes. Signs of vanadium toxicity were present for a longer period of time in vanadium-poisoned animals after $CaNa_2EDTA$ than after ascorbic acid administration. 4. One possible mode of detoxication of vanadium by ascorbic acid is suggested, on the basis of *in vitro* polarographic studies, to be reduction of the vanadium.

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1. Mitchell, W. G., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 346.

2. Proescher, F., Seil, H. A., and Stillians, A. W., *Am. J. Syphilis,* 1917, v1, 347.

3. Popovici, A., *et al.,* PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 415.

4. Lingane, J. J., and Meites, L., *J. A. C. S.,* 1947, v69, 1021.

5. Kolthoff, I. M., and Lingane, J. J., in *Polarography,* 2nd Ed., Interscience, 1952, v2.

6. Singer, T. P., Martell, A. E., and Rubin, M., in *Symposium on Equilibrium and Rate Behavior of Complex Ions,* Office of Naval Research Abstracts, 1951.

7. Mitchell, W. G., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 404.

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