B., Birkeland, S., and Evans, E. I., *ibid.*, 1953, v32, 578.

4. Shemin, D., and Rittenberg, D., *J. Biol. Chem.*, 1946, v166, 621.

5. Fischer, H., *Organic Syntheses*, 1941, v21, 53.

6. Fink, R. M., Enns, T., Kimball, C. P., Silberstein, H. E., Bale, W. F., Madden, S. C., and Whipple, G. H., *J. Exp. Med.*, 1944, v80, 455.

7. Abbott, L. D., Jr., and Dodson, M. J., *Analyt. Chem.*, 1952, v24, 1860.

8. Soloway, S., *ibid.*, 1951, v23, 386.

9. Schoenheimer, R., and Rittenberg, D., *J. Biol. Chem.*, 1939, v127, 285.

Received October 14, 1953. P.S.E.B.M., 1953, v84.

## Influence of pH on Toxicity of Vanadium in Mice. (20661)

WALTER G. MITCHELL. (Introduced by H. E. Stokinger.)

*From the Toxicology Section, Division of Occupational Health, U. S. P. H. S., Cincinnati, O.*

In testing various compounds as antidotes of vanadium(1,2), it was found that one of the substances (ascorbic acid) that afforded good protection against vanadium toxicity had a pH of 2.5 under the conditions of use. This suggested the possibility that H-ion concentration might alter toxic response to vanadium. Support for this possibility was found in a report that anionic vanadium is more toxic than cationic vanadium(3). Moreover, Aub *et al.*(4) showed long ago, and it has been amply confirmed since, that urinary lead excretion is increased following administration of ammonium chloride. More recently, Brown, Kolmer, and Rule(5) showed a similar effect for bismuth. It has further been postulated that an important factor in detoxication is the conversion of a weakly acidic substance, which the body excretes with difficulty, to a relatively strong acid which can be readily eliminated by the kidney(6). Accordingly, test were performed on mice to determine whether altering the pH in various ways would affect vanadium toxicity.

*Materials and methods.* Male mice of the Webster strain* weighing 20-29 g were used for this study. Each mouse was weighed individually and injected with the agents in doses according to body weight. The intraperitoneal route was used for all injections, since more consistent responses could be obtained by this route than by oral or subcutaneous administration. The mice were injected with substances suspected of altering vanadium toxicity into the left side of the peritoneal cavity, followed in 15 minutes by sodium metavanadate ($NaVO_3 \cdot H_2O$) injected into the right side of the peritoneal cavity. Solutions of sodium metavanadate were prepared in physiologic saline solution at a concentration of 4 mg/ml with a resultant pH of 7.0.

* Mice were obtained from Hamilton Laboratory Animals, Inc., Cincinnati, O.

TABLE I. Effect of Administering Ammonium Chloride and Bicarbonate, 15 Minutes Prior to Vanadium, on Vanadium Toxicity in Mice. Fatality rate expressed as ratio of No. of animals that died (numerator) to those used (denominator).

| mg/kg $NaVO_3 \cdot H_2O$ | mg/kg as V | Controls, no prior treatment | $NH_4Cl$, 400 mg/kg | $NaHCO_3$, 2 g/kg |
|---|---|---|---|---|
| 25 | 9.1 | 8/20 | | 17/20 |
| 30 | 10.9 | 12/20 | 5/20 | |

*Results.* Table I shows that ammonium chloride administered 15 minutes prior to vanadium resulted in a decrease of toxicity of $NaVO_3 \cdot H_2O$ at a level of 30 mg/kg. The mortality ratio of 12/20 (60%) was reduced to 5/20 (25%) by the acid chloride. Bicarbonate administered in the same manner conversely increased the toxicity of 25 mg/kg $NaVO_3 \cdot H_2O$ from 8/20 fatalities (40%) to 17/20 (85%). The doses of ammonium chloride and sodium bicarbonate used in the above tests, were the largest doses which could be used without producing signs of toxicity in the mice as determined by separate toxicity tests.

Table II shows that acidifying the $NaVO_3 \cdot H_2O$ solution to pH 1.8 before in-

TABLE II. Toxicity of Acid and Alkaline Solutions of $NaVO_3 \cdot H_2O$ and of a More Acidic Vanadium Salt. Fatality rate expressed as ratio of No. of animals that died (numerator) to those used (denominator).

| mg/kg as V | $NaVO_3 \cdot H_2O$ solutions | | | $NH_4VO_3$, |
|---|---|---|---|---|
| | pH 7.0 | pH 1.8 | pH 12.5 | pH 6.1 |
| 9.1 | 8/20 | | 7/20 | |
| 10.9 | 12/20 | 1/20 | | 2/20 |
| 14.6 | 17/20 | 0/20 | | 14/20 |

jection resulted in decreasing the toxicity of 10.9 mg/kg V (LD60)† and 14.6 mg/kg V (LD85)† doses to the point that these doses were practically nonlethal. Alkalinization of the vanadium solution prior to injection as seen in Table II had no effect on $NaVO_3 \cdot H_2O$ toxicity. As can be seen also in Table II, column 5, $NH_4VO_3$ is only slightly less toxic than $NaVO_3 \cdot H_2O$ at 14.6 mg/kg V; however, when the two compounds are compared at 10.9 mg/kg V, $NH_4VO_3$ is found to be lethal to 10% of the mice, whereas, $NaVO_3 \cdot H_2O$ is lethal to 60% of the mice. The solutions of $NaVO_3 \cdot H_2O$ and $NH_4VO_3$ used in this comparison were made to the same concentration in respect to vanadium and were not buffered.

*Discussion.* From the data presented, it appears that detoxication of vanadium may be affected by the bicarbonate reserve of the blood. Since alkalinization of a vanadium solution did not increase the toxicity, whereas injecting large amounts of bicarbonate into the mice did result in an increase in vanadium toxicity, it appears that excessive blood bicarbonate may retard the process of converting vanadium to a less toxic state. One of the means by which vanadium is detoxified appears to be acidification. This is further supported by the fact that ammonium chloride administered prior to vanadium decreased vanadium toxicity and acidification of vanadium solutions prior to injection, markedly reduced the toxicity.

On the basis of the foregoing results, it was predicted prior to testing that ammonium metavanadate would be less toxic than sodium metavanadate for 2 reasons: 1) Ammonium vanadate is more acid than sodium vanadate, and 2) it is also likely that $NH_3$ would be metabolized by the body to form urea, leaving $HVO_3$ a stronger acid as residue.

*Summary.* 1. Data have been presented to show that pH plays an important role in the toxic response of mice injected with vanadium. Shifting the pH of vanadium solutions toward greater acidity tended to decrease the toxicity. 2. One of the processes by which vanadium is detoxified is probably acidification. Increasing the blood bicarbonate conversely appears to retard detoxication.

Acknowledgment is herewith made to Dr. Herbert E. Stokinger for helpful suggestions and review of this manuscript.

† Values taken from experimentally determined points.

1. Mitchell, W. G., to be published, 1953.
2. ———, Proc. Soc. Exp. Biol. and Med., 1953, v83, 346.
3. Ricardi, P., *Lavori d. Cong. di med. int.*, 1910, v19, 401.
4. Aub, J. C., Fairhall, L. T., Minot, A. S., Reznikoff, P., *Medicine*, 1925, v4, 1.
5. Brown, H., Kolmer, J. A., Rule, A. M., *J. Syph.*, 1943, v27, 501.
6. Quick, A., *J. Biol. Chem.*, 1932, v97, 403.

Received October 14, 1953. P.S.E.B.M., 1953, v84.