brings about a widespread infection of the cell population resulting in an appearance quite dissimilar to that here described(9). The failure of these agents to produce obvious infection of suckling mice, in view of the high degree of susceptibility of this animal to the virus of herpes simplex(10), also indicates that they are not to be identified with the latter. We have no indication that a Rickettsia-like agent of the type described by Sprunt and Hirst(11) was present in our cultures.

Summary. The inoculation of roller tube tissue cultures of human tissues with vesicle fluid derived from patients with varicella has resulted in the isolation of six cytopathogenic agents. Two of the strains have been maintained for 10 tissue culture passages by employing tissue suspensions as the passage material. Histologically the lesions produced consist of focal accumulations of cells which become swollen, and then degenerate; characteristically, such cells contain intranuclear inclusion bodies. From the eruptive lesions of two cases of herpes zoster, cytopathogenic agents of a similar nature have been isolated and have been propagated serially in cultures of human tissue.

1. Rivers, T. M., *J. Exp. Med.*, 1926, v43, 275.
2. ———, *J. Exp. Med.*, 1927, v45, 961.
3. Goodpasture, E. W., and Anderson, K., *Am. J. Path.*, 1944, v20, 447.
4. Blank, H., Coriell, L. L., and Scott, T. F. M., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 341.
5. Weller, T. H., and Stoddard, M. B., *J. Immunol.*, 1952, v68, 311.
6. Robbins, F. C., Weller, T. H., and Enders, J. F., *J. Immunol.*, 1952, v69, 673.
7. Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 100.
8. Blank, H., Burgoon, C. F., Baldridge, G. D., McCarthy, P. L., and Urbach, F., *J. A. M. A.*, 1951, v146, 1410.
9. Enders, J. F., and Robbins, F. C., unpublished observations.
10. Kilbourne, E. D., and Horsfall, F. L., *J. Immunol.*, 1951, v67, 321.
11. Sprunt, K., and Hirst, G. K., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 178.

Received May 25, 1953. P.S.E.B.M., 1953, v83.

Antagonism of Toxicity of Vanadium by Ethylenediamine Tetra Acetic Acid in Mice. (20355)

WALTER G. MITCHELL. (Introduced by H. E. Stokinger.)
From the Toxicology Section, Occupational Health Field Hdqrs. U.S.P.H.S., Cincinnati, O.

It has been shown by a number of workers (1-3) that ethylenediamine tetra acetic acid (EDTA) forms complexes with many metals. This principle has been applied, to a very limited extent, to experimental lead and cobalt poisoning in animals(4-6).

Only occasional references to antidotes for vanadium are to be found in the literature. Priestly(7) found that potassium ferrocyanide and substances containing tannin were effective antidotes for vanadium. BAL on the other hand, according to Sjoberg(8), was not only ineffective in reducing vanadium toxicity by inhalation in rabbits, but actually worsened the effect.

In view of the peculiarly strong metal-complexing potentialities and low toxicity of EDTA, and the possible need for an effective antidote in acute cases of vanadium poisoning, the prophylactic value of EDTA was tested in vanadium-poisoned mice. This study constitutes a part of a larger program on vanadium toxicology currently in progress in these laboratories.

Materials and methods. The calcium disodium salt of EDTA* was used for this study, because it has been shown(9) that the sodium salts of this compound injected into an animal

* This salt was prepared by combining millimolecularly equivalent amounts of Na_2EDTA (Eastman) and $CaCl_2$. Grateful acknowledgement is made of the generous contribution of a solution of $CaNa_2EDTA$ by the Bersworth Chemical Co., used for part of this study.

TABLE I. Dosage-Mortality Data of Vanadium as $NaVO_3 \cdot H_2O$ in Mice.

mg/kg $NaVO_3 \cdot H_2O$	mg/kg as vanadium	No. of animal deaths to No. used	Mortality, %
20	7.3	3/20	15
25	9.1	7/20	35
30	10.9	14/20	70
40	14.6	18/20	90
50	18.3	19/20	95

in sufficient amount will combine with calcium, causing hypocalcemic tetany and death. The calcium disodium salt reduces this tendency to the extent that the toxicity is decreased 10-fold or more. *Colorless solutions* of hydrated sodium metavanadate ($NaVO_3 \cdot H_2O$) were prepared in physiologic saline at a concentration of 4 mg/ml with a resultant pH of 6.8. Male mice of the Webster strain were used.† Each mouse was weighed and given an intraperitoneal dose of EDTA and vanadium according to body weight. EDTA was first administered into the left side of the peritoneal cavity, followed by an injection of sodium metavanadate fifteen minutes later into the right side. Evidence for the validity of this procedure is presented below. An observation period of one week was used in all tests.

Results. Table I shows the dosage mortality data obtained from intraperitoneally administered vanadium, as $NaVO_3 \cdot H_2O$, at 5 dosage levels. The LD_{50} for 20-29 g white mice was 27.2 mg/kg $NaVO_3 \cdot H_2O$ (9.9 mg V/kg), obtained by plotting the data in Table I.

The validity of these data was checked at the LD_{70} and LD_{90} dosage levels.‡ In these tests, the mortality at the 30 mg/kg level of $NaVO_3 \cdot H_2O$ was 14/20 or 70%; at 40 mg/kg, the ratio was 19/20 or 95%.

Mice pretreated with $CaNa_2EDTA$, 1500 mg/kg, intraperitoneally and injected with 14.6 mg/kg and 18.3 mg/kg vanadium as $NaVO_3 \cdot H_2O$ fifteen minutes later, gave the results shown in column 3 of Table II. At the LD_{95} (18.3 mg V/kg) there was 45% protection; at the $LD_{92.5}$ (14.6 mg V/kg) the protection was 77.5%. The administration of 1500 mg/kg $CaNa_2EDTA$ intraperitoneally in a group of 20 mice not injected with vanadium resulted in one death. No symptoms of toxicity were observed at this dose level.

To be assured that the decrease in toxicity of vanadium in EDTA-pretreated mice was not simply a matter of local chelation in the peritoneal cavity, a group of 20 mice was injected with 1500 mg/kg $CaNa_2EDTA$, subcutaneously. Half of the group was injected with the LD_{70} dose of vanadium intraperitoneally 20 minutes later, and the other half was given the same dose of vanadium intraperitoneally 45 minutes later. Additional time was taken here to allow for the absorption of the EDTA salt from this site, which was slower than from the peritoneal cavity. Of the 20 mice prophylactically treated with the complexing agent, 4 mice of an expected 14 died, showing protection against vanadium administered by a different route, comparable to that when both agents are administered by the same route (Table II).

The optimal dose of EDTA to counteract vanadium toxicity was determined by injecting vanadium intraperitoneally at the LD_{70} level (10.9 mg V/kg). By maintaining the dose of vanadium constant and progressively reducing that of EDTA, in groups of 10 mice each, it was found that $CaNa_2EDTA$ at a dose of 62.5 mg/kg body weight protection first became significantly less than at higher dosage levels. At 30 mg/kg, $CaNa_2EDTA$ appeared to afford no protection (Table II, Col. 4-11). Additional groups of 10 mice each were used at certain critical points.

If 75 mg/kg is considered as the lowest dose of $CaNa_2EDTA$ which gives maximal protection against a vanadium dose 10.9 mg/kg, then it appears that approximately a 1:1 molar ratio of EDTA to vanadium is necessary for maximal protection prophylactically; seventy-five mg is one-fifth millimole of $CaNa_2EDTA$ (molecular weight, 374.3) and 10.9 mg is slightly more than one-fifth millimole of vanadium (atomic weight 51). Thus the optimal, prophylactic, protective dose of EDTA is one which is equal to or slightly greater than vanadium on a molar basis.

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

‡ Values taken at experimentally determined points.

TABLE II. EDTA Protection against Vanadium Toxicity in Mice. Values in table are ratios of number of mice that died to number treated.

Vanadium, mg/kg	Mortality ratios: Controls (no EDTA)	$CaNa_2EDTA$, mg/kg intraper., 15 min. prior to vanadium inj. 1500	1000	500	375	250	125	75	62.5	30
18.3	19/20	10/20								
14.6	37/40	6/40								
10.9	28/40	1/10	0/10	1/10	2/10	2/20	0/10	2/20	7/20	6/10

Discussion. The mice that had been pretreated with EDTA showed the same symptoms after vanadium administration as those that had not been pretreated with EDTA. The symptoms usually consisted of depression and respiratory distress. In the non-pretreated mice, this depression usually progressed to a coma with clonic convulsions frequently occurring before death. The pretreated mice usually recovered from this depressed state in about 2 hours and appeared to have returned to normal within 24 hours. From this, it is concluded that in the EDTA pretreated mice, the vanadium is absorbed and exerts its initial toxicity. Some time later (about 2 hours) the metal chelate of EDTA is probably formed in sufficient amount to reduce the vanadium to a nonlethal level except in a few individuals.

The fact that *subcutaneously* administered EDTA protects against vanadium administered *intraperitoneally* shows that local chelation probably is not a significant factor in these experiments.

Summary. 1. Calcium disodium ethylenediamine tetra acetate has been shown to prevent fatalities in mice injected with LD_{70}, $LD_{92.5}$, and LD_{95} doses of vanadium as $NaVO_3 \cdot H_2O$, 80 to 90% of the mice injected at the LD_{70} and $LD_{92.5}$ dose levels of vanadium surviving. 2. At least one millimole of EDTA must be present for every millimole of vanadium in order to show maximal prophylactic protection. 3. The time required for EDTA to detoxify an LD_{70} dose of vanadium in mice appeared to be approximately two hours.

Acknowledgement is herewith given to Dr. H. E. Stokinger, Chief Toxicologist, Division of Occupational Health, U. S. Public Health Service, Cincinnati, for helpful suggestions and review of the manuscript.

1. Plumb, R. C., Martell, A. E., Bersworth, F. C., *J. Phys. and Colloid Chem.,* 1950, v54, 1208.

2. Long, F. A., Jones, S. S., Burke, M., *Brookhaven Conf. Rep.* BNL-c-8.

3. Martell, A. E., and Calvin, M., *Chemistry of Metal Chelate Complex Compounds* (Prentice-Hall) 1952

4. Rubin, M., *Pharmacology, Physiology, Biochemistry, and Toxicity of Versenes, Tech. Bull.,* Bersworth Chem. Co., 1952.

5. Bauer, R. O., *et al., Fed. Proc.,* 1952, vII, 320.

6. Anonymous. *A.M.A. Arch. Ind. Hyg. and Occ. Med.,* 1953, v7, 137.

7. Priestly, J., *Phil. Trans.,* 1876, v166, 495.

8. Sjoberg, S., *Arch. Ind. Hyg.,* 1951, v3, 631.

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

Received May 26, 1953. P.S.E.B.M., 1953, v83.