Vanadium Concentration of Urine
Rapid Colorimetric Method for Its Estimation

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Vanadium compounds are used in dye, ink, and glass manufacture, as an alloy component in the steel industry, and as a catalyst in the production of sulfuric acid. Toxic exposures have been reported in the extraction of vanadium from ores and residues (1–5) and in the cleaning of burners fired with vanadium-bearing oil (6–8). The outstanding symptom associated with excessive inhalation of vanadium compounds, particularly the pentavalent forms, is acute irritation of the respiratory tract, sometimes attended by chemical pneumonitis (5). Irritation of the conjunctivae and eczematous lesions of the skin may also occur. Exposure to low levels of vanadium may cause metabolic disturbances as evidenced by a reduction in the fingernail cystine content (9).

Talvitie and Wagner (10) have shown that urinary vanadium concentrations constitute a reliable index to the degree of absorption of vanadium. Although vanadium in urine may be determined accurately with the method described by Talvitie (11), the need arose in toxicologic investigations for a faster screening test to provide pilot information on the absorption of vanadium. For this purpose a simple, colorimetric test for vanadium in urine was devised.

Necessary requirements for the test were (a) that only a small amount of urine be consumed in order to conserve the bulk of the sample for more accurate analysis, and (b) that the sensitivity be sufficient to detect minimal concentrations of vanadium. These conditions were met by basing the test on the catalytic effect of vanadium on the oxidation of organic chromogens by potassium chlorate. Szebelledy and Ajtai (12)
describe several of these catalytic reactions. Because its oxidized form has a deep, magenta color easily distinguished in the presence of urine, \( N,N\)-diethyl-\( p \)-phenylenediamine was selected as the chromogen.

**REAGENTS**

*Potassium Chlorate:* Saturated solution.

*Color Reagent:* 1% solution of \( N,N\)-diethyl-\( p \)-phenylenediamine (\( p \)-aminodiethylaniline) monohydrochloride (Eastman No. 1374) in glacial acetic acid. This reagent should be prepared fresh each day to avoid a high blank from air oxidation of the color agent.

*Standard Vanadium Solution:* One milliliter contains 100 \( \mu \)g. of vanadium. Dissolve 0.2296 Gm. of ammonium metavanadate in 25 ml. of 4N \( \text{H}_2\text{SO}_4 \) and dilute to 1 L. with distilled water. Make dilutions with distilled water each time as needed.

**PROCEDURE**

1. Transfer a 1-ml. portion of each of the urine samples to be tested into test tubes large enough to contain at least 15 ml.

2. Prepare a control by measuring 1 ml. of the urine of an individual having no exposure to vanadium into another test tube.

3. Dilute the urine samples and control to 11 ml. with distilled water.

4. Add 1 ml. of saturated potassium chlorate solution to each test tube.

5. Add 1 ml. of the color reagent to each test tube, mix, and place the tubes simultaneously into a boiling water bath.

6. The test tubes may be observed during heating for development of a red-to-magenta color. Because vanadium-free urine also develops a slight reddish coloration, the presence of vanadium is indicated by color in excess of that found in the control.

   After heating for 15 minutes, cool the tubes quickly to room temperature in a cold water bath. Additional color develops only slowly at room temperature and the colors are sufficiently stable to allow grading of the intensities.

7. The results may be quantified by comparing the samples with a simultaneously developed series of standards prepared by adding increments of standard vanadium solution to 1-ml. portions of vanadium-free urine. With a 15-minute heating period, a suitable color gradation is obtained with standards containing 0.0, 0.1, 0.2, 0.4, and 1.0 \( \mu \)g. of vanadium. The lower limit of sensitivity is 0.01 \( \mu \)g. of vanadium, whereas the colors developed by vanadium in excess of 1 \( \mu \)g. per ml. of urine (1 ppm) are so intense that they cannot be differentiated.
8. Numerical values may be assigned by reading the colors in a photometer at a wavelength of 540 mμ. The urine color may be conveniently compensated by reading the unknowns against duplicate samples prepared in the same manner as the unknowns except for the substitution of 1 ml. of distilled water for the potassium chlorate solution. A lower blank can be obtained by developing the colors at room temperature and after 1 hour of reaction sufficient sensitivity is obtained to cover the range from 0 to 1 μg. of vanadium per ml. of urine.

EXPERIMENTAL DATA

The method depends upon the catalytic effect of vanadium on the oxidation of N,N-diethyl-p-phenylenediamine by potassium chlorate. The rate of color development is proportional to the concentration of vanadium, but other factors which affect reaction rates, such as temperature, pH, and reagent concentrations, must be closely controlled if other than qualitative results are desired.

Volume of Urine

The optimum volume of urine for sensitivity was found to be 1 ml. Smaller volumes of urine tended to give higher blank values, whereas larger volumes of urine tended not only to mask the color but also to inhibit development of the color. In a series of urine: distilled-water dilutions of 1.0:9.0, 2.5:7.5, 5.0:5.0, 7.5:2.5, and 10.0:0.0 each containing the same amount of vanadium (1 μg. per sample) the most intense color was obtained with the 1.0:9.0 dilution. Also, when the urine from animals exposed to vanadium was tested, 10 ml. of undiluted urine gave only an amber color, while 1 ml. diluted to 10 ml. gave an intense magenta color. For convenience in measuring, the dilution in the test was standardized at 1 ml. of urine to 10 ml. of water.

Hydrogen-Ion Concentration

The effect of pH on the color development was determined with a series of urine samples containing vanadium in which only the pH was varied with acetic acid-sodium acetate buffers. The pH values were determined with a Beckman Model G glass electrode pH meter. Table 1 shows that stable colors increasing in intensity with decreasing pH develop in the pH range of 2.0–3.7. Because the lowest blank was obtained at a pH of 2.6, the acidity was adjusted to this value. It was found that this value is obtained with sufficient accuracy with the glacial acetic acid contained in the color reagent. Because an excess of the acid has little additional influence on the pH, urine specimens in which a sediment has
Table 1. Effect of pH on Color Development of Urine Samples

<table>
<thead>
<tr>
<th>pH</th>
<th>Blank</th>
<th>Test sample (0.1 µg V/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.9</td>
<td>Colors faded to grey</td>
<td>.</td>
</tr>
<tr>
<td>5.6</td>
<td>Colors faded to grey</td>
<td>.</td>
</tr>
<tr>
<td>4.7</td>
<td>Partial fading</td>
<td>.</td>
</tr>
<tr>
<td>3.7</td>
<td>67</td>
<td>135</td>
</tr>
<tr>
<td>3.4</td>
<td>88</td>
<td>175</td>
</tr>
<tr>
<td>2.6</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td>2.3</td>
<td>65</td>
<td>293</td>
</tr>
<tr>
<td>2.2</td>
<td>97</td>
<td>445</td>
</tr>
<tr>
<td>2.0</td>
<td>146</td>
<td>580</td>
</tr>
</tbody>
</table>

* Klett-Summerson photometer, with green filter #54.

formed may be clarified before the test by acidifying slightly with acetic acid.

Temperature and Time

The rate at which the color develops increases with temperature. At room temperature a color gradation suitable for colorimetric comparisons is obtained with concentrations varying from 0 to 1 µg of vanadium per ml of urine when the color is allowed to develop for 60 minutes. A hundredfold increase in rate of color development and sensitivity is obtained at the boiling temperature. The color continues to intensify as the heating time is extended beyond the 15 minutes specified in the procedure but the sensitivity is not increased materially thereby, because of a comparable increase in color in the control sample.

The color developed during the heating of the samples tends to fade slightly on cooling to room temperature. The relative intensities remain unaffected, however, and color comparisons can still be made 48 hours after removal of the samples from the water bath. If photometric readings are made, the time must be carefully standardized. The variations in readings with time at room temperature obtained with a Klett-Summerson photoelectric colorimeter are illustrated in Fig. 1. Qualitative comparisons or comparisons with a series of standards may be made at any time during the color development.

Specificity

Several metal ions are known to have a catalytic action similar to that of vanadium. Because of the possibility that these ions might appear in
the urine normally or through unusual exposures their effect upon the color reaction was determined. Of the metals tested (manganese, bismuth, copper, iron, uranium, and molybdenum) only copper and iron were found to produce a color or to interfere with the reaction. The color produced by copper was more intense than that produced by iron; however, in both instances, the color produced by 1 μg. of vanadium was much greater than that produced by 10 μg. of either copper or iron. The probability of interference from these metals, particularly iron, appears to be remote.

Attempts to prevent the interference by copper and iron by the addition of chelating or complexing agents such as EDTA, sodium fluoride, and 8-hydroxyquinoline were not successful. These agents either had no effect or suppressed also the color reaction produced by vanadium.

**Color Reagent**

*N,N*-diethyl-*p*-phenylenediamine was selected as the color agent although many of the indicators of low oxidation-reduction potential summarized by Koltchoff and Stenger (13) would also be suitable. Because the solution deteriorates rather rapidly as a result of air-oxidation, fresh solution must be prepared each day. A control must be run with
COLORIMETRIC ESTIMATION OF VANADIUM

Table 2. RESULTS OF COLORIMETRIC ESTIMATION OF VANADIUM IN URINE

<table>
<thead>
<tr>
<th>Species and study</th>
<th>Animal</th>
<th>Exposure</th>
<th>V in urine (µg./ml.)</th>
<th>Colorimetric estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>8</td>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NaVO₃ Diet</td>
<td>64</td>
<td>10 ppm V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>167</td>
<td>10 ppm V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>110</td>
<td>100 ppm V</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>123</td>
<td>100 ppm V</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Dog</td>
<td>7</td>
<td>Control</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>Inhalation of V₂O₅</td>
<td>5</td>
<td>0.5 mg. V/m²</td>
<td>3.6</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.5 mg. V/m²</td>
<td>0.7</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.5 mg. V/m²</td>
<td>1.3</td>
<td>++</td>
</tr>
<tr>
<td>Rabbit</td>
<td>1</td>
<td>Control</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>Skin absorption</td>
<td>2</td>
<td>ca. 0.02 mg. NaVO₃</td>
<td>3.7</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>&quot;</td>
<td>0.1</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>&quot;</td>
<td>0.0</td>
<td>-</td>
</tr>
</tbody>
</table>

* Analyses by method given in (11).
* Parts per million by weight.

Each set of unknown samples was compensated for the color formed due to air oxidation. The stability of the color agent was greater in acetic acid solution than in aqueous solution.

RESULTS AND DISCUSSION

Studies of the toxicity of vanadium currently in progress in this laboratory afford an opportunity to demonstrate the application of the method with urine samples known to contain vanadium. The results of the tests on these samples are summarized in Table 2 which lists the results of analyses by this procedure compared to accurate quantitative analyses. The table confirms the validity of this test in detecting vanadium excretion in the urine. The grading of the results in the table as negative (−), positive (+), or strongly positive (++) shows good correlation with the quantitative results as well as the magnitude of exposure.

This test is applicable for use in the detection of vanadium in quantities as low as 0.01 µg. per ml. of urine. That urinary vanadium concentrations constitute a reliable index to the absorption of vanadium has been demonstrated experimentally in animals by Talvitie and Wagner (10). Although precise data are lacking for absorption and excretion of air-
borne vanadium compounds by man, dogs repeatedly exposed daily for several months by inhalation to vanadium pentoxide dust at the suggested threshold limit value (14) of 0.5 mg. of vanadium per cubic meter of air were found to have a urinary excretion level of about 1 µg. of vanadium per ml. of urine. If a similar response obtains in man, this test would have sufficient sensitivity to detect exposures of individuals to air-borne vanadium at or above the suggested threshold limit value.

SUMMARY

A rapid, convenient test for the estimation of small amounts of vanadium in urine is described. The method is based on the catalytic effect of vanadium on the oxidation of \( N,N \)-diethyl-p-phenylene diamine by potassium chlorate. Only 1 ml. of urine is required and the test is sensitive to quantities of vanadium as low as 0.01 µg. per ml. of urine. Semiquantitative analyses can be made over a range of 0–1 µg. of vanadium per ml. of urine. The test is designed for application in rapid clinical analyses on small amounts of urine to determine possible vanadium absorption and is suggested for use as a screening test to determine on-the-job exposure of workers to vanadium compounds in excess of the suggested threshold limit value. Data showing the application of the test in several species are given.

REFERENCES