Technical methods

I wish to thank Dr. G. K. McGowan for reading this paper, Miss Joan Little for technical assistance, and the British Empire Cancer Campaign for full financial support.

REFERENCES


The determination of 3-methoxy 4-hydroxy mandelic acid in urine

R. J. GEORGES AND N. A. SMALL From the Department of Pathology, Southmead Hospital, Bristol

A simple colorimetric method for the determination of 3-methoxy 4-hydroxy mandelic acid (V.M.A.) in urine has recently been described by Woiwood and Knight (1961). In this method, urine, after acidification and saturation with sodium chloride, is extracted with ether and the extracted V.M.A. removed into aqueous solution with dilute alkali. After coupling with diazotized para-nitro aniline the azo derivative is extracted into chloroform from which it is re-extracted with sodium hydroxide as a red solution.

Trials in this laboratory have confirmed the suitability of this method for routine use, but we have found it helpful to introduce the following modifications into the technique:—

1 Ethyl acetate is substituted for ether as the first extracting solvent, two successive 10 ml. portions being employed.
2 The combined ethyl acetate extracts are washed with 2 ml. distilled water.
3 Extraction into aqueous solution is brought about by shaking with 25, 15, and 10 ml. portions of 0·01 M phosphate buffer, pH 7·6 (50 ml. 0·2 M KH₂PO₄ + 42·74 ml. 0·2 M NaOH, diluted 1 : 20 with distilled water).

The use of ethyl acetate is safer than ether in the routine laboratory, and the smaller volume of organic solvent makes subsequent handling easier. It was found that variable amounts of acid were being carried over in this first extraction; the washing step was found to remove 60 to 70% of this. The use of a buffer at pH 7·6 for the subsequent extraction avoided any large swing in pH during this step, together with the need for any final adjustment of the pH of the combined extract.

STANDARD CURVE

We have prepared a standard curve giving absolute optical density values for the azo-derivative of V.M.A. in alkaline solution, in order to calculate overall recoveries of quantities of V.M.A. taken right through the procedure. It is shown in Fig. 1 and is prepared as set out on page 389.

The optical density of the red solutions was measured on the Hilger Uvispek spectrophotometer at 510 mμ using 1 cm. cells.

Received for publication 15 January 1962.
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**TABLE I**

<table>
<thead>
<tr>
<th>No.</th>
<th>V.M.A. (5 ug./ml.)</th>
<th>Water (ml.)</th>
<th>Diazob</th>
<th>N/10 NaOH (ml.)</th>
<th>Equivalent Concentration (µg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>2.1</td>
<td>0.4</td>
<td>2.5</td>
<td>Blank</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>1.9</td>
<td>0.4</td>
<td>2.5</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>0.4</td>
<td>1.7</td>
<td>0.4</td>
<td>2.5</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>0.6</td>
<td>1.5</td>
<td>0.4</td>
<td>2.5</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>0.8</td>
<td>1.3</td>
<td>0.4</td>
<td>2.5</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>1.0</td>
<td>1.1</td>
<td>0.4</td>
<td>2.5</td>
<td>20</td>
</tr>
</tbody>
</table>

Table III shows the distribution of the results of 25 estimations of the urinary output of V.M.A. in 24-hour samples. All these specimens were from hypertensive patients, and had been submitted for screening for increased pressor amine output; all contained less than 180 µg. noradrenaline/24 hr. by the method of Hingerty (1957). A single case of surgically proved phaeochromocytoma was found to have excreted 29.4 mg. of V.M.A. in 24 hours.

Fig. 1. The standard curve for V.M.A.

**RESULTS**

Using the modified procedure we have investigated the recovery of various amounts of V.M.A. taken right through the method and the recovery of V.M.A. added to normal urine. The results are given in Tables I and II.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume Used (ml.)</th>
<th>V.M.A. Added (µg.)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>10</td>
<td>88</td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>15</td>
<td>87</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>10</td>
<td>98</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>10</td>
<td>98</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Cases</th>
<th>Range of V.M.A. Excretion (µg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-3</td>
</tr>
<tr>
<td>2</td>
<td>3-4</td>
</tr>
<tr>
<td>1</td>
<td>4-5</td>
</tr>
<tr>
<td>5</td>
<td>5-6</td>
</tr>
<tr>
<td>4</td>
<td>6-7</td>
</tr>
<tr>
<td>3</td>
<td>7-8</td>
</tr>
<tr>
<td>4</td>
<td>8-9</td>
</tr>
<tr>
<td>1</td>
<td>9-10</td>
</tr>
<tr>
<td>1</td>
<td>10-11</td>
</tr>
<tr>
<td>1</td>
<td>11-12</td>
</tr>
<tr>
<td>2</td>
<td>12-13</td>
</tr>
</tbody>
</table>

We wish to thank Dr. F. J. W. Lewis, Director of the Pathology Department at Southmead Hospital, for kind support and encouragement. One of us (N.A.S.) is in receipt of a salary grant from the National Spastics Society. Part of the expenses for apparatus have been met from the Ethel Showering Fund.

REFERENCES

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preparations can be made by drying the stained section in air and then mounting in Fluormount (E. Gurr, Ltd.), though this treatment sometimes causes a reduction in fluorescence.

**COMMENT**

The freeze-dried sections are equally suitable for identifying the autoantibody against thyroglobulin (Figure 1), and the CA2 antibody first described by Balfour et al. (1961) for which immunofluorescence provides the only known method of detection. The technique is also sufficiently reliable for use in reverse, so to speak, for studying the reactions of thyroid biopsy material with autoimmune sera of known specificity.

Because of the excellent preservation of stored sections, there is clearly no necessity for all laboratories interested in the detection of autoantibodies to thyroid to carry out the freeze-drying, embedding, and microtomy themselves. It should be possible to have embedded blocks or mounted sections prepared commercially for routine laboratory use.

This research programme has been supported by grants from the Medical Research Council and the Scottish Hospital Endowments Research Trust. We also wish to thank Dr. Deborah Doniach and colleagues for confirmatory tests with our stored sections in routine applications.

**REFERENCES**


**CORRECTIONS**

We regret that in the paper by J. A. Campbell and A. H. Cruickshank on 'Cystadenoma and cystadenocarcinoma of the pancreas' (*J. clin. Path.*, 15, 432-436) the legends to figures 8 and 9 on page 436 have been transposed.

Dr. Small regrets that there was an error in the third modification of the technique he describes in his paper on 'The determination of 3 methoxy-4 hydroxy mandelic acid in urine' (*J. clin. Path.*, 15, 388). It should read as follows:—

3 Extraction into aqueous solution is brought about by shaking with 25, 15, and 10 ml. portions of a phosphate buffer, pH 7-6 (50 ml 0-2 M KH₂PO₄ + 42-74 ml 0-2 M NaOH, diluted to 200 ml with distilled water). Dilute this solution 1:20 with distilled water for use.