The method for quantitative determination of serum sodium as described
by Vanatta and Cox (1) is adaptable to determination of urine sodium.
However, wider variations in sodium and potassium concentrations, and
the presence of ammonia and other interfering substances, necessitate
certain modifications of the procedure.

Apparatus and Reagents—The items of apparatus needed for the determi-
nation of urine sodium are those required for determination of serum sodium
with the following additions and exceptions.

1. The 50 cm. column of resin with a cross-sectional area of 0.93 to 1.04
   sq. cm. is increased to a length of 55 cm., the diameter remaining the same.
   A 50 cc. burette is adequate for either column.
3. A small glass boiling bulb which will fit the opening of the 250 cc.
   Erlenmeyer flask.
4. A stopper with a CO₂ absorption tube in it which will fit the 250 cc.
   Erlenmeyer flask.

The same reagents as for determination of serum sodium are required,
with the addition of the following reagents:

1. Nitrogen gas under pressure or compressed air washed in alkali or
   passed through soda lime.
2. 100 cc. of toluene with two or three crystals of thymol.
3. Soda lime or a similar CO₂ absorber.

Procedure

The urine is collected and toluene with thymol is immediately added in
sufficient quantity to form a thin layer on top of the sample. The analysis
is carried out according to the following procedure.

Step 1. Transfer of Urine to IR-112 Column—Place 1 or 2 cc. of urine
(depending on the sodium concentration as described below) on the IR-112
column in exactly the same manner that the serum sample is introduced.

* This study was supported by a grant from the American Heart Association in
cooperation with the Dallas Heart Association. Resins for this work were supplied
by the Rohm and Haas Company, Philadelphia 5, Pennsylvania.
Step 2. Chromatographic Elution of Sodium—The elution is carried out with 0.05 M BaCl₂ at a rate never exceeding 2 cc. per minute. The only difference from the serum sodium elution is that the effluent from 50 to 132.5 cc. is collected rather than the effluent from 40 to 122.5 cc. This is necessary because of the longer resin column. Substitution of the longer column and collection of the 50 to 132.5 cc. portion of the effluent in the serum method would allow the same column to be utilized for determination of either serum sodium or urine sodium.

Step 3. Precipitation of Barium as BaSO₄—This precipitation is carried out exactly as in Step 3 of the serum sodium determination.

Step 4. Conversion of NaCl to NaOH and Quantitative Determination of NaOH—10 cc. of IRA-400 are placed in the resin column and washed with water as described in Step 4 of the serum sodium procedure. When the resin has been washed as described, a 50 cc. aliquot of the supernatant fluid from Step 3, followed by two 10 cc. portions of deionized H₂O, is run through the IRA-400 column. The entire 70 cc. effluent is collected in a 250 cc. Erlenmeyer flask and boiled vigorously for 10 minutes by use of the boiling bulb, which will prevent loss of solution by spattering. The flask is immediately stoppered with the CO₂ absorber and cooled to room temperature by immersion in a cold water bath. Contamination of the solution with soda lime from the absorption tube gives marked excess in the titration. A source of such contamination may be the condensation of sufficient moisture inside the soda lime tube so that a drop falls into the liquid. If the entire flask is cooled rapidly, such contamination is less likely to occur.

Upon removal of the CO₂ absorber, a stream of N₂ gas or CO₂-free compressed air is immediately started bubbling through the solution and continued until the titration is completed. 5 drops of brom thymol blue are added, and the solution is titrated with 0.01 N HCl until the end-point is obtained. From the volume of 0.01 N HCl added, the sodium concentration may be calculated. This value for the sodium concentration must be corrected for two factors. (a) The blank titration of a saturated solution of BaSO₄ in approximately 0.13 N HCl and 0.02 N H₂SO₄ must be subtracted. Determination of the blank is described in Step 4 of the serum sodium method. (b) 6 per cent of the corrected value must be added to the corrected value as this is the amount of sodium lost by occlusion when the precipitation of BaSO₄ is carried out as specified in Step 3.

\[
\text{uncorrected m.eq. Na per volume of sample} = \frac{y \left( \frac{10^4}{\text{cc. of urine}} \right)}{\text{liter urine}} = \frac{\text{uncorrected m.eq. Na}}{\text{liter urine}}
\]
1.06 \left( \frac{\text{uncorrected m.eq. Na per liter urine}}{\text{blank in m.eq. per liter}} \right) = \text{Na m.eq. per liter corrected value}

### Table I

**Comparison of Determinations of Urine Sodium by Resin Method with Method of Butler and Tuthill**

All values in milliequivalents per liter; averages of duplicate determinations. The figures in parentheses represent differences of duplicate determinations.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Resin method (A)</th>
<th>Butler-Tuthill, urine ashed (B)</th>
<th>Butler-Tuthill, urine unashed (C)</th>
<th>( \frac{A - B}{B} \times 100 )</th>
<th>( \frac{C - B}{B} \times 100 )</th>
<th>( \frac{A - C}{C} \times 100 )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>60.7 (2.5)</td>
<td>61.1 (1.5)</td>
<td>61.4 (2.3)</td>
<td>-0.7</td>
<td>+0.5</td>
<td>-1.1</td>
</tr>
<tr>
<td>2</td>
<td>67.9 (2.1)</td>
<td>67.5 (1.8)</td>
<td>65.5 (0.5)</td>
<td>+0.6</td>
<td>-3.0</td>
<td>+3.7</td>
</tr>
<tr>
<td>3</td>
<td>132.6 (0.1)</td>
<td>131.8 (1.4)</td>
<td>130.3 (1.9)</td>
<td>+0.6</td>
<td>-1.1</td>
<td>+1.8</td>
</tr>
<tr>
<td>4</td>
<td>55.7 (1.1)</td>
<td>56.4 (0.6)</td>
<td>53.6 (0.2)</td>
<td>-1.2</td>
<td>-5.0</td>
<td>+3.9</td>
</tr>
<tr>
<td>5</td>
<td>154.3 (5.3)</td>
<td>158.8 (1.3)</td>
<td>154.1 (1.4)</td>
<td>-2.8</td>
<td>-3.0</td>
<td>+0.1</td>
</tr>
<tr>
<td>6</td>
<td>95.4 (2.2)</td>
<td>93.2 (3.7)</td>
<td>99.8 (2.9)</td>
<td>+2.4</td>
<td>+7.1</td>
<td>-4.4</td>
</tr>
<tr>
<td>7</td>
<td>174.4 (20.1)</td>
<td>172.8 (2.6)</td>
<td>173.8 (1.5)</td>
<td>+0.9</td>
<td>+0.6</td>
<td>+0.3</td>
</tr>
<tr>
<td>8</td>
<td>106.0 (2.2)</td>
<td>106.3 (1.7)</td>
<td>105.6 (0.2)</td>
<td>-0.3</td>
<td>-0.7</td>
<td>+0.4</td>
</tr>
<tr>
<td>9</td>
<td>227.9 (8.4)</td>
<td>230.4 (1.8)</td>
<td>225.0 (3.9)</td>
<td>-1.1</td>
<td>-2.3</td>
<td>+1.3</td>
</tr>
<tr>
<td>10</td>
<td>40.4 (1.9)*</td>
<td>42.7†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>53.8 (0.7)</td>
<td>53.2 (1.7)</td>
<td>50.0 (1.0)</td>
<td>+1.1</td>
<td>-6.0</td>
<td>+7.6</td>
</tr>
<tr>
<td>2</td>
<td>110.3 (1.2)</td>
<td>109.8 (2.6)</td>
<td>107.3 (3.3)</td>
<td>+0.5</td>
<td>-2.3</td>
<td>+2.8</td>
</tr>
<tr>
<td>3</td>
<td>137.3 (1.1)</td>
<td>137.6 (0.8)</td>
<td>133.1 (1.1)</td>
<td>-0.2</td>
<td>-3.3</td>
<td>+3.2</td>
</tr>
<tr>
<td>4</td>
<td>76.4 (2.1)</td>
<td>85.3 (1.0)</td>
<td>75.0 (0.8)</td>
<td>-10.4</td>
<td>-12.1</td>
<td>+1.9</td>
</tr>
<tr>
<td>5</td>
<td>36.3 (1.6)*</td>
<td>36.9 (2.3)†</td>
<td></td>
<td>-1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>129.4 (2.1)</td>
<td>129.1 (0.8)†</td>
<td>31.1 (3.1)</td>
<td>+0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31.6 (0.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 2 cc. sample analyzed.
† Single determination.
‡ 5 cc. sample analyzed.

### Results

Ten urine specimens from normal healthy individuals were analyzed in duplicate by the resin method, and the results were compared with those of duplicate determinations by both a modified and the original Butler-Tuthill methods (2). Also urine samples from six hospital patients with a sodium concentration over 20 m.eq. per liter were obtained, and duplicate determinations were run by all three methods (Table I).

The results of duplicate determinations by the resin method when compared with those of duplicate determinations by the modified Butler-Tuthill
method showed differences greater than 2 per cent in only four out of sixteen determinations, with an average algebraic difference of −1.09 per cent. The results of duplicate determinations by the resin method when compared with those of duplicate determinations by the original Butler-Tuthill method differed more than 2 per cent in six out of thirteen determinations, with an algebraic average difference of +1.65 per cent.

Four samples from hospital patients with low urine sodium concentration were run by the modified Butler-Tuthill method, and recovery of added sodium was tested by the resin method (Table II). Duplicate determinations were run on each sample with 0.1500 m.eq. of sodium added. Recovery in individual determinations ranged from 97.3 to 102.2 per cent, with an average recovery of 99.8 per cent.

The reproducibility of results of the resin method was evaluated by expressing the difference of duplicate determinations as per cent of the mean of the duplicates. This difference was 5 per cent or less in fifteen of the sixteen samples analyzed. We have no explanation for the variation of 11.5 per cent with Sample 7 of the series on normal urine. Reproducibility of the results of ten replicate determinations on a standard solution of 150 m.eq. per liter of NaCl to which potassium, calcium, and magnesium were added showed a standard deviation of 2.0 m.eq. per liter (1).

**Table II**

Recovery of Sodium Added to Urine Samples

All values in milliequivalents per 1 cc. of sample analyzed. 0.1500 m.eq. of Na added to each sample.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Na determined by modified Butler-Tuthill method (A)</th>
<th>Total Na by resin method (B)</th>
<th>( B - A )</th>
<th>Per cent recovery of added Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0310</td>
<td>0.1513</td>
<td>0.1503</td>
<td>100.2</td>
</tr>
<tr>
<td>2</td>
<td>0.0089</td>
<td>0.1502</td>
<td>0.1492</td>
<td>99.5</td>
</tr>
<tr>
<td>3</td>
<td>0.0098*</td>
<td>0.1522</td>
<td>0.1533</td>
<td>102.2</td>
</tr>
<tr>
<td>4</td>
<td>0.0052*</td>
<td>0.1533</td>
<td>0.1522</td>
<td>102.2</td>
</tr>
</tbody>
</table>

Average ................................................................. 99.8

* 5 cc. sample analyzed; values expressed in milliequivalents per 1 cc.
DISCUSSION

The original Butler-Tuthill method as applied to serum has been checked for accuracy by several workers (3–5). However, a review of the literature reveals no checks on this method as it is used to determine urine sodium concentration. Butler and Tuthill stated that they had studied the necessity of ashing urines, but present no results comparing dry ashed and unashed methods.

In checking our method against the original Butler-Tuthill procedure, we found an agreement of only 1.65 per cent, which raised the question of the accuracy of both of the methods. Since the results of the resin and the Butler-Tuthill methods were in agreement within 1 per cent on serum determinations, it was decided to modify the Butler-Tuthill method for urine by ashing the urine in the same manner as for the serum. The urine was ashed after the precipitation of the phosphates, and then the sodium was precipitated as sodium uranyl zinc acetate. When averages of results of duplicate determinations by the original and by our modified Butler-Tuthill method are compared, the differences are greater than 2 per cent in nine out of fourteen determinations, and all but one of these are in the direction of the modified method, giving results higher than the original method, with an average algebraic difference of −2.07 per cent. The ashed method is theoretically the more accurate because no sodium is lost in dry ashing as determined by ashing standards,¹ and organic substances which might interfere are removed. Therefore, if the Butler-Tuthill method is to be used for analyses of urine sodium, we recommend modification of the procedure by ashing as described above.

Larger amounts of potassium are present in urine than are found in serum; consequently the capacity of the IR-112 resin column had to be increased to 55 cm., which assures sodium-potassium separation. The sodium curve of the 55 cm. column begins at 55 cc. and ends at 130 cc. of effluent, as compared with the sodium curve on the 50 cm. column, which begins at 40 cc. and ends at 122.5 cc. of effluent. The potassium curve is moved from 122.5 cc. through 200 cc. on the 50 cm. column for 0.1 m.eq. of K to 137 cc. through 220 cc. on the 55 cm. column for 0.15 m.eq. of K. Thus the 55 cm. column gives a sodium-potassium separation margin of 7 cc. of effluent, which assures complete sodium recovery without contamination by potassium, even in the presence of concentrations of potassium of 150 m.eq. per liter if 1 cc. of urine is used or 75 m.eq. per liter if 2 cc. of urine are employed. We did not test the margin for separation at higher potassium concentrations.

¹ Unpublished data of the authors.
Ammonia, which is eluted at approximately the same rate as is sodium, is present in negligible quantities in serum. However, the ammonia concentration in urine is of sufficient magnitude to be a source of error and may be increased further by decomposition of urea. To prevent this decomposition, a preservative such as toluene with thymol must be added to the urine sample immediately after collection. Also, before titration with standard HCl the effluent from the anion exchange resin column must be boiled vigorously for 10 minutes in order to remove any ammonia present.

An undetermined substance found in some urines, which is eluted at approximately the same rate as sodium, causes extreme and varied positive errors by reacting with the indicator when heated. This was not observed in determination of serum sodium. To avoid this error, the indicator must not be added until the effluent from the anion exchange resin column has been boiled to remove ammonia and then cooled to room temperature. Since the solution may not be boiled again during the titration, in order to remove carbon dioxide all air coming in contact with the solution after boiling must pass through a CO₂ absorber such as soda lime. Then to prevent CO₂ absorption during the titration, nitrogen gas or CO₂-free compressed air is bubbled through the solution.

In urine the sodium concentration varies from 2 m.eq. per liter or lower to 200 or 300 m.eq. per liter. With sodium concentrations below 40 m.eq. per liter, the error in the end-point of the HCl titration results in deviations greater than 2 per cent of the total sodium present. The error in the end-point of the HCl titration also makes evaluation of the sodium occlusion inaccurate in dealing with quantities of sodium below 0.040 m.eq. of total sodium present in Step 3. Therefore, on urines with sodium concentrations from 20 to 40 m.eq. per liter, 2 cc. of the sample must be introduced into the column to obtain accurate results. We do not recommend the method for accuracy when applied to concentrations of sodium below 20 m.eq. per liter. We did not attempt to increase the volume further in samples with lower sodium concentrations.

The per cent of sodium occluded in the barium sulfate precipitation was determined with initial sodium concentrations of 50 to 200 m.eq. per liter and found to be a constant per cent of the sodium present over this range. It should be pointed out that the recovery experiments by the resin method depend upon the accuracy of the modified Butler-Tuthill method in determining low sodium concentrations. This was brought out in Sample 2 of Table II. Errors of +1.5 and +2.2 per cent were obtained when results by the modified Butler-Tuthill method were assumed to represent the true sodium concentration. When duplicate recovery determinations were performed by the original and the modified Butler-Tuthill methods, errors of +1.2 per cent resulted in both cases. The errors in the
recovery experiments by all three methods would be explained if the sodium concentration of 8.9 m.eq. per liter, determined by the modified Butler-Tuthill method, were 1.4 m.eq. per liter low. Similar errors may exist in the other three values of column (A), Table II.

**SUMMARY**

Modification of the resin method of Vanatta and Cox for serum sodium (1) was necessary in adapting the procedure to determination of urine sodium because of the higher potassium and ammonia content of urine, the more variable sodium content, and the presence of a substance in urine which interferes with the brom thymol blue indicator when the indicator is added before the solution is boiled.

Analyses of urine samples were carried out in duplicate by each of three procedures: (1) the resin method, (2) a modified (ashed) Butler-Tuthill method, and (3) the original (unashed) Butler-Tuthill method. Agreement within 2 per cent was found in twelve out of sixteen cases when the averages of results of duplicate determinations by Methods 1 and 2 were compared, while only seven out of thirteen cases agreed within 2 per cent when the results of Methods 1 and 3 were compared. Results by Methods 2 and 3 agreed within 2 per cent in only five out of fourteen determinations.

The average algebraic differences when the averages of results of duplicate determinations of Methods 1 and 2, Methods 1 and 3, and Methods 2 and 3 are compared are -1.09, +1.65, and -2.07 per cent respectively. It is evident that the resin method for determining urine sodium concentration agrees within 2 per cent with either of the Butler-Tuthill methods, though results by the two Butler-Tuthill procedures do not show close agreement.

The resin method was also checked for accuracy by testing the recovery of sodium added to urine samples of extremely low sodium concentration. Duplicate determinations were run on each sample with addition of 0.1500 m.eq. of sodium. An average sodium recovery of 99.8 per cent was obtained, and individual determinations ranged from 97.3 to 102.2 per cent.

**BIBLIOGRAPHY**

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John C. Vanatta and Catherine Carr Cox


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