Evaluation of Automated Urinary Iodine Methods: Problems of Interfering Substances Identified

Warwick May,1 Diana Wu,1 Creswell Eastman,1 Pierre Bourdoux,2 and Glen Maberly1

We evaluated automated methods for measurement of urinary iodine (UI) over a range expected in iodine-replete and iodine-deficient populations. Results obtained with Technicon AutoAnalyzer II systems, based on either dialysis or acid digestion, were compared with those obtained by a manual alkaline ashing technique. Results of automated dialysis were consistently higher than those obtained by the other methods. The apparently higher concentrations of UI we measured were due to interfering substances crossing the dialysis membrane and participating in the catalytic reaction. Thiocyanate (SCN) was one endogenous substance contributing to the increased measurement of UI. For urinary SCN concentrations of 5 to 15 mg/L, the amount of overestimation in the UI measurement attributable to SCN ranged from 21.8 to 61 μg/L. However, SCN may account for only 40–50% of the apparent increase in UI. In samples with lower UI (<50 μg/L), interfering substances produced a 100% error in results. We conclude that the automated dialysis system should not be used to assess iodine-deficient populations. This leaves a major dilemma for researchers wanting to assess the iodine status of populations, because the automated dialysis method is no longer commercially available.

Additional Keyphrases: dialysis and acid digestion techniques compared • analytical error • thiocyanate • nutritional status

Endemic goiter, resulting from nutritional iodine deficiency, is one of the oldest recognized disorders affecting whole populations. Recently, Hetzel (1) proposed the term “iodine-deficiency disorders” to reflect the broader spectrum of this condition, the effects of which include spontaneous abortion, increased infant mortality, and cretinism. In fact, the stunting of intellectual functioning in entire iodine-deficient communities has been demonstrated (2). The International Council for the Control of Iodine Deficiency Disorders has estimated that 800 million people worldwide, living mostly in Asia, Africa, and South America, are at risk of iodine-deficiency disorders (3).

Accordingly, many countries have made or implemented plans to set up centers to assess and monitor iodine deficiency. This usually includes automated laboratories for measuring urinary iodine (UI). Although UI excretion has been considered the definitive criterion for grading the severity of iodine deficiency in communities (4), over the past two decades this test has become rare in clinical chemistry departments in the developed world. Indeed, since modification of the earlier automated acid digestion system in 1973 (5), there has been no real progress in automated UI measurement, whereas methods for other biochemical determinations have advanced rapidly.

Our aim was to evaluate the performance of the automated UI system available since 1973, which is based on dialysis of urine (method A). Results from samples assayed at Westmead Hospital, Australia, were correlated with results from the Saint Pierre University Hospital, Belgium, which were obtained with an even earlier automated UI determination, based on acid digestion (method B). Results from these automated methods were also compared with results obtained by a more technically demanding manual technique (method C, performed at Westmead Hospital), based on the official method of the Association of Official Analytical Chemists (AOAC) (6), in which alkaline ashing is used to destroy organic matter in the sample before the final colorimetric measurement of iodine.

Materials and Methods

Apparatus

Automated measurement of UI was performed with a Technicon AutoAnalyzer II system (Technicon Instrument Corp., Tarrytown, NY) with either a dialysis module (method A) or acid digestion unit (method B). Ashing of urine samples (method C) was carried out in a special 102C muffle furnace (Scientific Equipment Manufacturers, Magill, South Australia). To measure absorbance, we used a Spectronic 20 spectrophotometer (Milton Roy, Rochester, NY).

Reagents

Glass-distilled de-ionized water, used to prepare all solutions and for dilutions, was passed through an ion-exchange column containing anion-exchange resin AG501-X8(D) (BioRad, Richmond, CA) to remove any traces of iodine. All other reagents were of analytical grade and were found to have negligible iodine content.

Urine Samples

Casual (untimed) urine samples from 51 patients and staff (Westmead Hospital) and from 11-year-old schoolchildren (Tasmania, Australia) were selected to cover the range of UI concentrations found in the Australian population. After collection, samples were acidified and refrigerated until assayed.

Procedures

Alkaline ashing. Urine samples (3 mL) were treated with 1 mL of 1 mol/L potassium hydroxide and ashed at 600 °C according to the method of Belling (7). We measured the iodine in the ashed solution by assessing the catalytic effect of iodine on the Sandell–Kolthoff reaction, as described by Potter et al. (8). Because the potassium hydroxide used for ashing the samples depresses the catalytic effect of iodine on the Sandell–Kolthoff reaction, the calibration curve obtained with nonashed aqueous standards is always steeper than the curve produced by adding iodine to ashed solutions. To compensate for this, we derived a correction

1 Australian Centre for the Control of Iodine Disorders, Westmead Hospital, Sydney, Australia.
2 Department of Nuclear Medicine, Saint Pierre University Hospital, Free University of Brussels, Brussels, Belgium.

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factor by adding known amounts of iodine (5 or 10 ng per tube), in triplicate, to a series of urine and blank samples (n = 17). In several assays (n = 9), we calculated the slope of the line obtained by plotting the amount of added iodine against the change in absorbance at 340 nm (a), and compared this with the slope of a line similarly produced with use of nonashed aqueous standards (b). We determined the correction factor (expressed as b/a) to be 1.31 ± 0.08 (mean ± SD); i.e., values obtained for urine samples by interpolation from a standard curve prepared with use of aqueous standards must be multiplied by 1.31 to obtain the correct result.

Analytical recovery of iodine after alkaline ashing was assessed in two ways. First, we prepared urine samples (n = 8) as usual, but added 15 μL of [125I]NaI to give about 70 000 counts/min. We counted the radioactivity before and after the ashing procedure and expressed the final count as a percentage of the initial counts. We also assessed the analytical recovery of nonlabeled iodine from urine after the ashing and assay stages as follows: 2, 4, or 6 ng of iodine was added, in triplicate, to 3-mL aliquots of urine (n = 4). After ashing, we determined the final concentration of iodine in each tube as described above. Recovery was calculated as the amount of added iodine measured, expressed as a percentage of the amount of iodine added.

Reproducibility was evaluated with use of a control urine specimen stored frozen in 3-mL aliquots. Aliquots of this control were then thawed, ashed, and assayed in duplicate with each run.

Automated analysis of urinary iodine. The Technicon continuous-flow system used for determining UI by method B is a modification of the procedure used for measuring protein-bound iodine in serum, in which an automated digester helix is used to digest the samples with concentrated mineral acids at 320 °C (9). UI measurements by method A were performed with the automated system described by Gary et al. (5), in which the digester unit, just described, is replaced by the simpler and safer dialysis module. Results were calculated by a personal computer programmed to correct for baseline drift and nonlinearity of the standard curve.

Analytical recovery of iodine from three urine samples (chosen with initial UI concentrations equivalent to the low, medium, and high regions of the standard curve) was assessed by adding increasing concentrations of iodine standard (25–125 μg/L) to an equal volume of urine.

For quality control of the automated dialysis method, we assayed 1-mL aliquots of these urine samples with low, medium, and high iodine concentrations. These quality-control samples were stored frozen, then thawed and assayed in duplicate with each set of 10–15 samples analyzed.

Interference of thiocyanate. To investigate the possible interference of urinary thiocyanate (USCN) on UI determinations by the different methods, we added potassium thiocyanate solutions (prepared in iodine-free water) to various samples to give a final concentration range of 1.25–20 mg/L (21.5–344 μmol/L), thus simulating the USCN concentrations commonly found in nonsmokers, 4.9 ± 2.3 mg/L, as well as the higher concentrations seen in smokers, 14.6 ± 7.1 mg/L (10). Concentrations of USCN >15 mg/L are also common in populations consuming cassava in their diet (10, 11). We also determined the effect of endogenous USCN for each method. Where sufficient sample remained (n = 38), we determined USCN by using a modified method of Aldridge (12) as described by Bourdoux et al. (13).

Data analysis. Statistical analysis—linear regression, Pearson's correlation coefficient (r)—was performed with StatView 512+™ (Brainpower Inc., Calabassas, CA) on an Apple Macintosh computer.

Results

Recovery and precision. The mean (± SD) analytical recovery we obtained in method C, after the addition of radioisotopic and nonlabeled iodine, was 100.3 (2.9)% and 97.8 (7.2)%, respectively. These results are in close agreement with the analytical recoveries published by others who used this method (7, 14). Similarly, method A gave a mean analytical recovery of 99% (SD 3.7%), which accords favorably with the mean recovery of 97% obtained by Gary et al. (5). Iodine-supplemented urine assayed by method B also showed an analytical recovery of 96–97% (authors' unpublished data).

The between-assay precision (CV) for the assay of a control urine with a mean UI concentration of 11.3 μg/L, in a total of 30 alkaline ashing assays, was 10.6%. In method A, the between-assay CV for the three urine controls with mean UI concentrations of 24, 45, and 94 μg/L was 3.8%, 2.5%, and 2.4%, respectively (n = 35 assays).

Discrepancy between methods. Figure 1 compares the UI results for the samples in each method. UI is plotted on a log scale, with results of the alkaline ashing method used to rank samples in decreasing order. This Figure demonstrates an increase in results obtained in automated dialysis, especially in samples with UI <200 μg/L. Regression analysis further highlights the bias between the ashing method (x) and automated dialysis (y), yielding a correlation coefficient (r) of 0.94, but a regression equation of y = 0.31x + 62.9. On the other hand, the correlation between ashing (x) and automated digestion (y) was very close for the range studied (r = 0.99, y = 0.93x + 7.9).

Interfering substances. To try to explain the apparent overestimation of UI in method A, we investigated the possible effect of thiocyanate, an ion that might cross the dialysis membrane and interfere with the Sandell–Kolthoff reaction (9, 15). Table 1 shows a significant increase in the UI concentrations (after the addition of increasing amounts of thiocyanate to urine) as measured in automated dialysis,
Table 1. Effect of Exogenous SCN on Iodine Determinations

<table>
<thead>
<tr>
<th>SCN added, mg/L</th>
<th>Mean (SD) UI, μg/L</th>
<th>SCN added, mg/L</th>
<th>I by digestion, μg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dialysis</td>
<td>Alk. ashing</td>
<td>Dialysis</td>
</tr>
<tr>
<td>0°</td>
<td>16 (0.3)</td>
<td>13.8 (2.9)</td>
<td>2.5</td>
</tr>
<tr>
<td>1.25</td>
<td>21 (0.4)°</td>
<td>16.4 (3.0)</td>
<td>5</td>
</tr>
<tr>
<td>2.5</td>
<td>26 (0.5)°</td>
<td>19.0 (1.1)</td>
<td>7.5</td>
</tr>
<tr>
<td>5</td>
<td>38 (0.8)°</td>
<td>19.4 (2.7)</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>59 (1.2)°</td>
<td>17.4 (0.8)</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* Added to an equal volume of urine. ° Added to an equal volume of iodine standard. † Urine diluted with an equal volume H₂O only (baseline). ‡ Statistically different from baseline (P < 0.0001). n = 3 each.

but not in alkaline ashing. The two columns at the far right of Table 1 indicate an absence of interference by SCN on iodine measurement in automated digestion, in which increasing concentrations of SCN solutions were mixed with an equal volume of the 100 μg/L iodine standard. Subsequent addition of increasing amounts of thiocyanate to 15 urine samples produced a consistent response in the apparent UI concentration measured in method A (Figure 2). Regression analysis of these data gives the following equation: \( y = 3.93x + 2.2 \) (\( r = 0.99 \), \( P < 0.0001 \)). In other words, a mean USCN concentration of 5 mg/L (nonsmokers) or 15 mg/L (smokers or cassetia consumers) will cause an overestimation in measured UI of 21.8 and 61 μg/L, respectively, when analyzed by automated dialysis.

The effect is also seen with endogenous USCN (Figure 3). The difference between UI measured in method A, minus the UI concentration measured in method C, is significantly correlated (\( r = 0.6 \), \( P < 0.0001 \)) to the USCN concentration in each sample. This relationship was absent in method B, the regression equation generated for the difference in UI (method B minus method C) \( y = 0.3x - 1.6 \) (\( r = 0.085 \), \( P = 0.62 \)). Furthermore, as Table 2 shows, the UI concentration in four urine samples measured by method A decreased after each sample was ashed to remove interfering substances such as SCN.

Finally, this overestimation in the UI measurement caused by USCN in method A is more critical in samples with low UI, because as the true amount of iodine in a sample declines, the proportion of apparent iodine measured that is caused by these interfering substances increases. Figure 4 illustrates a direct correlation between the UI concentrations by method A and the USCN content for samples with UI <100 μg/L (as determined by alkaline ashing). The correlation coefficient of 0.67 was highly significant for this relationship (\( P < 0.0001 \)). Furthermore, UI results obtained by method C and method B showed no significant correlation with USCN content.

**Discussion**

Comparing the UI results in three methods, we identified a serious bias in the Technicon AutoAnalyzer II system based on sample dialysis (Figure 1). The automated method based on acid digestion and a manual alkaline ashing technique were in close agreement for the studied range.

Because this difference could not be accounted for by any systematic iodine loss, we postulated that an interfering substance such as thiocyanate might account for the overestimation in UI measured by the dialysis method. Addition of thiocyanate solutions to samples assayed in each method showed a direct linear relationship between UI measured by automated dialysis and the USCN content (Figure 2), but not in the other two methods (Table 1).
If endogenous thiocyanate behaves similarly, the difference between the UI concentration in automated dialysis and alkaline ashing should be accounted for by the amount of USCN in each sample. Results showed that such a relationship did exist, but as seen in Figure 3, with the y-intercept value of 21.7 μg/L, USCN alone does not account for the total difference between UI concentrations measured by the various methods. Thus other substances may also cross the dialysis membrane and interfere with the catalytic reaction. At this stage we can only speculate as to the nature of these substances, but ions such as nitrite and ferrous iron have been shown to act as reducing agents in the Sandell–Kolthoff reaction (15). Whatever their nature, these other dialyzable interfering substances are responsible for up to 50% of the apparent increase in UI when measured by automated dialysis in samples with UI <100 μg/L. This is highlighted in Table 2, which shows the decrease in UI measured in automated dialysis, after the samples had been ashed to remove any interfering substances. For example, in the second sample the UI concentration decreased from 61 to 20 μg/L; therefore, 41 μg/L of the original UI concentration measured may have been due to interfering substances. The USCN concentration in this case was 5 mg/L, which produces an apparent iodine effect of 21.8 μg/L in automated dialysis (see Figure 2). Therefore, USCN accounts for only 53% of the overestimation in UI, whereas other substances may account for 47%.

Another important finding is the greater degree of overestimation or error due to the measurement of UI in dialysis in samples with low iodine concentrations (<100 μg/L). Using the regression equation generated in Figure 3, one can calculate an expected 55.5 μg/L difference between UI measured by dialysis and that by ashing for a sample with USCN of 10 mg/L. If this hypothetical sample had a true UI concentration of 50 μg/L, the overestimation of UI would be 111%. However, if this same sample had a true UI concentration of 300 μg/L, the overestimation is reduced to 18.5%. This accounts for the poor correlation observed when all UI concentrations obtained by automated dialysis are plotted against USCN (r = 0.148, P = 0.388), because the degree of overestimation becomes less noticeable in samples with normal to high UI concentrations (>100 μg/L). It may also explain the better correlation observed between digestion and dialysis by Gary et al. (5), whose range of analyzed UI was apparently 100–1000 μg/L.

Our study draws attention to the serious problems of using the automated dialysis method for determining UI; in its current form, this method should not be used as a monitoring tool for iodine deficiency. To our knowledge, there is no other fully automated system for UI analysis commercially available. The automated digestion system used in method B is more accurate, but lacks the simplicity and speed of dialysis and necessitates the use of corrosive concentrated mineral acids. Furthermore, the equipment used in method B is no longer available, having been superseded by the dialysis system. Therefore, the available options for monitoring UI in a large number of samples are limited. One possible alternative may be the use of a semi-automated technique, but this has the disadvantage of requiring manual digestion of samples before the final automated colorimetric analysis (16, 17). Because this preliminary digestion uses either acid digestion or alkaline ashing, the sample-processing capacity of a laboratory is limited. Furthermore, the safety issues in using a concentrated acid medium for sample digestion cannot be over-emphasized; hazards include the potentially explosive nature of perchloric acid, and the liberation of a considerable quantity of fumes, including toxic chlorine gas (18). Laboratories using acid digestion techniques should have adequate exhaust ventilation, preferably incorporating a fume hood specifically designed for use with perchloric acid.

In addition to these technical problems, there are other reasons why measurement of UI is not widespread. The validity of using Follis’s criteria of categorizing casual urine samples on the basis of iodine/creatinine ratio (4) is debatable, and Bourdoux has highlighted a number of disadvantages in using this index, especially in populations with lower body mass or protein intake (9). In addition, gastrointestinal factors may exacerbate the iodine deficiency, and measurement of UI excretion alone will not always reflect the severity of the effects of iodine deficiency in the population (9).

Using UI to investigate the effects of iodine deficiency on a population is compounded by the fact that such effects are related primarily to a lack of maternal and hence fetal thyroid hormone (20). The determinations of serum thyroid hormones and thyrotropin are more sensitive indices of the detrimental effects of iodine deficiency than is measurement of UI (21). Two new developments in measuring thyrotropin have made this analyte more attractive for routine surveillance of an iodine-deficient population. First, use of a small spot of blood dried on filter paper avoids the collection of serum, making this approach generally more acceptable (22). Second, the precision and sensitivity of thyrotropin measurements have been vastly improved by the use of isotopic- and nonisotopic-labeled monoclonal antibody methods (23, 24).

Clearly, the biochemical evaluation of the iodine status of communities and its application to the monitoring of iodine prophylaxis require refinement.

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References