Automated Measurement of Urinary Iodine with Use of Ultraviolet Irradiation

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We have modified an automated measurement system of urinary iodine (UI) and established a sensitive UI assay system by using ultraviolet (UV) digestion. The automated system is sensitive enough to detect concentrations of UI <0.78 μmol/L (<10 μg/dL) in a small volume of urine (500 μL). Sample throughput is >30/h, including a water washing. The within-assay imprecision (CV) was ≤10% in the UI range of 0.10–3.00 μmol/L; the between-assay CV was usually ≤15% in the same range. Analytical recovery of iodine added to urine samples was consistently >90%. The theoretical values were recovered when UV irradiation was used but not in its absence. High (supraphysiological) doses of thiocyanate or ascorbic acid, which are major interfering substances to the ceric–arsenious acid reaction, did not interfere with this system. The correlation between UI determined by this method and by the acid digestion method was linear (r = 0.994). For samples containing iodine at <1.00 μmol/L, the correlation between values by both methods was still significant (r = 0.937). UI in an iodine-deficient area in Ukraine, measured by this system, ranged from 0.06 to 1.63 μmol/L (median 0.44 μmol/L, n = 95), significantly lower in Japan (range 0.23–50.70 μmol/L, median 4.70 μmol/L, n = 84) and consistent with mild iodine deficiency. This modified automated assay system, therefore, is useful and applicable for screening UI in inhabitants of iodine-deficient areas.

Indexing Terms: population screening/nutritional status/acid digestion compared

Iodine, a requisite substrate for the synthesis of thyroid hormone, is known to cause pathological conditions when its intake is excessive or deficient (1). Excess iodine from iodine-rich foods or drugs gradually causes goiter and hyper- or hypothyroidism (2, 3). Endemic goiter is a major disorder related to deficient or excess serum concentrations of iodine (4). Cretinism, characterized by mental retardation with hypothyroidism, sometimes results from iodine deficiency; a major cause of the deficiency is lack of iodine intake from food or water, resulting in below-normal serum concentrations of iodine (5). Many other conditions manifest as hypothyroidism: e.g., agenesis of thyroid, dyshormonogenesis, thyroid hormone resistance syndrome, chronic thyroiditis, and iatrogenic state (6). To distinguish iodine deficiency from these fundamental diseases, one must check the iodine intake, the most valid index of which is excretion of urinary iodine (UI) (7).

Measurement of UI is particularly important for the differential diagnosis of the cause of goiter and hyper- or hypothyroidism. However, no conventional automated system for UI analysis is available (8). Therefore, we have established a new automated measurement system that uses the ceric–arsenious acid reaction and a modified digestion method. Although conventional acid digestion can be used to measure UI, it presents risks to the environment and to the user. Therefore, we substituted ultraviolet (UV) irradiation for acid digestion. Although the reactions involved have not been clarified, UV energy can produce oxygen and hydroxyl free radical from potassium persulfate in a four-step reaction in acidic conditions; the radicals can then react with inorganic iodide separated from iodine-containing organic compounds (9). We examined the sensitivity and reproducibility of this modified automated measurement of UI and evaluated the system by comparing the results with those obtained with earlier automated UI determinations based on acid digestion (10).

Materials and Methods

Apparatus

The AutoAnalyzer II system is produced by Bran+Luebbe, Norderstedt, Germany (before February 1987 it had been produced by Technicon Instrument Corp., Tarrytown, NY). The data handler is produced by Bran+Luebbe K.K. (Tokyo, Japan). The computer is from the NEC 9801 series (Japanese and English units are available). All parts, including UV unit and colorimeter, can be obtained from Bran+Luebbe K.K. Figure 1 is a flow diagram showing the connection of all units. The coil used in this system is made of quartz, to permit the passage of UV radiation; 2.0 mm (i.d.), it has a sample volume of ~14.5 mL. Sample residence time (i.e., exposure to UV) is 8–9 min. The UV lamp (General Electric low-pressure 14-W Hg lamp) contains a quartz envelope to permit the passage of the 185 nm Hg/ozone line. Radiation output is between 3% and 4% at this wavelength; life expectancy of the lamp is 7500 h. A 1.5 × 50 mm tubular flow cell and a 420-nm filter were used with the colorimeter.

Reagents

This detection system was based on the ceric–arsenious acid reaction, the recommended method of the International Council for Control of Iodine Deficiency...
Disorder (10). All reagents were of biochemical grade and purchased from Wako Pure Chemical Industries (Osaka, Japan). All solutions were prepared as follows:

Arsenious acid solution. Dissolve 7 g of sodium hydroxide in ~200 mL water and add 10 g of arsenic trioxide powder (As$_2$O$_3$). After dissolution and cooling, add 500 mL of aqueous sulfuric acid (H$_2$O/H$_2$SO$_4$ 60/40 by vol), 48 g of sodium chloride, and 1 mL of 300 mL/L Brij 35, and dilute to 1000 mL with water.

Ceric ammonium sulfate solution. Dissolve 4 g of ceric ammonium sulfate, Ce (NH$_4$)$_4$ (SO$_4$)$_4$·2H$_2$O, in 500 mL of the aqueous sulfuric acid, then dilute to 1000 mL with water.

Phosphoric acid solution. Dilute 40 mL of 85% phosphoric acid to 1000 mL with water.

Potassium persulfate solution. Dissolve 20 g of potassium persulfate in 1000 mL of water; prepare a fresh solution every week. Phosphoric acid provides appropriate acidic conditions to measure iodine without interfering with the ceric–arsenious acid reaction.

Iodine calibrators. Dissolve 168.5 mg of potassium iodate (KIO$_3$) in 1000 mL of water for the stock solution (I, 100 mg/L); dilute appropriately to prepare calibrators at concentrations of 0.078, 0.195, 0.39, 0.78, 1.56, and 3.9 μmol/L (1.0, 2.5, 5, 10, 20, and 50 μg/dL).

Samples

In August 1992, 95 samples (single, early morning urine specimens) were collected from children of Kiev City, Ukraine, and, for controls, 84 samples from people living in Nagasaki City, Japan. The samples were frozen until assay.

Procedures

After an instrument warm-up of 30 min, calibrators in ascending order of concentration and samples (500 μL each) were placed in separate chambers of each of 30 separate rotor cuvettes. Samples were warmed at 55°C in a heating block and flowed to the colorimeter to measure their absorbance at 420 nm. The system was run with a sample:wash ratio of 1:1, the sampling time being 80 and 40 s, respectively, and the throughput was 30 samples/h. All units were washed with water between samples to avoid carryover. An internal control was included every 10 samples. After the assay, the measured values were calculated and recorded by data-handling systems from Fourtec (Tokyo, Japan) and Bran+Luebbe K.K. The data handling is not complex, and a manual protocol can be obtained from Bran+Luebbe K.K.

Statistical Analysis

Stat View 512+™ soft (Brain Power, Calabassa, CA) was used for data analysis: linear regression, Pearson’s correlation coefficient (r). Some values (repeated measurements of the same sample) are presented as mean ± SD.

The median UI contents of the inhabitants in Japan and Ukraine are reported, along with the 95% confidence intervals.

Results

Analytical Performance

Linearity and recovery. A urine sample was serially diluted twofold, six times with distilled water. Recovery was calculated as 100 × the ratio of measured values to expected values. The results were highly linear with iodine concentrations (Table 1), suggesting

| Table 1. Linearity test of diluted urinary iodine by UV digestion method. |
|--------------------------|-----------------|-----------------|---------|
| Dilution ratio | Measured | Theoretical | Recovery, % |
| 1 | 1.746 | 1.746 | 100 |
| 1:2 | 0.905 | 0.873 | 104 |
| 1:4 | 0.411 | 0.437 | 94 |
| 1:8 | 0.221 | 0.218 | 101 |
| 1:16 | 0.109 | 0.109 | 100 |
| 1:32 | 0.060 | 0.055 | 110 |
| 1:64 | 0.036 | 0.028 | 129 |

Values are the means of duplicates.
a lack of interference by urine constituents in the assay for iodine concentrations >0.109 μmol/L. To assess analytical recovery, we added 100 μL of various concentrations of iodine (as KIO₃) to 400 μL of a urine sample containing iodine at 0.160 μmol/L. The measured values agreed well with the theoretical values (Table 2).

The samples containing thyroxine, triiodothyronine, diiodothyronine, moniodothyronine, and KIO₃ were measured with and without UV exposure. The theoretical values were recovered after UV irradiation was used but not when the UV lamp was turned off (Table 3).

**Precision.** Within- and between-assay CVs were determined with six replicate measurements of urine samples containing various concentrations of iodine (Table 4). The precision for urinary iodine concentrations >0.15 μmol/L ensures between-assay CVs of <10%.

**Comparison of methods.** We also measured urine samples (n = 36) by our method (y) and by the Technicon AutoAnalyzer II (x) method. The values measured by the Technicon system, which was based on automated acid digestion (10), correlates with results obtained by the manual alkaline ashing method (8), which is based on the official method of the Association of Official Analytical Chemists (11). Comparison of values for all 36 urine samples, with iodine concentrations ranging from 0.062 to 17.11 μmol/L, gave the following linear regression equation (Fig. 2A): y = 1.093x - 0.228 (r = 0.994, P < 0.0001, Sₓᵧ = 0.351, n = 36). Even for samples with relatively low iodine contents ranging from 0.028 to 0.899 μmol/L (Fig. 2B), the correlation between methods was significant: (y = 0.742x + 0.095, r = 0.937, P < 0.0001, Sₓᵧ = 0.071, n = 16).

<table>
<thead>
<tr>
<th>Added KIO₃, μmol/L</th>
<th>Measured</th>
<th>Theoretical</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.160</td>
<td>0.160</td>
<td>100</td>
</tr>
<tr>
<td>0.078</td>
<td>0.240</td>
<td>0.238</td>
<td>101</td>
</tr>
<tr>
<td>0.39</td>
<td>0.559</td>
<td>0.550</td>
<td>102</td>
</tr>
<tr>
<td>0.78</td>
<td>0.928</td>
<td>0.940</td>
<td>99</td>
</tr>
</tbody>
</table>

Values are the means of duplicates.

<table>
<thead>
<tr>
<th>Organic compounds, μmol/L</th>
<th>Measured Iodine, μmol/L</th>
<th>UV on</th>
<th>UV off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroxine, 13.1 (52.3)*</td>
<td>Mean b</td>
<td>51.1</td>
<td>30.7</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.07</td>
<td>1.18</td>
</tr>
<tr>
<td>Triiodothyronine, 13.5 (40.4)</td>
<td></td>
<td>38.6</td>
<td>16.4</td>
</tr>
<tr>
<td>Diiodothyronine, 35.0 (70.1)</td>
<td></td>
<td>68.6</td>
<td>20.7</td>
</tr>
<tr>
<td>Moniodothyronine, 56.6 (56.6)</td>
<td></td>
<td>52.1</td>
<td>58.6</td>
</tr>
<tr>
<td>KIO₃, 45.7 (45.7)</td>
<td></td>
<td>44.8</td>
<td>8.39</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are the theoretical iodine content in the organic compounds.

**Effect of thiocyanate and L-ascorbic acid.** To investigate the possible effects of thiocyanate and ascorbic acid, which interfere with the Sandell-Kolthoff reaction (12), we measured urinary samples before and after adding various concentrations of thiocyanate (SCN) or L-ascorbic acid. As shown in Table 5, neither substance interfered with the measurement of UI. When UV irradiation was not used, however, thiocyanate clearly affected the measurement (data not shown).

UI Contents in Japan and Ukraine

Measurement of 95 samples from people from an iodine-deficient area (Ukraine: Kiev region) and 84...
samples from people in iodine-rich areas in Japan gave median UI contents (and 95% confidence intervals) of 0.44 μmol/L (0.12–1.28) and 4.70 μmol/L (1.09–46.60), respectively. These values were significantly different (P < 0.0001) by the Mann–Whitney U-test. International organizations have described <0.78 μmol/L (<10 μg/dL) urinary iodine excretion as an indication of iodine deficiency—a value reached by 85.3% of the Ukrainian subjects and 2.4% of the Japanese (Fig. 3).

Discussion

Endemic goiter and cretinism due to iodine deficiency are still a public health problem worldwide. About 850 million people living in Asia, Africa, and South America are estimated to be at risk of iodine-deficiency disorders (13). Various assay methods have been used to survey iodine intake, i.e., manual assay (7), automated analyzers with acid digestion or dialysis systems (14), and iodine-selective ion electrodes (15). However, these are labor-intensive or inaccurate for low concentrations of iodine. Our system, a modified version of the automated analysis system, has at least three advantages. First, some inorganic or organic substances are more efficiently digested by UV digestion than acid digestion: e.g., UV digestion has been used to determine organic carbon in sea water or natural water (9, 16). Thyroxine, triiodothyronine, diiodothyronine, monoiodothyronine, and KIO₃ had analytical recoveries >90% after UV radiation. Also, thiocyanate (present in cassava and tobacco) and ascorbic acid are the main substances interfering with the Sandell–Kolthoff reaction (8, 17), such that thiocyanate concentrations >21.5 μmol/L or ascorbic acid concentrations >14.2 mmol/L lead to overestimation of UI in the automated dialysis method. Neither compound interferes with the measurement of UI in our system unless present at very high concentrations (i.e., 10.3 mmol/L and 14.1 mmol/L, respectively).

Second, this method is analytically sound, being based on the sensitive ceric ion–arsenous acid reaction (8). The dilution test showed high linearity for iodine measurement up to 0.109 μmol/L. The addition of iodine, 0.078 μmol/L, to urine was completely recovered. The precision for measuring UI >0.15 μmol/L ensured between-assay CVs of <10%. There was no discrepancy between our method and the acid digestion method, even for UI <1.0 μmol/L. The calculated detection limit (mean ± 2SD of zero calibrator) for iodine in this system was 0.1 μmol/L.

Third, this assay requires no manual pretreatment procedures and reduces the complicated laboratory work, whereas manual processing depends on the skillful technique of a technician for accurate measurement (18, 19). The liberation of harmful fumes is also a problem in the acid digestion technique because perchloric acid changes to toxic chlorine gas (20). Using potassium persulphate instead of perchloric acid means that no special fume hood is necessary. Finally, its running cost in this system is <2¢ (US) per sample.

Using this system to measure the UI contents of samples collected from the Kiev region, which is 120 km from Chernobyl and is thought to be an iodine-deficient area, we found that >85% of samples in this area had UI <0.78 μmol/L (10 μg/dL), similar to the previously reported low iodine content in water and food in the Ukraine (21). A previous report about iodine deficiency in Ukraine found that 23% of children living in Rovno and Chernigov regions, Ukraine, had UI values compatible with severe deficiency (22). The discrepancy between the severity of iodine deficiency found in our study and the previous report might result from a sampling bias such as time and geographical differences, or could merely reflect the absence or presence of supplementary iodine, the urine samples having been collected at random around the Kiev region.

International organizations have described three levels of iodine deficiency—severe, moderate, and mild—which Bourdoux (10) has related to the following three arbitrary ranges for urinary iodine excretion: <0.16, 0.16–0.39, and 0.40–0.78 μmol/L, respectively. The median iodine content of the samples in Ukraine

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**Table 5. Effect of exogenous thiocyanate or ascorbic acid on iodine determination.**

<table>
<thead>
<tr>
<th>Conc added, mmol/L</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiocyanate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.462 (0.012)</td>
<td>0.160 (0.002)</td>
</tr>
<tr>
<td>3.08</td>
<td>0.515 (0.030)</td>
<td>0.166 (0.011)</td>
</tr>
<tr>
<td>10.3</td>
<td>0.497 (0.021)</td>
<td>0.139 (0.006)</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.349 (0.022)</td>
<td>0.137 (0.057)</td>
</tr>
<tr>
<td>1.69</td>
<td>0.446 (0.022)</td>
<td>0.141 (0.017)</td>
</tr>
<tr>
<td>14.1</td>
<td>0.431 (0.050)</td>
<td>0.102 (0.009)</td>
</tr>
</tbody>
</table>

n = 3 each.

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**Fig. 3. Urinary iodine concentration of samples taken from an iodine-deficient area of Ukraine and an iodine-rich area of Japan.**

The median UI content (and 95% confidence interval): Ukraine, 0.44 μmol/L (0.12–1.28 μmol/L, n = 95); Japan, 4.70 μmol/L (1.09–46.56 μmol/L, n = 84). Horizontal lines (top to bottom) indicate UI values of 100 (78), 5050, and 200 (16) μg/dL (μmol/L). The percentage of the samples in each class of severity for iodine deficiency was 7.4% (severe), 40% (moderate), and 33% (mild) in the Ukraine and 1.1% (moderate) and 1.1% (mild) in Japan.
was 0.44 μmol/L, which correlates with mild iodine deficiency.

Kazakov et al. (23) reported that the number of children having thyroid nodules increased dramatically in Belarus after the Chernobyl accident. But other researchers have insisted on the necessity for more information about the thyroid cancers in children from Belarus and the need for further epidemiological studies, including the effect of iodine deficiency on the uptake and elimination rates of iodine (24, 25).

In conclusion, this new iodine assay provides precise, sensitive, and accurate quantification of iodine in urine. This system is useful as a monitoring tool for iodine intake, especially in iodine-deficient areas.

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References