NOTE

Measurement of Iodide in Urine Using the Iodide -selective Ion Electrode

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Abstract

A simple and rapid way to measure the concentration of iodide in urine with an iodide-selective ion electrode was described. Potentiometric equilibrium was attained in less than 5 min, and a linear calibration curve was obtained over the potassium iodide (KI) concentration range of 10^{-2} to 10^{-6} M. The coefficients of variation ranged from 6.2 to 10.0% within assay, and 5.4 to 14.4% between assays. The serial dilution of 3 urine samples with different concentration of iodide showed good linear correlations passing through zero. In practice, the chloride ions in urine did not cause serious errors in the measurement of iodide at molar ratios of chloride ion to iodide up to 2×10^4 . A good linear correlation was obtained between iodide concentrations in urine determined by the electrode method and by the conventional chemical method ($r=0.92$). A linear correlation was also observed between the iodide concentrations of 24 h collected urine and those of single morning urine ($r=0.91$). The normal iodide content in single morning urine specimens from 127 Japanese people was 5.3 to 62.0×10^{-6} moles/g creatinine.

Iodine deficiency is well known to be a major factor which contributes to endemic goiter, cretinism and impairment of thyroid function (Brush and Altland, 1952; Stanbury *et al.*, 1974; Sava *et al.*, 1984; Scriba, *et al.*, 1985). Excess iodine intake also has been a cause of goiter (Suzuki *et al.*, 1965) and thyrotoxicosis (Vagenakis *et al.*, 1972). Moreover, iodine rich drugs, such as amiodarone, have been reported to induce thyrotoxicosis, which is a therapeutic problem

in patients with tachyarrhythmia (Savoie *et al.*, 1975; Eason *et al.*, 1984; Leger *et al.*, 1984). In those cases, the measurement of the urinary iodide concentration is necessary for the diagnosis of goiter and thyroid dysfunction, because urinary excretion of iodide is the most valid index of iodine intake. Since the classic chemical procedures for measurement of iodine are tedious, time-consuming and sometimes inaccurate at low levels of iodine, a simplified, rapid, and accurate method is preferable. Recent success in the development of ion-selective electrodes has made it possible to determine low levels of iodide in milk (Lacroix and

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Wong, 1980; Gushurst *et al.*, 1984). We therefore examined the levels of iodide in urine samples with an iodide-selective electrode, and concluded that the simplicity and rapidity of this method would be useful as a laboratory procedure.

Materials and Methods

Subjects and specimens

Thirty-nine men, aged from 21 to 61, and 88 women, aged from 18 to 57, in the general population were examined. All of them were healthy and none was found to have a goiter.

Approximately 20 ml of morning urine from the first urination before breakfast was collected in a plastic vial containing 100 μ l of 10% sodium azide. These samples were stored at -20°C until used. For some experiments, the urine was collected for 24 h and also stored at -20°C .

Apparatus

An ION85[®] Ion Analyzer with an F1032I Iodide Selectrode[®] (Radiometer, Copenhagen, Denmark) was used to determine the iodide concentration in urine. The concentrations of urinary creatinine and chloride ions were measured using the Automated Stat/Routine Analyzer, Astra-8, (Beckman, Brea, U.S.A.), by the Jaffe rate method and the coulometric titration method, respectively.

Some urine samples were diluted 20 to 300 fold with deionized water, and the iodine concentration was determined by the chemical method described by De Visscher *et al* (1960) using an automated system (Auto-Analyzer[®], Technicon Instruments Co., Chauncey, U.S.A.).

Procedures

The iodide concentration in urine samples was determined by direct measurement according to the manufacturer's instructions. Standard solutions were prepared by serial dilution of the Iodide Standard[®] (S-3576, Radiometer, content: potassium iodide (KI), 1×10^{-1} M at 25°C) with deionized water to 1×10^{-2} , $\times 10^{-3}$, $\times 10^{-4}$, $\times 10^{-5}$, and $\times 10^{-6}$ M, respectively. All standards were freshly made up for every assay. Sodium nitrate (NaNO_3 ; Wako Pure Chemical Industries, Osaka, Japan) at 5M in deionized water was used as an ion strength adjuster. A calomel electrode (K701, Radiometer) was used as a reference electrode, and the temperature was monitored with a temperature sensor (T801, Radiometer). Five milliliters of 5M sodium nitrate was mixed with 10 ml of either standard solution or urine. The electrodes were immersed in each sample, and concentration readings were obtained after stable readings.

Urinary iodide content was expressed as moles of iodide per gram of creatinine times 1.0 (females) or 1.7 (males), according to the formula reported by Vought *et al.* (1963).

Fig. 1. Response behavior of the iodide-selective electrode. Standard solutions were used for the experiment. e. m. f.; electromotive force. a; KI: 5×10^{-5} M, b; 1×10^{-5} M, c; 5×10^{-6} M, d; 1×10^{-6} M, respectively.

Results

Response behavior of iodide-selective electrode

The response characteristics of the electrode were evaluated by exposing it in different standard iodide solutions ranging from 1×10^{-6} to 5×10^{-5} M. The change in the electromotive force vs. time is shown in Fig. 1. Equilibrium values were attained within 5 min in these standard solutions, and similar response curves were obtained when urine samples were measured. Therefore, 5 min was taken as electrode response time.

Calibration curve for iodide-selective electrode

A typical calibration curve for the iodide-selective electrode with 5 different standard solutions is shown in Fig. 2. Using standard solutions ranging from 1×10^{-6} to 1×10^{-2} M, a good linear calibration curve was always obtained, and the slope of the curve was 60.0 ± 1.1 mV per concentration decade ($n = 23$, and the mean temperature was $23.6 \pm 1.0^\circ\text{C}$).

Fig. 2. Calibration curve measured at 24.5°C .

Effect of dilution on the measurement of iodide concentration in urine

Three urine samples with different iodide concentrations were diluted to various degrees with deionized water and the effect of dilution on the measurement of iodide was then examined. As shown in Fig. 3, all three linear dilution curves passed through zero.

Effect of chloride ions

In order to examine the degree of interference of chloride ions with the electrode, potassium chloride was added to standard

Fig. 3. Effect of dilution on the measurement of iodide. ($\blacktriangle$ — $\blacktriangle$; 104.3×10^{-6} M, $\bullet$ — $\bullet$; 58.2×10^{-6} M, $\blacksquare$ — $\blacksquare$; 20.7×10^{-6} M, respectively). All samples were measured in the same assay at 24.5°C .

Fig. 4. Effect of chloride ions on the measurement of iodide. Potassium chloride was added to standard solutions with 4 different concentrations, ($\blacktriangle$ — $\blacktriangle$; 1×10^{-5} M, $\bullet$ — $\bullet$; 8×10^{-6} M, $\blacksquare$ — $\blacksquare$; 4×10^{-6} M, $\circ$ — $\circ$; 1×10^{-6} M, respectively). Dotted lines indicate the range of the coefficients of variation within assay. All samples were measured in the same assay at 25.0°C . Chloride-induced interference was expressed as $\Delta(I^-)/(I^-)_0 \times 100\%$, where $(I^-)_0$ and $\Delta(I^-)$ mean iodide concentrations without chloride ions, and the differences between those with chloride ions and $(I^-)_0$, respectively.

solutions with 4 different iodide concentrations, with molar ratios of chloride ion (Cl^-) to iodide (I^-) from 5×10^2 to 4×10^4 . As shown in Fig. 4, positive errors were seen in all standard solutions, but up to 2×10^4 of the $(\text{Cl}^-)/(\text{I}^-)$ ratio, the chloride-induced errors were less than 10% in 3 standard solutions except one with a low iodide concentration ($1 \times 10^{-6}\text{M}$).

Chloride ions in morning spot urine samples ranged from 12 to 279 mM with a mean of 131 mM, and iodide concentrations from 4.2 to $137.5 \times 10^{-6}\text{M}$ with a mean of $25.5 \times 10^{-6}\text{M}$. The calculated $(\text{Cl}^-)/(\text{I}^-)$ ratios were distributed between 1×10^3 and 2.2×10^4 , with a mean of 7.2×10^3 ($n=163$).

Reproducibility

The reproducibility of the assays performed within a day (within assay) and over a period of a week (between assays) is shown in Table 1. Within run re-

Table 1. Reproducibility of iodide determination in urine

Assays	Number of Determinations	Iodide Concentration* ($\times 10^{-6}$ M)	Coefficient of Variation (%)
Within assay	6	6.4 ± 0.4	6.2
	6	26.5 ± 2.6	10.0
	6	89.0 ± 6.6	7.4
Between assays	5	7.9 ± 0.9	11.8
	5	15.0 ± 0.8	5.4
	5	107.9 ± 15.6	14.4

*; expressed as mean $\pm$ SD.

producibility was 6.2–10.0% over the range 6.4 – $89.0 \times 10^{-6}\text{M}$, while day to day imprecision was 5.4–14.4% over the range 7.9 – $107.9 \times 10^{-6}\text{M}$.

Correlation between iodide concentrations measured by electrode method and by chemical method

Iodide concentrations of 21 urine samples were measured by both electrode (y) and chemical (x) methods (Fig. 5). A significant

Fig. 5. Correlation between iodide concentrations measured by the electrode method and by the chemical method. Values obtained by the chemical method (iodine concentrations, g/100 ml) were expressed as iodide concentrations ($\times 10^{-5}$ M) ($n=21$).

correlation was obtained in the following parameters ($r=0.92$, $n=21$, $y=1.26x+2.03 \times 10^{-5}$, $P<0.001$).

Correlation of iodide concentration in single morning urine samples and urine collected over a 24 hour period

Figure 6 shows a linear correlation of individual values for iodide concentration in single morning urine samples before breakfast (x) and urine collected over 24 h (y) ($r=0.91$, $n=18$, $y=0.99x+1.73 \times 10^{-5}$, $P<0.001$).

Normal iodide content in urine

The distribution of urinary iodide content corrected by Vought's formula showed a typical logarithmic normal distribution in both men and women, and there was no statistical difference between them (Student's t -test). We found normal iodide content in single morning urine samples before breakfast to be 5.3 to 62.0×10^{-6} moles/g. of creatinine ($n=127$).

Fig. 6. Correlation of iodide concentrations in single morning urine samples and urine collected over a 24 hour period ($n=18$).

Discussion

The simple and rapid way to measure the iodide concentration in urine samples with an iodide-selective electrode was described. No special treatment was required for urine samples except adding a bacteriostatic agent, 0.05% of sodium azide. Sodium azide itself did not interfere with the determination of iodide concentration. Samples were stable for at least 3 months under freezing at -20°C with tight stoppers (data not shown). Potentiometric equilibrium was always attained in less than 5 min, and the remarkable sensitivity and dynamic range of the electrode is evident from the calibration curve over the KI concentration range of 10^{-2} to 10^{-6} M (Fig. 2). Like other electrode methods, the iodide concentration measured with the Iodide Selectrode® varied according to the change in temperature.

However, if the difference between the reference temperature and that of the sample is greater than 2°C, the alarm is actuated. Although all assays were done at room temperature, the average slope of the calibration curve, 60.0 mV, was very close to the theoretical value, 59.2 mV. The reproducibility was sufficient for clinical use both within and between assays.

As Cooper and Croxson (1983) have already reported, the interference of chloride ions, which are major anions in urine, should be discussed. As shown in Fig. 4, with 2×10^4 of $(\text{Cl}^-)/(\text{I}^-)$ molar ratio, 10.4% of chloride-induced error was seen at the iodide concentration of 1×10^{-6} M. Among 163 urine specimens in the present study, the lowest iodide concentration was 4.2×10^{-6} M, and $(\text{Cl}^-)/(\text{I}^-)$ ratios ranged from 1×10^3 to 2.2×10^4 . The estimated chloride-induced errors in most of the specimens were approximately less than 6%, smaller than the coefficients of variation within an assay. Chloride interference less than 6% presents no serious problem for practical use, since iodide in urine from healthy volunteers was distributed widely between 4.2 and 137.5×10^{-6} M. Moreover, a good correlation between electrode and chemical methods was obtained (Fig. 5). Therefore, the electrode method described here could be recommended as a laboratory procedure instead of chemical methods, because of its simplicity and rapidity.

Since urinary excretion of iodide fluctuates according to the plasma concentration of iodide, 24 h collection of urine has been thought to be necessary in studies of urinary iodide excretion (Follis *et al.*, 1962). However, in Norway a single urine specimen was reported to be sufficient to estimate the 24 h iodine excretion (Frey *et al.*, 1973). It is noteworthy that a good correlation was obtained between single morning urine and 24 h collected urine in our present study in Japan, where a large amount of iodine is taken in the average diet.

It may be helpful to know the iodide concentration in urine when there is thyroid dysfunction associated with therapeutic and diagnostic iodine administration (Blum *et al.*, 1974; Savoie *et al.*, 1975; Rodesch *et al.*, 1976; Theodoropoulos *et al.*, 1979; Eason *et al.*, 1984; Leger *et al.*, 1984). The electrode method described here would be of value for routine use in clinical laboratories.

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