The Combined Measurement of the Four Stable Halides by the Ion-Selective Electrode Procedure Following Their Chromatographic Separation on a Strong Anion Exchanger Resin: Clinical Applications

by Guy E. Abraham, M.D.

Introduction

Group VII B of the periodic table contains five elements which are called halides in the reduced state and halogens in the oxidized state: fluorine (MW=19); chlorine (MW=36); bromine (MW=80); iodine (MW=127); and astatine (MW=210). In nature, the halogen astatine exists only in the radioactive form. The stable (non-radioactive) halogens and halides include the other four elements. The largest of the stable halogens, iodine is an essential element. It was called "The Universal Medicine" more than 100 years ago because of its safe, effective and widespread applications in clinical medicine for conditions not responding to other treatment modalities.[1-4]

Following World War II, iodophobic misinformation resulted in medical iodophobia.[2-4] As a result, iodine has been neglected in medical textbooks and vilified in endocrine publications. Medical iodophobia may have caused more human misery and death in the US than both World Wars.[5] After 60 years in the Dark Ages, iodine is experiencing a revival due to recent *in vivo* and *in vitro* research on this essential element. The observations of our medical predecessors regarding the role of iodine in clinical medicine are undergoing scientific validation.[1-18] With a resurgence of interest in its extra thyroidal functions,[13,19-22] iodine may soon regain its title as "The Universal Medicine" or more appropriately as the "Universal Nutrient."[1]

The halides fluoride and bromide block the uptake and utilization of iodine in target cells.[13] Bromide possesses goitrogenic, carcinogenic, and narcoleptic properties.[10] Iodine intake in adequate amounts increases the mobilization and urinary excretion of fluoride and bromide.[9,13] Chloride, like iodide, increases urinary excretion of bro-

mide.[14] Restriction of table salt decreases markedly the renal clearance of bromide by as much as tenfold, resulting in a corresponding tenfold elevation of serum bromide levels. Some subjects on a restricted salt diet with elevated serum bromide levels reported that they felt better the days they indulged in salty foods. Urinary excretion of chloride is a good index of intake, and its measurement should therefore be included in a comprehensive assessment of the metabolism and excretion of the goitrogenic, carcinogenic, and narcoleptic bromide.[14,15] In this author's experience, elevated serum fluoride levels (greater than 0.05 mg/L) are a rare occurrence whereas elevated serum bromide levels (greater than 12 mg/L) are common in our population.

The accurate, precise, and specific measurement of the essential element iodine and the toxic halides, fluoride and bromide, in biological fluids is essential for a comprehensive understanding of the metabolism of these elements and their interactions in health and diseases. Among the various techniques available for the measurement of the essential element iodine in biological fluids, the ion-selective electrode (ISE) procedure is the simplest, fastest, safest, and least expensive.[6,13,23,24] Quantification of iodide is performed by a potentiometric method, using an ISE. Urinary iodide is measured by the electromotive force (EMF) generated on the ISE due to the presence of iodide in the urine sample. Within a certain range of iodide concentrations, there is a linear relationship between the logarithm of iodide concentration and the EMF generated.

The ISE procedure for iodide was used in the US during the early 1980s to assess the concentration of this essential element in dairy[25] and human milk.[26] It was later applied in Japan by Yabu, *et al*,[23] in 1986, and by Konno, *et al*,[24] in 1993, to measure iodide in the urine of mainland Japanese. In all the above studies, iodide was measured directly in the biological fluids without prior purification.

As part of a project initiated by this author seven years ago, called The Iodine Project,[13] with the goal of re-evaluating the role of the essential element iodine in clinical medicine, the ISE procedure was applied to the measurement of iodide in urine,[6] serum,[12] and saliva[16] following chromatography on a strong anion exchange (SAX) resin. In order to assess whole body sufficiency for iodine,[10] an iodine/iodide loading test based on the ISE measurement of iodide in 24-hour urine collection following an iodine/iodide load was developed by this author. The daily amount of the essential element iodine needed for whole body sufficiency was named orthoiodosupplementation.[8]

The three biological fluids tested so far in the author's potentiometric R&D laboratory are urine, serum, and saliva. The chromatographic technique on SAX cartridges allowed the combined ISE measurement of iodide, fluoride, bromide, and chloride in these biological fluids.[9,13,16] These procedures were described in several publications[11,12,14,15] but not in detail. The combined measurement of the four stable halides in biological fluids are presented here in more detail. Health care professionals and commercial laboratories who wish to set up the ISE procedure will have access under one cover to practical guidelines for each phase of these techniques: the collection of biological fluids; the separation of the halides from each other by anion exchange chromatography; their measurements by the ISE procedure; and some clinical applications of these procedures.

Collection of Biological Fluids

Urine Collection: The procedure for urine collection was previously described.[6,27] For the sake of having all this information in one manuscript, the procedure is repeated here. Ingested iodates, inorganic iodine and iodide are quantitatively absorbed.[13] When the oral intake is not excessive, iodate is reduced to iodide in the intestinal tract.[28] Iodine is partly reduced in the intestinal tract, but a significant amount is absorbed as iodine.[29,30] Target cells have a mechanism to transfer peripheral iodide inside the cells by an active process.[16] However, recent evidence suggests that iodine is passively transferred across the cell membrane.[31] Peripheral iodide is cleared efficiently by the kidneys within 24 hours after ingestion, with a daily renal clearance rate of 43.5 L of plasma.[11]

The measurement of iodide in a spot urine sample is a rough estimate of inorganic iodate, iodine, and iodide intake. Spot urine iodide measurement is the procedure used in epidemiological studies. However, it must be emphasized that the spot test is not reliable for whole body iodine sufficiency.[10,11] The iodine/iodide loading test requiring a 24-hour urine collection following the iodine load has been standardized and correlates well with clinical response to orthoiodosupplementation.[10,13,17]

Prior to ingesting the iodine load, the first void sample is usually discarded. However, the iodide level in this spot urine may be used to confirm that the subject/patient has not ingested the iodine supplementation for 24-48 hours prior to the loading test. This is of value when the loading test is repeated following orthoiodosupplementation. If the subject/patient did not stop the iodine supplementation for at least 24 hours pre-load, a carryover effect of the iodine ingested the previous day will result in an overestimation of the percentage of the load excreted.

The following instructions for the 24-hour urine collection during the loading test apply to both pre- and post-orthoiodosupplementation.

1) Discard the first morning urine of Day 1. However, it may be used as a spot urine collection if the loading test is performed post-orthoiodosupplementation. A low iodide level in the spot sample confirms that the subject/patient has not ingested the iodine supplementation for 24-48 hours prior to the test.

2) Take four tablets of Iodoral® 12.5 mg with a glass of water.

3) Collect all urine samples for 24 hours following ingestion of the loading dose. Include the first morning urine on Day 2. A 3-liter plastic bottle appropriate for the 24-hour collection can be obtained from VWR Scientific Product (PN# 60872-564). The 16-ounce urine collection cup is available from General Bottle Supply Co., phone (323) 581-2001 (PN# T31216-1).

4) At the end of the 24-hour collection, shake the 3-liter bottle well. Measure the volume of the urine in the 3-liter bottle by looking at markings on the side of the bottle, and pour 1-2 oz. into a 2-ounce plastic bottle (VWR Scientific Product, PN# 16126-041). Two plastic 2-ounce bottles should be supplied in case the 24-hour urine volume is greater than 3 L. Have the patient return the other empty 2-ounce bottle with your package, if the total volume is 3 L or less.

5) If the 3-liter bottle is full before the end of the collection, the same 3-liter bottle can be used to continue collection after measuring the total volume:

A) Measure the volume of the urine in the 3-liter bottle by looking at the markings on the side of the bottle. Pour 1-2 oz. of urine in the 2-ounce plastic bottle. Write name, date and volume of urine on the 2-ounce bottle. Write on the label "Part 1 of 2 collections."

B) Discard the urine in the 3-liter bottle. Use the same 3-liter bottle to continue collection.

C) At the end of the 24-hour collection, measure the volume of the urine again and repeat step 5A. Write "Part 2 of 2 collections."

D) When the patient's information form is completed, under total volume, write: "Collected in 2 parts, both sample bottles enclosed."

(Continued on next page)

E) The concentration of iodide in the 24-hour urine collection will be the sum of both values obtained in the two specimens. Please note that in this case, you will be performing two measurements instead of one to compute the percentage load excreted. For example, you measure 6 mg/L in collection Part 1 with a volume of 3 L, and you measure 4 mg/L in collection Part 2 with a volume 1.5 L. The computation of the total amount of iodide excreted is: Part 1 = 6 mg/L × 3 L = 18 mg; Part 2 = 4 mg/L × 1.5 L = 6 mg; Total = 24 mg; The percentage excreted = 48%.

The food grade coloring FD and C Green #3 is added at a concentration of 1 ml of a 1% solution per liter of urine. It is used for its antiseptic properties and also as a marker for spent chromatography cartridges.[6] Sodium azide at a final concentration of 0.01% is usually the antiseptic used during collection of urine samples.[23] FD and C Green #3 (Warner Jenkinson Company, St. Louis, Missouri) and sodium azide (Spectrum PN# S1666) can both be used for maximum inhibition of mold and bacteria. In the author's research laboratory, the combined addition of FD and C Green #3 and sodium azide is used routinely for urine, serum, and saliva samples. An aqueous solution containing 15% sodium azide and 1% FD and C Green #3 is added to urine samples at one drop per ounce and to serum and saliva samples at one drop per 5-8 ml.

Serum Collection: Plasma contains fibrinogen which may coagulate and impair the flow of eluate through the cartridges during chromatographic separation of the halides. This causes an increased pressure in the cartridges which results in a distortion of the elution profile. For this reason, serum samples are recommended. After venipuncture and collection of 10-15 ml of whole blood in red top tubes without separator gel, let the blood clot and retract. Centrifuge the clotted blood. Decant 5-8 ml of serum in plastic tubes containing one drop of an aqueous solution containing 15% sodium azide and 1% FD and C #3.

Saliva Collection: After rinsing the mouth with water two to three times to remove any food particles, collection of saliva for a total volume of 5-8 ml can be done passively by letting it flow through a straw into a test tube or by spitting into a container with a wide opening. One drop of an aqueous solution containing 15% sodium azide and 1% FD and C Green #3 is added to the collecting vial. Xylitol and citric acid which increase saliva flow may be used in patients who cannot produce enough saliva within 5-10 minutes. Centrifuge saliva to remove coagulated mucus which interferes with the chromatographic separation of the halides.

Combining the loading test with the measurements of serum and saliva iodide 24 hours post-load gives an assessment of iodine sufficiency of the whole body and efficiency of the cellular uptake and utilization of peripheral iodide.[16] This is called the triple test for the combined assessment of sufficiency and efficiency. The biological fluids are stable for up to one week at room temperature when collected in containers with sodium azide and FD and C Green #3. However, it is best to freeze the samples if they are not processed within 48 hours of collection.

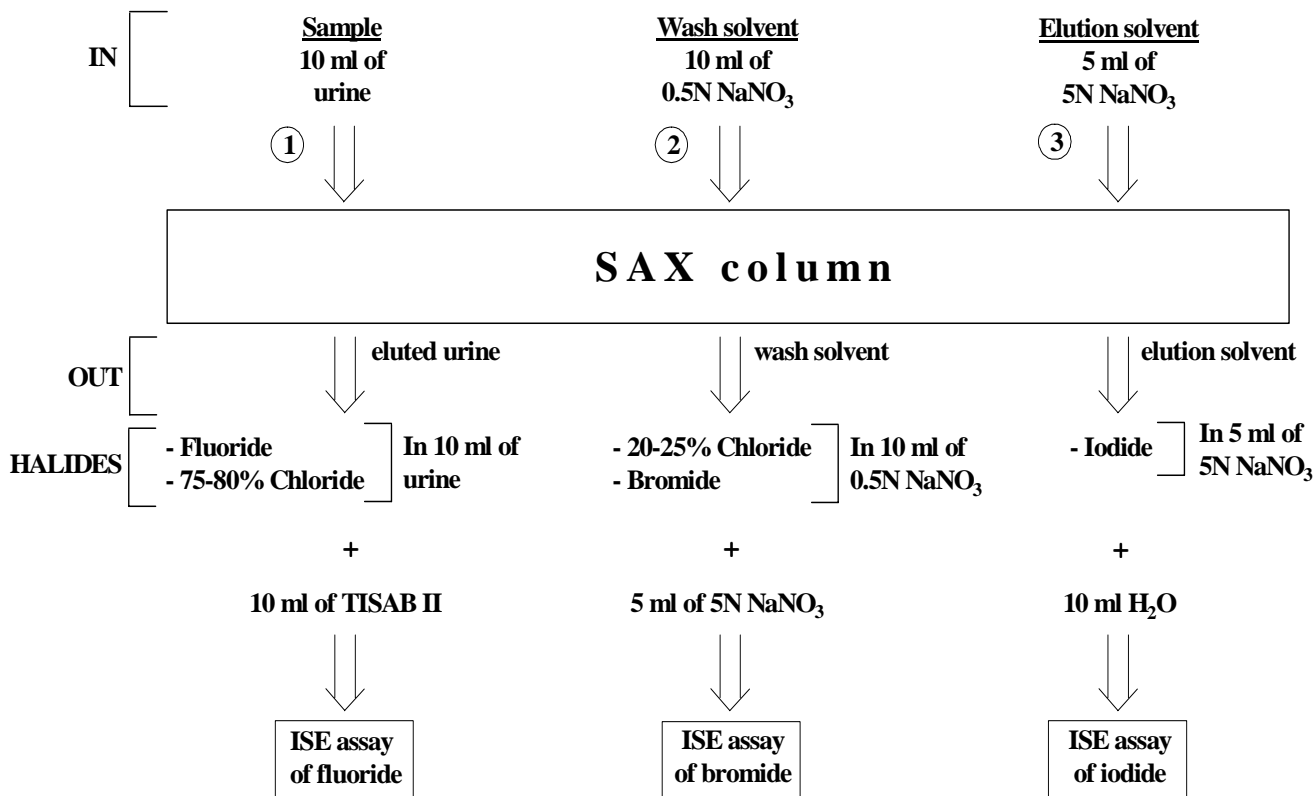**Chromatographic Separation of Halides from Biological Fluids**
In order to improve the specificity of the ISE procedure, the halides are separated from each other by solid phase partition on Strong Anion Exchanger (SAX) columns and cartridges prior to their ISE measurement.

Materials used in anion exchange chromatography are composed of three components attached together and placed in a column or a cartridge: the base or backbone support; the functional group or ion exchanger; and the counter ion available for exchange. For backbone, styrene divinyl benzene (SDB) was preferred over silica gel because it is more rugged, less sensitive to pH changes, and possesses a higher capacity. For example, SAX columns with silica backbone are available from Varian and Associates (Harbor City, California) with a capacity of 0.85 m Eq/gm of resin. However, with SDB backbone, Altech (Deerfield, Illinois) quotes a figure of 1.5 m Eq/gm, a 75% greater capacity to exchange anions. This translates into the ability to process a 75% greater volume of urine for the same amount of resin.

Strong anion exchangers are quaternary amines versus weak anion exchangers, which are primary, secondary and tertiary amines. Strong anion exchangers are always charged at any pH. Therefore, elution of the analate of interest could be achieved by increasing the ionic strength of the elution solvent without adding any acid. Data are available for strong anion exchangers regarding the relative selectivity of halides.[32] With fluoride as unity, chloride has a relative selectivity of 10; bromide 28; and iodide 87. The higher the number, the stronger the binding of the halide to the anion exchanger. The stronger the binding of the halide to the ion exchanger, the higher the ionic strength required for elution. By adding sodium nitrate to the biological fluid at an ionic strength high enough to elute fluoride, chloride, and bro-

Figure 1

**Flowchart Describing the Procedure Used in the Combined
Measurement of Fluoride, Bromide, and Iodide in the Same Urine Sample[6]**

	Sample 10 ml of urine	Wash solvent 10 ml of $0.5N\ NaNO_3$	Elution solvent 5 ml of $5N\ NaNO_3$
IN	① ⇓	② ⇓	③ ⇓

SAX column

	eluted urine	wash solvent	elution solvent
OUT	⇓	⇓	⇓
HALIDES	- Fluoride - 75-80% Chloride In 10 ml of urine	- 20-25% Chloride - Bromide In 10 ml of $0.5N\ NaNO_3$	- Iodide In 5 ml of $5N\ NaNO_3$
	+	+	+
	10 ml of TISAB II	5 ml of $5N\ NaNO_3$	10 ml H_2O
	⇓	⇓	⇓
	ISE assay of fluoride	**ISE assay of bromide**	**ISE assay of iodide**

mine with the biological fluid, but not high enough to elute iodide, a high degree of purity of the iodide fraction could be achieved in a 2-step procedure, even though the other halides were not separated from each other.

When the decision was made in early 2002, to measure urine iodide levels *in situ* and to use a chromatographic separation of the halides prior to measurement to improve specificity, the only available chromatographic manifolds affordable for a small laboratory were the positive pressure manifolds and the vacuum manifolds. Two vacuum manifolds capable of handling 10 samples and 30 samples were purchased by the Optimox Potentiometric R&D Laboratory from Applied Separation, Inc. (Allentown, Pennsylvania). To generate a vacuum in the vacuum manifold, the Benchtop Vacuum Station from Altech was chosen because it was capable of maintaining a preset vacuum. For the separation of halides, the SAX resin from Altech was tested in columns containing from 100-600 mg of resin. The 500-

milligram column with a 15-milliliter reservoir was chosen. A 3-step procedure using increasing concentrations of an aqueous solution of $NaNO_3$ yielded the halides shown in Figure 1. The eluted urine contained fluoride and 75-80% of the chloride. Some 20-25% of the chloride was retained on the column, together with bromide and iodide. A wash of the column with 10 ml of 0.5N $NaNO_3$ eluted the retained chloride and the bromide. Quantitative recovery of iodide (>95%) was achieved with 5 ml 5N $NaNO_3$.[6]

Although the iodide fraction did not contain the other halides, the chloride fraction was not completely separated from the fluoride and bromide fractions. This procedure was used to measure urine iodide levels during the early phase of The Iodine Project, and the specificity was confirmed by the high degree of correlation observed when values of urine iodide by the ISE procedure were compared with corresponding values by the ICP-MS technique.[6] The correlation was better with low iodide levels than levels post-orthoiodosupplementation.

In order to improve the specificity, precision and accuracy in the measurement of the other halides, the chromatographic purification of iodide from the other halides by solid state partition on anion exchange resins was modified. Instead of the 500-milligram column with a 10-milliliter reservoir (Altech #309750), the 600-milligram syringe cartridge (Altech #21907) was used.[12] Both chromatographic systems contain the same SAX resin.[6] The vacuum manifold connected to a vacuum pump was replaced with the Positive Displacement Manifolds (PDM-40, PDM-20, and PDM-6), capable of running 40, 20 and six samples, respectively, in the same batch. The PDMs were designed by the author with the assistance of precision machinists who built the units in aluminum.

The concentrations of $NaNO_3$ used for the elution of the halides were modified from a 3-step procedure to a 4-step procedure.[12] Pilot studies were performed with standards of the halides in order to optimize the new sys-

tem. In the 500-milligram column, fluoride was eluted with the biological fluid together with 75-80% of the chloride. With the 600-milligram cartridge, chloride came first with the eluted sample. The 4-step procedure using the 600-milligram cartridges had a much greater resolution power in separating the halides from each other than the 3-step procedure with the 500-milligram column. The sequence of the elution procedure used for the cartridges is displayed in Figure 2. This elution sequence resulted in an excellent separation of the halides with less than 2% overlap (Table 1).

As previously discussed, the stronger the binding of the halide to the ion exchanger, the higher the ionic strength required for elution. By increasing the ionic strength of the biological fluid with sodium nitrate to elute fluoride, chloride, and bromide with the biological fluid, but not high enough to elute iodide, a high degree of purity of the iodide fraction could be achieved in a 2-step procedure.

Figure 2

Flowchart Describing the Combined Measurement of Halides in the Same Urine/Serum/Saliva Sample
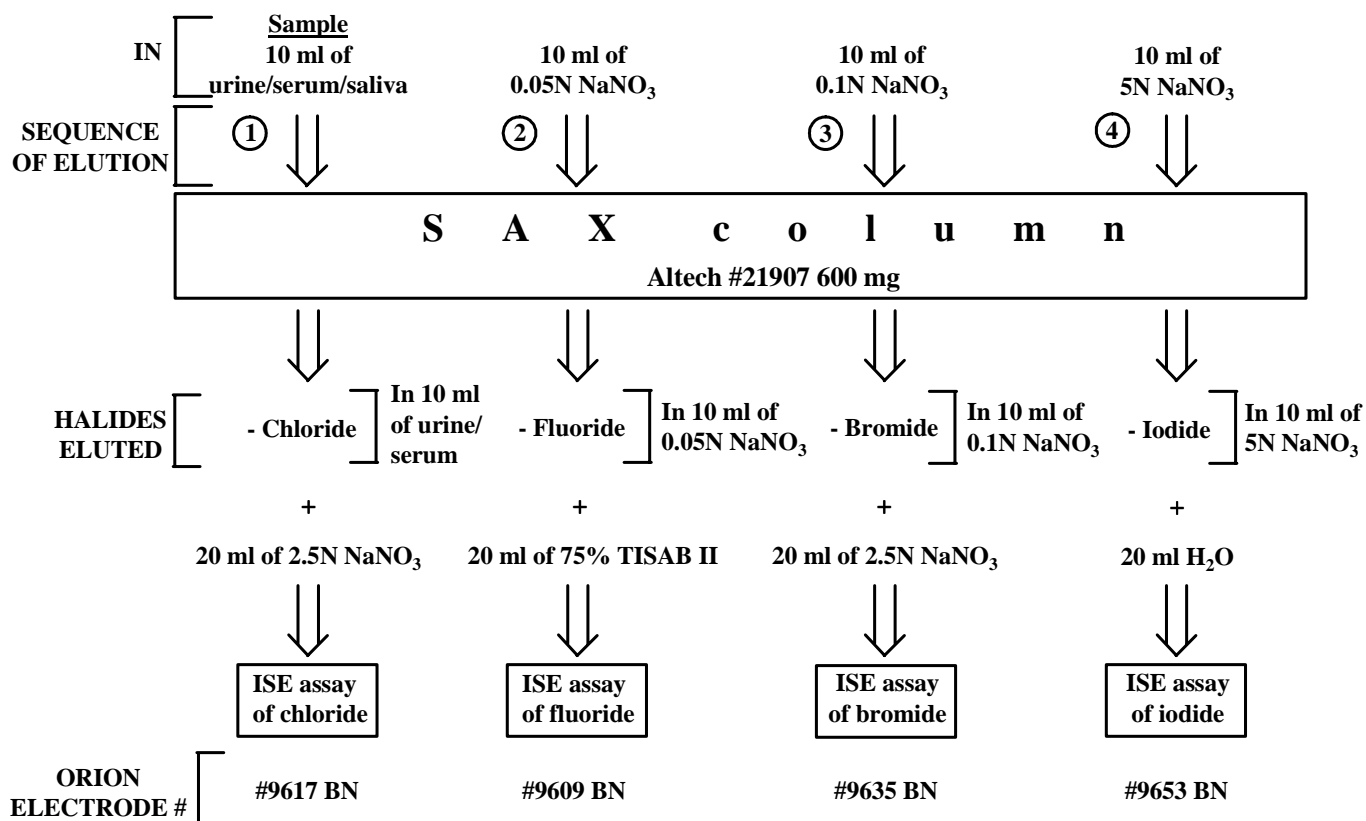
Chloride, fluoride, bromide, and iodide measured by prior chromatography on the anion-exchange resin cartridge SAX 600 mg fitted with 10-milliliter plastic syringes in a positive displacement manifold.[12]

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Table 1

Chromatography of Halides in Water:
Percent Recovery in Eluted Fractions of Increasing Ionic Strength

	Percent Recovery			
	Chloride	**Fluoride**	**Bromide**	**Iodide**
Eluate	≥98%	<1%	<1%	<1%
0.05N NaNO₃	<2%	≥98%	<1%	<1%
0.1N NaNO₃	<1%	<1%	≥98%	<1%
5.0N NaNO₃	<1%	<1%	<1%	≥98%

The eluted fractions were 10 ml each. For the chromatography an Altech 600mg SAX cartridge #21907 using a positive displacement manifold was employed.

If iodide is the only halide of interest, a 2-step procedure is adequate. Experiments were performed using increasing concentrations of $NaNO_3$ in urine samples. Complete elution of chloride, bromide, and fluoride with the eluted urine was observed when $NaNO_3$ was added to the urine sample at a concentration of 0.4 M (Figure 3). This 2-step procedure (Figure 4) was developed for the measurement of iodide exclusively.[27] The main application of this rapid procedure is in the measurement of percentage excretion of iodide following ingestion of the iodine/iodide load, in order to assess whole body iodine sufficiency.[10,13]

The 4-step procedure (Figure 2) with the PDM-20 or the PDM-40 and the 600-milligram SAX cartridges are used for the combined measurement of the four halides. It is best not to equilibrate the cartridges with water or buffer as recommended by Orion (Beverly, Massachusetts) prior to use. In the author's experience, their power of resolution is greater if the biological fluid is added to the dry cartridge without pre-equilibration. Keep at least one month supply of cartridges, mainly for commercial laboratories with a high volume of samples. The 4-step procedure using the PDM-40 is described below:

1) Turn the PDM-40 unit on.

2) Pull out the syringe rack to the stop position, and remove any used syringes.

3) Insert unused B-D 10cc plastic syringes (PN #BD301030) in the syringe rack. Make sure to remove the tips of the syringes. Secure syringes with aluminum plates.

4) Slide the syringe rack back into the PDM-40.

5) Remove the cartridge rack, discard used cartridges, and add new cartridges.

6) In the sample tray, add 10 ml of biological fluid (i.e., urine) in the 40 wells of the sample tray. If volume of the sample is less than 10 ml, complete volume to 10 ml with water.

7) Insert the sample tray into its position on the PDM-40 (same position as cartridge rack).

8) Set on cycle I (aspiration cycle), and push start to aspirate biological fluid in syringe.

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Figure 3

## Effect of Increasing
Concentrations of NaNO₃
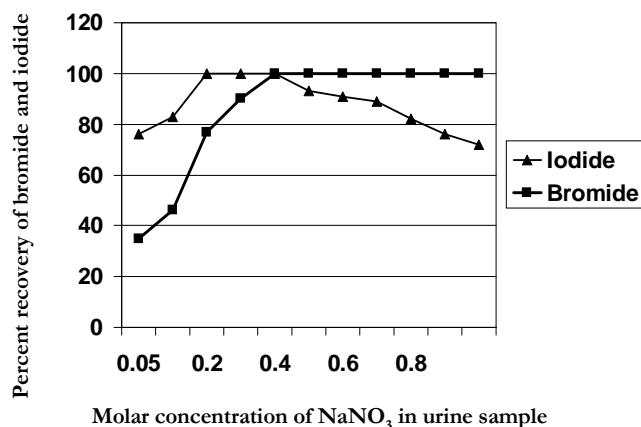
Effect of increasing concentrations of $NaNO_3$ in urine samples containing 300mM chloride, 12.5 mM bromide, 2 mM fluoride, and 0.1 mM of iodide on recovery of bromide in the eluted urine and the recovery of iodide in the second pass of 10 ml 5N NaNO3 through the Altech cartridges (#21907) using the PDM.[27]

9) The PDM-40 will stop at the end of each cycle and will disengage the syringe tip from the cartridge.

10) After the aspiration cycle is complete, remove the sample tray from the PDM-40. The sample tray, after use, should be soaked in 0.05-0.10% H_2O_2 containing the appropriate detergent in the right concentration. Rinse with water, dry and store for reuse.

11) Insert the cartridge rack into its appropriate location (same as sample tray).

12) Insert beaker tray in PDM-40 with 30-milliliter glass beakers or disposable 1-ounce plastic cups, corresponding to the locations of the new cartridges in the cartridge tray and of the used wells in the sample tray.

13) Switch to cycle II (elution cycle), and push start to reengage the syringes in the cartridges and to elute the biological fluid.

14) This fraction contains chloride with the eluted biological fluid.

15) After completion of cycle II, remove the beaker tray from the PDM-40. Add 20 ml of 2.5 M $NaNO_3$ to each beaker, and place the beaker tray in the Orion autosampler #AS 3000 for measurement of chloride using Orion electrode #9617BN. Place clean beakers in another beaker tray, and insert into the PDM-40.

Figure 4

The Simplified 2-Step Chromatographic Procedure
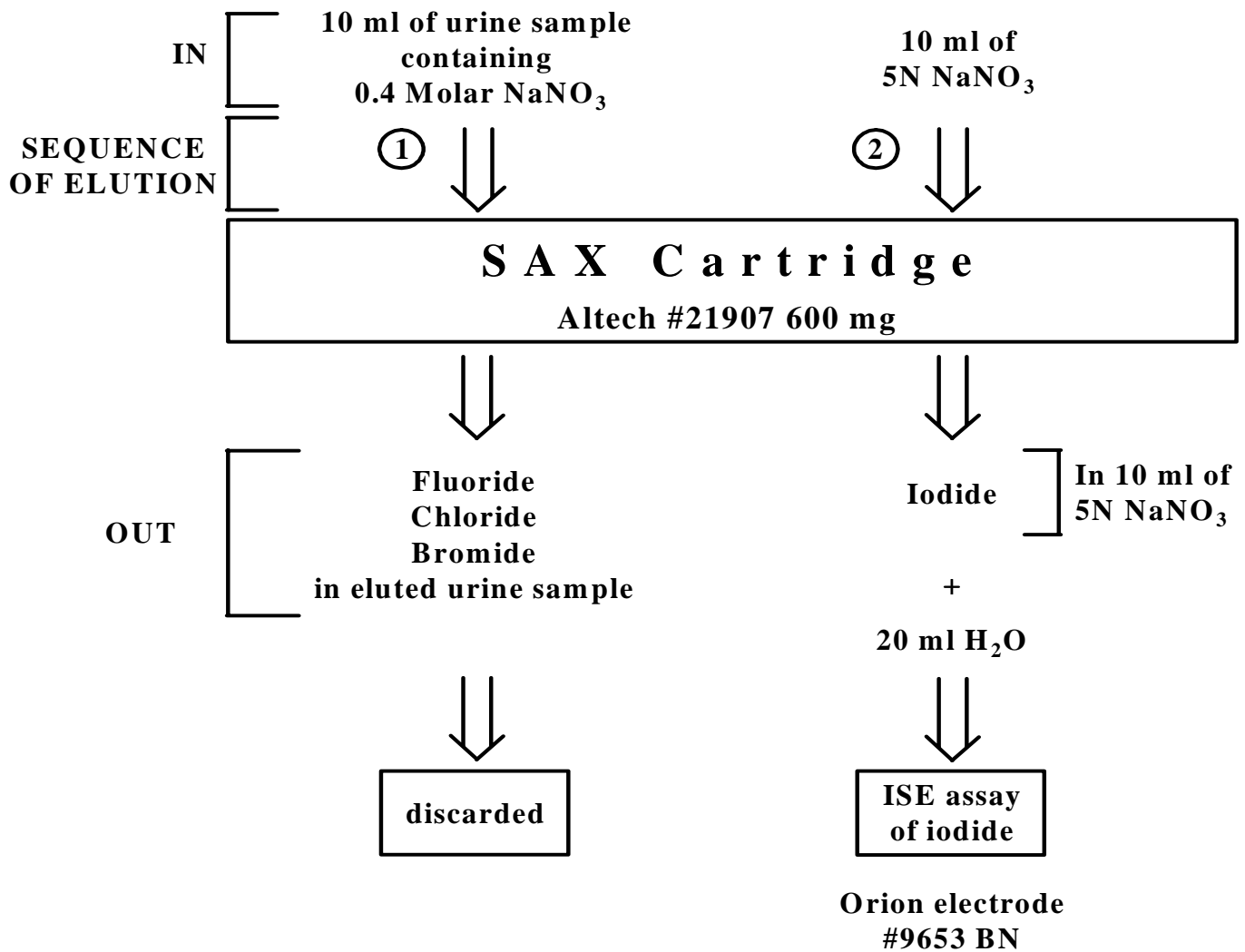
This procedure is used to separate the iodide fraction from the other halides prior to measurement by the ISE assay. $NaNO_3$ is added to urine samples to reach a concentration of 0.4 M.[27]

Table 2

Ion Selective Electrodes for Measurement of Halides:
Ion Plus Sure Flow Solid State from Thermo Orion

Halide	Orion #	Spectrum Chemicals #	Approximate Cost	Filling Solution	Phone #
Chloride	9617BN	246-25417	$630.00	B 90062* 246-24019**	1-800-772-8786
Fluoride	9609BN	246-25405	$630.00	A 90061* 246-24016**	1-800-772-8786
Bromide	9635BN	246-25429	$630.00	D 90063* 246-24022**	1-800-772-8786
Iodide	9653BN	246-25441	$630.00	D 90063* 246-24022**	1-800-772-8786

*Orion part number   **Spectrum part number

16) Add 10 ml of 0.05N $NaNO_3$ in the wells of another sample tray labeled "elution solvent tray" and insert into the PDM-40.

17) Switch to cycle I and push start to aspirate the eluate (0.05N $NaNO_3$) in the syringes.

18) Repeat steps 8-12.

19) This fraction contains fluoride. Remove the beaker tray containing the fluoride fraction. To each beaker add 20 ml of a solution containing three parts TISAB II to one part water. Place the beaker tray in the autosampler for measurement of fluoride using Orion electrode #9609BN.

20) Add 10ml 0.1N $NaNO_3$ to the wells of the elution solvent tray. It is not necessary to rinse the elution solvent tray prior to use for the 0.1N $NaNO_3$ and the 5.0N $NaNO_3$. Repeat steps 8-12.

21) This fraction contains bromide. Add 20 ml 2.5 M $NaNO_3$ to each beaker in the beaker tray, and place the beaker tray in the autosampler for assay of bromide using Orion electrode #9635BN.

22) Add 10ml of 5N $NaNO_3$ to the wells of the elution solvent tray and insert into the PDM-40.

23) Repeat steps 8-12.

24) This fraction contains iodide. Add 20 ml of water to each beaker and place the beaker tray in the autosampler for assay of iodide using Orion electrode #9653BN.

After use, the sample tray, all glassware and plasticware are soaked in warm water containing 0.05-0.1% H_2O_2 and the appropriate detergent for 30 minutes before rinsing with tap water followed by reverse osmosis (R/O) water. The beaker trays of the PDM-40 were designed to be compatible with the Orion Autosampler AS-3000. This autosampler has 45 positions. The first five positions are for washing beakers and the remaining 40 positions are for measurement of samples. The beakers in the beaker tray of the PDM-40 are coded with the same numerical sequence programmed in the 940/960 Model coupled to the Autosampler AS-3000, eliminating human error. Unfortunately, the AS-3000 is no longer available from Orion. The new version has a different configuration, and the beaker rack of the new Orion model cannot be installed in the PDM-40. So we are back to the drawing board to design a new PDM-42 that will be compatible with the new Orion autosampler.

Using the ISE Procedure to Measure Purified Halides
Although there are several suppliers of equipment and accessories for the ISE measurement of halides, the equipment and accessories discussed in this manuscript were obtained from Orion and were tested in the author's Potentiometric Laboratory. The results presented in this manuscript are from personal experience.

Potentiometric Equipment: In early 2002, this author decided to set up the ISE measurement of urine iodide *in situ* for better quality control in the performance of the iodine/iodide loading test.[13] The first instrument used

for the ISE measurement of iodide in urine samples was the Orion 720A[+] meter[6] which requires manual input for generating the standard curve and measurement of unknown samples. The procedure for generating a calibration curve and for measuring iodide in unknown samples with the 720A[+] meter was previously described.[6] This procedure was later modified and semiautomated in 2003 using the PDM40 for chromatographic separation of the halides using syringe cartridges[12,13] and the Orion 940/960 Meter coupled to the AS-3000 for measurement of the purified halides.

Electrodes: In Table 2, the ion-selective electrodes used to measure the four halides are displayed with the product number for both Thermo Orion and Spectrum Chemicals and their approximate cost. Although some manufacturers supply ISE electrodes at a lower cost, they are not as sensitive, as precise, and as simple to operate and maintain as the Orion electrodes displayed in Table 2. The filling solutions displayed in Table 2 can be drained easily for storage of the electrode. At the end of each day, it is best to drain the filling solution, rinse the inner chamber and the tip of the electrode with puri-

fied water, dry and store the electrode with the tip in its protective rubber cap. The Orion instruction booklet contains a troubleshooting checklist. Based on the experience obtained over the past five years at the Optimox Potentiometric Laboratory, the ISE electrodes for halides have a useful lifespan of 9-12 months with regular use, and the biological fluids are purified prior to their measurement with these electrodes. Some companies supplying similar electrodes at a lower cost claim a useful lifespan of six months. With new electrodes, the calibration curves performed monthly are superimposable. The slope and limit of detection are within a narrow range. Afterward 9-12 months of regular use, there is evidence of deterioration of performance as described below. First, attempt to correct the problems by following the recommendations from Orion, including polishing the tip of the electrode. If this fails, acquire a new electrode. The fluoride electrode shows decreased performance sooner than the others. It is time to replace these electrodes when:

- The slope of the calibration curve becomes less steep with decreased EMF per decade. If this deterioration occurs before the next calibration,

Figure 5

Effect of Aging of Iodide Electrode on the EMF Changes per Decade of Standard Iodide
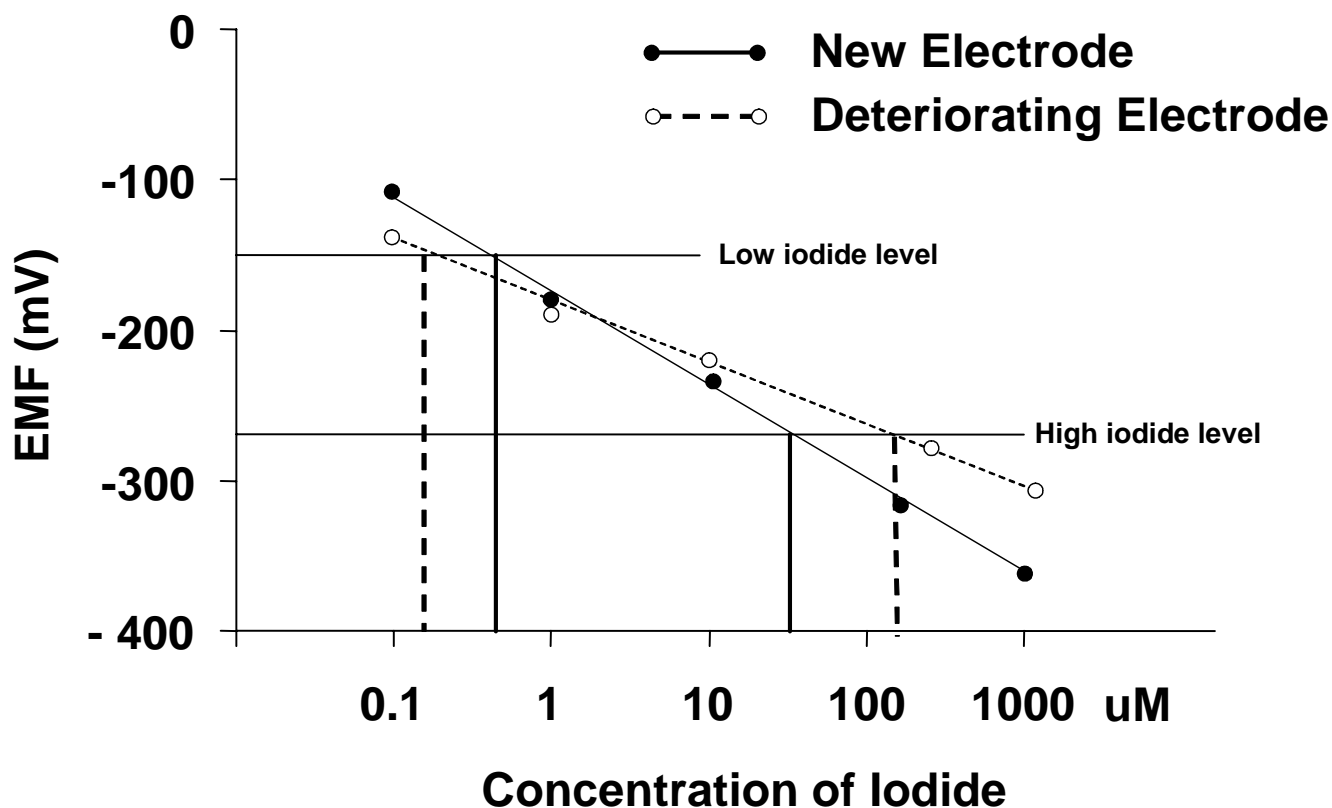
Decreased EMF changes/decade; underestimation of iodide at low levels and overestimation at high levels.

Table 3

Practical Limits of Detection of the Halide Electrodes in Descending Order of Sensitivities

Halide	Molecular Weight	Sensitivity*	
		Mg/L	µM/L
Iodide	127	0.006	0.05
Fluoride	19	0.04	2.1
Bromide	80	0.4	5.0
Chloride	36	13.0	360 (0.36 mM)

Tested in author's laboratory using 10 ml of biological fluid in a total volume of 30 ml

it causes underestimation at low halide levels and overestimation at high halide levels (Figure 5).

- The limit of detection (MUD value) has increased significantly with significant loss of sensitivity. With the Orion 940/960, this loss of sensitivity can be assessed during calibration since the MUD value will increase progressively over time.

- It requires a longer time during washing of the electrodes for a return of the EMF to baseline. Eventually, the EMF of the electrode does not return to baseline but remains above the expected value, therefore increasing the limit of detection. Using a new electrode, five washes of 10 seconds between measurements are adequate to prevent overestimation of a low halide sample when it follows a sample with concentration of halides 1000-fold higher. With deterioration of the electrode, a longer washing time becomes necessary. If this is not taken into consideration, a low halide sample following a high halide sample will be overestimated. If washing the electrodes becomes too time consuming to bring the EMF value to baseline, replace the electrode.

- Measurement of the pool samples with added standards results in underestimation of the halide levels in the pools with low concentrations of standards and overestimation of the halide levels in the pools with high concentration of halides (Figure 5). The accuracy of experiments performed in every batch is the best means of detecting a deteriorating electrode between calibration.

Any of the first three examples would justify replacing the electrode. Check every new electrode before use for the above. Compare performance with the previous electrode. Defective new electrodes can be detected early and exchanged. It may take up to two months to obtain new electrodes from Orion. For electrodes that are used frequently, mainly in commercial laboratories where turnaround time is critical, extra electrodes for halides most in demand should be kept in stock just in case.
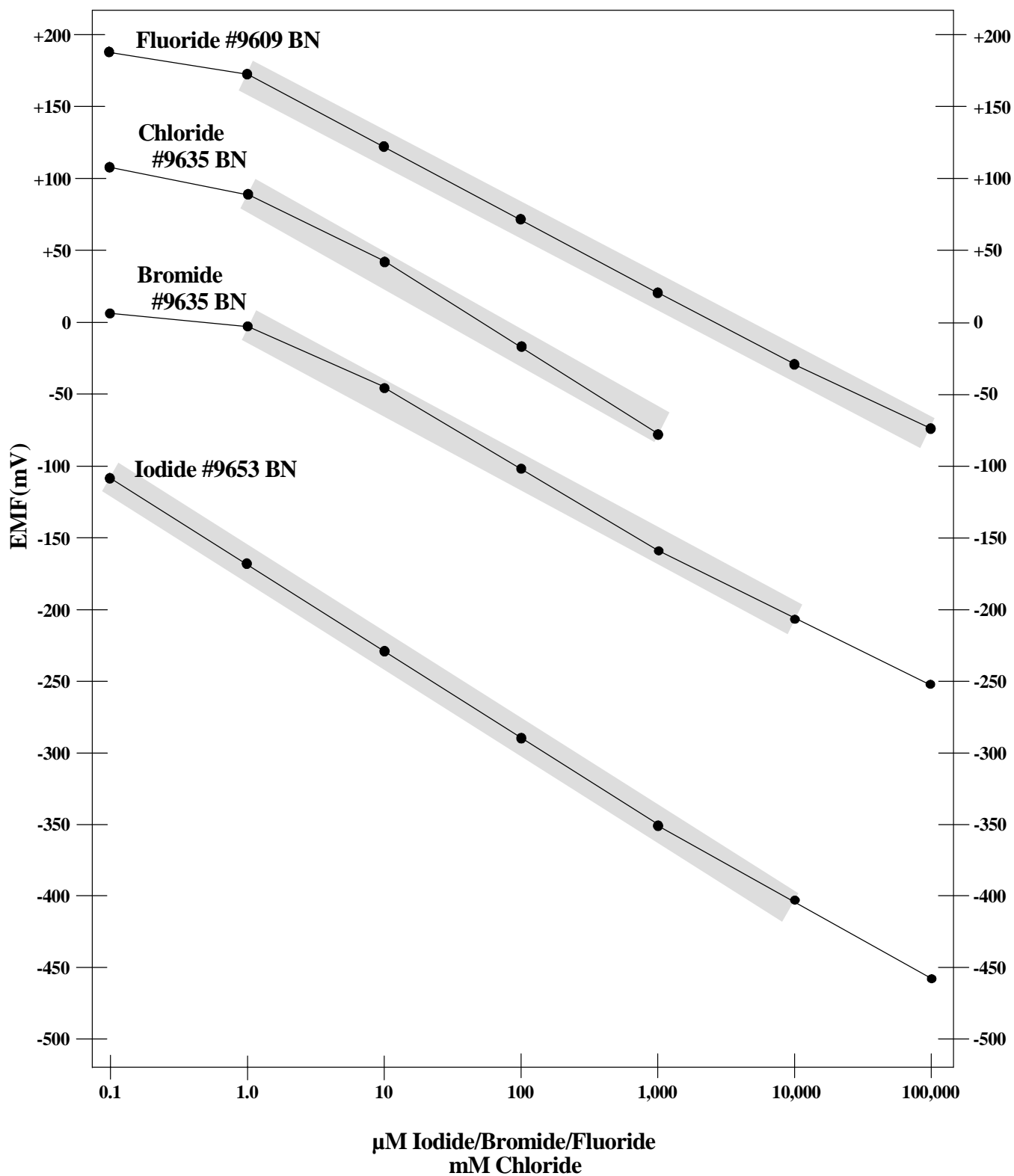
The sensitivity (limit of detection) of the standard curve obtained with these electrodes in the author's laboratory is displayed in Table 3, when 10 ml of biological fluid and 30 ml total volume are used in the assay. When a volume of less than 10 ml of biological fluid is used, and the total volume is maintained at 30 ml, the limit of detection increases proportionately. The values displayed in Table 3 are conservative and vary from batch to batch, but they represent average values. For example, the limit of detection for iodide can be as low at 0.002 mg/L with some electrodes.

Figure 6 displays the standard curves for the four halides. The linear or near linear portion of these curves are boxed in grey for easy identification. The two halides with the lowest limit of detection (iodide and fluoride) have the widest ranges of linearity. The iodide electrode is the most sensitive with limit of detection ranging from 0.002-0.006 mg/L, followed by fluoride at 0.04 mg/L then comes bromide at 0.4 mg/L, and lastly chloride at 13 mg/L. For urine samples, a volume of 10 ml is easily available. However, for serum and saliva, a volume of 3 ml diluted with 7 ml of water

Figure 6

Dose-Response Curve for the Four Halides Using Thermo Orion ISE Electrodes

The Thermo Orion part number is displayed next to the standard curve. The gray box outlines the linear or near linear portion of the standard curve.

is usually used in the assay. The limit of detection is therefore 3.3 times higher in the measurement of the halides in serum and saliva.

Standards: The halides can be purchased in the solid forms or dissolved in purified water. If an aqueous solution of the halide standards is used to generate the standard curves, make sure that these solutions are not beyond their expiration date. Some companies do not supply an expiration date, and it is best not to use standard solutions from these companies. The solid forms of the halides purchased from Spectrum Chemicals in the purest forms available are used in the author's laboratory. The Spectrum product number for the four halides are: chloride (sodium salt) PN# S1249; fluoride (sodium salt) PN# S0167; bromide (sodium salt) PN# S1195; and iodide (potassium salt) PN# P0185. Concentrated stock solutions of the halides are prepared every six months. For chloride, the stock solution contains 1 M/L. For the other halides, a concentration of 0.1 Molar is prepared. The iodide stock solution is stored in brown glass bottles and the other halides in opalescent plastic bottles. The concentration of the halide stock solution for each halide is displayed in Table 4.

For generating the calibration curve (standard curve) with the Orion 940/960, the owner's manual recommends a concentration of halide 10 times the arbitrary upper limit of detection set by the laboratory (Table 4).

For example, if one wishes to set an upper limit of detection of one mM/L (127 mg/L) for iodide, the concentration recommended in the stock solution for the Orion 940/960 is 10 mM/L. The calibration is performed every month. If the $720A^+$ meter is used, prepare standards in water at 10-fold dilutions within the linear portion of the standard curve. The 10-fold dilutions of standards to be used in the $720A^+$ model for the calibration curve can be expressed either as µM/L or as mg/L for iodide, fluoride, and bromide. For chloride, the measurement can be displayed either as mM/L or g/L. As many as five concentrations can be used for generating the standard curve with the Orion $720A^+$ meter. This meter performed a point to point calibration. The Orion 940/960 will compute the standard curve automatically by pumping increasing amounts of halides to an aqueous solution of the ionic strength adjuster appropriate for the halide measured (see Table 4).

Ionic Strength Adjuster (ISA): For best performance of the ISE electrodes, ISAs are used to bring standards and unknown samples within the same narrow range of pH and of ionic strength. For the measurement of chloride, bromide, and iodide, the ISA is an aqueous solution of sodium nitrate ($NaNO_3$) at a final concentration of 1.66 M. The ISA used in the assay of fluoride is an aqueous solution containing sodium acetate, sodium chloride, acetic acid, and citric acid. This ISA from Thermo

Table 4

Stock Solutions of Halides

Halide	Concentrations in Stock solution (Prepared fresh every six months)	Upper Limit of Detection in the 940/960 meter*	Concentration Used in the 940/960 Model for Calibration (Performed every month)	Final Concentration of ISA in the Assay Beaker
Chloride	1 Molar (36 g/L)	0.1 Molar (3.6 g/L)	1 Molar (36 g/L)	$NaNO_3$ 1.66 M
Fluoride	0.1 Molar (1.9 g/L)	0.001 Molar (19 mg/L)	0.01 Molar (190 mg/L)	TISABII 50% Aqueous Solution
Bromide	0.1 Molar (8 g/L)	0.005 Molar (400 mg/L)	0.05 Molar (4 g/L)	$NaNO_3$ 1.66 M
Iodide	0.1 Molar (12.7 g/L)	0.0005 Molar (63.5 mg/L)	0.005 Molar (635 mg/L)	$NaNO_3$ 1.66 M

*Arbitrarily set for practical reasons at the Optimox Potentiometric R&D Laboratory.

Orion is called TISAB I. Trivalent aluminum binds covalently to fluoride. Since the negative charge on the halides is required for detection by the ISE electrode, the covalent binding of aluminum would neutralize the negative charge of the halide, causing an underestimation of the levels of fluoride measured. In order to prevent interference from aluminum in the direct ISE measurement of fluoride, citric acid in the TISAB I is replaced with 1, 2-cyclohexane diaminotetraacetic acid (CDTA). This fluoride ISA from Thermo Orion is called TISAB II.

An aqueous solution of sodium nitrate 5 M concentration is available from Spectrum Chemicals (PN# 246-25150). In the author's laboratory, sodium nitrate FDC grade is obtained from Spectrum Chemicals in the solid form in bottles of 2,500 g (Spectrum PN# 501813). This quantity is dissolved in 6 L of R/O water to obtain a 5 M concentration. This preparation is then filtered through a 0.2 µm filter (Millipore PN# MPGL06 GHZ) and stored in 1-liter plastic bottles (Fisher Scientific PN# 05-712-224) for up to six months.

The Orion special ISA called TISAB II is obtained from Spectrum Chemicals already dissolved in water. The Orion product number for the TISAB II reagent is PN# 940909, and the Spectrum product number is PN# 246-25171. In the assay of fluoride, it is diluted with an equal volume of water or of the aqueous solution containing standards and purified biological fluids.

Validation of the ISE Procedure
The same procedure previously described for the validation of the radioimmunoassay of steroid hormones in biological fluids is used to validate the ISE procedure of the halides.[33,34] The criteria for acceptability includes reliability and practicability. The reliability of an assay depends on its specificity, sensitivity, accuracy, and precision. The practicability of an assay is judged by the skill required to perform it, the time involved in its performance, and the cost of the assay. The criteria for practicability of the ISE procedure are satisfied as previously discussed.[6]

Specificity: There are various ways of validating an assay in terms of its specificity, one of which is by comparison with an accepted method. Yabu, *et al*,[23] and Kono, *et al*,[24] validated the specificity of the ISE method for direct measurement of urinary iodide levels by comparison with the ceric ion-arsenious acid method.[35] The ISE procedure for urine iodide levels developed at the Optimox Potentiometric R&D Laboratory was previously validated by the high degree of correlation with value obtained using the ICP-MS technique.[6] The

specificity of the ISE procedure for the other halides is inerrant in the specificity of the ion selective electrodes themselves and in the chromatographic separation of each halide from the other interfering halides with a high degree of resolution. For example, the electrode for fluoride is so specific, that two commercial laboratories, Doctors Data and National Medical Services, measure urine fluoride directly with this electrode without prior purification.

Sensitivity: The sensitivity (limit of detection) of the assay in the measurement of halides in biological fluids is not identical with the sensitivity of the standard curve. The Orion 940/960 will compute the sensitivity of the standard curve which it identifies as "MUD." The MUD value is automatically subtracted from each sample measured. However, the limit of detection of the assay in the measurement of unknown samples must be calculated by running 4-6 replicates of the biological fluid devoid of the halide to be measured (i.e., stripped urine). A simple and practical way to obtain stripped urine for the assay of fluoride, bromide, and iodide is to elute 50 ml of urine per SAX 600-milligram cartridge. The eluted urine will be essentially devoid of halides, except chloride. The pooled urine samples should be collected from normal volunteers not on orthoiodosupplementation. A low chloride pooled urine can be obtained from these subjects if they are told to restrict salt during the period of urine collection.

Theoretically, no fluoride, bromide, or iodide should be present in the stripped urine samples. However, a value is measured which is called a blank. The mean ±SD of 4-6 blank samples are computed. The limit of detection in the measurement of unknown samples at the 95% confidence limit is equal to 2 SD of the mean blank after subtracting the mean blank value from the concentration measured in the unknown samples. At the 99% confidence limit, the sensitivity is equal to 3 SD of the mean blank value. If the blank value is below the MUD value, subtracting that blank is not required, since the Orion 940/960 automatically subtracts the MUD value. If the blank is higher than the MUD value, it is important to subtract the mean blank value, mainly when the levels of halides in the biological fluids are near the limit of detection. For example, when measuring iodide in random or morning urine samples prior to orthoiodosupplementation in the US population, some values will be near the limit of detection. It is not as critical to compute and subtract the mean blank value of urine iodide levels when the levels measured in unknown samples are 2-3 orders of magnitude above the blank, such as post- orthoiodosupplementation.

Table 5

Range of Halides Added to "Stripped" Biological Fluids* of Equivalents for the Assessment of Accuracy and Precision

Halide	Biological Fluid	Blank Fluid	Range of Added Halides
Chloride	Urine	Low chloride urine pool	0.1-10.0 g/L
Fluoride	Urine	Stripped urine pool*	0.1-10.0 mg/L
	Serum	3% BSA in NS**	0.05-2.00 mg/L
	Saliva	NS**	0.05-2.00 mg/L
Bromide	Urine	Stripped urine pool*	10-400 mg/L
	Serum	3% BSA in NS**	10-400 mg/L
	Saliva	NS**	1-40 mg/L
Iodide	Urine	Stripped urine pool*	0.05-50.00 mg/L
	Serum	3% BSA in NS**	0.05-10.00 mg/L
	Saliva	NS**	0.5-100.0 mg/L

*Urine collected following elution from the SAX cartridges. Only chlorine is eluted. The levels of fluoride, bromide, and iodide in stripped urine pool are below the limit of detection.

**BSA = Bovine serum albumin; NS = Normal saline

Accuracy: The accuracy is assessed by recovery experiments. Known amounts of the halide to be measured are added to pooled samples of the biological fluid or an equivalent aqueous solution devoid of the halides to be measured. The accuracy is expressed as percent recovery of the added halides at concentrations within the range expected in the unknown samples. An acceptable accuracy is a recovery rate of 90-110% of the added amount. The exact amount of halide added to the pooled blank samples can be estimated by transferring the same volume of halide standards in the ISA solution at the same total volume. For example, if one wishes to measure the recovery of 0.1 mM (12.7 mg iodide/L) added to the stripped biological fluid, pipette 10 μL of 0.1 M iodide/L standard solution in 30 ml of 1.66 M $NaNO_3$. In the protocol for the ISE measurement of halide, the volume of standards and purified unknown samples is 10 ml in a total volume of 30 ml ISA. Since 10 μL in 10 ml is 1,000-fold dilution of the 0.1 Molar stock solution of iodide, the amount added is 0.1 mM or 12.7 mg/L. Use the same pipette to add the same amount of iodide (10 μL of stock) to 10 ml of the stripped biological fluid prior to chromatography. The mean value of triplicate

direct measurement of iodide in the spiked ISA solution is taken as 100%.

Accuracy can also be evaluated by adding known amounts of halides to unknown samples. For example, if an unknown urine sample obtained post-iodine/iodide load is analyzed prior to, and after, adding 12.7 mg of iodide/L (100 μM), the difference between the values pre- and post-spiked samples should be 12.7 mg/L. Again, the acceptable range for spiked samples is a recovery rate of 90-110% of the added amount.

Precision: The intra-assay and inter-assay precision are evaluated by analysis of replicates of pooled samples without or with added halide in known amounts (4-6 replicates), and calculating the mean ±SD for within assay precision and duplicate measurement in several batches for between assay precision. The coefficient of variation is the standard deviation expressed as a percentage of the mean value. Taking the mean value as 100%, the acceptable limit for the coefficient of variation for a high degree of precision is 5%. That translates

Table 6

Quality Control Report

Halide Measured	Cl⁻ ☐ F⁻ ☐ Br⁻ ☐ I⁻ ☐		Pools with added standards. Date prepared		
Electrode Part#		Electrode SN#		Date first used	
Date QC test performed		Date last calibration of 940/960 Meter		MUD value _____ mg/L	

Pool I (blank) No halide added	Halide measured (mg/L)	Pool II Added standard _____ mg/L	Halide measured (mg/L)	Pool III Added standard _____ mg/L	Halide measured (mg/L)
1.		1.		1.	
2.		2.		2.	
3.		3.		3.	
4.		4.		4.	
5.		5.		5.	
X		X		X	
SD		SD		SD	
Limit of detection (2 × SD)		Accuracy (% Rec)		Accuracy (% Rec)	
		Precision (C.V.)		Precision (C.V.)	

Pool IV Added standard _____ mg/L	Halide measured (mg/L)	Pool V Added standard _____ mg/L	Halide measured (mg/L)	Pool VI Added standard _____ mg/L	Halide measured (mg/L)
1.		1.		1.	
2.		2.		2.	
3.		3.		3.	
4.		4.		4.	
5.		5.		5.	
X		X		X	
SD		SD		SD	
Accuracy (% Rec)		Accuracy (% Rec)		Accuracy (% Rec)	
Precision (C.V.)		Precision (C.V.)		Precision (C.V.)	

Q.C. Tests = Passed ☐   Failed ☐

into a 95% confidence limit from 90-110%. In general, the intra-assay precision is better than inter-assay precision; and the inter-assay precision in the same laboratory is better than inter-laboratory precision.

For a newly developed procedure, the above validation should be performed prior to analysis of unknown samples and every time the procedure is modified. For routine analysis, the accuracy experiments using single measurement of the pooled samples with added standard are adequate. It is a good practice to spread the pooled spiked samples evenly throughout the batch of unknown samples. When measurements are made near the detection limit, blank samples in 4-6 replicates are needed to assess the sensitivity of the assay. At the 95% confidence limit, the sensitivity would be equal to 2 SD of the blank; and at 99% confidence limit to 3 SD of the blank. Measuring blank samples is not necessary if the levels measured in unknown samples are 2-3 orders of magnitude greater than the blank value.

Acceptable limits of precision and accuracy should be based on the research and clinical applications of these tests. The range of halide concentrations used for the accuracy and precision evaluation should be within the range expected in biological fluids under normal and pathological conditions. For precision and accuracy experiments using serum and saliva, a 3% solution of Bovine Serum Albumin (BSA) in normal saline (NS) is used instead of stripped serum and normal saline is used instead of stripped saliva (Table 5).

A proposed protocol for the reliability testing of a new ISE procedure or a modification of an existing procedure, prior to measurement of halides in unknown samples, is outlined in Table 6.

Clinical Applications

During the 1930s, attempts were made to differentiate between euthyroidism, hyperthyroidism, and hypothyroidism by the effect of iodine supplementation on the basal metabolic rate and the profile of serial serum iodide levels.[36-38] At the Lahey Clinic,[38] an iodine/iodide "tolerance test" was used to assess thyroid functions. To quote Perkin, *et al*:[38]

> "The technique of the test may be reviewed briefly as follows: 10 cc of blood is withdrawn from the patient, following which a known amount of iodine as Lugol's solution (37.5 mg iodine used at present) in milk is given orally). At one-half hour, one hour, one and one-half hours and at two and one-half hour periods, two cc blood samples are taken ... From the above chart it may be seen that the blood iodine curve does not rise to such a high level in the hyper-

thyroid individual as in the normal or nontoxic goitrous patient."

The last paragraph of this manuscript reads: "An iodine tolerance test is described which may prove to be a valuable asset in establishing a diagnosis of hyperthyroidism."

Obviously, this prediction did not materialize. To this author's knowledge, the first published statement concerning whole body sufficiency for iodine was made by Thompson, *et al*, in 1930:[39] "The normal daily requirement of the body for iodine has never been determined."

This statement is still true today, more than 70 years later. We still don't know the iodine/iodide requirements for whole body sufficiency. The Iodine Project initiated by this author seven years ago[13] was an attempt to answer the question: What is the optimal intake and what is the optimal form of the essential element iodine for whole body sufficiency and for optimal mental and physical performances? Results obtained with the iodine/iodide loading test pre- and post-orthoiodosupplementation suggest that 100-400 times the RDA would be required for whole body iodine sufficiency.[10] At sufficiency, the human body retains approximately 1.5 g of iodine,[11] 50 times the medical textbooks' figure of 30 mg.

Background on the Metabolism of Halides: The stable halides are quantitatively absorbed by the gastrointestinal tract and renal clearance is the principal mode of excretion.[11,40-43] Chloride and bromide compete with each other for the extracellular fluid compartments and for renal tubular reabsorption. Chloride and bromide are mainly extracellular halides and, as such, are not stored. However, bromide can be oxidized to bromine in fat tissues and stored as organic bromine. Bromide has a high affinity for carbon-carbon double and triple bonds (unsaturated fatty acids). In the central nervous system, bromide is oxidized and organified to form a narcoleptic organobromide.[10] Fluoride is stored in bones and teeth, mainly in bones which contains 97% of the total body pool of fluoride.[43] Iodide is stored in target cells which contain the mechanism to transport peripheral iodide inside the cells.[10,16] The oxidized form of iodide, that is, iodine is mainly stored in fat tissues and muscles.[12] Next to fat tissue, muscle contains the largest amount of iodine. Fat and muscle combined contribute 70% of total body iodine in individuals who have achieved iodine sufficiency and, in such cases, the total iodine content in the thyroid gland represents only 3% of body iodine.[11,12] The organic forms of bromine, when stored in fat tissue,

Table 7

Some Clinical Applications of the ISE Procedure of Halides in Biological Fluids

| Halide	Biological Fluid	Applications
Chloride	Urine	• Assessment of salt intake
	Serum	• Serum chloride by ISE procedure pre- and post-chromatography may be useful in cases of suspected bromism
Fluoride	Urine	• Assessment of fluoride intake • Increases following orthoiodosupplementation
	Serum	• Below detection limit in 90% of the samples measured • Increases following the loading test • Decreases following orthoiodosupplementation
	Saliva	• Below detection limit in all samples measured so far • Sal/Ser ratio below 0.4
Bromide	Urine	• Depends on chloride intake • Increases following salt intake • Increases following orthoiodosupplementation
	Serum	• The best index of bromide excess • Increases following salt restriction • Decreases following orthoiodosupplementation and chloride load
	Saliva	• Lower concentration than serum • Sal/Ser ratios ranged is 0.1-0.8
Iodide	Urine	• Spot test is a rough estimate of iodine balance between intake and retention during the previous 24 hours. A significant difference was observed between female smokers and non-smokers. • The iodide levels in 24-hour urine collection post-loading test is a reliable assessment of whole bode iodine sufficiency.
	Serum	• Profile of serial serum levels following iodine/iodide load gives an indication of absorption of iodine/iodide and cellular uptake and utilization of iodine. • The 24-hour post-load serum level gives an estimate of whole body iodine sufficiency.
	Saliva	• Normal range of sal/ser ratio is 28-74 • Symporter inefficiency or inhibition associated with low sal/ser ration <28 • TPO inefficiency or inhibition associated with elevated sal/ser ratio >74

have half-lives of several years.[44,45] Organic bromine in fat tissue is slowly reduced to bromide which contributes to the bromide pool. Bromide increases the urinary excretion of iodide[46] and competes with iodide for uptake and utilization by the thyroid gland,[47-50] causing a relative iodine deficiency. Iodide increases mobilization and urinary excretion of bromide.[13] Based on this background information, some clinical applications of the ISE measurement of the stable halides in urine, serum, and saliva are discussed below. These data are summarized in Table 7.

Fluoride: Urine fluoride levels are a good index of fluoride intake. Urinary fluoride increases following orthoiodosupplementation.[9,13] Prior to orthoiodosupplementation, urine fluoride levels measured following chromatography in 24 normal subjects had a mean ±SD of 0.95±0.072 mg/24 hours and a post-loading test mean ±SD of 1.8±0.13/24 hours. The levels of urine fluoride measured in these subjects following chromatographic purification were not significantly different from levels obtained with the direct measurement of fluoride in the urine samples.[13]

In 250 normal subjects not exposed to fluoride, a mean serum fluoride of 0.018 mg/L with a range of 0.001-0.047 mg/L were reported by Torra, *et al*, in 1998.[51] Serum fluoride above 0.05 mg/L would therefore be above the normal range. Based on experience at the Optimox Potentiometric R&D Laboratory, serum fluoride concentrations measured in 10 ml of serum were below the limit of detection (<0.04 mg/L) in over 90% of the samples measured. Following ingestion of 50 mg of iodine in the form of a Lugol tablet by six normal women, serum fluoride increased slightly to reach mean levels ranging from 0.06-0.09 mg/L between 30 minutes and eight hours post-load.[27] By 24 hours post-load, the fluoride concentrations were below <0.04 mg/L (Figure 7).

The highest level of post-load serum fluoride measured so far was observed in a female patient with multiple allergies who consumed well water with elevated fluoride levels. Her serum fluoride was 0.32 mg/L and 24-hour urine fluoride was 6.4 mg/24 hours, giving a renal clearance rate of 6.4 mg/24 hr ÷ 0.32 mg/L = 20 L/day. Following orthoiodosupplementation at 50 mg of iodine/day and 3 g vitamin C/day, her serum fluoride decreased to 0.13 mg/L after six weeks and was below 0.04 mg/L by three months.[16] Her allergies improved markedly.

Saliva fluoride levels have consistently been below <0.04 mg/L in all samples measured so far, even post-

Figure 7

Serum Profile of Fluoride Levels post Iodoral®

Mean value of six normal female subjects[27]
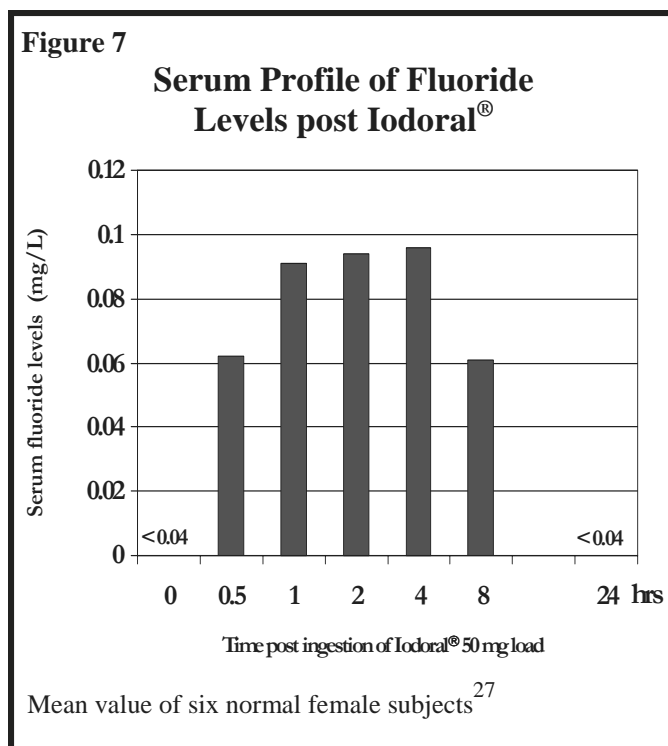
iodine/iodide loading, with serum samples increasing up to 0.1 mg/L. Saliva/serum ratio of fluoride is therefore below 0.4.

Chloride: The amount of chloride excreted in the 24-hour urine collection gives a good estimate of salt intake during the previous 24 hours. Since the atomic weight of chloride is 36 and the molecular weight of sodium chloride is 58, salt intake can be estimated by multiplying the amount of chloride excreted in 24 hours by 58/36, that is, 1.6.

In clinical chemistry, chloride is measured directly in serum by the ISE procedure. In cases of bromism, serum chloride is usually overestimated by the direct method due to interference from bromide.[52,53] Spurious elevation of serum chloride from bromide interference in a case of severe bromism was reported by Horowitz in 1997.[53]

Severe bromism should be suspected if the direct method of measuring serum chloride yields values significantly above the normal range. In these cases, the values obtained for serum chloride post-chromatography will be in the normal range. The chromatographic procedure described in this manuscript would assist physicians performing this procedure *in situ* and also commercial and hospital laboratories in the diagnosis of bromism.

Bromide: Both chloride and bromide are passively and

(Continued on next page)

quantitatively absorbed by the intestinal tract with an absorption rate faster for bromide than for chloride.[54,55] Chloride competes with bromide for tubular reabsorption, and the biological half-life of bromide can be decreased markedly by administration of chloride.[41,42] In studies performed on rats, the half-life of bromide was 25 days on a salt restricted diet and less than a day with chloride loading.

Compared to fluoride, chloride, and iodide with serum half-life of a few hours and a renal clearance rate of 20-50 L/day, the serum half-life of bromide averages 12 days (i.e., 288 hours), and the renal clearance of bromide is only 1 L/day. With salt restriction, the renal clearance rate of bromide can be as low as 0.2 L/day. Under chronic exposure to bromide, 40-50 days (4-5 half-lives) must elapse before a steady state serum concentration is achieved, assuming a narrow range of intake of bromide and chloride during that time period. In cases of low chloride intake (salt restricted diet), the time required for steady state is even longer. Because of the above, urinary bromide levels are not a reliable index of intake or of serum bromide levels, when there is recent exposure to bromide before steady state is achieved. Serum bromide levels are the best index of bromide toxicity.

Several authors have attempted to establish a normal range of serum bromide in otherwise normal subjects. Baselt[54] reported mean serum bromide levels of 3-5 mg/L in three groups of normal individuals. Sticht and Käferstein[55] reported a normal range for serum bromide of 1.0-7.5 mg/L. Jong and Burgrel[56] found a mean serum bromide level in 19 male and 19 female subjects of 5.4 mg/L. Miller and Capon[57] reported a range of 3.5-10.0 mg/L in eight normal adult subjects. Olszowy, *et al*,[58] in a manuscript published in 1998, did an extensive review of published data on bromide levels in biological fluids. To quote Olszowy:

"Much work has been carried out to establish a reliable reference concentration of bromide in the biological fluids of healthy humans, and some data are summarized in Table I … With the exception of the high cord serum result shown in Table I, bromide concentrations in all examined body fluids (whole blood, serum, and urine) are similar with a range of 3-12 mg/L and mean value around 5 mg/L, which is consistent with past routine clinical results of this laboratory."

In 183 random blood samples collected in healthy Australians, Olszowy, *et al*, measured a mean of 5.3±1.4 mg/L with a range of 2.5-11.7 mg/L. Based on the above data, the upper limit of the normal range for serum bromide would be 12 mg/L. By toxicological standards,

serum levels of bromide below 500 mg/L are considered non-toxic; 500-1,000 mg/L, possibly toxic; 1,000-2,000 mg/L, serious toxicity; 2,000-3,000 mg/L, coma; greater than 3,000 mg/L, possibly fatal.[53] There is a gray zone between the upper limit of normal serum bromide, that is 12 mg/L, and the lower limit of serum bromide considered toxic, that is 500 mg/L. This gray zone needs further investigation.

In Horowitz's patient previously mentioned, serum bromide was 3,180 mg/L, considered possibly fatal. Horowitz's patient experienced severe bromism following daily ingestion of a soft drink containing organic bromine.[53] Brominated oils are used frequently in soft drinks, and the label does not clearly indicate the amount of organic bromine present. As previously stated, organic bromine is stored in fat tissue and released slowly as bromide.

Horowitz stated that he was unable to measure the amount of bromine in the suspected drink because all the bottles were removed by the owner of the store who refused to have the product analyzed. The patient, a computer executive, experienced confusion, ataxia, headache, fatigue, and loss of concentration. The diagnosis of severe bromism was not made until the third visit with the same complaints. Hemodyalysis was required to improve the patient's clinical condition. All these problems originated from consumption of a cola drink. In several reported cases of chronic intoxication from ingesting an organic bromine-containing drug, serum bromide levels remained elevated even after serum levels of the drug were undetectable.[52] Chronic bromide intoxication from this drug is probably due to inorganic bromide released from the drug.

Physicians are encouraged to learn more about the symptoms of bromism and to request serum bromide levels in suspected cases. Chronic bromism causes symptoms that cover many medical specialties: psychiatry, neurology, gastroenterology, and dermatology. If the physician is not familiar with bromine toxicity, these symptoms will be misdiagnosed and mistreated. Ewing and Grant,[59] emphasizing the bromide hazard commented: "Physicians must participate in educating the public to the bromide hazard."

The lowest serum bromide levels that will result in significant mental and physical symptoms is at the present unknown. Fuortes[60] proposed 30 mg/L as the highest non-toxic level of serum bromide. In 1938, Clark[61] reported that small doses of bromide resulted in dullness, apathy, and inability to concentrate. Sangster, *et al*,[62] evaluated the effect of daily oral ingestion of bromide

for three months at 4 mg/kg BW and 9 mg/kg BW in seven male and seven female volunteers. At the 4 mg/kg BW level, five of the seven males complained of increased sleepiness and decreased ability to concentrate. Decreased amplitude of brain waves in the temporal and central areas were observed in these subjects. Mean serum bromide levels pre-intervention were 5.6 mg/L for the males and 4.8 mg/L for the female subjects. Under steady state conditions at 12 weeks post-intervention, the mean serum bromide levels were 160 mg/L for the seven male subjects and 240 mg/L for the seven female subjects. These levels of serum bromide associated with the above symptoms were much lower than the arbitrarily set limit of 500 mg/L for bromide toxicity.

How many patients with misdiagnosed bromism are currently treated with psychiatric drugs? Levin[63] in 1948, described four varieties of bromide psychosis: chronic brain syndrome from chronic bromism, delirium, schizophrenia, and hallucinosis. Decreased cerebral blood flow was reported in a case of bromide psychosis, being one-third of values observed in normal subjects.[64] Undiagnosed bromism is common in psychiatric patients.[59,65]

Bromide has been implicated in thyroid cancer.[9,10] Malenchenko, *et al*,[66] measured the content and distribution of iodine, chlorine, and bromine in normal and pathological thyroid tissue. No significant trend was observed for chlorine. Iodine levels were significantly lower in thyroid cancer, Hashimoto's thyroiditis, and goiter than normal tissue. Bromine concentrations were elevated in all thyroid pathologies. The highest bromine levels were found in thyroid cancer, being 50 times higher than normal thyroid tissue.

Rats fed brominated oils displayed thyroid hyperplasia, myocarditis, fatty liver, and testicular atrophy.[67-70] Decreased activity of glucose-6-phosphate dehydrogenase and glucose-6-phosphatase were observed before hepatic pathology was demonstrated histologically. Since the above enzymes are involved in the production of hydrogen peroxide by the thyroid gland, and hydrogen peroxide is the oxidant used by TPO to oxidize symported iodide,[10] mild bromism may be a factor in some forms of hypothyroidism caused by decreased oxidation and organification of intracellular iodide. In such cases, the exchangeable iodide pool of the thyroid and other target organs would be higher than normal.

Serum bromide levels measured at the Optimox Potentiometric R&D Laboratory prior to orthoiodosupplementation are rarely below 12 mg/L in "normal subjects." Values are usually 30-120 mg/L. These levels decreased markedly following orthoiodosupplementation in these

Figure 8

Serum Profile of Bromide Levels post Iodoral®

Mean value of six normal female subjects[27]
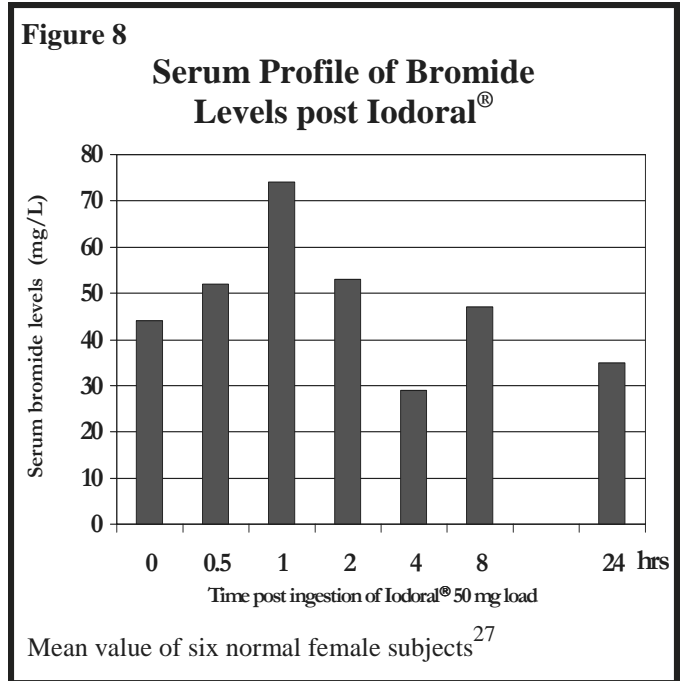
normal subjects who reported lifting of brain fog, less fatigue, and performing more in less time, that is, they became more efficient. In six normal female subjects evaluated following ingestion of 50 mg of iodine in the form of Lugol tablets, the mean serum level was 43 mg/L pre-load and increased significantly post-load with biphasic profile (Figure 8). This pattern is suggestive of an enterohepatic circulation of bromide like iodine.[12] The mean serum bromide levels in these six subjects prior to the iodine/iodide load were eight times higher than reported in the literature previously reviewed. This may be due to increased exposure to bromide and/or decreased salt intake. Based on the author's experience over the past 30 years with normal subjects participating in a wide range of clinical studies, normal subjects today are not what they used to be. "Normal" individuals often complain of brain fog, fatigue, and lack of motivation. Bromide is used extensively in our food and water supplies[15] and could play a role in these symptoms. Iodine and chloride increase the renal clearance of bromide and the above complaints are improved following orthoiodosupplementation[10] and salt loading.[14]

Iodide: In populations consuming iodine at RDA levels, random sampling of urine for ISE measurement of iodide gives a rough estimate of intake. A significant difference ($p<0.05$) was observed between spot urine iodide levels measured in female patients attending a breast clinic when female smokers were compared with non-smokers, and non-significant trend between patients with breast cancer and patients without breast cancer.[20] Spot urine sampling for iodide measurement may be of value

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in epidemiological studies, but it is not a reliable index of whole body sufficiency. The most useful application of the measurement of urinary iodide is in the 24-hour urine collection post-iodine/iodide load pre- and post-orthoiodosupplementation, using arbitrarily 90% of the load excreted as an index of whole body iodine sufficiency.[10] At 50 mg iodine/day, sufficiency is usually achieved within three months in normal female subjects but may take several months in patients with elevated bromide levels. In six normal subjects ingesting 100 mg iodine/day in the form of a Lugol tablet, body sufficiency was assessed by the loading test every two weeks for six weeks. All six subjects achieved sufficiency at six weeks.
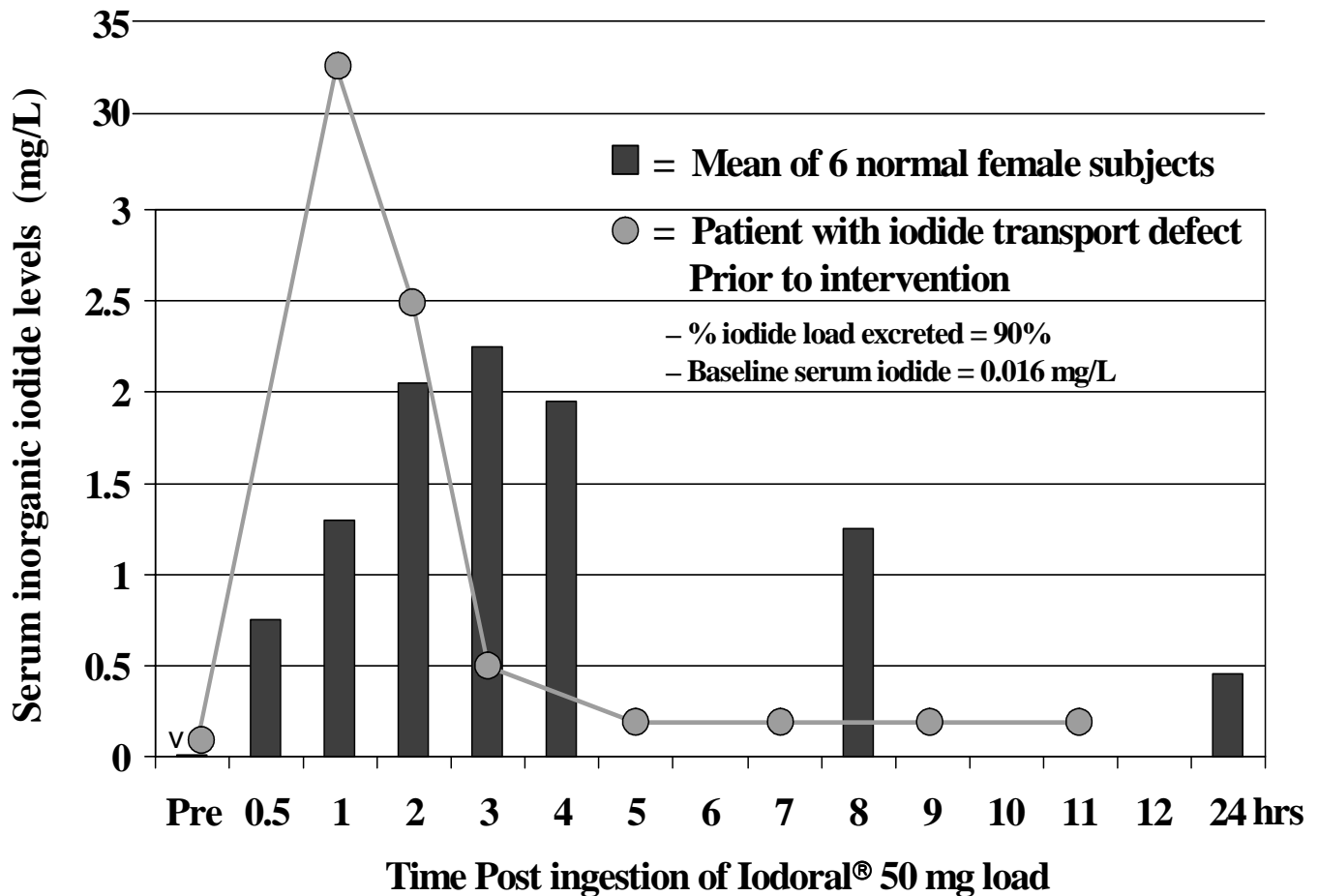
In certain circumstances, the iodine/iodide loading test may result in a high percentage of the iodine load recov-

ered in 24-hour urine collection from an iodine deficient patient, giving the false impression of whole body iodine sufficiency.[14,16] This can occur when the gastrointestinal absorption of iodine is very efficient, but peripheral iodide is not efficiently transferred inside the target cells or the symported iodide is not oxidized and organified.[14] Peripheral iodide is cleared very rapidly by the kidneys with a clearance rate of 43.5 L/day,[11] resulting in a high percentage of the iodine/iodide load excreted.

The saliva/serum iodide ratio 24 hours post-iodine/iodide load gives a reliable index of the efficiency of the iodide transport mechanism. We reported a normal range of 28-74 with a mean $\pm$SD of 44.2$\pm$13.7.[16] The 24-hour post-load mean serum iodide levels in these subjects were 0.76$\pm$0.17 mg/L. In a female patient with breast cancer and elevated serum bromide levels, or-
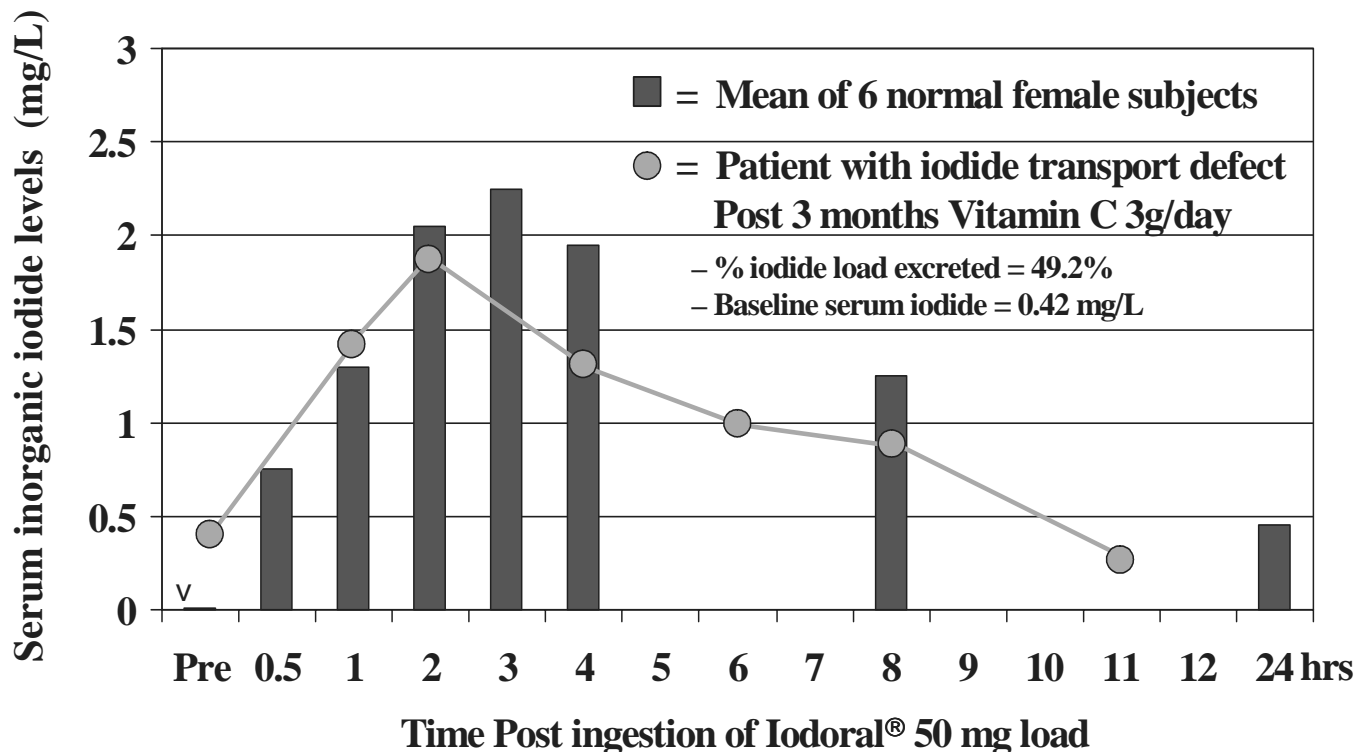
Figure 9

Serum Profile of Inorganic Iodide Levels Following the Iodine/Iodide Load

■ = **Mean of 6 normal female subjects**

● = **Patient with iodide transport defect Prior to intervention**

– **% iodide load excreted = 90%**
– **Baseline serum iodide = 0.016 mg/L**

X-axis: Time Post ingestion of Iodoral® 50 mg load (Pre, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24 hrs)
Y-axis: Serum inorganic iodide levels (mg/L)

50 mg in six normal female subjects and in a patient with iodide transport defect. Patient excreted 90% of the iodine load, but her basal serum inorganic iodide level was very low (0.016 mg/L). This pattern suggests a defect in the iodine retention mechanism.[14]

Figure 10

Serum Profile of Inorganic Iodine Levels Following Iodine/Iodide Load

= Mean of 6 normal female subjects

= Patient with iodide transport defect Post 3 months Vitamin C 3g/day
– % iodide load excreted = 49.2%
– Baseline serum iodide = 0.42 mg/L

(Y-axis: Serum inorganic iodide levels (mg/L))
(X-axis: Time Post ingestion of Iodoral® 50 mg load; Pre 0.5 1 2 3 4 5 6 7 8 9 10 11 12 24 hrs)

Iodine/iodide load was 50 mg for six normal female subjects, and for a patient with iodide transport defect following three months ov intervention with sustained-release vitamin C at 3 g/day. She excreted 49.2% of the iodine load and the baseline serum level ws 0.42 mg/L, evidence of improved function of the iodine cellular transport mechanism.[14]

thoiodosupplementation at 100 mg/day for six weeks resulted in a 10-fold drop in serum bromide and a 3-fold increase in the saliva/serum iodide ratio. At the beginning of the study, serum bromide level was 121 mg/L (normal range 3-12 mg/L), and the saliva/serum iodide ratio was 22 (normal range is 28-74). Following six weeks at 100 mg iodine/day, serum bromide decreased markedly to 12.7 mg/L, and the saliva/serum ratio increased to 61. These results were obtained without chloride loading.

In a female patient who presented with hyperthyroidism followed by hypothyroidism and elevated serum bromide, serial serum iodide measurement for 11 hours post-iodine/iodide load displayed a markedly elevated serum iodide level of 32 mg/L at one hour post-load followed by a marked drop to 2.5 mg/L at two hours and below 0.5 mg/L thereafter.[14] She could tolerate only 6.25 mg of the iodine supplement before because of side effects probably due to detoxification from bromism. Following 3 g of sustained release vitamin C/day, her serum profile normalized. She excreted

90% of the load prior to intervention and 49.2% three months post-intervention (Figures 9 and 10).

As previously mentioned, elevated bromide levels block TPO activity and could result in hypothyroidism. Besides bromide toxicity, other TPO inhibitors could produce the same end result. Since the salivary glands concentrate and organify peripheral iodide, blockage of oxidation of iodide would result in an elevated saliva/serum iodide ratio above 74. Elevated saliva/serum iodide ratio was observed in some patients with hypothyroidism and autoimmune thyroiditis. This interesting observation needs follow-up. This elevated ratio is suggestive of decreased oxidation and organification of iodide. Hydrogen peroxide (H_2O_2) administration or enhancing the H_2O_2 producing NADPH-oxidase system with vitamin B_3 would be expected to result in a beneficial effect. The author is currently assessing the organification of iodide by measuring the ratio of organic iodine over total iodide in saliva. By measuring saliva iodide before and after reduction with sodium metabisulfite and sodium borohydride, organic

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iodine can be estimated by the difference between the two values obtained.

The study of the metabolism of iodide and the other halides can be assessed using commercial laboratories, but physicians have not utilized these services because of the expense involved and also because physicians are not aware of the importance of these halides in medical practice. With the ISE procedure described in this manuscript, these studies can be performed in the physician's office. Consequently, the clinical applications of these procedures will become more widespread among the medical profession. Clinical research using nutrients instead of prescription drugs gives the physician the satisfaction of contributing to new knowledge in alternative medicine while at the same time improving patient care using nutrients instead of drugs.

About the Author

Guy E. Abraham, MD, is a former Professor of Obstetrics, Gynecology, and Endocrinology at the UCLA School of Medicine. Some 35 years ago, he pioneered the development of assays to measure minute quantities of steroid hormones in biological fluids. He has been honored as follows: General Diagnostic Award from the Canadian Association of Clinical Chemists, 1974; the Medaille d'Honneur from the University of Liege, Belgium, 1976; the Senior Investigator Award of Pharmacia, Sweden, 1980. The applications of Dr. Abraham's techniques to a variety of female disorders have brought a notable improvement to the understanding and management of these disorders.

Twenty-five years ago, Dr. Abraham developed nutritional programs for women with premenstrual tension syndrome and post-menopausal osteoporosis. They are now the most commonly used dietary programs by American obstetricians and gynecologists. Dr. Abraham's current research interests include the development of assays for the measurement of iodide and the other halides in biological fluids and their applications to the implementation of orthoiodosupplementation in medical practice.

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