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# *A Simple Procedure Combining the Evaluation of Whole Body Sufficiency for Iodine With the Efficiency of the Body to Utilize Peripheral Iodide: The Triple Test*

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## **Introduction**

A re-evaluation of the role of the essential element iodine in medicine, called The Iodine Project, was initiated seven years ago.<sup>1</sup> One of the goals of The Iodine Project was to assess the optimal daily requirement of iodine for whole body sufficiency and optimal physical and mental well-being.<sup>2,3</sup> In the process of testing the bioavailability of a tablet form of Lugol containing 12.5 mg elemental iodine, five normal subjects ingested 12.5 mg of the preparation, and iodide was measured in the 24-hour urine collection following ingestion of the tablet. The subjects excreted a mean of 20% of the ingested amount.<sup>4</sup> This low recovery of iodide in the urine samples could be due to either low bioavailability of the product tested or high retention in the body.

According to medical textbooks, urinary iodide excretion is the best index of iodine intake. Therefore, according to medical textbooks, this low recovery of iodide was due to low bioavailability of the product tested. In order to elucidate the cause of this low iodide excretion, we continued the administration of the supplement in those subjects for one month. Then, we repeated the 24-hour urine collection and iodide was measured again in the 24-hour urine samples. The percentage of the oral dose excreted in the 24-hour urine sample increased significantly, with a mean group value of 50%.<sup>4</sup> Contrary to medical textbooks, 80% of the iodine/iodide ingested was retained. After one month of supplementation, the body still retained 50% of the ingested amount. The iodine/iodide loading test evolved from these observations. However, instead of a one-month loading test, further studies were performed to shorten this test to a single ingestion of the preparation.<sup>3</sup>

For the loading test, the subjects ingest 50 mg of iodine/iodide and the percentage of the load excreted is evaluated by measuring the amount of iodide excreted in the 24-hour urine collection. Following orthoiodosupplementation at 12.5-50 mg/day, the

percentage of the load excreted progressively increased over several months to reach levels above 90% of the amount ingested. Because of the improved overall well-being reported by the subjects who achieved 90% or more iodide excretion, sufficiency was arbitrarily defined as 90%.<sup>3</sup> Implementation of orthoiodosupplementation based on the loading test revealed that sufficiency was not achieved in some subjects even after two years of iodine supplementation at 1-2 tablets/day (12.5-25 mg iodine/day). Following a daily ingestion of 50 mg Lugol in a tablet form, most normal subjects achieved sufficiency by three months, retaining 1.5 g of iodide at sufficiency.<sup>5</sup>

In some patients, the pre-supplementation loading test suggested whole body iodine sufficiency because the percentage of the load excreted was 90 or greater, but these patients did not display the beneficial effects expected from iodine sufficiency. That was unexpected. These patients reported significant improvement in cognition, energy level, breast pain, and bowel movement following orthoiodosupplementation at 50 mg/day. But the repeat loading test 1-3 months post-supplementation showed a marked drop in the percentage of the load excreted. The clinical improvement did not follow the usual expected increase in the percentage of the load excreted. That was also unexpected. Follow-up with loading tests revealed increased excretion of the load in these patients to eventually reach sufficiency 6-9 months post-supplementation.

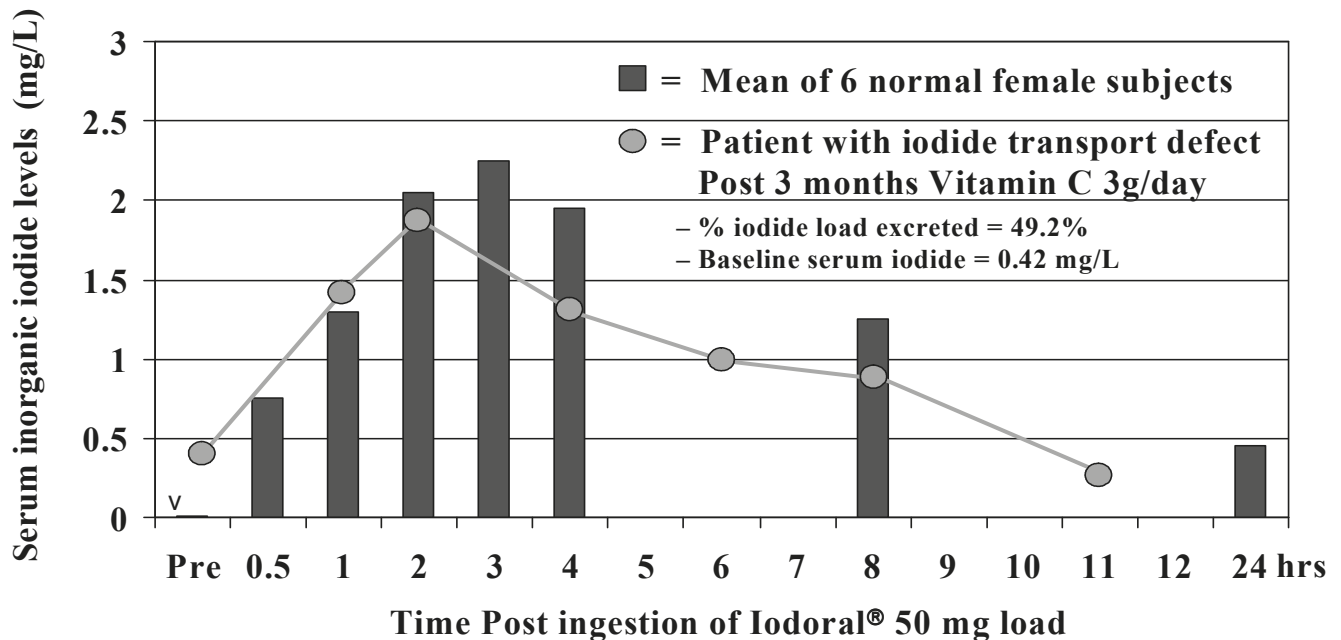
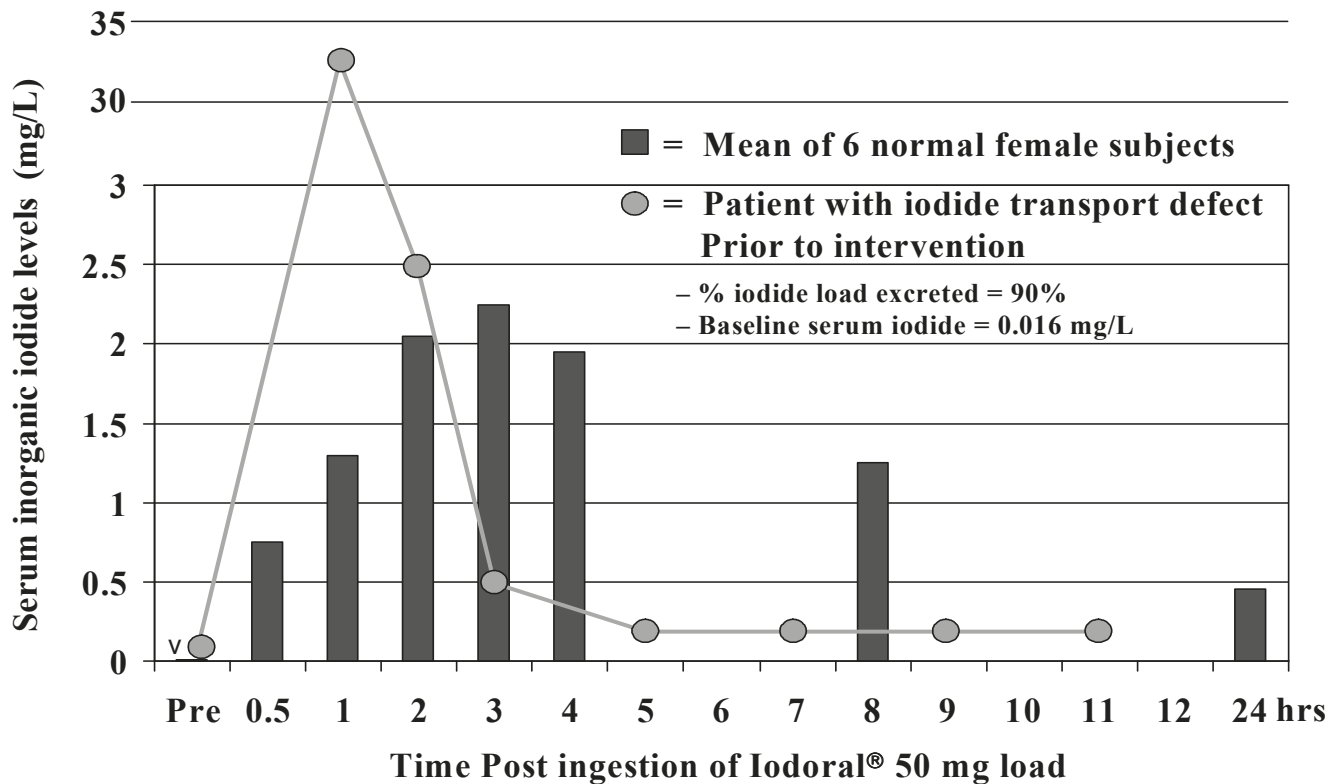
We evaluated one patient with high urinary excretion of the iodine load by collecting serial blood samples for 11 hours following the loading test.<sup>6</sup> The patient, a 52-year-old woman (height 64 inches; weight 140 lbs.), had a past history of hyperthyroidism followed by hypothyroidism and had taken Synthroid 50 µg/day for five years. She developed side effects to orthoiodosupplementation and could tolerate only half a Lugol tablet/day (6.25 mg iodine/day) due to detoxification from elevated bromide levels. She was evaluated with serial serum samples before and after three months on a sustained released form of vitamin C at 3 g/day.

Pre-vitamin C loading test showed 90% of the load excreted in the urine, but her baseline serum iodide level was only 0.016 mg/L, compared to the expected levels of 0.85-1.34 mg/L in normal subjects who achieved whole body iodine sufficiency.<sup>5</sup> The pattern observed in serum iodide levels pre- and post-vitamin C are displayed in Figure 1, superimposed on the mean value

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Figure 1

**Serum Profile of Inorganic Iodide Levels following the Iodine/Iodide Load in Six Normal Female Subjects and a Patient with Iodide Transport Defect**



The patient with the iodide transport defect 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. Following three months of intervention with sustained-release vitamin C at 3 g/day, she excreted 49.2% of the iodine load, and the baseline serum level was 0.42 mg/L, evidence of improved function of the iodine cellular transport mechanism.

observed in six normal women.

Prior to intervention with vitamin C, the sharp peak of serum iodide at 32 mg/L at one hour post-load followed by a rapid drop suggests that the gastrointestinal absorption of iodine was very efficient, but she was unable to transfer efficiently the serum iodide into the target cells. Following three months on vitamin C, the same test was repeated. The data revealed a normal profile of serum inorganic iodide levels. Her baseline serum inorganic iodide increased from 0.016 mg/L to 0.42 mg/L, and she retained 50% of the iodine load (49.2% recovered in 24-hour urine collection), compared to 10% of the load prior to supplementation with vitamin C.

To our knowledge, this was the first case report of a patient with evidence of a very defective retention mechanism for iodine who was studied with serial serum iodide levels prior to and following intervention. A combination of orthoiodosupplementation in amounts of iodine the patients could tolerate and administration of the antioxidant vitamin C via the oral route improved the performance of the iodine retention mechanism. Repair of a defective iodine cellular transport mechanism following orthoiodosupplementation combined with a complete nutritional program may explain our observation that in some cases a repeat loading test three months after orthoiodosupplementation resulted in a decreased percentage of the load excreted instead of the expected increase, even though the patients felt better on orthoiodosupplementation.

The milder forms of iodine retention inefficiency, either due to inefficient cellular uptake of peripheral iodide or inefficient utilization of intracellular iodide, will probably be overlooked until a more refined procedure is worked out to assess accurately the efficiency of the iodine transport and utilization mechanisms. Obviously, serial serum measurement of iodide would not be practical on a routine basis to evaluate patients with high percentage of the iodine load excreted prior to supplementation. A simple test was needed for the combined assessment of whole body sufficiency for iodine with the assessment of the efficiency of the body to utilize peripheral iodide.

#### **Uptake and Utilization of Peripheral Iodide**

The essential element iodine is present in every organ and tissue of the human body, not just the thyroid gland.<sup>7</sup> Several cells beside the thyrocyte concentrate peripheral iodide against a gradient. So far the list of these iodide concentrating cells besides the thyrocyte has increased to include: white blood cells, salivary and lacrimal glands,

ciliary body of the eye, renal cortex, the pancreas, the liver, gastric, small, and large intestinal mucosa, nasopharynx, choroid plexus, skin, adrenal cortex, mammary gland, placenta, uterus, and ovary.<sup>8</sup> In the target cells studied, the mechanism used to concentrate peripheral iodide involved an energy-dependant transport of one atom of iodide sandwiched between two atoms of sodium across the cell membrane.

Recently, a second mechanism for cellular transport of iodine has been reported by several investigators in the thyroid, mammary gland, and renal cortex, namely a chloride/iodide transporter identified as pendrin.<sup>8</sup> The iodine transporter, pendrin, was speculated to function at the apical membrane of the cell. Rodriguez, *et al.*<sup>9</sup> identified a third human protein, homologous to NIS at the apical membrane of the human thyrocyte. This new protein does not catalyze the accumulation of iodide like NIS, but mediates its passive transfer. It was designated as human Apical Iodide Transporter (hAIT).

Among target cells for iodide uptake and utilization, the predominant research has focused on the thyrocyte. In the thyrocyte, the sodium/iodide symporter (NIS) is located in the basolateral membrane. The peripheral iodide enters the thyrocyte via the symporter in the basal membrane and crosses the thyrocyte as iodide to exit the thyrocyte via the apical membrane transporter just prior to oxidation and organification (Figure 2).

Iodide must bind to a site called the halide symporter binding site before cellular uptake. Other substances compete with iodide for these binding sites. These competing substances are called goitrogens, because they sometimes cause goiter by creating a relative iodide deficiency in the thyroid gland. These substances interfere also with iodide transport and utilization in several organs besides the thyroid gland and a better term would be iodide transport inhibitors and iodide utilization inhibitors instead of goitrogens, depending on whether the inhibition is at the cell membrane transport system or at intracellular sites of iodide oxidation and utilization (Table 1).

#### **The Saliva/Serum Iodide Ratio**

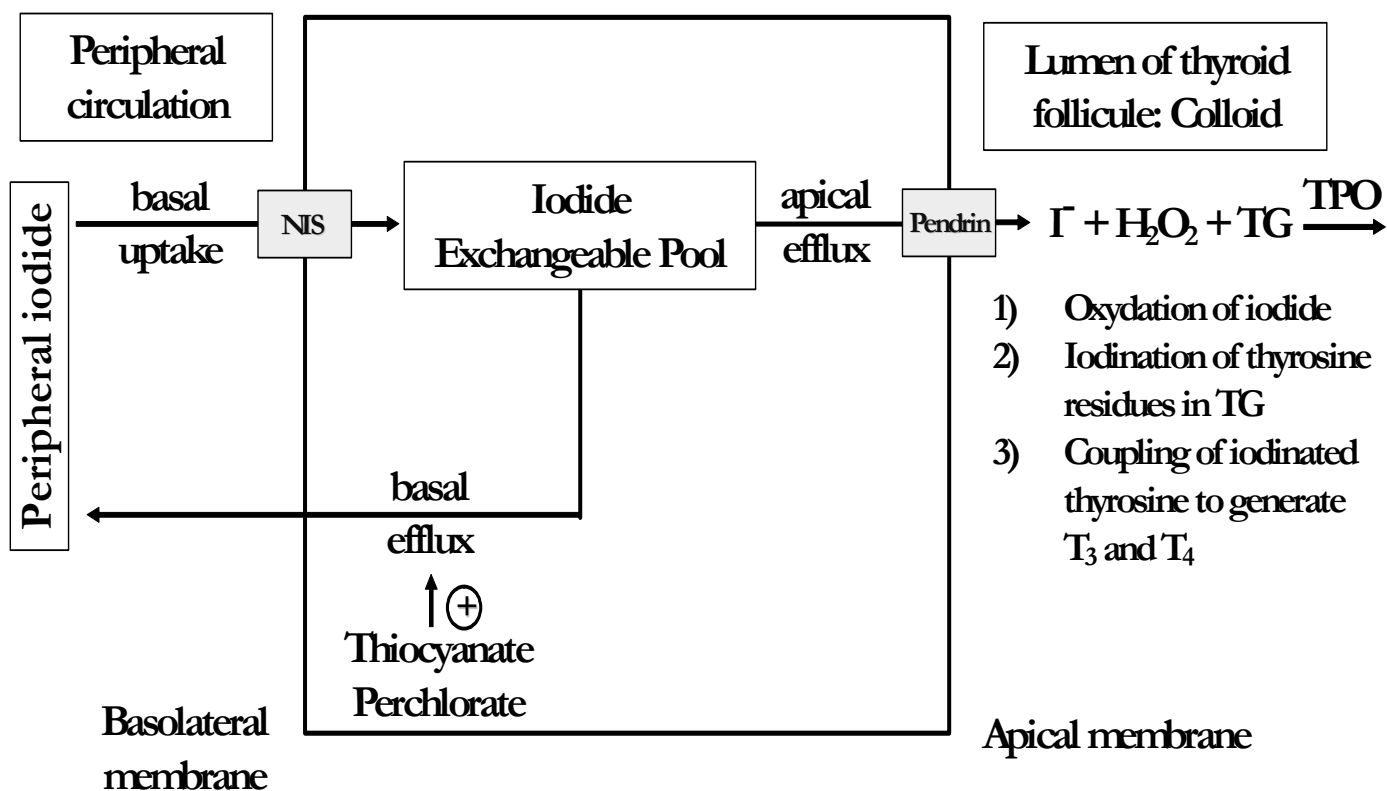
The salivary glands use a mechanism similar to the thyroid gland to concentrate peripheral iodide with subsequent oxidation and organification of iodide. Although the salivary glands can incorporate iodine in thyrosine to form mono- and di-iodothyrosine, they cannot couple iodinated thyrosine to form thyroid hormones.<sup>12</sup>

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Figure 2

Uptake of Peripheral Iodide by the Thyrocyte followed by Oxidation and Organification for the Synthesis of the Thyroid Hormones

# Thyrocyte



The saliva/serum iodide ratio measures the ability of the salivary glands to concentrate peripheral iodide. The assumption made is that the sal/ser ratio of iodide is an index of iodide uptake by target cells throughout the whole body.

The saliva radioiodide/serum radioiodide ratio is used in neonates with elevated TSH and low thyroid hormones in order to confirm a congenital iodide symporter defect.<sup>13</sup>

The procedure involves injecting radioactive iodide in the neonate and measuring the ratio of radioactivity between saliva and serum. According to Viljder and Vulsma, a ratio >10 is considered normal; 3-10, borderline; and <3 is considered abnormal.

Stable iodide instead of radioactive iodide to assess the efficiency of the iodide transport system has not been previously reported because of technical difficulties in measuring low levels of iodine in biological fluids. Measurement of stable inorganic iodide in serum and

saliva under standardized conditions seems the ideal procedure for fine tuning the assessment of iodide transport efficiency, and it is the least invasive way to assess response of the symporter function following intervention. This approach would obviate the need to expose the patient to radioactive iodide. A ratio near unity would indicate a severe defect/damage/inhibition of the symporter function.

We previously reported a procedure to measure saliva and serum inorganic iodide 24 hours following ingestion of 50 mg of iodine in the form of Lugol tablets.<sup>8</sup> The normal range of saliva/serum ratios was 28-74 with a mean of  $44.2 \pm 12.7$  in 14 normal subjects. Low saliva/serum ratios were observed in breast cancer patients with high serum bromide levels. Orthiodosupplementation at 50-100 mg/day resulted in decreased serum bromide and increased saliva/serum ratio.

In some patients with autoimmune thyroiditis and hypothyroidism, an unexpectedly high saliva/serum iodide ratio was observed (>74) concomitant with a high

pre-supplementation excretion of the iodine/iodide load.<sup>14</sup> The only explanation would be a normal transport but deficient oxidation and organification of iodide resulting in an increased exchangeable pool of iodide.

Currently, the procedure used to assess organification defect of the thyroid gland, called the thiocyanate or perchlorate discharge test, is to give the patient radioiodide followed a few hours later by an oral dose of 1 g potassium thiocyanate or 1 g of potassium perchlorate. The percentage of radioactivity in the thyroid is measured before and after iodide is discharged by thiocyanate or perchlorate.<sup>10,11</sup> Thiocyanate and perchlorate not only block the uptake of iodide by the thyroid gland but also cause a discharge of the inorganic iodide present in the thyroid. Under normal conditions, symported iodide is quickly organified and the amount of radioactive iodide discharged following thiocyanate and perchlorate is insignificant. When there is a blockage of organification of iodide, the percentage of radioiodide discharged from the thyroid gland is usually greater than 50% due to an increase in the exchangeable pool of iodine.

### The Triple Test

The expected levels and ratios of serum iodide and of the inorganic and protein bound forms of saliva iodine in patients with deficient uptake and/or utilization of iodide are displayed in Table 2. In the case of inefficient symport of iodide inside the cell, but with normal oxidation and organification of the symported iodide, the expected results displayed in Table 2 under Subsection A would apply. In the case of normal symport of iodide with inefficient oxidation and organification, the results displayed in Subsection B would apply. In the case of inefficiency of both symport and utilization of iodide, the results displayed in Subsection C would apply.

The advantages of the Triple Test procedure over the currently used thiocyanate<sup>10</sup> and perchlorate<sup>11</sup> discharge test to identify organification defect is that the Triple Test will obviate the need to inject radioiodide into the patient followed by administration of these iodine uptake inhibitors. Also, as displayed in Table 2, the radioiodide discharge test would be normal in patients with a combined inefficiency of uptake and utilization of iodide and also in patients with inefficient symport and normal organification, whereas the Triple Test could identify patients with these defects.

The Triple Test involves the collection of urine for 24 hours following ingestion of 50 mg of elemental iodine in the form of Lugol tablets. At the end of the 24-hour

period, serum and saliva samples are collected. Inorganic iodide is measured in the 24-hour urine collection, in the serum, and in the saliva samples by the ion-selective electrode procedure as previously described.<sup>13</sup> The saliva sample is processed further for the measurement of total iodine, both inorganic and protein bound iodine.

### Collection of Urine Sample

Discard the first morning urine of Day 1. Take four tablets of Iodoral<sup>®</sup> 12.5 mg with a glass of water. Collect all urine samples for 24 hours following ingestion of the loading dose. Include the first morning urine on Day 2.

At the end of the 24-hour collection, shake the 3-liter bottle well. Measure the volume of urine in the 3-liter bottle by looking at markings on side of bottle, and pour 1-2 ounces into a 2-ounce plastic bottle. 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.

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. Measure the volume of urine in the 3-liter bottle by looking at markings on the side of bottle. Pour 1-2 ounces of urine in the 2-ounce plastic bottle. Write the name, date, and volume of urine on the 2-ounce bottle. Write on the label, "Part 1 of 2 collections." Then discard the urine in 3-liter bottle. Use the same 3-liter bottle to continue collection. At the end of 24-hour collection, measure the volume of the urine again. Pour 1-2 ounces of urine into the second 2-ounce plastic bottle. Write the name, date, and volume of urine on the 2-ounce bottle. Write on the label, "Part 2 of 2 collections. When the patient's information form is completed, under *total volume*, write: "Collected in 2 parts, both sample bottles enclosed."

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, two measurements will be performed instead of one to compute the percentage of the load excreted. For example, if you measure 6 mg/L in Part 1 with a volume of 3 L, and you measure 4 mg/L in Part 2 with a volume 1.5 L. The computation of the total amount of iodide excreted is:

$$\text{Part 1} = 6 \text{ mg/L} \times 3 \text{ L} = 18 \text{ mg}$$

$$\text{Part 2} = 4 \text{ mg/L} \times 1.5 \text{ L} = 6 \text{ mg}$$

$$\text{Total} = 24 \text{ mg}$$

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**Table 1**  
**Some Examples of Interferences with Iodine Uptake and Utilization**

- A) Cellular Uptake Inhibitors<sup>1</sup>**
- Perchlorate
  - Fluoride
  - Bromide
  - Thiocyanate
- B) Cellular Utilization Inhibitors**
- 1) TPO inhibitors<sup>1</sup>
    - Thionamides (Antithyroid drugs)
    - Goitrin
    - Bromide
    - Thiocyanate
  - 2) Decreased H<sub>2</sub>O<sub>2</sub> production
    - FAD deficiency<sup>10</sup>
    - NADPH-cyt c reductase deficiency<sup>11</sup>

Percent excreted = 48%.

The food grade coloring FD&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.<sup>6</sup> Sodium azide at a final concentration of 0.01% is usually the antiseptic used during collection of urine samples.<sup>23</sup> FD&C Green #3 and sodium azide can both be used for maximum inhibition of mold and bacteria. The combined addition of FD&C Green #3 and sodium azide is used routinely for urine, serum, and saliva samples in our R&D Lab. An aqueous solution containing 15% sodium azide and 1% FD&C Green #3 is added to urine samples at one drop/ounce and to serum and saliva samples at 1 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&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&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. The biological fluids are stable for up to one week at room temperature when collected in containers with sodium azide and FD&C Green #3. However, it is best to freeze the samples if they are not processed within 48 hours of collection.

#### **Comments**

Combining the percentage of the iodine load excreted in the 24-hour urine collection with the measurements of serum and saliva inorganic iodide 24 hours post-load gives an assessment of iodine sufficiency of the whole body and also efficiency of the cellular uptake and utilization of peripheral iodide.

The iodide-selective electrode used in our laboratory is specific for inorganic iodide, and therefore, will not measure protein-bound iodine. The ICP-MS procedure measures total iodine and could be used to compute organic iodine by subtracting the amount of iodine measured by ICP-MS from the level measured by the ion-selective electrode procedure. Measuring protein-bound iodine (PBI) in saliva can also be performed directly by the appropriate technique. The value obtained by ICP-MS should be equal to the sum of the value obtained by the ion-selective electrode procedure and the value obtained by the PBI measurement.

We are currently collecting samples from normal subjects and patients with various clinical conditions in order to standardize the normal range of organic/total iodine ratio and to correlate these levels with clinical response following nutritional intervention in patients with abnormal ratios.

#### **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 orthiodosupplementation in medical practice.

David Brownstein, MD, is a family physician who utilizes the best of conventional and alternative therapies. He is the Medical Director for the Center of Holistic Medicine in West Bloomfield, Michigan. A graduate of the University of Michigan and Wayne State University School of Medicine, Dr. Brownstein is board certified by the American Academy of Family Physicians. He is a member of the American Academy of Family Physicians and the American College for the Advancement in Medicine. Over the past few years, he has had extensive experience in the use of orthiodosupplementation in his practice.

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## Table 2 Expected Finding in Cases of Inefficient Symport, Inefficient Utilization, and a Combination of the Two

### A) Inefficiency of iodide transport with normal oxidation and organification of iodide

- Decreased intracellular iodide
- Decreased exchangeable pool of iodide
- Decreased saliva iodide
- Decreased sal/ser iodide ratio
- Normal saliva organic iodine/total iodine ratio
- Normal nitrate and perchlorate discharge test
- Hypothyroidism and goiter in severe cases only

### B) Inefficiency of oxidation of iodide with normal transport

- Increased intracellular iodide
- Increased exchangeable pool of iodide
- Increased saliva iodide
- Increased sal/ser iodide ratio
- Decreased saliva organic iodine/total iodine ratio
- Increased discharge of iodide following nitrate and perchlorate
- Hypothyroidism and goiter in severe cases only

### C) Combined inefficiency in transport, oxidation, and organification of iodide

- Decreased intracellular iodide
- Decreased exchangeable pool of iodide
- Decreased saliva iodide
- Decreased sal/ser iodide ratio
- Decreased saliva organic iodine/total iodine ratio
- Normal nitrate and perchlorate discharge test
- Hypothyroidism and goiter in severe cases only