A Simplified Method for Butanol-Extractable Iodine and Butanol-Insoluble Iodine

Joseph Benotti and Samuel Pino

A simplified method is presented for the determination of butanol-extractable iodine (BEI). The results compare favorably with the original method as described by Man et al. Also available, as part of the procedure for BEI, is a direct chemical determination for butanol-insoluble iodine (BII) from the same specimen of serum. Heretofore, nonbutanol-extractable iodine has been studied only by means of radioactivity. This fraction merits further investigation.

The butanol-extractable iodine test (BEI) is a better chemical test for the measurement of thyroid function in the presence of iodide contamination than the protein-bound iodine test (PBI). The simplified procedure for BEI presented below, by nature of the method, yields another iodine measurement, butanol-insoluble iodine (BII), from the same specimen of serum. The latter compounds may be classified as iodinated proteins which may prove to be of value in the study of various other thyroid disorders.

The determination of iodine levels in blood serum has been widely accepted as a diagnostic test for thyroid function. PBI was the first chemical test to merit recognition in this field. In the absence of contamination, about 90% of the hormonal iodine is reflected in the PBI value.

Danowski et al. (1) have shown that feeding of iodide may give a falsely high PBI value, because of the formation of iodinated proteins which are nonhormonal. In view of these findings, it became obvious that a more reliable chemical test was needed for the measurement of hormonal iodine. Leland and Foster (2) were the first to extract thyroxine from digests of thyroid tissue with the use of butanol. Taurog and Chaikoff (3) also used butanol to extract thyroxine from serum. In-

From the Boston Medical Laboratory, Waltham Division, 15 Lunda St., Waltham, Mass. 02154.
We are grateful to Farah Maloof, M.D., for making available to us serum samples from patients with various thyroid disorders, and to Paul Plankey for his technical assistance.
Received for publication Feb. 17, 1966; accepted for publication May 5, 1966.
organic iodide and diiodotyrosine are removed from this solvent by means of an alkaline reagent as suggested by Blau (4).

It was on these principles that Man et al. (5) developed a quite precise method for determining thyroxine levels in serum. This determination is known in the clinical field as the butanol-extractable iodine test (BEI) for thyroid function. In spite of these advantages, the BEI has never become as popular as the PBI because it is quite time-consuming, more technically difficult to perform, and therefore more costly. It is for this reason that we have investigated the possibility of simplifying this procedure.

Experimental

Since iodide is the primary contaminant of serum in the measurement of thyroid hormone, we decided to remove it before separating the hormonal from the nonhormonal iodine compounds.

The removal of iodide necessitated a method of screening for iodide in order to be sure of its quantitative removal.

Screening Test for Iodide

Reagents

Arsenious acid Dissolve 2 gm. NaOH in 200 ml. H₂O. Add 3.0 gm. As₂O₃ and 87.5 gm. NaCl. In a separate beaker cautiously add 764 ml. concentrated H₂SO₄ to 1 L. water. When this solution is cool, add the acid to the salt mixture and dilute to 2 L. with water.

Ceric ammonium sulfate 2.5 gm. ceric ammonium sulfate per liter of 1 N H₂SO₄.

Procedure

Add 2 ml. of arsenious acid to the supernatant water wash, as noted under Methodology, and mix. Then add 1 ml. of ceric ammonium sulfate and mix again. The light yellow color must persist for 1 hr. at 37°. Any fading prior to this time indicates the presence of iodide; further washing with 10 ml. of water would be required. Each successive washing is screened as above until a negative reaction for iodide is obtained. Normally 1 additional washing with water will suffice to give a persistent yellow color.

Methodology-Butanol-Extractable Iodine (BEI)

To 0.5 ml. of serum in a 40-ml. round-bottom centrifuge tube add 10 ml. of water, 0.5 ml. of 10% zinc sulfate, and 0.5 ml. of 0.5 N sodium hydroxide with mixing, precipitating the proteins. After centrifugation pour the clear supernatant solution into another, similar tube and
screen for iodide as described above. After the final wash, the precipitate may be analyzed for PBI or further treated to separate the hormonal (BEI) from the nonhormonal iodine (BII). In the latter case the tube containing the proteins must be placed in an inverted position for 5–10 min. to remove as much water as possible.

To the washed protein precipitate, add 2 drops of 5 N sulfuric acid and mix. This will readily dissolve the precipitate completely. Add 8 ml of n-butanol and mix well on a vortex mixer to facilitate the extraction of extractable iodinated compounds (thyroxine) into the solvent. At this point it is important to ensure that there is only one liquid phase. If perchance two phases are visible, it means that too much water was left in the washed protein precipitate. The insoluble proteins are centrifuged, and the supernatant solution poured off into another 40-ml centrifuge tube. The insoluble protein is re-extracted with 4 ml of butanol. The mixture is stirred and recentrifuged. Since this precipitate may now be quite sticky, the use of a glass rod or a small applicator stick may be necessary. It will also help to scrape down the precipitate from the walls of the tube. The second butanol extract is added to the first, thus giving an approximate volume of 12 ml. To neutralize the acid present in the butanol, 2 drops of 1 N sodium hydroxide are added before proceeding with the evaporation.

The evaporation of butanol has already been described (6). However, it has been further simplified by closing both the upper and lower spaces in the back panel of the hood. Newer hoods may be procured with a crank arrangement which performs this operation. By cutting a hole in the lower back panel of the hood, a manifold may be inserted and thus receive the full suction. An existing hood may be closed by simply placing a piece of plywood over the upper and lower openings. We have designed a manifold, shown in Fig. 1, which has 16 small tubes in the top and has no bottom. It is placed over a glass tray to catch any condensate which may collect. The glass tubes, bent at a 120° angle, are placed directly into the test tube containing the butanol. An aluminum block 6 × 6 × 4 in. has been designed which contains 16 holes the size of the 40-ml centrifuge tube. The aluminum block is placed on an electric Thermolyne* heater set at 120°. The centrifuge tubes containing the butanol are placed in the aluminum block and connected to the suction hood by means of bent glass tubes, plastic tubing, and manifold. The vaporized butanol is immediately drawn out of the tubes by means of suction and thus refluxing is prevented. It takes approximately 30–40 min. to evaporate the butanol. Since the BII tubes have only a

---

*Temeo, Thermolyne Corp., Dubuque, Iowa.
small amount of butanol in them after decantation, they may be placed on the top of the aluminum block in a horizontal position, with no connection to the suction hood. Caution: Both the BEI and BII fractions must be completely freed of butanol before digestion with chloric acid, as the two together are quite explosive. The disappearance of final traces of butanol may be best detected by means of smell.

The final steps of digestion and colorimetry are performed as previously described (6).

Results

Direct comparison of our method for BEI with that of Man et al. (5) was made on 10 serum samples. The standard deviation was obtained and the t test for paired observations was performed according to the procedure outlined by Henry and Dryer (7). The standard deviation was 0.31 μg./100 ml. The t value for our data was 2.02. The normal value for BEI is 3.0–6.5 μg./100 ml. It has been our experience that the normal value for BII with this method is up to 1 μg./100 ml. (usually 0.5–1.0). We have found higher values in hyperthyroidism and nontoxic goiter, and in patients who were being given inorganic iodide for various therapeutic reasons.

Discussion

In the past, other investigators have treated the serum first with butanol, and then removed the iodide from the butanol by extracting
with alkali. Removing iodide first eliminates the necessity of extraction by means of two immiscible liquids in a separatory funnel.

The quantitative removal of iodide sometimes requires repeated washings. We investigated the solubility of thyroxine from various protein precipitates such as trichloroacetic acid (TCA), perchloric acid (HClO₄) and zinc hydroxide (Zn(OH)₂). By using a pooled serum in which the iodide content was low, it was possible to precipitate the protein of this serum by the 3 precipitants mentioned above. The TCA precipitates were washed from 1 to 6 times with 5% (w/v) TCA and analyzed for iodine; 5% HClO₄ was used in the same way. Figure 2 shows the decreasing iodine values with each successive wash. However, by precipitating the serum with Zn(OH)₂ and washing with water 6 times, no loss of iodine was observed. It is possible to wash Zn(OH)₂ protein precipitates as many times as may be necessary to remove iodide quantitatively.

In most laboratories specimens for routine PBI's are washed once or twice only to remove iodide. This is adequate when the iodide concentration is not grossly elevated. If the iodide concentration is high and the routine washing does not remove all the inorganic iodide, false high PBI values will result. If the proteins are to be separated further into BEI and BII, it is important to remove iodide quantitatively since we do not wash the butanol extract with alkali. It has been our experience with this screening method that occasionally it will be necessary to wash several times in order to remove all of the iodide.

**Fig. 2.** Lower set of 6 peaks shows gradual loss of iodine after successive washings with perchloric acid. Middle set likewise shows same effect after wash with 5% TCA. Upper set of peaks represents zinc hydroxide precipitates washed with water 1–6 times and analyzed for iodine: No loss of iodine may be observed even after 6 washings.
Theoretical

Now that a battery of chemical iodine determinations is available, such as BII, BEI, PBI, and total iodine (TI), a word about their relative merits may be of practical value.

The PBI assay alone as performed in the routine laboratory has little significance unless the lack of contamination can be substantiated chemically (8). Questioning of the patient and taking his history are sometimes unrewarding, since there are many drugs on the market that contain iodine, which the lay person has no way of knowing. The TI assay should always be performed in conjunction with the PBI assay, in order to establish whether a serum has been contaminated with iodine. If the differential between PBI and TI is less than 1 \( \mu g/100 \text{ ml.} \), there is no evidence for iodide contamination and the PBI assay will have more diagnostic value. Excess iodide ingestion gives a much greater differential between the PBI and TI assay.

As mentioned above, Danowski et al. have shown that excess iodide ingestion may give rise to a false high PBI value (1). This is because some of the proteins become iodinated to iodoproteins. This latter fraction is called BII and is nonhormonal. Consequently, the BEI or hormonal fraction value would have to be less than that of PBI, by the amount of the BII. It is for these reasons that a simplified procedure for the separation of hormonal (BEI) from nonhormonal (BII) iodine is important. The BII value may prove to have some importance in other thyroid disorders.

References