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Protein-Bound Iodine Determination by Activation Analysis

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INTRODUCTION

The most commonly-used test for determining the serum concentration of thyroid hormones is the protein-bound iodine analysis (PBI) which measures the serum's entire organic and some inorganic iodide concentration. Since it is desired to measure only that portion of the serum iodine which is in the thyroxine molecule, this test may be misleading. The method of PBI determination routinely used in most clinical laboratories is the dry ash method of Barker (1). It is one of the more complicated of the commonly-used clinical laboratory tests requiring skilled technicians and generally requiring a separate laboratory because of contamination problems. The presence in serum of organic iodine compounds used for radiographic and therapeutic purposes precludes the determination of PBI by the dry ash method for periods up to years (1).

The butanol-extractable iodine (BEI) method of serum iodine determination is recognized to be more reliable than the PBI, but it is also more difficult to perform and is therefore used less frequently (2,3).

Pileggi (3) has described a method of serum iodine determination using an ion exchange resin column which is reliable for the determination of thyroxine iodine in the presence of elevated inorganic iodine or iodotyrosine levels. When this method was compared with the PBI method, the column method also showed superiority in its ability to distinguish thyroxine iodine from organic iodine compounds. This method, subsequent to the ion column separation, suffers from the usual PBI analytic problems.

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In light of the PBI determination difficulties, it was decided to study the feasibility of developing a method of serum thyroxine iodine determination by using the column separation method followed by activation analysis for the actual estimation of the iodine. Several investigators (4-6) have used neutron activation analysis for iodine determination, but these are fairly lengthy, detailed procedures which could not meet the need for a simple rapid method. One activation procedure which is relatively quick and fairly simple to carry out has an unexplained positive bias in the PBI measured (6). The normal serum PBI is 3.5 μgms to 8.0 μgms/100 ml; it must be appreciated that extreme care to prevent contamination will always be required in the quantitative measurement of as small a concentration as 3.5 μgms iodine/100 ml of serum. For radioactivation methods in general, the contamination problem is less severe than in other trace element procedures. This is because fewer steps, less handling, and "tagging" of the key element early in the analysis are the essence of activation methods.

METHODS

A procedure utilizing an ion exchange resin column and successive washes with buffered acetic acid solutions of decreasing pH was used to separate thyroxine from serum. An aliquot of the final eluate and a standard solution of potassium iodide were irradiated and the intensity of the iodine activity in each was compared.

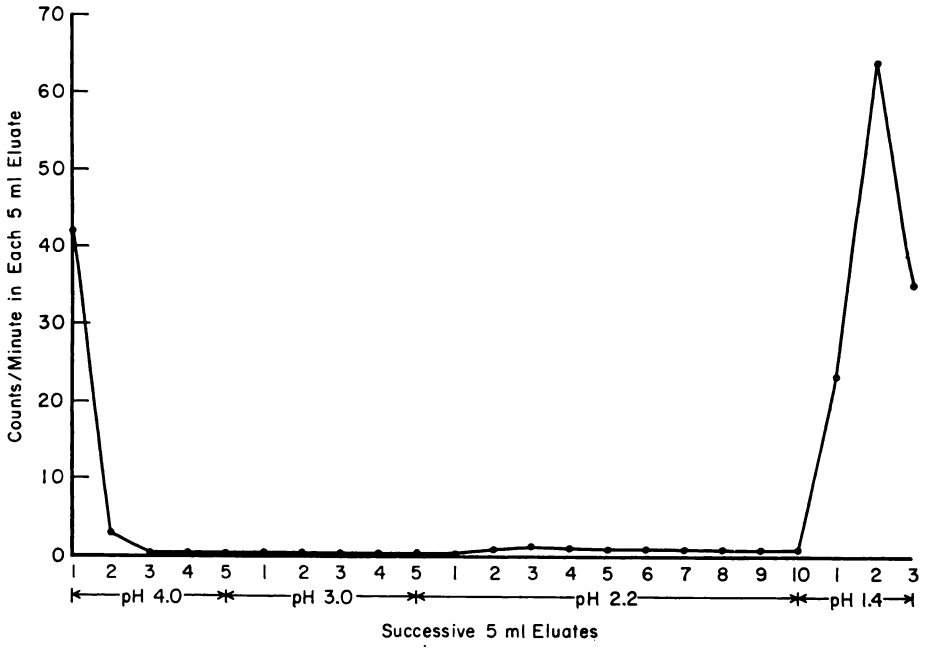

Fig. 1. Recovery of ^{131}I Tagged Thyroxine In Serum From Column.

The quantity of iodine in the original serum was determined by comparison of the relative peak heights in the gamma ray spectra from the iodine in the standard and from the iodine in the column eluate.

Preparation of Columns

The resin used was Dowex-1, X-2, 100-200 mesh, analytical grade in the Cl^- form. Two columns, 6 cm high x ½ cm diameter, were washed five times with 50 ml of 50 per cent acetic acid and then adjusted to pH 4.0 by washing with 50 ml of 0.2 M acetate buffer. The columns were kept under 0.2 M acetate buffer and were not allowed to dry between runs.

Preparation of Wash Solutions

1. Acetate buffer of pH 4.0, glacial acetic acid, 58.5 ml and sodium acetate, 38.5 gms, were made to 1 liter with distilled water.
2. Acetate buffer of pH 3.0. Glacial acetic acid, 88.5 ml and sodium acetate 2.2 gms made to 1 liter with distilled water.
3. Acetate buffer of pH 2.6. Glacial acetic acid 88 ml, sodium acetate 1.5 gms made to 1 liter with distilled water.
4. Acetic acid of pH 2.2. Glacial acetic acid 200 ml made to 1100 ml with distilled water.
5. Acetic acid of pH 1.4. Glacial acetic acid 500 ml made to 1 liter with distilled water.

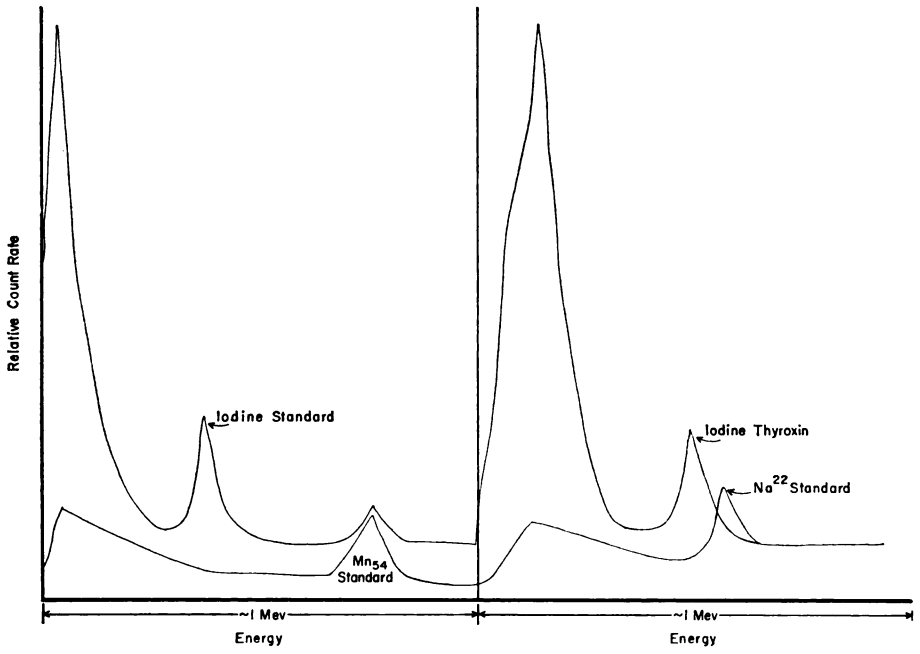

Fig. 2. Analysis of Spectra of Iodine Standard and Thyroxine.

All solutions were checked for final pH by means of a Beckman pH meter. The pH could be estimated to 0.02 and readings were reproducible to 0.02 units on this meter. All solutions were stable for long periods and large volumes could be prepared for long term use.

Extraction

Three to five ml of serum were adjusted to pH 4.0 by the addition of buffer solution of pH 2.6. This buffered serum was poured onto the resin column. When the serum had passed through the column, the following washes were made in this order: pH 4.0, 25 ml; pH 3.0, 25 ml; acetic acid pH 2.2, 50 ml; the final

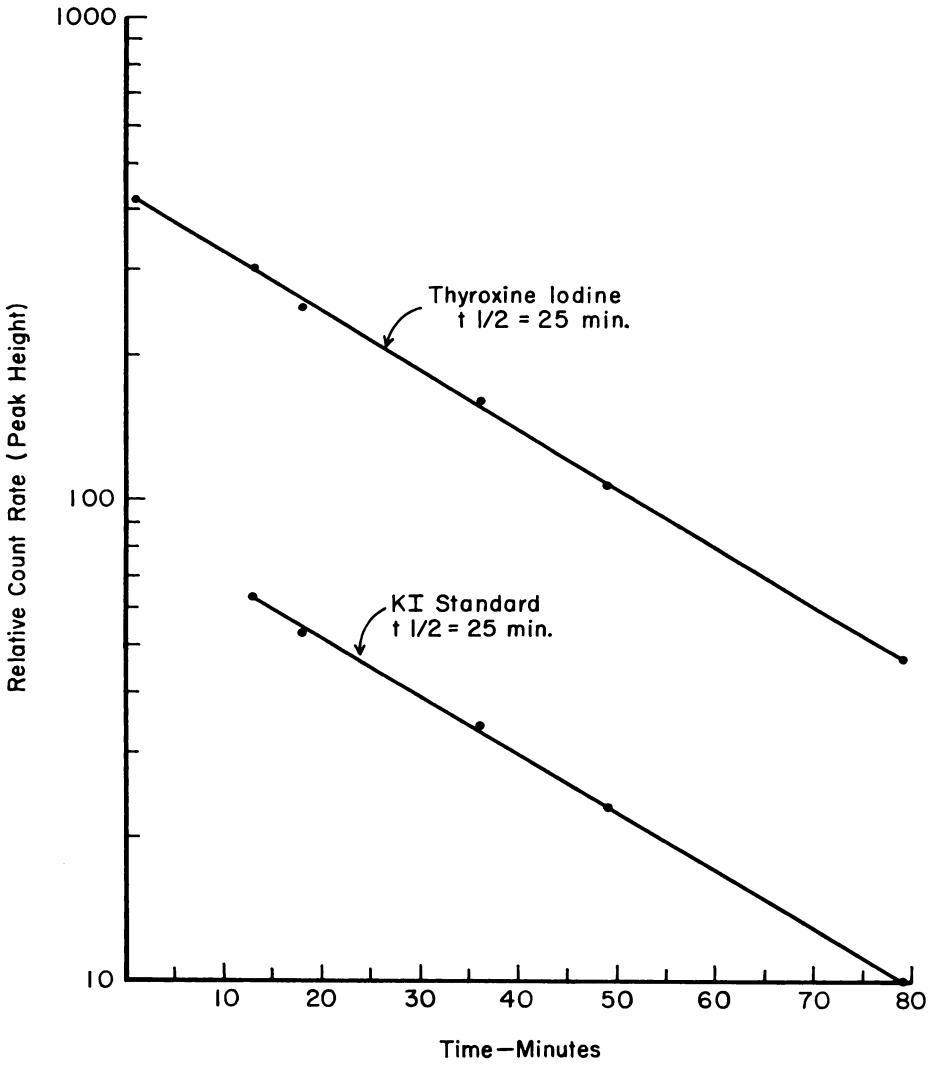

Fig. 3. Analysis of Decay of Standard Iodine and Thyroxine.

wash was the eluant, acetic acid, pH 1.4, 15 ml. This portion was collected for the final estimation. All rates of flow were 2 ml/min. The iodine content of the solution used for the final wash was negligible by activation check, and no further reagents were used. No blank correction was required.

Potassium iodide, analytical grade, was used as a standard. An aqueous solution containing 0.1 μgm I in 1.0 ml H_2O was made up for each set of analyses since these dilute solutions were not stable.

Irradiation and Analysis

The general procedures previously described (7) for the activation and analysis of the sample from the column extraction eluate were used. An aliquot of the final eluate from the resin columns and a standard, each in a polyethylene-sealed tube and taped together, were irradiated in the Walter Reed Research

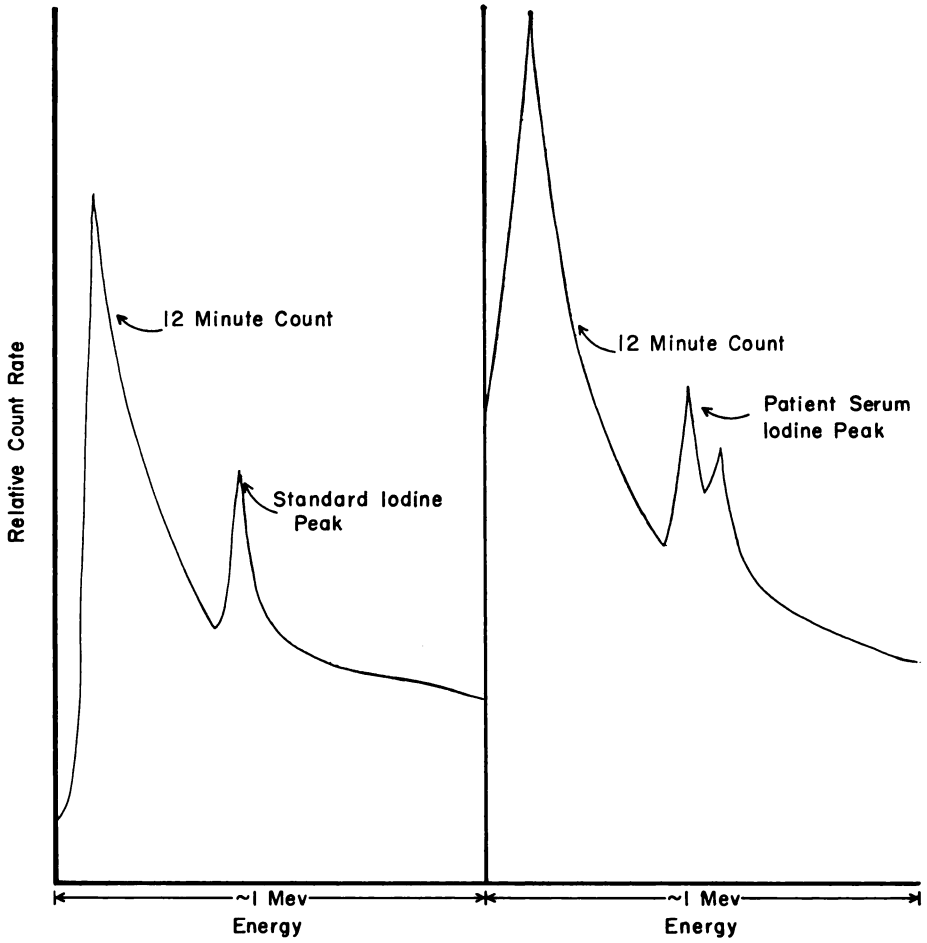

Fig. 4. Spectra of Iodine Standard (KI) and Patient Serum.

Reactor, a homogeneous reactor at a power level of 50 KW (th). The thermal neutron flux, as measured by gold foils, was 10^{11} neutrons/sq cm-sec.

The standard solution and the aliquot were then transferred to cellulose tubes and their radiation spectra were simultaneously obtained by means of a four hundred channel pulse height analyzer, using it in a split memory, two crystal input configuration (7).

Recovery of Radioactive Thyroxine

Iodine-131-labeled thyroxine with some inorganic iodine impurity was incubated with serum for two hours at 37°C. The serum was added to the resin column, washed and eluted in the manner described. The eluate from each added solution was collected in 5 ml portions and each analysed for radioactivity. A typical recovery curve is shown in Fig. 1. Ninety to 100 per cent of the radiothyroxine was eluted at pH 1.4 in a relatively small volume, while impurities were removed earlier at higher pH. This process is in agreement with Galton (8) and Pileggi (3).

Half-Life of Peak at Correct Energy Level

The gamma ray from ^{128}I is known to be at 0.46 MeV (9). In order to prove that the radioactivity being measured in this work was ^{128}I, the half life of its peak was measured. Since chemistry to separate the material had already been done, this procedure constituted a triple proof of identity. Irradiated iodine

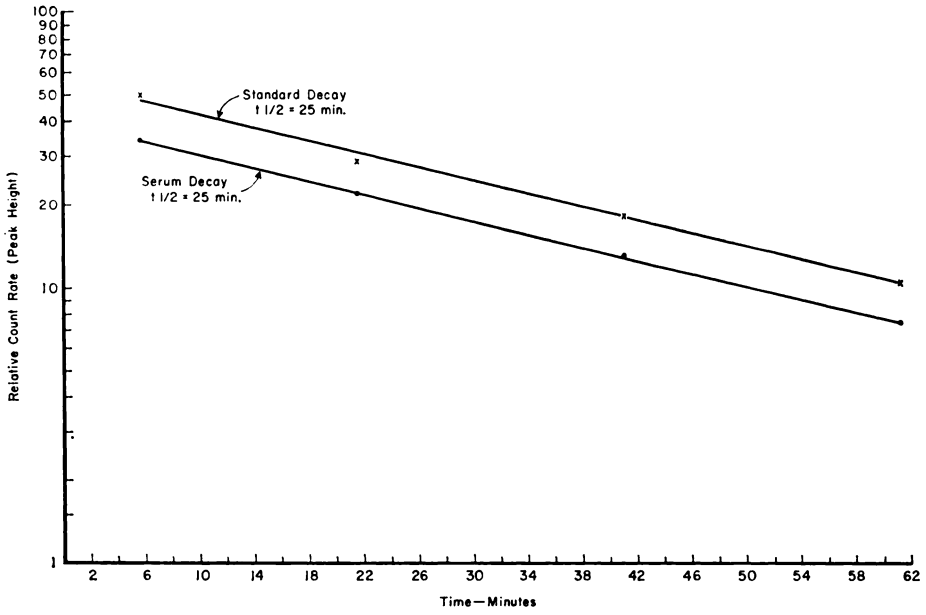

Fig. 5. Decay of Iodine Standard and of Patient Serum.

standard and thyroxine were checked for energy and for half life simultaneously by use of the dual crystal pulse height analysis arrangement (7). After their spectra had been obtained, ^{54}Mn(0.84) and ^{22}Na (0.51) MeV standards (9) were measured with precisely the same calibration of the analyzer and printed on the identical chart. This process is demonstrated in Fig. 2. The half-life of the two 0.46 MeV peaks was determined to be 25 min (Fig. 3).

Measurement of PBI in Patients' serum

Serum from eleven patients was analyzed for PBI concentration both by the modified Barker method (1)[1] and by activation analysis. Figure 4 is a typical pattern of spectra obtained for patient sera and standard. The decay of one sample and its standard are shown in Fig. 5. A comparison of results obtained on this set of patient sera by the two methods is given in Table I. The data obtained in the comparison of the clinical chemistry procedure and the activation procedure were statistically analysed (10).[2] A linear regression line and correlation coefficient were computed and are shown in Fig. 6.

[1]We are indebted to SSgt. Marjorie Glassbrook for analyzing a set of these sera by the Barker procedure in her clinical chemistry laboratory.

[2]We are indebted to Major M. K. Ginsberg, ANC, and to Specialist Edward Minkler, for their assistance in adapting the procedures of Natrella (10) to a computer and for carrying out the required computations for us.

TABLE I

COMPARISON OF RESULTS OF PBI DETERMINATIONS ON PATIENT'S SERA BY THE TWO METHODS

Patient	*Clinical Laboratory ugm/100 ml*	*Activation Analysis ugm/100 ml*	*Deviation From Clinical Laboratory*
1	9.5	9.1	−0.4
2	6.6	6.0	−0.6
3	5.8	5.7	−0.1
4	5.6	5.1	−0.5
5	5.2	4.7	−0.5
6	5.0	5.0	0
7	5.0	5.0	0
8	4.6	4.5	−0.1
9	3.3	3.2	−0.1
10	3.1	2.7	−0.4
11	1.8	1.9	+0.1

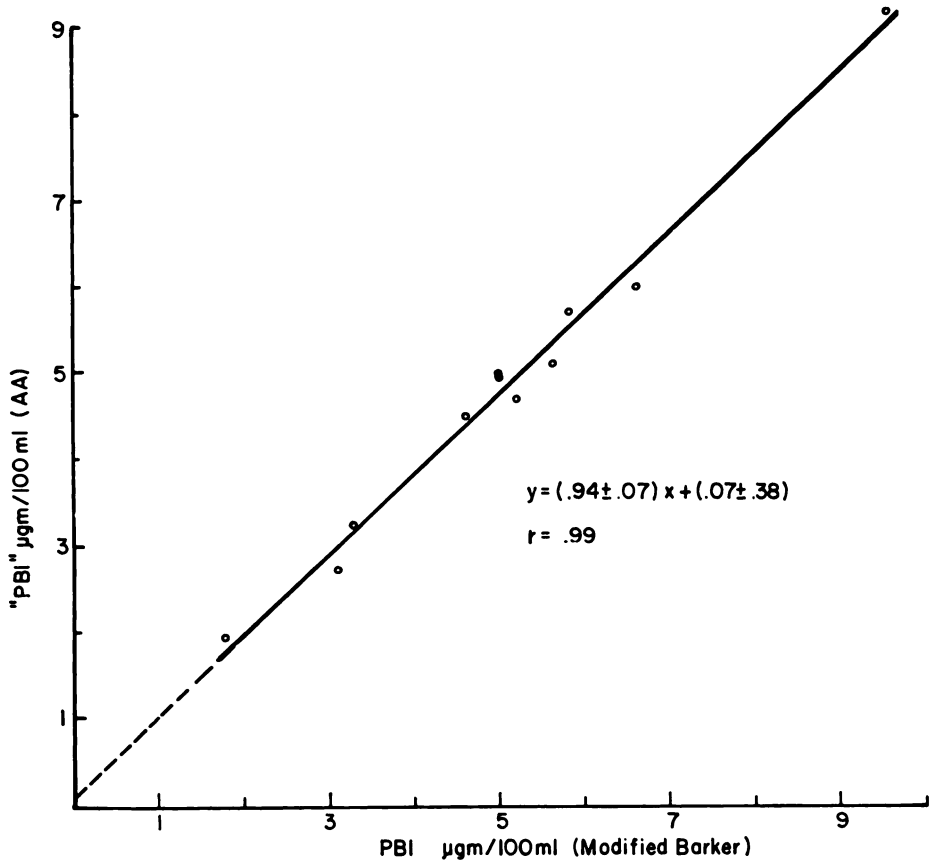

Fig. 6. Regression Line of Comparison of Analytical Results from Barker Procedure and Activation Procedure.

DISCUSSION

The combined use of a resin column and activation analysis for the determination of the serum PBI concentration offers advantages over other methods of analysis. The advantages of the ion exchange method previously demonstrated (3) are reliability in the presence of elevated inorganic iodide or iodotyrosine and increased ability to determine thyroxine iodine levels in the presence of some organic iodine compounds as compared with the dry ash method. With the addition of the radioactivation analysis, the final iodine estimation procedure becomes simple and rapid. For possible clinical laboratory use, it is believed that most of this procedure could be readily automated.

SUMMARY

A rapid simple method using anion-exchange resin column technique combined with radioactivation analysis has been described for the determination of serum protein-bound iodine concentration. Determinations made on patients'

serum were consistently close to results obtained by the usual dry ash method. The basic simplicity of the method and the rapidity with which it can be done were of primary consideration.

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CORRECTION NOTICE

In the November 1966 issue of the *Journal of Nuclear Medicine*, figures 7b and 7c (pp. 813 and 814) in the article entitled "Elimination of Liver Interference from the Selenomethionine Pancreas Scan" by Ervin Kaplan, M.D., Moshe Ben-Porath, B.S., Sidney Fink, M.D., Glenn D. Clayton, B.S. and Burton Jacobson, M.D., were printed upside down. This error has been corrected in the reprints of the article now available from Dr. Kaplan.