DETERMINATION OF SERUM THYROXINE BY SATURATION ANALYSIS OF THYROXINE BINDING PROTEINS

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Until recently protein-bound iodine (PBI) and butanol extractable iodine (BEI) estimations were the only methods for measuring thyroid hormones in serum. Unfortunately, these procedures involve chemical determination of iodine and do not belong to the field of nuclear medicine.

In 1964 Murphy and Pattee (*1*) described a new method for directly determining thyroxine (T_4) concentration in serum based on the saturation analysis of the thyroxine-binding proteins. This procedure involved the use of gel-filtration chromatography of labeled T_4 previously incubated in a standard pooled sera to which the endogenous T_4 extracted from the serum to be tested was added. Shortly after Nakajima and associates (*2*) tried to improve this technique by using a resin sponge and radioactive triiodothyronine (T_3).

In this paper we present our results with a similar method for estimating serum T_4 by using labeled T_3 and gel-filtration chromatography. This procedure seems to be accurate, simple and economical enough to replace PBI and BEI estimations, mainly when contamination from iodides or other iodinated compounds is presumed.

METHODS

This study was performed in sera from 487 subjects (136 euthyroid, 149 thyrotoxic, 98 hypothyroid, 42 nephrotic and 60 normal pregnant women). The euthyroid, thyrotoxic and hypothyroid individuals were chosen at random from the patients coming to this unit for routine thyroid-function tests. The nephrotic patients were chosen at random from the wards while the pregnant women were selected from those attending the obstetrics outpatients department who were pregnant for more than 6 months.

Patients whose serum was suspected of being contaminated with iodine-containing drugs were included in a separate group. In every subject studied, thyroid status was determined by clinical evaluation and by routine laboratory tests: PBI and/or BEI estimation, 24-hr ^{131}I-thyroid uptake, conversion ratio at 48-hr and *in vitro* binding of T_3 by a resin sponge and/or *in vitro* binding of T_3 by serum proteins.

Gel-filtration chromatography. The *in vitro* binding of labeled T_3 by serum proteins was estimated by gel-filtration chromatography by a modification of a method described elsewhere (*3,4*). Iodine-131-labeled T_3 (0.08–0.12 μCi) is added to 2 ml of serum and incubated for 60 min in an unstoppered glass test tube placed in a water bath with automatic shaker at 25°C. One milliliter of this serum is pipetted onto a chromatographic column containing 2 gm of Sephadex G25, medium grade (Pharmacia Corp.) mixed with 0.067 *M* phosphate buffer at pH 7.4. Elution is started with 3 ml of tris-maleate buffer (tris-hydroxymethylaminomethane and maleic acid) at pH 7.6, and the eluate is discarded. Then 5 ml of the same buffer is added to the column, and the resultant eluate containing serum-bound T_3 is collected in a glass test tube. Again 5 ml of buffer is added to the column, and the eluate which contains free radioactive iodides is collected in a glass test tube. The column is then washed with 5 ml of pooled normal sera, and the eluate is normally discarded, but it is occasionally collected and counted as a check; this eluate contains free T_3 which has been adsorbed on to the gel. Finally, the column is washed with 5 ml of buffer, and the eluate is discarded. The column is then ready for use again.

The remaining 1 ml of serum, mixed and incubated with labeled T_3, is diluted with distilled water up to 5 ml and assayed for radioactivity in a well scintillation counter. Radioactivity is also estimated

Received Nov. 4, 1968; original accepted Feb. 20, 1969.
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in the serum-bound T_3 and in the free-iodide fractions. The fraction of T_3 bound to serum is calculated by the following formula:

$$\text{Bound } T_3 = \frac{\text{cpm in bound } T_3 \text{ fraction} \times 100}{\text{cpm in 1 ml serum} - \text{cpm in free-iodide fraction}}.$$

The values obtained are compared with those procured at the same time with a sample of normal pooled sera used as a control, and the results are expressed as the test-to-control ratio.

Obtaining a standard calibration curve. Nonradioactive T_4 is dissolved in 95% ethanol with ammonia, and a serial dilution is made with the same solvent to obtain various known T_4 concentrations (from 0 to 20 μg/100 ml). Aliquots of each known T_4 solution are transferred to glass test tubes and dried under an air current at 30°C. The T_4 extract is then redissolved in 2 ml of a pool of normal sera diluted in saline solution (1:1 v/v) and incubated at 25°C during 15 min under constant shaking to insure homogeneous distribution of T_4 in serum. Radioactive T_3 is added and its binding to serum proteins is estimated by gel-filtration chromatography as described earlier. A graph is plotted relating the amount of nonradioactive T_4 added and the binding of T_3 by serum expressed as the test-to-control ratio (Fig. 1). This calibration curve can be used for a long time—as long as the same standard stock sera is being used.

Estimation of thyroxine in serum. Five milliliters of 95% ethanol, which has been made alkaline by adding a few drops of ammonia, is added to 2 ml of serum sample and mixed thoroughly. The mixture is allowed to stand for 5 min at room temperature and centrifuged at 1,800 rpm for 5 min. Five milliliters of the supernatant is transferred to a glass test tube, dried, redissolved in 2 ml of standard serum and the *in vitro* binding of labeled T_3 by serum is estimated according to the method described earlier.

Evaluation of ethanolic extraction of serum thyroxine. One milliliter of the serum sample to be tested is added to radioactive T_4 (0.08–0.12 μCi) and incubated at 25°C for 1 hr with constant shaking. Thyroxine is then extracted with 2.5 ml of 95% ethanol plus ammonia in a similar way to the procedure described above. The serum sample is assayed for radioactivity in a well scintillation counter before and after the extraction procedure, and the recovery of labeled T_4 is then calculated.

Calculations and corrections. Thyroxine content in 2 ml of serum is directly observed on the calibration standard curve (Fig. 1). The concentration of T_4 in 100 ml of serum is calculated by multiplying this value by 50. The result must be corrected by the efficiency of the ethanolic extraction of T_4 which is simultaneously estimated in one aliquot of the same sample as described above.

Because we were to compare our results with the PBI concentration, it was necessary to estimate the iodine content of T_4 in 100 ml of serum. This is easily calculated by multiplying the concentration of T_4 by the fraction of the molecule of T_4 corresponding to iodine (65.3%).

Effects of aging of the standard pooled sera on the *in vitro* binding of T_3 by serum proteins. To determine how long the same standard pooled sera can be used safely, the *in vitro* binding of T_3 by serum proteins and the thyroxine-binding globulin (TBG) capacities were estimated periodically over 12 weeks in aliquots of the same stock of pooled sera using fresh labelel T_3 solution in each case.

Free TBG binding capacity estimation. Free TBG binding capacity was estimated by the method described by Osorio *et al* (*5*) by adding tracer amounts of ^{131}I-tagged T_4 to serum and submitting the mixture to paper electrophoresis in tris-maleate buffer at pH 7.6. The proportion of the total radioactivity in the inter-alpha zone was determined, and the absolute amount of T_4 bound to TBG calculated by multiplying the percent of labeled T_4 in the TBG zone by the concentration of endogenous T_4 in the serum. This value represents the concentration of binding sites occupied by endogenous T_4 at pH 7.6. Free TBG binding capacity was obtained by subtracting this value from the total TBG binding capacity and expressed the concentration of non-occupied binding sites by endogenous T_4 at pH 7.6.

Estimation of total TBG binding capacity. Total TBG binding capacity for T_4 was estimated in tris-

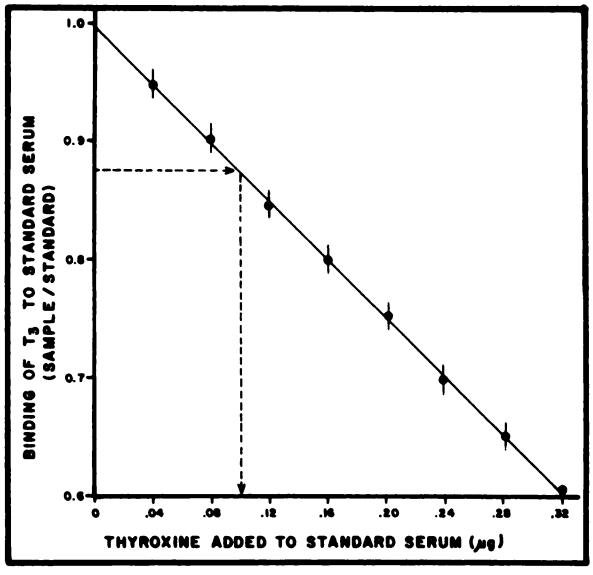

FIG. 1. Standard calibration curve. *In vitro* binding of labeled triiodothyronine by standard serum (expressed as test-to-control ratio) is plotted as function of amount of nonradioactive thyroxine added to serum.

maleate buffer at pH 8.6 from the amount of labeled T_4 present in the inter-alpha zone after paper electrophoresis of serum to which enough radioactive T_4 was added to give a concentration of 100 μg of exogenous T_4/100 ml of serum. Under these conditions TBG is saturated and the amount of T_4 bound to TBG can be calculated by multiplying the percentage of radioactive T_4 in the inter-alpha zone by the total concentration of T_4 in serum, including endogenous and exogenous T_4. The result was expressed as micrograms of T_4 bound to TBG in 100 ml of serum (*6*).

RESULTS

Figure 1 shows the *in vitro* binding of labeled T_3 at 25°C obtained when graded amounts of nonradioactive T_4 are added to 2 ml of stock normal pooled sera. A linear decrease was seen with concentrations of T_4 from 0 to 0.4 μg. This permits the use of the calibration curve for determining concentrations of T_4 up to 20 μg/100 ml of serum. This range is quite suitable for practical purposes since higher concentrations are very seldom observed in clinical work.

TABLE 1. EFFECT OF TIME ON BINDING PROPERTIES OF STANDARD SERUM

Age of serum (weeks)	Total TBG binding capacity (μg/100 ml serum)	Free TBG binding capacity (μg/100 ml serum)	*In vitro* binding of T_3 (test/control)
1	12.6	8.7	0.98
2	12.8	8.6	1.02
3	12.5	8.9	0.96
4	12.1	8.5	0.99
5	12.4	8.7	1.03
6	12.7	8.5	1.00
7	12.5	8.8	0.96
8	12.2	8.6	0.97
9	11.2	8.0	0.91
10	10.6	7.6	0.86
11	9.7	7.2	0.81
12	8.5	6.8	0.70

In a series of experiments, aliquots of the same normal diluted sera were assayed periodically for total and free TBG binding capacities and for the binding of T_3 by its proteins (Table 1). These experiments showed that the same stock of normal sera could be used safely for as much as 8 weeks without evident changes in its binding properties; after that time, both total and free TBG binding capacities were found to decrease, probably as a consequence of protein denaturation and therefore the *in vitro* binding of T_3 by serum proteins is proportionally reduced.

Table 2 shows the results of another series of experiments in which samples of the same standard serum were periodically incubated with aliquots of the same stock solution of labeled T_3 and submitted to gel-filtration chromatography. During the first week there was no evidence of deiodination of the radioactive hormone since the fraction of free radioactive iodides remained constant. However, after that time this fraction increased steadily from 3.0% to 7.9% in 2 weeks while the fractions corresponding to protein-bound and free radioactive T_3 decreased proportionally. Nevertheless, because the procedure lets one correct for free radioactive iodide present as a contaminant of the labeled hormone, the final result expressed either as percent of T_3 bound to serum or as the test-to-control ratio remained constant for at least 3 weeks.

Paired estimations of PBI and T_4-iodine concentration were performed in samples of serum from 487 subjects (Table 3). The mean serum PBI level was 5.45 μg/100 ml with 95% confidence limits from 3.0 to 7.9 μg/100 ml while the mean serum T_4-iodine level was 6.31 μg/100 ml with 95% confidence limits from 4.3 to 8.3 μg/100 ml. Both parameters were found raised in the thyrotoxic sub-

TABLE 2. EFFECT OF TIME ON ^{131}I-TRIIODOTHYRONINE STOCK SOLUTION

Age of T_3 solution (days)	Free T_3 fraction (%)	Bound T_3 fraction (%)	Total T_3 fraction (%)	Free I^- fraction (%)	Total ($T_3 + I^-$) (%)	Test (%)	Control (%)	Test / control
0	17.5	79.4	96.9	3.1	100.0	82.0	83.2	0.98
2	16.9	79.7	96.6	3.4	100.0	82.5	82.5	1.00
4	16.3	80.9	97.2	2.8	100.0	83.2	82.2	1.01
6	17.5	79.5	97.0	3.0	100.0	81.2	83.6	0.97
8	15.4	81.4	96.8	3.2	100.0	84.1	82.8	1.02
10	19.2	77.0	96.2	3.8	100.0	80.0	83.1	0.96
12	14.8	80.6	95.4	4.6	100.0	84.5	82.8	1.02
14	15.9	78.9	94.8	5.2	100.0	83.2	85.1	0.97
16	13.9	80.1	94.0	6.0	100.0	85.2	80.7	1.06
18	17.4	75.9	93.3	6.7	100.0	81.3	84.2	0.96
20	15.8	76.3	92.1	7.9	100.0	82.8	80.1	1.03

jects and in the normal pregnant women, while they were found reduced in the hypothyroid and in the nephrotic individuals. In every instance the T_4-iodine level was higher than the PBI concentration. Notwithstanding, Fig. 2 shows a highly significant positive correlation between the results obtained with both procedures (Correlation coefficient, r = 0.905).

One hundred and four patients were suspected of being contaminated with iodine and were not included in this correlation analysis. The data are analyzed in Table 4. When the patients considered to be contaminated with iodine-containing drugs were excluded (Table 4, top), the T_4-iodine level was compatible with the final diagnosis in 98.2% of cases, whereas the PBI concentration was compatible in 93.6%. However, when these patients were included (Table 4, bottom), the T_4-iodine concentration was consistent with the final diagnosis in 97.4% of cases, while the PBI was consistent only in 83.5%.

The good reproducibility of the procedure was shown by 40 triplicate determinations (Table 5). The range of differences of these triplicate analyses was from 0.0 to 1.8 μg of T_4-iodine/100 ml of serum (mean 0.58 ± 0.44).

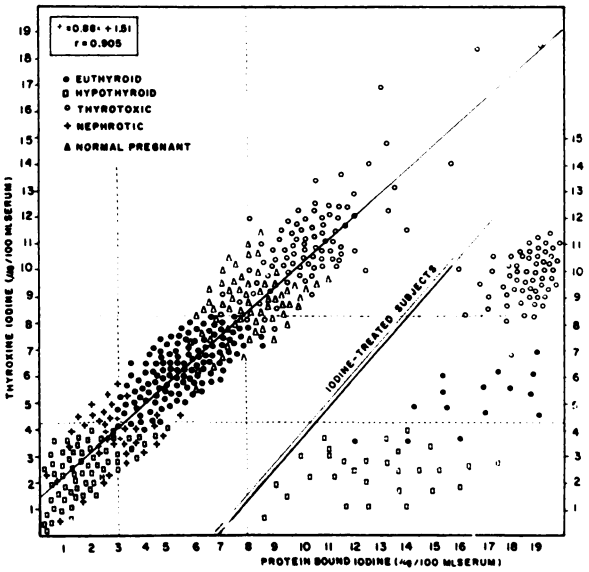

FIG. 2. Correlations found between protein-bound iodine and thyroxine iodine concentrations in sera from 487 subjects. Individuals suspected of being contaminated with iodine-containing drugs were excluded from statistical analysis and are shown in lower-right corner.

TABLE 3. PAIRED ESTIMATIONS OF PROTEIN-BOUND IODINE CONCENTRATION AND THYROXINE IODINE LEVEL IN SERA FROM 381 SUBJECTS*

Clinical group	No. of subjects	Protein-bound iodine (μg/100 ml of serum) Mean	Protein-bound iodine (μg/100 ml of serum) Standard deviation	Thyroxine iodine (μg/100 ml of serum) Mean	Thyroxine iodine (μg/100 ml of serum) Standard deviation
Euthyroid†	120	5.45	1.33	6.31	1.10
Hypothyroid	69	1.70	1.07	2.51	0.99
Thyrotoxic	90	10.53	1.90	11.14	1.80
Nephrotic	42	2.83	1.14	3.77	1.13
Normal pregnant	60	7.98	1.15	8.81	1.13

* Patients suspected of being contaminated with iodine-containing drugs were excluded.

† 95% confidence limits: for PBI 3.0–7.9; for T_4-iodine 4.3–8.3.

TABLE 4. RELATIONSHIP OF PROTEIN-BOUND IODINE AND THYROXINE IODINE LEVELS TO THYROID STATUS

Relation to normal limits	Protein-bound iodine: Hypothyroid	Protein-bound iodine: Euthyroid	Protein-bound iodine: Hyperthyroid	Thyroxine iodine: Hypothyroid	Thyroxine iodine: Euthyroid	Thyroxine iodine: Hyperthyroid
Cases suspected of iodine contamination excluded						
Above	0	4	87	0	4	119
Within	11	116	3	0	116	1
Below	58	0	0	69	0	0
Compatible	84.2%	96.6%	96.6%	100.0%	96.6%	98.9%
Incompatible	15.8%	3.4%	3.4%	0.0%	3.4%	1.1%
Total compatible		93.6%			98.2%	
Total incompatible		6.4%			1.8%	
Cases suspected of iodine contamination included						
Above	29	20	146	0	1	146
Within	11	116	3	0	129	3
Below	58	0	0	98	6	0
Compatible	59.2%	85.3%	97.9%	100.0%	94.9%	97.9%
Incompatible	40.8%	14.7%	2.1%	0.0%	5.1%	2.1%
Total compatible		83.5%			97.4%	
Total incompatible		16.5%			2.6%	

To assess the specificity of the method for T_4, paired estimations of T_4-iodine and PBI concentration were performed in samples of serum from 40 selected subjects before and after the administration of iodine-containing compounds (Fig. 3). Daily oral administration of 0.5 gm of KI during 1 week greatly increased PBI levels but caused no effect on the T_4-iodine concentration. Similar results were obtained with the administration of a single conventional dose of radiological iodinated contrast media (Telepaque, Hypaque, Pantopaque) and with the administration of mono- and diiodotyrosine (MIT and DIT), for a week. Daily administration of 100 μg of l-triiodothyronine for 10 days increased the PBI concentration in the serum from every subject studied but had quite a different effect on the T_4-iodine level; it decreased T_4-iodine concentration in sera from euthyroid subjects and had no evident effect on this parameter in sera from thyrotoxic patients. L-thyroxine and D-thyroxine administration, on the other hand, increased both PBI and T_4-iodine levels in every subject studied.

TABLE 5. ANALYSIS OF 40 TRIPLICATE ESTIMATIONS OF THYROXINE IODINE

Clinical diagnosis	Subjects	Thyroxine iodine (μg/100 ml of serum) I	II	III	Max. difference (μg/100 ml of serum)
Euthyroidism	1	5.5	5.5	5.5	0.0
	2	5.6	5.5	5.6	0.1
	3	5.7	5.4	5.3	0.4
	4	4.7	4.6	5.2	0.5
	5	4.6	4.3	5.1	0.5
	6	4.3	4.1	4.9	0.6
	7	4.4	4.4	5.2	0.8
	8	5.6	5.8	6.4	0.8
	9	6.2	6.4	5.3	0.9
	10	6.8	6.4	5.8	1.0
	11	6.7	6.6	7.7	1.0
	12	6.0	6.2	7.1	1.1
	13	6.8	6.3	7.5	1.2
	14	6.5	6.0	7.2	1.2
	15	6.8	5.8	5.5	1.3
Thyrotoxicosis	16	9.0	9.1	8.9	0.2
	17	9.3	9.0	9.4	0.4
	18	9.7	9.5	10.2	0.7
	19	10.0	9.8	10.5	0.7
	20	10.2	10.6	9.3	0.9
	21	10.4	10.9	9.6	1.3
	22	12.0	11.1	10.2	1.8
Hypothyroidism	23	0.5	0.5	0.4	0.1
	24	0.8	0.6	0.9	0.3
	25	0.9	0.5	0.9	0.4
	26	1.2	1.0	0.8	0.4
	27	1.7	1.5	2.1	0.6
Pregnancy	28	7.4	7.4	7.4	0.0
	29	7.9	7.9	7.9	0.0
	30	8.3	8.2	8.3	0.1
	31	8.4	8.6	8.8	0.4
	32	9.0	8.7	9.4	0.7
	33	9.2	9.0	8.5	0.7
	34	9.9	9.9	8.9	1.0
	35	10.3	10.2	11.4	1.1
Nephrosis	36	0.8	0.8	0.8	0.0
	37	0.9	0.9	0.9	0.0
	38	1.2	1.0	1.1	0.2
	39	1.4	1.5	1.2	0.3
	40	1.6	1.4	1.8	0.4
Mean		5.95	5.82	5.97	0.6
Standard deviation		3.38	3.39	3.32	0.4

DISCUSSION

The estimation of the thyroid hormone level in serum is usually thought to be the most useful single laboratory aid in evaluating thyroid function. The traditional procedure for this was based on the chemical determination of iodine in serum protein precipitates (*7*). This well-known method has been widely used for a long time, but it has the great disadvantage of being affected by contamination from iodides and other iodinated compounds (*8–11*). Since only rare organic iodides do not hamper the butanol-extractable iodine (BEI), this estimation is occasionally of assistance.

Attempts have been made to develop new techniques for the direct measurement of T_4 level in serum, but most of them are based on the chemical estimation of the iodine content of T_4 previously isolated from other iodinated compounds by thin-layer chromatography (*12*), solvent partition (*13*) or gel-filtration chromatography (*14*), and their methodologies are far from being suitable for a conventional radioisotope unit.

Nowadays most of the thyroid-function tests are performed by the radioisotope laboratories where every step of the metabolic cycle of iodine can be studied easily by the radioactive isotopes of the element. Actually, thyroid diagnosis is probably the largest activity of nuclear medicine, and it should be completed with a simple, accurate, economical and fast method for estimating thyroid hormones in serum.

Ekins (*15*) had this concern as early as 1960 and developed a method for estimating T_4 in serum by saturation analysis in which T_4 was first extracted three times into butanol and then measured electrophoretically by the shift of radioactive T_4 from TBG to the albumin fraction.

More recently, Murphy and Pattee (*1*) described a simpler and quicker method based on the same principle, in which the T_4 content is first extracted from the serum sample with ethanol and then measured according to its effect on the *in vitro* binding of labeled T_4 by serum proteins studied by gel-filtration chromatography. Later the same authors replaced this procedure by mixing the standard

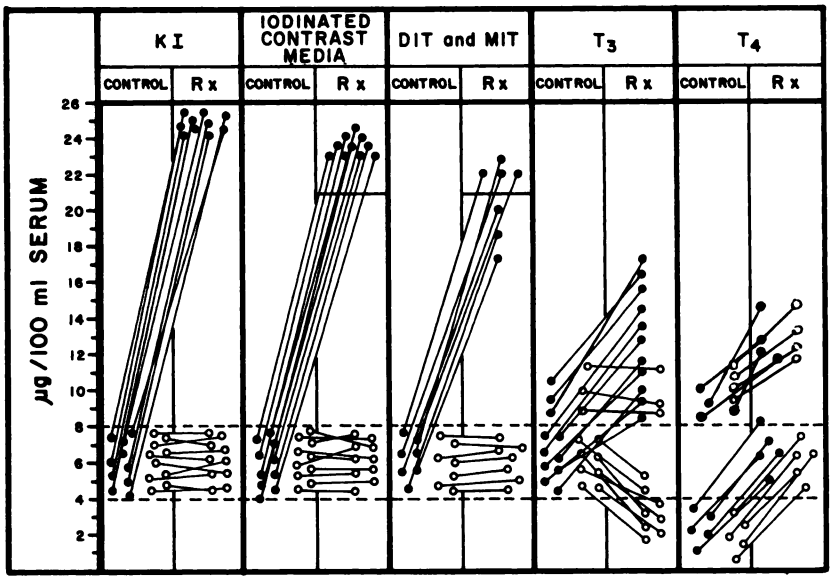

FIG. 3. Specificity of procedure for estimating thyroxine iodine concentration in serum. Effects of potassium iodide (KI), radiological iodinated contrast media (Telepaque, Hypaque, Pantopaque), mono- and diiodotyrosine (MIT, DIT), triiodothyronine (T_3) and thyroxine (T_4) on protein-bound iodine concentration and thyroxine iodine level in serum.

serum and the radioactive T_4 with an anion-exchange resin (*16*). Nakajima and associates (*2*) tried successfully to improve the technique by using a resin sponge and labeled T_3 instead of radioactive T_4.

We have made a compromise between these procedures. We have also taken advantage of the thyroid hormone binding properties of serum proteins, but we have used gel-filtration chromatography instead of a resin sponge and ^{131}I-tagged T_3 in place of labeled T_4. The advantages of these modifications will be discussed.

It has been shown elsewhere (*3,4,17–20*) that it is possible to estimate the binding of labeled T_3 by serum proteins by means of gel-filtration chromatography, eliminating some disadvantages of the use of erythrocytes, as the resin method does, but also allowing correction for free radioactive iodides present as contaminants. Previous experiments with this method (*17,18*) have shown that due to the higher affinity of thyroxine-binding proteins for T_4 than for T_3, when the concentration of T_4 is artificially raised in serum, the binding of T_3 by serum proteins decreases as this hormone is displaced from TBG to other weaker binding fractions. These experiments showed that for a given individual the binding of T_3 by serum depends on variations in T_4 level in serum, but also that their values are not necessarily the same for a given concentration of T_4 in different sera. Since the only two known factors directly influencing these values are the concentration of thyroid hormones in serum and the binding capacity of serum proteins, these particular variations are related to individual differences in T_4-binding capacity of the serum proteins. Nevertheless, if the T_4-binding capacities of serum are constant, as is so when aliquots of the same serum are used, the *in vitro* binding of labeled T_3 by serum depends only on variations on T_4 concentration. Thus, a standard calibration curve can be plotted, relating the *in vitro* binding of T_3 by the proteins in that particular serum and its T_4 concentration (Fig. 1).

However, a given stock of serum should not be used for more than 8 weeks because its proteins undergo denaturation in that span of time and a diminution in its binding capacities for the thyroid hormones ensues (Table 1). When changing to a new stock of serum it is necessary to draw a new calibration standard curve because its binding properties may be different to those of the previous one.

The stock solution of labeled T_3 remains stable for 1 week but at that time starts to release iodine probably by radiolysis (Table 2), increasing the fraction of radioactive iodides in the solution. However, gel-filtration chromatography separates free iodides from bound and free T_3, allowing correction for free radioactive iodides present as contaminants so the results are unaffected by them (Table 2). This correction is not possible when the resin sponge method is used. In this case the presence of large amounts of both organic or inorganic ^{131}I in serum from therapy patients quite often invalidates the results (*21*) and variations in the proportions of inorganic ^{131}I in the radioactive T_3 solution may alter the uptake by the resin because inorganic iodides are almost 100% bound by it (*22*).

The values obtained in this investigation for PBI and T_4-iodine concentrations in five different clinical groups (Table 3) are very similar to those reported

previously by others using diverse methods (*1,2,7, 12–14,16*). Both procedures had a very significant positive correlation when paired experiments were performed in sera from 381 patients not contaminated with iodinated compounds (Fig. 2). Since T_4-iodine concentration was corrected by the efficiency of the ethanolic extraction, its values were consistently higher than those of the corresponding PBI level, because this method does not normally include such a correction (Table 3).

When the patients known to be contaminated by iodine-containing drugs are excluded from the statistics, the overall diagnostic accuracy for both procedures were similarly high (93.6% and 98.2%), but when these patients are included, the overall diagnostic accuracy decreases to 83.5% for the PBI and to 97.4% for T_4-iodine concentration (Table 4).

The high incidence of observed invalidation of the PBI estimation due to iodine, i.e. 104 cases in 485 or 21.4%, testifies to the need of a new thyroid-function test not influenced by the ingestion of iodine-containing drugs. However, it should be noted that this incidence is not representative of the sample population usually sent to our nuclear medicine unit since it includes 42 nephrotic patients and 50 pregnant women specially selected for this study. If these subjects are excluded, the normal incidence of PBI invalidations due to iodine in our hospital increases to 104 cases in 383 or 27.1%.

A good reproducibility of the T_4-iodine estimation procedure was shown in 40 triplicate determinations (Table 5), and the specificity of the method was attested in 40 subjects where PBI and T_4-iodine concentration were estimated before and after the administration of iodides, iodinated radiological contrast media, thyroid hormone and its iodinated precursors, i.e. MIT and DIT (Fig. 3). This is a demonstration of the usefulness of this procedure in the control of treatment of hypothyroid patients with thyroid hormones and in the diagnosis of thyroid disease in iodine-treated subjects.

In previous work (*23*) we have shown that the index obtained by dividing the PBI concentration by the *in vitro* binding of T_3 by serum proteins studied by gel-filtration chromatography is directly correlated to free T_4 concentration in serum estimated by serum dialysis. Since free T_4 is the direct reflection of thyroid status, to get a better insight of thyroid function a similar index can be obtained by dividing the T_4-iodine concentration by the *in vitro* binding of T_3 by serum proteins. These estimates are performed in the same radioisotope laboratory with a similar methodology and can be obtained simultaneously.

Although based on the same principle, the procedure described here seems to be more accurate than the method proposed by Nakajima (*2*) since gel-filtration chromatography allows correction for free radioactive iodides contaminating the T_3 solution, the patients' serum or the glassware. It definitely seems to be more economical than the methods reported either by Murphy (*1,17*) or by Nakajima (*2*). Sephadex columns are cheaper and can be used over and over for hundreds of estimations, while the resin sponge is very expensive and can only be used once. The procedure described by Murphy (*1,17*) needs the use of labeled T_4, while the procedure herein described uses the same radioactive hormone used in the conventional *in vitro* thyroid function tests and consequently does not increase costs by requiring the purchase of a wider variety of radiopharmaceuticals.

We conclude that the procedure described here, a compromise between two previously reported methods (*1,2,17*), offers very special features for a small nuclear medicine unit.

SUMMARY

A new method for estimating thyroxine concentration in serum based on the saturation of the thyroxine-binding proteins is presented. This procedure was assessed in sera from 487 patients and proved to be simple, accurate, economical and fast. It is not affected by iodine contamination since it is not based on chemical estimations of this element. It offers a direct measurement of thyroxine and is specific for this hormone. Its methodology is suitable for a small nuclear medicine unit equipped with a conventional well scintillation counter since it does not involve any chemical procedure and is simple enough to be performed by any person used to basic radioisotope methodology. It is a low-priced method compared with those previously reported since a Sephadex column is cheaper than the resin sponge and the same column can be used several times during a long period of time. Furthermore, this procedure uses the same labeled hormone used by the traditional *in vitro* thyroid tests and consequently does not increase costs by requiring the purchase of a wider variety of radiopharmaceuticals. It seems to be more accurate than the methods based on the use of a resin sponge because this procedure automatically excludes the radioactive iodide contaminating the stock solution of labeled triiodothyronine. Since it is an *in vitro* test, it does not expose the patient to ionizing radiation. Finally, its overall diagnostic accuracy is higher than that of the protein-bound iodine estimation when used during routine clinical work due to the high incidence of

invalidation of protein-bound iodine estimation caused by iodine ingestion.

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