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RAPID DETERMINATION OF THYROXINE BINDING PROTEINS OF HUMAN SERUM

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Abstract: A simple method is described for determining thyroxine binding proteins in human serum by electrophoresis at pH 8.6, using cellulose acetate membrane as the supporting medium. The procedure had high reliability in sera of normal subjects, pregnant women and patients with decreased thyroxine binding capacity of thyroxine binding globulin.

The T_3-resin sponge uptake test (T_3-RSU) (1) and T_4-resin sponge technique (T_4-RST) (2) for determining serum thyroxine concentrations have been widely employed. The former depends on the principle that circulating thyroxine is bound to thyroxine binding globulin whereas added ^{131}I-triiodothyronine is less firmly bound to the same protein. Therefore, radiothyronine unbound to protein is taken up by exogeneous materials. The T_4-RST is based on *in vitro* competition between circulating thyroxine extracted with ethanol and tracer ^{125}I-thyroxine for binding sites of exogeneous thyroxine binding globulin. The resin sponge acts as a secondary binding site for unbound thyroxine.

A discrepancy is sometimes encountered between the procedures. Serum with an abnormal T_3-RSU value and a normal T_4-RST value should be examined for thyroxine binding capacity of thyroxine binding globulin. A decreased or an absence of serum thyroxine binding globulin has been reported since 1959 in certain cases (3, 4, 5). The genetic aspects of this defect have been analysed (6, 7).

Methods for determining thyroxine binding capacity of serum protein have been developed using reverse-flow paper electrophoresis (8, 9, 10), starch gel electrophoresis (9, 10, 11) and Laurell rocket immunoelectrophoresis (12). A simple method of electrophoresis on cellulose acetate membrane at pH 8.9 in Tris-acetate buffer was reported by Marshall and Tompkins (13).

We recently had the opportunity of studing two euthyroid patients who displayed elevated T_3-RSU. The purpose of this paper is to report a new method for rapid determination of thyroxine binding capacity of thyroxine

binding globulin. Decreased capacity was confirmed in the sera of the euthyroid patients. The method is simple to apply in a clinical laboratory with an electrophoretic system for routine serum protein fractionation.

MATERIALS AND METHODS

Subjects. The subjects were 20 euthyroids, 5 pregnant women and 2 euthyroids with increased T_3 RSU. A history of clinical thyroid disease was absent from all subjects.

Blood samples. Blood samples were obtained by venipuncture. The serum was separated and stored at −25°C until use.

Laboratory methods. T_3-RSU and T_4-RST were performed by using commercial products obtained from Abbott Laboratories, Oak Ridge. Tennessee.

^{125}I-labeled thyroxine in 50% propylene glycol (Abbott Laboratories) was diluted with water, extracted with n-butanol and repurified by an extraction procedure (8). Chromatographic analyses of these preparations revealed that thyroxine-^{125}I comprised from 85-90% of total radioactivity. Thyroxine-^{125}I thus purified was diluted to 3.87μg/316μCi/ml with cold L-thyroxine. The purity of L-thyroxine (Wako-Junyaku, Osaka) was verified in a silica gel TLC system of n-butanol : acetone : methanol : 2N-NH_4OH (3 : 5 : 1 : 1).

Assay of thyroxine binding capacity. Five microliters of ^{125}I-thyroxine solution were added to 50μl of serum and incubated for 60 minutes at 25°C. After incubation 1μl of the incubation mixture was applied to a cellulose acetate membrane (Separax, Jōko-Sangyo, Osaka), 1.0 cm×6.0 cm and electrophoresed in Veronal buffer at pH 8.6 with an ionic strength of 0.06 at a constant current of 0.473 mA for each strip for 30 minutes (14). After electrophoresis, proteins on the strip were stained by Ponceau 3R solution (Ponceau 3R : trichloroacetic acid : water, 0.8g : 6g : 100 ml) (14). Each protein fraction was removed, and the radioactivity was counted in an Aloka well-type scintillation counter.

Autoradiographs were prepared by overnight exposure to Fuji X-ray films. Their radioactivity on the strips was measured with an endwindow Geiger-Muller counter, employing a continuous-recording, counting-rate meter.

RESULTS

TBG was separated from thyroxine binding albumin (TBA) and thyroxine binding prealbumin (TBPA) by electrophoresis on cellulose acetate membrane in barbital buffer at pH 8.6 (Fig. 1). Radioactive-thyroxine distributions in serum are shown in Fig. 2. Radioactive bands corresponding to TBG, TBA and TBPA were present. Radioactive recovery after electrophoresis was 98% and the recovery after protein staining was 97%. The radioactivity of the protein fraction was counted after cutting out the bands.

Reproducibility of the method was determined by ten retests of the same sample for TBPA, TBA and TBG. The results are shown in Table 1.

When increasing quantities of thyroxine were added to the reaction

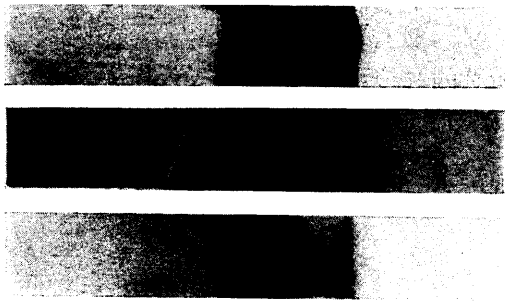

Fig. 1. Autoradiograph of thyroxine-binding proteins separated by cellulose acetate membrane electrophoresis. Anode is at the right. 1, normal serum; 2, serum from patient A; 3, serum from a pregnant woman.

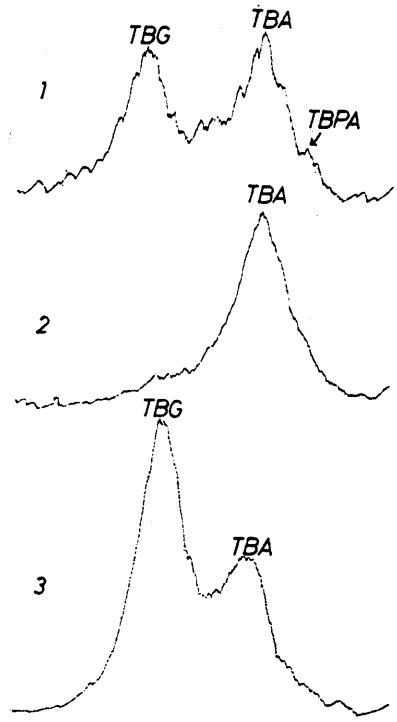

Fig. 2. Comparison of the distribution of ^{125}I-thyroxine in serum. Anode is at the right. 1, normal; 2, patient B; 3, a pregnant woman.

TABLE 1 REPRODUCIBILITY OF THE RAPID METHOD FOR DETERMINING HUMAN THYROXINE BINDING PROTEIN AT pH 8.6

	Fraction radioactivity (%)		
Retest no.	TBPA	TBA	TBG
1	4.8	49.6	45.6
2	4.1	51.9	45.0
3	3.0	51.3	45.7
4	3.9	51.2	44.9
5	5.0	50.0	45.0
6	4.1	50.7	45.2
7	3.0	51.3	45.7
8	4.0	50.8	45.2
9	3.9	49.9	46.2
10	3.0	51.7	46.3
Mean	3.8	50.7	45.5
S.D.	0.67	0.89	0.67

TABLE 2 DISTRIBUTION OF ^{125}I-THYROXINE IN NORMAL SERUM BY THE PRESENT ASSAY METHOD

Subjects	Fraction radioactivity			^{125}I-thyroxine (ng)*
	TBPA (%)	TBA (%)	TBG (%)	
1	5.4	45.4	49.2	
2	7.1	42.8	50.1	69.2
3	6.1	50.0	43.9	
4	8.2	42.6	49.2	67.9
5	7.4	44.1	48.5	70.0
6	5.0	42.1	52.9	77.9
7	5.9	50.0	44.1	
8	7.0	46.2	46.8	
9	5.4	40.8	53.8	63.4
10	5.4	58.7	35.9	
11	5.1	50.8	44.1	68.4
12	4.3	46.3	49.4	70.7
13	5.6	43.4	51.0	70.3
14	4.6	49.5	45.9	
15	5.9	49.3	44.8	64.1
16	4.6	44.4	51.0	
17	5.4	45.8	48.8	75.6
18	3.7	47.2	49.1	
19	6.5	39.2	54.3	
20	4.2	43.5	52.3	
Mean	5.6	46.2	48.2	69.8
S.D.	1.1	5.1	4.3	4.2

*The amount of ^{125}I-thyroxine in the TBG fraction per mg of α_1-globulin on available subjects.

TABLE 3 DISTRIBUTION OF ^{125}I-THYROXINE IN SERUM OF PREGNANT WOMEN BY THE PRESENT ASSAY METHOD

Case	Fraction radioactivity			^{125}I-thyroxine (ng)*
	TBPA (%)	TBA (%)	TBG (%)	
1	2.7	27.3	70.0	
2	7.5	31.3	61.2	78.2
3	4.0	16.0	80.0	96.2
4	5.6	20.4	74.0	82.6
5	4.3	30.4	65.3	
Mean	4.8	25.1	70.1	85.7
S.D.	1.7	7.8	2.1	

*The amount of ^{125}I-thyroxine in the TBG fraction per mg of α_1-globulin on available women.

mixture for patient A, a TBG plateau was obtained at a concentration of 0.2μg/ml of thyroxine, while the concentration of thyroxine bound to TBG of a normal subject was still increasing (Fig. 3).

Radiothyroxine distributions in serum obtained from normal subjects and pregnant women are shown in Tables 2 and 3, respectively. Data from two patients with discrepancies between T_3-RSU and T_4-RST are shown in Table 4. ^{125}I-thyroxine found in the TBG fraction per mg of α_1-globulin is shown in Table 2, 3 and 4. The amount bound was in the following order: pregnant women, euthyroids and the patients.

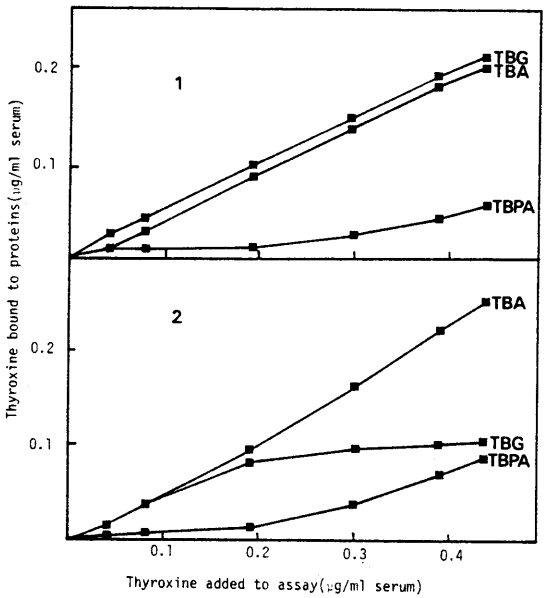

Fig. 3. Distribution of ^{125}I-thyroxine in serum protein as a function of radiothyroxine added to the assay. 1, normal serum; 2, serum from patient A.

TABLE 4 ABNORMAL SERUM DISTRIBUTION OF ^{125}I-THYROXINE BY THE PRESENT ASSAY METHOD AND THE T_3-RSU AND T_4-RST VALUES BY ROUTINE CLINICAL LABORATORY METHODS

Patient	Fraction radioactivity			^{125}I-thyroxine (ng)*	T_3-RSU (%)	T_4-RST (μg/100ml)
	TBPA (%)	TBA (%)	TBG (%)			
A	17.5	55.8	26.7	43.5	48.3	8.4
B	5.1	83.2	11.7	20.2	56.3	4.0

*The amount of ^{125}I-thyroxine in the TBG fraction per mg of α_1-globulin.

DISCUSSION

A clinical laboratory method is described for the rapid determination of thyroxine binding capacity of serum protein. This method uses the same electrophoretic system as that in routine laboratory procedures for serum protein fractionation. TBPA, TBA and TBG were separated (Fig. 1 and 2). The recovery of added radiothyroxine was 97% even after protein staining. The reliability of the method was confirmed for clinical usage (Table 1).

Marshall and Tompkins (13) described a method using an electrophoretic system on cellulose acetate membrane at pH 8.9, in which thyroxine binding proteins were separated, but electrophoresis at this pH is not usually performed in the clinical laboratory.

The method described in this paper was developed primarily to determine thyroxine binding capacity of TBG when a discrepancy between T_3-RSU and T_4-RST was found in serum. The capacity of TBG could be calculated after reaching a plateau from the following equation: capacity $=(a+b)\times c$, where a is the ^{125}I-thyroxine added to the assay, b is the endogeneous thyroxine determined by T_4-RST and c is the percentage of ^{125}I-thyroxine found in TBG fraction.

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