COMPARISON OF RADIOISOTOPIC AND COLUMN CHROMATOGRAPHIC ASSAY OF SERUM THYROXINE

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The determination of serum thyroxine has been widely accepted as a diagnostic test to evaluate the thyroid status of patients. At present the assays are usually carried out by column chromatography, which was originally reported by Pileggi *et al* and is based on the colorimetric determination of iodine (*1,2*). Recently a radioisotopic method of thyroxine assay based on competitive binding of nonradioactive and radioactive thyroxine to the binding sites of thyroxine-binding globulin has been developed (*3*). This method is reported to be specific for thyroxine and is not influenced by the presence of exogenous iodine or mercury. However, the comparison of this radioisotopic method with other methods of thyroxine assay has not been reported. The purpose of this study is to compare the column-chromatography and the radioisotopic method of thyroxine assay in the same sera and to evaluate the clinical value of the radioisotopic method.

MATERIAL AND METHODS

Sera were collected from 40 normal controls (21 males and 19 females) and 68 patients with various thyroid diseases. Among these patients 32 were finally diagnosed as hyperthyroid and 15 as hypothyroid on the basis of clinical evaluation and other thyroid-function tests. The other 21 patients were euthyroid but had some evidence of various thyroid abnormalities. The cases in which abnormality of thyroxine-binding globulin was suspected were excluded from the present analysis. In all of these collected sera, thyroxine was determined by both the column and the radioisotopic method. Assay using the column method was done as described by Pileggi (*1*) with modifications in the size of the column (*2*). Radioisotopic assay was performed using a commercially available assay kit (Tetrasorb, Abbott). The technique is essentially the same as described by Murphy (*3*) except for the use of a resin sponge to separate bound and free thyroxine.

Extraction of thyroxine from serum was carried out with absolute ethanol (U.S. Industrial Chemical Co.). One milliliter of serum was mixed with 2 ml of ethanol using a vortex mixer and then centrifuged to precipitate protein. The extraction efficiency using radioactive thyroxine was 77.2 ± 2.5%. The figure 77.2% was used to correct the extraction efficiency throughout the study.

The standard curve was constructed in two ways using standard thyroxine. With one set of standards, various amounts of standard thyroxine in ethanol were treated in the same way as the serum extract and the uptake of radioactivity by resin was calculated. With the other set, the effect of a "plasma blank" was tested using the serum which was passed through the resin column in the column method. Because the adsorption of thyroxine by this resin column, tested by radioactive thyroxine, was 99 ± 2%, it was assumed that this serum did not contain any thyroxine. The pH of the serum collected from the column was adjusted to 7.4 using hydrochloric acid. One milliliter of this serum was then mixed with 2 ml of ethanol and centrifuged; 0.3 ml of the extract was added to the second set of test-tubes with standard thyroxine. The results of the effect of this thyroxine-free serum on the standard curve are shown in Table 1. There was no difference in resin uptake of radioactivity between the set with standard thyroxine and the set to which serum extracts were added. The standard curve constructed with standard thyroxine was used for subsequent analyses.

RESULTS

Serum thyroxine values measured with the radioisotopic method are shown in Table 2.

Since the distribution of thyroxine values among control group showed some skewness, the normal range was defined as 5.6 to 12.6 from the 2-s.d.

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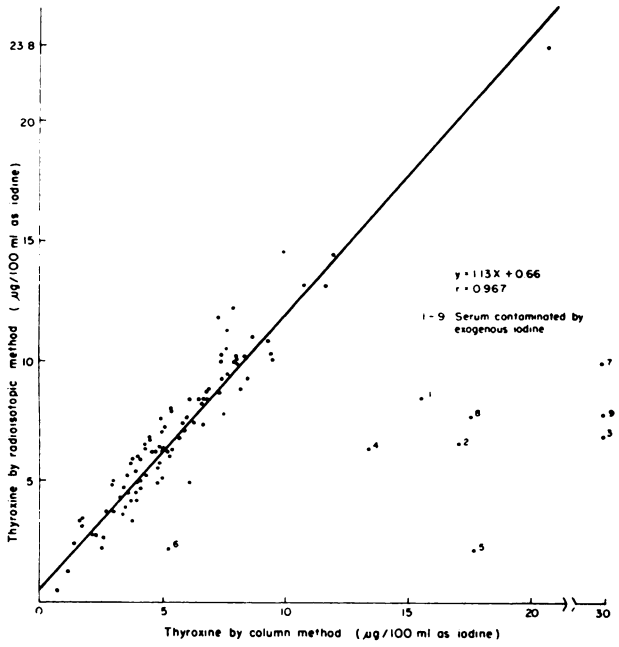

FIG. 1. Correlation between thyroxine determination by column method and radioisotopic method. Results of both methods are expressed as μg/100 ml of *iodine* to show relation between two methods. Circles with figures represent sera in which exogenous iodine was detected from elution pattern of column as well as from patient's history.

range of logarithmic transformation of thyroxine values of control group. By defining the normal values in this range, all hyperthyroid patients except two showed abnormally high serum-thyroxine values, and all hypothyroids except one showed abnormally low values. Two normal controls showed abnormally high values. In these two patients, no evidence of abnormality of thyroxine-binding globulin was detected.

The correlation of the radioisotopic method and column method of thyroxine assay is shown in Fig. 1. The radioisotopic determination showed slightly higher values compared with those determined by the column method. [Regression line: values by column method $= 1.13 \times$ (values by isotopic method) $+ 0.66$]. In the figure both results are expressed as micrograms of iodine per 100 ml. Even though a slight difference in the values is apparent, the correlation of the values by the two methods was satisfactory except in nine cases in which exogenous iodine was believed to be present because of the patients' history and the elution patterns of the column. The final diagnosis of thyroid state of these nine patients is shown in Table 3. In each case the thyroxine value determined by the radioisotopic method was lower than that determined by the column method and clinical evaluation of the thyroid state agreed with the results determined by radioisotopic methods. The correlation coefficient between the values obtained by the two methods excluding these nine patients was calculated to be 0.967.

DISCUSSION

In thyroid-function tests the contamination from exogenous iodine often causes a serious problem in interpreting the results. Thyroxine assay by the column method has the advantage that one can detect the presence of exogenous iodine from the elution pattern. But even with this method the accurate assay of thyroxine cannot be performed in the presence of exogenous organic iodine. The radioisotopic assay of thyroxine based on the competitive binding of radioactive and nonradioactive thyroxine to thyroxine-binding globulin is reported to be specific for thyroxine and to have no interference from exogenous iodine. Our results confirmed this in nine cases of iodine-contaminated sera. In all these cases the clinical diagnosis agreed with the results of radioisotopic thyroxine determination even when the amount of iodine was as high as 59 μg/100 ml.

Our results also showed that the radioisotopic thyroxine assay can be a good clinical test for the diagnosis of thyroid status. Although there was some overlapping between normal control and hyper- or hypothyroids, in more than 95% of the cases the results of thyroxine determination agreed well with clinical diagnosis. In the present analysis, however,

TABLE 1. EFFECT OF "PLASMA BLANK" ON STANDARD CURVE

Amount of standard thyroxine added	Uptake of radioactivity by resin	
	Thyroxine alone	With "plasma blank"
0	1.40 ± 0.1	1.53 ± 0.2
5 μg	32.0 ± 0.4	33.12 ± 0.4
10 μg	48.2 ± 0.5	49.9 ± 0.6
15 μg	58.9 ± 0.6	58.0 ± 0.8

TABLE 2. RESULTS OF RADIOISOTOPIC THYROXINE DETERMINATION

	No. of cases	Radioisotopic thyroxine determination	
		Mean ± 1 s.d.	Range
Normal control	40	9.0 ± 1.8	5.4 — 12.8
Euthyroids with thyroid abnormalities	21	8.8 ± 1.9	5.2 — 13.0
Hyperthyroids	32	16.1 ± 4.4	11.3 — 36.5
Hypothyroids	15	3.8 ± 1.4	0.7 — 5.8

TABLE 3. RESULTS OF THYROXINE DETERMINATION IN SERA CONTAMINATED WITH EXOGENOUS IODINE

Case No.	Diagnosis	Source of exogenous iodine	Thyroxine by column method	Thyroxine by radioisotopic method
1	Euthyroid	Cholecystography	15.6*	8.4*
2	Euthyroid	Salpingography	17.1	6.5
3	Euthyroid	Bronchography	59.0	6.8
4	Hypothyroid	Myelography ?	13.4	6.3
5	Euthyroid	IVP	17.7	2.0
6	Euthyroid	?	5.2	6.2
7	Euthyroid	Bronchography	32.1	10.0
8	Euthyroid	Cholecystography	17.7	7.7
9	Euthyroid	IVP	30.9	7.7

* μg/100 ml as iodine.

the cases in which abnormality of thyroxine-binding globulin was suspected were excluded. In these cases, the measured serum-thyroxine level can be deviated according to the level of thyroxine-binding globulin. The results of the T-3 resin-uptake test may be helpful in interpreting the results in these cases.

The results of radioisotopic assay of thyroxine showed slightly higher values than those determined by the column method. We first suspected that this might be due to the nonspecific displacement of iodinated thyroxine from thyroxine-binding globulin by some substance in the serum other than thyroxine. The experiments with standard curves using thyroxine-free serum were designed to test this hypothesis. Our results, however, did not show any effect of "plasma blank" on the standard curves. Because the "plasma blank" used in our experiments is the serum passed through the resin column, the effect of some substances that might have been adsorbed to the resin column with thyroxine could not be excluded. The reason for slightly higher values in the radioisotopic assay remains to be investigated.

SUMMARY

Serum thyroxine was determined in 108 sera by both the column and the radioisotopic method based on competitive binding of labeled thyroxine to thyroxine-binding globulin. Radioisotopic assay showed slightly higher values than the column method did. Correlation of the two methods was satisfactory (correlation coefficient = 0.967) except for nine cases in which exogenous iodine was believed to be present. In these nine cases, the results of thyroxine assay by the radioisotopic method agreed with the clinical diagnosis. The normal range for the radioisotopic thyroxine assay was 5.6–12.6 μg of thyroxine/100 ml. Five cases showed some overlapping between the normal and abnormal range, but all other cases showed good agreement with the clinical diagnosis.

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