

## Radioimmunoassay for Serum $T_3$

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A specific antibody to  $T_3$  (1-triiodothyronine) was made in rabbit immunization with  $T_3$  conjugated to bovine serum albumin with carbodiimide.

High specific activity (454 mc/mg) of  $^{125}\text{I}-T_3$  was kindly supplied by Dainabot Lab.

A cross reaction of the antibody for  $T_3$  to L-monoiodotyrosine, L-diiodotyrosine, or L-thyroxine was  $<0.001$ ,  $<0.001$  and  $0.16\%$  respectively.

For the determination of serum  $T_3$  values, two methods were examined.

### (1) In direct method

Ethanol extraction of thyroid hormone ( $T_4$  and  $T_3$ ) from test serum (0.1 ml) was dried, then human IgG (2 mg), 32 pg of  $^{125}\text{I}-T_3$  and anti- $T_3$  antibody (diluted 500 times as a final concentration) were added.

Using the double antibody technique (second antibody; sheep anti-rabbit gamma-globulin), a radioimmunoassay of  $T_3$  capable of detecting the range from 32 to 100 pg has been developed. The slope of the standard curve was abruptly changed between 32 and 600 pg, but became mild from 600 to 1000 pg.

Mean serum concentration in patients were as follows:

euthyroid subjects  $0.96 \pm 0.52$  ng/ml ( $n = 56$ )

hyperthyroid	$4.15 \pm 2.73$ ng/ml ( $n = 17$ )
hypothyroid	$0.49 \pm 0.24$ ng/ml ( $n = 21$ )
Hashimoto's thyroiditis	$0.60 \pm 0.23$ ng/ml ( $n = 8$ )

Thyroid cancer	$0.81 \pm 0.24$ ng/ml ( $n = 4$ )
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### (2) Direct method

By addition of salicylic acid ( $10^{-2}$  M, a final concentration) in test serum (0.1 ml) to inhibit binding of  $T_3$  to thyroxine binding globulin, serum  $T_3$  value was determined directly. The standard curve of a radioimmunoassay that could be detected the same amounts (range, 32–1000 pg) of  $T_3$ , was made using hypothyroid serum that had been removed endogenous  $T_3$  completely by dextran coated charcoal.

Mean serum concentration in patients were as follows:

euthyroid subjects	$1.32 \pm 0.43$ ng/ml ( $n = 51$ )
hyperthyroid	$7.57 \pm 4.49$ ng/ml ( $n = 15$ )
hypothyroid	$0.94 \pm 0.44$ ng/ml ( $n = 14$ )
Hashimoto's thyroiditis	$1.15 \pm 0.16$ ng/ml ( $n = 5$ )

Thyroid cancer	$1.01 \pm 0.14$ ng/ml ( $n = 3$ )
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The determined values by these two methods showed a little difference in various diseases.

## Experimental and Clinical Investigation on Thyopac-4 Test, and Thyopac Free Thyroxine

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The results from the experimental investigation were as follows: 1) the standard curve practically delineated as a straight line was found somewhat superiorly convex; 2) Thyopac-4

Value was little affected by various conditions of measurements; 3) Mean difference between the duplicate values was sufficiently small; 4) Examination time of the test a kit was able to

be less than one hour and so on.

Thyopac-4 and Res-O-Mat T4 test were examined on the same sera of 153 cases. Additionally in 112 cases (55 euthyroid, 26 hyperthyroid, 10 hypothyroid, 18 pregnancy and 3 nephrotic syndrome) of them, Thyopac-3 test was simultaneously performed for the purpose of the investigation on thyopac free thyroxine index (FT4I) calculated as Thyopac-4 value  $\times$  100/Thyopac-3 value.

Thyopac-4 values in various groups were as follows: 1) euthyroid,  $8.5 \pm 2.4$   $\mu$ g/dl (mean  $\pm$  SD); 2) hyperthyroid, ober  $20.0 \pm$  ober  $5.0$ ; 3) hypothyroid,  $2.5 \pm 1.5$ ; 4) pregnancy,  $13.3 \pm 2.4$  and 5) nephrotic,  $4.2 \pm 0.4$ . In the manner, there was almost no overlapping between euthyroid, hyperthyroid and hypothyroid while the values in pregnancy were generally high and

in nephrotic low as compared with euthyroid.

Correlation between Thyopac-4 and Res-O-Mat T4 values was sufficiently estimated (the coefficient of correlation = +0.80) although the former was frequently evaluated to be somewhat smaller than the latter.

Thyopac FT4I in the various groups were as follows: 1) euthyroid,  $7.6 \pm 2.3$ ; 2) hyperthyroid, ober  $27.3 \pm$  ober  $9.6$ ; 3) hypothyroid,  $2.1 \pm 1.1$ ; 4) pregnancy,  $9.8 \pm 2.4$  and 5) nephrotic,  $5.6 \pm 1.2$ . The values in pregnancy and nephrotic were all but inside the normal range which was supposed to be 3.0 to 12.2, and on the other hand in hyperthyroid and hypothyroid outside well. Accordingly, the value may indicate the true thyroid status, and may be a more accurate diagnostic aid than Thyopac-3 or -4 test alone.

## **Studies of Competitive Binding Radioassay Methods for Measurement of Thyroglobulin and Antithyroglobulin Antibodies in Human Serum**

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For the purpose of developing a sensitive solid-state radioimmunoassay for Thyroglobulin (TG) and anti-Thyroglobulin antibody (anti-TG), the purification of anti-TG was performed using an immunoadsorbent technique.

The immunoadsorbent was prepared by coupling purified TG to cyanogen bromide activated sepharose and was packed in a column.

IgG globulin fraction of human or rabbit antisera was passed through the column and the column was washed thoroughly.

Specific anti-TG bound to TG was then eluted with 0.17 M glycine-HCl, pH 2.3 or 4 M NaI.

From the cumulative bound percent of labeled material to a series of ten plastic tubes coated

with TG, eluted anti-TGs were found to have purities of 20 to 50%.

Measurement of anti-TG or TG using these relatively purified anti-TGs were attempted.

Anti-TG were measured by using cups coated with TG in the same fashion as described previously in the case of crude anti-TG IgG.

Using purified antibody IgG the sensitivity of the assay was improved by 10 times as former and 0.03  $\mu$ g of anti-TG IgG could be detected.

For TG determination, known amounts of TG or serum samples were added to the cups coated with crude antibody and were incubated over night at 4°C. After washing, uniform amount of labeled purified anti-TG was poured into