A Study on the Radioimmunoassay of Serum Triiodothyronine

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Preparing antibody for triiodothyronine (T3) by ourselves, the serum T3 concentrations were determined by radioimmunoassay and compared with those obtained with Dainabot’s T3 assay kit.

21 euthyroid normal control volunteers, 30 hyperthyroid patients, 12 hypothyroid patients and 2 patients with TBG deficiency were employed in this study.

The conjugate of T3 to human albumin was made by carbodiimide method of Oliver et al., emulsified in equivalent volume of complete Freund’s adjuvant, then injected into back muscles of rabbit every week and sera were drawn and served for assay successively at adequate intervals.

It was found that the relative activity of T4 to T3 was 0.25%, but there is no effect on T3 concentration when 10 or 20 ng of T4 was added to standard doses of T3 or 1, 10, 20 or 100 ng of T4 to 150 pg of T3. The relative activities of DIT and MIT to T3 were 0.009% and 0.008%, respectively.

The serum T3 concentration in various thyroid disorders was determined in duplicates and the average value was calculated. The assay procedure was similar to the conventional method, except for using 0.1M Veronal buffer of pH 8.6 and T3 free serum treated with ANS and expelling of T3 from TBG with ANS.

The precision and the reproducibility of this assay system were quite excellent, having C.V. of 4.9% for the former, and 8.6% for the latter. The rate of recovery was 127%.

The concentration of serum T3 was 136±12 (mean ±S.D.) ng/100ml for euthyroid normal volunteers, 570±93 ng/100ml for hyperthyroid and 52±10 ng/100ml for hypothyroid patients. In 2 patients with TBG deficiency they were 52 and 84 ng/100ml.

These results were comparable to the values obtained with Dainabot T3 radioimmunoassay kit.

It was concluded that the developed T3 radioimmunoassay system was very reliable and could be used for the routine T3 determination.

Radioimmunoassay of T3

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Radioimmunoassay of T3 in human serum has been studied. Antiserum to T3 was produced in rabbits by injection of a T3-Human serum albumin conjugate.

Cross reactivity of this antibody with T4 was less than 0.1%. T3-125 with high specific activity (600mCi/mg) was obtained, by the isotope exchange method, and 10pg of the labeled T3 was
used for each assay. 8-anilino-1-naphthale sul-
monic acid was used to block T₃-TBG binding.

For the separation of antibody bound T₃ from
free T₃, dextran coated charcoal was used, and
effects of temperature and protein concentrations
on adsorption of charcoal were examined.

From these results, assay conditions of T₃
radioimmunoassay were established and the
minimal detectable amount of T₃ with this sys-
tem was 0.25ng/ml.

The results of 100 serum samples assayed by
this method and double antibody method were
excessly agreed.

No significant difference in assay precision
was found between two methods.

Study of T₃-Radioimmunoassay

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The clinical usefulness of radioimmunoassay
for the determination of serum triiodothyronine
(T3) was studied with a comparative evaluation
of total T4 in various thyroid diseases. Influence
of the intravenous injection of TRH on the T3
was also studied. The assay was run with dupli-
cated method and the “bound” and “free” was
separated by dextrancoated charcoal. Of the
reproducibility of this method, the intraassay
precision was ±8.5 to 9.6% and the interassay
precision was ±3.8 to 4.1% (in low T3 range)
and was ±4.8 to 8.8% (in high T3 range).

In 108 serum samples obtained from 102 pa-
tients, T3 value showed a significant correlation
with total T4, indicating that T3-radioimmunoas-
say was an useful index for thyroid function.

The mean ±S.E. in 11 normal males aged 27yr.
in average was 1.33±0.07 ng/ml and was 1.23±
0.07 ng/ml in 13 normal females aged 26 yr. in
average. On the other hand in 34 aged subjects
from 65 yr. to 91 yr., it was 0.46±0.04 ng/ml,
and was significantly lower (p<0.001) than that
of young normal subjects. The data suggest that
serum level of T3 tends to decrease with aging.

In untreated 19 patients with hyperthyroidism,
the mean ±S.E. was 4.24 ±0.42 ng/ml, whereas
in those with hypothyroidism was 0.75±0.19
ng/ml.

In the study of influence of TRH on the serum
level of T3, the authors could not obtain any
conclusive results because of much individual
variations.