D3. Measurement D (In Vitro Assay, Thyroid and Parathyroid Hormone)

Radioimmunoassay of Thyrotropin Releasing Hormone (TRH) in Human Serum

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Radioimmunoassay of TRH in human serum has been developed. To prevent of inactivation of TRH immunoreactivity by serum, mixture of 8-Hydroxyquinoline and Tween 20 has been used. For extraction of TRH from serum methanol was used. Around 75% of TRH was extracted from one ml of serum by over 4.5 ml of methanol. In this assay system lowest sensitivity was calculated as 5.0 pg/ml. Dilution curve of high TRH serum after synthetic TRH i.v. administration was parallel to standard curve. Recovery experiment revealed 100%.

TRH levels in serum were under sensitivity to 70 pg/ml in normal subjects, were under sensitivity in all hyperthyroid patients, were 40 to 600 pg/ml in primary hypothyroid patients, were 100 to 800 pg/ml in secondary hypothyroid patients and were under sensitivity in all tertiary hypothyroid patients.

In hyperthyroid patients TRH levels in serum during antithyroid drug treatment increased 5.0 pg/ml or more in most cases, but remained under sensitivity in a few cases during observation period.

In primary hypothyroid patients TRH levels in serum during T4 treatment gradually decreased to normal range.

From above data it was suggested that measurement of TRH in serum might be useful tool to study the role of hypothalamo-pituitary -thyroid axis in clinical basis.

Studies on Radioreceptor Assay (RRA) of TSH

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The RRA of TSH is considered to be useful not only in evaluating TSH activity, but also in quantitating and analyzing the mechanism of the action of other abnormal thyroid stimulators, such as LATS and LATS-Protector, which are present in sera of patients with Graves’ disease.

The thyroid gland surgically obtained from patients with Graves’ dis. was homogenized and 10,000 x g fraction was used as the receptor. Human TSH was iodinated by using lactoperoxidase and
was purified by the receptor binding. The receptor (50 mg eq.), purified $^{125}$I-TSH and TSH or unknown samples were incubated at $37^\circ$C for 60 min. in a final volume of 300 $\mu$l. The binding was time and temperature dependent with optimal binding in the above condition. Monovalent cations, such as Na$^+$ or Li$^+$, inhibited the receptor binding. Addition of Ca$^{2+}$ or EDTA also inhibited the binding.

Studies of dissociation kinetics and Scatchard plot indicated that there were two classes of the receptors. High affinity constant was $1.5 \times 10^8$M$^{-1}$. Binding was inhibited by human, bovine and ovine TSH but was not inhibited by Insulin, HCG (50 IU), FSH-LH (1 IU−2.5 IU), PGE1!! (2×10M$^{-4}$), T$_3$ (10$^{-9}$M) and T$_4$ (10$^{-9}$M). Sensitivity was 50 $\mu$U/tube and 50% inhibition was observed at 500 $\mu$U/tube.

IgG prepared from sera containing high LATS activity completely inhibited the receptor binding of $^{125}$I-TSH. On the other hand, some IgG preparations from LATS negative Graves’ sera also inhibited the binding. Therefore this assay system appeared to be useful in evaluating the total thyroid stimulating activity in these sera.

Changes in Thyroxine and Triiodothyronine Concentration in Patients Treated with Radioactive Iodine

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It is well known that many patients treated with radioactive iodine for thyrotoxicosis eventually become hypothyroidism. Several years ago, we researched thyroid function in these patients who had alapsed for many years following$^{131}$I treatment and noticed that a resin sponge uptake of $^{131}$I-triiodothyronine decreased in considerable number of them, although no clinical signs and symptoms of hypothyroidism were detected. For explanation of low T$_3$ uptake test in eumetabolic patients after $^{131}$I treatment, either the status of impending hypothyroidism or so-called T$_3$ euthyroidism was considered.

The widespread availability of RIA for measurement of thyroid hormones now enables monitoring the changes in thyroxine and triiodothyronine following $^{131}$I therapy.

Triiodothyronine(T$_3$) was measured with radioimmunoassay kit, and its normal value was 136±54 ng/dl. (Mean±S.D.). Thyroxine (T$_4$) was determined with Res-O-Mat T$_4$ kit, its normal range was 8.9±2.8 $\mu$g/dl and normal T$_3$/T$_4$ ratio was 1.78±0.64 %.

In three cases, T$_3$, T$_4$ and T$_3$/T$_4$ ratio were measured before therapy at 2, 4, 6, 8 and 15 days following therapy, and there was no significant change.

In 26 cases of thyrotoxic patients, T$_3$, T$_4$ and T$_3$/T$_4$ ratio were monitored prior to therapy and monthly after therapy. T$_3$ changed in parallel to T$_4$ and T$_3$/T$_4$ ratio was influenced by change of T$_3$ than that of T$_4$.

Thyroid hormones were measured in 91 cases who had received $^{131}$I therapy from 1 to 18 years previously and attended to follow-up clinic. Most of them had received 60 $\mu$Ci. of $^{131}$I per gram of