Studies on Radioimmunoassay for Humna Thyrotropin —Some Observations on the Technique—

K. Miyai, M. Fukuchi and H. Abe

The First Department of Medicine

Y. Kumahara

The Central Laboratory For Clinical Investigation, Osaka University Medical School, Osaka

The plasma concentration of human TSH (HTSH) was determined by radioimmunoassay (ethanol-saline precipitation method) using a highly purified HTSH for labelling with ^{131}I or ^{125}I, a potent anti-HTSH serum and Human Thyrotropin Research Standard A. (These materials were given by Dr. W. D. Odell, N.P.A. and N.I.M.R.).

The method was sensitive to as little as 0.2 μU unlabeled HTSH. No effect was observed when HCG, HGH, FSH, ACTH and Bovine TSH were assayed. Dilutions of plasma from hypothyroid patients, highly purified HTSH (Condliffe's HTSH) and crude HTSH (acentone dried human pituitary powder) resulted in parallel curves to that obtained for the Standard HTSH.

Recovery of added HTSH to serum was $102 \pm 22.8\%$. Variation between assays was $\pm 5.4\%$. The HTSH were labeled with radioiodine by the chloramine-T method described by Hunter and Greenwood. Generally 5 μg HTSH and 5 mCi radioiodine have been used for the reaction. After iodination the labeled HTSH was separated from iodide by passage through a column of Sephadex G-75 measuring 0.8×18 cm. Most iodinations were carried out using ^{131}I (specific activities 336~670 mCi /mg) but ^{125}I was used in some experiments (specific activities 225 mCi/mg). The "damaged" labeled HTSH increased 2 to 3 times when ^{131}I or ^{125}I-HTSH was used 3~5 weeks after prepared. Thus, it is necessary to repurify the labeled HTSH by means of gel filtration (Sephadex G-100) when it is used more than one week after preparing.

For these reasons, we prefer to label the TS Hwith ^{131}I (within 24 hours after receiving) and use the ^{131}I-TSH within 1 week after prepared.

Studies on Radioimmunoassay for Human Thyrotropin —Clinical Application—

K. Miyai, M. Fukuchi, T. Matsuoka and H. Abe

The First Department of Medicine

Y. Kumahara

The Central Laboratory For Clinical Investigation, Osaka University Medical School, Osaka

By radioimmunoassay for human thyrotropin (TSH) the plasma levels in various clinical states were determined and its usefullness was reported.

The minimum detectable concentration of plasma TSH was as low as 1.0 μU/ml using H-TSH Research Standard A as standard TSH. Plasma TSH level ranged from undetectable to 2.5 μU/ml in six normal subjects, and the level was within normal range

in pregnant women and patients with nontoxic nodular goiter. The patients with nontoxic diffuse goiter showed the relatively high level of 2.5~7.5 μU/ml and those with Hashimoto's disease who had no apparent sign of hypothyroidism had the higher value of 2.5~20 μU/ml. The markedly high value was obtained in 36 patients with primary hypothyroidism (22~800 μU/ml) had in four with cretinism (200~700 μU/ml); on the other hand undetectable in most patients with panhypopituitarism, Graves' disease and Plummer's disease.

Following administration of thyroid hormone to two patients with primary hypothyroidism, plasma TSH level fell and then increased gradually up to the initial value. This phenomenon clearly indicates that the TSH secretion is regulated by the thyroid hormone. The plasma TSH simultaneously determined by McKenzie's bioassay showed the similar patterns to those obtained by radioimmunoassay.

In two euthyroid subjects, plasma TSH increased after treatment with antithyroid drug for 10~11 days, and decreased to normal value following administration of thyroid hormone. Two patients with Graves' disease with positive LATS but in euthyroid state showed the similar changes in plasma TSH following administration of antithyroid drug and then thyroid hormone, but no significant change was observed in LATS activity. These findings support the view that the feedback relation of pituitary-thyroid axis may remain in the patients with Graves' disease.

Thyroxine Turnover and Transport in Major Non-Thyroidal Illness

M. Inada, Y. Kazama, H. Takayama, T. Nakagawa and K. Torizuka

Endocrine Section, Department of Internal Medicine, Tenri Hospital, and Second Division, Department of Interal Medicine, and Central Radioisotope Service, Kyoto University, School of Medicine, Kyoto

Several laboratories have observed elevations of serum free thyroxine in patients with non-thyroidal illnesses, which was attributed to dimination of TBPA. No consistent abnormality of TBG has been reported.

Studies of the metabolism of thyroxine in patients with major non-thyroidal illnesses, mainly advanced malignancies, revealed a variety of deviations from normal. In additional to radiothyroxine turnover studies, the maximal binding capacities of TBG and TBPA have been reinvestigated by reverse flow paper electrophoresis in a glycine acetate system at pH 8.6. Free thyroxine was measured by the magnesium precipitation method. The following groups were studied: 8 "hospital controls", and 24 patients who divided into 9 "moderately ill" and 15 "severely ill" cases, based upon the clinical picture.

The "moderately ill" patients had variably diminished TBPA binding capacity in their sera but the TBG binding capacity was normal. The free thyroxine fraction was also normal; as expected, thyroxine half time did not differ significantly from the hospital control group.

TBPA was markedly reduced in the "severely ill" group (118 ± 28 μg per 100 ml, vs 244 ± 23 μg per 100 ml in the controls, $p<0.001$). TBG binding capacity was diminished in the "severely ill" group (16.7 ± 4.0 μg per 100 ml vs 19.9 ± 2.0 μg per 100 ml in the controls, $p<0.05$). The free thyroxine fractions were elevated in the "severely ill" group ($0.066 \pm 0.03\%$ vs $0.041 \pm 0.006\%$ in the controls, $p<0.05$).

The free thyroxine fraction bore a more significant inverse relation to the binding capacity of TBG ($\gamma = -0.66$, $p<0.001$) than that of TBPA ($\gamma = -0.44$, $p<0.05$). In the present group of sera, therefore, it was evident that TBG was the more important determinant.

The biological half-time of thyroxine turn-