

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

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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 $0.2 \mu\text{U}$ 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 (acetone 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 radio-

iodine by the chloramine-T method described by Hunter and Greenwood. Generally $5 \mu\text{g}$ 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 $0.8 \times 18 \text{ cm}$. Most iodinations were carried out using ^{131}I (specific activities $336 \sim 670 \text{ mCi/mg}$) 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—

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

The minimum detectable concentration of

plasma TSH was as low as $1.0 \mu\text{U/ml}$ using H-TSH Research Standard A as standard TSH. Plasma TSH level ranged from undetectable to $2.5 \mu\text{U/ml}$ in six normal subjects, and the level was within normal range