

## D. Measurement C (in vitro)

### TRH Radioimmunoassay

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TRH radioimmunoassay had been developed for unextracted human serum. Anti-TRH antibody was produced immunizing with rabbits TRH-bis-diazotized-benzidine-bovine serum albumin. This antibody was little cross-reacted with L or D,L Aze3-TRH, but not other TRH analogues, amino acids or pituitary hormones.

Radioiodination of TRH was performed by Greenwood-Hunter's method. Purification of TRH-I-125 was performed on a Sephadex G-10 column and three peaks were obtained. First peak was damaged protein, second peak was TRH-I-125 and third peak was I-125. Second peak was further divided to ascending (a), top (b) and descending portion (c). Immunoreactivity of each portion was 0.23 (a), 0.52 (b) and (c). Portion b or c were used this experiment. Immunoreactivity of TRH-I-125 was decreased with several times of freeze and thaw of TRH-I-125. Immunoreactivity was stable for 20 days if TRH-I-125

was divided to small amount.

It was found that TRH immunoreactivity was inactivated with serum. This inactivation could be prevented with adding of BAL.

In this system, recovery of known amounts of TRH were approximately 100%. Dilution curve of high TRH serum was parallel to standard curve. In this system sensitivity was 0.01ng/tube.

TRH levels measuring with this system were undetectable to 2.0ng/ml in normal subjects, undetectable in hyperthyroid patients or a tertiary hypothyroid patient and high in primary or secondary patients.

Urinary excretion of TRH following synthetic TRH iv. administration in a normal subject was about 3% for 120 min.

From above data it was suggested that this system is very useful tool to study the role of hypothalmo-pituitary thyroid axis in clinical basis.

### ACTH Radioimmunoassay

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The ACTH radioimmunoassay was announced to be established by R.C.C., England,