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$T_3$ ,  $T_4$ -free serum was prepared by incubating normal human serum with resin. 8-anilino-1-naphthalene sulfonic acid (ANS) has been used to inhibit binding of the hormone to TBG. 300  $\mu$ g of ANS per assay tube was employed in assays in which serum diluted 1:2. Barbitol buffer (pH 8.6) has been also used to inhibiting to thyroxine binding prealbumin.

The minimum detectable concentration of plasma  $T_3$  was as low as 20 ng/dl and plasma  $T_4$  was 100 ng/dl. In 26 normal subjects mean  $T_3$  concentrations was  $173.8 \pm 59.3$  ng/dl and mean  $T_4$  was  $7.72 \pm 2.24$   $\mu$ g/dl. In 25 patients with Graves' disease mean  $T_3$  was  $710.4 \pm 275.6$  ng/dl and mean  $T_4$  was  $16.8 \pm 2.92$   $\mu$ g/dl. In 15 hypothyroid patients mean  $T_3$  was  $49.5 \pm 13.2$  ng/dl and mean  $T_4$  was  $0.39 \pm 0.49$   $\mu$ g/dl.

## Solid State Radioimmunoassay for Human Thyroid Stimulating Hormone —Effect of carrier protein—

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Choice of carrier protein is one of the crucial points in the radioimmunoassay of polypeptide hormone.

Effects of various proteins were studied on the radioimmunoassay systems of HTSH in both solid-state and double antibody method.

Assay procedures of solid-state method had been detailed elsewhere, and 68/38 as standard TSH, anti-HTSH, and HTSH for iodination were the gifts from MRC and NIH. As to double antibody method, assay kits from Daiichi Radioisotope Co., which contained standard HTSH and anti-HTSH from Calbiochem, were used.

From the comparison of the maximal bound counts in solid-state system, 2% rabbit serum, whole rabbit, bovine and Graves' patient serum and 5% charcoal treated human serum as carrier revealed 115, 60.9, 50.7, 47.1 and 42.1%, respectively, of the counts obtained by 1% BSA.

On the other hand, 97.3% by bovine serum, 92.3% by serum of Graves' patients, 72.6% by charcoal treated human serum were observed in double antibody method. Solid-state method was

much more influenced by its carrier protein than double antibody method and was considered probably due to very small amount of antibody adhering on the surface of the plastic cups with which large amount of labelled TSH reacted.

From the results of the dilution and recovery check pooled untreated Graves' patient serum was found most suitable in both method. When 1% BSA was used as the carrier protein even in double antibody method, the sample serum seemed to show the false high value by the non-specific inhibition of the binding and the incomplete adsorption of anti-LH antibody.

Amount of unlabelled TSH, that displaced 50% of the labelled TSH from the antibody was 1.9  $\mu$ U/cup in the solid-state method and 6.2  $\mu$ U/tube in the double antibody method.

In the same 77 serum samples measured in both method, significant correlation ( $\gamma = 0.87$ ) was observed, but values in double antibody method were found approximately 5  $\mu$ U/ml higher than those in solidstate method.