

## Direct Comparison of Current Methods for Assay of Thyrotropin

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The *in vitro* bovine thyroid slice 4-hr.  $^{131}\text{I}$  release, the *in vitro* bovine thyroid slice weight change and the *in vivo* mouse blood  $^{131}\text{I}$  increase method were compared using the same USP reference standard thyrotropin (TSH) and the same mouse pituitary TSH (MTSH) preparation extracted by the ethanol-saline precipitation technique. The *in vitro* methods were performed using the same slices with the same dose of the same TSH preparation, and simultaneously,  $^{131}\text{I}$  release during the successive 18-hr. incubation period following the usual 4-hr. release was measured and expressed as percentage of the trapped  $^{131}\text{I}$  by the slice during the first 1-hr incubation period.

The TSH-dose response curve given by the usual 4-hr. release was reverse S in shape, and the successive 18-hr release observe S. The latter had better assay precision ( $\lambda=0.37$ ) than the former ( $\lambda=0.48$ ), however, the weight change of the slice passing through the all incubation period (23 hr.) was most excellent ( $\lambda=0.28$ ) and suitable for the assay method, while these were all inhibited nonspecifically by presence of 10% of human serum. The sensitivity of those was alike (0.004-0.006 mU/ml). When the *in vivo*  $^{131}\text{I}$  loaded mouse thyroid, which was treated previously with 10  $\mu\text{Ci}$  of  $^{131}\text{I}$  and thyroxine in

the same manner as that of the McKenzie's method, was samely incubated, almost complete liberation of the radioactivity into the incubation medium was observed without relation to TSH-dose added *in vitro*.

The McKenzie's technique also had considerable variation in assay precision and usable linear range of the curve. A inscrutable response of the mice of TSH-dose was seen 6 times in 68 assay performance, and ordinarily, when over 2 USP milliunits of the standard was injected to the test mouse, the 2-hr. response was rather decreased though the 8-hr. one was increased. The mean value obtained through the past 4 years was 190 in b (slope of the curve) and 0.35 in  $\lambda$  (assay error), and no marked seasonal variation was confirmed. The sensitivity (0.1 mU/0.3 ml) was apparently lower than each of those *in vitro* methods.

Parallel assay of the prepared MTSH using the above noted methods has indicated that the estimated potency ratio of the MTSH to the standard TSH (bovine origin) is highest in the McKenzie's, intermediate in the 18-hr.  $^{131}\text{I}$  release and lowest in the weight change method. This evidence may suggest that there is a grade of species specificity in biological action of TSH.

## Studies on the Free Thyroxine in Serum and Calculation of Free Thyroxine Index

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The present report deals with the relation between free thyroxine in serum and new calculation of free thyroxine index.

The free thyroxine in serum ( $\text{FT}_4$ ) was

determined from equilibrium dialysis of serum added to  $^{131}\text{I}$ -labeled thyroxine. The normal range of  $\text{FT}_4$  was from  $0.18 \times 10^{-2}$  to  $0.40 \times 10^{-2}$   $\mu\text{g}/100$  ml; hyperthyroidism and hypothy-