Radioimmunoassay of Thyroxine

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Thyroxine methyl-ester hydrochloride (T_4•MEH) was obtained by acid methanol method of Ashley and Harington and was conjugated to bovine serum albumin (BSA) by carbodiimide method of Oliver et al. 3.2 mg of T_4•MEH-BSA complex was dissolved into 0.8 ml of sterile physiological saline solution. This solution was emulsified in aliquot of complete Freund' adjuvant. 0.4 ml of this solution was injected into toe-pads of rabbit. Rabbit was boosted with 1.0 mg of antigen for one and half year. Sera were obtained one week after injection. T_4 in 0.1 ml unknown serum or standard T_4 added to the serum of cretinism or to the T_4-free serum which was obtained by using Amberlite IRA-400, was extracted with 2.0 ml of ethanol. After evaporation of ethanol, extracted T_4 was dissolved into 1.0 ml of barbital buffer (pH 8.6, 0.05 M) containing BSA in 0.5%. 0.1 ml of ^{125}I-T_4 (less than 25 pg of T_4, specific activity 230 μCi/μg, Malinchrodt.) and 0.1 ml of antiserum (1:500) were added to the assay system. The mixtures were incubated at 4°C for 18 hours. 0.2 ml of dextran-coated charcoal (dextran 0.5g and charcoal 5.0g in 200 ml of water) was added to them. One hour after, the mixtures were contrifuged and then radioactivities of supernatant and precipitate were counted in well-typed scintillation counter. Percentage of bound ^{125}I-T_4 was calculated. Good standard curve of T_4 was obtained by this method.

For direct radioimmunoassay, 0.9 ml of barbital buffer (pH 8.6) containing 0.5% of BSA and 100 mg/100ml of ANS (8-anilino-1-naphthalene-sulfonic acid) was added to 0.1 ml of unknown serum, and standard curve of T_4 was obtained, too. The relative activities of *I*-T_3 is 0,0029; T_4-form, 0,00050; Tetrac, 0,00060; T_3 form, 0,000021; T_3-prop, 0,000011; Triac, 0,0000-29; *I*-MIT, 0.000006; *I*-DIT, 0,0000007, respectively.

T_4 values obtained by RIA was well correlated these by competitive protein binding method (Tetrasorb-125, Abbott, Co).