A New Simple Method for the Determination of Thyroxine in Serum (Second Report)

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In a previous paper a new simple method for the determination of serum thyroxine (T4) was presented, which was devised by means of the resin uptake of 131I-triiodothyronine (131I-T3). Improvement and revision of the previous method was made by using resin sponge (Triosorb) in place of granular resin (IRA 400 formate form).

Method: (1) Two ml of test serum was added to 4 ml of 95% ethanol, mixed and centrifuged for 5 min at 1500 rpm. Four ml of the supernatant was evaporated to dryness in order to remove all the ethanol. The obtained material was dissolved in 0.5 ml of standard serum (pooled serum), 0.5 ml of distilled water and one ml of 131I-T3 in Tris buffer (PH 5.2), and incubated at 4°C for 30 min. The resin sponge (Triosorb) was then added and was kept at 4°C for 60 min. 131I-T3 resin sponge uptake was then counted in a well-type scintillation counter. (2) Known amounts of T4, i.e. 0.025, 0.050, 0.075, 0.100, 0.125, 0.150, 0.200, 0.250, 0.300, 0.400 µg were added to 0.5 ml of standard serum. The 131I-T3 resin sponge uptake in the serum was determined in the same way described above. A standard curve was plotted against the resin uptake and amount of T4. The standard curve from 0.025 µg to 0.2 µg indicated a straight line relationship. (Fig. 1) (3) The recovery of thyroxine in serum from the ethanol supernatant was determined by 2 ml of 95% ethanol added to one ml of the test serum containing 131I-T4. The radioactivity in 2 ml of the supernatant was counted. The mean recovery of 131I-T4 was 43.9% for 2 ml supernatant obtained from the initial volume of 3 ml. The recovery of T4 for 20 determinations in the same serum was approximately similar, i.e. ranged from 45.4 to 48.2%. (Table 1) (4) The amount of T4 in the test serum was determined by the standard curve according to the resin sponge uptake and multiplied by 50/R (R is recovery described above) to express it in µg/100 ml. The T4 iodine was calculated as 65.3% of this value.

Results: Fig. 2 represents T4 iodine values obtained by the present method. A good correlation of T4 iodine with PBI was obtained in most cases. (Fig. 4) The mean value for T4 iodine in the 39 euthyroid patients was 5.7±1.08 (Mean±S.D.) µg/100 ml, with a range of 3.8-7.8 µg/100 ml. Values in hypothyroid subjects ranged from 1.4-3.4, while those in hyperthyroid subjects ranged from 8.4-17.1 µg/100 ml. Values in nephrosis were very low, while in pregnant women they remained nearly normal. Table 2 gives the differences of duplicate determinations obtained in 36 cases. These values did not differ by more than 1.9 µg/100 ml.

These results proved that the above simple, rapid and reliable method is promising for routine clinical use in place of PBI.