A sensitive radioimmunoassay method was developed for measurement of 3',5'-diiodothyronine (3',5'-T2) in serum. Antisera to 3',5'-T2 were elicited in rabbits immunized with protein conjugated 3',5'-T2. I-125-3',5'-T2 was prepared by iodination of 3'-moniodothyronine (specific activity; 5000 mCi/µg). After 24 hours incubation of the reaction mixture, free I-125-3',5'-T2 was separated from the bound with polyethylene glycol (25%). Sensitivity ranged from 10 to 20 pg/ml. Among various thyroid hormone derivatives tested, only reverse T3 (rT3) cross-reacted significantly with the anti-3',5'-T2 antibody (10%). In order to eliminate the effect of cross-reaction with rT3 to the anti-3',5'-T2, there were following three methods: Method I. rT3 was removed from specimens by an addition of excess amount of anti-thyroglobulin serum which cross-reacts with rT3, but not with 3',5'-T2. Method II. Cross-reactivity of anti-3',5'-T2 serum with rT3 was abolished by absorption of anti-3',5'-T2 serum with rT3.

Method III. rT3 was removed from specimens by passing them through Sephadex G-25 (Super fine, 0.8 X 20cm) column. No significant differences of the results were observed among these three methods.

Changes in Serum Concentrations of 3,3'-Diiodothyronine in Various Thyroidal and Nonthyroidal Diseases.

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A sensitive radioimmunoassay was developed for measurement of reverse triiodothyronine (rT3) and 3,3'-diiodothyronine (T2) in unextracted serum using 8-anilineonaphthalene-6-sulfonic acid (ANS) to inhibit binding to serum proteins. Serum sample or standard, labelled hormone and antisera were incubated in bovine serum albumin containing ANS for 24 hr at 4°C, and then polyethylene glycol were added and the mixture was centrifuged to separate the precipitate. Serum levels of T4, T3, rT3 and 3,3'-T2 were measured in various thyroidal and nonthyroidal disorders, i.e. Graves' disease, hypothyroidism, complete starvation, anorexia nervosa, chronic hepatitis, liver cirrhosis, and in cord blood. All of these 4 iodothyronines concentrations were significantly higher in thyrotoxicosis and lower in hypothyroidism than those in normal subjects. Among nonthyroidal disorders, serum rT3 levels and the ratios of rT3/T2 levels were increased significantly in starvation, anorexia nervosa, liver cirrhosis and in cord blood. Serum levels of 3,3'-T2 were variable, but the ratios of 3,3'-T2/rT3 concentrations were significantly decreased in all of these disorders. 3,3'-T2 could be derived from T3 by 5 monodeiodination or from rT3 by 5' monodeiodination. However, in rabbits, serum 3,3'-T2 levels increased sharply after iv injection of rT3, but did not increase after T3 injection. In experiments to observe in vitro generation of 3,3'-T2 from T3 or rT3 in liver homogenates of rats, 3,3'-T2 was generated mainly from rT3. Therefore, the increase in rT3 levels appears to be accompanied by the decrease of 3,3'-T2 production from rT3. These results suggest that the fundamental change of T4 metabolism in starvation, anorexia nervosa, liver cirrhosis and cord blood is the inhibition of monodeiodination of outer ring iodine of iodothyronine resulting in increased ratios of serum rT3/T2 levels and decreased ratios of 3,3'-T2/rT3 concentrations.