

RADIOIMMUNOASSAY OF 3',5'-DIIODOTHYRONINE

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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 μ Ci/ μ g). 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. Shigenobu Nagataki, Hidenasa Uchimura, Hitoshi Ikeda, Nobuaki Kuzuya, Shoo Cheng Chiu, Michiko Maeda*, and Atsushi Takagi**, Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, *Department of Internal Medicine II, Tokyo Women's Medical College, and **Dainabot RI Laboratory.

A sensitive radioimmunoassay was developed for measurement of reverse triiodothyronine (rT₃) and 3,3'-diiodothyronine (T₂) in unextracted serum using 8-anilinoanthracene-6-sulfonic acid (ANS) to inhibit binding to serum proteins. Serum sample or standard, labelled hormone and antiserum were incubated in borate buffer, pH 8.6 containing bovine serum globulin and 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/T₃ 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/T₃ levels and decreased ratios of 3,3'-T₂/rT₃ concentrations.