Radioimmunoelectrophoretic Analysis of Thyroxine Binding Proteins

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Three thyroxine binding proteins, thyroxine binding prealbumin (TBPA), thyroxine binding globulin (TBG) and albumin, have been demonstrated by adding radiothyroxine to the sera and analyzing by various techniques. Conflicting reports have appeared regarding the additional thyroxine binding compornents in normal sera. The present study was designed to clarify how many thyroxine binding components exist in the normal serum by utilizing radioimmunoelectrophoresis.

Sera obtained from 28 healthy Japanese subjects and 12 occidental people were mixed with ^{131}I-thyroxine (^{131}I-T_4) and subjected to immunoelectrophoresis. After immunophoresis, each plate was washed, dried and autoradiographed.

When Biken's anti-whole human serum was used, at least 5 distinct radioactive arcs could be observed on autoradiogram in all sera, but only 3 or 4 radioactive arcs were shown by Hyland's or Behringwerke's antiserum.

The following findings may indicate that these 5 radioactive arcs are not artifacts but thyroxine binding proteins 1) fresh sera were used. 2) purified ^{131}I-T_4 was used. 3) ^{131}I-T_4 was added to the serum but no changes were observed in the protein pattern of immunoelectrophoresis. 4) the free ^{131}I-T_4 was eluted by washing: in fact neither precipitin arcs nor radioactive areas were shown when ^{131}I-T_4 was used without adding serum. 5) ^{131}I-T_4 was added to serum to give a low concentration of 0.05 μg/ml, and 6) phosphate buffer (pH 7.4) was used. These conditions were used to similate physiological condition except that thyroxine was added in vitro.

From the radioimmunoelectrophoretic pattern under the following conditions, these 5 thyroxine binding proteins may be identified as TBPA, Albumin, TBG, α_1-lipoprotein and β(or α_2)-lipoprotein 1) antiprealbumin, anti α_1-lipoprotein and anti β(or μ_2)-lipoprotein were used, 2) Lipoproteins were stained by Oil red O and Sudan Black B, TBG-deficient serum was used.

Studies on Iodoprotein in Simple Nodular Goiter and Thyroid Carcinoma

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After ^{131}I was administered orally to the patients with thyroid nodules 24 hours prior to operation, iodoproteins in nodular tissues were divided into soluble and insoluble iodoprotein by the Robbins method. Soluble iodoprotein/total iodoprotein ratio was determined and iodinated compounds in each iodoprotein were analysed by paperchromatography. Furthermore, soluble iodoprotein was divided into thyroglobulin and S-1 iodoprotein in the analytical ultracentrifuge, in order to determine the thyroglobulin/total soluble iodoprotein ratio.

The results obtained were as follows:

1) Soluble iodoprotein/total iodoprotein ratio was 97.4% on an average, ranging 93.5% to 99.8% in the normal thyroid tissue. On the other hand, the ratio was 96.3% on an average in colloid adenoma, 88.6% in tubular adenoma, 85.9% in trabecular adenoma, 83.6%

in papillary adenocarcinoma and 83.1% in anaplastic carcinoma. Namely, the ratio is observed to be decreased according to the grade of undifferentiation of the tumor.

2) Thyroglobulin/total soluble iodoprotein ratio was 78.3% on an average, ranging 72.3% to 92.5% in the normal thyroid tissue. On the other hand, the ratio was 66.0% on an average in colloid adenoma, 41.6% in tubular adenoma, 5.9% in trabecular adenoma, 8.6% in papillary adenocarcinoma and 0% in anaplastic carcinoma. Namely, thyroglobulin is observed to be decreased according to the grade of undifferentiation of the tumor.

3) The 27 S component was found in all the normal thyroid tissues and in the 5 of 12 colloid adenomas. However, it was not found in tubular adenoma, trabecular adenoma, papillary adenocarcinoma and anaplastic carcinoma.

4) From the above-mentioned results, it is concluded that thyroglobulin has a tendency to decrease in the tissue of thyroid nodules, especially remarkable in that of thyroid carcinoma, but there is no special component of iodoprotein in thyroid nodules. The differences in composition of iodoprotein in thyroid nodules appear to be closely related with a defects of thyroid hormone synthesis.

On the Determination of Free Thyroid Hormone in Serum by Activation Analysis

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We have studied on determination of free thyroid hormone (thyroxine + triiodothyronine) in the serum (Tf) by activation analysis.

In our previous experience, Tf value was more than that of equilibrium dialysis method.

Now, we examined on some points of analytical procedure and Tf value was determined by new method. Previously we separated Tf from serum by equilibrium dialysis and Sephadex G25 gel filtration, but 0.25% iodide was involved in Tf measured by those procedures. So, when we add column chromatography method of cathion exchange resin to this system, the recovery of Tf is 51.1% and the contamination of iodide is 0.0025%. Tf in solution form is irradiated with thermal neutron flux because of our results that in the recovery of extraction of Tf from the irradiation tube solution form (97.3%) is better than dryness form (44.5%).

After irradiation of Tf and extraction from the tube, iodinated compounds in the sample are made to iodide by oxidation and reduction on the basis of alternation of thyroxine to I^-, IO^-_2, IO^-_3 and many other organic iodinated compounds. Then Ag and serum are added to the sample, which is washed twice by 5% TCA in IN $NaNO_3$. Recovery rate by above procedures is 42.8% (serum only: 7.8%, Ag and serum: 23.4%).

We activated Tf extracted from euthyroid serum 50 ml and hyperthyroid serum 40 ml. ^{128}I from Tf was analysed with the decay curve counted by GM counter and well type scincillation counter, because of low value of ^{128}I measured by pulse height analysis. In this experience, Tf value is 1.2×10^{-10} g/ml, 2.0×10^{-10} g/ml. in euthyroid and 3.1×10^{-10} g/ml, 3.4×10^{-10} g/ml in hyperthyroid.