

Radioimmunoassay of Steroid Hormones

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Recently radiostereoassay (Murphy 1968) has been developed as the ultramicro-determination method (pg-ng) of steroid hormones in plasma and other body fluids in North America and Canada and has received widespread acceptance as routine clinical procedures.

Radiostereoassay method (Murphy 1968) can be classified into two: (1) competitive protein-binding analysis (Murphy 1963), using plasma and organ specific binding proteins as the specific binding reagents, and (2) radioimmunoassay (RIA) using steroid hormones as hapen. Steroid antibody has been obtained using steroids-BSA conjugates (Erlanger & Liebermann 1957) as antigen.

Radioimmunoassay methods for the measurement of plasma estradiol (Abraham 1969)

testosterone (Nugent et al 1970), and aldosterone (Nugent et al 1970) have been reported by this time.

We have established a radioimmunoassay for the ultramicrodetermination of plasma adrenal androgen-dehydroepiandrosterone sulfate (DHEA sulfate). The DHEA-17-oxime was coupled to bovine serum albumin, and the conjugate was used to obtain high-titer anti-DHEA rabbit serum.

We wish to report the details of practical procedure and application of this radioimmunoassay for the microdetermination of plasma DHEA sulfate and detailed results of investigation of radioimmunoassay for plasma aldosterone using antiserum of aldosterone which was kindly distributed by Dr. Nugent.

The Use of Radioimmunoassay of α -Fetoprotein for the Diagnosis of Hepatoma

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The usefulness of α -fetoprotein (α_f) concentration detectable is about 10 $\mu\text{g}/\text{ml}$.

By the Ouchterlony technique we could detect α_f in the sera of 52 out of 74 hepatoma patients. The serum levels in the patients determined by the single radial immunodiffusion method showed a very wide distribution, that is, 3,750-10 $\mu\text{g}/\text{ml}$. With the more sensitive technique such as radioimmunoassay, the higher positivity of α -fetoprotein, and the more definite diagnosis in the earlier stage of the disease may be expected.

Radioiodination of α -Fetoprotein: α -fetoprotein was isolated from antigen-antibody complex by gel filtration in low pH. The labeling of the purified preparation with ^{125}I was carried out by the Chloramine T method of Hunter and Greenwood. The iodinated α_f was separated from free radioiodine by gel filtration. The specific activity of the preparation was 16.6 $\mu\text{Ci}/\mu\text{g}$.

Antisera: The radioimmunoassay was carried out by double antibody technique using anti α_f rabbit serum prepared by immunizing rabbits with the pure α_f and anti rabbit

γ -globulin goat serum.

Assay System: One % horse serum albumin in PBS 0.5 ml, sample serum or standard serum 0.1 ml, ^{125}I - α_f 0.1 ml (c. 10,000 cpm), anti α_f (1:30,000) 0.1 ml, and normal rabbit serum (1:100) 0.1 ml were mixed in a tube and incubated for 24 h at 4°C. Then anti rabbit γ -globulin (1:10) 0.1 ml was added to the mixture and allowed to react for 24 h at 4°C. After centrifugation, the supernatant was removed and the radioactivity of the precipitate was measured by the well type scintillation counter.

The antiserum dilutions and the incubation periods were determined by preliminary experiments.

The amount of the first antiserum (diluted 1:30,000) corresponded to combine with 40% of the labeled α_f added and the amount of the second antiserum (diluted 1:10) was sufficient to precipitate all of the rabbit γ -globulin including the antigen-antibody complex in the system.

Standard serum was prepared by adding a definite amount of purified α_f to normal human serum which was confirmed to be free of α -fetoprotein by passing through an immunoadsorbent column of anti α_f antibody. The immunoadsorbent was prepared by coupling anti α_f antibody to Sepharose by the BrCN method of J. Porath.

By the addition of the standard serum (α_f concentration $500, 500 \times \frac{1}{2}, \dots, 500 \times \frac{1}{256}$ m μ g/ml) the precipitation of the labeled α_f was inhibited and a typical standard curve was obtained.

With fresh labeled α_f 2 m μ g/ml of could be detected with high reproductibility.

Levels in Various Disease: Sera of 194

patients with firm diagnosis and sera of 430 patients collected for liver function tests were examined. In this determination, the counting time was limited to 1 minute and the values above 20 m μ g/ml were taken as reliably positive.

Among the sera of 24 hepatoma patients: with the negative Ouchterlony test, 6 were above 500 m μ g/ml, 7 were 200-300 m μ g/ml and 7 were negative (below 20 m μ g/ml).

Other positive cases of the firmly diagnosed patients were as follows. Hepatitis: 27 out of 39 were positive, maximum 350 m μ g/ml. Cirrhosis: 16 out of 26 were positive, maximum 160 m μ g/ml. Metastatic liver cancer: 3 out of 9 were positive. Miscellaneous liver disease: 7 out of 19 were positive. Other malignancy: 3 out of 36 were positive. Heart disease and respiratory tracts disease: None of 10 and 9 were positive, respectively. Pregnant women: All of 14 in the second or the third trimester were positive but none of 8 in the first trimester were positive.

Among 430 serum samples for liver function test, 55 showed positive.

The 55 positive cases were pregnant women (5), babies (2), hepatitis (15), cirrhosis (4), hepatoma (1), other liver disease (4), other malignancy (8), miscellaneous disease (7) and without firm diagnosis (9).

The radioimmunoassay was as 5,000 times sensitive as the Ouchterlony test.

With this sensitive technique, α_f were detected not only in hepatoma patients but also in the sera of hepatitis and some other liver diseases, and pregnancy, but the serum levels were considerably lower than in hepatoma. Therefore, the early diagnosis of hepatoma is possible in the majority of the cases by following up the rapid increase of the serum α_f .