XII. Radioimmunoassay

A Study of Radioimmunoassay of Human Growth Hormone by Enzyme Proteolysis

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Principle:

Since the proteolytic enzyme, ficin or papain, destroys most of the free ^{125}I-HGH, and leaves autibody-bound hormone intact, the two fractions can be readily distinguished and measured by precipitation of antibody-bound ^{125}I-HGH with trichloroacetic acid solution.

Results:

(1) By the paper electrophoresis ,it was appeared that ficin was capable of degrading both free and antibody-bound ^{125}I-HGH. Increased concentrations of ficin resulted in appreciably more trichloroacetic acid solbule radioactivity after incubation with antibody-bound ^{125}I-HGH than did the weaker concentrations of ficin.

(2) For successful procedure of enzyme proteolysis method, it required more than 1 mg/ml concentrations of ficin.

(3) This assay system was not so affected by incubation temperatures (4°C–37°C).

(4) The quantity of nonspecific precipitated radioactivities depended on the concentration of proteins in the samples but not depended on the concentration of ficin. Therefore, the concentration of proteins in samples is of considerable importance for the reliability of this assay.

(5) This assay system was not affected by TSH and synthesized ACTH.

(6) Serum HGH levels of normal subjects at overnight fasting were 2.6 ± 0.5 mμg/ml (Mean ± SE) by this enzyme proteolysis method, and 2.3 ± 0.9 mμg/ml (Mean ± SE) by double antibody method.

(7) Dilution curve of serum from an acromegalic patient was linear.

(8) The recovery experiments revealed that high concentrations of HGH resulted in higher values.

(9) Samples of various sera were analyzed by enzyme proteolysis method and double antibody method. It seems that there was an exceptionally close correlation between the values by both two methods ($r = 0.85$), although the high concentrations of HGH resulted in higher values when measured by the enzyme proteolysis method.

(10) This enzyme proteolysis method required no special materials, no special technics and no longer times, and is very economical. The successful application of non-specific proteolytic enzymes to the assay of HGH suggests that the same principle can be employed to assay other hormones.