

Radioimmunoassay of adrenocorticotrophic hormone (ACTH)— with special reference to paradoxical binding phenomenon

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There is a phenomenon that the binding of ^{125}I -ACTH with its antibody is increased with an increase of unlabeled ACTH, when very small amount of ^{125}I -ACTH is added to relatively high concentration of antisera. We have studied further on this paradoxical phenomenon in order to clarify the reason for occurrence of the phenomenon.

Both macroglobulin and globulin fractions of antisera obtained by gel filtration gave similar paradoxical curves. Prolongation of incubation period or incubation with constant shaking did not affect the paradoxical binding phenomenon.

However, the paradoxical phenomenon disap-

peared and usual standard curve was observed when radioimmunoassay was performed with the first piece (Fab fraction) of papain-treated IgG fraction of antisera. This paradoxical phenomenon also disappeared with an increase in antibody titer caused by repeated immunization in an animal.

These findings suggest the presence of a kind of allosteric effect in antigen-antibody reaction with such specific antisera similar to that in enzyme-substrate interaction.

This phenomenon can be utilized to develop a sensitive and reliable radioimmunoassay for ACTH.

A sensitive radioimmunoassay for glucagon using talc method

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A sensitive radioimmunoassay for glucagon has been developed. Anti-glucagon antisera were prepared by immunizing rabbits and guinea-pigs with either pork glucagon-albumin conjugate or polymerized glucagon, the latter of which produced more sensitive antisera.

Glucagon was labeled with ^{125}I by the method of Hunter and Greenwood. The specific activity

of the labeled hormone ranged from 300 to 800 mc/mg. Bound and free hormones were separated by the talc method, which enable to check incubation damage easily. This method can measure as little as 40 pg/ml.

The antiserum used in this experiment cross-reacted with gut glucagon-like immunoreactivity (GLI). Cross-reactivity of porcine insulin, secretin,