D. Measurement C
(in vitro, immunoassay)

The Studies on Automation of the Radioimmunoassay Using a Minicomputer System


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We have some results in automatic procedure of the radioimmunoassay, using a minicomputer system.

In need of accuracy and simplification of the tests, by the increasing number of station and samples, two points were examined: I) which is superior the automatic pipetting or manual one? II) to obtain the standard curve in the case of two antibody style, the method of counting the supernatant, can be used insted of counting the precipitation.

Instruments used were micropipette (Eppendorf), automatic pipette (Bausch & Lomb, Shimadzu), auto-well counter and minicomputer system.

The results were: I) The coefficient of variation were, automatic pipette—0.81% and manual one—0.98%. II) The standard deviation of standard curve were 0.1310 m\(\mu\)g/ml (precipitation) and 0.1285 m\(\mu\)g/ml (supernatant).

In conclusion, the automatic pipette was useful for accuracy and simplification of the pipetting. And between the method of counting the precipitation and that of counting the supernatant, there were no great differences.

The Production of Sensitive Antisera to Small Peptides and Steroids by Immunization with Globulin Conjugates of the Appropriate Haptens for Use in Radioimmunoassay

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Antibodies to small peptides or steroids show specific interference to plasma proteins and cross reactivity to the other peptides or steroids.

The antibodies to vasopressin, triiodothyronine, angiotensin I or angiotensin II was produced by immunization with porcine-\(\gamma\)-globulin.
or bovine serum albumin conjugates of these compounds made by the use of carbodiimide. Cortisol, aldosterone and deoxycorticosterone were coupled to globulin by the method of chlorocarbonate. The conjugate was administered to rabbits by intramuscular injections at intervals of 2 weeks. Only 3-time injections could be produced a high titer of antibody to large peptides like as insulin or HGH. However, a long time (1.5-6 months) was required to produce antibody to the small peptides or steroids. Injection with conjugate of poly-L-lysine with peptide, or intra-lymph-node and intrasplenic injection absorbed on to microparticles of carbon black, resulted in poor titers in spite of a very long immunization period. The antibodies to vasopressin, angiotensin I, angiotensin II or triiodothyronine showed less than 0.1% of cross reactivity to other polypeptide hormone. Anti-angiotensin I serum showed 2.3% of cross reactivity to histidylleucine, released from angiotensin I by converting enzyme in plasma. Anti-aldosterone, cortisol or deoxycorticosterone sera had various grade of cross reactivity to the other steroids.

Antisera raised against peptide- or steroid-globulin conjugates contained antibodies to serum globulin as well as to the appropriate hapten. It was also observed that the presence of serum protein in the incubation mixture exerted a significant influence on the binding of the peptides or steroids by its homologous antibody. The boiling or extracting procedure was therefore used for elimination of the effect of plasma protein. Bovine serum albumin, used in the radioimmunoassay of angiotensin II, also markedly decreased sensitivity with standard curve of angiotensin I and triiodothyronine.

The sensitivity of selected antisera was such that the antisera could be used to measure small peptide or steroid hormone in low pg/ml range of concentration.

Dextran-coated Charcoal Technique to Make the Free Serum as a Diluent for Standard Curve of Radioimmunoassay

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Experiments were designed to make the hormone-free serum to serve for a standard curve of radioimmunoassay.

Adsorption rate of hormone by dextran-coated charcoal (D.C.C.) was analysed using radioactive hormones such as 3,5,3 triiodothyronine (T_3), thyroxine (T_4), insulin, corticosterone, angiotensin I and II, ACTH, growth hormone (GH), TSH and HCG in pooled human serum. In general, the hormone of a small molecular weight was easily adsorbed into D.C.C., but the hormone of a large molecular weight needed the large amounts of D.C.C. to remove hormone. Using the solution of high concentrated D.C.C. (20% charcoal and 2% dextran), T_3, insulin, corticosterone and angiotensin I and II were adsorbed by more than 80% into small amounts of D.C.C. solution (0.1 ml); T_4, GH and ACTH were adsorbed by more than 80% into relatively large amounts of D.C.C. solution (1 ml); and