Analysis of Plasma $^{131}$I-Insulin and Human Growth $^{125}$I-Hormone ($^{125}$I-HGH) Disappearance Curve in Diabetic Patients

H. KUZUYA, M. INADA, Y. KAZAMA and H. TAKAYAMA

Tenri Hospital, Tenri

Studies on kinetics of insulin and HGH metabolism were performed in 5 normal subjects, 6 patients with juvenile diabetes mellitus, and 7 patients with maturity onset diabetes mellitus. Patients who had abnormal renal and hepatic functions, and who had ever received insulin were excluded from the studies.

$^{131}$I-insulin and $^{125}$I-HGH were rapidly injected into antecubital vein simultaneously, and serial blood samples were obtained for 4 hours after injection. Plasma $^{131}$I-insulin and $^{125}$I-HGH concentration were estimated by double antibody immunoprecipitation method.

Plasma disappearance curve of both $^{131}$I-insulin and $^{125}$I-HGH was curvilinear when graphed on semilog paper. These curves could be resolved into sums of three exponentials by the methods of “peeling”. A three pool model was then formulated to describe the kinetics of plasma insulin or HGH disappearance, representing plasma (POOL 1), interstitial fluid (POOL 2), and all tissues in which insulin or HGH is utilized and degraded (POOL 3).

The appropriateness of this model was tested by computer analysis. The computer predicted values provided an excellent fit for experimental values. This indicates the postulated model is reasonable to describe the kinetics of insulin and HGH distribution and degradation.

The fractional turnover rates ($\lambda_{ij}$) were computed using the methodology described by Skinner, and compared to identify which parameter might most distinguish differences between the control group and the diabetic group.

In the diabetic group, especially in the patients with juvenile diabetes mellitus, increased fractional rate of insulin leaving interstitial fluid and entering pool 3 ($\lambda_{32}$) was noted ($0.045 \pm 0.006$ v.s. $0.034 \pm 0.004$ in the controls, $0.01 < p < 0.025$). As for HGH, increased fractional rate of HGH returning to plasma from pool 2 was characteristic in the diabetic group.

The formulation of the three pool model is reasonable and capable of yielding detailed informations relevant to insulin and HGH kinetics.

Fundamental Studies on Insulin Radioimmunoassay (2)

Y. TARUMI, Y. AZUMA, S. OTA, Y. KANASAKI and H. AKAGI

Department of Radiology, Osaka Medical College, Takatsuki

Our first report at Kinki Kakuigaku Kenkyukai, June, 1970, was concerned in the method including standard curves and its clinical usefulness of insulin radioimmunoassay.

The present studies are on precission of the measurement and simplification of the technique.

1) Leo, Stimmler reported in the Lancet 23, 1963; when the reciprocal of percentage of precipitation was plotted against the concentration of standard insulin, a straight line was obtained.

However the result of our data analysis, it was expressed by a curve of the second degree ($y = a + bx - cx^2$) rather than by a straight line ($y = a + bx$).
Calculating in our laboratory by least squares method, a, b and c were $1.87 \pm 0.37$, $0.345 \pm 0.88$, and $-0.0035 \pm 0.0017$ respectively.

2) As for reagents ($^{125}$I-insulin, antibodies, human serum etc.), use of 0.3 ml - 0.5 ml showed more excellent data than using original 0.1 ml standard method.

3) As a result of pipette sampling test, the most stabilized data was obtained by careful use of 0.5 ml all pipette.

Radioimmunoassay of Plasma Digoxin

H. KUBOTA and S. KAIHARA

Second Department of Internal Medicine, Tokyo University, Tokyo

H. KUROSAKI

Daiichi Radioisotope Laboratory, Tokyo

A rapid, sensitive method for measuring the plasma digoxin concentration has been developed with the radioimmunoassay technique.

Butler and Chen successfully raised digoxin-specific antibodies and Smith and Butler developed an immunoassay for digoxin. An immunoassay for digoxin has been developed and preliminary studies with this technique are reported here.

BSA-Dig conjugate was prepared as follows. Digoxin were added 0.1 M sodium periodate, absolute ethanal and dioxane. The entire reaction mixture was added to BSA in water. The mixture was stirred to maintain the pH in the 9.0-9.5 range. After 1 hr, sodium borohydride was added and the reaction mixture was set aside for 24 hr at room temperature. Approximately 1 M formic acid was added to lower the pH 5.5 considerable precipitation occurred. After 1 hr, 1 M NH$_4$OH was added to raise the pH 8.5. Some cloudiness persisted and the mixture was dialyzed overnight against running tap water. The pH was lowered to 4.8 by the addition of 0.1 N HCl with considerable precipitation of protein.

When examined spectrophotometrically in 83% H$_2$SO$_4$, this BSA-Dig preparation had absorption maxima at 390 and 470 m$_µ$. These absorption maxima appeared to be related to absorption maxima of digoxin.

Immunological procedure was as follows.

Rabbits were immunized by the injection of BSA-Dig, in complete Freund's adjuvant, nine injections in the foot pads over a 2-week period.

Assay procedure was as follows.

The assay was performed by incubation in small test tube, to which were added 0.5 ml pH 7.6 phosphate buffer, 1.0 ml of unknown or standard serum, 0.1 ml of $^{3}$H-Digoxin which was obtained from the New England Nuclear Corporation, and 0.1 ml of antiserum. The tubes were shaken and stood at 4°C for 16 hrs. Separation of bound from free labeled digoxin was achieved by the dextran coated charcoal, resulting in selective binding of free digoxin to the dextran coated charcoal, which was then separated by centrifugation. The supernatant phase was added to 15 ml of liquid scintillator, and was counted in liquid scintillation counter. Correction for quenching was made by Automatic External Standardization.

A standard curve was constructed for the solution of known concentration and the unknowns were read.

The results of assays performed upon venous plasma digoxin showed 0.5-4.5 ng/ml. There is a positive correlation between total daily dose and plasma digoxin concentration which is just significant. Three plasma sample were taken from patients not receiving digoxin; all gave results of less than 0.18 ng/ml.

The range of values determined by radioimmunoassay accords closely with that found after the administration of tritiated digoxin to patients with subsequent measurement of radioactivity in serum.