

Calculating in our laboratory by least squares method, a, b and c were 1.87 ± 0.37 , 0.345 ± 0.88 , and -0.0035 ± 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 hall pipette.

Radioimmunoassay of Plasma Digoxin

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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 ethanol 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_4OH 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_2SO_4 , this BSA-Dig preparation had absorption maxima at 390 and 470 $\text{m}\mu$. 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 ^3H -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 samples 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.