

$^{125}\text{I}$ -labeled antigens were prepared by the modified method of Greenwood et al using chloramin T. Digoxin-BSA conjugate in complete Freund's adjuvant were used to induce antiserum in rabbits, which is also used in  $^3\text{H}$ -digoxin RIA kit.

Our assay procedure was as follows: Add 0.1 ml of standard or sample, 0.1 ml each of diluted antiserum and carrier serum and 0.5 ml of phosphate buffer into test tube, and mix well.

Then, add 0.5 ml of dextran coated charcoal and mix well. After centrifugation of the mixture, remove the supernatant and count the radioactivity of precipitate in test tube by well type scintillation counter.

The present study is for evaluating mainly immunoreaction rate, amount of dextran coated charcoal and recovery rate of digitoxin and digoxin.

#### Results-digitoxin-

$^{125}\text{I}$  labeled digitoxin and digoxin antibody were equilibrated by 10 minutes of immunoreaction. The dextran coated charcoal having the charcoal concentration of 0.25–0.5%, and incubation time for 30 minutes after addition of it were adequate.

By adding labeled antigen at the final stage of procedure the best dose-response curve could be obtained. Moreover, the recovery rate was fairly good in that case. Five ng/ml of digitoxin could be detected.

-digoxin-

$^{125}\text{I}$  labeled digoxin and digoxin antibody were equilibrated by 5 minutes of immunoreaction.

The other results except for the sensitivity observed almost similar to those of digitoxin radioimmunoassay.

#### Discussion

Sensitivity and precision in these assay systems were good.

Those methods using  $^{125}\text{I}$  labeled antigens have an advantage to be able to omit the troublesome procedure of counting the sample required in the case of radioimmunoassay using  $^3\text{H}$  labeled antigens.

We consider that these methods could be adapted for digitoxin and digoxin radioimmunoassay in plasma.

Compounds for labeled antigens synthesized by Dr. S. Miyano and Dr. N. Abe (Faculty of Pharmaceutical Sciences, Fukuoka University) were utilized in our experiments.

## Radioimmunoassay for B-Methyldigoxin

### —Use of Digoxin RIA Kit—

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Recently, attention has been called for the clinical use of  $\beta$ -methyldigoxin because of its better absorbability from the intestines as compared with digoxine. With the purpose of evaluating the clinical use of  $\beta$ -methyldig-

oxine, Validation was made for the use of commercially available digoxin RIA kit to measure the serum concentration of  $\beta$ -methyldigoxine.

Digoxine RIA kit which was developed by

Kaihara et al and now available from Daiichi RI Reserch Institute was used for the following experiments.

1. Standard curves were obtained using known amounts of digoxine and  $\beta$ -methyl digoxine and the curves were practically identical.

2. The metabolites of  $\beta$ -methyl digoxine, i.e. digoxigenin-bis-digitoxoside and digoxigenine-mono-digitoxoside were proved to be measured with the nealy same sensitivity as  $\beta$ -methyl digoxine and digoxine.

3. Within assay error for the measurement of  $\beta$ -methyl digoxine were 3.9 and 9.6%

(coefficient of variation,  $n=10$ ) for two different sera.

4. Initial dose of 0.1mg of  $\beta$ -methyl digoxine in normal control caused significant peak in serum concentration at 0.5 - 1. hour after ingestion followed by rapid decline.

5. The tendency was noted that serum concentration of  $\beta$ -methyl digoxine is higher than that of digoxine with the same maintenance dose.

We concluded that it is feasible to use digoxin RIA kit for the clinical assessment of serum concnertration of  $\beta$ -methyl digoxine and its metabolites.

## The Radioimmunoassay Kit for Plasma Cortisol

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In the previous meeting, we have reported a simple radioimmunoassay of cortisol using cortisol-21-TME-<sup>125</sup>I. Now a radioimmunoassay kit for plasma cortisol was developed, which is much easier in procedure and gives greater precision than CPBA technique using <sup>3</sup>H-cortisol.

Florisil, dextran-coated charcoal, double antibody, resin strip, and polyethylene glycol method were examined to separate bound from free cortisol. And it was found that double antibody, resin strip, and polyethylene glycol method were available for a simple radioimmunoassay. Cortisol extraction from plasma with dichloromethane and alcohol, and denat-

uration of plasma CBG with alcohol were examined for sample preparation prior to a radioimmunoassay. Plasma cortisol concentrations measured by these methods agreed well with each other.

Plasma CBG denaturation method and polyethylene glycol method made it possible to produce the simplest radioimmunoassay kit for plasma cortisol measurement. This kit using these methods needs neither extraction nor chromatography, and only simple radioimmunoassay procedure is required.

The mean recovery of cortisol in concentration of 10.4, 25.6 and 50.0ug/dl, added to 9 kinds of plasmas which include cortisol of