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 β-methyldigoxine and the curves were practically identical.

2. The metabolites of β-methyldigoxine, i.e. digoxigenin-bis-digtoxoside and digoxigenine-mono-digitoxoside were proved to be measured with the nealy same sensitivity as β-methyldigoxine and digoxine.

3. Within assay arror for the measurement of β-methyldigoxine were 3.9 and 9.6% (coefficient of variation, n=10) for two different sera.

4. Initial dose of 0.1mg of β-methyldigoxine 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 β-methyldigoxine 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 concerntration of β-methyldigoxine 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-^{125}I. Now a radioimmunoassay kit for plasma cortisol was developed, which is much easier in procedure and gives greater precision than CPBA technique using ^{3}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 denaturation 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

5.68—22.6ug/dl were 103.0, 103.2, and 100.2% respectively. And the coefficient of correlation between the calculated values and the measured values was r=0.979 (p 0.001) and the regression line was (Mes.)=1.010 (Calc.)+0.358.

Respective values of plasma cortisol in 30 samples determined by both this kit and CPBA were in good correlation with each other. The coefficient of correlation war r=0.9260 (p 0.001) and the regression line was (RIA) —1.008 (CPBA) —1.060.

The intra assay precision in this kit was 11.9% in terms of coefficient of variation, when 10 subjects were 10 measured in n=9—29. The inter assay precision of 3 subjects in 6—26 separate assays was 10.6% in terms of coefficient of variation.

The procedure is simple, and accuracy and precision of this kit are satisfactory for routine determinations.

A Radioimmunoassay for Plasma Aldosterone by Immunologic Purification

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Antialdosterone serum (NIH 088) was diluted to 1 : 500 with borate buffer containing 0.5% albumin and 0.1% gamma globulin (S1). One-tenth ml of S1 was diluted with 8 ml of the same buffer, and 2 ml of protein-coated charcoal was added. The mixture was incubated for 4 hours at room temperature, then centrifuged, and the supernatant (1 : 50,000 dilution) was used for the purification (S2). S1 was diluted to 1:750,000 (S3) and this was used for radioimmunoassy. To 1—2 ml of plasma 1,000 cpm of ^{3}H-aldosterone was added, and extracted with methylene dichloride and dried. Half ml of S2 was added, and incubated overnight at 4°C. Then 0.5 ml of saturated ammonium sulfate was added, centrifuged, and the supernatant was discarded. The precipitate was dissolved in 0.2 ml of borate buffer, 0.3 ml of dextran-coated charcoal was added, then incubated for 5 minutes at 4°C, and centrifuged. The supernatant was extracted with 4 ml of methylene dichloride. One ml was used for recovery and two ml for assay. To each purified plasma samples and non-radioactive aldosterone standards 2,000 cpm of ^{3}H-aldosterone and 0.3 ml of S3 were added, and incubated overnight at 4°C. Then 0.3 ml of saturated ammonium sulfate was added, and centrifuged. When % bound for 0 pg was assumed to be 100%, corrected % bound for each weight of standard aldosterone plotted on a logit-log paper formed a straight line between 5 and 500 pg (standard curve). Recovery was 45—50%. Two ml water and one ml of adrenalectomized plasma gave less than 0.1 ng/100 ml and 0.30±0.11 ng/100 ml, respectively. Coefficient of variation was 11.3, 9.8 and 8.1% for 5,