

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 was $r=0.9260$ ($p 0.001$) 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

T. KONO, T. YOSHIMI

Second Division, Department of Internal Medicine, Kyoto University School of Medicine

J. ENDO

Central Clinical Laboratory, Kyoto University School of Medicine

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 radioimmunoassay. To 1–2 ml of plasma 1,000 cpm of ^3H -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 ^3H -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,

10 and 20 ng/100 ml. When 50, 100 and 200 pg of aldosterone was added to the adrenalectomized plasma, the assay values were 4.44 ± 0.50 , 9.67 ± 0.95 and 20.42 ± 1.66 ng/100 ml, respectively. Large amounts of other

competing steroids added to the adrenalectomized plasma gave no significant values. Seven supine normal males gave values of 7.5 ± 2.5 ng/100 ml at 9.00 AM.

Purification and Estimation of Plasma Aldosterone by Reversed Phase Partition Chromatography on Sephadex LH-20 and RIA

M. KIZAKI, Y. TERAYAMA, S. SATO

Section of Endocrinology, Kitasato Biochemical Laboratories.

(Bristol Laboratories of Japan.)

T. KOSHIMIZU

Department of Pediatrics, Kitasato University School of Medicine.

Crude Aldosterone (Aldo.) fraction extracted from plasma by CH_2Cl_2 was further purified by column chromatography on Sephadex LH-20 ($1 \times 30\text{cm}$), which was equilibrated and eluted with distilled water. It is demonstrated that Aldo. and Cortisol were separated clearly into first fraction (55ml to 72ml) and third (80ml to 100ml) respectively. Recovery of added ^3H -Aldo. was 55%–60% with constant yield.

RIA analysis of Aldo. was performed by using the Sorin test kit, which sensitivity was 1ng/dl Aldo. and the values of assay blank ranged from 0 pg to 20pg. The mean

recovery of added Aldo. (25pg–100pg/ml) was 117.5%. The intra-assay variation for each of 5 samples with triplicate determinations ranged from 6.5%–20.5% and inter-assay variation was 13.8%.

Normal values estimated is comparable with others reported (Adults; 6.1 ± 3.7 , Children; $6.5 \pm 1.2\text{ng/dl}$). However, newborns and infants presented on remarkable increase of Aldo. with range from 72.6–108.0ng/dl. Plasma Aldo. levels of clinical patients suffering from primary, secondary Aldosteronism, Adreno-genital syndrome and pregnant were determined also respectively.

Determination of Urinary Aldosterone by Radioimmunoassay

Y. TERAYAMA, M. KIZAKI, S. SATO

Section of Endocrinology, Kitasato Biochemical

Laboratories. (Bristol Laboratories, Japan)

A simple method for determination of urinary aldosterone-18-glucuronide has been