

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

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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

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

developed which appear to be applicable for clinical routine test. 5 ml of a 24 hr urine collection was pre-extracted with methylene chloride to remove free corticoids, hydrolyzed 24 hrs. at pH 1.0 and extracted into methylene chloride. The extract was washed successively with 0.1 N sodium hydroxide and acetic acid, then with water. The extracted aldosterone was followed by a rapid RIA analysis (Sorin).

High recovery of added cold aldosterone obtained from pooled urine of three different concentrations ranged from 75–90% and the sensitivity of 0.2ug/l. Reproducibility of 5.9–14.7% (c.v.) as a intra assay variation,

and 13.0% (c.v.) as a inter assay variation, are quite satisfactory using only methylene chloride extraction.

Correlation of aldosterone values determined between this simple method and Sephadex LH-20 column method showed highly significance ($r=0.96$, $y=0.75x+0.11$). The normal specimens of 62 gave 1–12 ug/day, and the patients suffering from primary and secondary aldosteronism showed elevated excretion of aldosterone which ranged 17.3–116 ug/day and 14.7–60.0 ug/day respectively.

Additionaly, cross reaction of aldactone (Aldactone-A, SC-14266) on the B/B. ratio of standard curve was discussed.

Measurement of Plasma 11-Deoxycorticosterone Levels by Radioimmunoassay in Man

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Radioimmunoassay procedure has been developed to measure 11-deoxycorticosterone (DOC) in human peripheral plasma. DOC-oxime was coupled with porcine gamma globulin and antibodies produced in rabbits. One to 3 ml plasma, with 1, 2 ^3H -DOC added for recovery, was extracted with dichloromethane and purification achieved by a silica gel column and by one paper chromatography. After overnight incubation of the antibody-steroid mixture at 4°C, bound and free fractions were separated using ammonium sulfate. The mean recovery of ^3H -DOC, after extraction and chromatography, was $84.6 \pm 7.4\%$. The method showed adequate specificity, precision and accuracy.

Normal plasma DOC levels were found to

be 4.4 ± 2.5 ng/100ml ($n=8$). Plasma DOC levels were almost normal (0.3–26.8 ng/100 ml) in fifteen patients with benign essential hypertension. The mean level of 8.1 ± 8.2 ng/100ml obtained in hypertensive patients with suppressed plasma renin activity, was not significantly different from normal. Plasma DOC showed a high level of 3.0–30.5 (11.4 ± 7.5) ng/100ml in 9 patients with primary aldosteronism. Four out of 8 patients with Cushing's syndrome were found to have elevated plasma DOC levels. The higher levels of 21.2 ± 15.8 ng/100 ml were found in 5 patients with adrenal hyperplasia than those of 12.3 ± 8.0 ng/100ml in 3 with adrenal adenoma. Plasma DOC levels were high levels of 113–176 ng/100ml in 2 patients with 17α -