

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  $^3\text{H}$ -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  $^3\text{H}$ -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  $4.4 \pm 2.5$  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  $8.1 \pm 8.2$  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 ( $11.4 \pm 7.5$ ) 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  $21.2 \pm 15.8$  ng/100 ml were found in 5 patients with adrenal hyperplasia than those of  $12.3 \pm 8.0$  ng/100ml in 3 with adrenal adenoma. Plasma DOC levels were high levels of 113–176 ng/100ml in 2 patients with  $17\alpha$ -

hydroxylase deficiency. ACTH administered to 5 subjects produced a mean increase in plasma DOC from 4.8 to 25.8ng/100 ml. Angiotensin II infused at a rate of 10ng/kg/min for 30 min into 4 subjects did not increase mean plasma DOC. Similarly, dietary sodium restriction or postural change did not increase plasma DOC.

These results confirm that DOC secretion is primarily under anterior pituitary control. From the basal level of 4.4ng/100ml and from its biological activity compared to aldosterone, the major mineralo-corticoid, it would seem that DOC plays a minor role in electrolyte homeostasis in normal man.

## **The Assay of Catecholamine by Radiometric Method (Report 1)**

### **Approach to Plasma Catecholamine Assay**

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In 1968, Engelman described double-isotope derivative method for plasma catecholamine assay and reported its concentration in normal resting adults and some other condition. He used column procedure in collecting plasma samples, but it needed relatively large amounts of plasma, 10 ml. And soon later, in 1973, Coyle assayed fetal and newborn rat cerebral catecholamines, using the same enzymatic procedures as Engelman, and its method could save the more time than his method. If we could apply his column procedure and Coyle's

assay to plasma catecholamine determination, we should save more time and plasma itself. This assay exhibited linearity with amounts of catecholamine ranging from less than 0.1 to 6 ng for norepinephrine.

This sensitivity is enough to assay plasma catecholamine concentrations which is contained in blood 0.13 to 0.52g/l according to Engelman, though norepinephrine and epinephrine cannot be distinguished one from the other by this procedure.