

### **Rapid Determination of Human Plasma Aldosterone**

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Method of rapid determination of human plasma aldosterone without chromatography was studied using CEA-IRE-SORIN kit. Half ml of plasma was extracted with 4 ml methylene dichloride by shaking for 2 minutes. After centrifugation supernatant was aspirated and 3 ml of the methylene dichloride layer was taken. This was evaporated under a nitrogen stream, and the residue was used for assay. Two-tenth ml and 0.3 ml of phosphate buffer was added to each aldosterone standard and each residue, respectively. Then 0.1 ml of  $^3\text{H}$ -aldosterone and 0.1 ml of anti-aldosterone serum which had practically no cross-reaction with other steroids were added to all the tubes, vortexed, and incubated for 30 minutes at  $37^\circ\text{C}$ , then for 2 hours at  $4^\circ\text{C}$ . Half ml of dextran-coated charcoal was added, vortexed, and 10 minutes later centrifuged at  $2,000 \times g$  for 15 minutes. Half ml of the supernatant was taken and counted (% bound). A linear standard curve was obtained between 12.5 and 400 pg on a logit-log paper when % bound for 0 pg was assumed to be 100%. Recovery of  $^3\text{H}$ -

aldosterone added to the plasma was 99% ( $n=4$ ). Half ml of plasma from an adrenalectomized woman showed values less than the sensitivity of the method. Coefficient of variation was 11.0, 7.0 and 5.5% for plasmas with mean values of 4.4, 14.8 and 26.3 ng/100 ml, respectively ( $n=5$ ). Half ml of the adrenalectomized plasma reinforced with 50, 100 and 200 pg of aldosterone showed values  $10.04 \pm 0.98$  (SD),  $20.38 \pm 1.51$  and  $39.92 \pm 2.38$  ng/100 ml, respectively ( $n=5$ ). Thirteen normal males in a supine position at 9.00 AM showed values 4.4 to 12.4 ( $7.23 \pm 2.86$  SD) ng/100 ml. Several of them showed increased values after infusions of angiotensin II, angiotensin III and 1-sarcosine, 8-isoleucine-angiotensin II. Patients with primary aldosteronism, idiopathic hyperaldosteronism, renovascular hypertension and Bartter's syndrome showed high values, and patients with benign essential hypertension showed normal values. With this method one technician could determine more than 50 plasma samples in one day.

### **Radioimmunoassay of II-Deoxycortisol in Unextracted Plasma after Metyrapone Test**

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To date, many methods have been used to estimate II-deoxycortisol in plasma after the admini-

stration of metyrapone including competitive protein binding and radioimmunoassay techniques,

but these methods required more extensive extraction and purification before proceeding to the assay stage. Using the specific antiserum for II-deoxycortisol, we have successfully applied the principle of the ethanol denaturation of binding proteins without extracting the II-deoxycortisol and without removing denatured protein from the radioimmunoassay incubation mixture.

In rabbits antibodies against II-deoxycortisol were produced by immunizing with a complex of II-deoxycortisol-3-oxime and bovine serum albumin. The specificity of the antiserum for II-deoxycortisol was evaluated by studying the cross reaction of major steroids known to occur in peripheral blood. It was observed that the tested steroids crossreacted less than 0.5 percent with the exception of II-deoxycorticosterone.

The assay was performed as follows: Dexamethasone treated plasma containing 0.1, 0.25, 0.5, 1, 2.5, 5 ng of II-deoxycortisol were prepared for standards. Add 50  $\mu$ l of absolute ethanol to 10  $\mu$ l of standards and each samples. Vortex-mix and add 500  $\mu$ l of phosphate buffer

containing  $^3\text{H}$ -II-deoxycortisol (4000 dpm/tube) and diluted antiserum (1:5000). Let stand for 2 hr at room temperature. Add 500  $\mu$ l of saturated ammonium sulphate and centrifuge (10 min, 1000  $\times$ g). Pour the total supernate into a counting vial containing 10 ml of the toluene based scintillation fluid.

The intraassay variation was  $\pm 2.2\%$  ( $n=5$ ); the interassay variation was  $\pm 8.7\%$  ( $n=14$ ). The sensitivity of the assay was about 25 pg.

After administration of a single dose of 30 mg/kg metyrapone, patients with normal pituitary function showed an 5.1–21.8  $\mu\text{g}/100$  ml with a mean of  $11.0 \pm 4.7$   $\mu\text{g}/100$  ml. These values estimated by present method were compared with those measured by radioimmunoassay with chromatography, the two values agreed well. All of the patients with pituitary or adrenal insufficiency had II-deoxycortisol values lower than 5  $\mu\text{g}/100$  ml.

It was concluded that plasma II-deoxycortisol levels in response to the metyrapone can be measured accurately, conveniently and rapidly by present method.

### **In Vitro Assay for ACTH-Releasing Activity Using ACTH Radioimmunoassay**

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In vitro assay of ACTH releasing activity utilizing pituitary incubation combined with ACTH radioimmunoassay was developed.

Rat half pituitary was preincubated in 2 ml Krebs Ringer bicarbonate buffer containing 0.2% glucose and 0.25% BSA (KRBG-BSA) for 1.5 hr (45 min  $\times$  2). The medium was replaced by 1 ml KRBG-BSA and incubated for 30 min. Then the medium was again replaced by 1 ml KRBG-BSA or KRBG-BSA containing test materials and

incubated for another 30 min. The amount of ACTH assayed by radioimmunoassay in the 2nd incubation was compared with that in the 1st incubation and expressed as percentage.

In ACTH radioimmunoassay, anti-ACTH serum was diluted to 1:1,500–3,000. The  $^{125}\text{I}$ - $\alpha^{1-24}$ ACTH-antibody system was not affected by lysine-vasopressin (LVP), arginine-vasopressin (AVP), rat's pituitary LH, GH, and prolactin. Human $^{1-39}$  ACTH was used as ACTH standard, and the dilution