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 µl of absolute ethanol to 10 µl of standards and each samples. Vortex-mix and add 500 µl of phosphate buffer containing ³H-II-deoxycortisol (4000 dpm/tube) and diluted antiserum (1:5000). Let stand for 2 hr at room temperature. Add 500 µl of saturated ammonium sulphate and centrifuge (10 min, 1000 × g). Pour the total supernate into a counting vial containing 10 ml of the toluene based scintillation fluid.

The intraassay variation was ±2.2% (n=5); the interassay variation was ±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 µg/100 ml with a mean of 11.0±4.7 µg/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 µg/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 × 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 ¹²⁵I-α¹–₃₉ACTH-antibody system was not affected by lysine-vasopressin (LVP), arginine-vasopressin (AVP), rat's pituitary LH, GH, and prolactin. Human¹–₃⁹ ACTH was used as ACTH standard, and the dilution
curve of incubation medium was paralleled with the standard curve. Repeatability of immunoassayable ACTH in withinasay was 174±5.0 pg/tube (CV=2.9%).

A log dose-response relationship was observed between the amounts of stalk median eminence extracts (SME: NIAMDD) added to the incubation medium and its ACTH releasing activities. The sensitivity of this assay method was at least 0.1 SME of 10 mU of LVP and AVP.

Using this method, it was found that LVP, AVP, norepinephrine (100 ng/ml-200 ng/ml) and 5-hydroxytryptophane (1 µg/ml) had ACTH releasing activities but LH-RH, TRH, glucagon, dopamine, phenolamine propranolol, haloperidol, prostaglandin E1 and indomethacin did not increase the release of ACTH.

Studies on a Radioimmunoprecipitation Method for the Determination of Insulin Antibody and the Binding Capacity of 125I-Insulin to Insulin Antibody

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We have studied on the several basic investigation of a radioimmunoprecipitation method of insulin antibody in human serum. The most suitable condition in this method was obtained follows: 0.10 ml of 125I-labeled insulin (0.02 ng/ml) was added to 0.05 ml of the 16-fold diluted human serum. This mixture was incubated for 72 hrs. at 4°C and to this, 0.20 ml of the 4-fold diluted anti-human IgG was added. Then, after the additional 48 hrs. incubation at 4°C, the total radioactivity (cpm) of the whole mixture was measured. Subsequently, the mixture was centrifuged at 3,000 rpm for 30 min, the supernatant was decanted and the precipitate was washed three times with phosphate-buffered saline, pH 7.5 to serve for the measurement of pure radioactivity in the precipitate. This method was superior in the specificity, reproducibility and sensitivity to those of the other method (PEG method).

Furthermore, in order to investigate the binding state of 125I-insulin to insulin antibody, we performed the following procedure. 0.1 ml of 125I labeled insulin (0.2 ng/ml) was added to 0.05 ml of human serum, and the mixture was incubated for 72 hrs. at 4°C. Then, 125I-insulin bound to the antibody was separated by gel-filtration through sephadex G-50 column (0.9×25) cm. The 125I-insulin-antibody complex solution obtained was fractionated by Pevikon electrophoresis and was precipitated by anti human γ-globlin. A peculiar phenomenon, in which 125I-insulin was liberated from 125I-insulin antibody complex during either electrophoresis or immunoprecipitation, was observed. However it is obscure that this phenomenon was caused by either some structural change in insulin due to iodination or co-existence of human antibodies with weak affinity to insulin.