

Measurement of Plasma Testosterone by Solid Phase Radioimmunoassay with Microtiter Tray

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A method of solid phase radioimmunoassay with microtiter tray was investigated to simplify the radioimmunoassay of plasma testosterone and to measure many samples at the sametime, and satisfactory results were obtained. Standard testosterone or extracted samples which had been dissolved by borate buffer containing ^3H -testosterone were incubated in cups of the disposable plastic microtiter tray precoated with diluted antiserum. After removal of the incubated solution, cups were cut and dissolved by toluene scintillator in counting vials, and radioactivity was counted with liquid scintillation counter.

In 7.0–8.0 pH, the maximal absorption of antibody from diluted antiserum by surfaces of microtiter tray was obtained. In order to obtain the best standard curve, precoating time and incu-

bation time required for 24 hours at room temperature, respectively.

Accuracy, precision and sensitivity in this method were satisfactory. Close correlation were founded between plasma testosterone levels obtained from this method and that from dextran coated charcoal separation technique. Although moderately high concentration of antiserum was needed, in this method that antiserum was able to recover and re-use. The plasma testosterone level in 31 normal male was 687.1 ± 48.3 (SE) ng/dl, and in 14 normal female was 46.08 ± 4.3 (SE) ng/dl.

According to the results obtained above this method is suitable for radioimmunoassay of plasma testosterone.

Radioimmunoassay of Serum Testosterone

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A simple and rapid method for radioimmunoassay of serum testosterone was presented. One tenth ml of serum for male adults or 1 ml for females and prepubertal children were extracted 3 times with 4 volumes of ethylether using a dry-ice acetone mixture. The dried extract in each

tube was dissolved in 1 ml of 1% bovine serum albumine (BSA) in 0.01 M phosphate buffered saline (PBS, pH 7.5) and a 200 μl aliquot of the solution was added with 200 μl of anti-testosterone-3-carboxymethyl-BSA and 100 μl of tritiated testosterone (approximately 3000 cpm).

The solution mixtures were incubated along with standard preparations at 4°C for 18 hours, and 500 μ l of a dextrancoated charcoal solution was added and incubated for further 30 min. After centrifugation at 3000 rpm for 15 min, 0.8 ml of the supernatant was pipetted into a liquid scintil-

lation vial and counted.

The sensitivity of the method was sufficient enough to detect as little as 40 pg/ml or 4 ng/dl of serum samples. Accuracy, precision and specificity of the method were discussed and some clinical data are also presented.

A Compositive Luteinizing Hormone Releasing Hormone (LH-RH) Loading Test on Male Sterility

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Plasma FSH, LH and testosterone were measured by radioimmunoassay technique in 15 cases with sterility and 5 normal males following intramuscular injection of compositive LH-releasing hormone (0.1 mg).

In almost all cases with sterility, increment of plasma FSH and LH values following LH-RH administration was seen. However, the reaction of pituitary to LH-Rh administration occurred a little slower in a group of azoospermia than the controls, while it seemed quite normal in a group of oligospermia.

On the other hand, the increase of plasma testosterone was lower than normal in patients

with sterility following LH-RH loading test.

As mentioned above, the pituitary reacted to LH-RH in male sterility, but LH-RH loading test resulted in scarcely any increase of plasma testosterone. These facts suggest that the interstitial cells themselves are not functioning normally in male sterility. The obtained results also show that LH-RH loading test is a far more surpassing method for examination with respect to its capability of diagnosis for the both conditions of pituitary and gonad at the same time, besides shortening the time for test, comparing with the conventional HCG test or the rapid HCG test recently applied.