Studies on Radioimmunoassay for Thyrotropin

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Radioimmunoassay for thyrotropin were studied using a highly purified human thyrotropin prepared by Drs. Bates and Condliffe (NIAMD) for labeling with $^{131}$I, and Human Thyrotropin Research Standard A. Anti-TSH sera were produced in rabbits by the injection of partially purified human TSH material which originally had a biological potency of 0.6-1.0 IU/mg in the McKenzie bioassay emulsified with complete Freund Adjuvant. Double antibody technique was used for separation of antibody-bound human TSH and free human TSH using a anti rabbits gamma globulin goat serum.

The method was sensitive to as little as 1.0 $\mu$U unlabeled human TSH. No effects was observed when HCG, FSH, ACTH and HGH were assayed using a anti-TSH serum absorbed with 20 IU HCG per assay tube.

Radioimmunoassay systems for TSH using highly purified human TSH and its anti sera which distributed by NIAMD and Calbiochem were also studied and comparison among each assay results was discussed.

Studies on the Radioimmunoassay of Parathyroid Hormone

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Antibodies against bovine parathyroid hormone (PTH) was prepared through repeated injections of 2 mg TCA-PTH (250 USP units/mg) in guinea pig with Freund complete adjuvant 5 times every other week. Bovine CMC-PTH (2,500 VSP units/mg) was labeled with $^{125}$I according to the method of Hunter-Greenwood. After the incubation of samples or standard with antibodies for 72 hrs at 4°C, $^{125}$I-PTH was added. The separation of bound and free fraction was performed 48 hrs later by means of adsorption to dextran-coated charcoal. Although the sensitivity of the present assay method is somewhat limited because of the difference in the immunological properties between human and bovine PTH, serum PTH in normal subjects was successfully determined and tends to decline with advancing age in both males and females. Serum PTH was increased in the females with postmenopausal osteoporosis as well as in the patients with primary or secondary hyperparathyroidism. The radioimmunoassay of PTH in the 8M-urea extract of rat parathyroid glands was performed by the same method as mentioned above. The dilution curve of PTH in rat parathyroid glands was coincident with that of bovine CMC-PTH, suggesting that they are immunochemically same. Organ culture of rat parathyroid glands was performed in glass vessels with 50% Eagle basal medium and 50% thyroparathyroidectomized rat serum under 95% air and 5% CO$_2$ at 37°C. Immuno-reactive PTH released into medium from rat parathyroid glands was increased by the decrease of calcium or magnesium concentration in the medium.