

used. ^{125}I -HGH was labelled with iodine-125 by the method of Greenwood, Hunter and Glover. Specific activities of 100-150 mCi/mg was utilized. The reactions were carried out as follows.

0.1 ml of plasma sample or standards are added to 0.4 ml 0.5% BSA veronal buffer in the incubation tube and mixed with 0.1 ml. ^{125}I -HGH (0.2-0.5 μg) and 0.1 ml of diluted guinea pig antihuman growth hormone serum with 0.1 ml of diluted (1:100) normal guinea pig serum. The tubes are incubated at 4°C for 72 hours. Then, 0.1 ml of diluted (1:2) rabbit anti guinea pig gamma-globulin are added and incubated again at 4°C for 24 hours. The tubes are then centrifuged at 3000 rpm for 30 min. and the supernatants are decanted and discarded. The paperchromatoelectrophoresis of the supernatants indicated complete paperchromatoelectrophoresis of the supernatants indicated complete separation of free HGH from bound HGH. The precipitates are counted in a well-type scintillation counter and the

percentage iodine-125 labelled human growth hormone is calculated. The standard curve is plotted and the amount of HGH in each sample is determined from the bound ^{125}I HGH % by comparison with the standard curve.

In this method it is possible to detect at least 0.2 μg /ml of human growth hormone in plasma and non-specific interference by plasma was negligible in this assay system. The reproducibility was satisfactory and excellent agreement was obtained in all ranges between the values of plasma human growth hormone measured by the double antibody method and by the paperchromatoelectrophoretic method of Berson et al. The double antibody method has several advantages compared with paperchromatoelectrophoretic method. The procedure is more simple and assay of many samples is possible in shorter period with high sensitivity and reproducibility. Moreover, long half-life of ^{125}I recommends it as an isotope for use, as a tracer.

Radioimmunoassay for Human Thyrotropin

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A radioimmunoassay for TSH in human plasma was applied to clinical investigation.

Anti-human TSH (HTSH) serum was obtained from rabbits immunized with crude HTSH which was extracted from pituitary glands by the method of Bates' percolation. The crude HTSH was purified by CM-Cellulose and Sephadex G-100 column chromatography. This purified HTSH, which yielded 6.9 IU/mg, was used for labeling of ^{131}I and standard HTSH. Bound and free ^{131}I -HTSH in incubated medium were separated by the method of Odell. This radioimmunoassay was capable of measuring 1 to 30 μg /ml of HTSH.

HCG was confirmed to give a considerable effect on measuring HTSH while FSH or

ACTH no effect. TSH level in different stages of menstrual cycle of normal women had no variation, but showed high value in menopausal or pregnant women.

HTSH levels in serum of 2 panhypopituitarism were undetectable, but those of 8 primary hypothyroid patients were high value (more than 45 μg /ml). Injection of T_3 (75 μg) to one of these hypothyroid patients induced an immediate and remarkable decrease of HTSH levels and then a slow elevation to the initial level in 96 hours. Treatment of 3 hypothyroid patients with desiccated thyroid powder led to a significant decrease in HTSH level. In these two changes of HTSH levels by T_3 or desiccated thyroid powder, a satisfactory parallel

correlation was found between the values measured by this radioimmunoassay and by McKenzie's bio-assay. The absolute value measured by the radioimmunoassay, however,

showed higher than that by bioassay. This discrepancy is probably caused by impurity of HTSH used as antigen or by cross-reaction between HTSH and LH.

The Studies on the Secretion and Metabolism of Androgens, using ^{14}C and ^3H labelled Androgens

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Added to the cases previously reported, the studies were made on the secretion and metabolism of androgens, using ^{14}C - and ^3H -labelled androgens.

The rates of secretion, production and interconversion of dehydroepiandrosterone (DHA) and dehydroepiandrosterone sulfate (DHA-S) in 2 normal elderly males, a 27-year-old woman with Leydig cell tumor of ovary and a 34-year-old woman with adrenocortical tumor (suspected cancer) were estimated by the method of Vande Wiele et al. (Recent Progr. Hormone Res., 19, 275, 1963).

The average value of the secretion rates of DHA in 3 normal elderly males was 2.6 ± 0.9 (S.D.) mg/day and showed statistically significant decrease ($p < 0.02$) compared with the average value in 4 normal males. The value of the secretion rate of DHA in a case with Leydig cell tumor of ovary was 90.9 mg/day and was markedly higher than the average value in 4 normal female. The value of the secretion rates of DHA and DHA-S in a case with adrenocortical tumor was 44.4 mg/day and 14.5 mg/day respectively and higher than the average value in 4 normal females.

Plasma concentration, metabolic clearance rate and blood production rate of DHA-S in normal subjects were calculated by the method of Conrad et al and the mathematical analysis of Tait et al. (Conrad et al J. Clin. Invest., 40, 947, 1961, Tait et al J. Clin. Invest., 40, 72, 1961).

The average values of plasma concentration were 206.0 ± 37.6 kg/100ml in 5 males and 25.40 ± 34.0 kg/100 ml in 3 normal females.

In 3 normal males, the average value of metabolic clearance rate was 1.69 ± 0.40 L/day and the average value of blood production rate was 3.62 ± 0.86 mg/day.

Urinary production rate (UPR) of testosterone were determined in 2 cases by the single isotope dilution using thin layer- and gas-chromatography (Lipsett et al J. Clin. Invest., 42: 1753, 1963). The first case was the patient above mentioned with Leydig cell tumor of ovary, who showed the signs of remarkable masculinization.

UPR of that case was strikingly increased (1970 mg/day). The second case was 14-year-old boy of hyperthyroidism. The UPR was 5.40 mg/day (within the normal limit).