

wise filtration around the center of filter membrane prevents a leakage of the solution.) (2); the larger size of filter membrane (at

least 26 mm in diam.), the better. (3); for washing procedure of the tubes high concentration of albumin solution should be used.

Radioimmunoassay of Insulin by Modified Method of Hales and Randle

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The purpose of this paper is to report the insulin blood level measured by the method modified by Hales and Randle.

a) Method

Mixture of 0.5 ml of standard insulin and/or assay sample, 0.5 ml of ^{125}I -Insulin and 0.5 ml of insulin binding precipitate is incubated for 24 hours at 4°C . 0.1 ml of normal G.P. serum and anti γ -globulin serum compound is added to it and the mixture is centrifuged for 15 min at 2500 γ pm to avoid supernatant.

The precipitate is washed by buffer solution and it is centrifuged twice. The precipitate is counted.

b) Calculation

B/B_0

Where B is counting rate and B_0 is counting rate at the insulin level zero.

B/B_0 is obtained by the standard curve made by the standard insulin and the insulin level of the material is calculated.

c) Insulin level of the normal individuals.

The insulin level at the fasting time measured by this method is 35.2 ± 4.0 ($\mu\mu/\text{ml}$). The level is 125.2 ± 8.4 ($\mu\mu/\text{ml}$) at one hour after ingestion of 50g of glucose, 80.2 ± 14.7 ($\mu\mu/\text{ml}$) at two hours and 44.6 ± 7.4 ($\mu\mu/\text{ml}$) at three hours.

It is found that the standard deviation of the blood sugar level is large in the same patients.

This method is simpler and easier than the original method.

Immunoassay of Human Growth Hormone with a Double Antibody Method

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The present report describes a double antibody procedure for radioimmunoassay of human growth hormone (HGH) utilizing ^{125}I human growth hormone tracer. All dilutions were made with veronal buffer, pH 8.6, containing 0.5% bovine serum albumin. Anti human growth hormone serum was obtained from guinea pig which received five weekly

subcutaneous injections of 1 mg HGH (Raben) emulsified in complete Freund's adjuvant. The appropriate dilution of antiserum (1:100000) was that which binds about 20-40% ^{125}I -HGH added when no unlabelled growth hormone is present.

Anti guinea pig gamma globulin serum was obtained from rabbit and dilution of 1:2 was

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