D. Measurement III
In Vitro Radioimmunoassay

Usefulness of Polyethylene Glycol in T3 Radioimmunoassay

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Routine laboratory test should better be simple and easy. This paper describes usefulness of polyethylene glycol (PEG) for the separation of bound and free in triiodothyronine radioimmunoassay. As to the incubation time, shorter duration of 2 hr at various temperatures was tested, and 2 hr at 25 C incubation was found to give almost similar binding as indicated 24 hr at 4 C. After addition of 1 ml 25% PEG centrifugation was performed at 0, 15, 30, and 60 min. The results did not differ significantly. Centrifuge at 2 to 4 C resulted in rather poor stability and room temeprature centifugation was found favorable. When added amounts of PEG were reduced to a half or increased to twice of indication, obtained standard curves did not show significant differences. All above results supported the simplicity and easiness of PEG method.

Furthermore, comparing to widely used dextran coted charcoal (D.C.C.) method, PEG showed lower experimental back ground and almost equal maximal binding, giving wider count range by the added standard T3. Clinically 48 determinations were performed by both methods, an excellent correlation ($r=0.94$ $Y=1.08X+0.07$) was obtained.

Clinical results by PEG methods in 52 cases with various thyroid disorders gave similar results as reported by other method, and 25 normal subjects ranged from 1.05 to 1.90 ng/ml average 1.48 ± 0.25 (s.d)

In conclusion, PEG method in T3 radioimmunoassay was found quite suitable and application in commercial Kit was thought quite favorable.

Studies on Measurement of Plasma Renin Substrate Concentration and Plasma Renin Concentration by Angiotensin I RIA

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1) Measurement of plasma renin substrate concentration

One m*l* of crude renin extracted from human kidney by Haas' method had biological potency equal to 0.2 mg of Angiotensin II. To 50 μl of normal plasma, 25 μl of the crude renin extract and 500 μl of enzyme inhibitor (Sorin Co. kit) were added and incubated at 37°C. Angiotensin I generation was increased with incubation hours and reached to the maximum at 12 hours. After 12 hours incubation, 0.1 m*l* of the mixture was diluted with 9.9 m*l* of tris acetate buffer (0.1 M, pH 7.4), and 0.1 m*l* of the diluted solution was used for Angiotensin I RIA. The better parallelism of a dilution curve of Angiotensin I between 100 to 800 pg to the standard curve was observed when Sorin kit (Midorijuji Co.) was used than Dainabot kit. The plasma renin substrate concentrations measured by this method ranged between 600 and 1100 ng/m*l* in patients with essential hypertension and there was no difference in low, normal and high renin groups. In four patients with Cushing syndrome, a high value (1530 ng/m*l*) was observed in only one pregnant

patient (20 W.). The plasma renin substrate concentration ranged between 600 and 700 ng/m*l* in four patients with primary aldosteronism and in four with renal hypertension.

2) Measurement of plasma renin concentration

Plasma renin substrate in the sample was destroyed and incubated with adequate amount of exogenous renin substrate (Skinner's method). Three kinds of renin substrate were tested for this purpose. In human pool plasma and nephrectomised sheep plasma, there were substances which generated Angiotensin I after 4 to 6 hours incubation at 37°C, however after Haas' treatment, there was no Angiotensin I generation. In one mg of Cohn fraction IV-4 there were also Angiotensin I generating substances.

Purification of ^{125}I-Glucagon by Microfine silica (Quso G-32)

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The ^{125}I-glucagon labelling by chloramin T method showed a damage of the hormone which bound to plasma protein non-specifically and migrated with the protein on hydrodynamic flow electrophoresis. In an aim to purify the labelled glucagon, separation was carried out on sephadex G-10 column, and the purified fraction was mixed with 20 mg of Quso G-32 in 3 m*l* of 0.2 M glycine buffer solution (pH 8.8).

The mixture was centrifuged and the precipitate was washed with 2 m*l* of the same buffer solution. The washed precipitate was then suspended in 2 m*l* of ethanol-0.5 N HCl (3: 1, v/v), and was confirmed to be pure chromato-electrophoretically.

The purified ^{125}I-glucagon reacted with a glucagon antibody (30K) satisfactorily.

The Fundamental Study of Competitive Binding Radioassay for 25 OH D_3 in Human Plasma

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The human plasma 25 Hydroxyvitamin D_3 (25 OH D_3) levels were successfully measured by a competitive protein binding assay, using Vitamin D deficient rat serum as Vitamin D binding protein, for which a rat had been fed with Vitamin D, calcium, phosphorous deficient meals for 3 months. We have investigated some fundamental problems on performing this assay systems, 1: on the stability of 25 OH D_3 in ethanol and in serum under different conditions; (i.e. temperature, N_2, O_2 and CO_2 gas, and ultraviolet irradiation) 2: on the effect of incubation time with binding protein, as well as incubation time with dextran coated charcoal (DCC), 3: variability of replacement curves obtained by changing the adding order of binding protein, radiolabeled and standard 25 OH D_3, 4: recovery, and 5: cross reactivity with other Vitamin D analogues. 25 OH D_3 in ethanol was considerably destroyed by ultraviolet irradiation and by CO_2 gas atmosphere, but was stable with temperature ranging from -20°C to 25°C under O_2 or N_2 gas as long as two days. Binding protein which was considered as Vitamin D transport protein had a high affinity to 25 OH D_3, reaching maximum binding (Approximately 60%) within 5 minutes. The adsorptive reaction of DCC continued as long as 4 hours. Standard curves were variable by changing the mixing order of binding protein, radiolabeled and standard 25 OH D_3, among those, the standard curve obtained by adding, : standard 25 OH D_3, : radiolabeled 25 OH D_3, and : binding protein; in this order, was the most practical and suitable for clinical use. Finary our assay system provided sensitivity of 0.1 ng/tube, excellent recovery (98.5 ± 3.0%), poor cross-reactivity with other Vitamin D analogues such as D_3, D_2, 1-25 $(OH)_2$ D_3, and 1α OH D_3, and was proved to be useful for clinical application.