Clinical Evaluation of ^{125}Tetrasorb (Abbott) as a Method of Measuring Serum Thyroxine

H. NAKAJIMA, T. HORIGUCHI, N. SASAKI and S. KUBO

Department of Pediatrics, Chiba University, School of Medicine, Chiba

The authors et al. (Nakajima et al.: J. Clin. Endocrinol. 26: 99, 1966) have extended the principle of saturation analysis as a simple method of measuring total serum thyroxine, by using ^{131}I-triiodothyronine resin sponge uptake. ^{125}Tetrasorb (Abbott's T_4 Diagnostic Kit) based on the same principle offers several advantages. The kit provides a combination package of Tetrasorb resin sponge, a solution containing Thyroxine Binding Globulin ^{125}I, and associated disposable laboratory equipment.

After 1 ml of the serum sample is deproteinized with 2 volumes of 95% ethanol, 0.3 ml of the supernatant is dried in a tube with Tetrasorb Evaporator. Add one ml of ^{125}I-TBG solution to the each tube, and let stand 5 minutes at room temperature. Then let stand for 5 minutes in ice-water bath, and add resin sponge to each tube. After incubation in ice-water bath for 60 minutes, aspirate fluid from the tube, then wash sponge by adding water, depressing sponge with plunger, and aspirating. Obtain final count on resin sponge, and compare with initial count after first 20 minutes of incubation. The uncorrected thyroxine value is determined by the working standard curve prepared by plotting sponge uptake versus known concentration of thyroxine. The uncorrected thyroxine value is corrected for extraction efficiency in ethanol supernatant.

Resin sponge uptake determined at different incubation times and incubation temperatures. The standard curve obtained at 4°C has a steeper rise than that obtained at room temperature. Between 40 and 70 minutes, sponge uptake increases approximately 0.33% per minute, and agrees well with that reported by Abbott Laboratories.

The values for thyroxine iodine (μg/100 ml) in various groups of disease were: 1) 27 euthyroid subjects, 3.8—8.1 (range), 6.0±1.2 (mean ±SD), 2) 5 hyperthroid patients, 9.2—15.5, 12.1±2.4, 3) 6 hypothyroid patients, 0.6—2.5, 2.1±1.0, 4) 3 nephrotic patients, 2.6—3.7, 3.2±0.5, 5) 6 pregnant women, 12.1—13.9, 13.1±0.6.

Satisfactory reproducibility was observed. Range and standard deviation ($\pm\sqrt{\sum d^2/2N}$) obtained from the results of 10 duplicate determinations was respectively 2.0–15.1 μg/100 ml and ±0.56 μg/100 ml.

These results proved that this method could be used as a routine clinical diagnostic test.

Radioimmunoelectrophoretic Analysis of Thyroxine Binding Proteins (Second Report)

K. MIYAI, K. F. ITOH and H. ABE

The First Department of Medicine

Y. KUMAHARA

The Central Laboratory for Clinical Investigation, Osaka University Medical School, Osaka

By means of radioimmunoelectrophoretic technique, five radioactvie arcs have been found and shown not to be artifacts but thyroxine-binding components in normal sera in our laboratory. These five components have been identified as thyroxine-binding preal-

bumin (TBPA), albumin, thyroxine-binding globulin (TBG), α_1-lipoprotein and β (or α_2)-lipoprotein from the radioimmunoelectrophoretic patterns with specific antisera added or stained for lipoproteins and using TBG- deficient serum. Paperchromatographic analysis of ethanolacetone extract of the immunoelectrophoretic plate indicated that only about 10% of inorganic ^{131}I was liberated from the ^{131}I thyroxine, and no distinct radioactive areas were demonstrated when equivalent amount of ^{131}I sodium was added to the serum and analyzed by radioimmunoelectrophoresis.

Diphenylhydantoin sodium (DPH) which has been reported to displace thyroxine from TBG to other thyroxine-binding proteins, was dissolved in solvent (propylene glycol, 40% V/V and ethanol, 10.5%V/V in H_2O, PH 12) and added to the serum to give a final concentration of 3.6×10^{-2}M and 1.4×10^{-1}M. DPH also decreased the arc represented the so-called "TBG" in our first report.

A freshly prepared serum was mixed with purified ^{131}I-T_4 to give a final concentration of 0.05 μg per ml of serum. The low density lipoprotein (L.D.L.) fraction and high density lipoprotein (H.D.L.) fraction were separted from the mixture by means of ultracentrifugation.

On radioimmunoelectrophoretic patterns of these fractions, bindings of ^{131}I-T_4 to β (or α_2)-and α_1-lipoprotein were observed. From the recovery of radioactivity and concentration of lipoproteins determined by immunodiffusion method in each fractions, the binding percentage of T_4 to β (α_2)-lipoprotein in whole serum was estimated as 1.5—3.5% and to α_1-lipoprotein 3.5—13%.

Correlationship between the Liver and Catabolism of Thyroidal Hormones

O. Chiaki, T. Ueno, K. Uchiyama, K. Anazawa and S. Miwa

The First Dept. of Internal Medicine, School of Medicine, Chiba University, Chiba

Six groups of the rats were used throughout these experiments. The liver of both group I and II was intact and thyroidectomy was performed only on those of group II. The group III rats were not thyroidectomized and were intoxicated with repeated CC14 administration. And those of group IV were thyroidectomized and were given CC14 also repeatedly. This hepatotoxic agent was injected to both group V and VI and these animals were sacrificed 48 hours after the administration, prior to this procedure, thyroidectomy was performed on only group VI rats. The liver of group III and IV showed septal fibrosis and that of group V and VI revealed central necrosis.

All experimental animals were administered with ^{131}I-T_4 intravenously immediately before collecting the following specimens.

T.C.=t/B×100 ("t" indicates the radioactivity per gm in tissue, "B" indicates initial dose per gm in total body) represents tissue concentration of radioactivity in the blood, bile, liver, kidney and muscle at eight hours after ^{131}I-T_4 administration.

In the liver and kidney among non-thyroidectomized groups, group V showed the highest concentration, and group I the lowest. Among thyroidectomized animals, this phenomenon was more remarkable in not only the liver and kidney, but also bile, blood, and muscle.

In order to analyse the ^{131}I-compounds in bile, the specimens from choledochus were collected at 1, 2, 3...., 8 hours after the isotope injection. They were studied by thin layer chromatography. And the radioactivity of each fraction on T.L.C. was determined and represented in percent dosis per milliliter.

In group I and II, the radioactivity from group I was generally lower than that or group II, and conjugates were detected only in the bile from group II.

In group III and IV, T_3 was not revealed in any fraction of T.L.C. The radioactivity of iodine in the bile from group IV was high-