mine by this method the intra-erythrocytic concentrations of this drug after preliminary extraction. After centrifugation, serum is separated and erythrocytes are washed two times with isotonic saline, then erythrocytes are hemolyzed at -18 °C. For the extraction, mix 1 mL of erythrocytes with five drops of glacial acetic acid and 5 mL of isopropanol in chloroform (5:95 by vol), vortex-mix for 3 min, and centrifuge (5 min, 1000 × g). Separate the chloroform layer, filter it through 500 mg of anhydrous Na₂SO₄, and evaporate it at 60 °C under nitrogen. Reconstitute the residue in 1 mL of distilled water and analyze by the immunoenzymatic method (Clin Chem 1977;23:1144–9) as for a plasma sample. We validated the extraction by assaying serum samples with the xer isomethyl system before and after extraction. For 25 sera from patients being treated with theophylline, the mean percentage extraction was 87.20% (SD 5.5%). For concentrations up to 35 mg/L, linearity of response was good (r = 0.969; y = 0.96x - 0.58).

We studied erythrocyte binding of theophylline in patients treated for at least two weeks, analyzing 17 samples before (serum) and after the extraction procedure (serum and erythrocytes). Mean ± SEM concentrations of serum and erythrocyte theophylline and the ratio of erythrocyte/serum theophylline were, respectively: 11.04 ± 1.21 and 7.15 ± 0.74 mg/L, and 0.695 ± 0.063. Our data indicate an erythrocytic binding of theophylline averaging 69.58% (SD 6.32), in agreement with those previously reported (Clin Chem 1985;31:1415–6), indicating that theophylline binds to both erythrocytes and serum proteins in approximately the same ratio.

However, recent results of our laboratory seem to indicate that the concentrations of drug in erythrocytes are greater than those of free drug in serum. We plan to clarify this particular point and to try to establish clinical correlations with values for total, free serum, or intra-erythrocytic theophylline.

C-Terminal Radioimmunoassays for Parathyryn Compared for the Diagnosis of Primary Hyperparathyroidism, Pedro Esbrit and Aurelio Rapado (Unidad Metabólica, Fundación Jiménez Díaz, Avda. Reyes Católicos 2, 28040 Madrid, Spain)

The existence of various circulating forms of parathyryn (PTH) complicates the assay of this hormone. The heterogeneity of the results obtained with different RIAs (1) and the lack of an available standard have, until recently, impeded measurements of PTH in human serum. Because use of iodinated C-terminal fragments as tracers in RIAs for human PTH (hPTH) improves assay performance (2), we have evaluated three different RIAs of C-terminal specificity to measure immunoreactive parathyryn (iPTH) in serum of 76 patients with surgically proved primary hyperparathyroidism.

In assay A we used purified bovine PTH (bPTH) 1–84 (Inolox, Chicago, IL) as standard, 125I-labeled bPTH 1–84 as tracer (3), and either antiseraum Ch 4M or GP 500M (provided by Dr. C.D. Arnaud, Veterans Administration Medical Center, San Francisco, CA). Both antisera cross react with b- or hPTH, although bPTH 1–84 appears to be more reactive with GP 500M. Assays B and C involve synthetic C-terminal PTH fragments as tracers (both from Immuno Nuclear, Stillwater, MN). In assay B the commercially available reagents include 125I-labeled (Tyr²⁴)-hPTH 65–84 as tracer, antiseraum raised to hPTH and reactive to the 65–84 sequence of hPTH, and a desalted parathyroid adenoma tissue culture medium that on dilution gives values paralleling those for human serum and hPTH 65–84 fragment (European PTH Study Group, EPG) as standard. Assays A and B were performed as described (4). In assay C (5), 125I-bPTH 37–84 and bPTH 1–84 (71/324; National Institute for Biological Standards and Control, London, U.K.) were tracers and standard, respectively; the antiseraum, 017-spring-78 (EPG), primarily showed mid-region immunoreactivity to the hPTH molecule.

For comparison, the iPTH values determined in each single assay were related to the upper limit of its normal range. The iPTH values obtained with assay B and assay C showed similar relative ranges, more than 75% of the values being more than twice the respective upper normal limits. Both assays gave normal iPTH values in 14 and 9% of the group of patients studied, respectively. In contrast, assay A gave lower values, with <30% exceeding the upper normal limit by more than twofold. Although a relatively high 20% of the iPTH values obtained with assay A were within the normal range, two of these (7% of total) were from patients having normal calcium (<105 mg/L) on the day iPTH was determined.

The correlation coefficient between the concentrations of calcium and iPTH in serum from hyperparathyroid patients was higher for the homologous assay B (r = 0.72) than for the other assays (r = 0.5).

These results suggest that an RIA for measuring PTH in human serum based on a synthetic carboxy-terminal fragment as tracer, especially if it contains a human sequence, might well be chosen as an aid for the diagnosis of primary hyperparathyroidism.

References


Aspartate Aminotransferase and Alanine Aminotransferase Double Kinetic Assay in a Single Channel of the Hitachi 705 Analyzer, Agustín Jiménez, Joaquín Arenas, Ignacio Santos, and Alberto Martínez (Departamento de Bioquímica, Hospital Primero de Octubre, Avda de Andalucía, Km 5.5, Madrid 28041, Spain)

One of the most interesting performance characteristics of the automated Hitachi 705 analyzer is the possibility of measuring two consecutive reactions in a single cuvet, thus increasing the number of determinations per unit of time.