CLIN. CHEM. 22/10, 1655–1658 (1976)

Automated Radioimmunoassay of Choriomammotropin (Human Placental Lactogen)

Albert Castro,[1] Isidro Prieto,[1] Christian Wunsch,[1] Gerhard Ertingshausen,[2] and Herbert Malkus[1]

We describe a totally automated procedure for radioimmunoassay of choriomammotropin, in which all phases of the assay are automated in a single system ("Centria"). This system permits the simultaneous incubation and separation of many samples in a nonequilibrium assay, and measurements are obtained in less than 30 min. Results for clinical samples by reference radioimmunoassay methodology and with the Centria system compared uniformly well: $y = 0.91x - 0.87$; $r = 0.94$. The coefficient of variation for samples run in duplicate on the same day was 5.2%, 7.4% for samples run on different days. The specificity, sensitivity, simplicity, and speed of this system makes it a useful new tool for kinetic, nonequilibrium immunoassay.

Additional Keyphrases: *assessing placental function* • *Centria system*

Choriomammotropin (formerly called human placental lactogen or HPL) is a protein hormone that is extremely similar in chemical and physical properties to human somatotropin (*1, 2*). It is secreted by the syncytiotrophoblast of the normal human placenta, and its measurement is a good index of placental function. Its concentrations in blood increase rapidly during the first 30 to 34 weeks of gestation, become constant, then decline slightly during the final week of pregnancy. It may also be found in the serum of patients with hydatidiform mole, choriocarcinoma, and some ectopic tumors.

[1] Department of Pathology, School of Medicine, University of Miami, Miami, Fla. 33152.

[2] Corporate Research Department, Union Carbide Corp., Tarrytown, N. Y. 10591. (Present address: Union Carbide, 4, Place des Etats Unis, Rungis, 94533 France).

Address correspondence to: A. Castro, Ph.D., University of Miami, School of Medicine, Department of Pathology, P.O. Box 520875, Biscayne Annex, Miami, Fla. 33152.

Received June 3, 1976; accepted July 8, 1976.

Methods for radioimmunoassay of this hormone have been described (*3–14*). Its concentrations in plasma, urine, and other biological fluids are readily measured, and the technique is sensitive to amounts of 50 pg or less.

The method we describe here is based on a novel automated system that in many respects is a radical departure from conventional ways of performing radioimmunoassays; all phases of the assay are done in an automated series of steps. The resulting simultaneous incubation and separation of many samples in a nonequilibrium assay allows measurement of the hormone in less than 30 min.

Materials and Methods

Reagents

Antigen. Highly purified choriomammotropin was supplied by the Medical Research Council Division of Biological Standards (NBS). Another batch was obtained from ICN Pharmaceuticals, Cleveland, Ohio 44128. Either material was used as a standard throughout the project. Dilutions were prepared in plasma from men, which contains no choriomammotropin. Fifty microliters of each dilution was prepared, divided into aliquots of 0.5 ml, and stored at −20 °C.

Antibody production. Four New Zealand rabbits were injected subcutaneously at three injection sites with 1 mg of choriomammotropin dissolved in 0.5 ml of physiological saline and emulsified with an equal volume of complete Freund adjuvant. After four weeks, a second injection of 1 mg in incomplete Freund adjuvant was given and repeated every two weeks. After 10 weeks, blood was collected via cardiac puncture and the serum assayed by radial immunodiffusion. One rabbit produced antibodies of high enough specificity to produce well-defined diffusion rings.

The antiserum was then characterized by radioimmunoassay. The titer was expressed as that dilution of antibody that will bind 50% of 2 ng of ^{125}I-labeled choriomammotropin, 100% being taken as the proportion of the label that was bound in antibody excess.

Assay buffer. This was a phosphate buffer (30 mmol/liter, pH 7.3) containing, per liter, 10 g of human serum albumin and 10 ng of sodium azide.

Preparation of ^{125}I-labeled choriomammotropin. We labeled the hormone with ^{125}I by a modification of the Chloramine T method of Greenwood et al. (*15*). Choriomammotropin, 20 μg, was dissolved in 20 μl of phosphate buffer, 30 mmol/liter, and added to 2 mCi of ^{125}I (New England Nuclear, Boston, Mass. 02118) in a small glass vial, followed by 35 μl of buffer containing 50 mg of Chloramine T. The reactants were mixed for 15 s. After adding 75 μl of the phosphate buffer containing 150 mg of sodium metabisulfite, the mixture was applied to a 0.9 × 18 cm column containing Sephadex G-75, to separate undamaged from damaged labeled material and unreacted ^{125}I.

The column was initially flushed with 2 ml of bovine serum albumin solution (20 g/liter). The purified reaction mixture was eluted from the column with assay buffer.

Specific activity of the labeled peptides during the project had a mean of 95–100 Ci/g.

The most suitable fractions were divided into small aliquots and stored at −20 °C.

Automated Equipment and Reagents

Centria system. A prototype of the "Centria" system (Union Carbide Corp., Tarrytown, N. Y. 10591) (*16*) was used for the experiments. Thirty analysis positions are provided in the system, consisting of three modules: a reagent and sample pipettor, an incubator/separator, and a counter/computer.

1. The Centria Pipettor and Transfer Disc. The Centria pipettor is designed around a turntable, which is first loaded with a ring containing 30 sample cups. A disposable plastic transfer disc, which consists of 30 radially aligned pairs of cavities, is then placed on the turntable. Standards or samples are automatically withdrawn from the cups and loaded into the outer cavities of the disc. The sample volume is either 15, 35, or 50 μl, depending on the assay. In each case the sample is diluted to 100 μl, to complete transfer of the measured volume and to minimize carryover problems. Before the next sample is aspirated, the outside of the sample tip is rinsed in a water reservior. While sample or standard is being loaded, antiserum (200 μl) and radioactive competing antigen (50 μl) are automatically withdrawn from reservoirs and dispensed into the radially aligned cavities—antiserum into the inner cavity and radioactive ligand into the outer cavity.

2. The Centria Incubator/Separator. This module is the heart of the system, where the reactants are incubated and separated. It consists of a variable-speed turntable, which is first loaded with a plastic ring holding 30 small, ready-to-use separating columns. They are positioned in test tubes, which in turn are suspended by their upper rims in ball seats in the plastic ring. The filled transfer disc is placed on the turntable and automatically keyed such that each pair of cavities is aligned with the opening of a separating column.

When the turntable is started to rotate, antiserum flows from the inner into the outer cavity, where all reactants are instantaneously mixed and held for a preset interval. At the end of the incubation period, the instrument automatically accelerates the turntable rotation to a higher speed, causing the incubated mixture to be transferred onto the columns, where it embeds in the separating medium. After it embeds, a pump is started, which dispenses a predetermined amount of liquid onto the spinning transfer disc, where it is divided into equal aliquots by the geometry of the disc. As the eluent is driving the incubated mixtures through the separating medium, the free radioactive tracer is either adsorbed or partitioned out on the column. The antibody-bound tracer in turn moves with the front of the eluent and is collected in the test tubes that support the spinning columns.

3. The Counter/Computer. The third module of the system consists of a three-channel gamma counter/computer combination, which counts the radioactivity in the test tubes and calculates concentrations based on standards run simultaneously with the clinical samples.

Ion-Exchange Column Preparation and Use.

Swelling. Phosphate buffer (pH 6.6, 0.2 mol/liter) was used for swelling. Ten to fifteen grams of diethylaminoethyl-Sephadex A-50 was suspended in 300 ml of the phosphate buffer, covered, and swelled overnight or until the slurry was thin enough to pipette. The slurry was degassed thoroughly before use.

Columns. Full-size Sarstedt columns (Walter Sarstedt, Inc., Princeton, N.J. 08540) were used. The bottom plug was a Porex chromic acid-treated hydrophilic plug, pre-wet with the pH 6.6 phosphate buffer. Slurry, 4 μl per column, was pipetted in with a 4-ml "Tip-a-tip" (Macalaster Bicknell, New Haven, Conn. 06507).

Columns were drained completely and washed with 20 ml of pH 6.6 phosphate buffer. Heights were checked and adjusted, then the columns were rewashed with the buffer and drained.

Elution. Columns were eluted in the centrifugal field with phosphate buffer (pH 7.0, 25 mmol/liter).

Other Analytical Details

Standard curve, choriomammotropin (Figure 1). The 200 mg/liter stock was further diluted 10-fold with serum from men. The following concentrations were then prepared by serial dilution: 10, 5, 2.5, 1.25, and 0.625 μg/ml.

Antibody dilution. Choriomammotropin antibody was diluted 8000-fold and used in the Centria pipetter module.

Choriomammotropin tracer. We used ^{125}I-labeled choriomammotropin, 0.8 ng/50 μl (16 μg/liter).

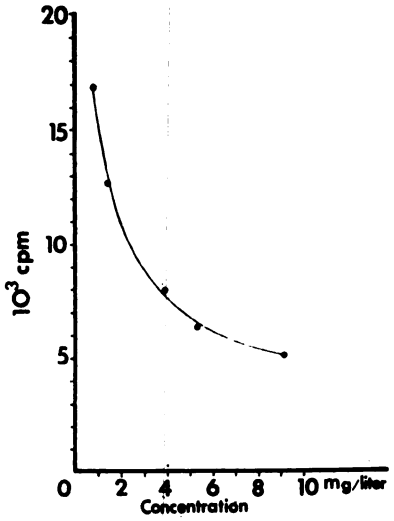

Fig. 1. Displacement of binding of ^{125}I-labeled choriomammotropin by nonradioactive choriomammotropin
The affinity constant was calculated to be 3.1×10^9

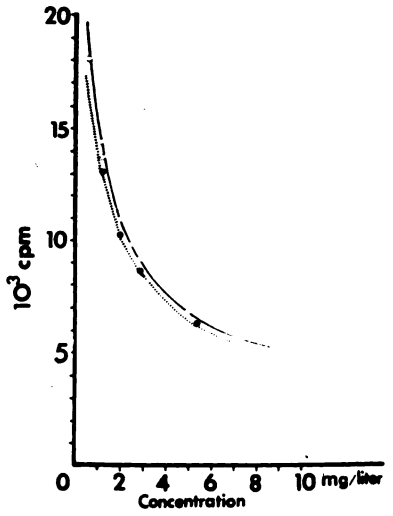

Fig. 2. Typical results of a parallelism study of different dilutions of two plasma samples

Radioimmunoassay. Unknowns and standards were loaded into the 30 sample cups of the turntable and the pipetter was programmed to aspirate the following: 50 µl of standard or unknown, 200 µl of choriomammotropin antiserum, 50 µl of ^{125}I-labeled choriomammotropin. Elution volume for the separation in the incubator/separator was 1.4 ml, and phosphate buffer (pH 7.0, 25 mmol/liter) was used as eluent.

Results

Blanks

Distilled water and pooled plasma from men were used for blanks. Blanks were not significantly different from zero.

Standard Curve

The variability between assays was not significant in 15 different runs. The percentage of ^{125}I-labeled choriomammotropin bound proportionally decreased as the mass of unlabeled choriomammotropin increased from 0.6 to 20 µg/ml (0.6 to 200 mg/liter), as shown in Figure 1.

Analytical Variables

Accuracy. In recovery experiments, an index to accuracy, we added 2, 4, 6, 8, 10, 12, 14, and 20 µg of choriomammotropin per milliliter to pooled plasma from men. The correlation coefficient (found vs. expected) was 0.997.

Sensitivity. In the standard curve plotted with every run, the percent bound for 0.6 ng was significantly different at the 95% confidence limit from that for 0 ng of choriomammotropin. The coefficient of variation at each point of the standard curve, assayed in duplicate, was always less than 5%.

Reproducibility. Reproducibility was examined by measurement of choriomammotropin in the same assay and in different assays of 10 replicate assays on the same day. The coefficient of variation was 5.2%. The coefficient of variation of 24 determinations, assayed on different days, was 7.4%.

Parallelism. We studied parallelism in samples of high choriomammotropin concentration. The samples were diluted two-, three-, five-, nine-, and 17-fold, to study the behavior of the antibody in comparison to a standard curve. The plot of plasma dilutions vs. trace binding (Figure 2) paralleled the standard curve, which indicates that the antibody was only reacting with choriomammotropin.

Comparability. Eighteen clinical samples were also analyzed by our manual choriomammotropin procedure in which charcoal is used in the separation step, and the results were compared to values obtained with the Centria system. The resulting regression equation was: $y = 0.91x - 0.87$; $r = 0.94$.

Discussion

In the present system, results of recovery studies were satisfactory. No systematic error was found in the procedure. Inter- and intra-assay precision of this method is very good. The coefficient of variation of the assay for samples run in duplicate the same day was 5.2%, and 7.4% for 24 samples run on different days. The sensitivity of this procedure was adjusted to 600 µg/liter, but may be increased to 10 µg/liter. However, this is not really necessary for this type of assay because of the concentrations of choriomammotropin in plasma.

The specificity of the radioimmunoassay depends upon the specific binding capacity of an antibody toward its specific antigen. Our anti-choriomammotropin antiserum has shown specific affinity for choriomammotropin, and the affinity constant (K_a) was 3.1×10^9 liter/mol.

In the Centria system we were able to assay more than 60 samples of choriomammotropin per hour with high sensitivity and specificity.

This work was supported in part by Grant 884M from the Council for Tobacco Research, U.S.A., Inc., New York, N. Y. 10022.

References

1. Sherwood, L.M., Similarities in the chemical structure of human placental lactogen and pituitary growth hormone. *Proc. Nat. Acad. Sci. U.S.A.* **58,** 2307 (1967).

2. Sherwood, L.M., Handwerger, S., McLaurin, W.D., and Lanner, M., Amino-acid sequence of human placental lactogen. *Nature, New Biol.* **233,** 59 (1971).

3. Grumbach, M.M., and Kaplan, S.L., On the placental origin and purification of chorionic growth hormone-prolactin and its immunoassay in pregnancy. *Trans. N. Y. Acad. Sci.* **27,** 167 (1964).

4. Beck, P., Parker, M., and Daughaday, W., Radioimmunologic measurement of human placental lactogen in plasma by a double antibody method during normal and diabetic pregnancies. *J. Clin. Endocrinol.* **25,** 1457 (1965).

5. Frantz, A.G., Rabkin, M., and Friesen, H., Human placental lactogen in choriocarcinoma of the male. Measurement by radioimmunoassay. *J. Clin. Endocrinol. Metab.* **25,** 1136 (1965).

6. Samaan, N., Yey, S., Friesen, H., and Pearson, O., Serum placental lactogen lack during pregnancy and in trophoblastic disease. *J. Clin. Endocrinol. Metab.* **26,** 1303 (1966).

7. Spellacy, W., Carlson, K., and Birk, S., Human placental lactogen levels as a variable of placental weight and infant weight. *Am. J. Obstet. Gynecol.* **95,** 118 (1966). .

8. Sciarra, J., Sherwood, I., Varma, A., and Lundberg, W., Human placental lactogen (HPL) and placental weight. *Am. J. Obstet. Gynecol.* **101,** 413 (1968).

9. Sexena, B., Refetoff, S., Emerson, K., and Selenkow, H., A rapid radioimmunoassay for human placental lactogen. Application to normal and pathologic pregnancies. *Am. J. Obstet. Gynecol.* **101,** 874 (1968).

10. Genazzani, A., Aubert, M., Casoli, M., et al., Use of human placental lactogen radioimmunoassay to predict outcome in cases of threatened abortion. *Lancet* **ii,** 1385 (1969).

11. Leake, N.H., and Burt, R.L., Solid phase radioimmunoassay of human placental lactogen. *Obstet. Gynecol.* **34,** 471 (1969).

12. Josimovich, J., Kosor, B., Boccella, L., et al., Placental lactogen in maternal serum as an index to fetal health. *Obstet. Gynecol.* **36,** 244 (1970).

13. Letchworth, A., Boardman, R., Bristow, C., et al., A rapid radioimmunoassay for human chorionic somatomammotrophin. *J. Obstet. Gynaecol. Br. Commonw.* **78,** 535 (1971).

14. Weintraub, B., and Rosen, S., Ectopic production of human chorionic somatomammotrophin by nontrophoblastic cancer. *J. Clin. Endocrinol. Metab.* **32,** 94 (1971).

15. Greenwood, F.C., Hunter, W.M., and Glover, J.S., The preparation of ^{131}I labelled human growth hormone of high specific radioimmunoassay. *Biochem. J.* **89,** 114 (1963).

16. Ertingshausen, G., Shapiro, S.I., Green, G., and Zborowski, G., Adaptation of a T_3-uptake test and of radioimmunoassays for serum digoxin, thyroxine, and triiodothyronine to an automated radioimmunoassay system—"Centria". *Clin. Chem.* **21,** 1305 (1975).

17. Berson, S.A., Yalow, R.S., Bauman, A., et al., Insulin I^{131} metabolism in human subjects: Demonstration of insulin binding globulin in circulation of insulin treated subjects. *J. Clin. Invest.* **35,** 170 (1956).