Use of Polyethylene Glycol in Radioimmunoassay of Human Placental Lactogen

Clifford L. Ermshar and David J. Gusseck

We describe a rapid radioimmunoassay for human placental lactogen in biological fluids, with use of polyethylene glycol to separate free from antibody-bound placental lactogen. The standard curve obtained, easily fitted to a logit-log transformation, is useful over a concentration range of 6 to 400 ug/liter. Within-assay variability is 1.97%, between-assay variability 2.20%. The relation is linear when lactogen added is plotted against that accounted for analytically, the actual recovery being 100.26%.

Human placental lactogen (hPL) is a simple polypeptide hormone secreted by the syncytiotrophoblast of the human placenta. Its production increases throughout normal gestation in a characteristic pattern, which is altered in certain trophoblastic disorders. Accordingly, its concentrations in maternal serum may serve as an index of placental function (7). In addition, hPL is sometimes synthesized ectopically by non-trophoblastic tumors.

Several RIA methods for the measurement of hPL have been previously described. Beck et al. (2) used a double antibody method for separation of free from antibody-bound hormone, while Saxena et al. (3), used dextran-coated charcoal. Chromatoelectrophoresis was used by Grumbach and Kaplan (4), and Letchworth et al. (5) used ethanol precipitation. Other methods have included ammonium sulfate precipitation (5) and solid-phase radioimmunoassay (6). It has been reported previously that polyethylene glycol is useful for separating free and antibody-bound peptide hormones in radioimmunoassays (7). Aqueous polyethylene glycol (PEG) causes the precipitation of antibody-bound hormone, while unbound antigen remains free in solution.

Our studies on hPL synthesis and secretion in vitro required that we have a rapid and reliable method for the radioimmunoassay of hPL. Because the polyethylene glycol method of RIA had been successfully applied to hormones of low relative molecular mass, such as insulin, somatotropin, and vasopressin (7), we extended this technique to the separation of free from antibody-bound hPL. This report describes the characteristics of the assay for the measurement of hPL concentrations in serum, plasma, and buffer. A straight-line plot for the standard curve is obtained by using the logit-log transformation.

Materials and Methods

Assay buffer. 0.1 mol/liter tris(hydroxymethyl)methylamino acetate, pH 7.4, containing 2.5 g of bovine serum albumin and 100 mg of sodium azide per liter, in de-ionized water.

Polyethylene glycol (PEG). A 260 g/liter-PEG (molecular weight 6000-7500 daltons) stock solution was prepared in de-ionized water and stored at 4 °C. An equal volume of this is added to each assay, bringing the final concentration of polymer to 130 g/liter.

Plasma buffer. Human plasma was prediluted 10-fold with assay buffer and 300 ul of the dilution was used per assay. The plasma provides carrier immunoglobulin, a necessity for complete precipitation of antibody-bound hormone by PEG (7).

Unlabeled hPL. Lyophilized hPL, stated to possess a purity in excess of 96% by the manufacturer, was obtained from the Nutritional Biochemicals Division of ICN Life Sciences Group, Cleveland, Ohio 44128. Standard solutions were prepared (0 to 400 ng/ml) with assay buffer as diluent.

Radiolabeled hPL. [125I]hPL was obtained from New England Nuclear, Boston, Mass. 02118, (specific acty. 26.5 Ci/g) and diluted with assay buffer containing 52.5 g of bovine serum albumin per liter to a final concentration of 0.48 mg/100 ml (4.8 g/liter) solution. All samples were counted with an automated gamma counter.

Antiserum to hPL. Rabbit antiserum to hPL (Antibodies Incorporated, Davis, Calif. 95616) was used at a dilution sufficient to precipitate 45% of the total counts per antibody control tube during a 30-min incubation at 37 °C.

Procedure for assay of hPL. All tests were run in duplicate in 12 x 75 mm polypropylene test tubes, according to the protocol indicated in Table 1. After addition of antiserum the contents of the tubes are vortex-mixed and incubated at 37 °C for 30 ± 5 min. After incubation, 260 g/liter PEG at 4 °C is added and the contents of each tube are vigorously vortex-mixed for 5 s. The tubes are then centrifuged at 4 °C, 6000 x g for 10 min. The tubes should be centrifuged within 30 min after PEG is added. The supernates are then aspirated, taking care to remove all liquid from the tube, and the radioactivity in the precipitates is counted.

Results and Discussion

Optimization of plasma concentration. Figure 1 shows the amount of radioactivity precipitated specifically and nonspecifically as a function of plasma concentration in the buffer. Consideration of several factors led us to choose a carrier plasma concentration of 100 ml/liter of buffer. First, a sufficient amount of carrier immunoglobulin needs to be present to completely precipitate the hPL antibody-bound complex by PEG while minimizing nonspecific precipitation of free
antigen. On the other hand, it is desirable to use as high a carrier plasma concentration as possible in order to minimize the additional nonspecific precipitation that might result when immunoglobulin present in a patient's serum or plasma (containing hPL) is added to the assay. Since these samples are diluted 100-fold for assay, they contribute a very small immunoglobulin increment when the carrier plasma concentration is high. Second, a high protein concentration gives a large, easily visible pellet, from which the supernate can be more efficiently removed. It is evident from Figure 1 that, at a plasma concentration of 100 ml/liter, precipitation of the antibody-bound complex is complete, and that nonspecific precipitation is relatively low.

**Determination of hPL concentration in biological fluids.**

Standard curves plotted in two different ways are shown in Figures 2 and 3. In both, the ordinate (B/B0 × 100) is shown as a percentage of the counts bound when a known amount of hPL (0 to 400 µg/liter), designated B, is added to the assay mixture relative to the counts bound by the antibody control or zero standard (containing no added unlabeled hPL), designated B0. In each case, the counts for nonspecific precipitation or blank are first subtracted from the counts bound by each standard (B) and the zero standard (B0). The "total counts control" tube is included with each assay to determine if 45% of the total counts present are being bound by the antibody control, B0, after correcting for nonspecific precipitation. Figure 2 is the simplest representation of the standard curve. The most nearly accurate determinations of hPL concentration fall in the linear portion of the dose-response curve. The least detectable concentration—i.e., the concentration resulting in a response two standard deviations away from the zero-dose response—is approximately 6 µg/liter. Figure 3 shows that the data fit a logit-log transformation from which one can easily interpolate the concentration of hPL in any unknown solution for a given % B/B0. The logit, which is plotted on the right-hand ordinate, is calculated as follows: logit P = log (P/100-P), when P equals the % B/B0.

**Determination of variability in results.** (a) Within-assay variability: For 10 plasma samples run in the same assay, the coefficient of variation was 1.97% for a mean hPL concentration of 115.48 ± 0.86 (SE) µg/liter. (b) Between-assay variability: For 10 plasma samples run in successive assays the coefficient of variation was 2.22% for a mean plasma value of 114.25 ± 1.27 (SE) µg/liter.

**Specificity of the antisera.** Rabbit antisera to hPL cross reacts to some extent with the structurally related peptide hormone, human somatotropin (8, 9). However, the importance of this cross reaction is minimized because the sensitivity of the antisera to hPL is 1000-10,000 times greater than to human somatotropin (10). In addition, the concentration of hPL in pregnancy sera is more than 100-fold the somatotropin concentration.

### Table 1. hPL Assay Protocol (Sequence of Addition of Reagents)

<table>
<thead>
<tr>
<th></th>
<th>Plasma buffer</th>
<th>Assay buffer</th>
<th>Known std. or unknown serum</th>
<th>[125I] hPL</th>
<th>Diluted antiserum</th>
<th>20% PEG</th>
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</thead>
<tbody>
<tr>
<td>Blank (nonspecific counts control)</td>
<td>300</td>
<td>200</td>
<td>—</td>
<td>100</td>
<td>—</td>
<td>600b</td>
</tr>
<tr>
<td>Antibody control (zero standard)</td>
<td>300</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>100a</td>
<td>600b</td>
</tr>
<tr>
<td>Standard solution (6.25–400 µg/liter)</td>
<td>300</td>
<td>—</td>
<td>100</td>
<td>100</td>
<td>100a</td>
<td>600b</td>
</tr>
<tr>
<td>Unknown serum or other fluid</td>
<td>300</td>
<td>—</td>
<td>100</td>
<td>100</td>
<td>100a</td>
<td>600b</td>
</tr>
<tr>
<td>Total counts control</td>
<td>—</td>
<td>1100</td>
<td>—</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* a Vortex-mix and incubate for 30 min at 37 °C at this step.
* b Vortex-mix vigorously for 5 s, centrifuge (8000 X g, 10 min), aspirate the supernate, and count the residue.

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**Fig. 1.** [125I] hPL precipitated specifically and nonspecifically as a function of human plasma concentration in the assay buffer.

**Fig. 2.** Semi-logarithmic representation of the standard curve. Data points are the average (±SE) of 10 replicates. B = Precipitated radioactivity in the presence of unlabeled hPL. B0 = Precipitated radioactivity in the absence of unlabeled hPL. B and B0 have been corrected for nonspecific precipitation.
**Table 2. Analytical Recovery Data**

<table>
<thead>
<tr>
<th>Vol. of unknown (patient's plasma, 100-fold dilution) added, μl</th>
<th>Conc. of std. added, μg/liter</th>
<th>Observed recovery, %</th>
<th>Expected recovery, %</th>
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<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>50</td>
<td>6.25</td>
<td>36.78</td>
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<td>50</td>
<td>25.0</td>
<td>47.74</td>
<td>47.97</td>
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<tr>
<td>50</td>
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<td>60.46</td>
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<tr>
<td>50</td>
<td>100.0</td>
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<td>85.46</td>
</tr>
<tr>
<td>50</td>
<td>200.0</td>
<td>142.34</td>
<td>135.47</td>
</tr>
<tr>
<td>50</td>
<td>400.0</td>
<td>230.20</td>
<td>235.47</td>
</tr>
</tbody>
</table>

*50 μl added in each case.*

**Antibody-bound hPL is completely precipitated with little nonspecific precipitation of free hPL. Virtually all radioactive hormone remaining in solution is unbound and there is no evidence for dissociation of antigen–antibody complexes upon addition of PEG. This method also avoids nonspecific effects of plasma and anticoagulants, which are ever present in double-antibody RIA methods.**

We thank C. K. Man for his technical assistance and Dr. George Lessard for helpful discussions. This work was supported by grants from the National Institutes of Health (HD09440) and the National Foundation—March of Dimes (6-86).

**References**


