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A rapid radioimmunoassay of human LH and HCG useful in early pregnancy problems

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A 5-hour radioimmunoassay for LH-HCG which proved to be useful in early pregnancy problems is described.

LH–HCG RIA; early pregnancy

Introduction

Rapid radioimmunoassays of LH–HCG using a solid phase system (Miyata, Taymor, Levesque and Lymeburner, 1970; Spona, 1973) to separate antibody bound from free hormone were hampered by aspecific serum interferences (Reuter, Hendrick, Sulon and Franchimont, 1973) although offering practical advantages. Soluble double antibody precipitation of the bound fraction (Vekemans and Robyn, 1974) minimalized the latter problem but was too time consuming for routine daily clinical application.

In order to combine the advantages of a solid phase system with the absence of aspecific serum effects (Koninckx, Bouillon and De Moor, 1976) a double antibody solid phase system with precipitation of the first antiserum before the incubation was investigated.

Materials and methods

The first antibody was a rabbit anti-HCG serum (Organon AS 350) while a ^{125}I-LH preparation (specific activity 100–150 μCi/μg) was used as tracer. The latter was purchased from IRE (Fleurus, Belgium) and repurified every fortnight on a Sephadex G-100 (1 X 60 cm) column. Our double antibody solid phase (DASP) was prepared as described previously (Koninckx et al., 1976) by coupling a goat anti-rabbit gammaglobulin serum to microcristalline cellulose activated by cyanogen bromide.

An amount of the first antiserum sufficient to run 500 determinations was incubated for 24 h at room temperature with an excess of DASP. This complex was immediately devided in assay tubes and stored frozen until use. The assay was performed by adding the tracer (0.1 ml, 30000 cpm) and the standard or unknown samples (0.1 ml) to freshly unfrozen tubes. After an incubation of 4 or 16 h at room temperature with continuous mixing, separation of antibody bound from free hormone were performed by centrifugation and decantation. The results were expressed in mIU/ml and the standards used were the 68-40 preparation of the M.R.C. and the LER-907 of the N.I.H. The detection limit was expressed as defined by Ekins, Newman and O'Riordan (1968).

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Results

As shown in Figures 1 and 2 the detection limit of the assay increased with the duration of the incubation from 4.8 mIU/ml (2 h) to less than 2 mIU/ml (16 h) and could further be improved by lowering the amount of tracer included. Using a 4-h incubation the within and between assay variances were 5.5 and 7.9% for 8 MIU/ml, and 6.2 and 7.5% for 128 mIU/ml, respectively; after a 16-h incubation they improved to 2.5 and 2.8% for 8 mIU/ml, and 2.7 and 5.7% for 128 mIU/ml, respectively.

Since about one year a 4-h incubation with inclusion of 30000 cpm of ^{125}I-LH was used for routine application and proved to be useful in clinical practice. Plasma LH–HCG concentrations of more than 128 mIU/ml were always associated with an intrauterine ($n > 500$) or extrauterine ($n = 3$) pregnancy, whereas slightly elevated levels (more than 20 mIU/ml) were difficult to interpret since several times ($n = 7$) a pregnancy could not be objectivated although LH peaks and menopause were excluded. When normal concentrations (2–10 mIU/ml) were found an extrauterine pregnancy, although clinically suspected, could always be excluded ($n = 12$).

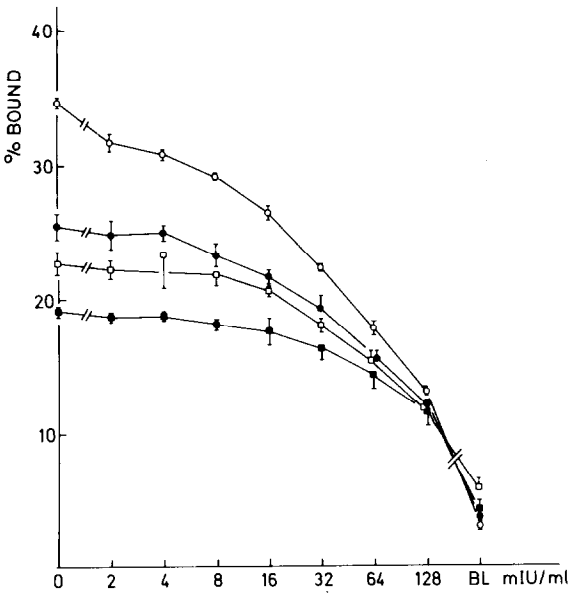

Fig. 1. Standard curves (mean ± SD) of LH for different durations of incubation (■ = 2-h; □ = 4-h; ● = 8-h; ○ = 16-h). The amount of ^{125}I-LH was 30000 cpm.

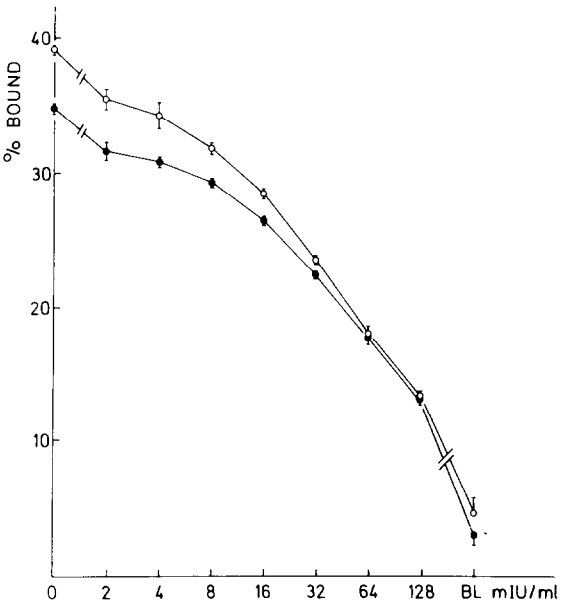

Fig. 2. Standard curves (mean ± SD) of LH for different amounts of ^{125}I-LH (● = 30 000 cpm; ○ = 10 000 cpm). The duration of incubation was 16 h.

Discussion

A rapid radioimmunoassay of LH–HCG is useful in clinical practice to confirm the diagnosis of an extrauterine pregnancy or to locate the midcycle LH peak (Goldstein, Miyata, Taymor and Levesque, 1972; Kosasa, Taymor, Goldstein, Levesque and Levesque, 1973; Spona, 1973). These indications implicate that the assay has to be performed daily and therefore it cannot be too time consuming. The latter was obtained by precipitating the first antibody before the incubation (Midgley, Rebar and Niswender, 1969) and by the solid nature of DASP, facilitating the separation of antibody-bound from free hormone. Delayed addition of tracer, or incubation at different temperatures, both known to enhance the sensitivity of a radioimmunoassay were considered too time consuming and were therefore not investigated.

A rapid radioimmunoassay requires not only a short incubation, but also rapid counting and processing of results. The latter was obtained by expressing standard curves and unknown samples in cpm while the increased amount of tracer allowed for a counting time of thirty seconds.

The detection limit was much lower than in our 7-day radioimmunoassay (Koninckx, De Moor and Brosens, 1975) but largely sufficient since the concentrations to be measured were anyway much higher than the normal adult basal levels. The diagnosis of an intrauterine pregnancy was accurately made from the missing period on and the clinical diagnosis of an extrauterine pregnancy could be rejected or confirmed in the majority of cases. However, drawing conclusions from slightly elevated plasma LH–HCG concentrations was difficult since seven women had a menstruation a few days later. We suggest that this were early abortions which would never have been diagnosed without LH–HCG radioimmunoassays since LH peaks were excluded on clinical grounds.

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