A SIMPLE TECHNIQUE FOR THE ASSAY OF PROGESTERONE IN PREGNANCY

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Diamond, Rust & Westphal (1969) originally showed that pregnant guinea-pig plasma binds progesterone about 100 times more firmly than cortisol. Work along this line yielded a competitive protein-binding method for the assay of progesterone in plasma during pregnancy.

Plasma was obtained from pregnant Casia guinea-pigs in the latter part of gestation. [1-2-3H]Progesterone (6 ng, 33 Ci/mnmol) in ethanol was evaporated to dryness and dissolved in 30 ml 0-04 m-phosphate buffer, pH 7-4. Fifteen microlitres guinea-pig plasma were added and mixed gently for 1 h at room temperature. To 100 μl plasma, 200 μl methanol were added and the mixture kept at −20 °C for 10 min. After centrifugation an appropriate volume of the supernatant was transferred to a small glass test tube and evaporated to dryness. Benzene (100 μl) and heptfluorobutyric anhydride (3 μl) were added to the residue and the sample was kept at 62 °C for 30 min. The solution was then evaporated to dryness. A blank was obtained by processing 100 μl distilled water in the same way. To glass tubes containing the unknown samples, the blanks, or known amounts of progesterone (standard curve), 1 ml of the protein-binding solution was added. After gentle shaking, the tubes were kept at 45 °C for 5 min and then at 4 °C for 30 min. To each tube was added 0-5 ml suspension of 0-25% charcoal and 0-025% dextran (w/v) in 0-04 m-phosphate buffer, pH 7-4. After mixing individually for exactly 10 s, all samples were kept at 4 °C for 15 min, after which they were centrifuged for 10 min at 3000 rev./min. The supernatant (500 μl) was transferred to a 20 ml vial and the radioactivity was counted after addition of 10 ml Bray scintillation mixture.

Sensitivity. In our system, 0-05 ng is significantly different from the zero value. Reagent blanks were never found to exceed 0-02 ng; the mean value was 0-015 ± 0-008 (s.d.) in 20 consecutive runs. This blank value is negligible at measurable plasma concentrations.

Specificity. We have studied the ability of the main steroids present during pregnancy to displace tritiated progesterone from diluted pregnant guinea-pig plasma. The highest binding affinity was obtained with 20α-dihydroprogesterone and testosterone. The cross reaction calculated according to Abraham’s (1969) formula gave 35 and 15% respectively for these two steroids. Heptfluorobutyratration decreased the binding affinity of these steroids to 10% for 20α-dihydroprogesterone and 4% for testosterone without changing the binding affinity of progesterone. Two aliquots of the same plasma samples were analysed, one by means of a column chromatographic

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separation (Murphy, 1970) and the other processed by our method. With our rapid technique the overestimation compared with purified samples was never found to exceed 20\% in estimation of 20 plasma samples.

**Accuracy.** The recovery of progesterone was studied by adding 0·1, 0·5, 1·0 and 2·0 ng progesterone in ethanol to test tubes and assaying the samples as described above. When 0·1 ng progesterone was added, 0·10 ± 0·02 (s.d.) ng was found; 0·5 ng gave 0·49 ± 0·09; 1 ng gave 0·96 ± 0·12 and 2 ng gave 2·10 ± 0·20. The recovery of 0·5 and 2·0 ng progesterone added to 0·1 ml follicular plasma was also studied. The results obtained were 0·42 ± 0·10 and 1·85 ± 0·20 ng respectively (10 replications for each experiment).

**Precision.** The coefficient of variation was dependent on the level of progesterone and varied from 10 to 20\%.

This method has been applied to the determination of progesterone from the 6th to the 40th week of pregnancy. The lowest value (15 ng/ml) was found at the 6th week, the highest (250 ng/ml) at the end of pregnancy.

We feel that this simple and sensitive method is a useful tool for studying human pregnancy. The small volume of plasma needed may be obtained by a simple finger puncture with a heparinized capillary tube. The practicability of the technique permits the determination of 20 samples in one day by one technician. The speed of the method is due to the fact that no extraction and no chromatographic purification are needed. Further purification of the pregnant guinea-pig plasma allowed us to attain sufficient sensitivity for assaying progesterone in small volumes of plasma during the menstrual cycle.

**REFERENCES**