we have monitored cardiotocographically 44.3% of the patients. There is actually 1 cardiotocograph available for every 400 deliveries per year.

The quality of the tracing, usually obtained by external monitoring, is qualitatively excellent in 88% of all cases, and in nearly 100% of all cases if you consider only internal monitoring.

Naturally the use of external monitoring permits us to control the whole duration of labor, even the begining of the dilatation period when with the first contractions there sometimes appears fetal distress from placental insufficiency.

Cardiotocography is not without error; in our statistics we had 9,8% false positives and 5,5% false negatives.

The false positives are eliminated by biochemical monitoring, which is done when indicated by electronic monitoring, on the average 3,7% of all cases.

More serious is the incidence of false-negative, due in some part to the bad quality of the tracings and for the rest due to the limits of cardiotocography.

Analysis of the results in terms of perinatal mortality however is very complex; you have to take into account that many patients are admitted only at the moment of delivery without an adequate monitoring of the pregnancy and thus the fetal mortality rate between the 28th week and delivery escapes any real possibility of control.

Even more difficult is the evaluation of perinatal morbility and most of all the outcome at some distance; for that reason there are in couse for those patients who were monitored during pregnancy and the delivery, evaluation of results up until 2 years.

In conclusion we feel that the monitoring of the pregnancy and delivery, even if it needs notable economic and technical assistance, and sometimes furnishes results constitutes an unrenounceable method in the field of modern obstetrical assistance.

Radial immunodiffusion: a new method for the evaluation in the serum of the placental lactogenic hormone

by

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The term placental lactogenic hormone (HPL or HCS) was coined in 1962 by Josimovich *et al.* (1) Subsequent studies have demonstrated that this hormone is synthetized and secreted by the cells of the placental trophoblastic syncytium (2). Spellacy *et al.* (3), had already recognized, in 1967, that analysis of HPL carried out on the serum of a pregnant woman could be used as a functional placental test throughout pregnancy. Many opinions to-day confirm that this can be done, although there may be some risk in certain circumstances due to functional in-

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volvement of the placenta. In such cases, according to Spellacy ([4,5]), the serum levels of HPL not only foretell the possible death of the foetus, but are correlated with the state of health of the newborn infant.

One problem that restricted the clinical use, up to a few years ago, of the serum analysis of HPL, was the fact that in order to obtain it, a very costly and sometimes complicated radio-immunological apparatus was essential. With the aim of solving this far from simple problem, a system of immunodiffusion was devised and developed.

This study, which follows that of Spellacy, has the aim of demostrating, further, that the radial immunodiffusion method is easy to carry out and provides results that can be correlated with those obtainable with the radio-immunological method; especially when the quantity of the HPL varies between 1-9 micrograms/ml of serum or plasma.

MATERIAL AND METHODS

The technique described here is that used with radial immunodiffusion, employing the kits available under the trade name « *Plac-♀-Gest* ».

1. Remove the plate from the protective plastic bag.
   Remove the wrapper containing the plate.
   N.B. The plastic bag and the plate wrapper are intended to avoid humidity and to protect the agar, prolonging the period of sterility of the contents, which will then remain effective for one year, and will also make it possible to use only one kit for the whole duration of pregnancy, thus eliminating problems of standardization.
2. Fill the small chambers contained in the plates with a certain amount of serum from the patient and with three reference HPL sera (3, 6 and 9 mcg/ml), using a capillary pipette and a small chamber of agar for each of the three quantities of HPL serum.
   N.B. The clarity and uniform volume of the agar, as well as its diameter and position, will ensure reliable results. The chambers are numbered to facilitate identification, while the plate is provided with sufficient space to write the name of the patient from whom the serum came.

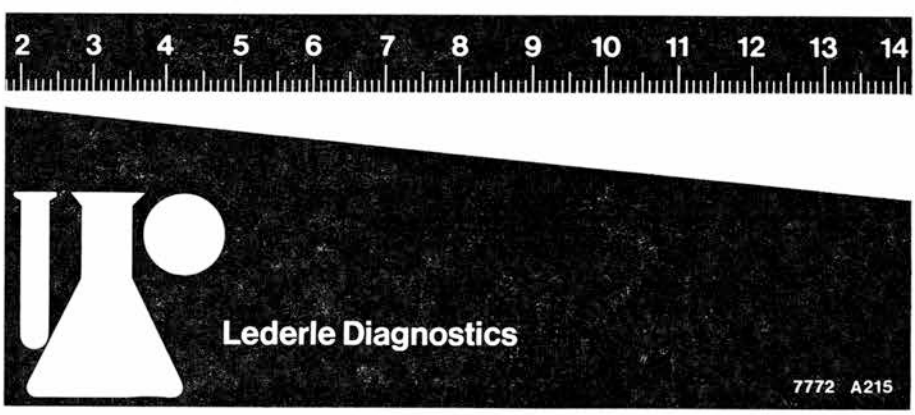

FIG. 1 - Instruments needed in order to measure the annular diameter of the precipitate.

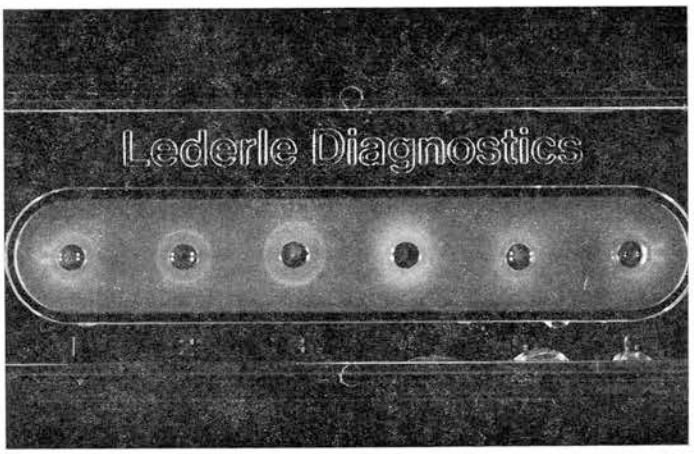

FIG. 2 - Example of annular precipitation.

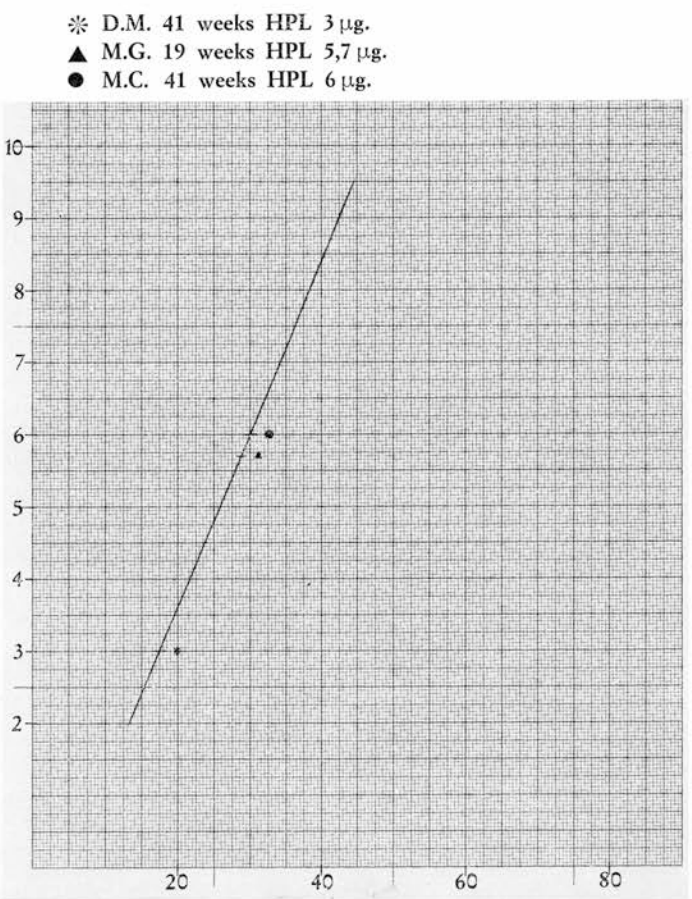

FIG. 3 - Standard curve for evaluation of analysis obtained with radial immunodiffusion.

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3. Incubate the whole for 18-20 hours at room temperature in a humid place.
   N.B. Use a humid room or re-seal the plastic bag with adhesive tape after having installed 2 or 3 drops of water into it.
4. Immerse the plate: I) into the triple buffer solution for 2 hours; II) into the copper sulphate solution for 30 minutes; III) into distilled water for 10 minutes.
   N.B. This is the first circular immunodiffusion process to be determined on a minimum of one microgram of antigen/ml.
5. Use the measuring instrument enclosed with the kit in order to measure the anular diameter of the precipitate (Figs. 1 and 2).
   N.B. The exact determination of the rings of the precipitate will simplify the reading and will make evaluation more accurate.
6. Prepare a standard curve, disregarding the concentration of the three reference HPL, with twice the diameters of their annular precipitates. Evaluate any serum not definitely shown, by reading the concentration in relation to the square of their diameters (Fig. 3).
   It is easy to deduce that the results will be obtained from the data given above in less than 24 hours, without any need to employ particularly sophisticated apparatus.

Samples of blood were obtained, at various times, during pregnancy (from the 9th to the 40th week), from 89 women. In 59 cases the HPL was determined simultaneously by both radial immunodiffusion and the radio-immunological method. The serum was obtained by centrifugation. In the remaining cases the HPL was determined exclusively by radial immunodiffusion.

Whenever the concentration of HPL was in excess of 9 μg/ml, due to the impossibility of measuring it exactly by means of radial immunodiffusion (the graph was arranged for evaluations not exceeding 9 μg/ml), any such statistics were discarded. However, if it is necessary to evaluate such data, this could be done by dilution of the serum, and attempting a fresh analysis.

RESULTS

The results obtained from a comparison between the radio-immunological determinations and those from radial immunodiffusion provide statistical support for the validity of the method studied here. In fact, in 59 cases in which the HPL was determined simultaneously with both methods, the coefficient of correlation was: $r = 0.593$ and $p < 0.0005$. These findings are confirmed by the results previously obtained by Spellacy ([6]): $r = 0.989$ and $p < 0.0001$.

CONCLUSIONS

In agreement with Spellacy's report ([6]) it may be concluded that the radial immunodiffusion test is a particularly exact method for the evaluation of the HPL especially when it is between 3 and 9 μg/ml, while for values less than 3 μg/ml or more than 9 μg/ml, the radio-immunological method is a more precise way of quantifying the hormone in question.

SUMMARY

The authors, in determining the HPL, took into consideration a method based on radial immunodiffusion. In 59 of their 89 cases, the HPL was determined at the same

time by the radial immunodiffusion method and by the radio-immunological technique. In these cases the coefficient of correlation between the two methods was: $r=0.593$ and $p<0.0005$.

The authors conclude that the radial immunodiffusion test is particularly precise for the evaluation of HPL in the serum when it is between 3 and 9 μg/ml, but for values less than 3 μg/ml or more than 9 μg/ml the radio-immunological method is more exact.

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Variations in acetylcholine esterase in relation to gestational age

by

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Acetylcholine esterase is undoubtedly one of the enzymes most studied with regard to its specificity and its physiological role. Various acetylcholine esterases exist, of different derivation, which differ from one another in their kinetic properties and their sensitivity to some inhibitors. There are « true » acetylcholine esterases, that have the well known physiological action of hydrolysing « non-specific » acetylcholine esterases (pseudo-cholinesterases, physostigmine-sensitive acetylcholine esterases). In order to obtain a correct determination of acetylcholine esterase activity, the esters of choline should be used as substrates and in addition sensitivity to physostigmine should be tested.

12-13 isoenzymes have been demonstrated in human serum, of which one fraction (ChE_7) constitues approximately 80% of the total activity ([1]). These isoenzymes are differentiated by their molecular weight and probably by the number of similar substrates.

The acetylcholine esterases belong to the group of enzymes already secreted in physiological conditions in the plasma, even though their biological function has not yet been identified. Recently it has been claimed by Chary *et al.* ([3]) that this enzyme has an important part to play in the metabolism of choline and indirectly in that of lecithin.

It has long been known that the diminution of acetylcholine esterase activity in the serum has the clinical significance of insufficient liver function; however, the blood level does not always reflect the capacity for hepatic synthesis. The normal

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