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variations in the alkaline phosphatase that are attributable to the placenta, the activity of the other enzymes was increased at the latter end of the pregnancy, probably in relation to the functional overload on the hepatocytes on the part of the steroids.

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Circadian rhythm of plasma progesterone in pregnant women at term

by
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The analysis, in the various maternal biological fluids, of protein hormones, steroid metabolites and enzymes of placental origin, made possible by increasingly precise and sophisticated analytical methods, is constantly gaining importance in the monitoring of the foeto-placental unit in normal and pathological pregnancy.

The controversial results obtained with other hormones (HPL, HCG, oestriol) have recently directed the interest of investigators towards progesterone and the very important part played by this hormone in the various stages of pregnancy, from the implantation of the ovum to delivery.

Clinically, the production of progesterone can be assessed by determining the amount of pregnanediol excreted in the mother's urine.

There are some factors that limit the value of this indirect method of evaluation of the metabolism of progesterone:

a) pregnanediol represents only 20% of the progesterone secreted ([1]); *b*) evident reduction of pregnanediol secretion is observed only in certain pathological situations, characterized by well defined disorders affecting the tissues that produce progesterone ([2]); *c*) its excretion remains apparently unchanged, even after the intra-uterine death of the foetus, in the presence of a retained, functioning placenta ([3]); *d*) the changes in its excretion under pathological conditions, e.g. in diabetes, are not rapid enough to allow intervention to prevent injury to the foeto-placental unit ([4]).

The inadequacy of urinary secretion of pregnanediol for the purposes of sufficiently exact evaluation of the changes in its metabolism, on the one hand, and the development of precise and rapid radio-immunological methods for the evaluation of progesterone levels in the blood, on the other, have in recent years directed the interest of investigators towards the analysis of progesterone in the plasma, on the assumption that such plasma values may be better correlated with actual production, whether in normal or in pathological pregnancy.

Since 1954, the year in which Zander succeeded in identifying and measuring

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progesterone in the peripheral blood of a pregnant woman, a number of studies have been carried out with the aim of stating more precisely its plasma concentrations during the various phases of pregnancy. But although we now have numerous data on the blood levels of this hormone before the 20th week of pregnancy ([5,6,7,8]), we have, on the contrary, only limited and contradictory information on the daily variations of the plasma concentrations of progesterone in pregnancy at term ([9,10]).

In this study we have set ourselves the aim of discovering whether daily variations in the blood levels of progesterone can exist in a group of pregnant women close to term, and if so, their extent.

MATERIAL AND METHODS

Our investigation was carried out on six pregnant women who had been admitted to the Obstetrical and Gynaecological Clinic of the University of Genoa, and were aged between 24 and 33 years (mean age, 28 years). Four of them were primiparae and two multiparae. The duration of pregnancy varied from 38 to 41 weeks. None of the subjects under consideration had presented with complications during the course of pregnancy; five were delivered normally by the vaginal route and one abdominally, due to anomalous presentation. All the newborn infants were in good health.

Samples of 10 ml of peripheral venous blood were obtained from each subject every 3 hours for 24 hours, starting at 10 a.m. This blood was immediately centrifuged and then stored at – 15° C until required for analysis.

We used the Sorin kit for the radio-immunological determination of progesterone in the blood. This procedure involves various stages, from the extraction of progesterone from the blood to the incubation of the text mixtures for 30 min. at 37° C and for 2 hrs at 4° C, the adsorption of free progesterone on to charcoal-dextran, and finally centrifugation at room temperature and calculation of the supernatant fluid.

In calculating the results, the basic mean for reckoning each group of test tubes was first determined, and then the binding capacity of the system of analysis was calculated as a percentage ratio between the mean relating to the « standard zero » point and the mean relating to total activity. The binding capacity was normally between 30 and 50%.

The percentages calculated for the standards were plotted on a linear-linear graph in relation to the progesterone content. In this way a calibration curve was calculated. Then the progesterone content of each sample being tested was read directly on the calibration curve.

All the determinations were made in duplicate. The coefficient of variation of the duplicates, calculated on the first 20 samples examined, was 7.2%. Any determinations with differences in the duplicates, as compared with the mean value, greater than ± 10.3%, were considered to be unreliable and were therefore repeated.

The values obtained were worked out by means of an « Intertechnique Multi 4 » computer.

RESULTS

Altogether about 150 determinations were made on samples of peripheral venous blood in six pregnant women. The results obtained are shown in Table I, while

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Table 1 - *Daily variations in plasma progesterone (ng/ml) in six pregnant women at term. a_i=mean individual daily value of plasma progesterone.*

Hours	10 $^{a}1$	13 $^{a}2$	16 $^{a}3$	19 $^{a}4$	22 $^{a}5$	1 $^{a}6$	4 $^{a}7$	7 $^{a}8$	10 $^{a}9$	
P.L.	24.4	25.5	30.3	27.8	33.6	37.3	39.2	45.5	28.5	32.45
P.E.	68.2	58.6	48.3	38.9	55.9	46.6	50.7	49.2	57.4	52.64
F.A.	39.2	32.7	31.1	39	43	48.6	47.9	45.7	38.3	40.61
L.R.	28.7	29.5	29.1	32	27.1	33.7	38.8	38.1	30.2	31.91
T.G.	26.4	29	23.5	32.4	28.6	25.3	34.9	26.3	29.2	28.39
S.C.	33.5	30.9	29.7	40.3	24.4	24.6	34.3	28.1	27.3	30.34
M.	36.73	34.36	32	35.06	35.43	36.01	40.96	38.81	35.14	36.05
D.S.	16.31	12.10	8.43	5.03	11.97	10.22	6.80	9.71	11.58	
E.S.	6.65	4.94	4.44	2.05	4.89	4.17	2.78	3.96	4.72	

M=mean D.S.=standard deviation ES=standard error

in Fig. 1 the daily variations in the plasma concentrations of progesterone are shown graphically for each of the six women.

In all cases a more or less marked tendency for the plasma progesterone to increase was noted from 4 p.m. to 4 a.m., followed by a gradual decrease from 4 a.m. to 4 p.m. The individual concentrations at 4 a.m. differed on average from those at 4 p.m. by a minimum of 4.96% and maximum of 54.01%. The mean maximum value in 24 hours, obtained during the night at 4 a.m., was significantly higher than the mean minimum value in 24 hours, obtained during the day at 4 p.m. ($p<0.030$).

The analysis of variance for the values of plasma progesterone was not statistically significant, either between the groups or within them.

It was interesting to note that the individual concentrations at 10 a.m., i.e. at

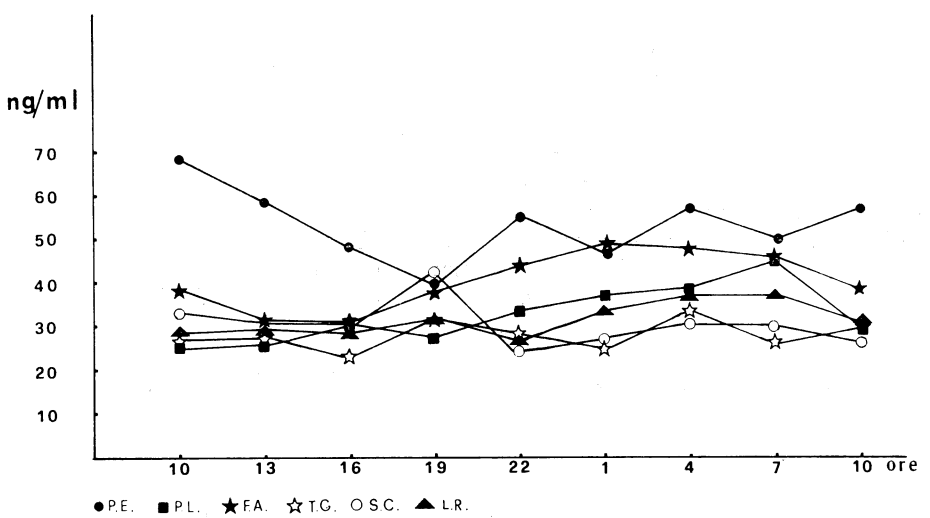

FIG. 1 - Daily variations in plasma progesterone during 24 hours in the six subjects examined.

the start of the test period, did not significantly differ from those recorded at 10 a.m. on the subsequent day, i.e. at the end of the test period, in all the subjects examined.

To prove that it was the intervals of time and not other factors that were not considered, which produced the variations encountered during the 24 hours, we made a comparison between the means by Neumann-Keuls' method. At the different hours considered, these differed significantly from one another ($p < 0.05$).

DISCUSSION

The findings reported by various authors on the daily variations in the plasma concentrations of progesterone in the last trimester of pregnancy are scanty and somewhat controversial.

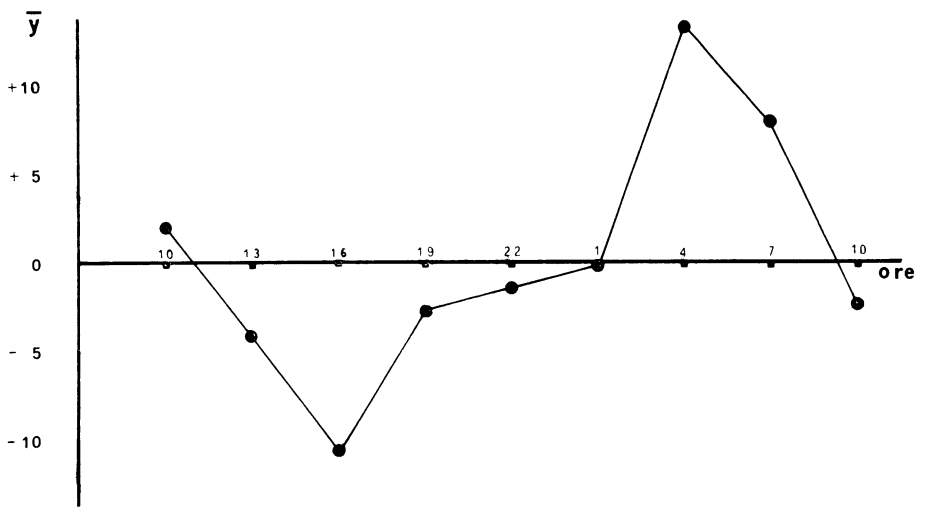

FIG. 2 - Daily variations in plasma progesterone expressed as a percentage of the mean value for all the subjects in 24 hours.

Wiest ([9]), in determining the concentrations of plasma progesterone every 4 hours for 24 hours, did not observe any significant daily variations. Craft *et al.* ([10]), in five pregnant women between the 36th and 40th week, found definite diurnal changes in the individual plasma progesterone values, but did not succeed in identifying any circadian rhythm in these variations.

Static evaluation on the basis of two factors of variance, on the values we obtained during 24 hours in the six patients studied, showed a statistically significant variation, especially between the maximum and minimum values, of the daily levels of plasma concentrations of progesterone.

In agreement with other authors ([11]), a circadian rhythm of progesterone in the blood was demonstrated, with maximum values at 4 a.m. and minimum values at 4 p.m. (Fig. 2). This rhythm is evident, despite the marked percentage variations in the hormone values that may be found among the subjects examined, during the different intervals of time considered.

These different periods of time, and the limited number of samples obtained

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in 24 hours from some of them, may partly explain the contrasting results obtained by various investigators. Certainly other factors may yet be responsible, but two of these seem to need special consideration: the sensitivity and the precision of the different methods of determination used.

But as regards the reasons for the daily variations in the levels of plasma progesterone in pregnancy at term, these are still far from clear.

The problem is further complicated by the fact that the exchange of progesterone between the different compartments of the foeto-placental unit (mother-foetus-placenta-amniotic fluid), characterized by selective permeability, makes the calculation of the different amounts of secretion extremely complex ([12]), so much so that at present there is practically no possibility of establishing correctly *in vivo* the amount of placental secretion.

In this connection, we cannot neglect the complex correlations existing between the plasma concentrations of progesterone and protein catabolism ([13]) and, most of all, between plasma progesterone and corticosteroid activity, so that some authors ([14,15,16]) have assumed progesterone to play an important role in the regulation of foetal steroid synthesis. It has been seen recently, at any rate, that the administration of dexamethasone and ACTH does not absolutely alter the concentrations of plasma progesterone either in the mother or in the foetus. This finding evidently provides confirmation for the fact that neither the maternal nor the foetal cortex contributes significantly to the production of progesterone in pregnancy at term.

Today, in fact, the majority of authors ([7,17,18]) agree that in advanced pregnancy the circulating progesterone is almost entirely produced by the placenta, starting chiefly from the maternal plasma cholesterol, to a lesser extent from other precursors (pregnenolone sulphate, etc.), whilst they consider that the foetal contribution is negligible.

It may, however, be conjectured that the daily variations in plasma levels of progesterone are none other than the reflex of analogous oscillations in the availability of these precursors. Against this hypothesis, the production of progesterone gradually increases during pregnancy in relation to the volume of the placenta, while only small modifications are produced because of fluctuations in the levels of serum cholesterol.

A theory that keeps closer to the fundamental and almost exclusive role of the placenta in the production of progesterone is that the circadian rhythm of the plasma progesterone is related to the variations in activity of the placental enzymes responsible for the conversion of the precursors of progesterone or to the modifications in activity of the plasma proteins that bind the progesterone.

We can neglect entirely the fact that in our case material the maximum values were obtained during the first hours of the morning, after the night-time rest. This could only have a positive effect on the placental blood flow, and cannot account for the changes encountered.

It seems to us, however, that the numerous correlations that exist between progesterone and other endocrine functions or aspects of protein, salt and water exchange, the lack of knowledge concerning the more intimate and complex aspects of the production, distribution and function of the hormone during pregnancy, do not enable us to state whether the circadian rhythm that we found is, or is not, the expression of an intrinsic rhythm of plasma progesterone.

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The diagnostic evaluation of iso-immunization due to the Rh factor

by

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The remarkable prophylactic successes obtained by means of IgG anti D in preventing immunization due to the Rh factor have recently resulted in a drastic reduction in the number of cases of Rh immunization. Further organizational progress and the formation of a more mature health knowledge at all levels leads one to think that cases of Rh iso-immunization will become still rarer.

Nevertheless, Rh iso-immunization cannot disappear altogether, and the obstetrician will still find himself in the position (even if very infrequently) of having to take decisions concerning a pathological condition of which everyday practice and his personal experience will more and more have lost sight.

We have therefore thought it worth while to collect together the results we have obtained in 4 years of studying the Rh problem, in order to define the limits of a grave diagnosis and the correctness of a therapeutic operation. This seems permissible statistically because of the large number of cases that we have observed, though what the future situation may be cannot be determined.

In assessing a case of iso-immunization due to the Rh factor, the obstetrician must establish whether the condition of the foetus is such as to allow the pregnancy to continue, or whether it justifies the induction of labour and possible

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