Urinary excretion of pregnanolone and pregnadiol and relative ratios of steroids to creatinine in urine during the 3rd trimester of pregnancy: the influence of diuresis and diurnal and day to day variations

by

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The discovery of the existence of a correlation between excretion of progesterone and its metabolites (pregnanalone, pregnandiol and pregnantriol) and placental function goes back to the nineteen thirties ([1, 2]) and represents the beginning of hormone chemistry in pregnancy ([3]). At that time, however, the determination techniques could not be carried out quickly and therefore did not allow the extensive study of cases; consequently, general, investigations carried out at that time are not accompained by complete statistical analysis.

The importance of the 21-carbon steroids, as a measure of foeto-placental function, has been reappreciated especially following studies that have shown that a considerable proportion of these end products present in the urine during pregnancy may be derived from the foeto-placental unit itself ([4, 5, 6, 7, 8, 9]). On the other hand, the use of levels of excretion of these end products as a parameter for measuring foeto-placental function presupposes the availablity in practical medicine of precise data regarding the characteristics of excretion of these hormones obtained from statistically valid analysis.

MATERIALS AND METHOD

6-hour urine specimens (7-13; 13-19; 19-1; 1-7) from pregnant patients between the 30th and 38th week of pregnancy, with values for hormonal excretion within normal limits, have been examined; in addition, from the second fraction, the sample taken between 13.00 hr and 15.30 hr was examined (rapid method = r). Pregnanolone (5 βpregnane-3α, olo-20 one) and pregnandiol (5 βpregnane-3α, 20α-diol) were estimated in the urine of each fraction and in the 24 hour pool with the semiautomatic gas chromatographic method of Scommegna-Chattoray ([10]). The results have been expressed as absolute values (mg/hr) and as the ratio of steroid to creatinine. The determination of creatinine was carried out by an adaptation of the Folin method (Carlo Erba kit).

STATISTICAL ANALYSIS AND RESULTS

The procedure adopted in these studies to evaluate the statistical reliability of the results is the same as that used by our group in previous studies on oestrogens ([11]).

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The problems which we needed to study for their practical importance were the following:

1) Investigation of the existence of a possible correlation between the extent of diuresis and that of hormone excretion.

2) Investigation of the possible presence of a daily rhythm in the urinary excretion of these hormones, and estimation of the daily variations.

3) Investigation of the presence and the extent of day to day variations.

1) The practical importance of seeing if there are correlations between the amount of diuresis and the quantity of hormone excreted is obvious, and is a subject on which very few data are available ([12, 13, 14, 15, 16]). We therefore calculated the coefficient of simple linear correlation between the values for diuresis and the absolute values for creatinine, pregnanolone and pregnandiol in the urine over 24 hours, and between diuresis and the steroid/creatinine ratio in the urine from the 13-15.30 fraction. This fraction is the way in which we usually express our findings. The data obtained are shown in Tables 1 and 2.

Table I. *Coefficient of simple linear correlation between diuresis and quantity of creatinine, pregnanolone and pregnandiol in 24 hour sample.*

Diuresis 24 hr.	⟶	Creatinine (g) 24 hr. =—0.3539
Diuresis 24 hr.	⟶	Pregnanolone (g) 24 hr.= +0.2749
Diuresis 24 hr.	⟶	Pregnandiol (g) 24 hr. = +0.0000

Table II. *Coefficient of simple linear correlation between diuresis and the ratio pregnanolone/creatinine and pregnandiol/creatinine in the 13-15.30 fraction.*

Diuresis 13-15.30	⟶	Pregnanolone/Creatinine 13-15.30= +0.0141
Diuresis 13-15.30	⟶	Pregnandiol/Creatinine 13-15.30 =—0.1286

Examination of the tables shows that none of the coefficients of correlation differs significantly (at a level of 5%) from a correlation value of zero. One can consider (with probability equal to 95%) that there is no correlation between the amount of diuresis and the quantity of hormone excreted with regard to the 24 hour specimen value and for the data obtained with the rapid method.

Testing of the Null Hypothesis (hypothesis of a lack of correlation between diuresis and the quantity of hormone excreted) has been carried out using the t test, given by the following expression:

$$t = \frac{r_{sample}}{\sqrt{\frac{1 - r_{sample}^2}{n-2}}}$$

where r_{sample} = value of the coefficient of correlation obtained for the sample; n = number of samples (in our case 27).

2) The importance of finding out if there is a daily rhythm in urinary excretion of these hormones also has considerable practical importance if one wishes to carry out determination on partial collection of urine (rapid procedure) in order to avoid the well known inconveniences of collecting urine over a 24 hour period. For determinations on partial collections to be valid, there must be a good correlation between values of the fractions used and values found in 24 hour samples. With this aim, we have carried out determinations of hormones and of creatinine

on five fractions of urine (7-13; 13-15.30; 13-19; 19-1; 1-7) and on the 24 hour pool.

Using statistical investigations we wanted to ascertain:

a) the existence of a possible correlation between values obtained in the five fractions and those for the 24 hour pool;

b) the possible existence, limited to the 5 fractions, of significant differences between the coefficients of correlation obtained.

For the first point (correlation of the various fractions with the values for the 24 hour pool), the coefficients of simple linear correlation were calculated for absolute values and for the steroid/creatinine ratio for the single fractions with corresponding data for the 24 hour pool. It is seen that the coefficients obtained were sufficiently high and always differed significantly from zero at a level of significance of 5% (Table III). For examination of the significance, the t test mentioned above was used.

Table III. *Coefficient of correlation between the single fractions and the 24 hour pool.*

	7-13	13-19	19-1	1-7	13-15.30	
Pregnanolone mg	0.65	0.72	0.76	0.71	0.71	T 0.594
Pregnandiol mg	0.67	0.62	0.72	0.78	0.75	T 1.361
Pregnanolone Creatinine	0.55	0.72	0.50	0.70	0.50	T 2.29
Pregnandiol Creatinine	0.52	0.77	0.52	0.63	0.64	T 3.162

With respect to the second point (possible significant differences between the coefficients of correlation) we went on to test the hypothesis of non-existence of a significant difference. The calculations carried out have confirmed the hypothesis at a level of significance of 5% with a t wich is approximately distributed according to χ^2 with K-I degrees of freedom. The experimental values for t are reported in Table (III) above. In addition, to obtain further evidence for the possible existence of a daily rhythm in the urinary excretion of steroid metabolites with 21 carbon atoms, we have calculated the percentage differences between the values of the prenanolone/creatinine and pregnandiol/creatinine ratios respectively for the single fractions and for the 24 hour collection.

The existence of a non-random distribution of the percentage variations of the 4 fractions (7-13; 13-19; 19-1; 1-7) around the mean zero could indicate the presence of a daily rhythm, assuming that there is uniform behaviour in the individual patients.

From the examination of data shown in figures 1 and 2 one sees in the intervals $M \pm 2 \times S.E.M.$ (where M is the mean of the percentage variations and S.E.M. is the standard error of the mean) that there is always the zero except in the first fraction, indicating the absence of a daily rhythm. All the results concerning the problem of daily variations correspond to those already found by our group for oestrogens ([17, 11]).

3) The practical importance of the determination of the presence and the extent of variations in the day to day excretion appear clear whenever studies are carried

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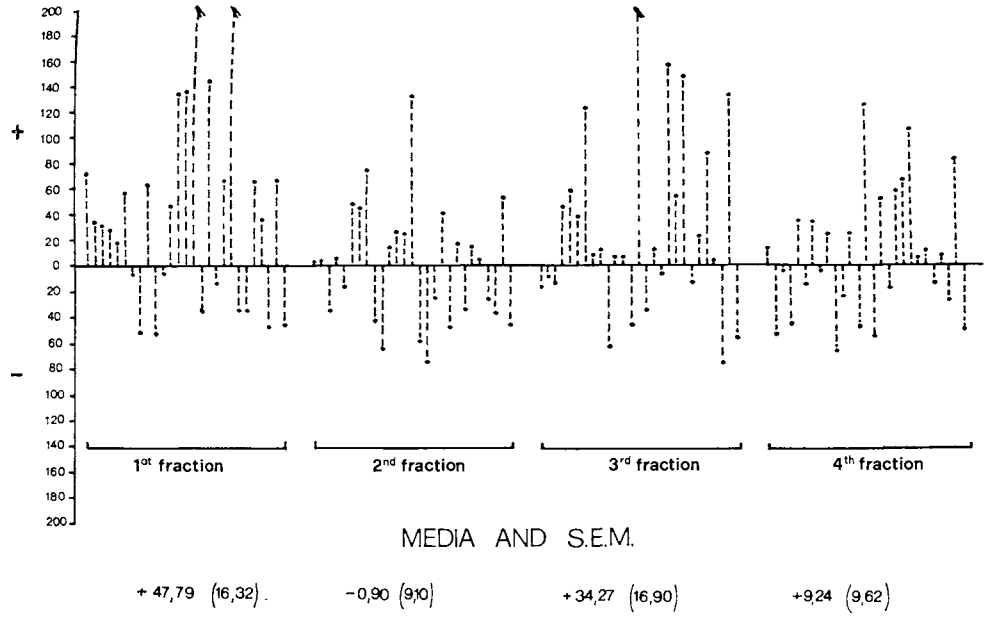

FIG. 1 - Percentage difference between the ratio pregnanolone/creatinine of the four daily 6-hour fractions and that of the 24 hour pool.

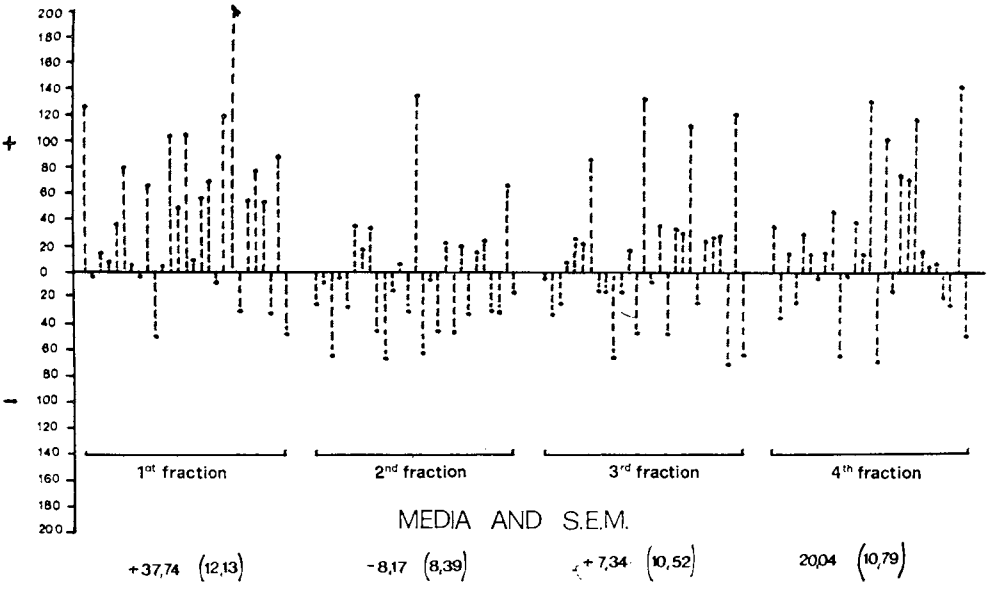

FIG. 2 - Percentage difference between the ratio pregnandiol/creatinine of the four daily 6-hour fractions and that of the 24 hour pool.

out on the development of pregnancy and whenever one wishes to use hormonal data as a parameter for the evaluation of possible therapies aimed at reestablishing a jeopardized foetal-placental function.

In such a case, in order to say that a therapy has been effective, it is necessary for the variations in the level of hormone excretion brought about by the treatment to exceed the value of the natural daily variations.

The statistical investigation aimed at establishing these variations for urinary pregnanolone and pregnandiol has been carried out on data obtained using daily determinations of these hormones in the urine of 6 women who were observed (on average) for 7 consecutive days.

The results have been expressed as absolute values (mg) and also as the steroid/creatinine ratio for the 24 hour sample and the steroid/creatinine ratio in the 2½ hour sample. On these data the following statistical indices have been calculated.

a) The relative percentage differences ($\Delta\%$) and their distribution in class intervals after having suitably eliminated the variations above the interval: arithmetic mean $\pm$ 2 times the mean square deviation.

b) The coefficient of variation, which constitutes a term of comparison of the absolute variability between the various parameters (Table IV).

c) The modal distribution of the variations (Fig. 3 and 4 and Table V).

From examination of Fig. 3 it appears that the day to day variations most

Table IV. *Coefficient of variation of the day to day variations for pregnanolone and pregnandiol expressed in absolute values and as the ration steroid/creatinine (24 hour urine).*

	P_1 mg	P_2 mg	Ratio P_1/C	Ratio P_2/C
Coefficient of variation	1.19	0.90	1.16	0.87

Table V. *Percentage frequency of the day to to day variations of the parameters: steroid mg/24 hr; steroid/creatinine for the 24 hr sample; steroid/creatinine for the sample collected over 2½ hr(r).*

	Frequency of the variations within:			
	50%	40%	30%	20%
Pregnanolone mg/24 hr	70.73%	65.85%	46.34%	24.39%
Pregnanolone/C 24 hr	63.41%	39.02%	31.70%	24.30%
Pregnanolone/C r	80.00%	75.00%	60.00%	25.00%
Pregnandiol mg/24 hr	80.48%	63.41%	46.34%	29.26%
Pregnandiol/C 24 hr	68.29%	51.21%	36.58%	21.95%
Pregnandiol/C r	77.27%	63.63%	45.45%	22.72%

Table VI. *Average day to day percentage variations*:

Pregnanolone mg/24 hr	53.25%
Pregnanolone/C 24 r	66.65%
Pregnanolone/C r	31.16%
Pregnandiol mg/24 hr	39.57%
Pregnandiol/C 24 hr	49.03%
Pregnandiol/C r	36.34%

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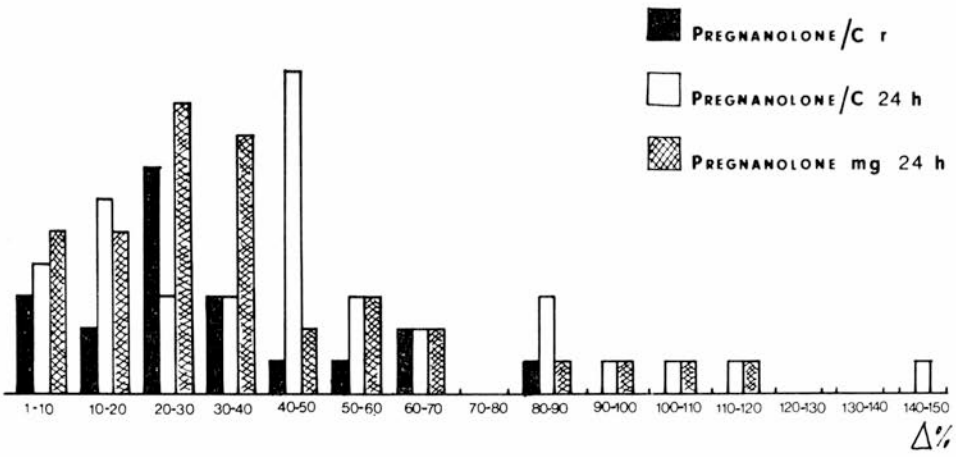

FIG. 3 - Frequency distribution of the percentage day to day variations in the excretion of pregnanolone in the 24 hr sample (absolute value and ratio steroid/creatinine) and in the 2½ hr fraction=r (ratio steroid/creatinine)

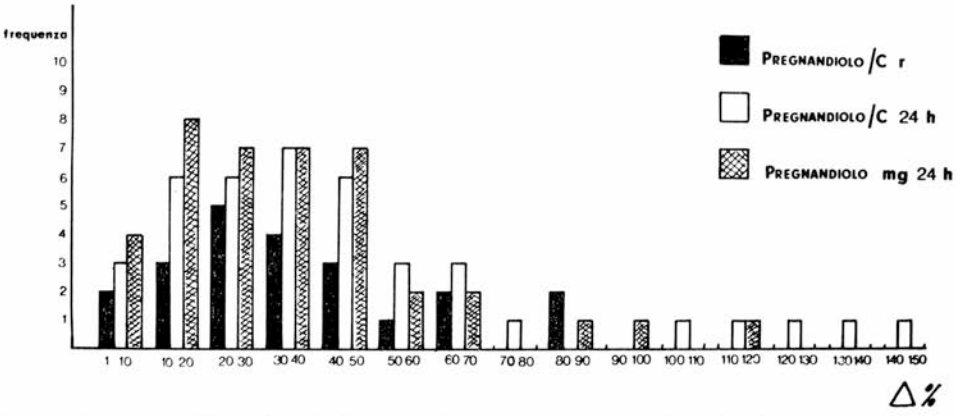

FIG. 4 - Frequency distribution of the percentage day to day variations in the excretion of pregnandiol in the 24 hour sample (absolute value and ratio steroid/creatinine) and in the 2½ hr fraction=r (ratio steroid/creatinine).

frequent are those included in the class interval 20-30% for pregnanolone mg/24 hours; in the class interval 40-50% for the ratio pregnanolone/creatinine on the sample collected over 2½ hours.

From examination of Fig. 4 it appears that the most frequent variations are those included in the class interval 10-20% for pregnandiol mg/24 hours; in the class interval 30-40% for the ratio pregnandiol/creatinine 24 hours and in the class interval 20-30% for the ratio pregnandiol/creatinine on the sample collected over 2½ hours.

In addition (Fig. 3 and 4) it can be seen that the distribution of the variations shows a positive asymmetry in the sense that most of the variations are included in the classes 1-10 and 40-50%.

d) The mean of the percentage variations (which appear in Table VI).

CONCLUSIONS

From the present investigations the following conclusion can be drawn:

1) The amount of pregnanolone and pregnandiol excreted in the urine is not influenced by the extent of diuresis, unless this is markedly altered by drugs or water intake.

2) There are no marked variations in the excretion of these two compounds over a 24 hour period.

3) On the other hand, the day to day excretion does not show the same consistency; this behaviour is common to all the steroid hormones in pregnancy and also reported for plasma pregnandiol ([18]). These variation which have been defined by the present investigation do not, however, undermine the validity of urinary estimation, especially in the field of mass screening and in serial studies during pregnancy, particularly since the variations found by using our rapid method, which is particularly useful in this type of investigation, were the lowest when compared with other means of expressing hormonal values.

SUMMARY

A statistical investigation has been carried out in order to establish if the urinary excretion of metabolites of progesterone (pregnanolone and pregnandiol):

- is correlated with the extent of diuresis;
- shows daily variations;
- shows day to day variations and to what extent.

The determination of hormones has been carried out by a semi-automated gas chromatographic method. The results of the determinations have been expressed as absolute values and as the ratio steroid/creatinine.

The following conclusions have been arrived at:

1) There is no correlation between the amount of diuresis and the quantity of hormone excreted.

2) The values obtained in the determination of each of the 5 fractions of urine taken over 24 hours are well correlated with the 24 hour pool.

3) There is no daily rhythm in the excretion of these hormones.

4) Day to day variations, however, do exist, of which the relative percentage differences and the distribution for class intervals are calculated.

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Flat metaplasia of the human uterine cervix revealed by the scanning electron microscope

by

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The squamo-columnar junction is of considerable importance in gynaecological oncology.

The pronounced restlessness of the two epithelia at their meeting point marks the spot where the development of a neoplastic transformation is most likely.

In our studies of the female genital tract by a scanning electron microscope (SEM) we have therefore examined the cervix with particular attention.

These transformation zones are among the most frequent and striking lesions in this area.

Until now it was thought that the repair process consisted largely of the replacement of ectopic cylindrical epithelium by flat epithelium.

However, our observations have shown that at least in some cases a metaplasia process (often pluricentric) begins mainly around the outlet of the glandular orifices.

The confluence of several metaplastic zones then brings about the transformation of the cylindrical epithelium into flat epithelium.

This metaplasia, seen under the scanning microscope ([1]), is characterised by the enlargement and flattening of the cell surfaces and by the appearance of marked cell borders, plasma bridges and microvilli.

The transformation process is initially more marked around the glandular orifices; at the end of its development it extends over the entire ectopic surface, which is covered by a definitely flat epithelium.

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